



Pharmacologie de la morphine chez les sujets obèses avant et après chirurgie de l'obésité

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UNIVERSITÉ PARIS DESCARTES
FACULTÉ DES SCIENCES BIOLOGIQUES ET PHARMACEUTIQUES
ÉCOLE DOCTORALE DU MÉDICAMENT

THÈSE

Pour l'obtention du grade de DOCTEUR DE L'UNIVERSITÉ PARIS DESCARTES

Présentée et soutenue publiquement par

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le 19 avril 2013

PHARMACOLOGIE DE LA MORPHINE CHEZ LES
SUJETS OBESES AVANT ET APRES
CHIRURGIE DE L'OBESITE

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" La volonté est tellement libre de sa nature, qu'elle ne peut jamais être contrainte."

Citation de René Descartes ; Les passions de l'âme - 1649.

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LISTE DES ABRÉVIATIONS

| | |
|-------|---|
| AMP | Adenonsine Monophosphate |
| ABC | ATP Binding Cassette Family |
| AC | Acide cholique |
| ACDC | Acide Chénodésoxycholique |
| Ahr | Récepteur aux hydrocarbures aromatiques |
| AUDC | Acide ursodéoxycholique |
| BCRP | Breast Cancer Resistance Protein |
| BHE | Barrière Hémato-Encéphalique |
| BMC | Masse Calcique |
| RYGB | Bypass Gastrique |
| C/EBP | CAAT/enhancer-binding protein |
| CAAT | cytidine-cytidine-adenosine-adenosine-thymidine |
| CAR | Récepteur aux androgènes |
| Cmax | Concentration Maximale |
| COMT | Catechol-O-Methyl-Transférase |
| CRP | C-Réactive Protéine |
| CYP | Cytochrome P450 |
| DEXA | Dual-X-Ray Absorptiometry |
| DFG | Débit de Filtration Glomérulaire |
| DR | Direct-Repeat |
| FEVG | Fraction d'éjection du ventricule gauche |
| FMO | Flavin containing |
| FPR | Flux Plasmaticque rénal |
| FXR | Récepteur X Farnésioïde |
| GLUT | GLUcose Transporter |
| GSTT | Glutathione-S-Transferase |

| | |
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| HNF | Hepatocyte Nuclear Factor |
| HO | Hème Oxygenase |
| IL | Interleukine |
| IMC | Indice de Masse Corporelle |
| IRM | Imagerie par résonnance magnétique |
| Kg | Kilogramme |
| LXR | Liver-X-Receptor |
| M3G | Morphine-3-Glucuronide |
| M6G | Morphine-6-Glucuronide |
| MCC | Monocyte chemoattractant protein-1 |
| MCP | Monocyte Chemotactic Protein |
| MG | Masse Grasse |
| MIP | Macrophage Inflammatory Proteins |
| MM | Masse Maigre |
| MRP | Multidrug Resistance Proteins, membres de la sous famille ABCC |
| MUG | 4-Methylumbelliferyl-b-D-glucuronide |
| NASH | Stéato-Hépatite Non Alcoolique |
| NFk B | Nuclear Factor Kappa B |
| NK | Natural Killer |
| Nrf2 | NF-E2-related factor 2 |
| OATP | Organic Anion-Transporting Polypeptide |
| Oct | octamer transcription factor |
| OPRM1 | mu-opioid receptor gene (orm1) |
| P-gp | P-glycoprotéine |
| PAI | inhibiteur de l'activateur du plasminogène |
| PBX | Pre-B-cell leukemia homeobox |
| PD | Pharmacodynamique |
| PG | Pharmacogénétique |
| PK | Pharmacocinétique |
| PPAR | Recepteur activé par les proliférateurs des peroxyosomes |
| PXR | Récepteur X des prégnanes |

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| PXR | Récepteur X des prégnanes |
| RE | reticulum endoplasmique |
| RXR | Récepteur X des rétinoïdes |
| SAA | Serum Amyloid A |
| SULT | Sulfotransférase |
| TBW | Total Body Water |
| TDM | Tomodensitométrie |
| TGF | Tumor Growth Factor |
| TNF | Tumor Necrosis Factor |
| UGT | Uridine Diphosphate Glucuronosyl-Transférase |

INTRODUCTION GENERALE

L'obésité, définie par un excès de masse grasse ayant des conséquences pour la santé (OMS 1996) est quantifiée par le calcul de l'indice de masse corporelle (IMC), rapport du poids sur le carré de la taille, exprimé en kg/m^2 , supérieur à 30. L'obésité morbide est définie par un IMC supérieur à 40. L'enquête épidémiologique ObÉpi-Roche 2012 menée dans la population adulte française (18 ans et plus) montre combien sa prévalence n'a cessé d'augmenter au fil des années ¹. L'indice de masse corporelle moyen (IMC) a progressé de $1,1 \text{ kg/m}^2$, passant de $24,3 \text{ kg/m}^2$ en 1997 à $25,4 \text{ kg/m}^2$ en 2012. Ainsi, la prévalence de l'obésité et du surpoids en France sont de 15 % et 32,3% en 2012 contre 8,5 % et 29,8% en 1997. L'obésité morbide affecte 1.2% de la population française. Cette augmentation de prévalence concerne de nombreux pays ². Entre 1980 et 2008, l'IMC du monde entier a augmenté de 0.4 kg/m^2 par décennie pour les hommes et 0.5 kg pour les femmes ². En 2005, le nombre de sujets en surpoids et obèses a été estimé à 1.6 milliards et 400 millions respectivement. Si cette tendance se confirme, 58% de la population adulte mondiale sera obèse ou en surpoids en 2030 ³.

L'excès pondéral est associé à un risque augmenté de complications métaboliques, ostéo articulaires, psychiatriques et de cancer, tel que davantage de patients obèses ont recours à des consultations médicales et à des prescriptions médicamenteuses, comme en témoigne l'augmentation des coûts pharmaceutiques et hospitaliers avec l'obésité ⁴⁻⁶. Malgré le nombre croissant de prescriptions médicamenteuses chez les sujets obèses, en rapport avec l'excès de complications mécaniques et métaboliques, les différences potentielles de métabolisme et d'effet des médicaments, en terme d'efficacité et de tolérance, sont peu connues des prescripteurs. On imagine volontiers que ces différences soient d'autant plus importantes que l'obésité est morbide.

La chirurgie de l'obésité est pratiquée de façon croissante afin de permettre une perte de poids importante et durable ⁷. Même si elle est associée à une réduction du nombre de complications de l'obésité, les sujets ayant bénéficié de cette solution thérapeutique restent susceptibles d'avoir recours à des traitements médicamenteux oraux. Ces besoins sont

comparables aux sujets de poids normal ou sont en rapport avec la persistance de complications de l'obésité.

Au cours de mon travail de thèse, j'ai souhaité m'intéresser aux facteurs de variabilité des médicaments chez les sujets obèses morbides avant et après chirurgie de l'obésité. J'ai choisi de m'intéresser plus particulièrement à la morphine, puisqu'il s'agit d'un médicament dont la marge thérapeutique est étroite et dont l'efficacité et la tolérance varient considérablement d'un patient à l'autre. Déterminer si l'obésité morbide et la chirurgie de l'obésité sont également sources de variabilité PK et PD de la morphine est un sujet incontournable compte tenu de la fréquence des douleurs rapportées chez les patients obèses, du recours croissant aux antalgiques morphiniques dans la population générale, et de leur intérêt pour le contrôle de la douleur chronique et aiguë post-chirurgicale ⁴. En absence de données PK et PD existantes, la prescription de morphine est effectivement redoutée par les cliniciens dans cette population exposée à des problèmes respiratoires. La prise en charge optimale et personnalisée des douleurs sévères des patients souffrant d'une obésité morbide permettrait pourtant de favoriser leur mobilité et d'aider leur prise en charge nutritionnelle.

Ce manuscrit s'articule en deux volets.

Le premier volet comprend des rappels bibliographiques abordant:

- 1) les modifications physiologiques associées à l'obésité et à la chirurgie de l'obésité
- 2) les modifications du métabolisme des médicaments chez les sujets obèses connues à ce jour
- 3) la morphine, son métabolisme et les acteurs de sa variabilité

Dans un deuxième volet, l'exposé de mes travaux permettra de répondre à plusieurs objectifs:

- Discuter les modifications possibles de la pharmacologie de la morphine chez les patients obèses
- Etudier la PG de la morphine chez les patients obèses
- Etudier la nociception chez les patients obèses, avant et après chirurgie de l'obésité
- Etudier la PK de la morphine orale chez les patients obèses

- Décrire l'expression entérocytaire des transporteurs et enzymes impliquées dans le métabolisme de la morphine
- Décrire l'évolution de la PK de la morphine orale après chirurgie de l'obésité de type by-pass gastrique

En annexe sont associés des cas cliniques portant sur la thématique de la pharmacologie de l'obésité (prescription d'une contraception progestative et d'un traitement anti-tuberculeux chez des patients respectivement obèse et en surpoids) et une revue de la littérature concernant la prescription d'opioïdes en post opératoire de chirurgie chez les patients obèses.

1. PHYSIOLOGIE DE L'OBESITE ET CHIRURGIE DE L'OBESITE

J'aborde, dans cette partie de l'introduction, les modifications physiologiques associées à l'obésité ainsi que les modifications physiologiques associées à la chirurgie bariatrique et à la perte de poids.

1.1. Composition corporelle et obésité

Les modifications de composition corporelle occupent une place importante dans la physiologie de l'obésité.

Rappelons que le poids (la « masse corporelle ») représente par un seul chiffre exprimé en kg un ensemble très hétérogène de composants. Outre l'augmentation de la masse grasse (MG), la composition corporelle du sujet obèse est caractérisée par une augmentation de la masse maigre (MM) qui porte à la fois sur la masse cellulaire active et sur le compartiment hydrique extracellulaire.

1.1.1. Modèles de représentation du corps humain

L'étude de la composition corporelle fait appel à des modèles ou systèmes de représentation du corps humain, qu'il convient de rappeler⁸.

a. Le modèle anatomique : les tissus

Le modèle anatomique, le plus ancien, sépare le corps en différents tissus : tissu musculaire, tissu adipeux, organes... Il s'agit d'un modèle descriptif qui permet de comprendre l'organisation spatiale des différents constituants. Les progrès de l'imagerie médicale avec la tomodensitométrie (TDM) et l'imagerie par résonance magnétique nucléaire (IRM) ont renouvelé l'intérêt de ce modèle.

b. Le modèle biochimique : les composants

Le modèle biochimique sépare les composants de l'organisme en fonction de leurs propriétés chimiques : l'eau, les lipides, les protéines, les glucides, les minéraux... Ce modèle introduit la notion de masse et de proportion des composants de l'organisme. C'est à partir des rares travaux biochimiques sur les composants et la mesure de l'hydratation moyenne du corps que la notion de densité moyenne puis de densité moyenne de la masse grasse et de la masse maigre a été introduite.

c. Les modèles physiologiques

Les modèles physiologiques reposent sur la notion de compartiments ⁹. Un compartiment regroupe des composants corporels qui sont fonctionnellement liés entre eux indépendamment de leur localisation anatomique ou de leur nature chimique. En médecine de l'obésité et en nutrition clinique, les modèles physiologiques les plus utilisés sont:

Le modèle à 4 compartiments :

C'est le modèle de référence. Les quatre compartiments sont :

- la masse cellulaire active qui correspond à l'ensemble des cellules des différents organes et muscles. Le fonctionnement de cette masse détermine les besoins énergétiques de repos, elle contient les réserves protéiques de l'organisme,
- l'eau extra-cellulaire qui correspond à l'ensemble des liquides interstitiels et au plasma ; elle constitue la masse liquidienne facilement échangeable,
- la masse grasse (MG) qui correspond aux triglycérides stockés dans les adipocytes quelle que soit leur localisation anatomique,
- la masse minérale osseuse, l'essentiel de la masse minérale de l'organisme.

Le modèle à 3 compartiments :

Il constitue une représentation simplifiée du précédent modèle: on regroupe sous le terme de masse maigre (MM) la masse cellulaire active et l'eau extra-cellulaire. Les autres compartiments étant la MG et la masse calcique.

Le modèle à 2 compartiments :

Ils oppose la MG et le "reste" appelé masse non grasse ("fat-free mass", FFM dans la littérature anglo-saxonne) et souvent abusivement appelé MM.

1.1.2. Méthodes d'analyse de la composition corporelle

Il existe différentes méthodes plus ou moins directes d'évaluation de la composition corporelle permettant d'appliquer les modèles cités précédemment. Ils sont rappelés dans le tableau 1.⁹ Certaines méthodes peuvent être associées afin de décrire la composition corporelle selon un modèle plus ou moins complexe. Leur caractère plus ou moins invasif, l'utilisation d'équations prédictives, leur reproductibilité, leurs avantages, leurs coûts et leurs limites sont résumés dans le tableau 2.

La limite principale de ces méthodes est qu'elles reposent fréquemment sur l'acceptation d'hypothèses telles qu'une hydratation ou densité constantes de la MM, une teneur moyenne en potassium de la masse cellulaire. Par ailleurs la prédiction n'est en général applicable qu'à un groupe comparable en tous points (âge, IMC, origine géographique...) au groupe de référence qui a servi à établir l'équation. On comprend que dès lors qu'une méthode d'analyse de la composition corporelle prévoit l'utilisation d'une équation de prédiction, elle soit critiquée lorsqu'elle est appliquée chez les patients obèses.

Le caractère très indirect de certaines méthodes (comme l'impédance bioélectrique) doit par ailleurs rendre extrêmement prudente l'interprétation des résultats à l'échelon individuel. La faible reproductibilité de certaines méthodes (impédance bioélectrique ou mesure des plis cutanés) expose à une variabilité telle qu'elles perdent leur intérêt dans l'analyse individuelle des sujets¹⁰. Ces méthodes, qui sont habituellement les moins invasives, conservent alors leur intérêt dans l'étude de la composition corporelle d'une population.

1.1.3. L'absorptiométrie bi-énergétique

Il s'agit de la Dual X-ray absorptiometry ou DEXA en abrégé. Cette méthode d'analyse de la composition corporelle a été utilisée au cours de mes travaux.

Initialement utilisée pour quantifier la densité osseuse, elle est utilisée depuis la fin des années 1980 pour évaluer la composition corporelle¹⁰. Elle est considérée actuellement comme l'une des techniques de référence, notamment chez le sujet obèse. La méthode

Tableau 1. Modèles et méthodes d'étude de la composition corporelle

| Modèle | Composants | Méthodes |
|---|--|--|
| Modèle anatomique | Séparation du corps en différents tissus : musculaire, adipeux, organes | TDM, IRM |
| Modèle biochimique | Séparation en fonction des propriétés chimiques des composants de l'organisme : eau, lipides, protéines, glucides, minéraux | Technique d'activation neutronique |
| Modèle physiologique | Séparation en compartiments : composants corporels liés entre eux indépendamment de leur localisation anatomique ou de leur nature chimique : -Modèle à 2 compartiments: MG et le reste: masse non grasse -Modèle à 3 compartiments: MM (masse cellulaire active et eau extra cellulaire), masse minérale osseuse et MG -Modèle à 4 compartiments (modèle de référence) : 1. Eau extracellulaire (liquides interstitiels et au plasma) 2. Masse minérale osseuse 3. MG 4. Masse cellulaire active | -Mesure de la densité corporelle : Hydrodensitométrie Pléthysmographie, méthode des plis cutanés -Mesure de l'eau corporelle totale: Impédancemétrie, Deuterium, Oxygène 18 -DEXA 1. Impédance, Méthodes de dilution 2. DEXA 3. DEXA, impédance, hydrodensitométrie ou pléthysmographie ou équation de prédiction de la MG * 4. DEXA, impédance, hydrodensitométrie ou pléthysmographie |
| * $\% \text{masse grasse} = 2.747 * \text{volume corporel} - 0.71 * \text{TBW} + 1.46 * \text{BMC} - 2.05 * \text{poids}$ (Volume= poids /densité ; TBWater : eau corporelle totale ; BMC : masse calcique) | | |

Tableau 2. Avantages et désavantages des méthodes d'analyse de la composition corporelle

| Méthode | Critère d'évaluation initial | Avantages | Désavantages |
|--|---|---|--|
| Mesures anthropométriques | Poids, taille Circonférences et plis | Simple, rapide, reproductible, non coûteux | Difficultés de réalisation chez les sujets obèses et en présence d'oedème, variabilité de mesure (pour les plis) |
| Hydrodensitométrie* | Volume corporel total, densité, masse grasse | Fiable, reproductible | Équipement lourd et coûteux, difficile chez les patients obèses |
| Pléthysmographie** | Volume corporel total, densité, masse grasse | Rapide, précis | Fiabilité non prouvée chez les patients obèses morbides |
| Absorptiométrie bi-énergétique (DEXA) | Masse grasse, masse maigre, masse calcique (mesures globales et régionales) | Simple, faible irradiation, précis | Matériel coûteux, techniciens entraînés nécessaire |
| Impédancemétrie bioélectrique | Eau corporelle totale, eau intra cellulaire et extracellulaire | Non coûteux, portable, simple, inoffensif et rapide | Manque de précision Rétention hydrosodée faussant les données de MM |
| Méthodes de dilution | Eau corporelle totale | Fiabilité, précision | Coûteux |
| Méthodes d'imagerie TDM ou IRM | Masse grasse, masse musculaire, contenu lipidique musculaire et hépatique (mesures globales et régionales) | Précis, reproductible | Coûteux |

*encore appelée « underwater weighing » : méthode consistant en l'immersion du corps dans une cuve d'eau afin de déterminer les modifications de volume de la cuve puis la densité corporelle.

**méthode consistant à la mesure du volume corporel dans une enceinte dont les volumes d'air sont mesurés.

consiste à effectuer un balayage de l'ensemble du corps avec un faisceau de rayons X à deux niveaux d'énergie. Le faisceau subit en traversant les tissus une atténuation qui va dépendre de la composition de la matière traversée. Le balayage du site à explorer s'effectue point par point et la mesure de l'atténuation des deux rayonnements permet d'obtenir une image digitalisée dont chaque surface élémentaire (ou pixel) est porteuse d'information. L'utilisation de deux niveaux d'énergie différents permet dans un premier temps de séparer la masse calcique osseuse des tissus mous, puis secondairement d'individualiser la MG et la MM au sein des "tissus mous". Il s'agit donc d'une méthode à trois compartiments. La coopération du patient se limite au maintien de la position allongée sur une table d'examen pendant 10 minutes.

Sa précision est excellente et elle permet une analyse segmentaire de la répartition des compartiments⁸. Les valeurs de masse grasse obtenues sont très bien corrélées avec celles de l'hydrodensitométrie ou obtenues à partir d'un modèle à 4 compartiments¹¹.

Cette méthode utilise des rayons X et malgré l'irradiation faible (très inférieure à celle d'une radio de poumons standard), elle est contre-indiquée chez la femme enceinte. L'absence de standardisation des algorithmes de calcul entre matériels de fabrication est à l'origine de différences entre les DEXA commercialisées et celles-ci sont donc non interchangeables.¹². Les installations sont coûteuses et le poids des patients ne doit pas excéder 200 kg et un diamètre abdominal de 60-65 cm.

1.1.4. Prédiction de la composition corporelle à partir de données cliniques

Les méthodes d'analyse de la composition corporelle ont souvent fait l'objet de travaux de recherche clinique, mais répondent difficilement à un besoin clinique immédiat comme par exemple celui de l'adaptation posologique des médicaments chez les patients obèses.

Il existe des moyens de décrire la corpulence ou de déterminer la composition corporelle au moyen d'équations de prédiction. En général, ces équations utilisent trois covariables clés, à savoir: la taille, le poids et le sexe et correspondent à des fonctions linéaires ou non de ces données (l'exception est le poids idéal tenant compte de la taille et du sexe). Elles présentent des limites que nous abordons ci-dessous.

a. Outils d'évaluation de la corpulence

L'indice de masse corporelle (IMC)

L'IMC comme outil de prédiction de la MG présente des limites, mais il est largement utilisé compte tenu de sa simplicité ^{13,14}. Sa valeur est hautement corrélée à la MG mesurée de façon directe sans être en mesure de la définir précisément. En effet l'IMC peut manquer de mettre en évidence les différences de MG qui sont réelles.

Age et indice de masse corporelle

Au fil des années, la part relative de MM des individus est réduite au profit de la MG, du fait de la sarcopénie et de l'augmentation de l'adiposité viscérale, malgré un IMC constant. La relation entre masse grasse et IMC est donc âge-dépendant.

La figure 1. Montre le pourcentage de MG en fonction de l'âge dans une population d'hommes de poids normal.

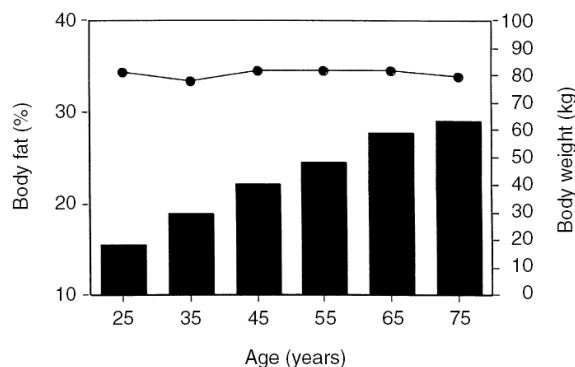


Figure 1. Masse grasse en fonction de l'âge

Origines géographiques et IMC

Une méta-analyse de données de composition corporelle dans des populations d'origines différentes montre qu'à IMC égal les sujets asiatiques ont plus de tissu adipeux que les sujets caucasiens, à l'inverse des sujets d'origine africaine ¹⁵.

Activité physique et IMC

L'activité physique modifie la composition corporelle. Les sujets sportifs ou entraînés présentent une faible adiposité en comparaison à la population générale pour un même IMC ¹⁶. C'est une des situations où l'utilisation de l'IMC afin d'estimer la MG des sujets est inappropriée. De la même façon, dans le suivi de la perte de poids, l'IMC et le poids ne

permettent pas de déterminer la proportion de perte de masse musculaire et de MG, et donc de juger de l'efficacité d'un entraînement physique associé ¹⁷.

La surface corporelle

Il s'agit de la surface externe recouvrant le corps. À l'aide de formules empiriques, la taille et le poids d'un humain servent au calcul de la surface corporelle.

Différentes formules existent, dont les plus utilisées sont celles de Mosteller et Dubois ¹⁸. Ces valeurs moyennes de la surface corporelle sont de 1.7m² chez l'adulte (1.6 chez la femme, 1.9 chez l'homme). La surface corporelle a été utilisée avec succès pour estimer la posologie de certains médicaments ¹⁹.

La formule de Dubois : $S = 0.007184 \times \text{taille}^{0.725} \times \text{Poids}^{0.425}$

La formule de Mosteller : $S = \sqrt{(\text{taille} \times \text{poids}) / 3600}$

Rappelons qu'elle est fréquemment utilisée en oncologie pour déterminer le dosage des anti cancéreux. Pourtant sa valeur maximale a été limitée à 2m² pour le calcul des doses de chimiothérapies depuis longtemps de façon empirique. C'est récemment que les oncologues ont remis en cause cette pratique chez les patients obèses ²⁰.

Le poids idéal et le poids idéal ajusté

Le concept de poids idéal a été développé par les assureurs au vu de la mortalité associée à l'excès pondéral. Devine et al. ont défini une formule de poids idéal, qui à l'inverse des autres outils, ne tient donc pas compte de l'excès de poids, par définition ²¹.

Poids idéal chez l'homme : $49.9 + 0.89 \times (\text{HT} - 152.4)$

Poids idéal chez la femme : $45.4 + 0.89 \times (\text{HT} - 152.4)$

L'intérêt actuel de cette mesure réside dans la possibilité de proposer une valeur intermédiaire qui tiendrait compte d'une part de la composition corporelle « attendue » d'un patient (poids idéal) et d'une partie de l'excès pondéral. On l'appelle le poids idéal ajusté.

Poids idéal ajusté = poids idéal + 0.4x (poids actuel - poids idéal)

b. Prédiction de la masse grasse

Equations de prédiction

Certaines équations de prédiction de la MG en fonction de l'âge ont été développées grâce à l'analyse de la composition corporelle par une technique de référence (underwater weighing) d'une large population de sujets sains âgés de 7 à 83 ans avec un IMC de 13.9 à 40.9 kg/m² (1229 sujets, 521 hommes et 708 femmes). Chez les adultes, la formule de prédiction obtenue est la suivante ²¹ :

Masse grasse% = 1,20 x IMC + 0,23 x âge - 10,8 x sexe - 5,4 (r² 0,79, SEE = 4,1% MG%)

Les données de MG obtenues présentent un degré de corrélation satisfaisant (r²=0.79) avec la mesure directe de la MG. Les auteurs précisait néanmoins que cette formule était moins valide chez les patients obèses où elle surestimait la masse grasse (Tableau 3.).

Tableau 3. Pourcentage de masse grasse moyenne selon l'IMC et différence de masse grasse observée entre valeur prédite et observé avec méthode de référence

| Catégorie de BMI | n | Pourcentage de MG | différence |
|------------------|-----|-------------------|------------|
| <20 | 540 | 18.2 | 0.1 |
| 20-25 | 531 | 23.2 | -0.1 |
| 26-30 | 109 | 32.4 | 0.3 |
| >30 | 49 | 39.4 | -0.5 |

Mesure des plis

L'examen clinique est susceptible d'informer sur la MG des patients. La mesure des plis cutanés est basée sur l'hypothèse que les sites choisis pour cette mesure représentent l'épaisseur moyenne du tissu adipeux sous-cutané et que celle ci reflèterait une proportion constante de la MG totale. A partir de la somme des 4 plis, une équation prédit la densité puis la MG totale (Tableau 4) ²². Cette méthode a ses limites en particulier chez les patients obèses où l'appréciation des circonférences musculaire est rendue difficile par l'adiposité ou l'oedème.

Densité corporelle

La mesure des plis renseigne sur la densité. Si une densité fixe est attribuée à chaque compartiment : 0.9g par mL pour la masse grasse et 1.1 pour la masse maigre, alors la proportion de chacun des compartiments peut être calculé.

Tableau 4. Mesure des plis cutanés et de la surface corporelle

| Mesure de la densité corporelle | Densité corporelle --->masse grasse % |
|---|--|
| Plis cutanés | |
| Densité corporelle = $C - (M * \log \sum 4 \text{ plis})$ § | |
| Ou équation de Lohman | |
| $= 1.0973 - (\sum 4 \text{ plis} \times 0.00815) + (\sum 4 \text{ plis})^2 \times 0.00000084$ | |
| ▪ pli cutané tricipital (à mi-hauteur du bras au niveau de la voussure du triceps) | Siri : $MG \% = (2,118/Db - 0,7 * TBW/WT - 1,354) \times 100$ |
| ▪ pli cutané bicipital (à mi-hauteur du bras au niveau de la voussure du biceps) | |
| ▪ pli cutané sous-scapulaire (1 cm sous l'angle inférieur de l'omoplate) | Brozecz : $MG \% = (4,57/Db - 4,142) * 100$ |
| ▪ pli cutané supra-iliaque (1 cm au dessus de la crête iliaque) | |
| | Gartner : $MG\% = (4.85/Db - 4.4) * 100$ |
| Hydrodensitométrie | |
| Pléthysmographie | |
| § Les coefficients C et M sont donnés par des tables | |
| MG : Masse grasse ; Db : densité | |

c. Prédiction de la masse non grasse

La masse non grasse (la masse musculaire, les organes, l'eau, la masse calcique) correspond à la soustraction de la MG au poids corporel total. Si certaines équations ont été développées afin de prédire la MG, certaines ont été développées afin de définir directement la masse non grasse. C'est à partir d'une mesure directe de la densité par des méthodes de référence, dans une population majoritairement féminine (104 femmes/24 hommes) incluant des patients obèses et dont les poids et tailles étaient variés (Hommes:

80.3±22.0 kg, 43.5-126kg ; 1.72± 0.07m et Femmes: 91.7±19.5kg, 42.3-133.5 ; 1.63±0.07m), qu'ont été élaborées les équations suivantes :

Masse non grasse (kg) = (poids x 0.285) + (12.1 x taille²) chez les hommes

Masse non grasse (kg) = (poids x 0.287) + (9.74 x taille²) chez les femmes

Ces formules de prédiction pourraient avoir un réel intérêt pour la prédiction de la juste posologie de médicaments hydrophiles compte tenu de leur absence de distribution dans la MG^{19,23}.

d. Prédiction de la masse maigre

La MM n'augmente pas de façon linéaire avec l'excès de poids mais représente 20 à 40% de l'excès de masse corporelle totale²⁴. Cette augmentation porte à la fois sur la masse cellulaire active (la masse musculaire et la masse des organes) et sur le compartiment hydrique extracellulaire.

Des équations de prédiction ont également été développées à partir de mesures directes de la composition corporelle dans des échantillons de sujets de poids normal (Tableau 5)¹⁹.

Tableau 5. Formules de prédiction de la masse maigre

| Masse maigre | |
|---------------------------|--|
| <u>James, 1981</u> | |
| Hommes | = (1.10 x Poids(kg)) - 128 x (Poids ² /(100 x Taille(m)) ²) |
| Femmes | =(1.07 x Poids(kg)) - 148 x (Poids ² /(100 x Taille(m)) ²) |
| <u>Humes, 1966</u> | |
| Hommes | =(0.32810 * (Poids(kg))) + (0.33929 * (Taille(cm))) - 29.5336 |
| Femmes | = (0.29569 * (Poids(kg))) + (0.41813 * (Taille(cm))) - 43.2933 |

e. Prédiction de l'eau corporelle totale

Grâce à la mesure directe de l'eau corporelle totale (Deutérium) dans un échantillon de plus de 1500 adultes de 18 à 90 ans, incluant plus de 300 sujets d'origine africaine, Chumlea et al. ont proposé des normes et des formules de prédiction de l'eau corporelle totale selon le sexe, l'âge, la taille, le poids et l'origine ethnique.

Ils ont notamment observé que les sujets d'origine africaine, à poids égal, présentent un volume d'eau corporel total plus important que les sujets caucasiens, et les hommes davantage que les femmes (Tableau 6). Notons que les auteurs observaient des valeurs d'eau corporelle totale fortement associées à la masse grasse et non grasse.

Tableau 6. Valeurs moyennes de l'eau corporelle totale

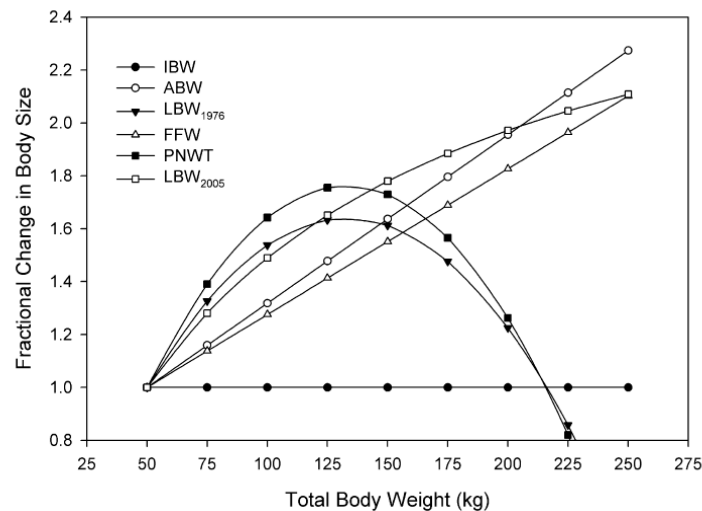
| Age years | Present study | | | | Fels 1999 | | Watson 1980 | | Cohn 1980 | | Norris 1963 | | Hume 1971 | |
|--------------|---------------|------|--------|------|-----------|------|-------------|------|-----------|------|-------------|------|-----------|------|
| | Whites | | Blacks | | | | | | | | | | | |
| | N | TBW | N | TBW | N | TBW | N | TBW | N | TBW | N | TBW | N | TBW |
| Men | | | | | | | | | | | | | | |
| 20-29 | 137 | 45.6 | 21 | 50.9 | 90 | 41.9 | 171 | 43.3 | 24 | 46.9 | 4 | 39.4 | | |
| 30-39 | 98 | 47.5 | 20 | 50.7 | 57 | 43.3 | 93 | 44.1 | 10 | 41.0 | 23 | 41.7 | | |
| 40-49 | 101 | 45.7 | 26 | 50.8 | 72 | 43.9 | 59 | 41.2 | 10 | 44.7 | 35 | 41.6 | 5 | 46.2 |
| 50-59 | 87 | 46.9 | 19 | 46.9 | 57 | 43.8 | 68 | 39.7 | 10 | 45.2 | 30 | 39.9 | 16 | 39.9 |
| 60-69 | 74 | 44.8 | 19 | 46.4 | 30 | 42.9 | 33 | 36.7 | 10 | 41.0 | 26 | 41.7 | 7 | 35.3 |
| 70-79 | 59 | 44.1 | 23 | 44.2 | | | 23 | 33.2 | 9 | 40.3 | 21 | 38.6 | | |
| 80-89 | 48 | 42.5 | | | | | | | | | 4 | 39.1 | | |
| Women | | | | | | | | | | | | | | |
| 20-29 | 124 | 32.0 | 15 | 39.1 | 85 | 30.7 | 100 | 32.2 | 10 | 32.2 | | | | |
| 30-39 | 130 | 33.2 | 27 | 36.6 | 80 | 31.0 | 48 | 31.4 | 10 | 33.1 | | | | |
| 40-49 | 104 | 33.0 | 33 | 37.8 | 88 | 30.7 | 37 | 32.1 | 10 | 31.5 | | | 9 | 30.9 |
| 50-59 | 135 | 32.9 | 41 | 38.7 | 69 | 30.0 | 43 | 33.2 | 10 | 32.0 | | | 12 | 30.2 |
| 60-69 | 117 | 31.4 | 44 | 34.1 | 29 | 27.8 | 19 | 32.6 | 14 | 28.5 | | | 5 | 39.8 |
| 70-79 | 91 | 30.9 | 30 | 32.9 | | | 5 | 25.8 | 8 | 26.6 | | | | |
| 80-89 | 71 | 30.2 | | | | | | | | | | | | |

f. Intérêt des outils de corpulence et de prédiction de la composition corporelle et obésité morbide

La figure 2 illustre la raison pour laquelle les équations prédictives de la composition corporelle peuvent perdre de leur intérêt chez les patients présentant une obésité morbide ²³.

Les connaissances actuelles suggèrent que la juste posologie d'un médicament et les modifications PK chez ces patients ne dépendent pas que d'un seul outil descriptif de la corpulence ou de la composition corporelle ¹⁹.

Figure 2. Relation entre le poids et les outils descriptifs de la corpulence (d'après Pai et al.)



Abbreviations: IBW, ideal body weight (poids idéal); ABW, adjusted body weight (poids ajusté); LBW₁₉₇₆, lean body weight (masse maigre) selon la formule publiée en 1976; FFW et PNWT, fat free weight (masse non grasse); LBW₂₀₀₅, lean body weight (masse maigre) selon une formule publiée en

1.2. Modifications physiologiques avant chirurgie

1.2.1. La fonction cardiaque et vasculaire

a. Vascularisation du tissu adipeux

Les adipocytes sont entourés d'un réseau de capillaires, caractérisés par une haute perméabilité et une pression hydrostatique plus basse, favorisant le transport de molécules entre adipocytes et plasma. Le débit sanguin artériel au repos est généralement de 2 à 3 mL / min par 100 g de tissu adipeux mais peut être augmenté d'un facteur 10. Ce débit augmente après l'ingestion d'un repas, de façon variable d'un patient à l'autre. Il est réduit chez les patients présentant un syndrome métabolique, mais il reste néanmoins toujours inférieur à celui observé dans le muscle squelettique (50 à 75 mL / min par 100 g) ²⁵.

L'eau de l'espace interstitiel du tissu adipeux représente 10% de celui ci. Ce compartiment pourrait avoir des répercussions importantes chez les personnes obèses souffrant

d'insuffisance cardiaque, si ce supplément de volume était redistribué dans la circulation. Cependant la modulation du débit sanguin dans le tissu adipeux empêche généralement que cela ne se produise et ce secteur reste peu accessible.

b. Le débit cardiaque

Le débit cardiaque augmente avec la MG totale. Sachant que la perfusion par unité de tissu adipeux diminue avec l'augmentation de la MG, puisqu'elle est respectivement de 2,36 et 1,53 mL/ min par 100 g de tissu adipeux chez les patients ayant une MG de 15-26% et supérieure à 36%, ce ne sont pas les exigences vasculaires accrues du tissu adipeux qui expliquent l'augmentation du débit sanguin systémique ²⁶. Les augmentations concomitantes de la MM, du volume systolique, du débit cardiaque et de la masse ventriculaire gauche avec l'obésité interviennent probablement dans la détermination de ce phénomène ²⁵.

En effet, l'obésité s'accompagne d'une augmentation du volume sanguin total et du débit cardiaque car la demande métabolique est accrue avec l'excès de poids. Elle entraîne donc une augmentation du travail cardiaque qui est davantage lié à une augmentation du volume d'éjection car le rythme cardiaque n'augmente pas avec l'obésité. La majoration progressive de la pression de remplissage ventriculaire gauche et de son volume peut favoriser la dilatation, puis l'augmentation de la masse myocardique et une hypertrophie ventriculaire gauche, elle même à risque de dysfonction diastolique. L'IMC détermine le degré d'hypertrophie ventriculaire gauche chez les sujets hypertendus en surpoids ou obèses indépendamment des autres covariables ²⁷.

Par ailleurs, chez les humains et la plupart des modèles animaux, l'obésité s'accompagne de dépôts lipidiques importants au niveau des organes, susceptibles d'altérer leur fonction de 2 manières possibles: (1) l'épaisseur du tissu adipeux autour des organes clés peut modifier leur fonction, soit par compression physique simple ou parce que les adipocytes sécrètent diverses molécules agissant localement (2) l'accumulation de lipides dans les cellules peut conduire à un dysfonctionnement cellulaire ou la mort cellulaire, un phénomène connu sous le nom de lipotoxicité avec développement de cellules anormales pouvant altérer les cardiomyocytes et favoriser une cardiomyopathie. L'accumulation peut affecter le ventricule droit jusqu'au phénomène d'« adipositas cordis », un remplacement de l'ensemble du

myocarde par le tissu adipeux favorisant des troubles du rythme et une cardiopathie restrictive²⁸.

c. Autres

De nombreux dysfonctionnements vasculaires surviennent plus fréquemment chez les patients obèses et je les citerai uniquement: insuffisance veineuse, thromboses veineuses et embolies pulmonaires, dysfonction endothéliale, hypertension artérielle, augmentation des résistances vasculaires périphériques, hypertension artérielle pulmonaire, accidents vasculaires cérébraux, coronaropathie, arythmies cardiaques . Le syndrome d'apnée du sommeil est par ailleurs en lui même susceptible de favoriser un dysfonctionnement cardiaque de façon directe, une élévation de la tension artérielle, des arythmies nocturnes, une hypertension artérielle pulmonaire, une insuffisance cardiaque droite et gauche, des infarctus du myocarde, des accidents vasculaires cérébraux et une mortalité plus élevée.

1.2.2. La fonction hépatique

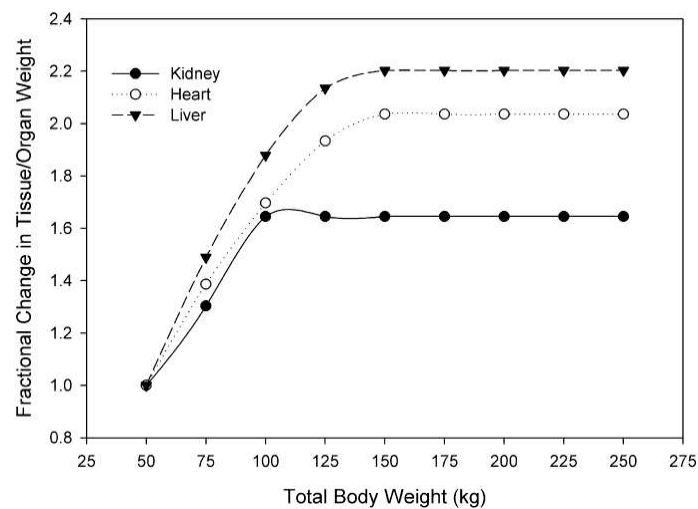
L'obésité est associée à un risque accru de stéatose hépatique qui survient quand le taux d'absorption des acides gras hépatiques à partir du plasma et la synthèse de novo d'acides gras est supérieur au taux d'élimination et de transport des acides gras via les lipoprotéines²⁹. La surcharge lipidique explique en grande partie l'augmentation du volume du foie. L'excès de triglycérides intrahépatiques, désignée par le terme de « stéatose hépatique », est associé à de nombreuses anomalies du métabolisme du glucose, des acides gras et des lipoprotéines, qui participent avec l'excès de tissu adipeux, au développement de l'insulino-résistance, des dyslipidémies et d'autres facteurs de risque cardiométaboliques associés à la stéatose hépatique. Cependant, on ne sait pas actuellement si la stéatose hépatique engendre le dysfonctionnement métabolique ou si le dysfonctionnement métabolique est responsable de l'accumulation des triglycérides, ou s'il s'agit de l'association des deux.

La stéatose hépatique est associée à une inflammation du tissu hépatique et est susceptible d'évoluer vers la Non-alcoolique-stéatohépatite (NASH) qui associe une stéatose hépatique et des altérations des hépatocytes telles qu'une ballonnisation ou une nécrose, la présence d'agrégats de cytokératine, un infiltrat inflammatoire lobulaire contenant de façon

prédominante des polynucléaires neutrophiles. A un stade plus évolué, une fibrose sinusoïdale ou centro-lobulaire s'installe, encore appelée cirrhose, associée au risque d'altération de la fonction hépatocellulaire, pathologie dont la prévalence ne cesse de croître.

1.2.3. La fonction rénale

Figure 3. Augmentation de la taille du cœur, du foie et des reins en fonction du poids



L'obésité sévère est associée à une augmentation de la pression artérielle systémique, du débit plasmatique rénal, du débit de filtration glomérulaire (DFG), et du taux d'excrétion d'albumine^{30,31}. Le lit capillaire glomérulaire est effectivement soumis à un gradient de pression transcapillaire hydrostatique élevé résultant en une hyperfiltration, et tel que l'obésité favorise le syndrome néphrotique et l'insuffisance rénale³². La glomérulopathie liée à l'obésité a été définie morphologiquement comme une glomérulomégalie avec ou sans glomérulosclérose segmentaire et focale³³. Des données expérimentales et cliniques suggèrent que l'hyperfiltration et la glomérulomégalie peuvent conduire à des lésions glomérulaires.

La figure 3 montre l'augmentation de la taille des différents organes (reins, foie et cœur) avec le poids³⁴. L'augmentation n'est pas linéaire, en particulier l'augmentation de la taille des reins n'est pas aussi importante que celle des autres organes, dont l'augmentation de taille est en partie liée aux dépôts lipidiques.

1.2.4. Protéines de l'inflammation, Adipokines et obésité

La prolifération et l'hypertrophie du tissu adipeux sont associés à la présence de cellules inflammatoires en son sein. Par ailleurs les adipocytes produisent une quantité notable de molécules bioactives (lipidiques et protéiques) identifiées comme étant des « adipokines » dont certaines de ces molécules ont un statut d'hormone. Elles sont énumérées dans le tableau 7.

a. Inflammation de bas grade

L'obésité est considérée depuis peu comme un état inflammatoire chronique évoluant à bas bruit, à l'instar de nombreuses pathologies qui lui sont associées comme l'athérosclérose, le diabète de type II et certaines maladies hépatiques entre autres. Ce concept repose sur le fait que les sujets obèses présentent une augmentation modérée, mais chronique, des taux circulants de médiateurs de l'inflammation eux-mêmes associés à une augmentation des risques cardiovasculaires.

Classiquement, le foie et les organes lymphoïdes sont considérés comme les sources principales de production de facteurs inflammatoires. Cependant, une série de données récentes montre que le tissu adipeux exprime également de nombreux facteurs pro- et anti-inflammatoires et contribue vraisemblablement à l'augmentation de leurs taux circulants. Le tissu adipeux produit donc des cytokines inflammatoires, incluant des protéines de la phase aigue de l'inflammation : Tumor Necrosis Factor (TNF) α , Tumor Growth Factor (TGF) β , interféron γ , Interleukine (IL)1, IL6, IL10 et IL8, Serum Amyloid A (SAA), l'inhibiteur de l'activateur du plasminogène (PAI)-1, la C Reactive Protein (CRP), le fibrinogène, des facteurs du complément, des chimiokines, comme les Macrophage Inflammatory Proteins (MIP) 1 α et les Monocyte chemoattractant protein (MCP)-1³⁵. A l'inverse, l'obésité est associée à une diminution de la production d'adiponectine. L'accroissement du tissu adipeux conduit donc à un déséquilibre dans la production et la sécrétion de molécules anti et proinflammatoires, en faveur des facteurs pro-inflammatoires³⁶. De plus, l'accumulation de macrophages dans le tissu adipeux chez le sujet obèse peut vraisemblablement contribuer à l'augmentation des concentrations systémiques de certaines cytokines inflammatoires.

Tableau 7. Productions du tissu adipeux

Production par les adipocytes et cellules de la fraction stroma-vasculaire. Principaux rôles dans le contrôle des grandes fonctions.

| | |
|---|--|
| <p>Métabolisme des lipides et des lipoprotéines</p> <p>lipoprotéine lipase</p> <p>protéine stimulant l'acylation (<i>acylation stimulating protein</i> / ASP)</p> <p>prostaglandine E2, prostacycline</p> <p>autotaxine (lysophospholipase D) + phosphatidylcholine__acide lysophosphatidique</p> <p>protéine de liaison du rétinol (RBP)</p> <p>protéine de transfert des esters de cholestérol (CETP)</p> <p>Prise alimentaire et activation du système nerveux sympathique</p> <p>leptine</p> <p>Métabolisme et homéostasie énergétique</p> <p>leptine</p> <p>adiponectine</p> <p>résistine</p> <p>interleukines -6 et -8</p> <p>visfatine</p> <p>Vaisseaux et angiogénèse</p> <p>facteur de croissance de l'endothélium vasculaire (<i>vascular endothelial growth factor</i> / VEGF)</p> <p>monobutyrine</p> <p>leptine</p> <p>apeline</p> <p>protéine angiopoïétine-like-4 / FIAF (<i>fasting-induced adipose factor</i>) / PGAR (<i>peroxisome proliferator-activated receptor γ angiopoietin-related gene</i>)</p> <p>angiopoïétine -2</p> <p>angiotensinogène/angiotensine-2</p> | <p>Métabolisme de la matrice extracellulaire</p> <p>collagène de type 6</p> <p>inhibiteur-1 de l'activateur du plasminogène (<i>plasminogen activator inhibitor-1</i> / PAI-1)</p> <p>métalloprotéases (gélatinases mmP-2 et mmP-9)</p> <p>inhibiteurs tissulaires des métalloprotéases (TIMP - 1 à -3)</p> <p>Système immunitaire et protéines de la phase aiguë</p> <p>facteur de nécrose des tumeurs-α (<i>tumor necrosis factor-α</i> / TNF-α)</p> <p>interleukines 1β, -6, -8, -10</p> <p>antagoniste récepteur interleukine-1 (<i>interleukin-1 receptor antagonist</i> / IL-1Ra)</p> <p>adipsine, facteurs C3, B et D du système alterne du complément</p> <p>protéine de chimio-attraction des monocytes (<i>monocyte chemotactic protein-1</i> / MCP-1)</p> <p>α1-glycoprotéine acide</p> <p>sérum amyloïde A 3 (SAA3)</p> <p>haptoglobine</p> <p>pentraxine-3</p> <p>lipocaline 24p3</p> <p>métallothionéine</p> <p>cathepsine S</p> <p>Sensibilité à l'insuline du muscle de l'hépatocyte et de l'adipocyte</p> <p>leptine</p> <p>adiponectine</p> <p>résistine</p> <p>visfatine</p> <p>interleukine-6</p> <p>adipsine/ASP</p> |
|---|--|

b. Leptine

Première adipokine identifiée, la leptine, qui ressemble à une cytokine, est sécrétée quasi-exclusivement par les adipocytes du tissu adipeux blanc (soit le type de tissu adipeux le plus abondant de l'organisme) et surtout par ceux du tissu adipeux sous-cutané. La leptine, en se liant à des récepteurs de la famille des cytokines, inhibe la prise alimentaire en agissant au niveau de l'hypothalamus ³⁷. Elle diminue donc la consommation alimentaire et augmente la consommation énergétique au niveau des mitochondries. Néanmoins et à l'inverse des obésités génétiques liées à la leptine (mutations de la protéine ou de son récepteur), l'obésité idiopathique serait associée à une résistance à la leptine.

De plus en plus de travaux montrent que cette hormone n'est pas seulement un agent régulateur de la prise alimentaire et de la balance énergétique. Elle a aussi une fonction polyendocrinienne et métabolique ³⁸. Il est maintenant bien établi qu'elle est impliquée dans le métabolisme du glucose, dans la maturation des organes de la reproduction, dans les mécanismes de la reproduction et dans la régulation de l'axe hypothalamo-hypophysaire.

La leptine favorise l'angiogénèse, a un effet pro-inflammatoire au niveau vasculaire, en agissant sur la production de TNF α et sur l'activation des macrophages, et pro-fibrosant au niveau hépatique en étant produite par les cellules myofibroblastiques activées ^{39,40}. Son taux est élevé dans l'obésité est corrélé à l'IMC, mais il ne semble pas corrélé à l'insulinorésistance de façon indépendante ³⁹.

c. Adiponectine

Cette protéine produite exclusivement par le tissu adipeux est présente en concentration élevée dans le sérum à un taux 1000 fois supérieur à la leptine. Elle est exprimée plus fortement dans le tissu adipeux sous-cutané que viscéral et elle présente différentes formes circulantes. Il n'est pas encore établi quelles sont les formes actives ni si les deux récepteurs clonés, exprimés par de nombreux tissus mais en particulier dans le foie et les muscles, sont les seuls récepteurs actifs, ni par quelles voies ils transmettent le signal de l'adiponectine. Dans les cellules, l'adiponectine active l'enzyme Adénosine Monophosphate (AMP) Kinase et augmente ainsi l'oxydation des acides gras. Elle inhibe l'expression des enzymes de la

néoglucogenèse hépatique et donc inhibe la production hépatique de glucose alors que, dans le muscle, elle favorise le recrutement des transporteurs de glucose GLUT4 à la membrane favorisant l'entrée du glucose ⁴¹. Ainsi, l'adiponectine exerce des effets insulinomimétiques. De plus, l'adiponectine a des effets protecteurs sur la paroi vasculaire et antiathérogènes: elle diminue l'expression des molécules d'adhésion des cellules endothéliales, empêche l'activation des macrophages et inhibe la prolifération des cellules musculaires lisses de la paroi artérielle ⁴². Elle inhibe enfin la production de TNF α par les macrophages.

Les taux d'adiponectine sériques sont diminués chez les patients obèses et inversement corrélés à la masse grasse viscérale. En situation de résistance à l'insuline, en particulier dans le syndrome métabolique, l'adiponectine plasmatique est abaissée et inversement corrélée à la résistance à l'insuline, suggérant le rôle de l'adiponectine comme modulateur de l'insulinorésistance ⁴³.

1.2.5. Microbiote intestinal et obésité

Le microbiote intestinal, anciennement dénommé "flore intestinale", n'est pas distribué de façon homogène le long du tractus digestif humain. Sa présence est relativement discrète dans les deux premiers segments de l'intestin grêle où le transit est rapide. Il augmente fortement dans l'iléon pour atteindre des niveaux de populations cent fois supérieurs au grêle proximal dans le colon et le rectum ⁴⁴. Le microbiote digère à son profit les résidus en transit dans le colon. Il peut produire de nombreux métabolites assimilables par la muqueuse digestive, dégrader certains résidus alimentaires nocifs pour l'homme et il joue un rôle majeur dans la stimulation permanente du système immunitaire. Cet équilibre dynamique est perturbé au cours de certaines maladies inflammatoires intestinales.

Le microbiote joue un rôle dans la prise de poids et l'obésité. L'équilibre entre les populations bactériennes est différent entre les microbiotes "obèses" et "minces". En effet, le microbiote de souris obèses semble plus efficace que celui des souris minces dans la récupération d'énergie. Il favorise la vascularisation entourant l'intestin grêle (angiogenèse), permet une meilleure digestion des résidus alimentaires et stimule l'assimilation des lipides

⁴⁴ .

Chez l'Homme, lors de la perte de poids consécutive à des changements de régimes alimentaires, ce rapport se modifie dans le même sens que celui observé chez les souris, suggérant une relation entre l'équilibre du microbiote humain et l'obésité.

1.3. Chirurgie de l'obésité

1.3.1. Rappel concernant le tube digestif

Il comprend les différents segments : œsophage, estomac, intestin grêle, côlon, rectum et canal anal. L'œsophage est un tube rectiligne et flexible qui réunit le pharynx à l'estomac. Il a une longueur de 25 cm et un calibre de 2 à 3 cm; il traverse le diaphragme et s'ouvre sur l'estomac au niveau du cardia. Les différentes parties anatomiques de l'estomac sont le cardia, le fundus, le corps, l'antrum pylorique et le pylore.

L'intestin grêle a un diamètre de 45 mm pour une longueur de 6 m et comporte le duodénum (0,25 m), le jéjunum (2,5 m), et l'iléon (3,5 m). Son principal rôle est une fonction d'absorption des nutriments. Il présente plusieurs dispositifs de niveaux d'amplification de surface : les valvules conniventes correspondent aux replis transversaux de la muqueuse intestinale de 1 à 2 cm de hauteur, les villosités intestinales sont des plis muqueux de 1 mm de hauteur environ, séparées par des cryptes qui constituent la zone de prolifération comprenant des cellules souches et des progéniteurs. Enfin les microvillosités intestinales, au pôle apical de l'entérocyte, constituent la bordure en brosse. La superposition des valvules, villosités et microvillosités multiplie la surface d'absorption par 600 pour aboutir à une très large surface d'échange de 200m^2 , permettant d'assurer et d'optimiser l'absorption des nutriments et des médicaments.

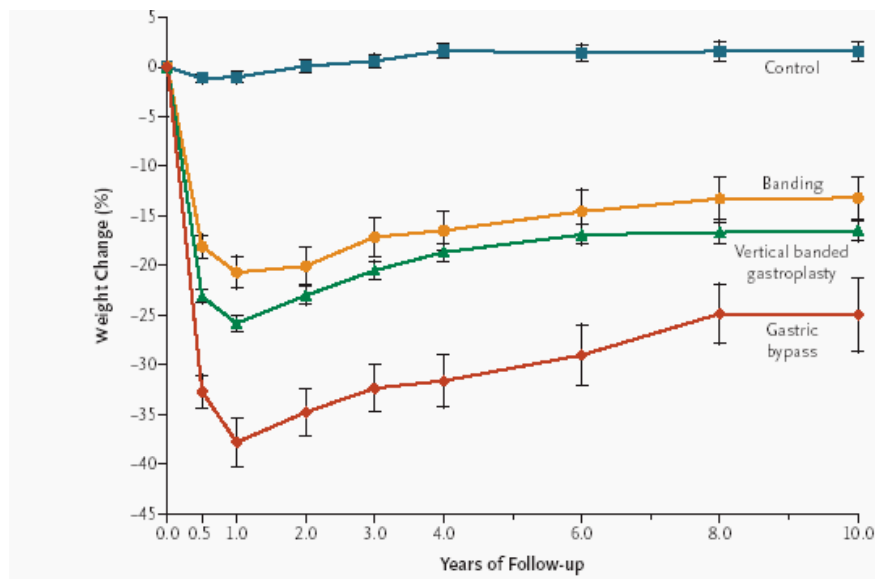
De calibre plus large que l'intestin grêle, le gros intestin ou colon mesure environ 1,5 m de long et comporte le côlon ascendant, le transverse et le descendant suivi du sigmoïde prolongé par le rectum. A son niveau, il n'y a pas d'anse intestinale mais un cadre colique, pas de valvule connivente, des villosités de petite taille et peu de colonocytes. Les fonctions du côlon sont la déshydratation du bol alimentaire (absorption de l'eau et des électrolytes),

la digestion terminale de la cellulose par la flore intestinale et l'évacuation des déchets alimentaires.

1.3.2. Traitement des obésités sévères

Les traitements classiques de l'obésité (activité physique, régimes alimentaires) s'adressent davantage aux obésités modérées qu'aux formes sévères où seule la chirurgie a montré une réelle efficacité sur le long terme. L'étude SOS (Swedish Obesity Study) a comparé la perte de poids observée sur une période de dix années chez des patients traités de façon conventionnelle et les patients opérés, tous types de chirurgie confondus. Au terme de dix ans, le poids a augmenté de 1.6% dans le groupe médical et il a chuté dans tous les groupes chirurgicaux : 25% dans le groupe opéré d'un bypass gastrique (RYGB) et 13.2% dans le groupe opéré d'un anneau gastrique (Figure 4.)⁷.

Figure 4. Evolution de la perte de poids après chirurgie de l'obésité



Banding=anneau gastrique ; Vertical Banded Gastroplasty =anneau gastrique ajustable

Les interventions sont toutes réalisables sous laparoscopie et peuvent être classées en deux types : les interventions dites restrictives visant à réduire la capacité gastrique et donc la prise alimentaire et les interventions de malabsorption.

1.3.3. Les techniques restrictives

Trois techniques restrictives ont été couramment pratiquées :

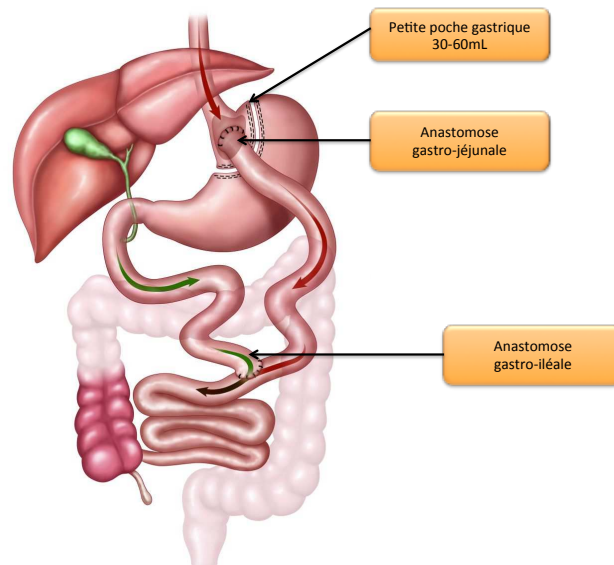
- La gastroplastie vertical calibrée : elle réalise une partition de l'estomac avec une petite poche gastrique se vidant dans le reste de l'estomac par l'intermédiaire d'une zone calibrée. Elle est rarement réalisée aujourd'hui.
- La gastroplastie ajustable : elle consiste à encercler la partie supérieure de l'estomac par un anneau délimitant ainsi une petite poche de 20ml qui se déverse dans le reste de l'estomac à travers un chenal étroit réglable.
- La sleeve gastrectomy consiste en l'exérèse des deux tiers de la partie gauche de l'estomac, transformant la partie résiduelle en un tube d'une capacité d'environ 200cc. Elle est réalisée de façon croissante aujourd'hui.

1.3.4. Les techniques de malabsorption

Les techniques de malabsorption pures ont été abandonnées et elles s'associent de nos jours à une restriction gastrique

- Le Bypass gastrique (RYGB) avec anse en Y comporte une partition de l'estomac en une petite poche supérieure de 30 à 60 mL selon les équipes et le reste de l'estomac est exclu du circuit alimentaire. La petite poche gastrique est ensuite raccordée au tube digestif par l'intermédiaire d'une anse digestive prélevée au niveau du jéjunum, réalisant une anse en Y de longueur variable. L'absorption des aliments n'est possible qu'au delà de l'anastomose du pied de l'anse lorsque les sécrétions gastrique, biliaire et pancréatique arrivent au contact du bol alimentaire. Cette technique chirurgicale représente 85% des chirurgies de l'obésité aux Etats Unis actuellement.
- La diversion biliopancréatique associe une gastrectomie et un bypass intestinal distal ne laissant qu'un court segment d'iléon pour l'absorption des nutriments. Cette technique a été modifiée avec une gastrectomie de type sleeve et switch duodéal. Cette technique s'adressant aux super-obèses n'est que peu pratiquée en France.

Figure 5. Le Roux-en-Y bypass gastrique



En pratique courante, trois interventions sont couramment pratiquées en France : la gastroplastie par anneau ajustable, la gastrectomie de type sleeve et le bypass gastrique. Le choix de la technique repose sur l'analyse des avantages, inconvénients et résultats des différentes interventions. Au cours de mon travail de thèse, je me suis intéressé à l'effet du bypass gastrique sur l'absorption des médicaments puisque la malabsorption associée à cette procédure fait redouter des modifications de l'absorption des médicaments.

1.4. Modifications de composition corporelle et physiologiques après chirurgie

1.4.1. La composition corporelle après chirurgie

L'effet attendu d'une perte de poids, avec le suivi d'un régime alimentaire et après chirurgie bariatrique, est une diminution de la MG. De nombreux travaux cliniques ont confirmé cet effet et ont révélé des modifications associées de la MM.

a. Masse grasse et masse maigre

Un échantillon d'études concernant l'effet du RYGB sur la MG et l'IMC est rapporté dans le tableau ci dessous. Les études s'accordent sur la perte significative de poids, d'IMC et de MG en valeur absolue avec quelques différences en partie imputables à la méthode d'évaluation de la composition corporelle.

Tableau 8. Evolution de la masse grasse après RYGB

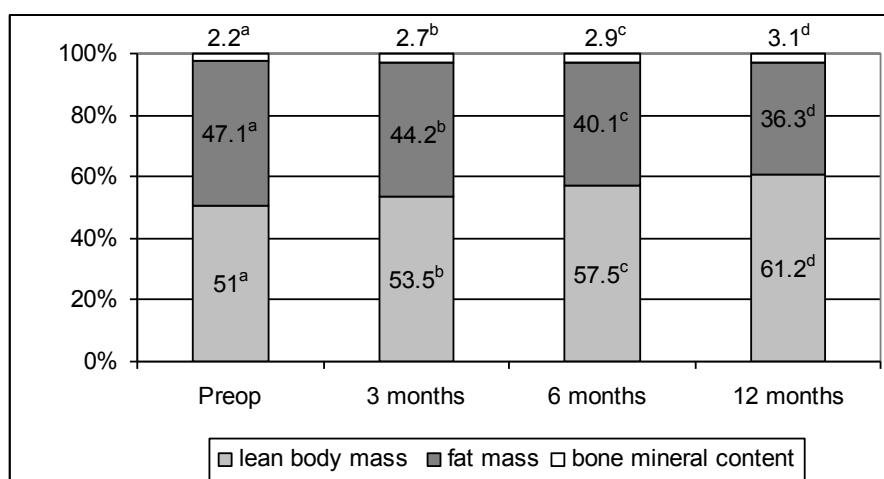
| | Poids, kg | | IMC, kg/m ² | | Masse grasse, kg | | |
|---|-----------|-------|------------------------|-------|-------------------------------------|---------|-------------------------------------|
| | Initial | Final | Initial | Final | Méthode | Initial | Final |
| <u>Levitt et al</u> n=20, 12 mois | 132.4 | 87.8 | 47.7 | 31.69 | Eau Corporelle* | 71.27 | 33.34 $\Delta FM/\Delta Wt=0.85$ |
| <u>Carrasco et al</u> n=38, 12 mois | 114.9 | 82 | 44.0 | 31.6 | Eau Corporelle* | 60.0 | 34.0 $\Delta FM/\Delta Wt=0.79$ |
| <u>Das et al</u> n=20, 14 mois | 124.3 | 79.6 | 47.8 | 30.5 | 3C | 64.4 | 28.7 $\Delta FM/\Delta Wt=0.8$ |
| <u>Carey et al</u> n=19, 12 mois | 140.8 | 89.9 | 48.7 | 30.8 | Densité corporelle | 67.0 | 28.7 $\Delta FM/\Delta Wt=0.75$ |
| <u>Carrasco et al,</u> n=42, 12 mois | 113.9 | 74.5 | 45 | 29.5 | DXA | 54.9 | 26.3 $\Delta FM/\Delta Wt=0.73$ |

Mesure de l'eau corporelle par dilution au deutérium ; DXA : Dual X absorptiometry ; 3C : 3 compartiments ; IMC : indice de masse corporelle ; $\Delta FM/\Delta Wt$: perte de masse grasse sur la perte de poids totale

Une vision longitudinale de la composition corporelle après chirurgie de l'obésité de type RYGB montre que la perte de poids mensuelle diminue au fil du temps et qu'elle est en moyenne de 6.4 ± 1.8 , 3.2 ± 1.7 et 1.2 ± 1 kg respectivement sur les périodes de 0-3, 3-6 et 6-12 mois suivants la chirurgie. La perte de MM respective est de 2.3 ± 1.2 , 0.5 ± 0.7 et 0.2 ± 0.4 kg/mois et celle de MG de 4.1 ± 1.7 , 2.6 ± 1.4 et 1.0 ± 0.7 kg/mois⁴⁵. Ainsi la perte de MG est plus importante que celle de MM qui par ailleurs était maximale en post opératoire

immédiat supposant une part de perte hydrique non négligeable. La part relative de la MG des patientes est donc diminuée avec le temps au profit d'une augmentation de la part relative de masse maigre. La figure 6 montre les parts respectives de perte de masse maigre, de masse grasse et de masse calcique à 3, 6 et 12 mois après la chirurgie de type RYGB.

Figure 6 Composition corporelles à 3, 6 et 12 mois après chirurgie de type RYGB



Lean body mass : masse maigre ; Fat mass : masse grasse ; Bone mineral Content (masse calcique)
(d'après Ciangura et al.)

La composition corporelle des femmes 12 mois après chirurgie en comparaison à celle d'une population de patientes obèses appariées sur l'âge n'ayant pas maigri montre que les masses musculaire, calcique, maigre des membres inférieurs et tronculaire (en kg) sont supérieures chez les patientes ayant maigri ⁴⁵. Ainsi il apparaît que certaines modifications physiologiques associées à l'obésité persistent après perte de poids, avec une masse maigre en valeur absolue plus importante que le groupe contrôle, portant notamment sur la masse musculaire des membres inférieurs.

b. Eau corporelle

Das et al. ont étudié la composition et l'eau corporelle de 20 femmes après chirurgie de type bypass gastrique (39±10 ans, IMC initial=37.5 à 76.4 kg/m²) ⁴⁶. Les résultats de cette étude sont résumés dans le tableau 9. Au moyen d'un modèle à 4 compartiments, alors que la perte de poids est stabilisée (14 mois après la chirurgie), on observe que l'hydratation de

la masse non grasse chez les patientes diminue avec la perte de poids, au niveau intra et extra-cellulaire. Das et al. montrent que, malgré la perte de poids, l'eau corporelle totale est supérieure à celle d'une population de poids normal. L'augmentation de la part relative d'eau extracellulaire par rapport à l'eau intracellulaire est par ailleurs observée chez les patients obèses, avant et après perte de poids.

Tableau 9. Evolution de la composition corporelle et de l'eau corporelle totale après RYGB

| | Avant | A 14 mois | Δ |
|--|--------------|--------------|---------------|
| IMC, kg/m ² | 47.8 ± 8.8 | 30.5 ± 7.0† | -17.3 ± 5.6 |
| Poids, kg | 124.3 ± 24.1 | 79.6 ± 19.1† | -44.7 ± 14.6 |
| Masse grasse, kg | 64.4 ± 16.0 | 28.7 ± 14.7† | -35.7 ± 12.2 |
| Masse non grasse, kg | 60.0 ± 9.5 | 50.9 ± 7.7† | -9.1 ± 4.1 |
| Masse grasse, % | 51.4 ± 3.6 | 34.6 ± 9.3† | -16.8 ± 8.5 |
| Masse cellulaire active, kg | 33.1 ± 6.0 | 28.0 ± 5.0† | -5.1 ± 3.4 |
| Eau corporelle totale, kg* | 45.5 ± 7.3 | 38.2 ± 5.8† | -7.3 ± 3.2 |
| Eau corporelle extra cellulaire, kg | 26.0 ± 5.6 | 21.8 ± 3.9‡ | -4.2 ± 4.8 |
| Eau corporelle intra cellulaire, kg | 18.8 ± 4.7 | 15.6 ± 3.3‡ | -3.2 ± 3.6 |
| Eau corporelle extra cellulaire /Intra cellulaire | 1.48 ± 0.57 | 1.44 ± 0.30 | -0.04 ± 0.64 |
| Eau corporelle totale /masse non grasse | 0.756 ± 0.01 | 0.747 ± 0.03 | -0.009 ± 0.03 |

† :p<0.001 ; ‡p<0.01

Certains auteurs proposent qu'il existe une anomalie de la régulation des fluides chez les patients obèses persistante après perte de poids, tandis que d'autres suggèrent que l'excès de tissu cutané et dont la part d'eau extracellulaire est importante (rapport ECW/ICW= 0.42) contribue au maintien de ce rapport élevé.⁴⁷ Une autre hypothèse est que la part d'eau extra cellulaire du tissu adipeux augmente alors que la taille adipocytaire diminue. Des biopsies de tissu adipeux ont montré que les sujets de poids normal ont une part d'eau extracellulaire plus importante que les sujets obèses et que par ailleurs elle augmente après perte de poids dans ces deux groupes de patients⁴⁸.

1.4.2. La fonction cardiaque et vasculaire après chirurgie

La perte de poids induite par la chirurgie est responsable d'une diminution de la consommation d'oxygène au repos et du débit cardiaque proportionnelle à la perte de poids²⁵. Le volume et le travail diastolique et systolique sont réduits parallèlement à la diminution du volume sanguin et du volume cardiaque. L'hypertension artérielle pulmonaire et systémique diminuent mais les résistances systémiques sont peu modifiées.

Garza et al. ont observé une diminution de la masse ventriculaire gauche (HVG) de 239.9g à 208.2g à 3.6 années de la chirurgie tandis qu'une augmentation de masse est observée dans une population obèse non opérée (251.1 à 258.8g)⁴⁹. Une diminution de la taille du septum ventriculaire de 12.0 à 10.6 mm est associée à la diminution de masse du ventricule gauche. La perte de poids n'explique qu'une partie de la diminution de la masse ventriculaire gauche (14% à 25%).

Les modifications de pression artérielle, du système renine angiotensine, de production de neurohormones, impliquées dans la genèse de l'hypertrophie ventriculaire gauche y participent également⁵⁰. En effet la perte de poids réduit l'activité sympathique (noradrénaline plasmatique urinaire), et s'accompagne d'une réduction de l'activité rénine plasmatique et des taux d'aldostérone. Par ailleurs l'hyperinsulinisme et la résistance à l'insuline, facteurs indépendamment associés à la survenue d'une hypertrophie ventriculaire gauche chez les sujets obèses non diabétiques normotendus, sont également diminués. Le mécanisme exact expliquant l'association entre l'HVG et l'insulino-résistance n'est pas connu, mais il est possible que l'hyperinsulinémie ait un rôle de croissance sur les cellules ventriculaires gauches. L'amélioration du syndrome d'apnée du sommeil participerait également aux modifications de volume du ventricule gauche.

Ainsi la prévalence de patients dont la fraction d'éjection du ventricule gauche est inférieure à 50% diminue avec la perte de poids, alors qu'elle augmente chez les sujets non opérés. Différentes observations sont en faveur d'une amélioration de la fonction cardiaque chez les patients insuffisants cardiaque bénéficiant d'une chirurgie de l'obésité⁴⁹.

1.4.3. La fonction hépatique après chirurgie de l'obésité

La diminution rapide de la taille du foie, après réduction des apports lipidiques ou rapidement après chirurgie bariatrique, suggère que l'excès de graisse au sein du parenchyme et plus particulièrement de l'hépatocyte, explique majoritairement son volume et son évolution après perte de poids ⁵¹. L'évaluation directe de la stéatose hépatique par biopsie chez 16 patients candidats à un RYGB après chirurgie montre une disparition complète de la stéatose hépatique avec une disparition des stigmates d'inflammation chez 15 sujets à deux années de suivi. La fibrose hépatique, présente chez 4 patients initialement, avait totalement disparu chez un patient après RYGB, était améliorée chez l'un d'eux ou restée stable chez les 2 autres ⁵².

1.4.4. La fonction rénale après chirurgie de l'obésité

La réduction de l'hyperfiltration glomérulaire par la perte de poids pourrait prévenir ou retarder le développement de la maladie rénale chez les personnes obèses. L'évolution du DFG (évalué par la mesure de la clairance de l'inuline) et le flux plasmatique rénal (FPR) (mesure de l'acide para amino hippurique) chez 8 sujets présentant une obésité sévère ($IMC=48,0\pm2,4\text{kg/m}^2$) avant et après la perte de poids en comparaison à 9 sujets sains témoins va dans le sens de cette hypothèse ³². En effet, dans le groupe de sujets obèses, le DFG ($145\pm14\text{ ml/min}$) et le FPR ($803\pm39\text{ml/min}$) sont significativement supérieurs en comparaison aux sujets contrôles ($90\pm5\text{ ml/min}$ et $610\pm41\text{ ml/min}$). Après perte de poids ($IMC=32,1$) les DFG et FPR sont significativement diminués à $110\pm7\text{ ml/min}$ et $698\pm42\text{ ml/min}$ respectivement et l'excrétion d'albumine est diminuée (de 16 mg/min à 5 mg/min $P<0,01$). L'ensemble de ces observations va dans le sens d'un effet bénéfique de la perte de poids sur la fonction glomérulaire et d'une réduction de la filtration glomérulaire.

1.4.5. Adipokines après chirurgie de l'obésité

La perte de poids induite par une restriction calorique plus ou moins sévère diminue les taux circulants de CRPus, TNF et d'IL6. Une années après chirurgie de l'obésité, et après une

perte de poids de 30% du poids initial en moyenne, il existe une forte diminution du fibrinogène et la CRPus (marqueurs non spécifiques), du SAA, de l'orosomucoïde, de l'IL6, du TNF et une élévation de l'adiponectine^{53,54}. La cinétique de diminution est différente d'une molécule à l'autre. Par exemple, alors que le SAA et la CRPus diminuent rapidement et de façon parallèle, l'IL6 diminue plus tardivement⁵⁵. L'amaigrissement se caractérise donc par une diminution des marqueurs de l'inflammation circulants qui pourrait favoriser l'amélioration des complications cardiovasculaires et la résistance à l'insuline⁵⁶.

La leptine diminue de façon importante après chirurgie et sa réduction est significativement corrélée à la diminution de l'indice de masse corporelle⁵⁷. L'adiponectine augmente de 30 à 40% après la chirurgie de type RYGB, tandis que les valeurs d'insulinémie et l'insulinorésistance diminuent d'un facteur 5⁵⁷.

2.Effet de l'obésité et de la chirurgie de l'obésité sur le devenir des médicaments

2.1. Les étapes de la pharmacocinétique des médicaments

On distingue schématiquement 3 étapes dans la pharmacocinétique (PK) d'un médicament : son absorption, sa distribution et son élimination, phase incluant elle-même son métabolisme et son excrétion.

2.1.1. L'absorption

L'absorption est le processus par lequel le médicament inchangé passe de son site d'administration à la circulation générale. Il dépend donc de sa voie d'administration.

a. Caractéristiques physiologiques du milieu

L'absorption n'est possible que dans certaines conditions. En effet un médicament ne peut être absorbé que sous forme dissoute et la forme galénique constitue donc un déterminant de l'absorption⁵⁸. Par ailleurs, seule la forme non ionisée et liposoluble peut être absorbée par diffusion passive et les caractéristiques physicochimiques du principe actif, définies notamment par son pKa (constante d'acidité) et sa liposolubilité (solubilité dans les graisses), vont alors influencer l'absorption.

Enfin, les caractéristiques physiologiques du milieu, c'est à dire le pH, la surface d'absorption, la vascularisation, le flux sanguin splanchnique et le péristaltisme intestinal, conditionnent également l'absorption et expliquent que l'absorption soit différente en fonction du milieu considéré.

Le tableau 10 rappelle les caractéristiques physico-chimiques plus ou moins favorables des segments du tube digestif dans l'absorption des médicaments.

Les caractéristiques anatomiques de l'intestin grêle, abordée dans le chapitre précédent, expliquent en partie qu'il soit un lieu privilégié d'absorption des médicaments.

Tableau 10. Voie orale et absorption digestive

Estomac

Faible surface (1 m^2)

Epaisse muqueuse

Faible vascularisation (0.2L/min)

Temps de latence modéré (vidange gastrique)

pH acide: 1,5 à 3,5

Dégradation de certains composés

Mais milieu favorable:

Pour les médicaments acides faibles : forme non ionisée permettant leur absorption

Pour certains médicaments bases faibles (meilleure dissolution).

Pour la transformation de certains principes actifs.

Phase biopharmaceutique: estomac (sauf formes gastro-R).

Toute modification de la vidange gastrique modifie la vitesse à laquelle les médicaments arriveront au niveau de l'intestin grêle où ils seront absorbés.

Intestin grêle

Absorption favorisée par de nombreuses sécrétions:

Pancréatiques : enzymes protéolytiques.

Biliaires: les sels biliaires indispensables pour l'absorption des molécules liposolubles.

Intestinales: nombreuses enzymes présentes dans la lumière intestinale et /ou la membrane entérocytaire: disaccharisades, dipeptidases, entérokinase, cytochromes

Duodénum/Jéjunum : Lieu plus favorable

Surface importante, pH moins acide

Présence de bile favorisant la dissolution des principes actifs

Concerne la majorité des formes per os

Intestin grêle (iléum)

Surface, longueur, élevées : 200 m^2 , 4-5 m

pH 5 à 8 : favorise la forme non ionisée.

Forte vascularisation (1 L/min), villosités, capillaires lymphatiques

Transporteurs actifs

Bile, surfactant: accroît la solubilisation des principes actifs

Concerne la majorité des formes per os

Colon

Faible surface d'échange (pas de villosités)

Surface et longueur plus faibles

Absorption plus lente mais résidence importante

Intéresse essentiellement les composés à dissolution lente et les formes galéniques à délitement progressif

b. Métabolismes pré et post-entérocytaire

L'absorption intestinale est déterminée par le métabolisme pré-entérocytaire, la perméabilité cellulaire et le métabolisme entérocytaire et post-entérocytaire des médicaments. Le métabolisme correspond à la dégradation par voie enzymatique du médicament en métabolites.

Métabolisme pré-entérocytaire

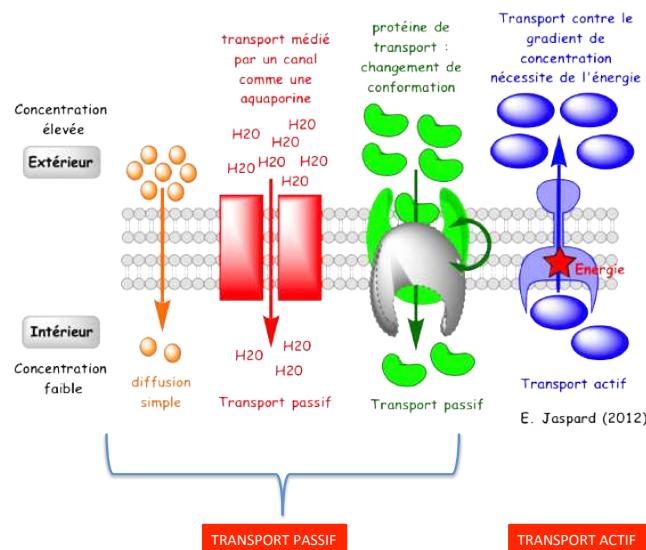
La flore bactérienne intestinale et les glandes exocrines de la muqueuse intestinale sécrètent des enzymes métaboliques lumenales susceptibles d'intervenir dans le métabolisme ou la réabsorption de composés endogènes et exogènes⁵⁹. Tandis que la flore intestinale augmente du duodénum jusqu'au colon, les enzymes sécrétées par les glandes exocrines sont plus actives dans les parties proximales de l'intestin grêle et sont souvent inactivées par les bactéries du côlon^{60,61}.

La bordure en brosse contient elle aussi de nombreuses enzymes dont l'activité est plus importante dans le duodénum et le jéjunum que dans l'iléon, et à l'origine de la mauvaise biodisponibilité de nombreux peptides thérapeutiques tels que l'insuline ou des hormones peptidiques, mais elles n'occasionnent pas de variabilité interindividuelle d'absorption. Le métabolisme des médicaments dans la lumière intestinale est relativement faible si on le compare à celui des entérocytes ou du foie⁶².

Perméabilité entérocytaire

Les cellules entérocytaires sont liées les unes aux autres par des jonctions plus ou moins serrées et reposent sur une membrane basale, elle-même plus ou moins perméable aux molécules. La perméabilité est la propriété que possède la surface cellulaire d'absorber directement des substances du milieu extra cellulaire et d'y éliminer d'autres substances. Elle peut prendre deux formes : la perméabilité paracellulaire et transcellulaire, incluant elle-même la perméabilité passive dans le sens du gradient de concentration (simple sous la dépendance des lois physicochimiques) ou facilitée par l'intervention de protéines et active contre le gradient de concentration (impliquant la participation de la cellule par un apport d'énergie métabolique).

Figure 7. Illustration schématique des types de transports membranaires



D'après Emmanuel Jaspard, 2012

Métabolisme entérocytaire et post-entérocytaire

Le métabolisme au niveau de la lumière et/ou de la muqueuse intestinale d'une molécule administrée par voie orale correspond à l'effet de premier passage intestinal. Arrivée dans la circulation porte, la molécule transite par le foie où une proportion plus ou moins importante sera transformée en métabolites ou éliminée directement dans la bile : c'est l'effet de premier passage hépatique.

Ainsi, l'intestin et le foie jouent un rôle de barrière en limitant la biodisponibilité des médicaments absorbés par voie orale puisqu'une partie de ceux-ci est dégradée à leurs niveaux et que des protéines d'efflux limitent le passage systémique des médicaments. Nous définirons ultérieurement les effecteurs du métabolisme des médicaments.

La fraction restante arrive au cœur par la veine cave et est véhiculée vers les poumons où elle peut subir un effet de premier passage pulmonaire. Revenant au cœur, la quantité résiduelle est distribuée par le système artériel dans les tissus où elle pourra exercer son effet thérapeutique.

En général, l'effet de premier passage est plus important au niveau hépatique qu'au niveau pulmonaire ou intestinal. Il sera déterminé par trois facteurs : le métabolisme des composés dans les organes traversés, le débit sanguin irriguant ces organes.

La biodisponibilité représente la fraction de principe actif qui, après administration, atteint la circulation générale et la vitesse avec laquelle elle l'atteint. Elle est conditionnée par la quantité absorbée, la vitesse d'absorption et la quantité éliminée par l'effet de premier passage. Elle est appréciée le plus souvent par la concentration plasmatique ou sanguine maximale (C_{max}), le temps nécessaire pour atteindre cette concentration (t_{max}) et par le facteur de biodisponibilité F exprimé en pourcentage.

2.1.2. La distribution

Une fois la circulation sanguine systémique atteinte, les médicaments se distribuent dans l'espace extracellulaire (volume plasmatique + volume interstitiel) et éventuellement dans l'espace intracellulaire. Cette distribution tissulaire dépend de différents facteurs : (i) la perfusion sanguine tissulaire, (ii) la diffusion tissulaire dépendante elle-même des caractéristiques physico-chimiques du médicament (degré de lipophilie) et des mécanismes de passage transmembranaire (par exemple, le cerveau et la barrière hémato-encéphalique ont une paroi vasculaire composée de capillaires continus difficilement franchissable) pouvant impliquer des transporteurs membranaires, (iii) la liaison aux protéines plasmatiques et enfin (iiii) de l'éventuelle élimination du médicament par le tissu considéré.

2.1.3. L'élimination

Cette phase du devenir du médicament comporte son métabolisme et son excrétion.

a. Le métabolisme

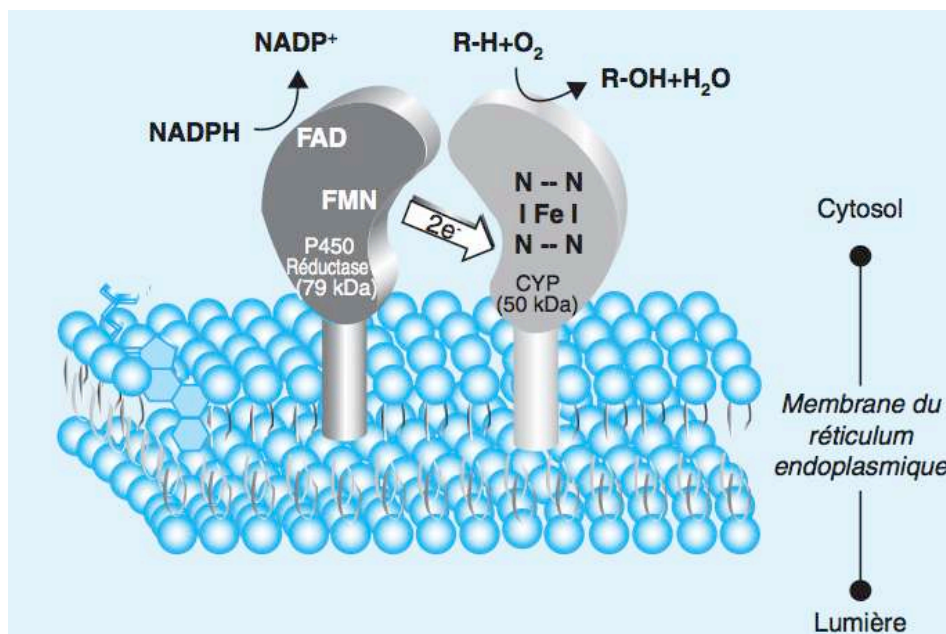
Les réactions de phase I

Le but de cette phase du métabolisme est de rendre les médicaments (ou substrats) plus hydrophiles afin de favoriser leur élimination dans les urines ou la bile en y introduisant un

groupement polaire grâce à l'oxygène atmosphérique (ex. oxydation aromatique ou aliphatique) ou en dégageant des groupements hydrophiles, comme des amines primaires (-NH₂), sulfhydryles (-SH) ou carboxyles (-COOH) déjà présents sous forme masquée sur le médicament initial (appelé composé « parent ») pour augmenter sa polarité (ex. désalkylation, déhalogénéation).

Les enzymes responsables de la phase I sont principalement les cytochromes P450 (CYP). Ces derniers sont des monooxygénases dites aussi « hémio-thiolates », apportant donc des électrons au substrat. La transformation d'un composé chimique par ces enzymes a lieu le plus souvent sur la face externe du réticulum endoplasmique (RE) où est ancrée l'enzyme (Figure). Le site actif du cytochrome P450 contient un atome de fer (Fe) fixé par des liaisons de coordination. Deux électrons, provenant de molécules de NADPH, sont transférés à l'hémoprotéine par diverses enzymes dont certaines sont des enzymes de type flavoprotéine (FAD-FMN) en présence d'une molécule organique (R-H) et d'un atome d'oxygène. Le composé organique est oxydé et un atome d'oxygène moléculaire est incorporé au produit chimique (R-OH).

Figure 8. Exemple de transformation d'un composé chimique par les Cytochromes P450



R : médicaments, acides gras, stéroïdes, polluants. Flavoprotéine : FAD-FMN, molécule organique : R-H

Les réactions de phase II

La phase II du métabolisme des xénobiotiques comprend les réactions de conjugaison, définies par la liaison covalente d'une molécule endogène (polaire) à un groupement fonctionnel d'une molécule substrat (hydrophobe). Bien que le substrat contenant un groupement fonctionnel approprié puisse directement subir la phase II du métabolisme, la conjugaison se produit souvent consécutivement à une réaction de phase I, durant laquelle la molécule endogène est ajoutée au métabolite de phase I. Parmi les enzymes responsables des réactions de phase II, notons les sulfotransférases, les N-acétyltransférases, les glutathion-S-transférases, les catéchol-O-méthyltransférases et surtout les enzymes uridine diphosphate glucuronosyl-transférases (UGT).

Ces enzymes ont pour point commun de permettre au médicament de perdre toute activité pharmacologique, sauf pour quelques exceptions comme le métabolite actif de la morphine. La glucuronidation sera abordée de façon plus approfondie, ultérieurement dans le chapitre intitulé « La morphine ».

b. L'excrétion

Le foie, organe épurateur principal, participe largement à l'excrétion des médicaments hors de l'organisme par le biais du système biliaire, de même que le rein via la filtration glomérulaire et la sécrétion tubulaire (sous forme inchangée ou sous forme de produits de dégradation). Des protéines, appelées transporteurs d'efflux ou de phase III, participent au transport au travers des membranes des médicaments et surtout de leurs dérivés conjugués. Ces transporteurs influencent également la PK des médicaments dans l'organisme en intervenant dans les phases d'absorption et de distribution, puisqu'ils sont présents au niveau de plusieurs membranes cellulaires.

Ils sont divisés en deux catégories : la superfamille des transporteurs ABC (ATP-binding cassette) et la superfamille des transporteurs de solutés (solute carriers [SLC]).

c. Les transporteurs de la famille ABC

Ce sont des pompes d'efflux qui dépendent de l'hydrolyse de l'ATP afin d'activer le passage des substrats au travers des membranes biologiques. Ces transporteurs agissent en limitant

l'accumulation de composés cytotoxiques aussi bien dans les cellules tumorales que dans les tissus sains. Ce phénomène cellulaire, dans les cellules tumorales, est appelé *multidrug resistance* (MDR).

Le plus connu est le transporteur ABCB1, la P-glycoprotéine, produit du gène *ABCB1*, que je décrirai davantage dans le chapitre suivant. Les transporteurs Multidrug Resistance-associated Protein (MRP) MRP2 et MRP3, produits des gènes *ABCC2* et *ABCC3*, le seront également puisqu'ils transportent les métabolites de la morphine.

Tableau 11. Membres de la superfamille des récepteurs ABC

| ABCA | ABCB | ABCC | ABCD/E/F/G |
|--------|--------------|--------|------------|
| ABCA1 | ABCB1 | ABCC1 | ABCD1 |
| ABCA2 | ABCB2 | ABCC2 | ABCD2 |
| ABCA3 | ABCB3 | ABCC3 | ABCD3 |
| ABCA4 | ABCB4 | ABCC4 | ABCD4 |
| ABCA5 | ABCB5 | ABCC5 | ABCE1 |
| ABCA6 | ABCB6 | ABCC6 | ABCF1 |
| ABCA7 | ABCB7 | CFTR | ABCF2 |
| ABCA8 | ABCB8 | ABCC8 | ABCF3 |
| ABCA9 | ABCB9 | ABCC9 | ABCG1 |
| ABCA10 | ABCB10 | ABCC10 | ABCG2 |
| ABCA12 | ABCB11 | ABCC11 | ABCG4 |
| ABCA13 | | ABCC12 | ABCG5 |
| | | | ABCG8 |

ABC : ATP Binding Cassette Family

d. Les transporteurs de la famille SLC

La famille SLC comprend 55 familles de transporteurs classés selon leur séquence en acides aminés. Ceci représente environ 300 membres chez l'homme. Ils assurent généralement la capture cellulaire des nutriments comme le glucose ou les acides aminés, soit par mécanisme de transport facilité où le substrat est déplacé selon le gradient de

concentration, soit selon un mécanisme secondaire de transport actif, où la translocation du substrat contre le gradient de concentration est couplée à un flux d'ion (gradient électrochimique).

Les substrats transportés incluent notamment des cations et anions inorganiques (ex. H^+ , HCO_3^- , Cl^- , Na^+ , K^+ , Ca^{2+} , Mg^{2+} , etc.), des métabolites énergétiques tels que les acides aminés, des oligopeptides, du glucose et d'autres sucres. Des carboxylates et d'autres anions organiques, des acides gras, des lipides ainsi que des composés vitaux tels que des vitamines, des nucléosides et des neurotransmetteurs, entre autres, peuvent aussi être transportés.

Comme les transporteurs ABC, les transporteurs SLC jouent un rôle important dans la pharmacocinétique (absorption, distribution et élimination) d'un large panel de médicaments, de toxines, de composés endogènes et de leurs métabolites.

A noter qu'après excrétion, le médicament peut être réabsorbé au niveau digestif ou rénal: il s'agit du cycle entéro-hépatique ou de la réabsorption tubulaire.

2.2. Transporteurs et enzymes entérocytaires des médicaments

Les différents travaux concernant l'expression entérocytaire des enzymes du métabolisme des médicaments, encore appelés effecteurs, seront abordés dans ce chapitre. Il s'agit de notions importantes permettant de comprendre les déterminants de l'absorption orale des médicaments et la difficulté de prédire le devenir de l'absorption des médicaments après chirurgie de l'obésité induisant des modifications profondes du circuit digestif.

Les segments d'intestin ayant fait l'objet de ces travaux ont été le plus souvent collectés chez des patients souffrant de pathologies digestives variées, pour des raisons éthiques évidentes. Les effecteurs du métabolisme entérocytaire ont en commun de présenter une variabilité interindividuelle significative.

2.2.1. Les transporteurs entérocytaires des médicaments

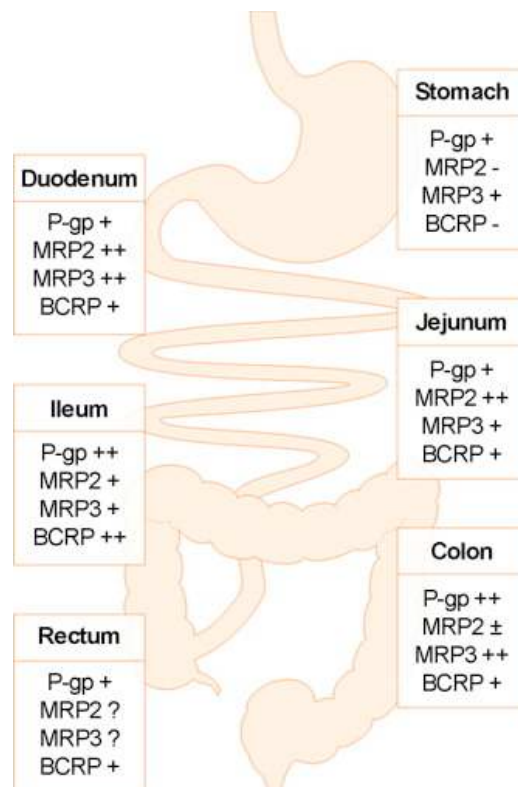
L'expression transcriptionnelle (ARNm) des transporteurs entérocytaires est mieux caractérisée que l'expression protéique. Concernant les transporteurs, l'expression du gène

ABCC3 de la protéine MRP3 est moins connue que celle des gènes *ABCC2* et *ABCB1/MDR1* codant respectivement pour les protéines MRP2 et P-gp. L'expression en ARNm doit faire l'objet d'une interprétation prudente car elle n'est pas toujours corrélée à l'expression protéique, en particulier pour la P-gp⁶³. Notons par ailleurs que les travaux décrivent dans la majorité des cas l'expression entérocytaire mais abordent rarement l'activité catalytique/métabolique entérocytaire de ceux-ci.

a. La protéine P-gp, codée par le gène *ABCB1/MDR1*

La plupart des travaux s'accordent sur le fait que les niveaux d'expression (en ARNm et protéines) de *MDR1/ABCB1* augmentent le long de l'intestin grêle, suggérant que les substrats de la P-gp soient moins absorbés en cas d'absorption distale par rapport à une absorption proximale⁶⁴⁻⁶⁷.

Figure 9. Variabilité de l'expression des transporteurs le long de l'intestin



P-gp : P-glycoprotéine, *MRP* : Multi-Drug- Resistance Protein, *BCRP* : Breast Cancer Resistance Protein

Ces données sont en accord avec une étude phénotypique révélant une réduction de l'exposition de 50% du talinolol s'il est instillé au niveau distal par rapport à une instillation proximale ⁶⁴⁻⁶⁶. Toutefois une stabilité du contenu protéique en P-gp le long de l'intestin a été décrite ⁶³. Effectivement Berggren et al. montrent que les niveaux d'expression du gène sont inférieurs au niveau de l'iléon à ceux du jéjunum et qu'ils diminuent encore au niveau colique. Ils montrent que le contenu en P-gp est comparable entre l'intestin grêle proximal et le colon tandis qu'il est légèrement diminué au niveau de l'iléon en comparaison à ces segments.

La figure 9. synthétise la variabilité d'expression de différents transporteurs en fonction des segments d'intestin considérés.

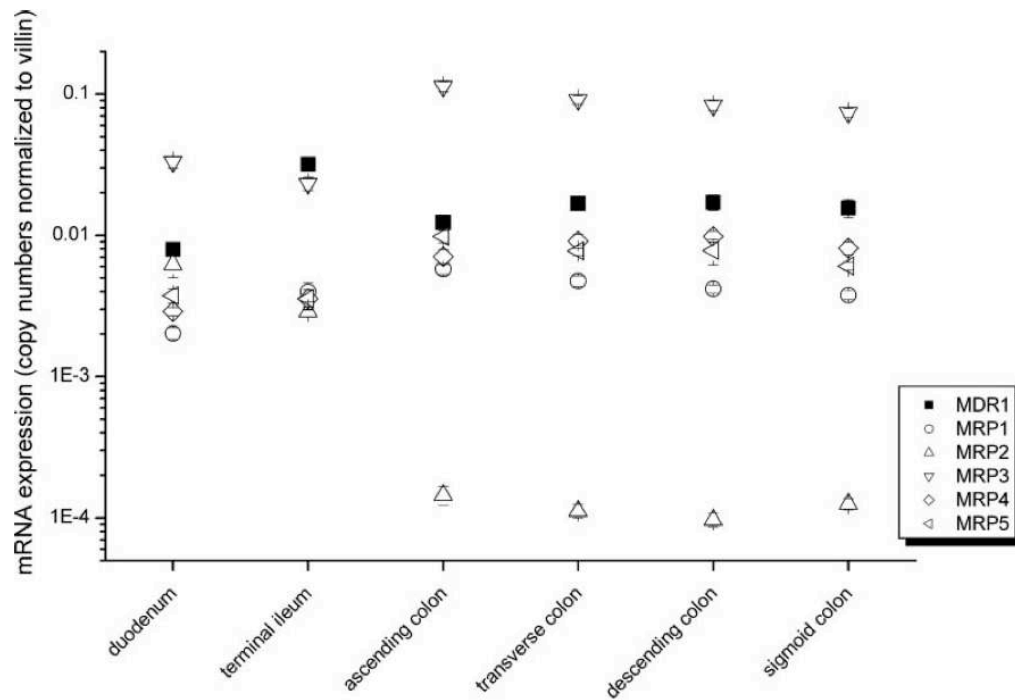
b. MRP2, MRP3, codés par les gènes ABCC2 et ABCC3

L'expression (en ARNm et protéines) de *ABCC2* est maximale au niveau des duodénum et jéjunum et diminue vers les régions plus distales ^{63,66}. En revanche différents travaux s'accordent sur le fait que l'expression de *ABCC3* est relativement stable le long de l'intestin ⁶⁶.

c. Expression relative des transporteurs au niveau entérocytaire

Zimmermann et al. ont étudié l'expression transcriptionnelle des gènes *MDR1/ABCB1* et des gènes codant pour les transporteurs *MRP1-5* au niveau des différents segments intestinaux (duodénum, iléon terminal, et différents segments du colon) ⁶⁸. Les résultats de ces travaux sont représentés sur la figure 10. Ils montrent que le gène *ABCC3* codant pour la protéine *MRP3* a un niveau d'expression transcriptionnel plus élevé que les autres gènes codant pour les autres transporteurs, quel que soit le segment intestinal considéré en dehors de l'iléon terminal où le niveau d'expression de *MDR1/ABCB1* est supérieure. De façon constante le long de l'intestin, le niveau d'expression du gène *ABCC2* est moindre que celui du gène *MDR1/ABCB1*, en particulier au niveau colique où il est à peine détectable.

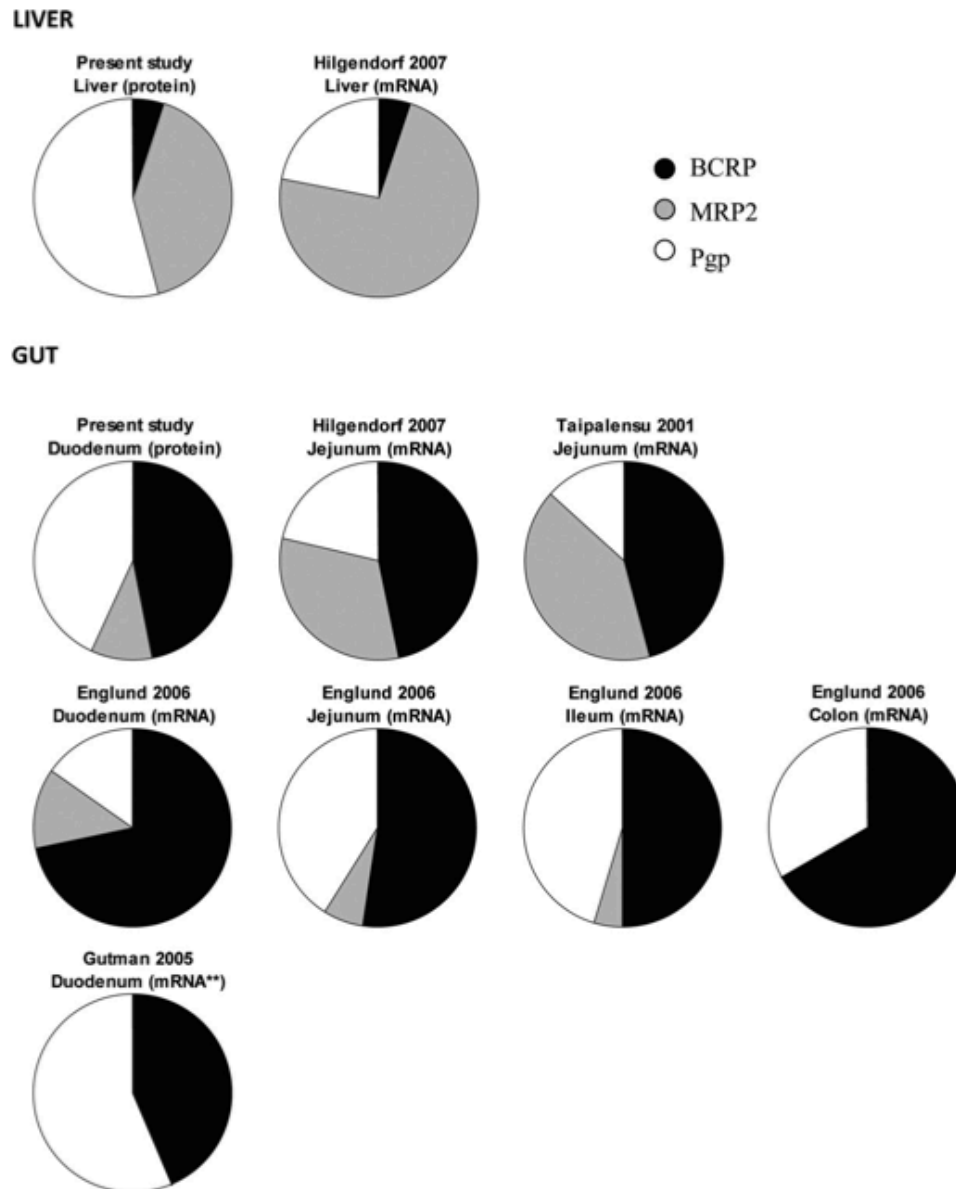
Figure 10. Niveau d'expression transcriptionnelle des gènes MDR1/ABCB1 et MRP1 à 5 dans différents segments de l'intestin.



Résultats normalisé sur l'expression de la villin chez 10 sujets sains. MDR/MRP : Multidrug Resistance Protein

Tucker et al. ont comparé les niveaux d'expression transcriptionnelle et protéique des gènes des gènes codant pour les protéines BCRP, MRP2 et P-gp au niveau hépatique et intestinal⁶⁹. Le contenu protéique en P-gp et BCRP est 4 fois supérieur à celui de MRP2 au niveau duodénal, tandis que la P-gp prédomine au niveau hépatique. La comparaison de ces données à celles de la littérature permet de constater leur variabilité des résultats des études, possiblement en rapport avec la technique d'extraction et de quantification de l'ARN extrait, mais également selon le segment intestinal considéré. Cette comparaison est illustrée par la figure 11.

Figure 11. Variabilité des niveaux d'expression des transporteurs BCRP, MRP2 et P-gp selon différentes études



BCRP : Breast cancer Resistance Protein ; MRP2 : multidrug resistance protein 2, P-gp : P-glycoprotein.

D'après Tucker et al.

2.2.2. Les enzymes entérocytaires des médicaments, exemple du CYP3A4

Au niveau intestinal, le CYP3A est l'enzyme la plus abondante avec un contenu spécifique moyen représentant entre 50 et 70% de la quantité des CYP ⁷⁰. Il convient de signaler toutefois que peu de données existent quant à l'importance et l'abondance des autres CYP dans l'intestin.

Le contenu microsomal entérique en CYP3A, l'expression (en ARNm et protéines) aussi bien que l'activité catalytique associée, sont généralement plus importants au niveau du duodénum proximal et ensuite déclinent nettement vers l'iléon distal pour disparaître au niveau colique ^{66,71-73}. Elle est faible au niveau gastrique ⁶⁶. Bien que la quantité totale de CYP3A dans l'intestin grêle soit estimée à environ 1% de celle du foie, les études chez l'homme montrent que le CYP3A peut contribuer significativement, et dans certains cas à parts égales avec le CYP3A hépatique, au métabolisme de premier passage par voie orale de plusieurs médicaments (ex. ciclosporine, midazolam et vérapamil) ⁷⁴⁻⁷⁶.

Néanmoins, le rôle relatif des activités des CYP3A hépatique et intestinale dans la clairance des médicaments après administration orale reste difficile à évaluer. Une telle estimation a été généralement basée sur les déterminations *in vitro* des taux d'expression de CYP3A ou des activités au sein de microsomes intestinaux ou hépatiques, ou encore par des modèles de prédiction en incorporant les données expérimentales obtenues *in vitro* et/ou *in vivo*. Il est néanmoins certain que le foie est déterminant dans le métabolisme du premier passage des substrats du CYP3A4 ⁷⁷. Notons que les activités CYP3A hépatique et intestinale ne sont pas forcément corrélées et peuvent même être inversement corrélées au niveau individuel ^{78,79}.

2.2.3. Régulation de l'expression des transporteurs et enzymes par les récepteurs nucléaires

Les récepteurs nucléaires forment une superfamille de facteurs de transcription. Ils régulent une variété de fonctions biologiques, dont la croissance, le développement, la reproduction,

le métabolisme, et ce, chez une multitude d'organismes vivants. Ils sont également impliqués dans la régulation de l'expression des transporteurs et enzymes des médicaments. Ces protéines exercent un contrôle direct sur l'expression de gènes cibles en réponse à différents signaux. En se liant à l'ADN au niveau de séquences spécifiques, les éléments de réponse aux hormones (HREs pour « hormone response elements »), elles participent ainsi au contrôle de différents mécanismes cellulaires dont la prolifération, l'apoptose, la différenciation et l'homéostasie. Bien que pour plusieurs de ces facteurs de transcription une interaction à un ligand soit nécessaire à leur activation, il existe un ensemble de récepteurs, définis comme orphelins, pour lesquels aucun ligand n'a été identifié.

L'interaction du ligand peut avoir lieu dans le cytoplasme ou le noyau. Les ligands, de diverses catégories, peuvent être d'origine extracellulaire (sécrétion endocrine ou paracrine) ou intracellulaire (intermédiaire métabolique).

Les récepteurs nucléaires auraient un ancêtre commun. Une analyse de la séquence des protéines a permis d'établir une classification des récepteurs nucléaires en six sous-familles.

-La classe I regroupe les récepteurs des hormones thyroïdiennes (TRs), des acides rétinoïques (RARs), de la vitamine D (VDRs), des prostaglandines et des acides gras polyinsaturés « Peroxisomes proliferator activated-receptor » (PPAR). Cette classe comprend également plusieurs autres récepteurs nucléaires notamment, « Pregnane X receptor » (PXR), « Constitutive androstane receptor » (CAR), « Liver X receptor » (LXR), « Farnesoid X receptor » (FXR), « Reverse ErbA » (RevErb) et « Retinoid Z receptor » (RZR/ROR).

-La classe II contient les récepteurs des acides rétinoïques-9-cis (RXRs). Elle regroupe également les récepteurs « chicken ovalbumine upstream regulators » (COUPs), « hepatocyte nuclear factor 4 » (HNF4), « Testis receptors » (TR2), « Tailless-related receptor » (TLX) et « Photoreceptor-specific nuclear receptor » (PNR).

-La classe III regroupe les récepteurs des hormones stéroïdiennes incluant les récepteurs des glucocorticoïdes (GR), des hormones androgènes (AR), de la progestérone (PR) et des hormones oestrogènes (ER). Elle comprend également les « Estrogen-related receptors » (ERR).

-Les récepteurs nucléaires orphelins « » (NOR-1), « NGF-induced clone B » (NGFI-B) et « Nur-related factor 1 » (NURR 1) forment la quatrième classe tandis que les récepteurs « Stéroïdogenic factor 1 » (SF-1/FTZ-F1) et « Germ cell nuclear factor » (GCNF) appartiennent respectivement aux classes V et VI.

-Une dernière catégorie de récepteurs nucléaires, formant la classe 0, comprend les protéines «Small heterodimeric partner» (SHP) et «Dosage-sensitive sex reversal» (DAX-1).

2.3. Absorption et obésité

Il n'existe pas de travaux, à ce jour, chez l'homme, permettant de savoir spécifiquement si l'absorption des médicaments oraux chez les sujets obèses est différente de celle des sujets de poids normal.

L'expression des protéines entérocytaires pourrait être modifiée par rapport aux sujets non-obèses par des facteurs physiologiques associés à l'obésité tels que le microbiote intestinal, l'inflammation, ou encore des anomalies d'expression de protéines favorisant à la fois la surcharge pondérale et une variabilité de métabolisme entérocytaire des médicaments. Néanmoins ces hypothèses n'ont pas fait l'objet de travaux cliniques.

Il sera décrit, dans le chapitre « élimination des médicaments et obésité », les travaux en rapport avec le métabolisme et l'excrétion des médicaments chez les sujets obèses, susceptibles de concerner le métabolisme entérocytaire et post-entérocytaire des médicaments.

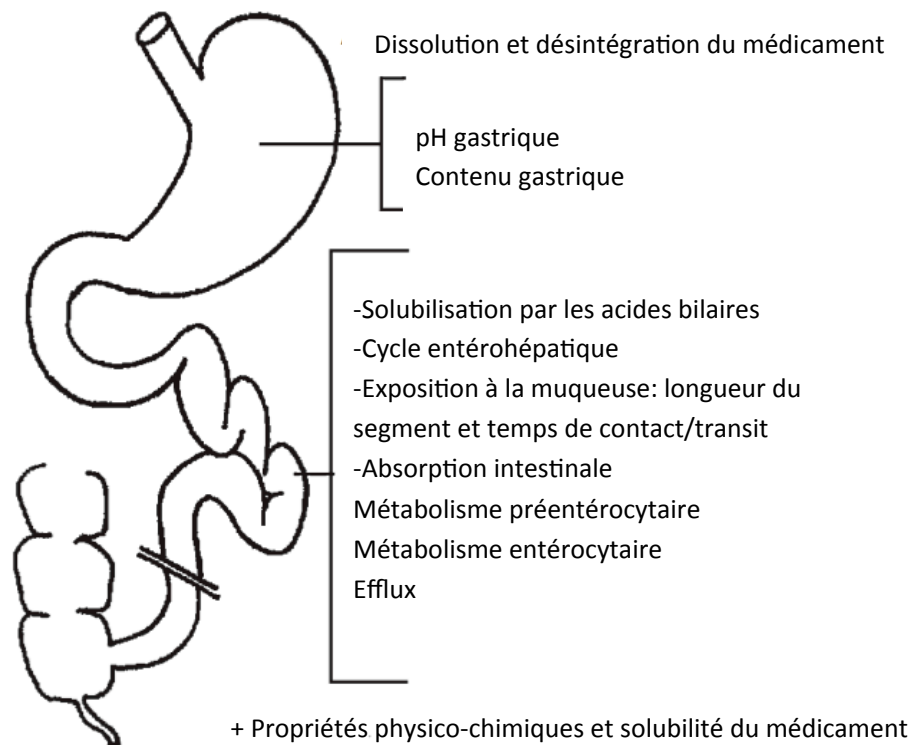
2.4. Absorption et chirurgie de type bypass gastrique

Compte tenu de la multiplicité des facteurs déterminants l'absorption des médicaments, on conçoit volontiers que la chirurgie de type RYGB la modifie.

2.4.1. Effets théoriques de la chirurgie sur l'absorption des médicaments

Les facteurs à l'origine de l'absorption des médicaments chez l'individu normal sont rappelés dans la figure 12.

Figure 12. Facteurs influençant l'absorption



a. Effet de la réduction de la poche gastrique

Désagrégration des médicaments

Pour mémoire, la désagrégration d'un médicament varie considérablement selon les différentes préparations et peut constituer un facteur limitant dans l'absorption de formes galéniques solides ⁵⁸. La désagrégration est favorisée par le mélange du médicament au contenu gastrique, et cette étape est réduite dans les chirurgies de type restrictives. L'administration d'un médicament sous une formulation liquide ou en poudre est susceptible d'atténuer ce phénomène.

Par ailleurs, l'estomac nouvellement créé exclue les parties de l'estomac contenant la plupart des cellules productrices d'acide. Or l'augmentation de pH peut réduire la désagrégration de formes galéniques solides ⁸⁰.

La dissolution

La dissolution du médicament peut être potentiellement altérée dans les procédures restrictives qui augmentent le pH gastrique. Théoriquement, l'augmentation du pH gastrique doit augmenter la solubilité de médicaments basiques (devenu moins ionisés) et de diminuer la solubilité de médicaments acides (plus ionisés) ⁸¹. Bien que la dissolution des médicaments n'ait pas été examinée directement in vivo, son analyse in vitro simulant les effets de la chirurgie bariatrique, révèle que 10 des 22 médicaments psychiatriques exposés présentent une dissolution moindre tandis que deux médicaments présentent une dissolution meilleure ⁸².

Absorption et pH gastrique

Il convient de noter que les changements de pH affectent peu l'absorption gastrique, habituellement limitée compte tenu de la surface réduite de l'estomac par rapport à celle de l'intestin grêle. Néanmoins, le pH gastrique, via son influence sur la solubilisation, peut modifier l'absorption entérocytaire. Si l'on prend l'exemple des suppléments martiaux, parfaitement solubles dans un environnement acide, on observe qu'elles sont mieux absorbées lorsqu'elles sont co-administrées avec de l'acide ascorbique après chirurgie restrictive ⁸³.

L'impact clinique des modifications de l'estomac et de son pH sur l'absorption des médicaments après chirurgie est probablement spécifique au médicament et à sa forme galénique.

Vidange gastrique

L'effet de la moindre vidange gastrique des solides après RYGB n'est pas déterminé ⁸⁴. La réduction de la vidange gastrique pourrait réduire la vitesse mais pas la quantité totale de médicament absorbé. Les études portant sur l'effet des procédures réduisant la vidange gastrique, chez les sujets non obèses, objectivent une diminution variable de l'absorption des médicaments ⁵⁸.

b. Effet de la technique de malabsorption

Les procédures qui impliquent un shunt intestinal sont susceptibles de réduire l'absorption de médicaments, principalement en raison de la modification de leur solubilité, de la réduction de la longueur de l'intestin, des temps de transit intestinal et d'exposition à la muqueuse plus courts. Elles sont aussi associées à un déversement plus tardif des acides biliaires au niveau entérocytaire.

Sels biliaires

La solubilité des médicaments fortement lipophiles dépend souvent de la disponibilité des acides biliaires. Ces mêmes médicaments subissent souvent un cycle entéro-hépatique. Le shunt de l'intestin grêle et la dérivation des sels biliaires dans un intestin plus distal limite le mélange des substances avec des acides biliaires à un segment de l'intestin grêle plus distal, plus court et peut conduire à la diminution d'absorption de médicaments tels que la cyclosporine, la phénytoïne, la rifampicine, la thyroxine, le tacrolimus, qui sont tous des médicaments lipophiles en dehors du tacrolimus qui subit un cycle entérohépatique ⁸⁵.

Longueur d'intestin

Le GBP réduit la longueur fonctionnelle de l'intestin grêle et l'exposition globale du médicament à la muqueuse, bien qu'il possède la plus grande surface totale par unité de longueur du tractus gastro-intestinal.

Les médicaments ayant des propriétés de dissolution lente, une libération prolongée ou ayant une galénique enrobée risquent d'être moins absorbés compte tenu de la diminution de la longueur de l'intestin. Cependant le temps de transit intestinal, plus lent au niveau du colon par rapport à l'intestin proximal, peut favoriser une absorption distale de médicaments de dissolution plus rapide et habituellement absorbé par l'intestin grêle avec un temps de contact avec la muqueuse moindre ⁵⁸.

Métabolisme entérocytaire

L'effet propre du shunt de segments de l'intestin grêle impliqués dans le métabolisme et le transport entérocytaire des médicaments est difficile à distinguer des phénomènes précédemment cités ⁸⁶. Son effet dépend probablement des voies métaboliques empruntées habituellement par les médicaments. Par exemple, la cyclosporine est un substrat pour la P-

gp, qui agit dans le sens d'une diminution de l'absorption. Sachant que son expression augmente de la partie proximale de l'intestin à sa partie distale, le shunt de l'intestin grêle proximal peut accroître l'influence de la P-gp sur l'absorption de la cyclosporine⁶⁵.

Au total, au vu du nombre de facteurs décrits précédemment, le devenir de l'absorption des médicaments après chirurgie de type bypass gastrique est difficile à prévoir⁸⁷. La galénique du médicament, les propriétés physicochimiques du principe actif ou encore la variabilité inter individuelle de la barrière intestinale, participent aux modifications de l'absorption après RYGB.

L'épithélium de l'intestin, constitué à 80% d'entérocytes est une structure extrêmement dynamique qui se renouvelle dans son intégralité tous les 4 à 5 jours⁸⁸. Différentes études suggèrent qu'il existe un phénomène d'«adaptation intestinale» après résection du grêle tel qu'une hypertrophie de la muqueuse intestinale restante compense la diminution de la capacité d'absorption au fil du temps chez les patients souffrant d'une grêle court^{89,90}. Toutefois, la façon dont ce phénomène affecte l'absorption du médicament après chirurgie bariatrique est inconnue, en particulier la façon dont il évolue à moyen et long terme après chirurgie.

2.4.2. Devenir de l'absorption digestive après bypass gastrique

Les observations concernant le devenir des médicaments après RYGB sont la conséquence d'un effet du montage chirurgical sur leur absorption et de la perte de poids susceptible de modifier leur distribution et indirectement leur élimination. Les données rapportées ici ne permettent pas de distinguer leurs responsabilités relatives.

a. Cas cliniques

Quelques cas cliniques rappellent les risques d'une méconnaissance de l'effet de la chirurgie de l'obésité sur l'absorption des médicaments.

L'échec du traitement oral d'une cystite sensible à l'amoxicilline et à la nitrofurantoïne chez une patiente âgée de 29 ans enceinte de 9 semaines, 7 années après un RYGB, fut compliqué

d'une pyélonéphrite aigue résolue grâce à l'administration d'une antibiothérapie par voie systémique (ceftriaxone) ⁹¹.

Le doublement de la posologie d'halopéridol fut nécessaire, de façon transitoire et immédiate après chirurgie de type RYGB, chez une femme de 51 ans dont la schizophrénie était habituellement contrôlée par 20mg d'halopéridol ⁹². L'augmentation de la posologie d'immunosuppresseurs en rapport avec une diminution de leurs concentrations est observée après RYGB ⁹³.

Le premier cas rappelle combien la méconnaissance du devenir des antibiotiques peut engendrer un retard d'efficacité thérapeutique et un risque médical majeur. Le deuxième et le troisième soulignent l'intérêt d'un monitoring systématique des concentrations de médicaments pris au long cours n'ayant pas de marqueur pharmacodynamique immédiat.

b. Etudes cas contrôles

Metformine

La PK d'une dose unique de metformine (2cp de 500mg) a été réalisée chez 16 patients non diabétiques ayant bénéficié d'un RYGB (nombre d'années après RYGB non précisé) et 16 patients témoins appariés sur le sexe et l'IMC (âge moyen de 40 ans et IMC 39.2 kg / m²) ⁹⁴ (Tableau12).`

Tableau 12. Etude cas contrôle comparant la PK de la metformine avant et après RYGB

| Variable | Gastric bypass subjects | Control subjects | Mean difference (95% CI) | P |
|--|-------------------------|------------------|--------------------------|--------|
| <i>n</i> | 16 | 16 | | |
| Pharmacokinetic outcomes | | | | |
| AUC _{0-∞} (μg/h/mL) | 13.7 (6.0) | 11.4 (3.6) | 2.3 (-1.3 to 5.9) | 0.20 |
| Bioavailability (%) | 41.8 (16.2)* | 27.8 (10.4) | 14.0 (4.1-23.9) | 0.007 |
| AUC _{0-24 h} (μg/h/mL) | 13.4 (5.7) | 11.1 (3.6) | 2.2 (-1.3 to 5.6) | 0.20 |
| C _{max} (μg/mL) | 2.0 (0.86) | 1.8 (0.61) | 0.2 (-0.3 to 0.8) | 0.32 |
| T _{max} (h) | 3.0 (1.5-3.0)† | 3.0 (1.5-3.0)† | 0 (0)† | 0.89† |
| Half-life (h) | 3.9 (0.74) | 4.0 (0.87) | -0.1 (-0.7 to 0.5) | 0.66 |
| Urinary recovery (0-24 h) (mg) | 326 (126) | 217 (81) | 140 (41-239) | 0.007 |
| Weight-normalized urinary recovery (0-24 h; mg/kg) | 3.1 (1.2)* | 2.0 (0.78) | 1.5 (0.5-2.5) | 0.003 |
| Renal clearance (mL/min) | 461 (199)* | 337 (131) | 125 (1.5-248) | 0.047 |
| Weight-normalized renal clearance (mL/min/kg) | 4.3 (1.6)* | 3.0 (1.0) | 1.3 (0.3-2.3) | 0.009 |
| Weight-normalized volume of distribution (L/kg) | 1.4 (0.4) | 1.0 (0.4) | 0.4 (0.1-0.7) | 0.02 |
| Pharmacodynamic outcome | | | | |
| AUC glucose _{0-8 h} (mmol/mL/h) | 35.8 (3.7) | 41.7 (4.1) | 5.9 (3.1-8.8) | 0.0002 |

Data are means (SD), unless otherwise indicated. *Sample size was 15. One sample was lost. †Numbers are median (range). Mann-Whitney U statistic used.

D'après Padwal et al.

Par rapport aux sujets témoins, les auteurs montrent une augmentation non significative de l'exposition de la metformine de 21% (13,7 contre 11,4 pg/mL/h). Ils décrivent aussi une augmentation significative de la biodisponibilité de 50% (41,8 vs 27,8%). Néanmoins, on s'interroge sur les critères permettant aux auteurs de définir et de comparer la biodisponibilité sachant qu'il s'agit d'une étude cas/contrôle et qu'aucune étude de PK de la metformine par voie intraveineuse n'est réalisée.

Les Cmax et Tmax sont superposables dans les deux groupes, tandis que l'augmentation de la clairance de la metformine persiste après ajustement sur le poids, faisant suggérer une augmentation du métabolisme de la metformine chez les sujets obèses opérés, non liée au poids.

Azythromycine

Une étude réalisée par le même auteur sur l'absorption de l'azythromycine chez 14 femmes opérées d'un RYGB depuis plus de trois mois, versus 14 femmes non opérées de même IMC (36, 4kg/m²), montre une diminution de l'exposition de 32% chez les patients opérées sans modification du temps et de la concentrations maximum⁹⁵.

Sertraline

Une étude de méthodologie superposable compare la PK d'une dose unique de sertraline 100mg chez 5 patients ayant bénéficié d'un RYGB 9 à 15 mois plus tôt et 5 patients n'ayant pas été opérés⁹⁶. L'exposition moyenne plasmatique est significativement plus faible après chirurgie (124,4 ± 55,5 ng-hr/mL) par rapport au groupe témoin (314,8 ± 129,6 ng-h / mL) et le Cmax est significativement plus faible.

c. Etudes pharmacocinétiques avant/après

Inhibiteurs de la recapture de la sérotonine

Une étude prospective PK concernant les inhibiteurs de la recapture de la sérotonine et les inhibiteurs de la recapture de la sérotonine et de la noradrenaline chez 12 patients candidats au RYGB, montre une diminution de l'exposition de 54% à un mois de la chirurgie chez 8 des 12 sujets. Six de ces 8 patients retrouvent des valeurs comparables à la période préopératoire après 6 mois. Une exacerbation des symptômes dépressifs associée à la

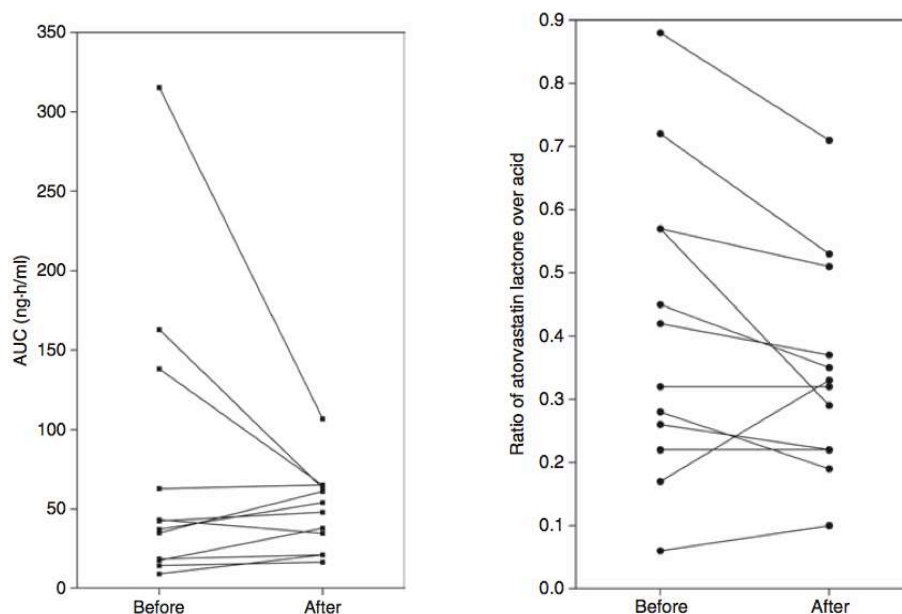
diminution de la concentration des antidépresseurs est observée chez 4 patients. Il est intéressant de noter que parmi ces 4 patients, trois ont pris du poids ou n'en ont pas perdu dans les 6 à 12 mois suivants la chirurgie, soulignant l'effet négatif d'un mauvais contrôle des symptômes dépressifs après la perte de poids.

Cependant la diminution de concentration concerne davantage les inhibiteurs de la recapture de la sérotonine que les inhibiteurs de la recapture de la sérotonine et de la noradrénaline.

Atorvastatine

L'atorvastatine a également fait l'objet d'une étude PK chez 12 patients souffrant d'obésité morbide traités par 20 à 80 mg d'atorvastatine et candidats à un RYGB, la veille de la chirurgie et 5 semaines après ⁹⁷. L'évolution de l'exposition systémique à l'atorvastatine varie de façon considérable d'un patient à l'autre et son évolution semble liée à l'exposition préopératoire. Effectivement une majoration de l'absorption de l'atorvastatine est observée chez 8 des 9 sujets ayant des AUC inférieures à 100ng.h/mL. A l'inverse, les 3 sujets dont l'AUC est supérieure à 130 présentent une forte réduction de l'exposition(Figure 13).

Figure 13. Exposition plasmatique de l'atorvastatine orale et du ratio de ses formes lactone et acide avant et après RYGB.



D'après Skothheim et al.

Ces résultats témoignent d'une part de la difficulté de prédire le devenir de l'absorption interindividuelle des médicaments et d'autre part de définir le rôle du duodénum et du jéjunum proximal dans la variabilité interindividuelle d'absorption des médicaments. En effet, il semble qu'après RYGB la variabilité d'exposition tend à diminuer. Sachant que le contenu en CYP est supérieur dans l'intestin grêle proximal, le shunt de ce segment détermine probablement la réduction de la variabilité d'absorption ⁷³.

Une étude de design comparable décrit les variations de la PK de l'atorvastatine après diversion biliopancréatique, soit après un shunt presque complet de l'intestin grêle ⁹⁸. L'absorption est multipliée d'un facteur 2 à 3, tandis que le Tmax va dans le sens d'un allongement (1.2 h avant chirurgie et 2.3 h après chirurgie), en rapport avec une moindre rapidité d'absorption des médicaments au niveau colique mais une augmentation de l'absorption supposant une augmentation de la durée de contact de l'atorvastatine avec une muqueuse pauvre en CYP, favorable à l'absorption.

Antibiotiques

La biodisponibilité orale de la moxifloxacine chez 12 sujets à distance d'un RYGB (administration orale et intraveineuse) est de 88%, superposable ou légèrement inférieure à la valeur observée chez les sujets indemnes de RYGB ⁹⁹. La comparaison des expositions systémiques n'était pas réalisée

2.5. Distribution des médicaments et obésité

Différents facteurs peuvent modifier la distribution des médicaments chez les sujets obèses.

2.5.1. Liaison aux protéines des médicaments

Les concentrations d' α 1-glycoprotéine acide peuvent doubler chez le sujet obèse par rapport à celles observées chez les sujets de poids normal ¹⁰⁰. Ceci entraîne une diminution de la fraction libre, active, des agents faiblement basiques qui se lient à cette protéine soit

par exemple l'érythromycine, la lidocaïne, la bupivacaïne, le propranolol, l'alfentanil, le fentanyl (en partie), le sufentanil ou le vérapamil.

2.5.2. Le volume de distribution

a. Déterminants

Les modifications du volume de distribution induites par l'obésité sont multifactorielles et influencées par les modifications de la composition corporelle abordées dans le premier chapitre de l'introduction. Elles dépendent en effet de l'augmentation de la masse grasse, de la masse maigre, du volume sanguin et de la taille des principaux organes, modifications à l'origine d'une augmentation du volume du compartiment central. Ceci nécessite d'administrer une dose initiale plus grande d'un médicament pour obtenir le même effet pharmacologique. Chez les sujets obèses, l'augmentation du volume de distribution à l'équilibre d'un composé donné dépend de l'affinité relative du composé pour les différents tissus.

b. Médicaments hydrosolubles

La distribution des agents hydrosolubles, dont les volumes de distribution sont souvent moins importants que ceux des agents liposolubles, n'est en règle que peu modifiée ¹⁰¹. Cependant, l'étude des aminosides, de l'ibuprofène, de l'acétaminophène par exemple, montre une nette augmentation du Vss (Volume de distribution à l'état stable) dans la population obèse ¹⁰¹⁻¹⁰⁴. Cette augmentation a été attribuée à la contribution relative des liquides extra-cellulaires dans le tissu adipeux et à l'augmentation de la masse maigre. L'étude de la distribution de l'antipyrine, utilisée comme marqueur de l'eau totale montre que si le volume de distribution de l'antipyrine rapporté au poids corporel est significativement plus bas chez les sujets obèses, ce même volume rapporté au poids idéal est statistiquement plus élevé, ce qui traduit bien une augmentation de l'eau totale, mais qui n'est pas exactement parallèle à l'augmentation du poids corporel ¹⁰⁴. Par conséquent on doit s'attendre chez un sujet obèse à une augmentation de la distribution des agents hydrosolubles (curares par exemple) sans que l'on puisse cependant utiliser les schémas

posologiques habituels rapportés au poids corporel. Certains auteurs utilisent comme facteurs de corpulence, entre le poids idéal et le poids réel, un poids ajusté prenant en compte 40% de l'excès pondéral, afin de prédire les données pharmacocinétiques de médicaments hydrosolubles¹⁰⁵.

c. Agents liposolubles

La distribution des agents dans le tissu adipeux dépend de leur liposolubilité exprimée le plus souvent par le coefficient P de partage octanol/eau, bien que ce paramètre ne soit peut-être pas toujours un très bon reflet de la liposolubilité in vivo¹⁰⁶. Certains agents avec un coefficient P traduisant une bonne affinité pour les lipides et la capacité de franchir les barrières lipidiques, ne présentent pas obligatoirement une distribution importante dans le tissu adipeux, comme par exemple le propofol ou l'alprazolam dont le coefficient Log P est inférieur à 20^{106,107}. Le Vss de ces agents est augmenté chez le sujet obèse, de façon proportionnelle à celle du poids corporel. D'autres agents se distribuent de façon préférentielle dans le tissu adipeux, tels que le midazolam (LogP=34), le thiopental (LogP=89), le diazépam (LogP=309)^{106,108}. L'augmentation du Vss est dans ce cas proportionnellement plus importante que celle du poids corporel.

2.6. Elimination des médicaments et obésité

Il sera abordé dans ce chapitre les liens entre obésité et activité des enzymes et transporteurs des médicaments. Les données concernant leur expression sont essentiellement issues de données chez l'animal.

2.6.1. Influence de l'obésité sur l'activité des enzymes du métabolisme des médicaments chez l'homme

Certains médicaments sont hautement métabolisés par certains cytochromes. Leur PK informe sur l'activité des enzymes du métabolisme. Le tableau 13 illustre les résultats de ces différentes études.

Tableau 13 Effet de l'obésité sur l'activité des enzymes de phase I et II

| Médicaments étudiés | | Influence de l'obésité | Ajustement sur le poids |
|------------------------------|---|--|--|
| Enzymes de phase I | | | |
| CYP1A2 | Caféine (n=5) Théophylline (n=3) | Tendance à une augmentation de la clairance des substrats | Résultats contradictoires |
| CYP2C9 | Glimepiride (1) Glipizide (1) Ibuprofène (1) Phénytoïne (1) | Légère augmentation de la clairance des substrats | Légère diminution de la clairance |
| CYP2C19 | Diazepam (1) Demethyldiazepam (1) | Résultats contradictoires | Légère diminution de la clairance |
| CYP2D6 | Dexfenfluramine (n=2) Nebivolol (n=1) | Tendance à une augmentation de la clairance des substrats | Absence de données |
| CYP2E1 | Chlorzoxazone (n=5) Enflurane (n=2) Sevoflurane (n=2) Halothane (n=1) | Augmentation significative de la clairance des substrats | Clairance égales ou supérieures |
| CYP3A4 | Taranabant (n=1) Docetaxel (n=2) Carbamazepine (n=2) Erythromycine (n=2) Midazolam (n=1) Alprazolam (n=1) Ciclosporine (n=2) Trazodone (n=1) Alfentanil (n=1) | 7/13 études : diminution de la clairance des substrats 4/13 études : diminution non significative de la clairance | Diminution de la clairance des substrats |
| Xanthine Oxydase | Caféine (n=1) 6 mercaptopurine (n=1) | Augmentation significative de la clairance des substrats | |
| Enzymes de phase II | | | |
| UGT1A9, 1A6, 2B15 | Paracétamol (n=4) | Augmentation significative de la clairance des substrats | Clairance égales ou légèrement diminuées |
| UGT1A9, 2B7, 2B15 | Oxazepam (n=1) | Augmentation significative de la clairance des substrats | Augmentation significative de la clairance |
| UGT sulfo-conjugaison | Garenoxacine (n=1) | Augmentation significative de la clairance des substrats | Clairance égales ou légèrement diminuées |

D'après la review de Padwal et al.

a. Le CYP1A2

Il est impliqué dans le métabolisme d'environ 5% des médicaments. La caféine et la théophylline en sont des substrats hautement spécifiques. Plusieurs études ne montrent pas d'effet de l'obésité sur la clairance de la caféine ¹⁰⁹⁻¹¹². Néanmoins la clairance de la théophylline est réduite après perte de poids modérée et une corrélation entre la clairance de la théophylline et le poids corporel total a également été rapporté, laissant suggérer un effet de l'obésité sur la clairance des substrats du CYP1A2 ^{113,114}.

Après correction sur le poids corporel, les données concernant l'effet du poids sur la clairance restent cependant contradictoires ¹¹⁵.

b. Le CYP2C9

Le métabolisme médié par le CYP2C9 représente environ 10% du métabolisme des médicaments de phase I chez l'homme. Quatre substrats du CYP2C9 (ibuprofène, phénytoïne, glimépiride et glipizide) nous informent sur son activité.

Il existe une augmentation significative de la clairance de l'ibuprofène chez les patients obèses ^{103,116}. Celles du glimépiride et du glipizide ne le sont pas de façon significative mais l'excrétion urinaire des métabolites du glimepiride est augmentée de 30% ($p < 0,05$) chez les sujets obèses par rapport aux non-obèses, après avoir reçu doses comparables en faveur d'une clairance augmentée ^{117,118}. Après ajustement sur le poids, la clairance médiée par le CYP 2C9 est réduite chez les sujets obèses, supposant que l'augmentation de la clairance ne soit pas linéaire avec le poids ¹¹⁵.

c. Le CYP2C19

Il est responsable du métabolisme de 5% des médicaments et son activité est soumise à une importante variabilité influencée par les polymorphismes génétiques. Une seule étude phénotypique, utilisant comme substrat le diazépam montre une clairance plus élevée chez les sujets obèses, sans différence concernant la clairance des métabolites ¹¹⁹. Cependant l'ajustement de la clairance sur le poids révèle une réduction de celle-ci, à l'image du CYP2C9.

d. Le CYP2D6

Le métabolisme via le CYP2D6 concerne 10 à 15% du métabolisme des médicaments de phase I chez l'homme et diffère selon ses polymorphismes génétiques. Deux substrats du CYP2D6, le dexfenfluramine et le nébivolol, ont fait l'objet d'études PK comparatives chez les sujets obèses et non obèses. Il existe une clairance augmentée du dexfenfluramine, parallèlement à une augmentation du ratio métabolites / parent chez les sujets obèses ¹²⁰. De la même façon, la clairance hépatique du nébivolol est plus élevée chez les sujets obèses ¹²¹. Cependant, sachant que la clairance du nébivolol est élevée ($> 1 \text{ L / min}$), l'augmentation de clairance est peut être davantage le reflet d'une augmentation du flux sanguin hépatique plutôt que d'une augmentation du métabolisme du CYP ¹²².

e. Le CYP2E1

Bien que le métabolisme médié par le CYP2E1 ne représente qu'environ 5% du métabolisme des médicaments de phase I, l'impact de l'obésité sur l'activité du CYP2E1 a fait l'objet de plusieurs études, dont certaines incluaient des patients souffrant d'obésité morbide.

La chlorzoxazone, substrat hautement sélectif du CYP2E1, présente un métabolisme augmenté chez les patients obèses, comme le démontre l'augmentation de la formation de sa forme hydroxylée (40%) et l'augmentation de sa clairance orale (multipliée par un facteur 3) ¹²³⁻¹²⁵. Emery et al. montrent également des différences de clairance selon que la stéatose affecte plus ou moins 50% des hépatocytes. En effet il semble exister une tendance à une augmentation de la clairance corrélée avec le degré de stéatose, suggérant l'influence de la stéatose sur l'activité des cytochromes, la diminution de la clairance après chirurgie de type RYGB est en faveur de cette hypothèse ¹²⁶.

Les anesthésiques volatils, tels que l'enflurane, l'halothane, le sévoflurane sont des marqueurs fiables de l'activité du CYP2E1 ¹²⁷. A l'exception d'une étude concernant le sévoflurane, les concentrations des dérivés sont significativement plus élevées chez les sujets obèses en comparaison aux non-obèses ¹¹⁵.

Ainsi une augmentation constante et significative de la clairance de différents substrats du CYP2E1 est observée chez les sujets obèses en comparaison aux sujets non obèses. Après normalisation sur le poids corporel, la clairance est plus ou moins égale entre ces deux

populations, en faveur d'une augmentation de l'activité du CYP2E1 avec le poids corporel et l'infiltration stéatosique du foie ¹²⁶.

Il est à noter que les métabolites toxiques de l'acétaminophène sont issus de l'activité du CYP2E1 et que deux études concernant le paracétamol chez les sujets obèses ne permettent pas de savoir si la production de métabolites est accrue ^{102,128}.

f. Le CYP3A4

La PK de plusieurs substrats du CYP3A4 chez les sujets obèses en comparaison aux sujets non-obèses ont été rapportés ¹¹⁵.

Le test respiratoire à l'érythromycine marquée au carbone 14 permet de mesurer l'activité du CYP3A4. Son utilisation a permis de montrer que l'obésité est significativement associée à un métabolisme plus lent reflétant une réduction de l'activité du CYP3A4 chez les hommes et les femmes ($r^2 = 0,91$ et $r^2 = 0,90$, respectivement) ^{129,130}. De même, la clairance du triazolam est significativement plus faible chez les patients obèses ^{131,132}. Concernant les pharmacocinétiques du midazolam, de l'alprazolam et de la ciclosporine, une diminution non significative de la clairance est observée, le manque de significativité pouvant être en rapport avec le faible effectif de patients inclus dans ces études ¹³¹⁻¹³⁴.

Au delà des études phénotypiques, des études PK concernant des substrats essentiellement métabolisés par le CYP3A4 montrent une tendance à un moindre métabolisme des médicaments tels que la carbamazépine, tandis qu'une perte de poids importante s'associe à une augmentation significative de sa clairance, suggérant les rôles de la stéatose hépatique dans la réduction du métabolisme chez les sujets obèses et/ou la diminution du flux sanguin comme déterminant de l'activité du CYP3A4 ^{135,136}. De la même façon il est observé un moindre métabolisme du fentanyl et du taranabant chez les patients obèses, réduit d'un facteur deux chez les sujets obèses par rapport aux sujets non-obèses ^{137,138}. Seuls la clairance du trazadone et du docétaxel ne sont pas modifiés par l'obésité ^{20,139,140}.

Ainsi plus de la moitié des études PK et phénotypiques sont en faveur d'une clairance plus faible des médicaments substrats du CYP3A4 chez les sujets obèses, et cette différence persiste après ajustement sur le poids corporel ¹¹⁵. Notons que les effectifs de patients avec une obésité morbide ($IMC > 40\text{kg/m}^2$) étaient faibles dans ces études.

g. Les enzymes de phase II

Une augmentation significative de la clairance du paracétamol est observée chez les sujets obèses en comparaison aux sujets non-obèses, en faveur d'une augmentation de la glucuronidation ^{102,128}.

Une analyse PK de population du Garénoxacine, substrat majeur des UGT, montre une augmentation de sa clairance avec le poids corporel total ¹⁴¹. L'oxazépam et le lorazépam, également substrats des UGT, présentent des valeurs de clairance significativement plus élevées chez les sujets obèses par rapport aux sujets témoins ¹⁴²⁻¹⁴⁴. Sur la base des observations les concernant, une augmentation de la capacité de conjugaison proportionnelle au poids corporel total a été suggérée.

Par ailleurs, la N-acétylation de la procaïnamide, marqueur de l'activité de la N-acétyltransférase (NAT), est supérieure, mais de façon non significative, chez les adultes obèses en comparaison avec les non-obèses ¹⁴⁵. Dans une étude incluant des patients acétylateurs lents, l'activité métabolique de la NAT évaluée par un test à la caféine, est multipliée d'un facteur 5 chez les enfants obèses par rapport aux enfants non obèses ¹⁴⁶.

Chez des patients obèses ou en surpoids, la clairance orale du busulfan, marqueur de l'activité du glutathion, est augmentée de façon significative par rapport aux sujets non obèses, mais elle est inférieure lorsqu'elle est ajustée sur le poids corporel ¹⁴⁷.

h. La P-gp

Le substrat spécifique de la P-gp le plus connu est la digoxine. S'agissant d'un médicament à marge thérapeutique étroite, des études PK ont tenté de prédire sa « juste posologie » selon des critères clinico-biologiques. Le poids a été proposé comme déterminant des concentrations de digoxine dans différents travaux. Cependant la clairance totale de la digoxine est comparable entre sujets de poids normal et sujets obèses ¹⁴⁸. Par ailleurs, la perte de poids, et notamment la perte de MG, ne modifie ni les concentrations, ni la clairance de la digoxine administrée par voie intraveineuse, supposant l'absence d'influence de la masse grasse sur l'élimination majoritairement rénale (sécrétion tubulaire) de la digoxine et sur l'activité de la P-gp ¹⁴⁹.

2.6.2. Influence de l'obésité sur l'expression des enzymes du métabolisme des médicaments

La modification de l'activité des enzymes des médicaments chez les patients obèses peut être la conséquence d'une expression différente. Certains travaux, notamment chez l'animal, montrent l'effet de la stéatose hépatique et de la stéato-hépatite non alcoolique, pathologies fréquemment associées à l'obésité, sur l'expression des enzymes hépatocytaires.

Une revue des études concernant l'effet de la stéatose hépatique *in vivo* chez l'animal et chez l'homme, et *in vitro* dans des modèles cellulaires, est synthétisée dans la figure ci-dessous ¹⁵⁰.

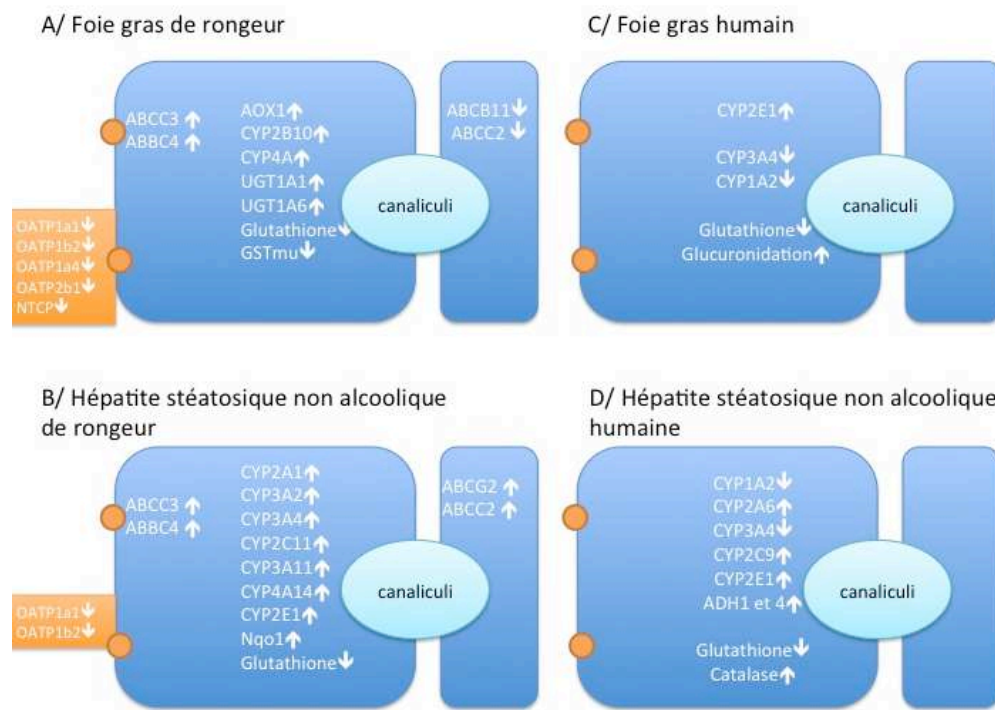
Certaines données suggèrent que la régulation de l'expression de différents cytochromes ou transporteurs sous l'effet de l'obésité, de la stéatose hépatique, ou de facteurs physiologiques leur étant associés, ait pour objectif de diminuer de la surcharge lipidique intra hépatocytaire et de modifier les conséquences métaboliques de l'obésité.

Ainsi, l'activité du facteur de transcription Nrf2 et l'expression des gènes *ABCC2*, *3* et *4* sont augmentés dans le foie des rongeurs ayant une NASH suggérant que Nrf2 soit impliqué dans l'induction d'expression de ces trois transporteurs ABC ¹⁵¹. Par ailleurs, le traitement de souris obèses (dites *ob/ob*, par mutation du gène de la leptine) par l'agoniste de CAR augmente l'expression du CYP2B10 et du CYP3A11 et améliore la tolérance au glucose. Un traitement prolongé diminue par ailleurs la stéatose hépatique, suggérant qu'une augmentation de l'activité de CAR observée dans la stéatose hépatique ait un rôle de protection vis à vis de l'accumulation de lipides intrahépatocytaires ¹⁵². Une augmentation de l'hème oxygénase (HO-1), régulée par le facteur de transcription Nrf2, est observée chez les patients atteints de NASH et est corrélée à la sévérité de la maladie ^{153,154}.

Par ailleurs, la comparaison de l'expression de gènes au sein de biopsies hépatiques de patientes souffrant d'obésité morbide opérées d'un RYGB et de femmes subissant une cure de hernie abdominale une année après RYGB montre des différences concernant les profils d'expression hépatique de 154 gènes dont 28 gènes candidats intervenant dans le métabolisme lipidique et la régulation de l'inflammation, mais aussi le transport des acides biliaires et le métabolisme des xénobiotiques ¹⁵⁵. Leur expression est effectivement réduite

chez les femmes obèses morbides en comparaison aux patientes ayant maigri (SULT2A : Sulfotransferase family, cytosolic, 2A, member 1; FMO 5 : Flavin containing monooxygenase 5; CYP2B7P1 Cytochrome P450; GSTT2 : Glutathione S-transferase; CYP1A2 ; CYP1A1; ABCP11).

Figure 14 Effet de la stéatose hépatique que l'expression des protéines impliquées dans le métabolisme hépatique des médicaments



Foie gras chez le rongeur (A), Hépatite stéatosique non alcoolique chez le rongeur (B), Foie gras chez l'homme (C), stéatose hépatique non alcoolique chez l'homme (D).

2.7. Influence de l'obésité sur le débit vasculaire hépatique

L'influence de l'obésité sur le débit sanguin hépatique n'est pas complètement définie. La stéatose hépatique est associée à un excès de triglycérides hépatiques, favorisant le rétrécissement des capillaires sinusoidaux et une altération de la morphologie fonctionnelle du foie ¹⁵⁶. Cependant l'augmentation du volume plasmatique et du débit cardiaque peuvent maintenir le débit sanguin hépatique.

Les produits à clairance intrinsèque élevée (supérieure à 1,5L/min) sont très dépendants du flux sanguin hépatique et en sont un marqueur potentiel. Leur cinétique est modifiée par les pathologies associées à des variations hémodynamiques.

L'étude PK de médicaments dont la clairance est supérieure à 1,5 L/min (propofol, sufentanil et le paclitaxel), montre une augmentation ou une stabilité de la clairance chez les patients obèses ^{20,157-159}. En revanche la normalisation de la clairance sur le poids révèle une forte diminution de celle-ci, traduisant l'influence de manifestations mécaniques locales, volontiers compensées par l'augmentation des débits sanguins. Ainsi, les clairances par kilogramme de poids du propranolol et de la lidocaïne sont pratiquement diminuées de moitié en cas d'obésité.

2.8. Influence de l'obésité sur la clairance rénale

2.1.1. La filtration glomérulaire

De nombreux médicaments, tels que les antibiotiques et les héparines de bas poids moléculaire éliminés par filtration glomérulaire, ont une clairance augmentée chez les patients obèses ^{160,161}. Les clairances de la vancomycine et du carboplatine sont effectivement corrélées au poids corporel total ^{162,163}. Toutefois les différences de clairance apparaissent significatives, dans certains cas, uniquement lorsque le degré d'obésité est important ^{164,165}. Après normalisation sur le poids, l'obésité n'est pas liée à un excès de filtration glomérulaire, supposant qu'il n'y ait pas de facteur autre que l'excès de masse corporelle dans la détermination de la filtration glomérulaire des patients obèses.

L'estimation de la clairance de la créatinine afin d'estimer la filtration glomérulaire est souvent utilisée pour prédire l'élimination des médicaments, ajuster leurs posologies et de définir leurs intervalles d'administration. Cependant les modifications physiologiques liées à l'obésité, tel que le débit de filtration glomérulaire, n'augmentent pas de façon linéaire avec le poids corporel total, de la même façon que la masse musculaire n'augmente pas de façon proportionnelle avec le poids. Ainsi la clairance de la créatinine (Clcr) estimée à l'aide de l'équation de Cockcroft-Gault (CG) et corrélée au poids surestime la clairance des patients obèses. A l'inverse l'équation MDRD tient compte de l'âge, du sexe, de la créatininémie et de

l'ethnie, et risque de sous estimer l'augmentation du DFG lié à l'obésité.

Clairance créatinine selon Cockcroft-Gault (CG) (ml / min)

$[(140 - \text{âge (années)}) \times \text{Poids (kg)} \times A] / \text{créatininémie } (\mu\text{mol/l})$

A = 1,23 chez l'homme et 1,04 chez la femme.

L'équation MDRD

Formule abrégée = GFR = $186 \times ([\text{Créat}/88.4]^{-1.154}) \times (\text{âge})^{-0.203} \times (0.742 \text{ si sexe féminin}) \times (1.210 \text{ si sujet noir})$

Certains auteurs ont trouvé que la surface corporelle était un bon outil d'évaluation de la corpulence permettant d'ajuster des posologies de médicaments. Effectivement la surface corporelle n'augmente pas linéairement avec l'obésité, à l'image de l'excès de masse non grasse déterminant l'excès de filtration glomérulaire. Pour les mêmes raisons, une mesure de la masse musculaire ou de l'eau corporelle totale sont pertinents pour expliquer les variations de clairance des médicaments à clairance rénale.

2.1.2. La sécrétion tubulaire

Certains médicaments sont en partie éliminés par sécrétion tubulaire et ont fait l'objet d'études chez les sujets obèses: la procainamide, la ciprofloxacine, le cisplatine, le topotécan et la digoxine. Environ 50% de la procainamide est éliminée sous forme inchangée par filtration glomérulaire et sécrétion tubulaire active¹⁶⁶. Une étude décrit une augmentation de sa clairance rénale chez les patients obèses en comparaison avec des sujets non-obèses, via une élévation de la sécrétion tubulaire puisque la clairance de la créatinine des 24h était comparable entre les deux groupes^{145,167-169}. Le cisplatine et de la ciprofloxacine font également l'objet d'une sécrétion tubulaire plus importante^{145,167-169}. Dans ces études, les clairances normalisées par le poids corporel sont comparables ou légèrement inférieures chez les patients obèses en comparaison aux patients non-obèses.

2.1.3. La réabsorption tubulaire

Peu d'études nous renseignent sur l'influence de l'obésité sur la réabsorption tubulaire des médicaments. La réabsorption du lithium chez les patients obèses serait réduite, puisque sa clairance est significativement augmentée malgré une filtration glomérulaire comparable aux sujets non obèses¹⁷⁰.

2.9. Transporteurs des médicaments et physiopathologie de l'obésité

Il est intéressant de décrire des travaux récents concernant les liens entre le transporteur P-gp et obésité. La P-gp est impliquée dans le mouvement à travers les membranes de molécules tels que le cholestérol (et en particulier le cholestérol exogène), les phospholipides et les sphingolipides et une variété de stéroïdes^{171,172}. Inversement, la teneur en cholestérol des membranes affecte l'activité de transport de la P-gp¹⁷³. Des travaux suggèrent que la P-gp soit impliqué dans le stockage adipocytaire et dans la physiopathologie de l'excès pondéral.

Le polymorphisme 2677G->A/T du gène *ABCB1* codant pour la P-gp est associé à une prise pondérale sous antipsychotiques^{174,175}. Les auteurs suggèrent que ce polymorphisme soit associé à une augmentation de la concentration intra cérébrale des neuroleptiques. Néanmoins, dans une population japonaise, ce variant est lié à l'indice de masse corporelle ($p = 0,01$) puisque les sujets porteurs du variant T ont un IMC supérieur aux sujets ayant le génotype GG (IMC moyen 23.52 versus 23.22)¹⁷⁶.

Des travaux in vivo chez l'animal vont dans la sens d'une augmentation du stockage des lipides en cas de déficit en P-gp. Après absorption entérocytaire d'une charge orale de cholestérol, les souris déficientes en P-gp présentent une augmentation de la concentration hépatique en ester de cholestérol¹⁷⁷. Par ailleurs, les patients hypercholestérolémiques porteurs de l'haplotype G2677T et C3435T de *ABCB1* ont des taux de cholestérol-LDL supérieurs aux autres patients¹⁷⁸. Une équipe française a récemment démontré que des souris habituellement pourvues d'un patrimoine génétique leur conférant une résistance aux régimes riches en graisses, ont un excès pondéral, une hypertrophie du tissu adipeux, une

stéatose hépatique, des taux plasmatiques élevés d'insuline et de glycémie lorsqu'elles sont rendues déficientes en P-gp¹⁷⁹. L'augmentation de l'activité des facteurs de transcription CAR et PXR et l'augmentation de l'expression de gènes impliqués dans la synthèse d'acides biliaires sont observés parallèlement¹⁷⁹.

Ainsi un dysfonctionnement de la P-gp semble s'accompagner d'une hypertrophie adipocytaire et d'une stéatose hépatique mais le rôle protecteur ou délétère de l'activation de CAR et PXR reste à déterminer^{180,181}.

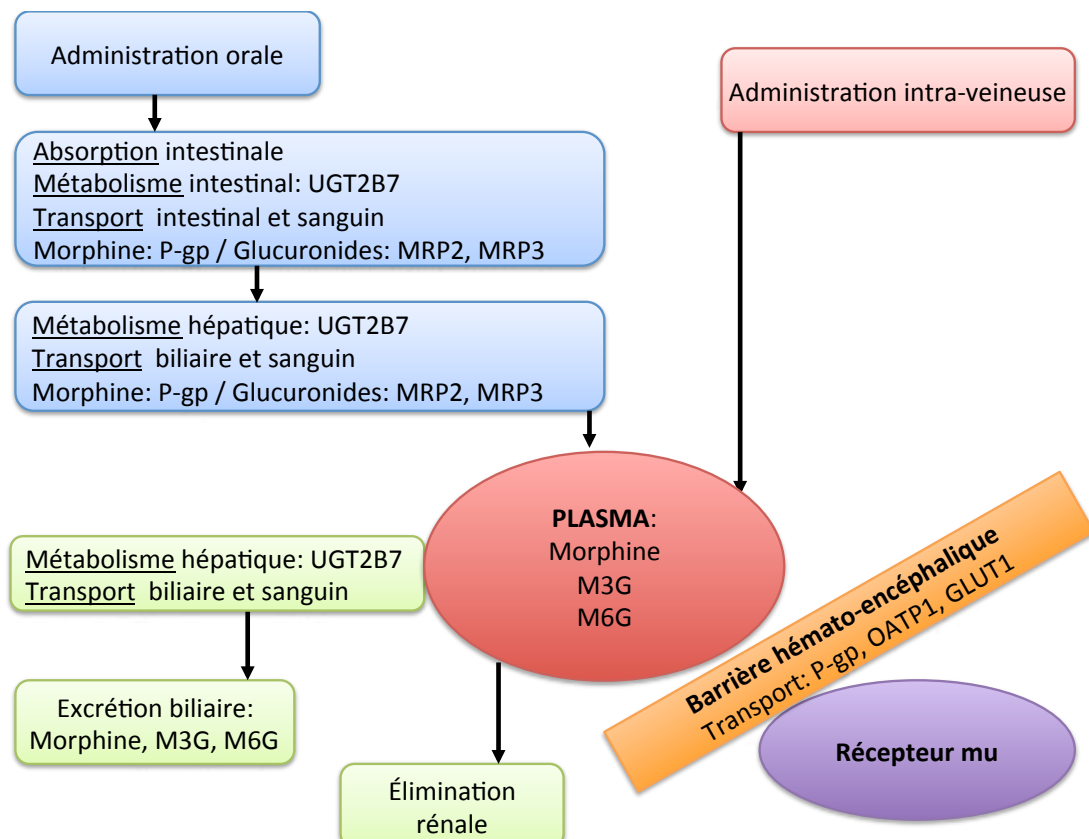
3. La Morphine

La morphine est un médicament présentant une importante variabilité interindividuelle d'un point de vue pharmacocinétique et pharmacodynamique (efficacité et tolérance)^{182,183}. Dans la pratique clinique, il en résulte une grande variabilité des besoins en morphine d'un individu à l'autre. Cette variabilité rend complexe sa prescription et fait redouter la survenue d'effets indésirables tels que la dépression respiratoire, les nausées, les vomissements et la constipation, susceptibles de compromettre l'observance du traitement et son efficacité.

Dans ce chapitre seront détaillés les acteurs ou effecteurs du métabolisme de la morphine (l'enzyme UGT2B7, les transporteurs P-gp, MRP2 et MRP3), les données PK et PD de la morphine chez l'homme et ses facteurs de variabilité.

3.1. Les effecteurs de la pharmacocinétique de la morphine

Figure 15. Métabolisme de la morphine



3.1.1. La glucuronidation de la morphine : l'enzyme UGT2B7

La morphine est métabolisée selon une réaction de glucuronidation, par une enzyme de phase II : l'enzyme UDP-glucuronyl-transférase (UGT) 2B7. Elle n'est pas métabolisée par les enzymes de phase I.

Le métabolite obtenu par glyconoconjugaison du groupe OH en position 6 est un agoniste actif, la morphine-6-glucuronide (M6G) tandis que le métabolite résultant de la conjugaison du groupe OH en 3, la morphine-3-glucuronide (M3G), est un antagoniste.

a. La glucuronidation

La glucuronidation est quantitativement la plus importante réaction de phase II et elle se réalise, à l'échelle cellulaire, au niveau du réticulum endoplasmique ¹⁸⁴. Ses substrats sont souvent des dérivés résultant des réactions précédentes d'oxydation, dites de phase I. Elle intervient dans la détoxification de l'organisme de médicaments de toutes classes thérapeutiques, des produits chimiques issus de l'environnement, et de composés endogènes tels que la bilirubine, les acides gras, les hormones stéroïdes ^{185,186}.

Ses produits sont désignés sous les noms de glucuronides ou glucuroconjugués et sont excrétés activement via des transporteurs, par le rein dans les urines et par l'hépatocyte dans la bile.

b. Lieu de la glucuronidation

La glucuronidation a lieu principalement dans le foie, mais sa présence est détectée dans d'autres tissus tels les reins, la muqueuse intestinale (œsophage, estomac, colon, intestin, pancréas) et les poumons ¹⁸⁷. L'activité métabolique de glucuronidation spécifique de la muqueuse intestinale est la deuxième plus importante dans l'organisme après celle du foie ^{188,189}. Les glucuronides sont en effet formés tout au long du tractus gastro-intestinal et la plus forte activité de glucuronidation apparaît dans la région jéjunale ¹⁸⁹.

c. Les UDP-glucuronosyltransférases (UGT)

La glucuronidation est catalysée par des UGT qui favorisent la fixation de l'acide UDP-glucuronique (forme activée de l'acide glucuronique) sur un atome d'oxygène, d'azote ou de soufre d'une molécule ¹⁸⁴.

Il existe deux familles d'UGT, la famille 1A et la famille 2 qui elle-même comprend les familles 2A et 2B ¹⁹⁰. Les UGT partagent plus de 50% d'homologie entre les membres des familles 2A et 2B, et un peu moins de 50% entre les UGT1A et UGT2.

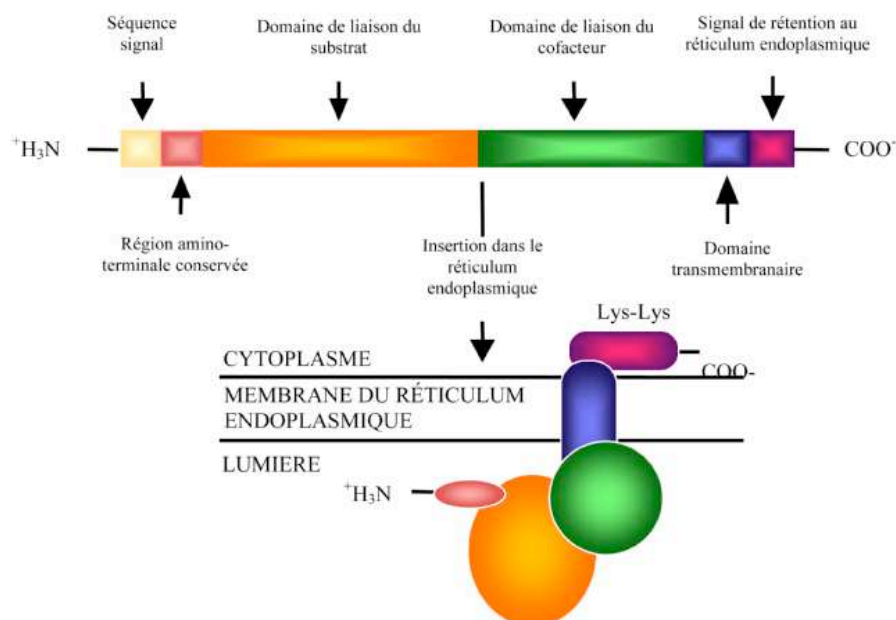
Gènes

Les gènes codant pour les UGT2B sont tous situés dans la région q13-21.1 du chromosome 4. Ils sont constitués de 6 exons et il existe 7 gènes fonctionnels chez l'homme (*UGT2B4*, *UGT2B7*, *UGT2B10*, *UGT2B11*, *UGT2B15*, *UGT2B17* et *UGT2B28*) ainsi que 5 pseudogènes (*UGT2B24p* à *UGT2B27p* ainsi que *UGT2B29p*) ¹⁹¹. Le premier exon code pour le domaine de liaison au substrat (domaine N-terminal), tandis que les autres exons codent pour la zone de liaison à UDPGA (domaine C-terminal) et des domaines transmembranaires ¹⁹¹.

Modulation de l'expression du gène des UGT

Les différences interindividuelles d'expression et d'activité des UGT sont nombreuses d'un organe à l'autre et résultent probablement de la variabilité locale d'expression des gènes. Plusieurs facteurs de transcription impliqués dans la régulation des gènes UGT ont été identifiés. Il s'agit des facteurs tels que le facteur nucléaire des hépatocytes 1 (HNF1), le CAAT-Enhancer Binding Protein (C/EBPs), le facteur de transcription octamère 1 (Oct-1) et le pre-B-cell leukemia homeobox 2 (PBX2). De plus, l'expression des gènes UGT est aussi modulée par les hormones, les médicaments et d'autres produits chimiques exogènes à travers l'action des facteurs nucléaires qui détectent la présence de ces produits ¹⁹². Ces protéines incluent le récepteur aux hydrocarbures aromatiques (AhR), les membres de la superfamille des récepteurs nucléaires, tels que le récepteur des androgènes (CAR) et le récepteur X des pregnanes (PXR), le récepteur X farnésyle (FXR) et les récepteurs activés par les proliférateurs de peroxyosomes (PPAR) ¹⁹²⁻¹⁹⁴.

Figure 16. Structure de l'enzyme UGT2B7



Protéines

Les UGT sont des enzymes composées de 529 à 534 acides aminés intégrées dans le réticulum endoplasmique (RE) de la cellule ¹⁹⁵. À l'exception d'UGT1A10, la région amino-terminale est composée d'une séquence signal permettant de diriger la protéine vers le RE ¹⁹⁵.

Suite à la séquence signal on retrouve une région très importante impliquée dans la spécificité du substrat ¹⁹⁵. Par ailleurs, la région carboxyl-terminale comprend une région très conservée qui est impliquée dans la liaison à l'acide UDP-glucuronique et qui se termine par un court domaine transmembranaire suivi d'une courte chaîne de 19 à 26 acides aminés présent dans le cytosol. Cette chaîne est impliquée dans le signal de rétention au RE ainsi que dans la maintenance de l'activité catalytique des UGT par de possibles interactions avec des protéines provenant de la région cytoplasmique ¹⁹⁵.

d. L'enzyme UGT2B7

Substrats, inhibiteurs et inducteurs

Les nombreux substrats de l'enzyme UGT2B7 sont abordés dans le tableau 13¹⁹⁶. Il existe de nombreux inhibiteurs de UGT2B7 in vitro. Aussi, différentes études PK ont été menées in vivo chez l'homme afin de déterminer les conséquences potentielles de ces interactions sur le métabolisme des substrats de UGT2B7. Le table 14 résume ces études PK¹⁹⁶. L'effet inhibiteur de certaines molécules est vérifié, soit de façon indirecte par une diminution de la formation des glucuronides, soit par une diminution de la clairance du médicament (ou parent) et une augmentation de son exposition.

A l'inverse des travaux montrent une induction de la glucuronidation par UGT2B7 par différentes molécules. Ces études sont résumées dans le tableau 15.

Tableau 14. Les médicaments substrats de UGT2B7

| Médicaments substrats de UGT2B7 | |
|--|---|
| Médicaments à visée cardiologique | Médicaments à visée rhumatologique et anti inflammatoire |
| almokalant (antiarythmique de classe III) | rofecoxib |
| carvedilol | benoxaprofène, ibuprofène, ketoprofène, |
| acide clofibrigue (hypolipémiant) | zaltoprofène |
| ezetimibe | diclofénac, indométhacine, acide tiaprofénique |
| gemfibrozil, pивastatine | diflunisal , fenoprofène, naproxène, zomepirac, |
| | flurbiprofène ¹⁹⁹ |
| Médicaments psychotropes et analgésiques | Antiépileptiques |
| buprénorphine | carbamazépine |
| codéïne, clonixine (antalgique) | |
| morphine, naltrexone,nalorphine | Autres |
| oxazepam, 1'- et 4'hydroxymidazolam | tacrolimus |
| halopéridol ¹⁹⁷ , topiramate ¹⁹⁸ | cyclosporine |
| | 5,6-Dimethylxanthénone-4-acetic acid DMXAA |
| | zidovudine, efavirenz ²⁰⁰ , lersivirine ²⁰¹ |
| | prodrug PR-104A ²⁰² |
| | chloramphenicol ²⁰³ |
| | rofecoxib ²⁰⁴ |

L'induction de la glucuronidation accélère le métabolisme/la clairance du médicament (parent) et augmente la production de glucuronides, voire augmente le ratio glucuronides/parent, tout en diminuant l'exposition au médicament parent.

Modulation de l'expression du gène UGT2B7 et de l'activité enzymatique

Il existe peu de données spécifiques à la variabilité d'expression et d'activité de UGT2B7. Les données relatives au métabolisme de la morphine seront abordées plus loin dans l'introduction.

Le gène *UGT2B7* est soumis à des polymorphismes génétiques dont le variant le plus étudié est UGT2B7*2 (C802T ; exon 2 ; rs7439366), qui engendre une enzyme avec soit une tyrosine, soit une histidine à la position 268 (Tyr268His)²⁰⁵. L'impact de cette mutation sur l'activité catalytique de la protéine reste controversé. Sa distribution varie selon l'origine ethnique des patients. Ainsi, Bhasker et al. ont décrit une distribution à peu près égale de sujets homozygotes pour chaque allèle dans la population caucasienne. Les sujets homozygotes pour l'allèle UGT2B7 * 1 sont 10 fois plus répandus au Japon que les sujets homozygotes pour UGT2B7 * 2. Les fréquences des allèles UGT2B7 * 1 et * 2 étaient de 0,511 et 0,489 chez les sujets Caucasiens, et de 0,732 et 0,268, respectivement chez les sujets japonais²⁰⁶.

A l'aide de microsomes (fragments de membranes du réticulum endoplasmique) et in vivo, l'utilisation de différents substrats n'a pas permis de montrer un impact important du variant UGT2B7 * 2 sur l'expression et l'activité de glucuronidation²⁰⁶⁻²¹⁰. Des travaux plus récents ont étudié l'influence d'haplotypes, groupe d'allèles de différents gènes situés sur un même chromosome et habituellement transmis ensemble, dans la variabilité d'expression du gène *UGT2B7*. Innocenti et al., grâce au séquençage du gène *UGT2B7* au niveau d'échantillons hépatiques, ont découvert 10 nouveaux haplotypes dont le 4e est associé à une augmentation de l'expression transcriptionnelle et de l'activité enzymatique²¹¹.

Par ailleurs, il semble que l'activité de l'enzyme UGT2B7 soit directement modulée par les cytochromes. Récemment Takeda et al. ont étudié l'effet du CYP450 sur la glucuronidation de la morphine au niveau microsomal, dans l'objectif de démontrer une interaction directe entre les protéines UGT et CYP450²¹². Ils ont ainsi démontré que l'action catalytique de UGT2B7 est spécifiquement modulée par l'enzyme CYP3A4, jusqu'à modifier la

régiosélectivité de UGT2B7 de telle façon que la formation de M6G est augmentée par rapport à celle de M3G ²¹². Inversement la glucuronidation de facteurs transcriptionnels par UGT2B7 est susceptible de moduler l'expression de différentes enzymes. Effectivement, l'acide phytanique et l'acide docosahexaénoïque, substrats de UGT2B7 au niveau microsomal, hépatique, intestinal, notamment au niveau de l'intestin grêle, interviennent dans la régulation de l'expression des enzymes ²¹³.

Tableau 15. Inhibition de UGT2B7 et conséquences pharmacocinétiques

| Inhibiteur potentiel | | Conséquence |
|--|------------------|---|
| Antalgiques et opioïdes | | |
| Codéine ²¹⁴ | Diclofenac | Absence de modification en dehors d'une élévation très modérée du glucuronide C-6-G |
| Morphine ²¹⁵ | Propanolol | Aucune |
| Morphine ²¹⁶ | Ranitidine | Absence de modification en dehors d'une diminution modérée du ratio M6G/M3G |
| Acétaminophène ²¹⁷ | Cisapride | Diminution du Cmax des glucuronides Augmentation de l'AUC et du Cmax de l'Acétaminophen (et non le Tmax) |
| Acétaminophène ²¹⁸ | Probénécide | Diminution franche de l'excrétion des glucuronides et augmentation de l'excrétion des dérivés sulfatés (excrétion globale stable) Augmentation nette de la T1/2 et diminution de la clairance de l'Acétaminophen |
| Acétaminophène ²¹⁹ | Probénécide | Diminution de l'excrétion urinaire des glucuronides et autres dérivés Augmentation du Cmax, Tmax, T1/2, diminution du Vd, mais exposition comparable |
| Acétaminophène ²²⁰ | Propanolol | Diminution de la clairance des glucuronides et autres dérivés Augmentation du Tmax, T1/2, diminution de la clairance du parent |
| Methadone ²²¹ | Codéine | Diminution de la concentration plasmatique des métabolites de la morphine Absence de modification des concentrations de morphine |
| Anti inflammatoires non stéroïdiens | | |
| Diflunisal ²²² | Acide Valproïque | Diminution de l'exposition aux deux types de glucuronides Augmentation de la clairance isolée du diflunisal |
| Indométhacine ²²³ | Probénécide | Diminution nette de la clairance rénale de l'indométhacine Augmentation nette de l'exposition et diminution de la clairance du parent |

| | | |
|-------------------------------|----------------------------------|--|
| Indométhacine ²²⁴ | Diflunisal | Diminution nette de la formation des glucuronides Augmentation franche de l'exposition, du Cmax, diminution de la clairance et du Vd |
| Naproxène ²²⁴ | Acide valproïque | Absence de modification concernant les glucuronides Augmentation modérée de l'exposition et diminution modérée de la clairance |
| Antirétroviraux | | |
| Azidothymidine ²²⁵ | Atovaquone | Diminution modérée du Cmax et de l'exposition des glucuronides Augmentation de l'exposition et diminution de la clairance du parent |
| Azidothymidine ²²⁶ | Fluconazole | Diminution de la formation des glucuronides Diminution de la clairance du parent, augmentation du Cmax, de l'exposition et de la demi-vie du parent |
| Azidothymidine ²²⁷ | Indométhacine, Naproxène | Naproxène : diminution isolée de l'exposition aux glucuronides Absence de modification de la PK du parent |
| Azidothymidine ²²⁸ | Probénécide | Diminution nette de la clairance rénale des métabolites Augmentation nette de l'exposition au parent |
| Azidothymidine ²²⁹ | Probénécide | Augmentation de l'exposition et de la T _{1/2} des glucuronides, diminution de la clairance rénale des glucuronides Augmentation nette de l'exposition au parent et sa T _{1/2} , réduction de la clairance |
| Azidothymidine ²³⁰ | Acide valproïque | Diminution de Cmax et de l'exposition aux glucuronides Augmentation de l'exposition et diminution de la clairance du parent |
| Lersivirine ²⁰¹ | Ketoconazole Acide valproïque | Augmentation de l'exposition et de la concentration maximale |
| Anticonvulsivant | | |
| Carbamazépine ²³¹ | Acide valproïque | Diminution de la formation des métabolites, plus souvent sous forme époxyde Pas de modification PK du parent |
| Lamotigine ²³² | Cimétidine | Absence d'effet |
| Lamotigine ²³³ | Felbamate | Augmentation modérée de la Cmax et de l'exposition du parent, sans modification des métabolites |

| | | |
|--------------------------------------|------------------|---|
| Lorazepam ²³⁴ | Acide valproïque | Diminution de la formation des glucuronides Réduction modérée de la clairance du parent |
| Lorazepam ²³⁵ | Acide valproïque | Diminution franche de la formation des glucuronides Augmentation modérée de l'exposition et du Cmax |
| Temazepam ²³⁵ | Probénécide | Diminution modérée de la formation des glucuronides Pas de modification PK du parent |
| Autres | | |
| Olanzapine ²³⁶ | Probénécide | Diminution de la formation des métabolites Augmentation de la Cmax, du ka, de l'exposition |
| Mycophenolate mofetil ²³⁷ | Tacrolimus | Diminution nette de l'exposition aux glucuronides Augmentation de la Cmin et de l'exposition au parent |

Tableau 16. Les inducteurs potentiels de UGT2B7

| Inducteur potentiel | | Conséquence |
|-----------------------------------|---|--|
| Antalgiques | | |
| Codéine ²³⁸ | Rifampicine | Augmentation de la clairance de la codéine via une augmentation de la formation des glucuronides chez les métaboliseurs rapides, parallèlement à une diminution des effets dépresseurs respiratoires et une diminution du myosis. (suggérant une induction préférentielle de la N-déméthylation) |
| Morphine ²³⁹ | Rifampicine | Augmentation de la clairance de la morphine sans diminution de la clairance des métabolites. Diminution de l'exposition aux glucuronides et réduction de leur excrétion urinaire, suggérant que d'autres mécanismes que l'induction d'UGT2B7 ne soit responsable de l'interaction. |
| Acetaminophène ²⁴⁰ | Rifampicine Carbamazépine Phénobarbital Phénytoïne | Augmentation du ratio glucuronide/parent et augmentation de l'excrétion urinaire de glucuronides, sans modification de l'exposition aux glucuronides et aux dérivés sulfates, supposant l'intervention d'autres systèmes enzymatiques. |
| Acetaminophène ²⁴¹ | Phénytoïne | Augmentation du ratio glucuronide/parent, diminution des taux urinaires d'acetaminophène |
| Acetaminophène ^{242 243} | Ethynil estradiol | Augmentation de la clairance et de l'excrétion urinaire des glucuronides, sans modification de la clairance du parent et de son dérivé sulfate. Augmentation de la clairance et diminution de la T1/2 de l'acetaminophène suggérant ne augmentation de la clairance. |
| Autres | | |
| AZT ^{244 245} | Rifampicine | Augmentation de l'exposition et de la clairance des glucuronides. Diminution de l'exposition à l'AZT |
| Lamotrigine ²³² | Rifampicine | Augmentation de la clairance de la lamotrigine et diminution de la T1/2 et de l'exposition |

3.1.2. Un transporteur de la morphine: la P-glycoprotéine

La P-glycoprotéine (P-gp) transporte la morphine au niveau de différentes membranes cellulaires.

a. Localisation de la P-gp

La P-gp a dans un premier temps été décrite au niveau des cellules tumorales, où elle participe au phénomène de résistance aux chimiothérapies anticancéreuses. Elle agit comme une protéine transmembranaire qui expulse le médicament hors des cellules. On la trouve localisée sur la surface apicale des cellules épithéliales du côlon et du jéjunum, sur la membrane canaliculaire des hépatocytes, sur la surface apicale des cellules épithéliales du pancréas et des tubules proximaux rénaux ²⁴⁶. L'expression de la P-gp à ces points stratégiques contribue à réduire l'absorption de xénobiotiques à partir du tractus gastro-intestinal et favorise l'élimination de substances toxiques endogènes et exogènes par l'urine et la bile. Elle est également retrouvée sur la surface luminale de l'endothélium des capillaires sanguins du cerveau, du testicule et du placenta ^{247,248}. Elle protège ainsi ces territoires en contribuant aux fonctions de la barrière hémato-encéphalique (BHE) et placentaire. On la retrouve dans les cellules souches hématopoïétiques de la moelle osseuse, les cellules sanguines mononucléaires périphériques, les macrophages matures, les cellules *Natural Killer* (NK) et les lymphocytes T et B. Le rôle de la P-gp au niveau des systèmes immunitaires et hématologiques est encore hypothétique, mais elle pourrait être impliquée dans le transport de certaines interleukines (IL-2 et IL-4) et d'interféron gamma, ainsi que dans la protection des cellules souches contre des composés endogènes et des xénobiotiques ²⁴⁹.

b. Le gène MDR1/ABCB1

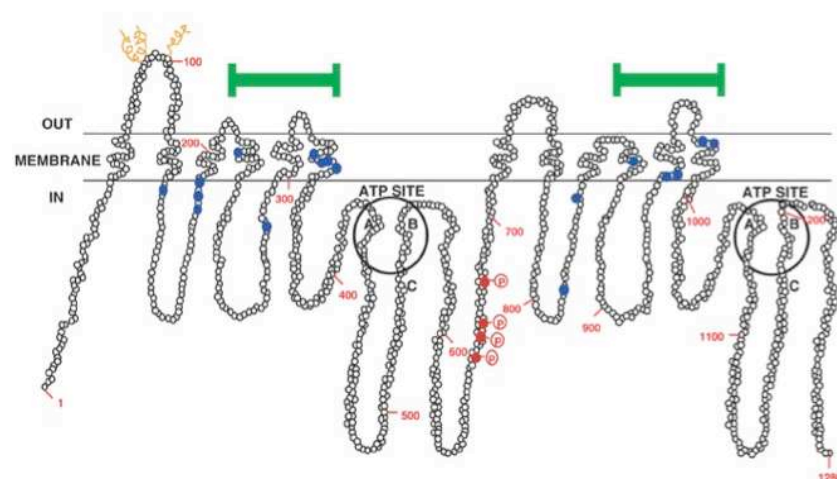
La P-gp appartient à la superfamille des transporteurs ABC. Celle-ci compte aujourd'hui 7 sous-familles composées elles-mêmes de différents membres dont les structures primaires présentent un certain nombre d'homologies. La P-gp est le produit du gène MDR1/ABCB1,

situé sur la région chromosomique 7q21 et consistant en 28 exons codant pour une protéine de 1280 acides aminés (environ 170 kDa).

c. La protéine

La P-gp est une glycoprotéine phosphorylée de 170 kDa composée de 2 domaines transmembranaires et de 2 domaines de liaison à l'ATP (Figure 17). Les travaux de Shapiro et Martin ont permis d'identifier au moins quatre sites de liaison répartis sur les différents domaines (trois sites seraient destinés au transport, le quatrième à la régulation)^{250,251}. La fixation des substrats sur la P-gp peut se faire sur plusieurs sites en même temps²⁵². Il en résulte une grande complexité et une grande diversité d'interactions possibles. Les substrats se lient à différents domaines du pore, provoquant l'exposition des sites de liaison nucléotidique. Un ATP vient ainsi se lier sur un de ces domaines, provoquant un changement de conformation des domaines transmembranaires résultant en l'ouverture du pore et l'expulsion du substrat. Les domaines nucléotidiques fonctionnent de façon alternée, la liaison de l'ATP à un des sites inhibant le deuxième. Ainsi, la P-gp nécessite un ATP par substrat pour l'éjecter.

Figure 17. Représentation schématique de la P-glycoprotéine en 2 dimensions



D'après Ambudkar et al., 2003

Son activité est saturable et répond au principe de l'équation de Michaelis-Menten. Une interaction peut avoir lieu entre deux substrats soit en se liant au même site de fixation (compétition) soit en modifiant la conformation du site de liaison, entraînant ainsi une diminution de l'efflux d'un des deux substrats.

d. P-gp, substrats, inducteurs et inhibiteurs

La P-gp transporte une large variété de substrats qui ont en commun d'être hydrophobes, neutres ou chargés positivement, en ayant une masse moléculaire située entre 200 et 1800 Da. Parmi les médicaments transportés par cette protéine, on trouve notamment des agents anticancéreux, des médicaments cardiaques, des inhibiteurs de la protéase du VIH, des antibiotiques, des immunosuppresseurs et des analgésiques opioïdes ⁸⁶. On estime qu'environ un médicament sur deux actuellement sur le marché est efflué par la P-gp. De nombreux travaux chez l'homme ont démontré les interactions médicamenteuses mettant en jeu la P-gp seule ou la P-gp et le CYP3A4 qui agissent dans le même sens.

Le tableau 16 montre la variété de substrats de la P-gp, des inducteurs et inhibiteurs potentiels.

e. Modulation de l'expression du gène *MDR1/ABCB1* et de l'activité enzymatique de la protéine

La P-gp montre une grande variabilité interindividuelle d'expression et de fonction, ceci ayant une influence directe sur la disponibilité systémique de ses substrats. Etant impliquée dans des phénomènes de chimiorésistance, de nombreuses équipes ont tenté de comprendre les mécanismes intervenant dans sa régulation ²⁵³. La variabilité d'expression et d'activité de la P-gp est en partie due à un polymorphisme génétique auquel est soumis son gène.

A ce jour, près de trente mutations du type SNP (*single nucleotide polymorphism*) ont été identifiées. La plupart sont introniques ou silencieuses et ne modifient pas la séquence d'acides aminés de la protéine. Le premier criblage systématique du gène MDR1 a été effectué par Hoffmeyer et coll. en 2000 ²⁵⁴. Les 28 exons et la région promotrice ont été amplifiés par PCR (*polymerase chain reaction*) chez 188 individus caucasiens. Par ce moyen,

quinze SNP ont été détectés dont six localisés dans la région codante. Seules neuf de ces mutations altèrent la séquence d'acides aminés de la P-gp.

Les mutations les plus étudiées sont la G2677T/A, localisée au niveau de l'exon 21, dans le deuxième domaine transmembranaire et conduisant à un changement d'acide aminé Ala pour Ser ou Thr, et la mutation silencieuse C3435T localisée au niveau de l'exon 26, cette dernière mutation étant soumise à une transmission autosomale récessif.

Des variabilités significatives de la fréquence d'expression des polymorphismes génétiques de la P-gp existent entre les populations d'origines ethniques différentes, particulièrement pour la mutation C3435T. Chez les Caucasiens et les Asiatiques, la fréquence des individus homozygotes pour l'allèle C ou T est de 25%, alors que chez les Africains, la fréquence du génotype 3435TT n'atteint guère plus de 6%^{255,256}. Le génotype sauvage CC s'exprime avec une fréquence allant jusqu'à 80% environ dans la population africaine²⁵⁷.

Hoffmeyer et al. ont rapporté, pour la première fois, une association entre un polymorphisme génétique de la P-gp et son taux d'expression dans le duodénum chez l'homme²⁵⁴. Le génotype 3435TT a été associé à une expression plus faible du transporteur que les génotypes CT et CC et à une augmentation de concentrations plasmatiques du substrat. En revanche, de nombreuses études ont succédé à ce travail et ont parfois montré des résultats discordants. Les plus récentes ont décrit l'effet des haplotypes plutôt que celui du polymorphisme C3435T de *ABCB1*. Différents travaux sur l'effet des polymorphismes de la P-gp sur son expression intestinale sont présentés dans le tableau 17.

Des facteurs de transcription interviennent également dans la variabilité de l'expression du gène de la P-gp. La première voie de régulation mise en évidence fait intervenir le PXR. En effet, une induction de *MDR1/ABCB1* est observée en présence d'agonistes de ce récepteur (rifampine, clotrimazole, nifédipine, cisplatine, paclitaxel etc...) dans différents modèles animaux et dans des hépatocytes humains^{253,258-261}. Cette régulation serait due à une liaison de PXR à un élément de réponse DR-4 (pour Direct Repeat) présent dans la région promotrice de *ABCB1*²⁶⁰. Par ailleurs, d'autres récepteurs tels que CAR et LXR peuvent se lier à cet élément de réponse et moduler l'expression de *MDR1/ABCB1*^{253,259,262}.

Le facteur de transcription NF-κB (pour Nuclear Factor-kappa B) interviendrait aussi dans la régulation de *MDR1/ABCB1* par l'intermédiaire de cytokines produites dans un contexte d'inflammation²⁶³. La voie du stress oxydant interviendrait dans la régulation de *MDR1* via une activation de la voie de l'AMPc/PKA par les prostaglandines²⁶⁴.

Tableau 17. Substrats, inhibiteurs et inducteurs de la P-gp

| Substrats | Inhibiteurs |
|---|--|
| Anticancéreux | Anticancéreux |
| Amsacrine, Colchicine, Docétaxel, Etoposide, Imatinib, Irinotécan, Ivermectine, Paclitaxel, Téniposide, Topotécan, Vinblastine, Vincristine, Vinorelbine, Vindésine | Etoposide, Ivermectine, Tamoxifène, Vinblastine, Vincristine |
| Antibiotiques | Antibiotiques |
| Actinomycine, Azithromycine, Ciprofloxacine, Dactinomycine, Daunorubicine, Doxorubicine, Epirubicine, Arythromycine, Levofloxacine, Mitomycine, Mitoxantrone, Rifampicine, Sparfloxacine, Tétracycline, | Azithromycine, Clarithromycine, Daunorubicine, Doxorubicine, Erythromycine |
| Cardiovasculaires | Cardiovasculaires |
| Acébutolol, Atorvastatine, Céliprolol, Digitoxine, Digoxine, Diltiazem, Losartan, Lavsastatine, Mibéfradil, Phénytoïne, Quinidine, Talinolol, Vérapamil | Acébutolol, Amiodarone, Atorvastatine, Céliprolol, Dipyridamole, Félodipine, ovastatine, Mibéfradil, Nicardipine, Quinidine, Quinine, Simvastatine, Talinolol, Vérapamil |
| Antiviraux | Antiviraux |
| Amprénavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Zidovudine | Indinavir, Nelfinavir, Ritonavir, Saquinavir |
| Opioides | Antifongiques |
| Lopéramide, Méthadone, Morphine | Itraconazole, Kétoconazole |
| Immunosuppresseurs | Opioides |
| Ciclosporine, Sirolimus, Tacrolimus | Lopéramide, Méthadone |
| Hormones | Immunosuppresseurs |
| Cortisole, Déxaméthasone, Estradiol, Hydrocortisone, Prednisolone | Ciclosporine, Tacrolimus |
| Autres | Antidépresseurs |
| Cimétidine, Dompéridone, Fexofénadine, Méfloquine, ondansétron, Phénobarbital, Ranitidine, Terfénadine | Fluoxétine, Paroxétine, Sertraline |
| | Autres |
| | Bromocriptine, Fexofénadine, Jus de pamplemousse, Méfloquine, Progestérone, Spironolactone, Terfénadine |
| | Inducteurs |
| | Déxaméthasone, Efavirenz, Millepertuis, Résérpine, Rifampicine |

f. Rôles physiologiques de la P-gp

Bien que le rôle physiologique exact de la P-gp ne soit pas totalement élucidé, elle pourrait prévenir l'accumulation de substances potentiellement nuisibles dans l'organisme. L'expression de la P-gp pourrait influencer la susceptibilité ou protection face à des maladies. La fréquence élevée du génotype CC observé chez les Africains pourrait, par exemple, offrir une protection sélective contre des infections gastro-intestinales endémiques sévissant dans ces pays ²⁵⁷. Il a été proposé que la flore intestinale endogène soit impliquée dans la pathogenèse de l'inflammation intestinale et qu'une perturbation de la barrière cellulaire protectrice encourage le développement d'une maladie inflammatoire, telles que la colite ulcéreuse ou la maladie de Crohn. En participant à la barrière intestinale, la P-gp pourrait prévenir l'entrée de toxines bactériennes dans la muqueuse de la paroi intestinale et réduire une réponse immunologique exagérée vis-à-vis de la flore endogène. Il a été montré sur une cohorte de 149 patients souffrant de rectocolite et de 126 patients avec une maladie de Crohn, que l'allèle 3435T est significativement plus fréquent par rapport aux sujets contrôles ²⁶⁵. Des études épidémiologiques ont révélé une incidence de colite ulcéreuse plus faible dans les populations africaines connues pour avoir une fréquence de l'allèle T plus faible ²⁶⁶.

Tableau 18. Effet des polymorphismes de *MDR1* sur l'expression intestinale de la *P-gp*

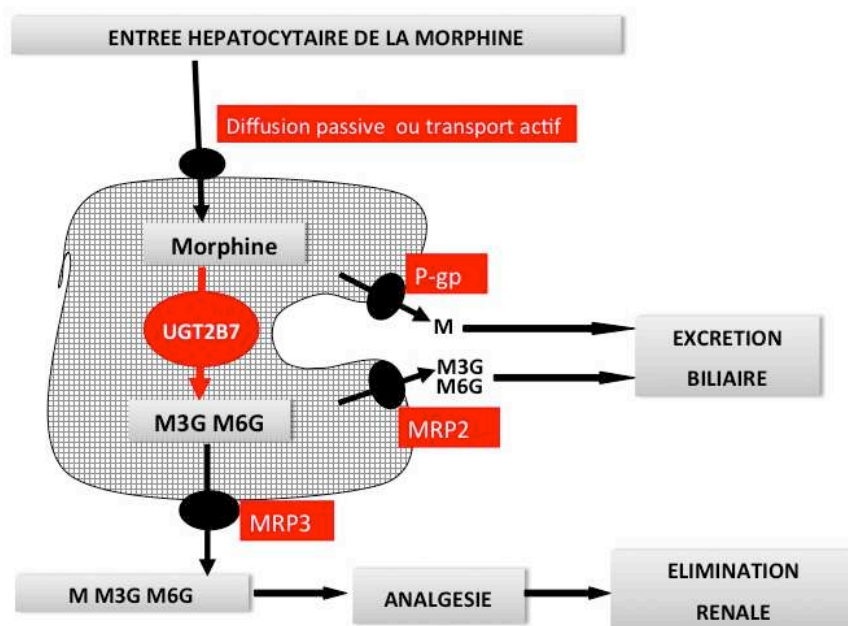
| | Méthodes | Résultats |
|---------------------------------------|--|---|
| Hosohata, 2009 ²⁶⁷ | Expression jéjunale de <i>CYP3A4</i> mRNA | mRNA: valeurs plus élevées chez les patientes portant l'haplotype 2677TT-3435TT que les 2677GG-3435CC et 2677GT-3435CT |
| 63 patients (28 femmes) | PK du tacrolimus | Absence d'effet de l'haplotype sur l'expression de <i>CYP3A4</i> ni sur la PK du tacrolimus |
| Etude japonaise (greffés) | Analyse de l'Haplotype de <i>MDR1</i> G2677T/A et C3435T | |
| Larsen, 2007 ²⁶⁸ | Expression duodénale de <i>MDR1</i> | Rifampicine: augmentation de l'activité de la <i>Pgp</i> et son expression |
| 32 volontaires | (mRNA RT-PCR et WB) | Association expression <i>Pgp</i> et activité |
| Etude danoise | PK digoxine orale | Activité plus importante pour les porteurs de CC |
| | Analyse de l'Haplotype de <i>MDR1</i> G2677T/A et C3435T | |
| | Traitement en cross-over rifampicine/ketoconazole | |
| Mendoza, 2007 ²⁶⁹ | Etude cas contrôle | Chez les non répondeurs |
| 76 patients Crohn | Répondeurs versus non-répondeurs | Fréquence plus élevée du génotype 2677TT |
| | Analyse de l'Haplotype de <i>MDR1</i> G2677T/A et C3435T | Fréquence plus élevée du génotype 3435TT |
| Etude espagnole | | Fréquence plus élevée de l'haplotype 2677T/3435T (29.4% versus 20.2%) |
| | | 2677G/3435C plus fréquent chez les répondeurs (58.3% versus 47.1%) |
| Bernsdorf, 2006 ²⁷⁰ | Avant et après traitement par simvastatine | Simvastatine: pas d'influence de l'expression duodénale de <i>MDR1</i> et <i>ABCC2</i> |
| 18 volontaires | PK talinolol | pas d'influence sur la PK du talinolol |
| Etude allemande | | Expression duodénale de <i>MDR1</i> mRNA corrélée de façon significative avec l'exposition du talinolol ($r = 0.627$, $P = 0.039$) et la $C(\max)$ ($r = 0.718$, $P = 0.013$) |
| | | Polymorphismes génétique de <i>ABCB1</i> and <i>ABCC2</i> : sans influence sur les PK |

| | | |
|---|---|--|
| Siegmund, 2002 ²⁷¹ | 10 plus fréquents polymorphismes | Absence d'effet des polymorphismes sur l'expression duodénale de <i>MDR1</i> |
| 37 : effet du génotype sur l'expression <i>MDR1</i> | <i>MDR1</i> mRNA RT-PCR et IHC | Absence d'effet des polymorphismes sur la PK talinolol |
| 55: effet sur exposition talinolol | | |
| Etude allemande (volontaires) | | |
| Goto, 2002 ²⁷² | 10 plus fréquents polymorphismes | Absence d'effet des polymorphismes sur l'expression duodénale de <i>MDR1</i> ou sur les concentrations de tacrolimus |
| 46 sujets: effet du génotype sur expression <i>MDR1</i> | <i>MDR1</i> et <i>CYP3A4</i> mRNA RT-PCR | |
| 69 sujets: effet du génotype sur concentrations | Concentration de tacrolimus | Influence du génotype sur l'expression du <i>CYP3A4</i> : CC: 8 fois moindre chez les TT que les CC |
| Etude japonaise (transplantés) | | |
| Moriya, 2002 ²⁷³ | Expression duodénale de <i>MDR1</i> et <i>ABCB1</i> , <i>ABCC2</i> (mRNA RT-PCR rapportés à l'expression de la villine) | Expression de <i>MDR1</i> plus élevée chez les génotypes TT que chez les CT et que les CC |
| 13 volontaires | Et influence de différents génotypes de <i>MDR1</i> et <i>ABCC2</i> | Absence d'effet des polymorphismes sur l'expression de <i>ABCB1/MDR1</i> et <i>ABCC2</i> |
| Etude japonaise | | |
| Nakamura, 2002 ²⁷⁴ | Expression duodénale de <i>MDR1</i> (mRNA RT-PCR) | Augmentation non significative de l'expression de <i>MDR1</i> chez les sujets TT en comparaison avec les CT et CC |
| 13 volontaires | | Corrélation entre les expressions de <i>MDR1</i> et <i>CYP3A4</i> |
| Etude japonaise | | Concentrations plus faibles de digoxine chez les porteurs de T |
| Hoffmeyer, 2000 ²⁵⁴ | Expression duodénale de <i>MDR1</i> (mRNA IHC et WB) | Expression de <i>MDR1</i> supérieure chez les C/C par rapport aux T/T |
| 21 volontaires | PK digoxine orale | Exposition à la digoxine plus faible chez les C/C |
| Etude allemande | | |

3.1.3. Les transporteurs des glucuronides : MRP2 et MRP3

Les transporteurs MRP2 et MRP3 transportent les glucuronides M3G et M6G de la morphine. La littérature scientifique les concernant est moins riche que celle concernant la P-gp.

Figure 18 . Métabolisme et transport hépatocytaire de la morphine et de ses métabolites



P-gp : Pglycoprotéine, M :Morphine ; M3G :Morphine-3-Glucuronide, M6G :Morphine-6-Glucuronide ; MRP : Multidrug Resistance Protein

a. Localisation des MRP

Le transporteur MRP2 est principalement exprimé au niveau de la membrane canaliculaire des hépatocytes. Il est également exprimé au niveau des membranes apicales des cellules de l'épithélium tubulaire rénal proximal et au niveau de la barrière hématoencéphalique, notamment lors de la prescription de médicaments anti-convulsivants. Dans l'intestin, MRP2 est présent dans le duodénum proximal, le jéjunum, et peu au niveau de l'iléon distal ²⁷⁵. Une distribution similaire de différentes enzymes de conjugaison de phase II suggère que ces enzymes agissent de façon coordonnée dans l'excrétion des substrats ²⁷⁶.

Le transporteur MRP3 est présent au niveau des organes suivants : foie, reins, intestin grêle, côlon, glandes surrénales, pancréas, vésicule biliaire, rate, vessie, poumon, estomac, et amygdales ²⁷⁷. Dans le foie normal, MRP3 est localisé au niveau de la membrane basolatérale des hépatocytes ²⁷⁸. Il est surexprimé en cas de déficit en MRP2 et en cas de cholestase extrahépatique. Leurs expressions sont inverses dans de nombreuses conditions. MRP3 a un rôle compensatoire dans la sécrétion hépatique de conjugués anioniques lorsque la sécrétion biliaire est altérée ²⁷⁷. MRP3 est également impliqué dans la réabsorption des acides biliaires de la lumière intestinale et contribuent à leur cycle entérohépatique ^{66,279}. Ainsi, les transporteurs MRP2 et MRP3 sont les MRPs majoritaires au niveau du foie. Ils assurent une fonction importante puisqu'ils permettent l'élimination d'acides biliaires et peuvent compenser l'absence de BSEP (Bile Salt Export Pump), exprimée également au pôle canaliculaire de l'hépatocyte ²⁸⁰. De plus, il est probable qu'ils jouent des rôles importants au niveau intestinal en raison de leurs taux d'expression élevés en comparaison aux autres MRP ²⁸¹.

b. Gènes ABCC2 et ABCC3

Le transporteur MRP2 est le produit du gène *ABCC2* situé sur la région chromosomique (10q23-24) comprenant 32 exons et d'une taille est de 65Kb. Le déficit constitutif bi-allélique du transporteur MRP2 est le syndrome de Dubin-Johnson. Cette maladie génétique rare concerne 0,5 à 1 individus sur 100 et est caractérisé par une hyperbilirubinémie à prédominance conjuguée d'évolution chronique sans hémolyse.

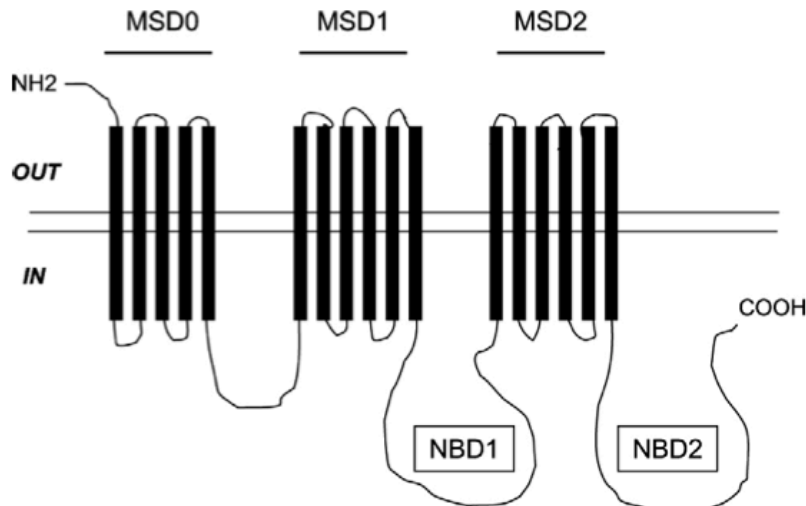
Le transporteur MRP3 est le produit du gène *ABCC3* situé sur la région chromosomique (17q22) comprenant 31 exons et d'une taille est de 57Kb.

c. Protéines MRP2 et 3

Les protéines MRP2 et 3 sont constituées respectivement de 1545 et 1527 acides aminés. Il s'agit de protéines comprenant 17 hélices transmembranaires distribuées sur trois domaines transmembranaires, incluant deux domaines de liaison transmembranaires, qui forment un canal permettant l'export des substrats, et deux domaines cytoplasmiques qui lient l'ATP et contiennent les motifs caractéristiques des protéines ABC. L'activité de transport de leurs

substrats nécessite l'énergie d'hydrolyse de l'ATP par les domaines cytoplasmiques ²⁸². La protéine MRP3 représente l'isoforme basolatérale de MRP2.

Figure 19. Structure de la protéine MRP2



MSD, membrane-spanning domain. NBD, nucleotide-binding domain. D'après Fardel et al.

d. MRP2 et 3, substrats, inducteurs et inhibiteurs

Les nombreux substrats de MRP2, endogènes et exogènes, sont représentés de façon non exhaustive dans le tableau 19.

MRP3 peut transporter des composés organiques conjugués tels que le glutathion, le sulfate, le glucuronate et les sels biliaires et des composés exogènes tels que le méthotrexate. En effet, MRP3 joue un rôle dans la physiologie des sels biliaires et de défense contre les anions organiques toxiques ²⁰⁴. Ses inhibiteurs et inducteurs sont moins documentés que MRP2.

Tableau 19. Substrats, inhibiteurs et inducteurs de MRP2 et substrats de MRP3

| Substrats de MRP2 | |
|---|--|
| Substrats endogènes | |
| Glutathion, Leucotriènes C4, D4, E4, Stéroïdes (17β-glucuronosyl estradiol), bilirubine | |
| Substrats exogènes | |
| Anticancéreux | |
| doxorubicine, étoposide, méthotrexate, mitoxantrone, cisplatine, vincristine, vinblastine, camptothécine | |
| Antirétroviraux | |
| indinavir, ritonavir, saquinavir, adefovir, didanosine, zalcitabine, zalcitabine, nelfinavir | |
| Antibiotiques | |
| ampicilline, cefodizime, ceftriaxone, grepafloxacin, irinotecan, azithromycine | |
| Autres | |
| pravastatine, temocaprilate, dérivés conjugués (acetaminophène, indométhacine, phénobarbital, sulfinpyrazone) | |
| Toxiques | |
| S-glutathionyl-2,4-dinitrobenzene, S-glutathionyl ethacrynic acid, ochratoxin A, 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, α-naphthylisothiocyanate, métaux lourds (arsenic, glutathione, Sb, Zn, Cu, Mn, Cd) | |
| Colorants | |
| fluoresceine-3-carboxy, dichloro fluoresceine, sulfobromophthaleine | |
| Inhibiteurs | |
| Composés a, b carbonyles insaturés, azithromycine, Benzoylated taxinine K, curcumin, cyclosporine A, Flavonoïdes, Jus de fruits, Glibenclamide, Isoniazide, Phénobarbital, MK-571, PK-104P: 2-[4-(Diphenylmethyl)-1-piperazinyl]-5-(trans-4,6-dimethyl-1,3,2-dioxaphosphorin-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinecarboxylate, P-oxides ; Progestatifs (norgestimate, progesterone) Probénécide, Furosémide ; Ritonavir, Saquinavir ; Lamivudine, Abacavir, Emtricitabine, Efavirenz, Delavirdine, Nevirapine, Cidofovir, adefovir, tenofovir | |
| Inducteurs | |
| Sels biliaires, Glutathion, Acide Ursodeoxycholique, Gentamicine, Indométhacine, Sulfanitran, Hormones : glucocorticoïdes, endotheline-1, Cytokines : Interleukine-6 et 1-β, TNFα, Xénobiotiques : Métaux (arsenic, antimoine, cisplatine), ligands de PXR (rifampicine, spironolactone, nifedipine, ritonavir, hyperforine, RU486), Agents carcinogènes (2 acétylaminofluorene), autres (tamoxifène, phénobarbital, genipin) | |
| Substrats de MRP3 | |
| Glucuronides des composés suivants: E217βG, Ethinylestradiol, Etoposide, vincristine, MTX, Morphine, E3040, Acetaminophène, Sels biliaires et conjugués: Hyodeoxycholate, Hyocholate, Leucotriènes, etc | |
| Conjugués du glutathion | |
| Inducteurs | |
| Ethinylestradiol | |
| Inhibiteurs | |
| Etoposide, MTX | |

e. Modulation de l'expression de *ABCC2* et *ABCC3* et de leur activité de transport

Effet des polymorphismes

Différents polymorphismes de *ABCC2* et *ABCC3* ont été décrits^{283,284}.

Haenisch et al. ont étudié l'effet de polymorphismes de *ABCC2* sur la régulation de l'expression intestinale de *ABCC2* dans une cohorte de 374 sujets d'origine caucasienne²⁸³. Les fréquences alléliques sont les suivantes : 18.3% pour -24T, 21.1% pour 1249A, 1.4% pour 1446G, 0.1% pour 3542T, 4.5% pour 3563A, 34.2% pour 3972T, 4.4% pour 4544A. Le polymorphisme -24T est fortement lié à 3972T, et 3563A avec 4544A, tandis que 1249A est rarement lié à d'autres polymorphismes. Aucun des polymorphismes n'influence l'expression entérocytaire du gène²⁷³. Pourtant le polymorphisme 1249G>A est associé à une diminution significative de la biodisponibilité orale du talinolol, substrat de MRP2, et une augmentation de la clairance du talinolol intra veineux, suggérant son rôle dans la clairance hépatique du substrat²⁸³.

Concernant *ABCC3* au sein de trois groupes ethniques différents, 61 variants de *ABCC3* ont été décrits avec des fréquences faibles (au maximum 4,7%) et variables selon le groupe. Aucun ne modifie l'expression de *ABCC3* dans des échantillons de foie humain ou ne modifie la pharmacocinétique du 4-MUG (4-méthylumbelliferyl-alpha-D-glucoside), un substrat de MRP3²⁸⁵. A l'inverse, dans une population caucasienne, Lang et al. décrivent un lien entre le polymorphisme -211C> T dans la région promotrice du gène et son expression hépatique²⁸⁶. Le rôle potentiel des polymorphismes de *ABCC3* dans la PK et PD des médicaments nécessite davantage de travaux cliniques. En effet, le variant T du polymorphisme A189 est associé à une augmentation du risque de récives de leucémie aigüe lymphoblastique au niveau cérébral et à une moindre toxicité médullaire, supposant une majoration de l'efflux du methotrexate en présence de ce variant allélique²⁸⁷.

Autres facteurs

Les expressions des gènes *ABCC2* et *ABCC3* sont souvent modifiées lors des pathologies cholestatiques et les modifications varient selon la pathologie cholestatique considérée, son stade, son association à une inflammation ou non²⁸⁸.

Par ailleurs l'expression interindividuelle de *ABCC2* est très variable et modulée par différents facteurs ²⁸⁹. Elle est induite par la rifampicine, la dexaméthasone et la carbamazépine, tous ligands de PXR ²⁹⁰⁻²⁹². Par ailleurs des acides biliaires comme l'acide cholique (AC), l'acide ursodéoxycholique (AUDC) et l'acide chénodéoxycholique (ACDC), via une activation du complexe FXR/RXR α , augmentent l'expression de *ABCC2* ²⁹³. A l'inverse, des inhibiteurs tels que le probénecide et la cyclosporine, réduisent la sécrétion de conjugués ²⁹⁴. Une étude réalisée par Kast et al. synthétise ces résultats en montrant que Mrp2/MRP2, qu'elle soit d'origine humaine, murine ou de rat, est régulée par les différents récepteurs nucléaires CAR, PXR et FXR, et que cette régulation porte au niveau d'un élément de réponse commun aux différents complexes et avec RXR α ²⁹⁵.

Les travaux permettant de connaître les facteurs de variabilité de l'expression de *ABCC3* sont plus récents et peu nombreux. Cherrington et al. ont montré que son expression est induite par le phénobarbital de façon indépendante au facteur de transcription CAR, supposant une activation de RXR α indépendante de CAR ²⁹⁶.

3.2. La pharmacocinétique et pharmacodynamique de la morphine

3.2.1. La pharmacocinétique

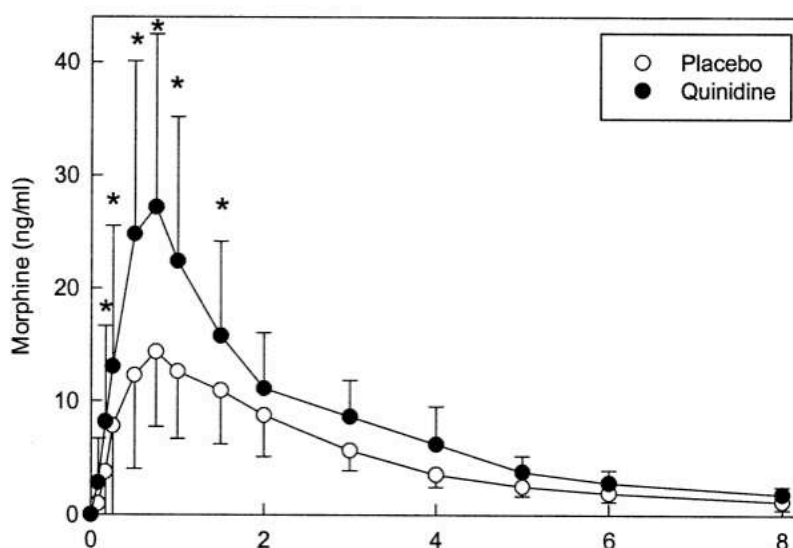
a. Absorption

La PK de la morphine orale montre que son absorption par l'intestin est de 82 \pm 14% ²⁹⁷. Néanmoins, seuls 42 \pm 8% échappe à l'élimination de premier passage hépatique, la biodisponibilité de la morphine orale étant de 30 à 35% environ ^{297,298}. Sa concentration plasmatique maximale est observée en 1 heure. Les métabolismes entérocytaire et hépatique de la morphine et son efflux probable vers l'intestin sont impliqués dans cette faible biodisponibilité.

Le rôle de la P-gp entérocytaire dans la variabilité de la morphine orale a été souligné par Kharasch et al. et rappelé dans des travaux plus récents. Dans une étude en cross over et en double-aveugle versus placebo chez des sujets volontaires sains, Kharasch et al. démontrent

que l'administration de quinidine, un puissant inhibiteur de la P-gp, ne modifie ni les concentrations ni l'effet de la morphine administrée par voie intra-veineuse. En revanche, l'exposition à la morphine orale est multipliée d'un facteur 2 en présence de quinidine malgré une élimination comparable, et avec davantage d'effets thérapeutiques²⁹⁹. La figure 20 et le tableau 20 montrent les résultats des études PK en présence ou non de quinidine. Nawa et al. montrent que les effets analgésiques et les concentrations cérébrales de morphine orale sont significativement augmentées chez les rats rendus diabétiques par l'administration de streptozotocine³⁰⁰. A l'inverse aucune modification n'est notée lorsque la morphine est administrée par voie sous-cutanée, suggérant le rôle prédominant de la variabilité d'expression de la P-gp entérocytaire dans l'efficacité de la morphine orale³⁰⁰. Okura et al. observent qu'un inhibiteur puissant de la P-gp, la quinidine, augmente les concentrations plasmatiques de morphine d'un facteur 5.2 et 1.7 après administration orale et intraveineuse respectivement, en faveur d'une meilleure absorption et d'une diminution de la clairance systémique de la morphine (40%). L'absence de modification du ratio entre concentrations moyennes cérébrales et plasmatiques est également en faveur du rôle majeur de la P-gp entérocytaire dans la détermination des effets analgésiques³⁰¹. L'absorption de jus de pamplemousse et de l'itraconazole, de façon plus discrète, s'accompagnent d'une augmentation de l'absorption de morphine et de ses effets analgésiques^{302,303}.

Figure 20. Effet de la quinidine sur les concentrations de morphine après son absorption orale



D'après Karasch et al.

Tableau 20. Effet de la quinidine sur les paramètres pharmacocinétiques et pharmacodynamies de la morphine orale

| | Placebo | Quinidine |
|-----------------------------------|-----------|------------|
| Données pharmacocinétiques | | |
| Tmax (h) | 1.1±0.8 | 1.1±1.0 |
| Cmax (ng/mL) | 16.9±7.4 | 31.8±14.9* |
| AUC ng.h.mL⁻¹ | 40.8±14.1 | 65.1±21.5* |
| T1 /2 (h) | 2.1±0.6 | 1.8±0.3 |
| AUC metabolite/parent | | |
| M6G | 14.4±4.5 | 9.0±2.4* |
| M3G | 134±51 | 80±28* |
| Données pharmacodynamiques | | |
| Tmax (h) | 2.7±2.2 | 2.7±1.2 |
| Myosis (mm) | 2.8 ±1.1 | 3.7±1.5 |
| AUC (mm.h) | 10.8±6.5 | 16.8±9.3* |
| Keo (h-1) | 0.89±0.85 | 1.0±0.2 |

D'après Karasch et al.. Les valeurs sont exprimées en moyenne ± SD

Cmax : concentrations plasmatiques maximales; t1/2, demi-vie; M6G, morphine-6- glucuronide; M3G, morphine-3-glucuronide; ke0, constante d'absorption de premier ordre. P <0 .05

Il existe une augmentation de la biodisponibilité de la morphine chez les patients souffrant de cirrhose, néanmoins, les rôles respectifs de la glucuronidation entérocytaire et hépatocytaire dans la faible biodisponibilité de la morphine ont été rarement étudiés³⁰⁴.

b. Distribution de la morphine

Après son administration, la morphine se distribue dans trois compartiments, le compartiment central/ plasmatique, un compartiment périphérique, composé essentiellement des muscles des viscères et du tissu adipeux, et enfin un dernier compartiment, probablement le compartiment effecteur qui représente le système nerveux central où elle va développer la majorité de ses effets. Le volume de distribution de la morphine est compris entre 1,0 et 4,7 L/kg et il peut varier d'un facteur 1 à 4 chez les sujets cancéreux. Il s'agit d'une molécule considérée comme peu lipophile et elle traverse mal la barrière hémato-encéphalique.

Avant de se lier aux récepteurs aux opiacés (au niveau du système nerveux central), la morphine est en partie liée aux protéines plasmatiques (30 à 35 %).

c. Caractéristiques pharmacocinétiques de la morphine

Elles sont variables selon les études (selon les doses, voies et galéniques d'administration, techniques de dosage) et un panel de ces études PK est présenté dans la tableau 21. Sa demi-vie varie de 1.4 à 3.7 h mais elle a été décrite 5 fois plus élevée dans une étude, où après administration IV, les auteurs observaient une phase terminale d'élimination plus lente de la morphine²⁹⁸. Sa clairance varie de 0.5 à 1.5L/h/kg selon les études.

d. Métabolisme

Les glucuronides M3G et M6G sont formés à des quantités différentes *in vitro* et *in vivo*^{305,306}. Après administration orale, 57% et 10% de la dose de morphine sont transformés en M3G et M6G, avec des expositions (ou aire sous la courbe AUC) respectivement 50 et 9 fois supérieures à celle de la morphine^{298,306}. 71±7% de la M6G sont formés lors du premier passage entérocytaire et hépatique de la morphine tandis que 29±7% le sont à partir du métabolisme systémique de la morphine²⁹⁷. Après 36 heures, les proportions de M6G et de morphine excrétées dans l'urine correspondent à 92+/-17% et 9+/-3 respectivement de la morphine absorbée²⁹⁷.

Après administration orale et IV de morphine, les ratios de M6G/ morphine sont de 3,6±1,2 et 0,7±0,3, respectivement²⁹⁸. Les chiffres correspondants pour les ratios M3G/morphine sont de 29,9±6,8 et 7,7±1,4²⁹⁸.

e. Caractéristiques PK de la M6G

C'est un modèle à trois compartiments qui permet de décrire la PK de la M6G³⁰⁷. Son volume de distribution (0,20 L/kg) et sa clairance (variable selon les études) sont réduits d'un facteur 10 environ par rapport à la morphine^{298,308,309}. La variabilité interindividuelle de ses paramètres PK est moindre que ceux de la morphine avec des coefficients de variation allant de 11% à 30% selon les études^{298,308,309}.

Chez l'homme, une perfusion de M6G s'accompagne de peu voire d'une absence de formation de M3G et de morphine, dont les infimes quantités seraient en rapport avec un

faible métabolisme entérocytaire de la M6G après son élimination biliaire, elle même très faible en comparaison avec l'excrétion rénale majoritaire du M6G sous forme inchangée²⁹⁸.

f. Elimination biliaire de la morphine et de ses métabolites

Après leur formation au niveau hépatocytaire, les glucuronides sont transportés à travers la membrane apicale (canaliculaire) dans la bile *via* le transporteur MRP2 et à travers la membrane basolatérale (sinusoïdale) dans la circulation sanguine via le transporteur MRP3³¹⁰. Des données chez l'animal montrent que MRP2 et MRP3 sont impliqués dans le transport de la M3G³¹¹. L'expression importante de MRP3 chez des souris ne possédant pas de transporteur MRP2 augmente le transport sinusoïdal et les concentrations plasmatiques du métabolite M3G par la MRP3, suggérant que ces transporteurs fournissent des voies alternatives pour l'excrétion des substrats glucuronoconjugués afin d'en limiter l'accumulation intra-hépatocytaire^{311,312}. A l'inverse, les souris déficientes en MRP3 ont des valeurs intra-hépatocytaires et biliaires de M3G augmentées d'un facteur 50 tandis que les souris déficientes en ces deux transporteurs présentent une accumulation intra-hépatocytaire du métabolite dont l'élimination (majoritairement urinaire) est ralentie^{310,311}. Les travaux de Van de Wetering et al. ont proposé que MRP1 soit aussi impliqué dans le transport sinusoïdal de M3G même si son expression hépatique basale reste faible en condition physiologique³¹¹.

Les mécanismes de régulation de ces transporteurs sont confirmés dans un modèle de rats rendus diabétiques par l'administration de streptozotocine, où une augmentation de l'activité de glucuronidation de la morphine est observée parallèlement à une augmentation de l'expression de MRP3 et une diminution de l'expression de MRP2. L'excrétion biliaire de M3G est réduite et ses concentrations plasmatiques sont plus élevées chez les rats diabétiques par rapport aux témoins³¹³.

L'effet des transporteurs sur la clairance de la M6G est moins connue car elle n'est pas formée chez l'animal. Cependant, une diminution de l'effet anti-nociceptif de la M6G est décrite chez les souris déficientes en transporteur MRP3, en rapport avec une moindre excrétion biliaire³¹⁰.

Tableau 21. Résumé de paramètres pharmacocinétiques après une dose unique de morphine

| | Dose de Morphine | Exposition (AUC) | Vd | T1/2 | Clairance plasmatique | Biodisponibilité | Cmax | Tmax |
|-----------------------------------|------------------------------|--|----------------|-----------------|-----------------------|------------------|------------------------|-------------------|
| | | mg/L.h | | (h) | totale | % | (ng.ml ⁻¹) | (h) |
| Lotsch ²⁹⁷ | 0.14 mg/kg IV | ND | ND | | 133.4 (26.4) L/h | | ND | |
| (n=5) | 90mg PO (MST) | | | | | 34 (9) | | |
| Kharasch ²⁹⁹ | 30mg PO (IR) | AUC ₀₋₈ = 40.8 (14.1) | ND | 2.1 (0.6) | ND | ND | 16.9 (7.4) | 1.1 (0.8) |
| (n=12) | | | | | | | | |
| Hoskin ³¹⁴ | 5mg IV | 91.9 (6.3) | ND | 1.9 (0.2) | 1.4 (0.24) L/h/kg | 23.8 (4.9) | 340.2 (47.3) | |
| (n=6) | 10mg PO (IR) | 22.2 (5.3) | | | | | 10.6 (2.15) | 0.75 |
| Säwe ³¹⁵ | 0.037 to 0.066 mg/kg IV | ND | 2.08(1.18)L/kg | 3.1 (2.3) | 0.55 (0.25) L/h/kg | | | |
| (n=7) | 0.231 to 0.495 mg/kg PO (IR) | | 1.73 (1.19) | 3.4 (1.93) | | 38.2 (17.1) | | |
| Hasselström ²⁹⁸ | 5mg IV | | 2.9 (0.8) L/kg | 15.1 (6.5) | 1.2 (0.2) L/h/kg | | 22 (11.7) | 1.1 (1.1) |
| (n=7) | 20mg PO (IR) | | | | | 29.2 (7.2) | | |
| Hasselström ³¹⁶ | 30mg PO Solution orale | AUC ₀₋₁₂ = 72.5 (38.7) ** | ND | ND | ND | | 22 (11.7) | 1.1 (1.1) |
| (n=11) | | | | | | | | |
| Drake ³¹⁷ | 30mg PO Solution orale | AUC ₀₋₂₄ (à jeun) = 71.9 (22.2) | | A jeun=3.65±2.6 | | | A jeun=13.9 (5.9) | A jeun= 1.2 (0.3) |
| (n=24) | | AUC ₀₋₂₄ (repas)= 89.7 (35.5) | | Repas=3.17±1.19 | | | Repas=15.2 (5.6) | Repas= 1.4 (0.7) |
| Osborne ³⁰⁶ | 11.7mg PO | AUC ₀₋₁₂ = 40.0 (12)*** | | 1.44 (0.44) | | | 19 (7) | 0.8 (0.3) |
| (n=8) | | | | | | | | |

MST: sulfate de morphine; IR: immediate release : forme à libération immédiate; IV: intraveineux; PO: oral; T1/2: demi-vie terminale

Données exprimées en moyennes (deviation standard=SD); ND: absence de donnée

** données initiales en nmol/L obtenues après une dose orale de 10mg de sulphate de morphine ** et corrigée pour une dose de 30mg en supposant une PK linéaire entre 10 et 30mg.*

3.2.2. La pharmacodynamique

a. Effets analgésiques de la morphine

La morphine est agoniste des récepteurs mu, mais aussi delta et kappa dans le système nerveux, tant au niveau supraspinal, spinal que périphérique ³¹⁸.

Des récepteurs aux opiacés sont présents au niveau spinal sur les terminaisons des fibres C (fibres permettant la transmission du message douloureux jusqu'à la moelle épinière). Leur stimulation par la morphine permet de bloquer la libération de la substance P, neuropeptide habituellement libéré au niveau de la corne postérieure en cas de stimulus douloureux ³¹⁸. Ainsi, la morphine déprime immédiatement la transmission des messages nociceptifs. Au niveau supraspinal, la morphine agit en bloquant l'action des Contrôles Inhibiteurs Diffus déclenchés par des stimulations nociceptives. Elle permet ainsi de diminuer l'information nociceptive et de réduire la sensation douloureuse ³¹⁸. Enfin, les récepteurs aux opiacés sont contrôlés dans certaines régions du cerveau comme par exemple le système limbique, responsable des émotions et des sensations de plaisir. A ce niveau, la morphine module l'activité dopaminergique en mimant l'action des enképhalines et est susceptible de modifier les circuits de récompense ³¹⁹.

b. Effets associés de la morphine

Au delà de ses effets analgésiques, la morphine diminue le rythme et l'amplitude respiratoire, *via* une diminution de la sensibilité des centres respiratoires au CO₂. Par ailleurs son action sur les récepteurs centraux et périphériques s'accompagne d'une diminution du péristaltisme digestif (gastrique et intestinal) et de la sécrétion hydrique, responsable d'une constipation. Les nausées et les vomissements induits par la morphine sont liés à la stimulation de la «chemoreceptor trigger zone», et non à l'effet spasmogène direct. La morphine présente encore d'autres effets variés, au niveau dermatologique (prurit) et endocrinien (troubles hypothalamo-hypophysaires au long cours).

c. Effets des métabolites de la morphine

Le principal métabolite de la morphine, la M3G, bien qu'inactif sur le plan analgésique, serait susceptible d'antagoniser la morphine et de favoriser l'apparition d'effets neuroexcitateurs, tels que les myoclonies, les convulsions et l'allodynie³²⁰. En revanche, le métabolite mineur, la M6G, présente des capacités analgésiques supérieures à la morphine, sans majoration des effets indésirables^{321,322}. Par rapport à la morphine, la M6G a 4 fois moins d'affinité pour le récepteur opioïde mu, une affinité similaire pour le récepteur opioïde delta, et 20 fois moins d'affinité pour le récepteur opioïde kappa³²². Certains travaux suggèrent l'existence de plusieurs récepteurs mu, dont l'affinité serait différente pour la morphine et la M6G et à l'origine des effets pharmacodynamiques différents entre les molécules³²³⁻³²⁵.

Cependant, alors que l'activité analgésique de la M6G est puissante après administration intracérébroventriculaire, elle est nettement inférieure à celle de la morphine après une administration systémique^{321,326,327}.

d. Transport de la morphine et des métabolites au niveau cérébral

Il est admis que le transport de la morphine et de ses métabolites, et en particulier de la M6G, au niveau de la barrière hémato-encéphalique, conditionne en partie son efficacité et sa tolérance.

Différents travaux, portant notamment sur la pharmacogénétique (PG) de la morphine, suggèrent que la P-gp détermine les concentrations intracérébrale de morphine et indirectement de ses deux principaux métabolites, puisque la M6G pourrait être formée directement dans le système nerveux central^{328,329}. Cependant, certains auteurs ne valident pas cette hypothèse. En effet, l'administration de la quinidine affecte les concentrations plasmatiques de morphine orale mais elle ne modifie pas ses effets centraux après administration intraveineuse³³⁰. L'administration du Valspodar, un autre inhibiteur de la P-gp chez des volontaires sains donne des résultats superposables³³¹.

Meineke et al. ont montré que les concentrations maximales de morphine, M3G et M6G au niveau du liquide céphalo-rachidien étaient observées à 103, 417 et 443 minutes respectivement après une injection intraveineuse de 0.5mg/kg de sulfate de morphine en 30 minutes chez des patients ayant nécessité la pose d'un cathéter ventriculaire pour des raisons médicales³²⁸. Le passage de la M6G au travers de la barrière hémato-encéphalique

(BHE) relativement lent par rapport à la morphine est à l'origine du manque d'activité analgésique après administration intraveineuse^{328,332,333}. Certains travaux suggèrent que la M6G prenne une structure lui permettant de masquer ses groupes polaires et d'augmenter sa lipophilie pour passer la BHE³³⁴. Par ailleurs la M6G est un substrat au niveau de la BHE pour les transporteurs de l'anion organique 2 polypeptide (OATP2) et du glucose GLUT-1 qui pourraient participer à son transport³³⁵.

Certains auteurs proposent également que le délai de réponse à la M6G soit en rapport avec des différences de distribution intracérébrale et de liaison aux récepteurs par rapport à la morphine^{327,336}.

3.3. Facteurs de variabilité de la morphine

Les répercussions cliniques des variations du métabolisme peuvent être importantes lorsqu'il s'agit d'un médicament présentant une marge thérapeutique étroite. Ainsi la morphine fait l'objet de nombreux travaux scientifiques et cliniques dont l'objectif commun est de déterminer des facteurs prédictifs de réponse et de survenue d'effets indésirables, afin de les prévenir.

3.3.1. Facteurs physiologiques

a. Facteurs physiologiques et pharmacocinétique de la morphine

L'analyse des concentrations de morphine et de ses métabolites chez 300 sujets traités au long cours par morphine montre que la dose administrée explique les concentrations sériques de morphine ($r = 0,69$), de M6G ($r = 0,76$) et de M3G ($r = 0,76$)³³⁷. La dose et la voie d'administration de la morphine prédisent les concentrations sériques (la voie orale étant associée à une augmentation de formation de métabolites), alors que la dose et la fonction rénale prédisent celles de M6G et M3G. Il s'agit effectivement des facteurs physiologiques habituellement pris en compte pour ajuster la prescription de morphine puisque l'altération de la fonction rénale (fréquente avec l'âge) s'associe à une moindre clairance de la M6G. Il existe une augmentation de la biodisponibilité de la morphine chez les patients souffrant de

cirrhose³⁰⁴. A l'inverse le poids et le sexe ne sont pas des facteurs connus de variabilité PK de la morphine et de ses métabolites³³⁷.

b. Facteurs physiologiques et pharmacodynamique de la morphine

Il n'y a pas de relation simple entre les effets cliniques et les concentrations plasmatiques de morphine, ni avec ses métabolites ou le ratio morphine/métabolites.

De nombreux facteurs physiologiques sont impliqués dans la réponse à la morphine et interviennent en pratique clinique dans la détermination des besoins et de la tolérance à la morphine.

Sexe

Les seuils d'apparition et de tolérance de la douleur sont plus bas chez les femmes que chez les hommes. Toutefois ces observations sont plus nettes lorsqu'il s'agit de stimuli mécaniques par rapport à des stimuli thermiques/électriques³³⁸. Par ailleurs, il existe des différences au niveau de l'intégration et de l'activation des circuits neuronaux entre hommes et femmes au cours de la douleur³³⁹.

Une conséquence de l'excès de nociception chez la femme est une plus forte consommation de morphine en postopératoire immédiat^{340,341}. Cependant, la fréquence de nausées et vomissements en post-opératoire de chirurgie est aussi plus élevée chez les femmes³⁴². A l'inverse les autres effets secondaires, tels que l'insuffisance respiratoire ou les effets psychomoteurs sont comparables.

Age

L'âge modifie la sensibilité à la douleur. Les seuils douloureux aux signaux thermique et électrique sont augmentés tandis qu'il existe une diminution des seuils douloureux à la pression mécanique³⁴³. Une diminution des besoins en morphine est observée avec l'âge en post-opératoire de chirurgie³⁴⁴.

Les nausées et vomissements sont deux fois plus fréquents chez les enfants et les adultes jeunes par rapport aux adultes³⁴².

Le tabac

La consommation de tabac est associée à une augmentation des besoins en morphine en

post opératoire immédiat de chirurgie (dans les 48 heures) ³⁴⁵. Cependant elle est considérée comme un facteur protecteur de la survenue de nausées et vomissements dans les mêmes circonstances ³⁴².

3.3.2. Les interactions médicamenteuses

Quelques interactions médicamenteuses dont l'enzyme UGT2B7 fait l'objet ont été décrites avec des conséquences sur les concentrations de morphine. Hara et al. ont testé l'effet de 21 médicaments sur la glucuronidation, au moyen de microsomes hépatiques, et ont décrit une diminution de la formation de M3G et M6G en présence de 10 médicaments : tamoxifène, tacrolimus, diclofenac, carbamazépine, imipramine, clomipramine, amitriptyline, diazepam, lorazepam and oxazepam ³⁴⁶. Même si certains d'entre eux ne sont pas substrats de UGT2B7, ils en sont volontiers des inhibiteurs.

Effectivement, certaines interactions ont été observées *in vivo*. C'est le cas du diclofenac ^{347,348}, de la clomipramine et de l'amitriptyline qui augmentent l'exposition à la morphine d'un facteur 2 *via* une réduction de la glucuronidation de la morphine ³⁴⁹. Le ketoconazole a également un effet inhibiteur sur la glucuronidation de la morphine, indépendamment du CYP450 ³⁵⁰. En revanche, peu d'études permettent d'évaluer l'effet inhibiteur d'autres médicaments sur la glucuronidation *in vivo*.

En dehors des travaux de recherche expérimentaux chez l'homme et l'animal, montrant l'effet d'inhibiteurs spécifiques de la P-gp (quinidine, valsopodar, jus de pamplemousse) et non spécifiques (étoposide, cisplatine) ayant en commun d'augmenter l'exposition et les effets de la morphine, les interactions médicamenteuses faisant intervenir la P-gp et concernant spécifiquement la morphine sont peu connues dans la pratique clinique ^{299,301,302,331}.

Par ailleurs, des études sur les interactions médicamenteuses concernant l'administration de la morphine au long cours sont sans doute nécessaires, comme le rappelle un travail sur l'étoposide. En effet, l'étoposide, administré une fois, inhibe la P-gp et augmente l'effet analgésique des opioïdes, tandis qu'après administrations répétées, la même molécule favorise l'expression de la P-gp et diminue l'effet analgésique de la morphine orale ³⁵¹.

3.3.3. La pharmacogénétique

a. UGT2B7

Sachant que les patients traités par opioïdes au long cours ont des concentrations de M6G supérieures à celles de la morphine, que ce métabolite lie le récepteur mu et que son potentiel analgésique est supérieur à celui de la morphine, on imagine volontiers que la variabilité d'activité de UGT2B7 et les ratio morphine/métabolites expliquent une variabilité de réponse à la morphine ³²¹.

Les polymorphismes génétiques de *UGT2B7*, abordés dans le chapitre précédent ne modifient pas ou peu les ratio M3G/morphine, M6G/morphine ou M3G/M6G et les besoins en morphine ^{207,210,352-355}. Cependant le variant -840G>A est associé à une diminution de la clairance hépatique de la morphine chez des patients drépanocytaires ³⁵⁶. Par ailleurs, le variant UGT2B7*2 est associé à une plus grande fréquence d'évènements indésirables chez des sujets cancéreux, alors qu'il n'influence ni les besoins de morphine ni la survenue de nausées en post opératoire de chirurgie colique ^{353,354}.

b. MDR1/ABCB1

En situation expérimentale, les effets du lopéramide, opioïde dont les effets centraux sont inhibés par la présence de P-gp au niveau de la BHE, sont comparables entre patients porteurs du génotype 3435TT et ceux porteurs du 3435CC. Ils deviennent différents en présence de quinidine, puisque les sujets porteurs du génotype TT présentent alors un myosis plus prononcé que les autres sujets, suggérant un moindre efflux de la morphine ^{357,358}. Les sujets homozygotes mutés TT ont des concentrations cérébrales plus élevées de morphine après administration intraveineuse (sujets explorés en neurochirurgie) ³²⁸. En revanche, l'efflux de la M6G à travers la barrière hémato-encéphalique dépend d'un transport inhibé par le probénécide, mais indépendant de la P-gp ³⁵⁹.

En pratique clinique, le variant 3435T ne détermine pas les doses de morphine administrées en post opératoire d'une chirurgie colique mais un besoin supérieur en anti-émétiques ³⁵³. En revanche, l'haplotype G2677/T3435 prédit la survenue d'évènements indésirables de type nausées et vomissements, puisque les sujets homozygotes GG/CC y sont moins exposés que les autres ^{353,354}. Les évènements indésirables de type confusion, hallucinations, sont

aussi moins fréquents chez les sujets cancéreux porteurs de l'allèle G du polymorphisme G2677T de *ABCB1* ³⁶⁰.

L'association de différents variants génétiques portant sur des protéines impliquées dans la PK et la PD de la morphine explique volontiers la variabilité de la morphine. Ainsi, Campa et al. ont montré que les polymorphismes génétiques associé à un meilleur efflux de la P-gp et à une moindre sensibilité du récepteur mu répondaient moins à la morphine et nécessitaient la prescription de doses plus importantes ³⁶¹. Le classement des patients en trois groupes selon le génotype (très bons répondeurs, répondeurs et non-répondeurs) permet de prédire les doses de morphine avec une sensibilité de 100% et une spécificité de 70% ³⁶¹.

3.3.4. Expression et activité des effecteurs

Aucune étude n'a porté sur les liens entre expression et contenu tissulaire des effecteurs et PK de la morphine.

On conçoit volontiers que cette étude soit difficile au niveau de la BHE. En revanche, même si des travaux montrent que l'intestin contribue au premier passage de la morphine et à la limitation de sa biodisponibilité, il n'existe pas de travaux sur les relations entre expression de *MDR1* ou contenu intestinal en P-gp et variabilité PK ou PD de la morphine.

Mais les pathologies relevant d'un traitement par opioïdes, comme le cancer peuvent contribuer à la variabilité de réponse aux traitements. La modulation de facteurs de transcription, la production de médiateurs de l'inflammation ou encore les traitements sont susceptibles de modifier l'expression ou l'activité de la P-gp. L'administration de VEGF au niveau du tissu cérébral animal, malgré la stabilité de l'expression de la protéine, s'accompagne d'une diminution de l'activité de la P-gp et d'une augmentation de la distribution intracérébrale de morphine ³⁶². Un inhibiteur de la P-gp augmente davantage l'effet analgésique de la morphine chez des rats pré traités par cisplatine par rapport à des rats contrôles ³⁶³. Par ailleurs, dans un modèle animal soumis à une douleur inflammatoire, une augmentation de l'activité de la P-gp au niveau de la BHE est associé à une diminution des concentrations intra cérébrales de morphine, alors qu'elle n'est pas observée en absence d'inflammation ³⁶⁴.

Aucun travail chez l'homme ne permet de déterminer la place des transporteurs MRP2 et 3 dans la variabilité PK et PD de la morphine et de ses métabolites.

3.3.5. Variabilité pharmacodynamique de la morphine

a. Le récepteur mu

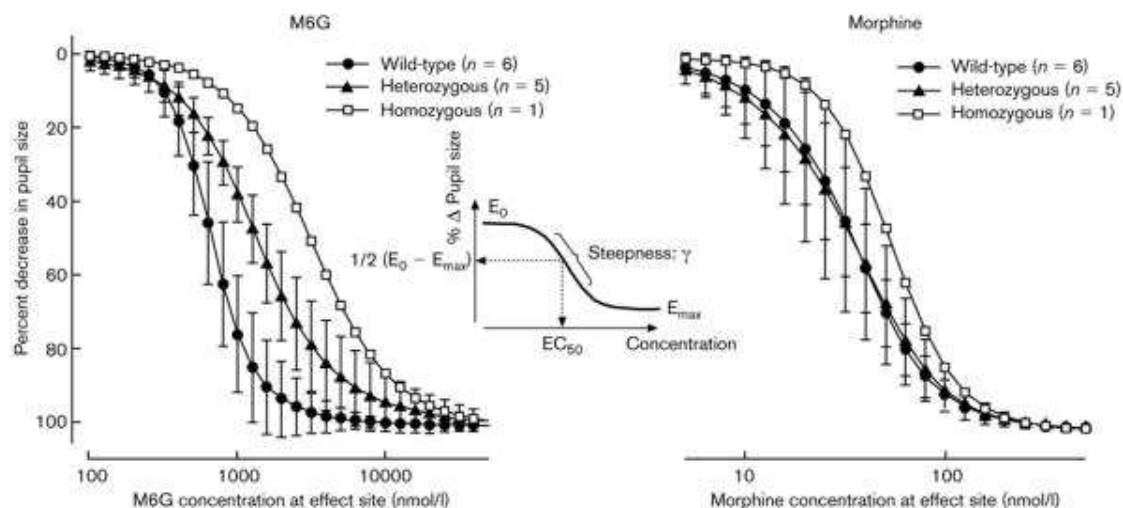
Le récepteur mu, codé par le gène *OPRM1*, est le principal site de liaison des opioïdes. Approximativement 100 variants ont été identifiés à ce jour ³⁶⁵. Le plus connu est le SNP A118G, dont la fréquence allélique est variable en fonction de la population étudiée (2 à 48%). Il est responsable d'un changement d'acide aminé en position 40 (asparagine>aspartate), conduisant à la perte d'un site de N-glycosylation dans la partie extra cellulaire du récepteur. Le récepteur muté conduit à une affinité supérieure pour les b-endorphines (3.5 fois). Une moindre expression gène observée dans le tissu cérébral humain chez les sujets porteurs du variant G est en faveur d'un défaut de production de la protéine ^{366 367}. Le récepteur mu est un facteur de variabilité de la morphine bien connu puisqu'il intervient d'une part dans la détermination des effets des opioïdes et d'autre part dans la perception de la douleur.

En utilisant la taille pupillaire (ou myosis) comme marqueur PD, Lötsch et al. ont montré que la valeur de l'EC50 (concentration ayant 50% de l'effet escompté) pour le M6G, mais non celle de la morphine, est augmentée d'un facteur quatre chez le sujet homozygote en comparaison avec les sujets ayant deux allèles sauvages, et d'un facteur 2 chez les sujets hétérozygotes ³⁶⁸. Ces résultats sont représentés dans la figure 21.

Ce polymorphisme diminue aussi l'effet de la morphine et de la M6G après administration intraveineuse (myosis, nausées et vomissements) ³⁶⁹.

En utilisant comme facteur PD la sensibilité à un courant électrique chez des sujets sains, une diminution de l'effet de la M6G est observée chez les sujets porteurs de l'allèle G en comparaison aux sujets porteurs du génotype sauvage ^{307,370}. A l'inverse, les effets dépresseurs respiratoires ne sont pas modifiés par le génotype ³⁷⁰. Pourtant les sujets homozygotes pour l'allèle G nécessitent des concentrations sanguines de fentanyl 10 à 12 fois plus élevées que les autres patients pour obtenir un même degré de dépression respiratoire ³⁷¹.

Figure 21. Effet du Polymorphisme A118G du gene du récepteur mu sur la pharmacodynamique de la Morphine et de la M6G



D'après Lötsch et al.

M6G : Morphine-6-Glucuronide ; l' EC_{50} : concentration ayant 50% de l'effet escompté

De plus, plusieurs études ont étudié l'effet du polymorphisme A118G sur l'efficacité ou la dose nécessaire d'opioïdes en pratique clinique. Les sujets traités au long cours par morphine dans le cadre d'une pathologie cancéreuse et homozygotes pour l'allèle muté (n=4 sujets) requièrent des doses de morphine 2.3 fois plus élevées que les sujets présentant le génotype sauvage (n=78 sujets) ³⁷². Après différents types de chirurgie (digestive, orthopédique, gynécologique) et en cancérologie, des doses de morphine plus élevées sont nécessaires afin de soulager la douleur chez les sujets porteurs du variant G ^{353,372-375}. Des cas de sujets porteurs de l'allèle G à l'état homozygote résistants à des doses élevée de morphine (2g/jour) ou tolérants à la morphine malgré une insuffisance rénale ont été décrits ^{376,377}. Cette diminution d'efficacité s'accompagne aussi d'une diminution des effets secondaires ^{378,379}.

Ainsi, le polymorphisme A118G est généralement associé à une meilleure tolérance de la morphine concernant la survenue de nausées et vomissements, une diminution de l'effet des opioïdes et la nécessité de prescrire des doses plus élevées de morphine pour soulager la douleur. Par ailleurs les études expérimentales, chez les volontaires sains, suggèrent que

l'effet du polymorphisme A118G du récepteur mu dépend de la molécule opioïde utilisé (morphine, M6G et autres) et semble spécifique de certains effets pharmacodynamiques. Le polymorphisme A118G influence également la sensibilité à la douleur expérimentale, aiguë, et chronique. Parmi les sujets non douloureux, les porteurs de l'allèle 118G, les seuils de survenue de la douleur à la pression sont plus élevés que les porteurs de l'allèle A³⁸⁰. Les porteurs de l'allèle 118G présentent également des réponses corticales moindres aux stimuli douloureux expérimentaux³⁸¹. Toutefois, les femmes porteuses de l'allèle 118G sont plus douloureuses que les femmes homozygotes pour le 118A dans les 24 premières heures suivant une césarienne^{375,382}. En effet, il existe une interaction significative entre le sexe et le génotype A118G comme le suggèrent des travaux sur concernant l'évolution de la douleur au cours des 12 mois suivant une hernie discale³⁸³. Les femmes porteuses de l'allèle G sont 2,3 fois plus douloureuses et présentent une récupération plus lente que les hommes porteurs de l'allèle G. En revanche, les hommes et femmes ayant le génotype A/A ont des évolutions superposables.

b. COMT

Enfin, la catechol-O-methyl transferase (COMT) métabolise les catécholamines (noradrenaline, adrenaline, et dopamine). De nombreux travaux suggèrent que les voies dopaminergiques et adrénergiques interagissent avec la neurotransmission du signal douloureux³⁸⁴. Cette enzyme intervient effectivement dans la sensibilité à la douleur et la réponse à la morphine³⁸⁴⁻³⁸⁶. Son gène présente différents polymorphismes, largement étudiés dans le cadre de pathologies psychiatriques, dont le plus étudié est le SNP en position 122 (substitution d'une adenosine en guanosine), responsable d'une substitution en acides aminés (valine>methionine) au sein de la protéine³⁸⁷. Le variant 158Met ou A1947 diminue l'activité de la protéine d'un facteur allant de 3 à 4, augmente la concentration intra-synaptique des catécholamines et augmente le signal douloureux qu'il s'agisse de douleurs aiguës ou de douleurs chroniques^{384,388,389}.

Zubieta et al. ont effectivement décrit une réduction de la réponse du système opioïde régional à la douleur et une augmentation de la sensibilité à la douleur chez les sujets homozygotes pour l'allèle 158Met³⁸⁴. Cependant une augmentation des capacités de liaison des récepteurs mu et de leur densité est susceptible d'accroître la réponse aux opioïdes³⁸⁴.

Les sujets homozygotes pour l'allèle Met ont effectivement des besoins réduits en morphine, en particulier en cancérologie ^{386,390,391}. Un lien entre un haplotype (deux SNPs situés dans l'intron 1 du gène codant pour l'enzyme) de la COMT affectant 10.4% des individus et la survenue d'évènements indésirables centraux de type hallucination, confusion sous morphine, indépendamment de son effet sur la sensibilité a également été décrit ³⁶⁰. Loggia et al. ont récemment démontré l'impact de ce polymorphisme grâce à l'IRM fonctionnelle ³⁹². Les sujets homozygotes pour l'allèle Met présentent une activation de zones cérébrales impliquées dans la perception de la douleur, notamment émotionnelle, et dans la régulation négative des signaux douloureux lorsqu'ils sont soumis à des stimuli répétés ³⁹².

Travaux personnels

Manuscrit 1. Facteurs de variabilité de la morphine chez les patients obèses

Les modifications du devenir des médicaments chez les patients obèses sont possibles et il existe de nombreux facteurs de variabilité de la morphine.

Afin de déterminer les objectifs de ma thèse, j'ai effectué une revue de la littérature abordant la question de la variabilité de la morphine chez les patients obèses.

Cette synthèse bibliographique a fait l'objet d'une publication dans la revue Clinical Pharmacokinetics (article 1.).

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Clinical Pharmacokinetics

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Pharmacology of Morphine in Obese Patients

Clinical Implications

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Abstract

Morphine is an analgesic drug used to treat acute and chronic pain. Obesity is frequently associated with pain of various origins (e.g. arthritis, fibromyalgia, cancer), which increases the need for analgesic drugs. Obesity changes drug pharmacokinetics, and for certain drugs, specific modalities of prescription have been proposed for obese patients. However, scant data are available regarding the pharmacokinetics and pharmacodynamics of morphine in obesity. Prescription of morphine depends on pain relief but the occurrence of respiratory adverse effects correlates with obesity, and is not currently taken into account. Variations in the volume of distribution, elimination half-life and oral clearance of morphine, as well as recent advances in the respective roles of drug-metabolizing enzymes, catechol-*O*-methyltransferase and the μ opioid receptor in morphine pharmacokinetics and pharmacodynamics, may contribute to differences between obese and non-obese patients. In addition, drug-drug interactions may alter the disposition of morphine and its glucuronide metabolites, which may either increase the risk of adverse effects or reduce drug efficacy.

Obesity is recognized as a major public health problem worldwide. The WHO estimates that 400 million people were obese in 2005. In 2015, the number of obese adults is expected to reach 700 million and the number of those overweight, approximately 2.3 billion.^[1] The prevalence of obesity (body mass index [BMI] >30 kg/m²) doubled in the US between 1980 and 2002 in adults older than 20 years.^[2] Similar trends are observed in Europe, where the prevalence of obesity exceeds 20% in certain countries.^[3] In the US, one out of 20 obese subjects is morbidly obese (BMI >40 kg/m²), and in Europe too, the prevalence of morbid obesity dramatically increased between 2000 and 2006.^[2,4]

Obesity is associated with a high prevalence of pain, due to the increased prevalence of many chronic diseases (including musculoskeletal diseases and cancer) and with poor health status and poor quality of life.^[5] An effective treatment for pain is therefore of paramount importance for a substantial number of patients, especially during weight loss management and cardiovascular disease prevention. Moreover, morphine is commonly used in the treatment of cancer pain, and the prevalence of cancer is higher in obese than lean subjects.^[6] In the series of obese patients reported by Raebel et al.,^[7] 21% used narcotic analgesics for pain.

The use of narcotic analgesics in obesity is particularly difficult because it has been shown that adverse effects are more frequent in obese populations; thus, the incidence of post-operative nausea and vomiting was 65% in obese patients compared with 35% in non-obese patients in a study involving 1181 subjects. Of the 98.1% of patients who were over 17 years of age, 3.6% were obese and 29% were overweight.^[8] It is hard to determine a morphine dosage regimen that provides adequate pain relief, as morphine may lead to severe adverse effects, including respiratory depression.^[9] Obesity increases the potential for respiratory depression with sleep apnoea syndrome, respiratory failure and the use of sedative medications. Hence, obese patients are at higher risk of admission to an intensive care unit after surgery, and seem to be at higher risk of morphine adverse effects.^[10] Variability in opioid-induced antinociception has also been reported in the morbidly obese after surgery, and the 10-fold variation observed in opioid requirements was not related to body surface area, sex, age, dose per injection or anaesthetic agent.^[11]

The use of morphine in obesity therefore raises several questions, such as whether the adequate initial dosage should be adjusted to the actual or ideal bodyweight (IBW), and whether, in obesity, the influence of bodyweight, and the respective effects of fat and lean mass, gastric bypass, pharmacogenetics, pain sensitivity and potential drug-drug interaction

are due to the increased number of medications prescribed or to the variability of morphine disposition.^[7,12] Better knowledge of the potential differences in morphine metabolism in obese compared with lean subjects could help to identify the adequate balance between pain control and the avoidance of sedative or respiratory depressant adverse effects. The aim of the present review is therefore to address different aspects of morphine metabolism and drug-drug interactions involved in the wide intra- and interindividual variability of analgesia and opioid-induced toxicity in morbidly obese patients.

1. Pharmacokinetics and Pharmacodynamics of Morphine in Normal-Weight Subjects

1.1 Pharmacokinetics: Absorption, Distribution, Metabolism and Excretion of Morphine

After oral administration, morphine is almost completely absorbed by the gastrointestinal tract.^[13] In animals, the fastest absorption of morphine takes place in the medium of the jejunum and duodenum rather than in the stomach.^[14] The pharmacokinetics of morphine and its main glucuronide metabolites are in particular driven by their interaction with both drug transporters and drug-metabolizing enzymes, which may be responsible for their pharmacokinetic interindividual variability. Several drug transporters are located in several healthy tissues, such as the liver, small intestine, kidneys and several barriers such as the blood-brain barrier (BBB), and are involved in the pharmacokinetics of drugs. With drug-metabolizing enzymes, they may reduce oral bioavailability of drugs that are substrates either by effluxing them out of the gut or by eliminating them into the bile during the hepatic first-pass.^[15-17] Although morphine is a well known substrate of the drug efflux transporter P-glycoprotein (P-gp), the influence of P-gp on its oral absorption needs to be ascertained since morphine is well absorbed by the gastrointestinal tract. P-gp is richly expressed in the intestine but its impact on the *in vivo* oral absorption is difficult to measure.^[15,18] Nevertheless, Kharasch et al.^[19] have reported increased absorption of oral morphine in patients receiving quinidine, a well-known P-gp inhibitor, suggesting that intestinal and biliary P-gp may affect absorption and systemic exposure of oral morphine. Among the various members of the multidrug resistance protein (MRP) [ABCC] transporter family, MRP2 (ABCC2) and MRP3 (ABCC3) actively transport morphine glucuronides. However, the role of MRP2 in counteracting intestinal absorption of drugs is limited and it appears to play a more significant role in efflux of chemicals

from the systemic circulation into the bile rather than an absorptive barrier.^[17,20] Most drug metabolism occurs within the liver and, to a lesser extent, the proximal small intestine, where drug metabolizing enzymes are also located.^[21] Morphine is primarily metabolized in the liver by uridine diphosphate glucuronosyltransferase (UGT) enzymes, and has a specific affinity for the UGT2B7 isoenzyme. UGT, a phase II metabolism enzyme family with several isoforms, has been found to be active in the liver, kidneys and epithelial cells of the lower intestinal tract and more recently in the brain.^[22] Sixty percent of an oral dose of morphine 20–30 mg is glucuronidated to morphine-3-glucuronide (M3G), and 6–10% to morphine-6-glucuronide (M6G).^[23,24]

Morphine pharmacokinetics after a single dose in normal-weight subjects are summarized in table I.^[13,19,25–27] Hasselström and Säwe^[27] reported oral bioavailability of $29.2 \pm 7.2\%$ after administration of a single oral 20 mg dose of morphine to seven healthy subjects, whereas others studies have pointed towards the important variability in morphine oral bioavailability from 15% to 64%.^[25,26]

M6G has a very different distribution, metabolism and excretion profile than that of morphine. Using a three-compartment model, Romberg et al.^[28] reported the pharmacokinetic parameters after an M6G bolus dose of 0.3 mg/kg in a homogenous group of healthy subjects.^[28] In comparison with intravenous morphine, the volume of distribution (V_d) of M6G was smaller by a factor of about 10 (0.20 L/kg). The smaller V_d of M6G as compared with morphine indicates that M6G distributes less

well than morphine into tissues, probably related to its lower lipophilicity as compared with morphine.^[28] In addition, the interindividual variability in the V_d of M6G is smaller than that of morphine, with the coefficient of variation ranging from 11% to 30%.^[28]

In healthy subjects, Kharasch et al.^[19] reported pharmacokinetic data on oral morphine disposition (oral morphine sulphate 30 mg): the time to reach the maximum concentration (t_{max}) was 1.1 ± 0.8 hours, the maximum concentration (C_{max}) was 16.9 ± 7.4 ng/mL, the area under the plasma concentration-time curve (AUC) was 40.8 ± 14.1 ng•h/mL and the terminal elimination half-life ($t_{1/2}$) was 2.1 ± 0.6 hours.^[19] Similarly, Hoskin et al.^[25] compared the pharmacokinetic parameters after intravenous (5 mg) and oral (10 mg) morphine, respectively; the average t_{max} ranged from 0.25 to 1.0 hour for the oral morphine, whereas the C_{max} ranged from 274 to 574 ng/mL after intravenous morphine and from 3.9 to 16.4 ng/mL after oral morphine, the AUC ranged from 74.7 to 107.0 ng•h/mL after intravenous morphine and from 11.9 to 46.5 ng•h/mL after oral morphine, and the $t_{1/2}$ ranged from 1.5 to 2.5 hours after intravenous morphine administration.^[25] However, a pronounced interindividual variability in the $t_{1/2}$ of morphine was previously reported.^[26,29–32]

The mean plasma AUC values for M6G were 209.0 ± 27.6 and 183.7 ± 20.2 ng•h/mL after oral and intravenous morphine administration, respectively.^[25] When morphine was given orally to patients with normal renal function, the mean M3G/morphine AUC ratio was 24.3 ± 11.4 while the M6G/morphine

Table I. Summary of pharmacokinetic parameters after a single dose of morphine in non-obese subjects^a

| Study and subjects | Dose of morphine | Route of administration | V_d (L/kg) | $t_{1/2}$ (h) | CL (L/h/kg) | F (%) | C_{max} (ng/mL) | t_{max} (h) |
|--|-------------------|-------------------------|-----------------|------------------|---------------------------|-------------|----------------------|------------------|
| Lötsch et al. ^[13] (n=5) | 0.14 mg/kg | IV | | | 133.4 (26.4) ^b | 34 (9) | | |
| | 90 mg | PO (MST) | | | | | | |
| Kharasch et al. ^[19] (n=12) | 30 mg | PO (IR) | | 2.1 (0.6) | | | 16.9 (7.4) | 1.1 (0.8) |
| Hoskin et al. ^[25] (n=6) | 5 mg | IV | | 1.9 (0.2) | 1.4 (0.24) | 23.8 (4.9) | 340.2 (47.3) | 0.75 |
| | 10 mg | PO (IR) | | | | | 10.6 (2.15) | |
| Säwe et al. ^[26] (n=7) | 0.037–0.066 mg/kg | IV | 2.08 (1.18) | 3.1 (2.3) | 0.55 (0.25) | 38.2 (17.1) | | |
| | 0.231–0.495 mg/kg | PO (IR) | | 3.4 (1.93) | | | | |
| Hasselström and | 5 mg | IV | 2.9 (0.8) | 15.1 (6.5) | 1.2 (0.2) | 29.2 (7.2) | | |
| Säwe ^[27] (n=7) | 20 mg | PO (IR) | | | | | | |

a Values are expressed as mean (SD).

b L/h.

CL=apparent total body clearance; C_{max} =maximum plasma concentration; F=absolute bioavailability; IR=immediate release; IV=intravenous; MST=morphine sulphate, 5H₂O sustained-release tablet, equivalent to MST 90 mg; PO=oral; $t_{1/2}$ =terminal elimination half-life; t_{max} =time to reach the C_{max} ; V_d =volume of distribution.

ratio was 2.7 ± 1.4 .^[26] The $t_{1/2}$ values of morphine, M3G and M6G reported by Hasselström et al.^[23] were 15.1 ± 6.5 hours, 11.2 ± 2.7 hours and 12.9 ± 4.5 hours, respectively.

The mean systemic plasma clearance of morphine reported by Hasselström and Säwe^[27] was 21.1 ± 3.4 mL/min/kg (1.27 ± 0.20 L/h/kg), in agreement with other studies.^[23,25,26,28] The clearance values of morphine to form M3G and M6G were 57.3% and 10.4%, respectively, and renal clearance represented 10.9% of total systemic plasma clearance.^[27] The major route of elimination for M3G and M6G in subjects with normal renal function appeared to be renal excretion and was influenced by renal function.^[33-35] The increased polarity of both morphine glucuronides relative to the parent aglycone limits their diffusion through biological membranes, and it has been suggested that specific transporters may mediate their transport.^[36,37] MRP2 and MRP3 may play a role in the urinary elimination of M3G and M6G.^[36,38]

More than one-fifth of a dose (20.8%) remained as unidentified residual clearance and pharmacokinetic parameters reported by Hasselström and Säwe^[27] are highly suggestive of enterohepatic cycling. MRP2 is localized both at the apical side of enterocytes and at the canalicular membrane of hepatocytes and thus may be responsible for biliary and intestinal secretion of the predominant inactive morphine metabolite M3G, as recently shown in knockout mice.^[17,20,39] Interestingly, in the study by van de Wetering et al.,^[39] the loss of biliary M3G excretion in MRP2 knockout mice resulted in its increased sinusoidal efflux from hepatocytes to blood and prolonged exposition in plasma that could be attributed to its transport into the bloodstream by MRP3, which is exclusively expressed at the basolateral membrane of hepatocyte.^[39] Indeed, MRP3 can easily transport M3G and M6G from the liver into the bloodstream, as recently shown using *in vitro* and MRP3 knockout mice studies.^[39]

To date, not much information has been available about the physiological function of MRP3 and MRP2 and their role in the pharmacokinetics and pharmacodynamics of morphine in humans. In conclusion, all of these pharmacokinetic studies pointed out that at least three ABC transporters (P-gp, MRP2 and MRP3) and one drug-metabolizing enzyme (UGT2B7) may be determining factors affecting the pharmacokinetics of morphine and its glucuronide metabolites.

1.2 Morphine Pharmacodynamics

To be a potent opioid agonist, morphine must penetrate the BBB to reach the brain parenchyma, but its penetration is rather limited compared with that of many other drugs, although it permeates the BBB well.^[40] The relatively poor brain

penetration of morphine has been linked to its active efflux from the brain to the blood by the P-gp at the BBB.^[41] Furthermore, a significant negative correlation between the analgesic effects of morphine and P-gp expression in the cortex was recently reported in mice.^[42]

M3G lacks analgesic properties, but M6G is an effective analgesic, and might have a more favourable adverse effect profile than morphine, causing less nausea and respiratory depression.^[24,43-45] Studies in animals suggested that M3G is a functional antagonist of the antinociceptive effects of morphine and M6G, possibly due to its interaction with receptors other than the known opioid receptors.^[46] When we consider the blood-effect site equilibration half-life ($t_{1/2ke0}$), human studies indicate that M6G equilibrates slowly with the postulated effect-site within the CNS. Romberg et al.^[28] reported a mean $t_{1/2ke0}$ of 6.2 (3.3) hours in 20 healthy subjects receiving intravenous M6G 0.3 mg/kg in a study evaluating pain tolerance with increasing transcutaneous electrical stimulation. In comparison, Lötsch et al.^[13] measured the central opioid effect using the pupil size in eight healthy subjects who received morphine 0.5 mg as a loading dose followed by 10.7 mg as an infusion over a period of 4.7 hours, and M6G 10.2 mg as a loading dose followed by M6G 39.1 mg given over a period of 3.7 hours. The estimated median $t_{1/2ke0}$ of M6G was 6.4 hours, and that of morphine was 2.8 hours. In another study, significant differences in pharmacodynamics between ten men and ten women receiving intravenous morphine (a 0.1 mg/kg bolus dose followed by an infusion of 0.030 mg/kg/h for 1 hour) were observed.^[47]

Meineke et al.,^[37] who studied morphine, M3G and M6G transfer from the central compartment into the cerebrospinal fluid in a population of neurosurgical patients after an 0.5 mg/kg intravenous administration of morphine over 30 minutes, found that transfer of the metabolites M3G and M6G was slower than that of morphine, as the maximum concentrations occurred at 417 minutes and 443 minutes for M3G and M6G, respectively, compared with 102 minutes for morphine. The brain uptake of M6G measured in the rat, killed 30 minutes after a morphine intravenous injection, was 32-fold lower than that of morphine in an *in vivo* study, and the BBB permeability surface area product of M6G was 57-fold lower than that of morphine.^[48] The investigators reported that the liposolubility of M6G was 187-fold lower than that of morphine.^[48] Brain uptake in rats was also measured by the internal carotid perfusion technique and after intravenous bolus injections; the BBB permeability to M6G was 32-fold lower than that of morphine.^[49] The rate of M6G through the BBB is generally assumed to be slower than that of morphine because

of the hydrophilic nature of M6G.^[48,49] The poor BBB permeability to M6G combined with the high concentrations of M6G found in the brain have not yet been explained.^[48-50] GLUT-1 and a digoxin-sensitive transporter (probably organic anion transporting polypeptide-2 [OATP2] or SLCO1B1) may be involved in the M6G transport.^[50] In addition, MRP2 has been found in human cerebral endothelial cells in patients with refractory epilepsy but the presence of MRP2 at the healthy BBB is still debated since it has not been found by immunofluorescence in human brain vessels from patients with different brain pathologies.^[51,52]

Morphine, as well as M3G and M6G, has an affinity primarily for the μ opioid receptor, a product of the opioid receptor mu 1 (*OPRM1*) gene and, to a lesser degree, for the κ and the δ opioid receptors. M6G might have a lower affinity for the μ and the κ opioid receptors than morphine, but may have slightly higher analgesic efficacy and might induce fewer respiratory adverse effects than morphine.^[45,53] The μ opioid receptor modulates the responses to mechanical, chemical and thermal nociception at the supraspinal level, and the κ opioid receptor modulates spinally mediated thermal nociception and chemical visceral nociception. Following inflammation, μ opioid receptors are found at the periphery of pre- and postsynaptic sites in the dorsal horn of the spinal cord, and in the brainstem, thalamus and cortex, which together constitute the ascending pain transmission system.^[54] In addition, μ opioid receptors are found in the midbrain periaqueductal grey substance, the nucleus raphe magnus and the rostral ventral medulla, where they constitute a descending inhibitory system that modulates spinal cord pain transmission.^[55] At the cellular level, opioids reduce calcium ion entry, thus also reducing the release of presynaptic neurotransmitters such as substance P, which is released from primary afferents in the dorsal horn. They also enhance potassium ion efflux, resulting in the hyperpolarization of postsynaptic neurons and a decrease in synaptic transmission. A third mechanism of opioid action is the inhibition of GABAergic transmission in a local circuit (e.g. in the brainstem, where GABA inhibits the action of a pain-inhibitory neuron). This disinhibition of the action of the dopamine system causes dopamine release in the nucleus accumbens and has the net effect of exciting a descending inhibitory circuit.

The opioid receptors are part of the endogenous opioid system, which includes a large number of endogenous opioid peptide ligands. Three distinct families of classical opioid peptides have been identified: the enkephalins, endorphins and dynorphins.^[56] The physiological roles of the endogenous opioid peptides are not completely understood. They appear to function as neurotransmitters, neuromodulators and,

in some cases, neurohormones. They play a role in some forms of stress-induced analgesia and constitute part of an endogenous pain modulatory system. In addition, catechol-O-methyltransferase (COMT), an enzyme metabolizing catecholamines, has recently been implicated in the modulation of pain. Low COMT activity leads to increased pain sensitivity via a β_2 - and β_3 -adrenergic mechanism.^[57]

The individual variability of opioid pharmacology suggests that genetic factors may influence the response to opioids. This view is strongly mediated by observations of variation among ethnic groups with respect to the opioid response.^[58,59]

Interindividual variability in morphine efficacy can be related to variations in the interaction between M6G and the μ opioid receptor.^[58] The genetic complexity of the *OPRM1* gene was shown by Hoehe et al.,^[60] who identified 43 allelic variants. Their consequences have been studied in healthy subjects.^[61,62] The frequency of the most common single nucleotide polymorphism (SNP), A118G, is about 10–14% in Caucasians.^[60] This polymorphism has been associated with reduced opioid effects and can lead to the need for 2- to 4-fold higher concentrations of alfentanil to control pain, and for 10- to 12-fold higher concentrations to obtain respiratory depression compared with the wild-type allele in healthy subjects.^[63,64] In studies enrolling cancer patients, homozygous carriers for 118G required about twice as much morphine as those homozygous for the wild type A118 allele to achieve adequate pain relief.^[65-67] Human subjects with one or two 118G copies exhibited decreased papillary constriction after M6G administration, while the 118G variant may be protective against M6G toxicity.^[68,69] The A118G SNP of the *OPRM1* gene and C3435T SNP of the human *ABCB1/MDR1* exert strong but independent effects on responsiveness and pain relief, but not on the occurrence of adverse effects.^[67] Other recently identified variants have not been found to influence morphine efficacy. Among cancer patients, homozygous carriers of both 118G *OPRM1* and 158Met *COMT* allelic variants required the lowest morphine dose to achieve pain relief.^[64,70]

Recent reports have suggested that Val158Met, a functional polymorphism of the *COMT* gene, partially influences cognitive performances, some psychiatric affections, fibromyalgia, experimental pain sensitivity and morphine efficacy in cancer pain treatment morphine requirements.^[57,71-76] Functional polymorphisms in the *COMT* gene result in 3- to 15-fold reductions in *COMT* activity.^[57,73-76] Lower *COMT* activity is associated with heightened pain sensitivity.^[77] The frequency of the 158Met allelic variant, associated with lower activity of *COMT*, is about 50% in Caucasians, 18% in Han Chinese and 29% in Japanese.^[77-79] In addition, among patients

Table II. Proteins involved in the control of nociception

| Protein | Gene | Role |
|---|--------------|--|
| μ opioid receptor | <i>OPRM1</i> | Mediates endorphin effects in the physiological pain protective system |
| δ_1 opioid receptor | <i>OPRD1</i> | Mediates enkephalin effects in the endogenous opioid system |
| Catechol-O-methyltransferase | <i>COMT</i> | Degrades catecholamines and mediates adrenergic, noradrenergic and dopaminergic neuronal transmission |
| Transient receptor potential cation channel | <i>TRPV1</i> | Mediates pain induced by heat or capsaicin |
| Transient receptor potential cation channel subfamily A | <i>TRPA1</i> | Mediates cold sensation and pain |
| Fatty acid amide hydrolase | <i>FAAH</i> | Degrades the fatty acid amide family of endogenous signalling lipids, including the endogenous cannabinoid anandamide, involved in the suppression of pain |
| GTP cyclohydrolase 1 | <i>GCH1</i> | Contributes to the regulation of biogenic amine and nitric oxide synthesis |
| IL-1 receptor antagonist | <i>IL1RN</i> | Competitive inhibitor of IL-1 bioactivity |
| IL-1 α | <i>IL1A</i> | Cytokine-inducing apoptosis |
| IL-1 β | <i>IL1B</i> | Cytokine involved in the inflammatory response and in a variety of cellular activities, including cell proliferation, differentiation and apoptosis |

GTP = guanosine triphosphate; **IL** = interleukin.

with cancer who received morphine, another allelic variation in the COMT enzyme (a SNP in intron 1 (-4873G) present in 10.4% of the population) was independently associated with central adverse effects.^[80]

In addition, it is well known that the response to painful stimuli varies between individuals and this could be the consequence of individual differences to pain sensitivity that may be related to genetic factors. The proteins involved are briefly reported in table II.

2. Pharmacokinetics and Pharmacodynamics of Morphine in Obese Subjects

2.1 Clinical Observations

Interindividual variability in opioid pharmacology leading to variability in dose requirements for pain relief was observed in an obese population who used patient-control anaesthesia (PCA).^[11] In a sample of 1181 patients using PCA, more obese than non-obese patients experienced postoperative nausea and vomiting.^[8] Furthermore, in a post-anaesthesia care unit, obesity was significantly associated, over a period of 33 months, with a larger number of critical respiratory events than in non-obese subjects, in a cohort of 24 157 consecutive patients given a general anaesthetic.^[10] In this cohort, anaesthetic risk factors ($p < 0.05$) included, among others, opioids used in premedication (odds ratio = 1.8) and fentanyl used in combination with morphine (odds ratio = 1.6). These observations raise questions concerning opioid pharmacokinetics and morphine pharmacodynamics in obese populations.

Drug concentration and elimination rates depend on metabolic activity and interindividual variability in metabolism affects drug action. We review the factors involved in the variability of metabolism and the efficacy of morphine and study them in the case of obese subjects. They are summarized in table III.

2.2 Drug Absorption and Consequences of Bariatric Surgery

Absorption of drugs does not appear to be significantly modified in the presence of obesity.^[133] Genetic factors and drug-drug interactions may constitute a source of inter-individual variation in drug transporter and drug metabolizing enzymes, and thus in oral bioavailability.

Little is known about the consequences of bariatric surgery on intestinal absorption of drugs, especially that of morphine.^[88,89] Drug solubility, the surface area of drug absorption and gastrointestinal blood flow may affect oral drug bioavailability. Most drugs are absorbed in the jejunum rather than in the stomach, duodenum or ileum, whereas drug efflux, especially P-gp-mediated efflux, occurs mainly in the ileum and the colon. Conversely, MRP2-mediated efflux seems to occur all along the small intestine.^[134-137] Tablets and capsules must disintegrate and dissolve before absorption, and the time required for disintegration and dissolution affects the amount of drug absorbed and/or the rate of its absorption. Once a drug is solubilized, it is absorbed through the jejunum epithelium by paracellular and/or transcellular passive diffusion or active uptake transport. Drugs in aqueous solutions are more rapidly absorbed than those in oily solutions, suspensions or solid

form. Half of the total mucosal area is found in the proximal quarter of the gut, which has the greatest capacity for drug absorption.^[138]

Roux-en-Y gastric bypass is one of the most frequently performed surgical techniques and combines restrictive and malabsorptive procedures. A 30–60 mL pouch is created at the top of the stomach to restrict food intake. The small intestine is cut by 45–150 cm from the stomach, and the intestine is connected to the pouch at the top of the stomach. The small pouch produces much less hydrochloric acid than the entire stomach. Subsequently, this increase in gastric pH may affect drug absorption of medications that depend on drug ionisation.^[139] For instance, it increases absorption of weak bases such as ketoconazole.^[140–142] When there is a reduction in the total

intestinal surface area for absorption, drugs with long absorptive phases may have decreased bioavailability.

It is, however, possible that mechanisms for compensatory absorption by other sites intervene, although this requires confirmation. The stagnation of weight loss after bypass may account for such an adaptative mechanism of the intestinal barrier to nutrient malabsorption, but whether or not these modifications also impact on drug absorption has never been tested, to the best of our knowledge. Drug pharmacokinetics before and at different times after surgery may be helpful to describe such an adaptive mechanism of the remaining small intestinal mucosa.

Bariatric surgery may also increase the risk of adverse drug effects due to removal of the epithelial intestinal barrier.^[18] Because of its extensive glucuronidation by UGT2B7, which is expressed in the small intestinal mucosa, morphine absorption may be modified after bariatric surgery.^[143] In the very few studies including patients who had a jejunoileal bypass, phenazone absorption and hepatic drug metabolizing capacity appeared to be unaffected for up to 57 months after intestinal shunting.^[90] No permanent effect on the rate or amount of sulfisoxazole absorption was observed after intestinal bypass surgery in four morbidly obese women (110–150 kg).^[144] However, unlike morphine, these drugs do not undergo intestinal first-pass. Therefore, it would be clinically relevant to describe the consequences of gastric bypass on morphine systemic exposure and pharmacodynamics in obese patients.

2.3 Hepatic Drug Metabolism in Obese Subjects

Among liver diseases, non-alcoholic steatohepatitis is frequently reported in obesity and may progress to cirrhosis and end-stage liver disease.^[145] The inflammatory infiltrate and cytokine expression play a role in the development of fibrogenesis.^[146,147] Different stages of non-alcoholic steatohepatitis may influence morphine pharmacokinetics.^[100–102] In human percutaneous biopsy samples, a decrease in UGT messenger RNA (mRNA) levels, which correlated with inflammation scores, was observed in patients with various forms of acute liver disease.^[100–102] However, despite contradictory results, it was generally accepted that glucuronidation capacity is unaffected by most liver disease, especially steatohepatitis. However, during end-stage liver disease, patients with a portal shunt are at risk of drug toxicity because the shunt diverts much of the blood away from the liver and therefore away from most metabolizing enzymes. Hasselström et al.^[23] found significantly lower plasma clearance, a longer $t_{1/2}$ and higher oral

Table III. Putative factors between obese and normal-weight subjects that may affect morphine pharmacokinetics and pharmacodynamics

Pharmacokinetics

Absorption

Genetic factors

Intestinal flora^[81]

Drug-drug interactions^[29,82–87]

Bariatric surgery^[88–90]

creation of a 30–60 mL pouch

increase in gastric pH

reduction in the total intestinal surface area

Distribution

Increased adipose tissue and lean body mass^[91]

High cardiac output^[92–94]

Increased total body water^[91,95–97]

Expansion of the extracellular compartment relative to the intracellular compartment^[97–99]

Higher hydration of the fat-free mass^[92]

Metabolism

Genetic factors

Non-alcoholic steatohepatitis^[23,100–103]

Inflammation^[100,104–112]

Oxygenation^[113]

Elimination

Increased glomerular filtration rate^[114,115]

Genetic factors

Pharmacodynamics

Genetic factors^[116–119]

Endocrine factors^[120–126]

Psychological factors^[127]

Nociception^[128–132]

bioavailability of morphine in seven patients with cirrhosis than in patients with normal hepatic function.

Glucuronidation is the main metabolic pathway of morphine. Factors affecting glucuronidation include cigarette smoking, age, sex and obesity.^[103] Glucuronidation has been shown to be increased in obese subjects but no specific information is available on UGT2B7, which metabolizes morphine. Likewise, whether steatohepatitis has a specific effect on UGT2B7, P-gp and/or MRP2 or MRP3 is currently unknown. Morphine has a high total plasma clearance (21.1 ± 3.4 mL/min/kg) mainly due to UGT2B7-mediated metabolism, which classifies morphine as a high-extraction drug.^[23] Thus changes in hepatic blood flow occurring in obese subjects may increase its hepatic plasma clearance.

In addition, for the drug-metabolizing enzymes to function normally, a sufficient supply of oxygen and nutrients is necessary. Changes in oxygen delivery due to pulmonary or cardiovascular disease may alter metabolism.^[113] In the case of chemotherapeutic agents, susceptibility to drugs is greatly affected by hypoxia, which enhances resistance to these agents.^[148] Collectively, hepatic, inflammatory and pulmonary consequences of obesity (apnoea syndrome and Pickwick syndrome) may thus alter drug metabolism and morphine pharmacokinetics.

2.4 Distribution and Renal Elimination in Obese Subjects

Dosage modifications in obesity are driven by routine determination of drug concentrations in plasma. Drug distribution into tissue is affected by body composition, regional blood flow and physico-chemical properties of the drug such as lipophilicity and plasma protein binding. Body composition is dramatically different in obese versus non-obese subjects. The increased adipose tissue and lean body mass characterizing obesity is associated with high cardiac output, increased blood volume and an increased glomerular filtration rate.^[91-94,98,99,114,115,145,149] In non-obese subjects, approximately 65% of total body water is intracellular versus only 35% in the extracellular compartment. An increase in total body water, with expansion of the extracellular compartment relative to the intracellular compartment, is observed in obese patients.^[91,95,96] Waki et al.^[97] reported an increase in total body water by 12.9 litres in obese compared with normal-weight women. Moreover, hydration of the fat-free mass appears to be significantly higher in obese versus non-obese subjects.^[92] Extracellular water, hydration of the fat-free mass and adipose tissue may influence the V_d of drugs. Various studies have described the differences between obese and non-obese

subjects in drug pharmacokinetics. We report some of them in table IV.^[150-160] The differences in morphine pharmacokinetics in obese versus non-obese subjects has never been reported.

Previous studies have focused on antimicrobial and anaesthetic drugs.^[161,162] Hydrophilic drugs generally have a low or moderate affinity for adipose tissue and hence exhibit no increase or a moderate increase in their V_d , which in obesity and in the case of some drugs correlate with an increase in lean body mass; adjustment of aminoglycoside and ciprofloxacin dosage should therefore be based on adjusted body weight (including IBW +40% of excess weight).^[155,163-166] However, total body-weight was a better predictor of the V_d in the case of vancomycin, and a double dose of cefazolin was found to be more effective than a single dose in decreasing postoperative infections in obese patients.^[154,167,168]

In the case of lipophilic drugs, including benzodiazepine and opioids, a larger V_d is usually observed in obese versus non-obese patients, and correlates with the degree of obesity. For example, Abernethy and Greenblatt^[133] reported a V_d of 158 L in obese subjects and 63 L in lean subjects after administration of a 15 mg chlorazepate capsule, and the value of the V_d remained greater after correction for bodyweight. But in the case of thiopental sodium and remifentanyl, the V_d was more closely related to lean body mass and cardiac output than to total body water.^[88,151,169-174] The estimates of the distribution volumes for remifentanyl (mean central volumes of distribution of 7.5 L and 6.8 L in the obese and lean groups, respectively, and mean peripheral compartment volumes of distribution of 8.7 L and 7.6 L in the obese and lean groups, respectively) are somewhat less than expected for lipid-soluble molecules and revealed only modest distribution into body tissues.^[173] Morphine has an intermediate V_d in humans (ranging from 0.95 to 3.75 L/kg), probably related to its lipophilicity.^[26] The question of the role of adipose tissue on morphine tissue distribution, which in turn may affect its pharmacokinetics, has not been investigated.

Obesity affects the glomerular filtration rate, which may alter clearance of antibacterials that are eliminated unchanged through the kidney.^[175] Obese kidney donors have a larger glomerular planar surface area than non-obese donors, thus confirming the concept that a higher BMI is associated with larger glomeruli in humans.^[114,115] Therefore, in the case of hydrophilic drugs, obese patients may require more frequent drug administration.^[155,163-166]

A prolonged $t_{1/2}$ is observed with lipophilic drugs.^[133,150,162,174] For example, diazepam $t_{1/2}$ was greatly prolonged in obese subjects (82 vs 32 hours in non-obese subjects), with no change in total metabolic clearance.^[133] Differences in drug

Table IV. Examples of drug pharmacokinetics in obese and non-obese subjects^a

| Drug dosage and subjects | t _{1/2} (h) | | V _d | | CL | | Reference |
|--|----------------------|----------------------|--|---|---|---|-----------|
| | obese | control | obese | control | obese | control | |
| Examples of drugs used in anaesthesiology | | | | | | | |
| Sufentanil 4 µg/kg: single IV bolus; 8 obese and 8 control | 3.5 (1.4)* | 2.25 (0.7) | 9 (2.8) L/kg IBW* | 5 (1.7) L/kg IBW | 32.9 (12.5) L/kg IBW | 26.4 (5.7) L/kg IBW | 150 |
| Midazolam 2.5–5 mg IV bolus; 20 obese and 20 control | 8.4 (0.84)* | 2.7 (0.34) | 311 (27) L = 2.7 L/kg TBW* | 114 (7) L = 1.7 L/kg TBW | 472 (0.38) mL/min* | 530 (34) mL/min | 151 |
| Vecuronium 0.1 mg/kg IV bolus; 7 obese and 7 control | 2 (0.7) | 2.21 (0.9) | 0.8 (0.3) mL/kg IBW | 0.9 (0.3) mL/kg IBW | 4.65 (0.89) mL/min/kg IBW | 5.02 (1.13) mL/min/kg IBW | 152 |
| Examples of anti-infectives drugs | | | | | | | |
| Daptomycin 4 mg/kg TBW IV infusion; 6 obese and 6 control | 8.12 (21) | 8.04 (29) | V _z : 0.18 (18.1) L/kg IBW* = 0.09 (12.9) L/kg TBW* | V _z : 0.12 (14.0) L/kg IBW = 0.11 (11.9) L/kg TBW | 0.27 (0.45) mL/min/kg IBW = 0.13 (0.33) mL/min/kg TBW* | 0.18 (0.53) mL/min/kg IBW = 0.17 (5.1) mL/min/kg TBW | 153 |
| Vancomycin 1 g IV over 40 min; 6 obese and 4 control | 3.2* | 4.7 | 43.0 L = 0.26 L/kg TBW* | 28.9 L = 0.39 L/kg TBW | 187.5 mL/min* | 80.8 mL/min | 154 |
| Ciprofloxacin single 400 mg IV dose over 1 h; 17 obese men and 11 control | 4.26 (0.66) | 4.0 (0.34) | V _{ss} : 269.17 (51.64) L* V _{ss} /kg: 2.46 (0.42) L/kg | V _{ss} : 219.03 (35.80) L V _{ss} /kg: 3.06 (0.31) L/kg | 897.44 (159.57) mL/min* | 744.44 (120.51) mL/min | 155 |
| Others | | | | | | | |
| Paracetamol (acetaminophen) single 650 mg IV dose; 21 morbidly obese and 21 control | OM: 2.55 OW: 2.32 | CM: 2.76 CW: 2.66 | OM: 108.5 L* = 0.81 L/kg TBW* OW: 61.4 L* = 0.71 L/kg TBW* | CM: 77.0 L = 1.09 L/kg TBW CW: 51.6 L = 0.95 L/kg TBW | OM: 484 mL/min* OW: 312 mL/min* | CM: 323 mL/min CW: 227 mL/min | 156 |
| | | | | | No difference between groups after correction for TBW | | |
| Continued next page | | | | | | | |

Continued next page

Table IV. Contd

| Drug dosage and subjects | $t_{1/2}$ (h) | | V_d | | CL | | Reference |
|---|---------------|------------|----------------------------|---------------------------|-----------------------------|-----------------------------|-----------|
| | obese | control | obese | control | obese | control | |
| Cyclophosphamide 7 overweight and 5 obese | 8.5 | 4.8 | 37.1 L | 34.5 L | 60.6 mL/min | 91.3 mL/min | 157 |
| Isofosfamide 4 obese and 12 control | 6.4* | 4.9 | 42.8 L* = 0.55 L/Kg TBW | 33.7 L = 0.53 L/Kg TBW | 76.0 mL/min | 72.2 mL/min | 158 |
| Lithium (oral lithium citrate) 31.4 mEq; 10 obese and 8 control | 18.7 (4.0)* | 25.6 (9.6) | 0.42 (0.1) L/Kg TBW* | 0.66 (0.15) L/Kg TBW | 33.9 (7.0) mL/min* | 23.0 (6.2) mL/min | 159 |
| Glipizide 5 mg single dose 12 obese and 8 control | 5.0 (2.3) | 5.2 (2.0) | V_d/F : 17.2 (4.3) L | V_d/F : 19.5 (4.4) L | CL/F: 0.18 (0.06) mL/min | CL/F: 0.12 (0.06) mL/min | 160 |

a Values are expressed as mean (SD).

CL = apparent total body clearance; CL/F = apparent oral clearance; CM = control men; CW = control women; IBW = ideal bodyweight; IV = intravenous; OM = obese men; OW = obese women; $t_{1/2}$ = terminal elimination half-life; TBW = total bodyweight; V_d = apparent volume of distribution; V_d/F = volume of distribution after oral administration; V_{ss} = steady-state volume of distribution; V_z = apparent volume of distribution during the terminal phase; * $p < 0.05$ vs control.

lipophilicity in morbidly obese populations may also explain differences in postoperative recovery after anaesthesia with desflurane versus sevoflurane.^[176]

Morphine has relatively low renal clearance compared with its total plasma clearance, suggesting that modification of glomerular filtration occurring in obese subjects may only weakly affect its total clearance. However, M6G and M3G are mainly eliminated by renal clearance and the higher glomerular filtration in obese subjects may increase renal clearance of M6G and M3G, leading to decreased M6G pharmacological activity.

The pharmacokinetics of drugs are, in general, affected to various degrees by obesity, and the extent of this effect is difficult to predict.^[161] The situations thus created illustrate the differences between drug distribution in obese versus non-obese subjects, as well as the need for predictive markers that could be used routinely to individualize drug dosage.

2.5 Inflammation and Drug Metabolism

Obesity is a state of chronic low-grade inflammation.^[146,177-179] Adipose tissue is considered as a secretory organ that produces adipokines (leptin and adiponectin) and other cytokines such as interleukin (IL)-6, tumour necrosis factor (TNF)- α and vascular endothelial growth factor.^[179,180] It has been suggested that inflammation and infection may increase drug bioavailability.^[100,104-112] Inflammatory agents increase the production of interferon, TNF and mainly IL-1 and IL-6.^[181] TNF and IL-1 induce the production of IL-6, which inhibits drug metabolism *in vitro*. A recent study conducted in six bone marrow transplant recipients showed that the peak serum concentration of IL-6 after transplantation was systematically followed by an increase in ciclosporin serum concentrations.^[182]

Liver and intestinal P-gp and UGT2B7 are the two major proteins involved in the intestinal and hepatic first-pass of morphine in humans. One study revealed a trend towards downregulation of most UGTs in the mouse liver during acute inflammation.^[104] A decrease in UGT mRNA levels that correlated with inflammation scores has been observed in human tissue samples from percutaneous liver biopsies.^[100] In addition, expression and activity of P-gp were decreased by IL-6, IL-1, IL-10 and TNF *in vitro* and in animal studies during inflammation in the CNS and intestinal tract.^[105-110] Hartmann et al.^[106] also reported a 40–70% reduction in the expression and mRNA levels of P-gp in the livers of IL-6-treated mice. Buyse et al.^[109] reported an increase in P-gp expression in the non-inflamed intestine of rats with colitis, which may reflect the existence of an adaptative mechanism to compensate for a loss

of P-gp functionality. A study of the long-term consequences of continuous exposure of rat brain capillaries to low levels of TNF α and endothelin-1 showed a rapid decrease in P-gp transport activity followed by an increase in this activity and P-gp protein expression.^[111] In humans, Fakhoury et al.^[112] compared P-gp mRNA and protein levels and functionality in 19 non-inflamed duodenal biopsies from children with Crohn's disease with control duodenum, and found higher P-gp levels in the children with Crohn's disease, although the disease was silent at the time of the study.

MRP2 (another transporter involved in the biliary, intestinal and renal transport of morphine and its glucuronidated metabolites) mRNA levels were also lower during sepsis or hepatitis C infection, and cytokines (IL-1 β , TNF α , IL-6) may be involved in reducing the expression level of MRP2, as shown in animals and *in vitro*.^[17] To date, transporter activity has not been specifically studied in obesity, although this clinical setting may reflect chronic inflammation and alter morphine pharmacokinetics and pharmacodynamics due to alteration in morphine metabolism transport.

2.6 Nociception, the μ Opioid Receptor and Obesity

The most frequent type of pain in obesity is joint pain, mainly due to osteoarthritis.^[183] It remains unclear whether or not differences in pain perception exist between obese and non-obese patients and influence morphine requirements. Many factors may influence nociception, including pain mechanisms (mechanical factors and possibly inflammation in the case of obesity), smoking, alcohol (ethanol) consumption, pathological conditions, psychological and genetic factors.^[127,184]

Few studies have reported contradictory results regarding nociception in obese populations and differences in the methods of assessment used may account for the mixed findings. In humans, Pradalier et al.^[128] using a nociceptive flexion reflex (the sapheno-bicipital reflex), reported increased pain, with a significantly lower threshold in obese patients than in non-obese patients. McKendall and Haier^[129] also found lower mechanical pain thresholds in obese subjects, as assessed by a constant force applied to the finger. Conversely, in a sample of 206 healthy subjects, Khimich,^[130] who used a method based on dosage pressure by a needle on the forearm, found that obese patients had a higher pain sensitivity threshold and then felt less pain. Zahorska-Markiewicz et al.^[131] using transcutaneous electrical stimulation, found an elevated pain threshold in obese subjects. However, Raymond et al.^[132] detected a significantly higher pain threshold in obese subjects with binge-eating disorder than in those without binge-eating disorder but the BMI

and pain threshold were not correlated, suggesting abnormal physiological painful stimuli in patients with binge-eating disorder.

Interestingly, a recent study in parturient women showed that obese patients required smaller amounts of intrathecally administered analgesics than lean patients. Several factors might account for this, including polymorphisms of the μ opioid receptor, reduced analgesic efflux or the anatomy of the CNS, characterized by increased intrathecal pressure in obesity.^[185]

Moreover, common circuits are involved in food behaviour and in nociception, which may explain differences in nociception and the responses to morphine analgesia in obese patients: endogenous opioid, central melanocortin and dopamine systems.^[120-126] Interestingly, a mutation was recently identified in a subject with severe obesity, impaired learning and memory, who also had impaired nociception, illustrating the possibility that genetic factors may predispose to both obesity and impaired nociception.^[186-188] Pain perception, the efficacy of morphine and its adverse effects, the responses to addictive opioid drugs, the rewarding properties of opioid compounds and the responses to stress mediated by the hypothalamic pituitary adrenal axis, are all controlled by the μ opioid receptor. Different genotypes of this receptor may modify these different responses.^[189,190] Recent studies support the possibility that the μ opioid receptor may have a role in behaviour and suggest that in obesity, the opioid system is deregulated which, if true, would lead to differences in morphine pharmacodynamics between obese and non-obese patients.^[26,191-198]

Since there are associations between the frequency of *OPRM1*, *COMT* and *MDR1* polymorphisms and morphine efficacy and tolerance, as well as vulnerability to dependence on addictive substances, and because similarities between obesity and addictions have been reported, the prevalence of the aforementioned genetic polymorphisms may be clinically relevant variables to study in obese versus non-obese patients.^[199] Some studies have recently reported a relation of some polymorphism of these genes and obesity or weight gain. A stronger influence of the *MDR1* (G2677T and C3435T) polymorphisms on risperidone-induced weight gain has been recently reported among 108 female schizophrenic patients.^[116] Among 5448 Japanese individuals, the G2677T polymorphism was also significantly associated ($p=0.0003$) with obesity.^[117]

Xu et al.^[118] recently reported that tagging SNPs (tSNPs) in the *OPRM1* gene (rs1799971 in exon 1, and rs514980 and rs7773995 in intron 1) were significantly associated with the BMI in a Uyghur population. Recently, Davis et al.^[200] reported a significant difference in the prevalence of the G allele between the population of obese patients with binge eating

(allele G=0.18; mean BMI=35.6 kg/m²) and the population of obese patients without binge eating (allele G=0.10; mean BMI=39.2 kg/m²), suggesting that binge eating is a genetically determined subtype of obesity. It has also been suggested that *COMT* polymorphism may play a role in the risk of obesity following antipsychotic drug usage and in the general population. In a cohort of 240 Swedish men, homozygous subjects for the low-activity allele (met) displayed higher blood pressure, heart rates, waist-to-hip ratios and abdominal sagittal diameters as compared with heterozygous subjects.^[119]

3. Conclusions and Perspectives

This review has not been designed to present all current aspects of opioid pharmacology but rather to highlight the lack of pharmacokinetic and pharmacodynamic data on morphine in obese subjects and to focus on some selected findings that may be clinically relevant to the morbidly obese population. Obesity resulting from environmental and genetic factors is associated with changes in body composition, endocrine signals, inflammatory status and morbidity. These changes may affect drug disposition and may partly explain interindividual variations in morphine efficacy and toxicity. We think that all these parameters merit investigation. Studying morphine pharmacokinetics and pharmacodynamics in obese patients and incorporating the currently known morphine pharmacogenomic aspects would provide very useful clinical information on issues such as nociception and the influence of body composition, inflammation and concomitant medications on morphine pharmacokinetics and analgesia. Several issues such as the initial dosages in obesity and gastric bypass or the consequences of drug-drug interactions are still unresolved.

Further studies are therefore needed to determine the influence of P-gp, UGT2B7, MRP2, COMT and OPRM1 on oral morphine disposition and the dose-effect relationship in obesity. In addition, pharmacological studies before and after bariatric surgery may highlight the role of the intestinal barrier in the disposition and clinical efficacy of morphine. A better understanding of the sources of pharmacokinetic variability may improve the use of opioids in the clinical management of obese patients, especially in morbidly obese subjects undergoing bariatric surgery.

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Manuscrit 2. Pharmacogénétique de la morphine chez les patients obèses

Les déterminants de l'obésité humaine sont multiples. D'un extrême à l'autre, il existe des formes purement génétiques, liées à de rarissimes mutations ou à des formes purement comportementales. Entre ces deux extrêmes, toutes les situations se rencontrent, mais la règle est une interaction de facteurs environnementaux, comportementaux et génétiques. Ainsi, les sujets souffrant d'obésité sont différents sur le plan génétique des sujets de poids normal.

J'ai souhaité déterminer la fréquence de polymorphismes génétiques impliqués dans la variabilité de PK et de PD de la morphine dans une cohorte de sujets obèses.

109 sujets présentant une obésité morbide ($IMC=49.1 \pm 7.7 \text{ kg/m}^2$) ont été génotypés pour trois polymorphismes : c.A118G de *OPRM1* (codant pour le récepteur mu), p.Val158Met de *COMT* (codant pour l'enzyme Catechol-O-Methyl-Transferase) et c.C3435T de *ABCB1* (codant pour le transporteur P-gp).

Ce travail a fait l'objet d'une publication dans la revue *Obesity Surgery* (article 2.).

Pilot Study Examining the Frequency of Several Gene Polymorphisms Involved in Morphine Pharmacodynamics and Pharmacokinetics in a Morbidly Obese Population

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Abstract Morbidly obese patients are at significantly elevated risk of postsurgery complications and merit closer monitoring by health care professionals after bariatric surgery. It is now recognized that genetic factors influence individual patient's response to drug used in anesthesia and analgesia. Among the many drug administered by anesthe-

tists, we focused in this pilot study on morphine, since morphine patient-controlled anesthesia in obese patients undergoing gastric bypass surgery is frequently prescribed. We examined the allelic frequency of three polymorphisms involved in morphine pharmacodynamics and pharmacokinetics in patients with body mass index (BMI) >40. One hundred and nine morbidly obese patients (BMI=49.1±7.7 kg/m²) were genotyped for three polymorphisms c.A118G of mu opioid receptor (*OPRM1*), c.C3435T of the P-glycoprotein gene (*ABCB1*), and p.Val158Met of catechol-O-methyltransferase gene (*COMT*). Allelic frequencies were 118G—0.22, C3435—0.55, and 158Met—0.5 in our whole population and 0.23, 0.5, and 0.47 in Caucasian population. Allelic frequencies did not differ according to gender. Mean BMI did no differ according to the allelic variant. *OPRM1*118G allele was more frequent in our population than in most previously described European populations. Since the concept of “personalized medicine” promises to individualize therapeutics and optimize medical treatment in term of efficacy and safety, especially when prescribing drugs with a narrow therapeutic index such as morphine, further clinical studies examining the clinical consequences of the *OPRM1* c.A118G polymorphism in patients undergoing gastric bypass surgery are needed.

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Keywords Morphine · Pharmacogenetics · Obesity ·
OPRM1 · *ABCB1* · *COMT* · Analgesia

List of abbreviations

| | |
|--------------|--|
| <i>ABCB1</i> | ATP-binding cassette, subfamily B, member 1 |
| BMI | body mass index |
| COMT | catechol-O-methyltransferase (enzyme and gene) |
| DNA | deoxyribonucleic acid |
| EDTA | ethylenediaminetetraacetic acid |

| | |
|--------------|--------------------------------|
| M6G | morphine-6-glucuronide |
| MAO-A | monoamine oxydase A |
| MAO-B | monoamine oxydase B |
| MOR | mu opioid receptor |
| <i>OPRM1</i> | mu opioid receptor gene |
| P-gp | P-glycoprotein |
| SNP | single nucleotide polymorphism |

Introduction

Bariatric surgery is increasingly used to obtain substantial weight loss and to reduce obesity-related comorbidities. Concerns about the safety of bariatric surgery have grown along with its increasing popularity [1, 2]. Morbidly obese subjects, with or without obstructive sleep apnea, experience frequent oxygen desaturation episodes postoperatively despite supplemental oxygen therapy suggesting that perioperative management strategies in morbidly obese patients undergoing laparoscopic bariatric surgery should include measures to prevent postoperative hypoxemia [3].

The concept of “personalized medicine” promises to individualize therapeutics and optimize medical treatment in terms of efficacy and safety, especially when prescribing drugs displaying narrow therapeutic index such as morphine [4]. Morphine has been widely used within the fields of anesthesia and acute chronic pain for many years, and its use is characterized by large interpatient variations in dose requirements and by occurrence of side effects [5, 6]. A better understanding of opioid response’s variability could help to identify the adequate balance between pain control and the avoidance of sedative or respiratory depressant side effects; moreover, it will improve clinical management of these patients. The following genes have been reported to affect either the pharmacokinetics or the pharmacodynamics of morphine: *OPRM1*, *ABCB1*, and *COMT* encoding for the mu opioid receptor (MOR), the drug transporter P-glycoprotein (P-gp), and the catechol-O-methyltransferase (COMT), respectively.

MOR is the primary target of opioid drugs. Genetic polymorphisms resulting in changes in receptor density and function may partially explain interpatient variations in opioid response [4]. Genotype distribution and allelic frequencies of the *OPRM1* c.A118G single nucleotide polymorphism (SNP) varies between ethnic groups [7–10]. This polymorphism is associated with lower MOR and mRNA levels in human autopsy brain tissues due to a transcription defect [11]. In healthy subjects, G allele is associated with decreased efficacy of morphine and morphine-6-glucuronide (M6G), the need for two to four times higher concentrations of alfentanil to control pain and with 10–12 times higher concentrations to obtain the same respiratory depression than in wild-type patients [12, 13].

The occurrence of adverse events such as nausea and vomiting following M6G administration is lower among subjects carrying the 118G allele [12, 14]. Although 118G allele carriers are less sensitive to mechanical pain, this allele is associated with lower analgesia and higher morphine requirement to achieve pain relief [9, 15–18]. Interestingly, this polymorphism may predict clinical response to naltrexone in alcohol-dependent individuals, suggesting greater sensitivity to morphine antagonist in G carriers patients [19–25].

Drug transporters facilitate the passage of opioid drugs across biological membranes in liver, kidney, intestine, and at the blood brain barrier. Systems involved in both efflux and uptake of drugs can potentially influence absorption, distribution, and elimination of opioids. Genetic polymorphism in these transporters may therefore account for some of the interpatient variability in response to opioid drugs. Morphine is a well-known substrate of the drug efflux pump P-gp (encoded by *ABCB1*) that modulates its oral bioavailability, elimination, and brain-to-blood efflux [26–28]. Interindividual variability in P-gp expression and activity is important and may be partly explained by the c.C3435T SNP [29–34]. Indeed, this SNP has been previously associated with variations in morphine cerebrospinal fluid concentrations, suggesting its role in morphine efficacy and tolerance [35]. Campa et al. reported that pain relief variability was significantly associated with both *ABCB1* c.C3435T and *OPRM1* c.A118G polymorphisms in Italian patients [36]. Recent studies also suggested that the effect of the c.C3435T polymorphism was reinforced by the association with other polymorphisms within the same gene [17, 37].

The COMT metabolizes catecholamines and several studies suggested some links between dopaminergic and adrenergic systems and the pain signal transmission [38]. A common polymorphism in *COMT*, p.Val158Met (present in about 50% of Europeans [39, 40]) causes a valine (Val) to methionine (Met) substitution at codon 158 in the COMT enzyme, leading to a three- to fourfold reduced activity. Therefore, this SNP may explain part of the interindividual difference in the adaptation and response to pain and may be involved in morphine dosing requirements and side effects [38, 41, 42]. The homozygous carriers of the variant allele may require significantly lower doses of morphine to achieve pain relief as compared to wild type subjects [27, 42]. Reyes-Gibby et al. studied the influence of *COMT* p.Val158Met and *OPRM1* c.A118G on dose requirements to achieve cancer pain relief: Homozygous patients with *OPRM1* AA and *COMT* Met/Met genotypes required the lowest morphine dose (87 mg/24 h) compared to wild type patients (147 mg/24 h) [42].

In the present study, we aimed to study genes implied in opioid pharmacokinetics and pharmacodynamics in patients with body mass index (BMI) over 40 kg/m² candidate for bariatric surgery. We examined three SNPs in three genes

coding for mu opioid receptor (*OPRM1*), P-glycoprotein (*ABCB1*), and *COMT*.

Patients and Methods

Subjects and Anthropometric Data

This study enrolled 109 morbidly obese subjects, candidate for bariatric surgery with BMI ≥ 40 kg/m², and consecutively admitted to the Department of Nutrition at La Pitié Salpêtrière Hospital (Paris, France) between July 2007 and January 2009. Written informed consent for the genetic study was obtained from all patients and the local Research Ethics Board approved the study protocol.

Body weight was measured to the nearest 0.1 kg with subjects in indoor clothing and no shoes. Height was measured to the nearest 0.5 cm with a wall-mounted stadiometer, in the same conditions. BMI was calculated as weight (kg) divided by height squared (m²).

Ethnicity was a data available for most of the patients. In only three cases, ethnic descents were not reported in the patient medical file.

Genetic Analysis

DNA was extracted from EDTA whole blood samples using the Wizard Genomic DNA Purification Kit (Promega). The samples were genotyped for the following SNPs: *OPRM1* (c.A118G; rs1799971), *COMT* (c.G472A p.Val158Met; rs4680), and *ABCB1* (c.C3435T; rs1045642). Genotyping was performed using Real Time PCR Taqman assays (StepOne plus, Applied Biosystems, Foster City, USA) following the manufacturer's instructions. Water control, previously genotyped samples, and genomic DNA were included in each experiment to ensure the accuracy of genotyping.

Control Populations

We searched for recent studies focusing on the allelic frequency of *OPRM1* c.A118G, *ABCB1* c.C3435T, and *COMT* p.Val158Met in control Caucasian population [7, 40, 43]. We screened Caucasian populations and selected in the corresponding articles the data necessary to compare allelic frequencies between control Caucasian populations and our morbidly obese Caucasian population.

Statistical Analysis

Statistical analysis was implemented in Statview v4.0 (SAS Institute, Cary, NC, USA). Quantitative data were presented as mean \pm standard deviation. Comparisons between genotypes were performed by means of Fisher's PLSD

Table 1 Patients characteristics according to genotype (mean \pm SD)

| | Population <i>n</i> =109 | <i>OPRM1</i> c.A118G (rs1799971) | | | | <i>ABCB1</i> c.C3435T (rs1045642) | | | | <i>COMT</i> p.Val158Met (c.G472A, rs4680) | | | |
|--------------------------|-----------------------------|----------------------------------|--------------------|-------------------|----------|-----------------------------------|--------------------|--------------------|-------------------|---|-------------------------|-------------------------|----------|
| | | AA <i>n</i> =66 | AG <i>n</i> =38 | GG <i>n</i> =5 | <i>p</i> | CC <i>n</i> =34 | CT <i>n</i> =51 | TT <i>n</i> =24 | <i>p</i> | Val/Val <i>n</i> =29 | Val/Met <i>n</i> =51 | Met/Met <i>n</i> =29 | <i>p</i> |
| Genotypic frequency (%) | | 60.5 | 34.9 | 4.6 | | 31.2 | 46.8 | 22 | | 26.6 | 46.8 | 26.6 | |
| Caucasians (<i>n</i>) | 94 | 55 | 34 | 5 | | 23 | 48 | 23 | | 21 | 46 | 27 | |
| Age (years) | 37.1 (11.1) | 36.5 (11.0) | 37.8 (11.3) | 39.5 (12.6) | ns | 39.6 (12.2) | 37.6 (11.4) | 36.2 (9.1) | ns | 35.0 (11.8) | 37.9 (10.5) | 37.8 (11.6) | ns |
| Height (m) | 1.67 (0.1) | 1.68 (0.1) | 1.65 (0.1) | 1.70 (0.1) | ns | 1.69 (0.1) | 1.67 (0.1) | 1.66 (0.1) | ns | 1.68 (0.1) | 1.66 (0.1) | 1.68 (0.1) | ns |
| Weight (kg) | 138.6 (27.1) | 141.0 (27.5) | 132.4 (24.4) | 151 (35.1) | ns | 134.6 (25.9) | 141.0 (30.1) | 139 (21.9) | ns | 138.5 (21.6) | 137 (29.7) | 141.5 (27.9) | ns |
| BMI (kg/m ²) | 49.2 (7.7) | 49.5 (7.4) | 48.1 (7.4) | 52.1 (12.9) | ns | 46.7 (5.9)* | 50.3 (8.4)* | 50.1 (8.1) | * <i>p</i> =0.359 | 48.7 (6.1) | 49.1 (8.1) | 49.7 (8.7) | ns |

BMI body mass index (weight in kilograms divided by height squared in meters), ns no statistically significant difference

Table 2 Allelic frequencies in the whole population including Caucasian and non-Caucasian patients

| Gene | Polymorphisms | Allele | Allelic frequency (%) |
|--------------|----------------------|--------|-----------------------|
| <i>OPRM1</i> | c.A118G (rs1799971) | A | 0.78 |
| | | G | 0.22 |
| <i>ABCB1</i> | c.C3435T (rs1045642) | C | 0.55 |
| | | T | 0.45 |
| <i>COMT</i> | p.Val158Met (rs4680) | Val | 0.5 |
| | | Met | 0.5 |

test. Khi-2 test was performed to compare allelic frequencies. A *p* value of 0.05 or less was considered significant.

Results

Patients' characteristics are summarized in Table 1. Overall, 82 of the 110 subjects studied were women (74.5%). Mean age of the population was 37.1 ± 11.1 years and the mean BMI was 49.2 kg/m^2 (range: 40.1–76), with no significant differences neither between genders nor genotypes. Allelic frequencies in the whole population including Caucasian and non-Caucasian patients are presented in Table 2. Genotypes distribution according to ethnic groups and gender for the different polymorphisms are summarized in Table 3. Allelic frequencies were *OPRM1* 118G—0.22, *ABCB1* C3435—0.55, and *COMT* 158Met—0.5 in our whole population and 0.23, 0.5, and 0.47 in Caucasian population. These frequencies did not differ according to gender. Mean BMI did not differ according to the allelic variant. *OPRM1* 118G allele was more frequent in our patients population than in most previously described

European populations. Comparisons between allelic frequencies in our obese Caucasian population and Caucasian populations are reported in Table 4. No significant differences with the Hardy–Weinberg expected values were observed.

Discussion

Variability in morphine's pharmacodynamics and/or pharmacokinetics may have clinical consequences since morbid obesity is frequently associated with respiratory diseases [3, 6]. The molecular basis of this variability is not well defined. To the best of our knowledge, this is the first study describing three distinct SNPs involved in morphine variability in a population of morbidly obese patients.

The *OPRM1* 118G frequency in our Caucasian subgroup (0.234) is significantly higher than most of the previously reported frequencies (6/8 studies) [7, 25, 44–50]. *OPRM1* may have implications in the vulnerability to develop obesity, however this hypothesis remains debated. The reward system may modulate motivated and consummatory behavior and SNPs altering dopamine and serotonin availability have been involved in human obesity [51–53]. *OPRM1* was also involved in many drug abuses and in obesity [21, 54, 55]. Davis et al. examined *OPRM1* genotypes distribution in obese individuals in relation to different patterns of overeating [56]. The obese patients with binge eating had a greater frequency of the “gain of function” G allele of the *OPRM1* SNP (allele G=0.18; mean BMI=35.6 kg/m^2) as compared to obese controls (allele G=0.10; mean BMI=39.2 kg/m^2). The authors hypothesized that the tendency to binge eat would be magnified in G allele carriers, responsible for an increased responsiveness to opiates and alcohol, and their higher risk for addiction to these substances. The G allele is more

Table 3 Genotype distribution according to ethnic group and gender

| Genotype | Ethnic group | | | | Gender | |
|---|---------------------------|-------------------------|----------------------|-------------------|------------------------|----------------------|
| | Caucasian <i>n</i> =94 | African <i>n</i> =10 | Asian <i>n</i> =2 | NA <i>n</i> =3 | Female <i>n</i> =82 | Male <i>n</i> =28 |
| <i>OPRM1</i> c.A118G (rs1799971) | | | | | | |
| AA | 55 | 10 | 0 | 1 | 48 | 18 |
| AG | 34 | 0 | 2 | 2 | 29 | 9 |
| GG | 5 | 0 | 0 | 0 | 5 | 1 |
| <i>ABCB1</i> c.C3435T (rs1045642) | | | | | | |
| CC | 23 | 8 | 1 | 2 | 23 | 11 |
| CT | 48 | 1 | 1 | 1 | 40 | 12 |
| TT | 23 | 1 | 0 | 0 | 19 | 5 |
| <i>COMT</i> p.Val158Met (c.G472A, rs4680) | | | | | | |
| Val/Val | 21 | 6 | 2 | 0 | 23 | 6 |
| Val/Met | 46 | 3 | 0 | 2 | 37 | 14 |
| Met/Met | 27 | 1 | 0 | 1 | 22 | 8 |

NA data not available

Table 4 Comparisons of allelic frequency of the mutant allele between our Caucasian population and previously published control populations

| Population | Reference | n | Allelic frequency | χ^2 | p |
|---|-------------------------|-------|-------------------|-----------|-------|
| <i>OPRM1</i> c.A118G (rs1799971) | | | | | |
| Our study (obese Caucasian population) | | 94 | 0.234 | | |
| European American | Bergen et al. [44] | 80 | 0.125 | 28.91429 | ns |
| European American | Bond et al. [45] | 52 | 0.114 | 116.3319 | <0.01 |
| European American | Crowley et al. [25] | 100 | 0.153 | 9.676474 | ns |
| European American | Luo et al. [47] | 179 | 0.137 | 88.58994 | <0.01 |
| European American | Schinka et al. [48] | 297 | 0.136 | 282.3692 | <0.01 |
| German | Franke et al. [49] | 365 | 0.121 | 408.2335 | <0.01 |
| Swedish | Bart et al. [50] | 170 | 0.074 | 106.8096 | <0.01 |
| Finnish | Bergen et al. [44] | 184 | 0.111 | 102.7786 | <0.01 |
| <i>ABCB1</i> c.C3435T (rs1045642) | | | | | |
| Our study (obese Caucasian population) | | 94 | 0.500 | | |
| European American | Komoto et al. [60] | 99 | 0.430 | 4.073695 | ns |
| European Netherlands | Aardnouse et al. [61] | 89 | 0.490 | 0.641254 | ns |
| European UK | Roberts et al. [62] | 190 | 0.480 | 97.15958 | <0.01 |
| Turkish European | Bebek et al. [63] | 174 | 0.510 | 73.60386 | <0.01 |
| German | Fiedler et al. [64] | 1,005 | 0.470 | 1,651.648 | <0.01 |
| German | Cascorbi et al. [65] | 461 | 0.460 | 584.581 | <0.01 |
| German | Hoffmeyer et al. [29] | 188 | 0.520 | 94.15064 | <0.01 |
| <i>COMT</i> p.Val158Met (c.G472A, rs4680) | | | | | |
| Our study (obese Caucasian population) | | 94 | 0.532 | | |
| European American | Strous et al. [70] | 87 | 0.464 | 4.893594 | ns |
| European American | Egan et al. [71] | 55 | 0.454 | 63.17802 | <0.01 |
| European UK | Daniels et al. [46] | 78 | 0.53 | 6.567438 | ns |
| European UK | Karayiorgou et al. [72] | 129 | 0.488 | 20.04963 | ns |
| European UK | Norton et al. [73] | 334 | 0.542 | 344.9319 | <0.01 |
| Canadian European | Joober et al. [74] | 96 | 0.500 | 0.833333 | ns |
| French European | De Chaldee et al. [75] | 137 | 0.533 | 26.99331 | ns |
| Turkish European | Herken et al. [76] | 65 | 0.577 | 28.14116 | ns |
| German | Gallinat et al. [77] | 170 | 0.556 | 68.19721 | <0.01 |
| German | Rujescu et al. [78] | 323 | 0.500 | 324.935 | <0.01 |
| Finnish | Illi et al. [79] | 94 | 0.521 | 0.089748 | ns |

ns no statistically significant difference

frequent in our population than in previously reported populations of obese patients even in the population suffering from binge eating (allele G=0.10; mean BMI=39.2 kg/m²) [56]. Based on the hypothesis of Davis et al., the G allele may lead to a tendency to weight regain in patients after severe food restriction, as frequently reported in the history of morbid obese patients candidate to bariatric surgery [56]. Interestingly, supporting this hypothesis, Raymond et al. reported a decrease in pain perception in obese patients only in the case of eating disorders [57].

Xu et al. recently reported, in a Chinese Uyghur population with a mean BMI of 26.5±4.39 (18.5–43.1), that subjects carrying the G allele had a 25% reduced risk of getting obese than those carrying the common A allele, suggesting this allele might prevent obesity due to a possibly less active MOR [58]. However, the G allele is two- to fourfold more present in

patients of Asian descents than in Caucasians [7]. Moreover, many environmental factors, which may differ between Caucasians and Asians, are necessary to the development of obesity and could be implied in this apparent discrepancy.

Regarding the *ABCB1* c.C3435T, none of the alleles is significantly overrepresented in our population as compared to controls [29, 43, 59–65]. Some studies yet reported a relationship between this polymorphism and obesity or weight gain. The *ABCB1* (c.G2677T and c.C3435T) polymorphisms have been associated to risperidone-induced weight gain in 108 schizophrenic women [66]. Among 5,448 Japanese individuals, the c.G2677T polymorphism was also significantly associated with obesity ($p=0.0003$), but these results have not yet been confirmed in Caucasians; moreover, molecular basis of such an association may need further investigation [67]. Our study does not

highlight any association between *ABCB1* c.C3435T and obesity in a population without neuroleptics.

Dopamine is involved in motivation and reward circuits and is a potent neuromodulator of ventral striatum reactivity, widely implicated in reward processing [51, 53, 68]. It has been suggested that dopamine deficiency in obese individuals may perpetuate pathological eating as a means to compensate the decreased activation of these circuits [68]. Dopamine availability is largely controlled by two enzymes: the COMT and the monoamines oxidase (MAO-A and MAO-B) and by the dopamine transporter [69]. These genes have well-characterized functional variants but only *COMT* polymorphisms have been implicated, until now, in nociception and morphine pharmacodynamics [40, 42].

Neither the Val nor the Met alleles of the p.Val158Met SNP was overrepresented in our patients as compared to controls [46, 70–79].

Several phenotypes of obesity (metabolic consequences in men, loss of fat mass after exercise intervention in menopausal women) have been associated to this polymorphism, which is involved in estrogen and androgen metabolism [80, 81]. However, without distinction of the phenotypes of obesity, the *COMT* p.Val158Met polymorphism may play the same role in morphine pharmacodynamics as in the general population.

In conclusion, in this population of morbid obese patients candidate to bariatric surgery, the *OPRM1* 118G allele was more frequent as compared to previously published controls. This pilot study identifies a candidate gene to explore the interindividual variability in morphine response and requirements in morbidly obese population. Further clinical investigations focusing on this allele are needed to identify its consequences in the clinical setting. Further clinical studies are also needed to explore the role of this SNP in morphine pharmacodynamics in patients undergoing gastric bypass in order to individualize morphine prescription and optimize the concept of “personalized medicine” in this population at risk of postsurgery complications.

Conflict of interest None.

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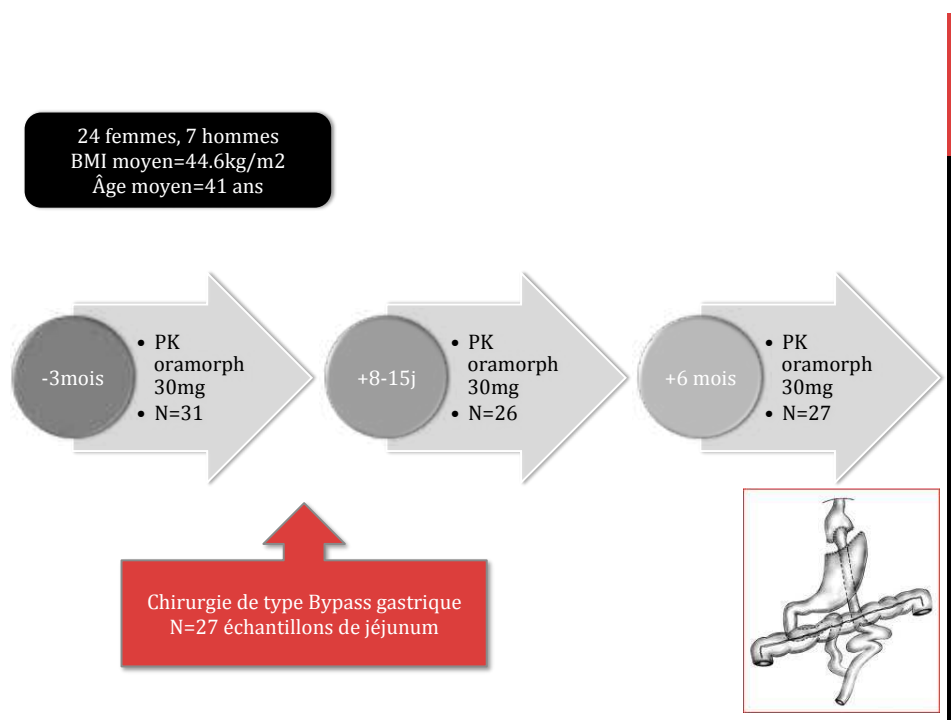
Le protocole OBEMO

L'étude OBEMO, Obésité et morphine, a pour objectif de déterminer la pharmacocinétique de la morphine avant et après chirurgie de l'obésité de type bypass gastrique.

J'ai inclus 31 patients volontaires pour une étude pharmacocinétique de 12 heures suivant l'administration d'une dose unique de morphine orale 30mg, avant la chirurgie, immédiatement après et six mois après chirurgie. Parallèlement au déroulement de la pharmacocinétique, j'ai surveillé les patients concernant leur tolérance à la morphine et évalué leur seuil de sensibilité et de douleur à un stimulus électrique douloureux progressivement croissant.

Par ailleurs, des prélèvements intestinaux (jéjunum), considérés comme déchets chirurgicaux, ont été collectés au cours de l'intervention chirurgicale de 27 sujets inclus.

Cet étude a préalablement été autorisée par le CPP Ile de France I, l'ANSM (anciennement AFSSAPS) et déclarée sur le site Clinical Trial.



Manuscrit 3. Nociception/ pharmacodynamique de la morphine chez les patients obèses

L'obésité s'accompagne de douleurs articulaires et musculaires. Des travaux antérieurs ont montré des modifications de la sensibilité à différents types de douleurs expérimentales chez des patients obèses avec des résultats discordants.

Nous avons comparé le seuil de sensibilité et le seuil douloureux à un stimulus électrique chez des patients obèses avant et après perte de poids induite par la chirurgie de l'obésité, et nous avons comparé ces résultats à ceux de sujets de poids normal.

Ces résultats ont fait l'objet d'une publication dans la revue « Clinical Journal of Pain » (Article 3.).

Sensory Impairment in Obese Patients? Sensitivity and Pain Detection Thresholds for Electrical Stimulation After Surgery-induced Weight Loss, and Comparison With a Nonobese Population

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Introduction: Obese patients have a high prevalence of painful musculoskeletal disorders that may decrease after massive weight loss. Pain thresholds may be different in obese participants.

Objectives: To assess the sensitivity and pain detection thresholds, through the application of an electrical sensitivity, before and after massive weight loss, and to compare the thresholds obtained with those in a control population.

Methods: The sensitivity and pain detection thresholds obtained in participants subjected to electrical stimulation were determined in 31 obese individuals (age: 40.3 ± 10.5 y) before (body mass index: 45.7 ± 6.8 kg/m²) and 6 months after a mean weight loss of 32 kg induced by gastric bypass. The results obtained were compared with those for 49 nonobese control participants (38.5 ± 11.2 y; body mass index: 22.6 ± 2.6 kg/m²). Body composition and metabolic biomarkers, such as leptin, adiponectin, insulin, and interleukin 6, were assessed and single-nucleotide polymorphisms of the mu opioid receptor [*OPRM1* (c.118A > G) and *COMT* (p.Val158 Met)] were genotyped in obese patients.

Results: Sensitivity and pain detection thresholds (3.9 ± 1.1 ; 11.6 ± 6.0) were significantly higher in obese than in nonobese participants (3.1 ± 1.1 ; 6.0 ± 3.0), respectively ($P < 0.0001$), and were not affected by drastic weight loss (mean change: 32 kg). Pain thresholds in obese participants were not correlated with any of the clinical and biological variables studied. The obese participants in the highest quartile for both sensitivity and pain detection thresholds were significantly older than those in the lowest quartile.

Conclusions: Further studies are required to explore sensory dysfunction in obese individuals and to investigate the implications of this dysfunction for pain management.

Key Words: obesity, bariatric surgery, pain, threshold, pain matcher, *OPRM1*, *COMT*

(*Clin J Pain* 2013;29:43–49)

According to the World Health Organization, obesity is a major public health problem that is increasing in prevalence, with 1.5 billion obese adults worldwide.¹ The joint and muscle pain associated with obesity complicates the management of obese patients by limiting compliance with current physical activity guidelines.^{1,2} Various musculoskeletal disorders have a higher prevalence among obese individuals than in the general population, and this prevalence decreases with weight loss.^{2,3}

Differences in susceptibility to pain in obese persons have been discussed in various studies. One hypothesis is that common factors, including susceptibility genes and hormonal factors, determine the susceptibility of a given individual to both pain and obesity.^{4–7} This hypothesis is supported by the sensitivity and pain threshold modifications found in certain genetically linked types of obesity.^{8,9} Conflicting results have been obtained in studies of nociception in obese and non-obese participants, but these studies differed in their methodology, particularly in terms of the experimental tests and control populations used.^{7,10–13}

Differences in pain sensitivity may have implications for pain management, as they may account in part for the variability in analgesic requirements between individuals. Improvements in our understanding of the pain threshold would make it possible to adjust and to predict the need for prescriptions of drugs with a narrow therapeutic margin, such as morphine, particularly in obese patients.¹⁴

The pain matcher is a device for the assessment of pain experimentally induced with an electrical signal. Pain level assessments with the pain matcher are strongly correlated with those obtained with the visual analog scale in patients experiencing pain.¹⁵ Sensitivity and pain detection thresholds can also be evaluated with this electrical signal, and the level of pain felt in clinical situations can be predicted from these numerical values.^{15–17} Lundblad et al¹⁶ reported an inverse

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correlation between pain threshold assessments with the pain matcher and the risk of persistent joint pain 18 months after total knee replacement for osteoarthritis. Nielsen et al¹⁷ observed similar results for pain after cesarean section, at rest, and on movement. This study was designed to determine whether experimental values for sensitivity and pain detection thresholds obtained with an electrical sensitivity differ between nonobese and severely obese participants and whether sensory dysfunction is modified by weight loss.

MATERIALS AND METHODS

Participants

Obese Participants

Participants eligible for bariatric surgery were included in a clinical trial studying the pharmacokinetics of oral morphine before and 6 months after bariatric surgery (the "Obesity and Morphine," OBEMO protocol). Participants did not have diabetes, alcohol dependence, neuropathy, and neurological disease, and were not taking analgesics at the time of inclusion. The decision to proceed with bariatric surgery (Roux-en-Y gastric bypass) was made on the basis of the Interdisciplinary European Guidelines for Surgery for Severe Obesity.¹⁸ The participants were followed up regularly in the Department of Nutrition, Pitié-Salpêtrière Hospital (Paris, France), where clinical and routine biological assays were performed, including determinations of leptin, adiponectin, insulin, interleukin 6, and free T4.¹⁹

One participant finally underwent sleeve gastrectomy (a surgical procedure in which the stomach is reduced to about 25% of its original size), whereas the others underwent a Roux-en-Y gastric bypass (a surgical procedure causing restriction and malabsorption, in which most of the stomach and some of the small intestine is bypassed). Each participant gave written informed consent for this study. This study was approved by the Ile de France I ethics committee, Paris. The OBEMO study is registered at ClinicalTrials.gov (NCT), with an EudraCT number 2009-010670-38.

Control Population

Control participants with a body mass index (BMI) below 28 (37 women and 12 men) were enrolled by the Department of Internal Medicine team at Lariboisière Hospital. These participants did not have diabetes, alcohol dependence, neuropathy, and neurological disease, and were not taking analgesics at the time of inclusion. Male control participants had to be over the age of 30 years, and female control participants over the age of 20 years, to generate a control population with an age distribution similar to that of the obese population. All volunteer control participants were in good health and gave informed consent for participation in this study.

Sensitivity and Pain Detection Thresholds

The same investigator, in each case, used an electrical device (pain matcher) to deliver 2 sequences of 3 stimuli to each of the participants.¹⁵ The first threshold measured corresponded to the detection of an electrical sensitivity: the participants were instructed to hold an electrode box between the thumb and the index finger of their nondominant hand. Electrical stimulation was initiated, with electrical pulses delivered at a random velocity and with increasing intensity. The participants were asked to release the box immediately from their fingers when they first sensed the electrical stim-

ulation. The second threshold corresponded to the threshold for pain detection. In this test, the same procedure was followed, but the participants were asked to release the box when they first felt pain. They were asked not to resist pain. A numerical value was automatically recorded when the participants released their fingers from the box. The pain threshold was determined twice in each sequence. The total sequence of the 3 measurements (1 electrical sensitivity and 2 pain detection thresholds) was performed twice, and the thresholds were calculated as averages. We defined the sensitivity detection threshold as the mean of the first value from each of the 2 sequences and the pain threshold as the mean of the remaining 4 values from the 2 sequences (mean of the 2 pain thresholds obtained by 2 different measurements in each of the 2 sequences).

Body Composition

Anthropometric data and information about body composition were obtained by the same investigator in each case. Weight was measured to the nearest 0.1 kg using the built-in weight scale included in a bioelectrical impedance analysis (BIA) device (Tanita BC-420MA, Tanita Ltd., France), and BMI was calculated as weight (kg) divided by the square of height (m) (kg/m^2).

Obese Sample

Body composition was estimated by whole-body dual-energy x-ray absorptiometry (DEXA) scanning (Hologic Discovery W, software v12.6, 2; Hologic, Bedford, MA), as previously described.²⁰ For each pixel, the equipment calculates weight, bone mineral content, and fat mass (FM) and integrates the measurements for the whole body and different body areas. The variable from DEXA used in the analysis was FM as a percentage of weight.

Control Subjects

FM was estimated by BIA. The Tanita BC-420MA leg-to-leg BIA device looks like a traditional bathroom scale and may be used for participants weighing up to 270 kg.²⁰ The electrodes on the metal foot plates provide a small alternating voltage of 90 μA (50 kHz). Body composition estimates are derived from total body water by proprietary equations not supplied by the manufacturer and on the basis of the segmental resistance index. The BIA variable used in the analysis was FM as a percentage of weight.

Genetic Analyses

DNA was extracted from blood cells using a semi-automatic Promega extractor, as recommended by the manufacturer (Promega, France). DNA concentration was determined using a Nanodrop spectrophotometer (Nanodrop, Wilmington).

Obese participants were genotyped for single-nucleotide polymorphisms in *OPRM1* (c.118A > G; rs1799971) and *COMT* (c.675G > A -p.Val158Met-; rs4680). This genotyping was based on Taqman real-time polymerase chain reaction assays (StepOne plus; Applied Biosystems, Foster City) carried out according to the kit manufacturer's instructions. A control (water), previously genotyped samples, and a genomic DNA were included in each experiment to verify the accuracy of genotyping.

Statistical Analyses

Statistical analysis was performed using Statview v4.0 (SAS Institute, Cary, NC). Quantitative data were

presented as mean (SD). Quantitative data were compared using Fisher PLSD test. The χ^2 test was performed to compare qualitative data. Associations of anthropometric and biological data with detection and pain thresholds were tested using the Spearman rank correlation test. Characteristics of the participants were compared between quartiles of detection and pain thresholds. A P -value ≤ 0.05 was considered significant.

RESULTS

Characteristics of the Study Population

Thirty-one obese individuals (24 women and 7 men) were enrolled in the OBEMO study and evaluated before surgery; 27 of these were also evaluated 6 months later (2 withdrew from the study, 1 did not undergo surgery, and 1 died). Before surgery, the mean age was 40.3 ± 10.5 years and the mean BMI was 45.7 ± 6.8 kg/m². Painful knee arthritis was reported by most obese participants, but without the need for long-term analgesic treatment ($n = 16$, 51.6%). Other pain reported by obese participants included low back pain ($n = 16$, 51.6%), hip pain ($n = 4$, 12.9%), and neck pain ($n = 3$, 9.7%). Only 6 obese participants reported an absence of arthralgia, and the characteristics of these participants were similar to those of the rest of the population (Table 1).

Six months later, the mean weight loss of this population was 32 kg and the mean BMI had decreased to 34 ± 5.6 kg/m². Percent body fat measured by DEXA was significantly reduced (39.6% vs. 46.3%).

The control sample included 49 healthy participants with a similar sex ratio as the surgical group (75% women). The mean age was 38.5 ± 11.2 years and was not significantly different from that of the group of obese sample. These participants had a mean BMI of 22.6 ± 2.6 kg/m² and a mean percentage body fat of 23.1%, both these values being significantly lower than those for the obese population either before or after weight loss.

Sensitivity and Pain Detection Threshold Measurement With an Electrical Sensitivity

The sensitivity (3.9 ± 1.1) and pain (11.6 ± 6.0) detection thresholds were significantly higher in obese participants than in nonobese participants (3.1 ± 1.1 and

6.0 ± 3.0 , respectively; $P < 0.0001$). These thresholds were not significantly modified by weight loss. The number of obese participants with a sensitivity threshold above 10 was significantly higher among obese participants before and after weight loss (64.5% and 85.2%, respectively) than control participants (12.2%) (Fig. 1, Table 1).

Quartile-based Analyses of the Sensitivity and Pain Detection Thresholds

Obese participants in the first and last quartiles did not differ in terms of sex ratio, BMI, FM, or allelic frequencies of the polymorphisms studied (Table 2). The obese participants in the last quartile were significantly older than those in the first quartile, whichever threshold was considered: 49.7 ± 7.3 versus 34.4 ± 6.9 years for the sensitivity detection threshold ($P = 0.0026$) and 44.4 ± 9.7 versus 35.6 ± 6.9 years for the pain detection threshold ($P = 0.05$). A similar difference between these 2 quartiles was observed only for the sensitivity detection threshold in the control population (44.2 ± 12.0 vs. 32.2 ± 8.3 y; $P = 0.01$).

Interindividual Variability in the Pain Detection Threshold Between Obese Participants

For obese participants, no significant correlation was observed between pain threshold and the following clinical and laboratory data: weight, BMI, arthralgia, FM, leptin, adiponectin, insulin, interleukin 6, and free T4 concentrations. The allelic frequencies of the alleles studied in the obese population were 0.15 for *OPRM1* 118G and 0.61 for *COMT* 158Met (c., rs4680). Despite a trend toward higher pain thresholds in participants with the Met/Met genotype, no significant difference in sensitivity or pain detection threshold was found between genotypes (Table 2). Carriers of the Met allele of the *COMT* gene and the 118G allele of *OPRM1* had pain detection thresholds similar to those carrying the Val and A118 alleles, respectively.

DISCUSSION

In this study, on the basis of the use of an electrical device, we observed a difference in sensitivity and pain detection thresholds between obese and nonobese participants. Previous studies on pain thresholds in obese patients were performed on a smaller scale, with different experimental tests (Table 3).^{7,10-13} Our results are consistent with those of

TABLE 1. Electrical Stimulation (Pain Matcher) Sensitivity and Pain Thresholds in Obese and Normal-weight Participants Before and 6 Months After Bariatric Surgery

| | Obese Participants | | Normal-weight Participants n = 49 |
|---------------------------------|--------------------------|-------------------------|--------------------------------------|
| | Before Surgery n = 31 | After Surgery n = 27 | |
| Age | | 40.3 (10.5) | 38.5 (11.2) |
| Height (cm) | | 165.5 (8.2) | 166.9 (9.0) |
| Weight (kg) | 126.1 (25.9) | 94.1 (21.8)** | 63.3 (11.2)* |
| BMI (kg/m ²) | 45.7 (6.8) | 34 (5.6)** | 22.6 (2.6)* |
| FM (%) | 46.5 (3.5) | 39.6 (4.6)** | 23.1 (7.0)* |
| Sensitivity detection threshold | 3.9 (1.1) | 4.0 (1.1) | 3.1 (1.1)* |
| Pain threshold | 11.6 (6.0) | 12.6 (5.9) | 6.0 (3.0)* |
| Pain threshold > 10 (n) | 20 | 23 | 6* |

Mean values are reported as means (SD).

*Significant difference between obese (before and after weight loss) and normal-weight participants ($P < 0.0001$).

**Significant difference between obese participants before and after weight loss ($P < 0.0001$).

BMI indicates body mass index; FM, fat mass.

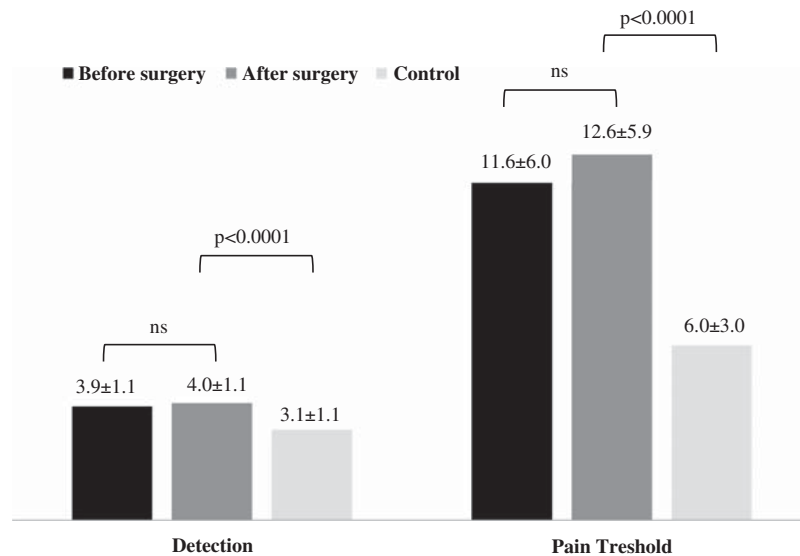


FIGURE 1. Sensitivity and pain thresholds assessed by the pain matcher before and 6 months after bariatric surgery in an obese population and in a normal-weight population. The sensitivity and pain threshold values were obtained with a pain matcher (numerical value). Results are expressed as means with SD. NS indicates not significant.

Zahorska-Markiewicz et al,¹³ who also measured pain thresholds with an electrical sensitivity and reported a higher pain threshold. Pradalier et al¹² reported an earlier saphenobicipital reflex in response to an electrical signal in a group of obese patients; however, the perception of pain, beyond the reflex, was not analyzed further. Using other models of experimental pain, McKendall and Haier¹¹ found that the pain threshold in response to mechanical pressure was lower in obese patients than in the control population. In a more comprehensive approach, Miscio et al⁷ evaluated vibration pain thresholds in several experimental tests, and compared motor and sensory nerve conduction in obese patients without diabetes ($n = 21$) with those in normal-weight participants. They reported that vibration sensitivity and pain thresholds were lower in the obese group, but that these patients had a lower compound muscle action potential amplitude of the tibial and peroneal nerves and a lower sensory action potential amplitude for all nerves.⁷

Our observations, consistent with those of Miscio et al,⁷ suggest that many physiological changes associated with obesity may affect pain pathways. Miscio et al⁷ found no direct correlation between BMI and vibration sensitivity or thresholds, despite significant differences between obese and normal-weight participants. The hypothesis that pain signals may be modulated by the thickness of subcutaneous tissue was not supported by this work or by our findings. No correlation was found between pain threshold and body composition, and fat loss was not associated with changes in sensitivity or pain

thresholds. However, Miscio et al⁷ reported that neurological latencies were correlated with BMI and that only thermal sensitivity was correlated with insulin resistance. Many studies in insulinopenic diabetic rats have shown that insulin deficit, rather than hyperglycemia, plays a role in painful diabetic neuropathy and that insulin may prevent a decrease in pain threshold in rats, through an endogenous opioid mechanism.^{21,22} In our study, pain threshold was not correlated with fast insulin or other biological factors, the values of which are usually different in obese and normal-weight participants (adiponectin, insulin, and interleukin 6).

After significant weight loss (mean loss: 32 kg), the sensitivity and pain detection thresholds measured with an electrical sensitivity remained higher in obese participants than in normal-weight participants and were similar to those obtained before weight loss. There are 2 possible explanations for this finding: first, this population remained overweight 6 months after surgery, despite massive weight loss [BMI:34 (5.6) kg/m²] and, second, the factors responsible for differences in sensitivity before surgery may persist after weight loss. Buskila et al¹⁰ also reported that nonarticular tenderness in obese women was not affected by weight reduction (Chatillon dolorimeter). The lack of change in pain thresholds after weight loss suggests persistent abnormalities in pain pathways, due to constitutional, biological, and hormonal factors. Our results do not go further than the previously published papers cited in the manuscript but several factors, which have not been analyzed in this study, may contribute to altered pain sensations.

TABLE 2. Pain Thresholds According to Genotype for the *OPRM1* (c.118A>G; rs1799971) and *COMT* (c.472G>A -p.Val158Met-; rs4680) Polymorphisms

| Genotype | <i>OPRM1</i> c.118A > G (rs1799971) | | | <i>COMT</i> c.472G > A -p.Val158Met- (rs4680) | | |
|----------------|--|------------|------------|--|------------|---------|
| | GG | AG | AA | Met/Met | Val/Met | Val/Val |
| n | 0 | 9 | 22 | 10 | 18 | 3 |
| Frequency | 0 | 0.3 | 0.7 | 0.27 | 0.62 | 0.1 |
| Pain threshold | — | 10.1 (1.9) | 12.2 (1.3) | 13 (1.9) | 11.6 (1.4) | 7 (3.4) |

TABLE 3. Pain Threshold in Obese Participants: A Review of the Literature

| References | Participants | | Methods | Results |
|---|--|--|--|---|
| | Obese | Control | | |
| Miscio et al ⁷ | 21 nondiabetic obese patients BMI = 41.06 ± 4.74 | 20 nonobese participants BMI = 22.71 ± 2.88 | TSA-II Neuro Sensory Analyzer and VSA-3000 | Lower cold and heat-induced pain thresholds in the obese group Lower sensory action potential for all nerves in the obese group Lower amplitude for the tibial and peroneal nerves in the obese group |
| Buskila et al ¹⁰ | 42 women (before bariatric surgery) BMI = 42.4 ± 4.5 | 42 women (after massive weight loss) BMI = 31.3 ± 5.4 | Chatillon dolorimeter | No difference in pain threshold before and after weight loss |
| McKendall and Haier ¹¹ | 26 patients (18 women and 8 men) BMI ≥ 130% of the ideal value/weight | 34 participants (14 women and 20 men) Less than 130% of ideal body weight | Pressure device | Lower pain threshold in the obese group |
| Zahorska-Markiewicz et al ¹³ | 20 women BMI = 38 | 20 women BMI = 20.6 8 women in underweight BMI = 18 | Electronic device with intensity generator | Increase in pain threshold in the obese group |
| Pradalier et al ¹² | 30 women BMI = 35.7 | 20 women BMI = 23 | Neurophysiologic method of study of saphenofemoral biceps reflex | Decrease in pain threshold in the obese group |

TSA-II Neuro Sensory Analyzer and VSA-3000: computerized quantitative device for assessing various sensations; Chatillon dolorimeter: a pressure device. BMI indicates body mass index.

Some rare genetic diseases are associated with a decrease in pain threshold and with eating and energy homeostasis disorders.^{8,9} The p.Val66Met polymorphism in the brain-derived neurotrophic factor (*BDNF*) gene is associated with obesity in various populations^{23,24} and with a modulation of electrical pain threshold in patients experiencing chronic pain.²⁵ Plasma *BDNF* concentration decreases with massive weight loss, but residual neuromodulator concentrations may nonetheless affect nociceptive pathways.^{26,27} In animal models, *BDNF* is involved in modulating pain thresholds and inflammatory hyperalgesia but does not seem to affect the development of neuropathic pain.²⁸

Several other hypothalamic and peripheral factors involved in energy homeostasis, appetite, and satiety may modulate pain pathways through interaction with the central opioid system. Oxytocin is a mammalian hormone secreted from the pituitary gland that acts primarily as a neuromodulator in the brain. The oxytocinergic system and its interaction with the opioid system has been found to decrease the pain threshold effectively in animals.²⁹ Plasma oxytocin levels were 4 times higher in the obese participants than in the control participants. They had decreased significantly 6 months after gastric band surgery (mean weight loss of about 30 kg), but remained higher than those in the control participants.³⁰ Interestingly, postnatal oxytocin injections cause sustained weight gain and an increase in nociceptive thresholds in male and female rats.³¹ The oxytocinergic system in obese patients may account for their higher pain threshold.

Melatonin is a hormone that is secreted by the pineal gland in the brain, regulates other hormones, and maintains

the circadian rhythms of the body. Various studies in animals have shown melatonin to have anti-inflammatory and antinociceptive properties.^{29,32} Furthermore, the prescription of melatonin during the preoperative period also decreases the need for opioid analgesics after surgery.³³ It has recently been shown that exposure to light after the onset of darkness may affect weight gain by suppressing melatonin production, thereby decreasing brown adipose tissue metabolism.³⁴ The potential of melatonin as an antiobesity drug is still being discussed. In addition, a parabolic relationship between pain threshold and urinary sulfate-melatonin concentration has been observed in women with eating disorders ($r = 0.6299$, $P < 0.05$).³⁵ The authors speculated that severe eating disorders may decrease both melatonin levels and pain sensitivity.³⁵

Other hormonal factors may also play a role. Ghrelin is an orexigenic hormone of gastric origin, the levels of which are inversely correlated with obesity. The concentrations of this hormone thus increase in conditions of fasting, weight loss, and bariatric surgery.^{36–38} Studies in animals have shown that ghrelin decreases various types of experimental pain (mechanical and inflammatory).^{39,40} Similarly, leptin, an adipocyte hormone that reduces appetite by acting on receptors in the hypothalamus of the brain, has been shown to be present at higher levels in obese patients and animals than in controls. These studies have demonstrated that leptin decreases pain thresholds when peripherally administered in mice.⁴¹ Thus, the ghrelin and leptin levels of obese patients may be associated with a lower pain threshold. Further studies are required to clarify the relationship between ghrelin, leptin, and nociception.

All of these hormones may influence pain pathways by interacting with the central opioid system, which may itself account for differences in pain threshold. Interestingly, this system may be a useful therapeutic target.⁴² Opioid agonists and antagonists have been shown to stimulate and to inhibit feeding, respectively.⁴² The central opioid system may be involved in hyperreactivity to the hedonic properties of food, and the number of studies suggesting a role for opioids in the regulation of food intake and comparing eating behavior with addiction is steadily increasing.^{43,44} Pain threshold may be a marker of β -endorphin concentration in obese patients or of a genetically different opioid system in normal-weight participants, and may be sensitive to the prescription of opioid antagonists in both cases.⁴⁵

No difference in pain threshold was found between genotypes or between carriers of the same variant. We were unable to draw conclusions about the allele frequencies of the polymorphisms studied from this sample of patients. The trend toward an increase in the pain matcher threshold in patients carrying the Met allele is consistent with published results concerning this polymorphism, which is generally associated with an increase in pain threshold.^{25,46} In addition, Vossen et al²⁵ reported a role for this polymorphism in the modulation of acute pain solely in patients experiencing chronic pain. The study confirms data obtained in other studies and fails to establish a connection between the reduced pain thresholds in obese patients and the frequency of the allele studied. The observed effect of age on sensitivity and pain detection in obese patients is consistent with previous studies but could not account for the differences between groups in our study, as there was no significant difference in age between groups.⁴⁷

Nevertheless, the electrical sensitivity delivered by the pain matcher seems to be of clinical interest for predicting the occurrence of postoperative pain and may be used to predict the analgesic needs of obese patients.^{15–17} The limitations of this work are the small sample size, particularly for the assessment of possible correlations with genetic factors, and the lack of precise assessment of chronic pain and its progression. Furthermore, the exclusion of patients already taking analgesics may have resulted in the selection of a subgroup of patients with hypoesthesia, and factors protecting against pain.

CONCLUSIONS

Although pain, especially of musculoskeletal origin, seems to be more prevalent in obese individuals, the thresholds for the detection of a cutaneous electrical sensitivity and for pain caused by that sensitivity were found to be significantly higher in obese than in control participants. This sensory dysfunction appears unchanged by weight loss and does not seem to be related to a number of hormonal and genetic factors. Further studies are warranted to explore sensory dysfunction in obese patients and to investigate the implications of this dysfunction for pain management in such patients.

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Manuscrit 4. Pharmacocinétique de la morphine avant chirurgie de l'obésité

La morphine est un médicament de marge thérapeutique étroite et tout élément clinique permettant d'expliquer la variabilité la pharmacocinétique de la morphine est utile.

L'obésité est associée à des modifications de la composition corporelle et de la physiologie susceptibles de modifier le devenir des médicaments.

Les premiers résultats des dosages de morphine ont fait l'objet d'une analyse pharmacocinétique de population. Nous avons cherché à savoir si des outils de description de la corpulence et la mesure de la composition corporelle pouvaient prédire les données pharmacocinétiques de la morphine.

Notre article, présenté ici, a été soumis à la revue « European Journal of Clinical Pharmacology» (Article 4.).

European Journal of Clinical Pharmacology

Population Pharmacokinetic Modeling of Morphine after single oral dose in Obese Volunteers, Influence of body composition.

--Manuscript Draft--

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| Full Title: | Population Pharmacokinetic Modeling of Morphine after single oral dose in Obese Volunteers, Influence of body composition. |
| Article Type: | Original Article |
| Section/Category: | Pharmacokinetics and Disposition |
| Abstract: | <p>Purpose: Morbid obesity may influence drug pharmacokinetics. Knowledge of morphine pharmacokinetics and its determinants in morbid obese patients may help clinicians in their prescription. We aimed to investigate morphine pharmacokinetics in order to optimize individual treatment in morbid obese subjects and to identify patient characteristics that are predictive of variability in the pharmacokinetic parameters.</p> <p>Methods: 268 concentrations were analysed following an oral administration of 30 mg of morphine sulfate solution in 31 obese subjects. A population pharmacokinetic model was developed to describe the concentration-time course of morphine and to estimate inter-patient variability. The influence of body composition, assessed by dual-energy X-ray absorptiometry scanning and bioelectrical impedance, and different size descriptors were tested using the likelihood ratio test.</p> <p>Results: Pharmacokinetics parameters expressed as median (range) were 0.8 h (0.5 - 1.5) for Tmax, 12 mg/L (6 - 21) for Cmax, 52 mg/L.h (19-76) for the AUC0-24. None of the covariates measured or predicted of body composition explained the inter-subject variability.</p> <p>Conclusions: A population pharmacokinetic model was developed and validated to describe morphine concentration-time data. In this cohort of morbid obese patients, morphine pharmacokinetics is not influenced by body composition. Further studies are necessary to individualize morphine dosage in obese patients.</p> |
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| Author Comments: | <p>Paris, March, 13th, 2013 Dear Editor,</p> <p>On behalf of my co-authors, I submit the enclosed original article entitled: Population Pharmacokinetic Modeling of Morphine after single oral dose in Obese Volunteers, Influence of body composition. for consideration by "European Journal of Clinical Pharmacology".</p> <p>This work is original. It has not been published in this or a substantially similar form (in print or electronically, including on a web site), nor accepted for publication elsewhere, nor is it under consideration by another publication.</p> <p>All authors have read and approved the manuscript, have met the criteria for authorship as established by the International Committee of Medical Journal Editors and have completed the Unified Competing Interest Form. None of them received support from any organization for the submitted work, or have financial relationships with any organization that might have an interest in the submitted work in the previous 3 years, or have other relationships or activities that could appear to have influenced the submitted work.</p> <p>We suggest you two reviewers: Pr Jules Desmeules: Jules.Desmeules@hcuge.ch Pr Nicolas Simon: nicolas.simon@univmed.fr</p> <p>We sincerely hope that you will find this work of interest, and will be happy to provide you with any further information you may require. We are looking forward to your response. Sincerely yours, Célia Lloret Linares, MD and Deborah Hirt PhD</p> <p>Corresponding author : Dr Célia Lloret-Linares, MD University of Paris Cité-Descartes, (INSERM U705, CNRS UMR 8206) Hôpital Lariboisière- Service de Médecine Interne A 2 rue Ambroise Paré 75010 Paris - France Tel: 33-1-49 95 81 27 Fax: 33-1-49 95 84 46 Email: celialloret@yahoo.fr</p> |

Population Pharmacokinetic Modeling of Morphine after single oral dose in Obese Volunteers, Influence of body composition.

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Conflict of interest: none

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ABSTRACT

Purpose: Morbid obesity may influence drug pharmacokinetics. Knowledge of morphine pharmacokinetics and its determinants in morbid obese patients may help clinicians in their prescription. We aimed to investigate morphine pharmacokinetics in order to optimize individual treatment in morbid obese subjects and to identify patient characteristics that are predictive of variability in the pharmacokinetic parameters.

Methods: 268 concentrations were analysed following an oral administration of 30 mg of morphine sulfate solution in 31 obese subjects. A population pharmacokinetic model was developed to describe the concentration-time course of morphine and to estimate inter-patient variability. The influence of body composition, assessed by dual-energy X-ray absorptiometry scanning and bioelectrical impedance, and different size descriptors were tested using the likelihood ratio test.

Results: Pharmacokinetics parameters expressed as median (range) were 0.8 h (0.5 – 1.5) for T_{max}, 12 mg/L (6 – 21) for C_{max}, 52 mg/L.h (19-76) for the AUC₀₋₂₄. None of the covariates measured or predicted of body composition explained the inter-subject variability.

Conclusions: A population pharmacokinetic model was developed and validated to describe morphine concentration-time data. In this cohort of morbid obese patients, morphine pharmacokinetics is not influenced by body composition. Further studies are necessary to individualize morphine dosage in obese patients.

INTRODUCTION

The prevalence of obesity has dramatically increased in recent years and obesity is frequently associated with pain of various origins (e.g. arthritis, fibromyalgia, cancer), which increases the need for analgesic drugs [1]. Interindividual variability in opioid pharmacology leading to variability in dose requirements for pain relief has been observed in both normal-weight and obese patients [2].

Morphine pharmacokinetics (PK), pharmacodynamics and pharmacogenetics, as well as pain threshold, contribute to morphine variability in terms of efficacy and safety, and data are lacking regarding the case of morbid obesity [3, 4]. However, the knowledge of morphine variability is of paramount importance in this particular population where pain relief is challenging: pain is more severe with morbid obesity and may require morphine prescription [5]. However this raises several therapeutic and clinical concerns, since adverse events following analgesia (nausea, respiratory depression) and respiratory diseases are more prevalent in obese subjects than in normal weight patients [6-9].

The physiological differences between normal-weight and morbid obese patients may modify both the volume of distribution and the clearance of many drugs, with clinical consequences [10, 11]. Specific data in morbid obese subjects do not exist for the majority of drugs and the prediction of PK differences is difficult. An optimal body size descriptor for dose calculation should be identified on prospective specific studies, as it has been performed for some drugs such as anticancer drugs and antibiotics [10, 12, 13].

Better knowledge and prediction of morphine variability may improve its use in morbid obese patients, the choice of loading and maintenance doses, to avoid suboptimal therapeutic management or respiratory depression side effects. We therefore aimed to develop a morphine PK model in morbid obese patients and to identify body size descriptor or body composition characteristics that are predictive of variability in PK parameters.

PATIENTS AND METHODS

Obese Volunteers

Thirty-one obese volunteers, candidates for a gastric-bypass surgery, have been enrolled in the OBEMO study (Obesity and Morphine Study) in the Nutrition Department of La Pitié Salpêtrière Hospital (Paris, France). Subjects with diabetes, renal or hepatic dysfunction, untreated Obstructive Sleep Apnea Syndrome or usually treated with sedative or analgesic drug were not eligible for this study. None of the subjects had a history of allergic response to morphine or other opioid. All subjects gave their written informed consent. The protocol was approved by the regional ethics committee of Paris, France (CPP Ile de France I) and registered at ClinicalTrials.gov, with an EudraCT number 2009-010670-38.

Pharmacokinetic Study

The obese subjects were given a single oral dose of 30 mg of morphine sulphate oral solution (Oramorph 5mL, Roxane Laboratories, Inc, Columbus, Ohio), in the morning after an over night fast. A standard meal schedule was given approximately 4 hours after dosing. No food or beverage was allowed before the meal.

Blood samples were drawn in heparinized tubes. Plasma was separated immediately by centrifugation, frozen, and stored in a labelled container at -20°C pending analysis. Ten blood samples per patient were collected for morphine assay, at 0, 0.5, 1, 2, 4, 6, 8 and 10 or 12 hours for each patient after morphine administration. Additional blood samples were drawn at 0.25, and 0.75 in 8 subjects.

Body composition

Height (HT) was measured to the nearest 5 mm, without shoes, using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using the in-built Bioelectrical Impedance Analysis (BIA) as a weight scale and body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m²).

Total body water (TBWater) was assessed using the Tanita BC-420MA leg-to-leg BIA (<http://www.tanita.eu/products/catagory/detail/professional-body-composition-analyzers/74-bc-420-ma-high-capacity-body-composition-analyser-with-integral-printer.html>, last access 27th of January 2013). Fat mass (FM) and lean body mass (LBM), in percentage and in kilogramm, were estimated by whole-body dual-energy X-ray absorptiometry (DEXA) scanning (Hologic Discovery W, software v12.6, 2; Hologic Bedford MA), as previously described [14].

Body size descriptor

Body size descriptors were calculated using total body weight (TBW in kg), height (HT in cm) and the following formulas:

- Body mass index (BMI) in kg/m² = BW / (HT/100)² [15]

- Body surface area (BSA) in m² = (BW)^{0.425} x (HT)^{0.725} x 0.007184 [16]
- Lean bodyweight 1 (LBW1) [17]
 - LBW1 in kg = 1.10 x (TBW) - 0.0128 x (BMI in kg/m²) x (TBW) for males
 - LBW1 in kg = 1.07 x (TBW) - 0.0148 x (BMI in kg/m²) x (TBW) for females
- Lean bodyweight 2 (LBW2) [18]
 - LBW 2 in kg = 9270 x TBW / (A + B x BMI)
 where A and B respectively = 6680 and 216 for males, 8780 and 244 for females.
- Ideal Body Weight (IBW) [19]
 - IBW in kg = 45.4 + 0.89x(HT -152.4) for females
 - IBW in kg = 49.9 + 0.89x(HT -152.4) for males
- Adjusted body weight (ABW) in kg = IBW + CF x (TBW - IBW) with CF = correction factor of 0.4 [20]

Analytical method

The method utilized reversed phase ion-pairing high performance liquid chromatography (HPLC) coupled with coulometric electrochemical detection [21]. All reagents were HPLC grade. Briefly, Liquid-Liquid extraction of morphine was performed as follows: the buffer was made by adding 500 µL of plasma sample and 500 µL of borate buffer adjusted at pH 8.9 and spiked with 40 ng of naloxone (100 µL internal standard). The mixture was extracted with 5 mL of chloroform:isopropanol (95:5, v/v) and vortexed for 20s. The organic phase was collected, then dried with anhydrous sodium sulfate and evaporated under a stream of nitrogen at 35°C. Residue was dissolved in 200 µL of the mobile phase. 50 µL of the solution was injected into the liquid chromatograph Lichrocart using Lichrospher® 60 RP-select B C18 chromatographic column (125 x 4 mm ID, 5 µm). The mobile phase contained tetrahydrofurane-acetonitrile-0.005 M, sodium heptanesulfonate and 0.05 M phosphate buffer (pH 4.8) (1/10/89, vol/vol). The HPLC column was run at a flow rate of 1 mL/min and separations were performed at room temperature. The retention times for morphine and internal standard were approximately 4.5 and 9.4 min, respectively. A Coulochem® II 5200A Electrochemical Detector with High Sensitivity Analytical Cell Model 5011 (ESA, Bedford, USA) was used. Oxidative potentials were applied at 100 mV and 600mV for E1 and E2 cell, respectively.

A 10-point calibration curve was built over the range of 1 ng/mL to 100 ng/mL. Based on quality control samples, the within-day and between-days coefficients of variation (CVs) were less than 10%. The lower limit of quantification (LOQ) was 1 ng/mL. Morphine dosage was expressed in morphine base from sulfate salt.

Modelling strategy and population pharmacokinetic model

The few concentrations below the limit of quantification were censored [22]. Data were analysed using the nonlinear mixed effect modelling software program Monolix software version 41s (MONOLIX : MOdèles NOn LInéaires à effets miXtes; <http://software.monolix.org/index.php>) [23, 24].

PK parameters were estimated by computing the maximum likelihood estimator of the parameters as previously described [25]. A one-compartment model is able to describe PK parameters when the concentrations distribution is homogenous. In this study, a 2-compartment and a 3-compartment models with zero-order or first-order absorption and first-order elimination were tested to describe morphine data. An exponential model was used to describe the inter-individual variability. Morphine concentration profiles were simulated and compared with the observed data thanks to prediction-corrected visual predictive check in order to validate the model (Figure 1.) [26]. The visual predictive plots demonstrated that the model adequately described the PK (data not shown).

Individual primary PK parameters were estimated from the final model: morphine maximal concentrations (C_{max}), time to maximal concentrations (T_{max}), absorption rate constant (k_a), apparent total clearance from plasma after oral administration (CL/F), apparent volume of the peripheral compartment (V_2/F), and apparent central volume of distribution (V_3/F), where F represents the oral bioavailability. The secondary PK parameters: area under the curve from 0 to 8, 12 and 24 hours (AUC_{0-8h} , AUC_{0-12h} , AUC_{0-24h}) and the intercompartmental clearance (Q/F), were estimated from primary PK parameters.

Effect of covariates

The effects of the continuous covariates (CO) as age, weight, TBW, HT, BMI, BSA, LBW1, LBW2, IBW, ABW, TBwater, LBM and FM were tested on Bayesian Empiric estimates of K_a , CL/F , V_2/F and Q/F from the basic model.

RESULTS

Demographic data

Data from 31 obese subjects (24 women and 7 men) were available for morphine pharmacokinetic evaluation. Table 1 summarizes the subjects' characteristics. Eleven subjects were taking proton pump inhibitor, 10 antihypertensive drug, 11 vitamin supplementation, and 6 oral contraceptive method.

Population pharmacokinetics

A total of 268 concentrations time points in obese subjects were available for the PK analysis. Thirty concentrations (5%) were below the limit of quantification (LOQ) and censored.

A 2-compartment model with first-order absorption and elimination best described morphine data. The available data were not sufficient to estimate the inter-subject variability for V_3/F and fixing the variance of this random effect to zero had no influence on the objective function values (OFV) that is linked to the likelihood ratio test (LRT). A covariance was significant between CL/F and V_2/F . Residual variabilities were best described by proportional error model.

The model performance could be validated thanks to the prediction-corrected visual predictive check. This evaluation method provided good proof for the model adequacy.

PK primary parameters expressed as median (range) were $T_{max}=0.8$ h (0.5 – 1.5), $C_{max}=12$ mg/L (6 – 21), $k_a=2.08$ h⁻¹ (0.83 - 5.07), $CL/F=268$ L/h (146 - 1048), $V_2/F=1135$ L (777 - 2134). PK secondary parameters were $AUC_{0-24}=52$ mg/L.h (19-76), $AUC_{0-8h}=37.1$ mg/L.h (16.9-54.4), $AUC_{0-12h}=40.9$ mg/L.h (17.7-61.3). Table 2 summarizes the final population PK estimates for a median obese patient and their inter-subject variability estimates (ω). Table 3. shows morphine mean (standard deviation) C_{max} , T_{max} and AUC in our population and those of control populations previously reported in the literature.

Correlations between V_2/F and body compositions, TBWater (L), LBM and FM (in kilogram) and were not significant ($r=0.3$ to 0.4). None of the covariates significantly decreased the $-2 \times \log$ -likelihood, decreased the inter-subject variability and improved the correlation between observed and predicted concentrations from the model.

DISCUSSION

In the current study we developed and validated a population PK model in order to describe oral morphine pharmacokinetics (PK) in morphine obese patients. We evaluated the influence of body composition (TBWater, LBM and FM) and body size descriptor on PK parameters, in order to highlight clinical parameters that may help to personalize morphine prescription in morbid obese patients.

Morphine PK was satisfactorily described by a two-compartment model with first-order absorption and elimination. A two-compartment model has already been used to describe morphine after an oral administration whereas morphine data after an IV administration was better described by a three-compartment model [27, 28]. The validity of our model is supported by the good correlation between the population predicted and the observed morphine concentrations and by the satisfactorily prediction-corrected visual predictive check method. With our model and one or two concentrations of morphine in morbid obese subjects following its oral administration, morphine PK may be predicted using the Bayesian estimation method and help to adjust doses if necessary.

Few studies have reported oral morphine PK in healthy volunteers, using the same analytical method [29-32]. These studies are reported in the table 3.

In comparison with the other studies, the C_{max} was always lower in our population, despite similar or reduced T_{max}. A lower C_{max} may result from a decreased morphine oral bioavailability or absorption, and/or an increased volume of distribution. An increased intestine and liver first-pass metabolism may decrease morphine oral bioavailability. P-glycoprotein (ABCB1, MDR1, P-gp) and UDP-glucuronosyltransferase isoform 2B7 (UGT2B7) are morphine transporter and metabolizing enzyme respectively. These effectors are involved in the low and variable oral bioavailability of immediate release form of morphine (23.8 to 38.3 %) [3, 32]. Physiologic changes in obesity, as low grade but long-term inflammation, hyperinsulinemia, dietary factors or changes in intestinal microbiota, may influence their activity [33, 34]. For example, a high duodenal *MDR1* mRNA and P-gp levels were observed in children with Crohn's disease, and an increased *MRD1* mRNA expression was showed in the placenta of women with gestational diabetes mellitus [35, 36]. In addition, little is known about the intestine glucuronidation in obese subjects, but inflammation and obesity are associated with increased UGT mRNA levels in human and animal liver respectively [11, 37, 38]. Since gastric emptying rate appears to influence the oral absorption, a delayed gastric emptying in morbid obese patients may be involved in the decreased C_{max} [39].

Morphine volume distribution was not significantly correlated with FM, LBM and FM ($r=0.3-0.4$). In addition these clinical data were not clinically relevant, as they did not decrease the inter-subject variability in morphine PK parameters. It is possible that our study failed to demonstrate an effect of body composition on morphine volume of distribution that is real. Indeed, despite the number of patients, only morbidly obese patients were included in our study. Additional PK data of subjects with various BMI may highlight this effect. Actually despite a relatively low fat distribution of morphine,

an excess of bodyweight is frequently associated with an increased volume of distribution of many drugs [10].

A trend toward a lower morphine exposure in obese subjects may result from a lower bioavailability, as previously discussed, and / or an increased morphine clearance. The consequence of obesity on liver blood flow is not clearly specified. Fat disposition in the liver of obese patients may reduce hepatic blood flow whereas high liver mass and cardiac output may increase it. However, it has yet been reported that clearances of drugs displaying a high-extraction-ratio drug are increased in obese patients. In addition, an increased glucuronidation of different UGT substrates is described in obese patients in comparison with normal-weight patients [11]. The physiological changes associated with obesity may also increase morphine renal clearance although this route of elimination represents 10.9% of total systemic clearance [10, 40]. Finally, the differences in exposure appear small between obese and non-obese subjects, suggesting that the clearances are slightly modified and that morphine does not seem to accumulate in obese patients.

None of the body size descriptor predicted morphine exposition. Body size descriptor, for which the formulas were developed in normal-weight patients, are inappropriate to account for the differences in body composition and physiology in morbid obese patients, and then unable to highlight an influence of body composition on morphine metabolism [13, 18]. However the assessment of body composition analysis by DEXA and BIA did not provide a better way to individualize morphine prescription. Our results suggest that morphine metabolism is influenced by many factors different than body composition in morbid obese patients [3]. Moreover, this study illustrates how it is difficult to predict the changes of drug metabolism in these patients.

Hence, morphine dosage should not be adjusted according to body weight, body size descriptor or body composition in morbid obese patients.

Regarding the variability in morphine PK parameters, its prescription remains challenging in this population. In addition, both morphine efficacy and tolerance are not entirely explained by PK and are influenced by many physiological and genetic factors, some of which could be directly linked to obesity [4]. Hence, further studies are needed to determine the influence of covariates that can explain variability in exposure, efficacy and tolerance of morphine as single dose and at steady state.

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Figures legends

Figure 1

Prediction corrected visual predictive check for morphine concentrations.

Footnotes: The grey lines show the 10th, 50th and 90th of observed data; the areas represent the 90% confidence interval around the simulated percentiles.

**Table 1. Characteristics of the morbid obese subjects enrolled in the pharmacokinetic study
OBEMO (n=31)**

| Covariates | Median (Min-Max) |
|----------------------------------|---------------------|
| Age (years) | 41 (18 – 62) |
| Body size descriptor | |
| Height (cm) | 165 (151 - 188) |
| Bodyweight (kg) | 116.9 (94.1 – 180) |
| Body mass index (kg/m) | 44.6 (35.4 – 62.2) |
| Body surface area (m) | 2.21 (1.95 - 2.91) |
| Lean bodyweight 1 (kg) | 50.7 (25.9 - 80.7) |
| Lean bodyweight 2 (kg) | 56.7 (48.3 – 94.4) |
| Ideal bodyweight (kg) | 56.6 (44.2 – 81.6) |
| Adjusted bodyweight (kg) | 81.3 (67.5 – 121.0) |
| Body composition analysis | |
| Total body water (L) | 40.3 (34.9-75) |
| Body fat mass (kg) | 56.2 (37.4-91.3) |
| Body fat mass (%) | 46.7 (38.3-52.2) |
| Lean body mass (kg) | 64.7 (48.6-101.6) |
| Lean body mass (%) | 51.3 (45.9-57.9) |

Footnotes: Min=minimum value and Max=maximum value

Table 2. Population pharmacokinetic parameters of morphine for a morbid obese patient after receiving 30 mg of sulfate morphine (22.6 mg of morphine) oral solution.

| Structural model | | Statistical model | |
|--------------------|------------------|---------------------|------------------|
| Parameter | Estimate (RSE %) | Parameter | Estimate (RSE %) |
| k_a (h^{-1}) | 2.35 (20) | ω_{ka} (%) | 67 (17) |
| CL/F (L/h) | 288 (14) | $\omega_{CL/F}$ (%) | 57 (12) |
| V_2/F (L) | 1120 (10) | $\omega_{V2/F}$ (%) | 36 (24) |
| Q/F (L/h) | 346 (13) | $\omega_{Q/F}$ (%) | 30 (30) |
| V_3/F (L) | 6401 (27) | | |

Footnotes: RSE%, relative standard error; k_a , absorption rate constant; CL/F, apparent elimination clearance from the central compartment; V_2/F , apparent central volume of distribution; Q/F, intercompartmental clearance; V_3/F , apparent peripheral volume of distribution; ω , between subject variability estimates; σ , residual variability estimates (proportional error model).

Table 3. Pharmacokinetic parameters comparisons between obese patient and normal bodyweight patient after receiving a unique sulfate morphine oral administration, corrected to a 30 mg dose of sulfate morphine, corresponding to a 22.6 mg dose of morphine.

| | | C _{max} (ng/mL) | T _{max} (h) | AUC (ng/mL.h) |
|-----------------------|----------------|-----------------------------|-------------------------|---|
| <u>Our study</u> | | | | |
| Obese patients | oral solution, | | | AUC ₀₋₂₄ = 52.5 (14.7) |
| Mean weight (kg) | n = 31 | 12 (3.8) | 0.8 (0.3) | AUC ₀₋₁₂ = 41.1 (10.9) |
| =116.9 (94.1 – 180) | | | | AUC ₀₋₈ = 37.5 (9.5) |
| <u>Drake et al.</u> | | | | |
| Mean weight (kg) | oral solution, | Fasting=13.9 (5.9) | Fasting = 1.2 (0.3) | AUC ₀₋₂₄ (Fasting) = 71.9 (22.2) |
| =ND | n=24 | Fed=15.2 (5.6) | Fed = 1.4 (0.7) | AUC ₀₋₂₄ (Fed)= 89.7 (35.5) |
| <u>Hasseltrom</u> | | | | |
| Cancer Patients | oral solution, | | | |
| Mean weight (kg) | n=11 | 22 (11.7) | 1.1 (1.1) | AUC ₀₋₁₂ = 72.5 (38.7) ** |
| =60 (47-69) | | | | |
| <u>Osborne et al.</u> | | | | |
| Healthy Volunteers | oral tablet, | | | |
| Mean weight (kg) | n=8 | 19 (7) | 0.8 (0.3) | AUC ₀₋₁₂ = 40.0 (12)*** |
| =72 (63-83) | | | | |
| <u>Kharasch</u> | | | | |
| Healthy Volunteers | oral tablet, | | | |
| Mean weight (kg) | n=12 | 17 (7.4) | 1.1 (0.8) | AUC ₀₋₈ = 40.8 (14.1) |
| =ND | | | | |

Footnotes:

Data are presented in mean \pm SD.

ND: no data; Maximal Concentrations: C_{max}, Time to Maximal Concentrations: T_{max}; Area Under the Curve from 0 to 8, 12 and 24 hours:

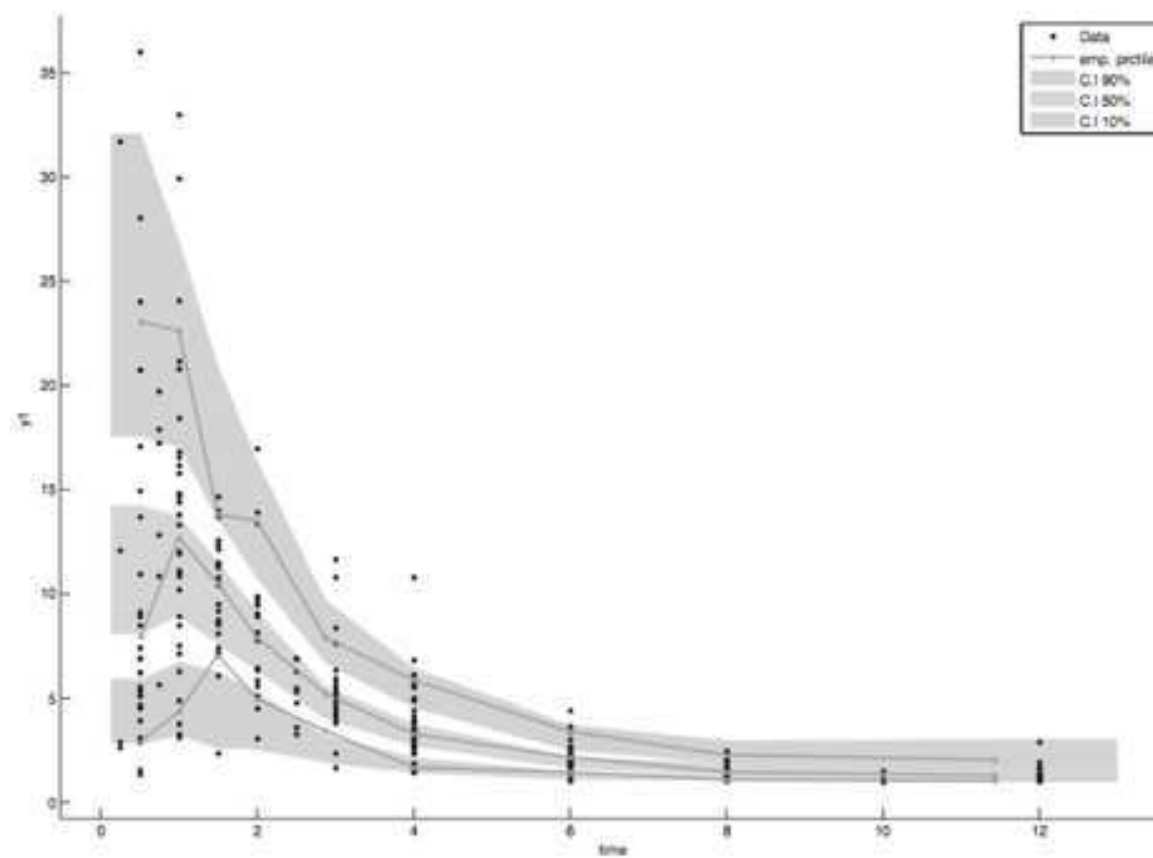
AUC_{0-8h}, AUC_{0-12h} AUC_{0-24h}.

** Initial data in nmol/L were obtained with a 10mg sulphate morphine dose and corrected for a 30mg dose assuming a linear PK between 10 and 30mg.

*** Initial data in ng/mL were obtained with a 20mg sulphate morphine dose and corrected for a 30mg dose assuming a linear PK between 10 and 20mg.

Figure

[Click here to download high resolution image](#)



Manuscrit 5. Expression intestinale d'enzymes du métabolisme et de transporteurs des médicaments chez les sujets obèses

Chez les patients obèses, l'expression intestinale des enzymes du métabolisme et des transporteurs des médicaments pourrait être différente compte tenu de modifications physiologiques associées à l'obésité telles que des modifications du microbiote intestinal, l'inflammation de bas grade, la production d'adipokines.

Nous avons étudié les niveaux d'expression des gènes suivants au sein des échantillons de jéjunum prélevés durant la chirurgie :

- ABCB1
- ABCC2
- ABCC3
- CYP3A4
- UGT2B7

Nous avons étudié les corrélations entre les niveaux d'expression et les données biologiques d'inflammation et les dosages d'adipokines dont nous disposons en pré-opératoire de chirurgie.

Nous avons comparé les données d'expression et le contenu protéique entérocytaire de ces mêmes effecteurs pharmacocinétiques pour 15 prélèvements .

Le manuscrit en rapport avec ce travail est en cours de rédaction. Nous attendons des données protéomiques de 12 prélèvements.

Les résultats préliminaires sont présentés (Article 5).

Drug-metabolizing enzymes in the small intestine of obese subjects

Célia Lloret-Linares, ... , Jean-François Bergmann, Xavier Declèves

Introduction

The prevalence of obesity, defined by a body-mass index (BMI) (the weight in kilograms divided by the square of the height in meters) of 30.0 or more, has increased substantially worldwide over the last decades (mc gee). In 2007-2008, the prevalence of obesity was 32.2% among adult men and 35.5% among adult women in the United States ¹. Obesity increases risk for many diseases such as diabetes, atherosclerosis, fatty liver disease and certain cancers, which increases the need for drugs, including oral drugs ²⁻⁴. Physiologic changes associated with obesity, such as chronic inflammation or changes in the composition and metabolic function of the gut microbiota, may affect epithelial integrity and hence lead to impaired oral absorption of drugs ^{5,6}.

It has yet been reported that obesity is associated with differences in drug-metabolizing enzymes (DME) expression and activity ^{7,8}. The small intestine is recognized as an important site for first-pass effect and constitutes the first defence against xenobiotic absorption. In particular, the jejunum is considered as the most important site for oral xenobiotic absorption. Cytochrome P450 (CYP) and UDP-glucuronosyl transferases are abundantly expressed in the human small intestine and affect the oral bioavailability of endogenous and exogenous compounds ⁹⁻¹³.

Among the metabolizing enzymes present in the small intestinal mucosa, the CYPs are of particular importance, being responsible for the majority of phase I drug metabolism reactions. Among the CYP3A subfamily, CYP3A4 is the most important, both quantitatively and qualitatively and is encoded by the *CYP3A4* gene ¹⁴. Although the enteric content of CYP3A4 is much lower than that in the liver, approximately 1% of liver content, it has been established that the intestine contributes equally to the metabolic first-pass effect for CYP3A4 substrates especially due to its high expression in the proximal small intestinal ^{14,15}. In addition, it has been shown that CYP3A4 cooperates with the drug efflux ATP-binding cassette (ABC) transporter ABCB1/P-gp to efficiently decrease the oral absorption of drugs that are substrates of both proteins ^{9,16}. Approximately 35% of phase II drug metabolism reactions are UGT-catalyzed glucuronidation ¹⁷. The human UGT proteins are a superfamily of enzymes that conjugate a variety of endogenous and exogenous compounds with glucuronate ¹⁸. The UGT2B7 is one of the most UGTs proteins expressed in the intestine, especially in the duodenum and jejunum ^{18,19}. However little is known about its expression relative to others DME and its interindividual variability ¹⁹.

DME are not the only key elements altering the oral absorption of drugs. Some drug efflux pumps of the ABC superfamily have yet to be considered as major proteins able to limit the

oral bioavailability of drugs. ABCB1/P-gp is encoded by the *MDR1* gene (*ABCB1*) and was the first ABC transporter identified in human cancer cells and responsible for the multidrug resistance (MDR) phenotype²⁰. It is also expressed in several healthy tissues in human including at the apical membrane of enterocytes of the small and large intestine and in hepatocytes at the bile canalicular membrane. P-gp has been evidenced as a key element for oral absorption of drugs that are substrates, such as digoxin or talinolol, either by transporting them from the apical membrane back into the intestinal lumen and/or by clearing them as unchanged parent drug into the bile during the first pass effect²¹⁻²³. P-gp shows a large interindividual variability of expression and function which is partly due to single nucleotide polymorphism altering oral drug bioavailability^{9,10,12,14,24-30}. The intestinal P-gp mRNA and protein content is higher than in the liver in most studies, suggesting that its intestinal location may be the main factor limiting oral bioavailability of its substrates³¹.

ABCC2/MRP2 and ABCC3/MRP3, two other ABC transporters respectively encoded by *ABCC2* and *ABCC3* genes, are also expressed in several tissues involved in the pharmacokinetics of drugs where they are mainly involved in the transport of endogenous and drug conjugates²⁰. ABCC2 is located in the intestine at the apical membrane of enterocytes and at the biliary membrane of hepatocyte whereas ABCC3 is expressed at the basolateral membrane of enterocytes and at the sinusoidal membrane of hepatocytes²⁰. ABCC2 plays a major role in hepato-biliary elimination of several drugs mainly as drug conjugates whereas its role in the small intestine may be less important due to its two-fold lower expression in the duodenum and jejunum than in the liver^{10,30,32}. ABCC3 is predominantly expressed in the intestine and in liver, where it is thought to be involved in bile acid absorption due to its basolateral expression in enterocytes and to export a wide range of organic anions from the liver, back to the blood, thereby decreasing hepatic exposure and toxicity to liver^{28,29,33,34}. Their intestinal expression level has been reported with contradictory results^{28,29,34}.

Expression of DME and ABC transporters remains to be determined in obese patients since a modification of their expression may have consequences on oral absorption of drugs and their effect. Here, we reported for the first time abundance at both mRNA and protein levels of ABCB1, CYP3A4, UGT2B7, ABCC2 and ABCC3 in human jejunal samples from obese subjects. We aimed to investigate whether or not physiological and biological changes associated with obesity are determinants of their interindividual intestinal expression.

2. Materials and Methods

2.1. Patients

The subjects were severely obese patients ($\text{BMI} > 35 \text{ kg / m}^2$) candidates for a Roux-en-Y gastric bypass surgery (RYGB). Subjects with diabetes, renal or hepatic disease, untreated obstructive sleep apnea syndrome or usually treated with sedative or analgesic drug were not eligible for this study. Most patients were receiving chronic medication (Eleven subjects were taking proton pump inhibitor, 10 antihypertensive drug, 11 vitamin supplementation, and 6 oral contraceptive method), but none was known to induce the enzymes studied. Each patient had complete assessment of body composition using dual-energy X-ray absorptiometry, as previously described, to determine the percentage of fat mass (FM) and the percentage of troncular fat mass (TFM) that is the ratio of the troncular FM on the total FM³⁵. They had complete clinical biochemistry, including liver and renal function assessment (aspartate aminotransferase (AST), alanine aminotransferase (ALT), (gamma-glutamyl transferase (GGT), serum creatinin), nutritionnal assessment (albumin, prealbumin), thyroid function assessment (serum TSHus and free T4) and dosage of inflammatory markers (IL6, CRPus, orosomucoid). Dosage of adipocytokines (adiponectin and leptin) and insulin were also performed.

All subjects gave their written informed consent. Decision for operation was performed by a multidisciplinary team including physicians, surgeon, anesthetists, dieticians, nurses, and psychologist following guidelines for the management of obese patients issued by consensus conferences³⁶. The protocol was approved by the regional ethics committee of Paris, France (CPP Ile de France I) and registered at ClinicalTrials.gov, with an EudraCT number 2009-010670-38.

2.2. Intestinal Tissues

Jejunal segments were obtained from patients undergoing RYGB, which was performed in the same department of surgery and using the same laparoscopic technique³⁷. A fragment of jejunal mucosa located about 2 meters after the usual gastroduodenal junction and considered as a surgical waste was preserved during surgery. Immediately after resection the intestinal segments were snap frozen using liquid nitrogen and stored at -80°C .

2.3. RNA extraction

Total RNA (100 μg approximately) was extracted from each mucosal sample using the Rneasy micro kit (Qiagen GmbH, Hilden, Germany) according to the supplier's

recommandations. Proteinase K was used to lyse the basement membrane surrounding the enterocytes and samples were treated with Dnase I (Rnase-Free Dnase Set; Qiagen SA) to remove genomic DNA. The concentration and purity of the RNA samples were assessed spectrophotometrically at 260 nm and 280 nm using the Nanodrop ND-1000 instrument (NanoDrop Technologies, Wilmington, DE, USA). Ratio of absorbance at 260 over 280 nm was higher than 1.8 for all the samples. Concentrations of total RNA extracted from intestinal mucosa were in the range 130- 750 ng/ μ L.

2.4. Reverse transcription and qRT-PCR

Reverse transcription (RT) was performed on the RNA extracted from the intestinal mucosa of each patient, using 1 mg total RNA in a final reaction mixture (20 μ L) containing 500 mM of each dNTP, 10 mM DTT, 1.5 mM random hexanucleotides primers (Amersham Biosciences, France), 20 U Rnasin ribonuclease inhibitor (Promega, France) and 100 U superscript II Rnase reverse transcriptase (Invitrogen, France). All samples were incubated at 25 °C for 10 min, then at 42°C for 30 min and at 99°C for 5min on a thermal cycler (PTC-100 programmable thermal controller, MJ research INC, USA). cDNAs were stored at -80 °C.

qPCR was performed in a final reaction mixture of 20 μ L containing 8 μ L of cDNA (5 μ L of cDNA for each patient /95 μ L of RNase free), 10 μ L of SYBR Green, 1 μ L of Forward Primer, 1 μ L of Reverse Primer on a Light-Cycler1 instrument (Roche Diagnostics, Meylan, France). All the primers used for pRT-PCR analysis were checked on positive controls (human liver RNA, Clontech Laboratories, USA) and all samples were run in duplicate. The genes of interest were *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4* and *UGT2B7* and the gene of the reference protein: *villin*. The primer sequences used for the qPCR are given in Table 1. All the primers were tested on an ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA) using SYBR Green fluorescence detection.

2.5. Relative expression

The relative transcript levels were determined using the comparative Ct method (the $\Delta\Delta$ Ct method). The background was proportionally adjusted and the cycle at which the log-linear signal was distinguishable from the background was taken as the crossing-threshold value (Ct) for each sample. Villin gene expression was used as a reference transcript for each sample, in accordance with previous studies^{28,29,34}. The expression profiles of the gene were then established using the following formula: $\Delta Ct = (Ct \text{ Target gene} - Ct \text{ the Villin gene})$ and were determined from the $2^{-\Delta Ct}$ values. Hence, relative expression of gene of interest was

determined and normalized for each patient with villin mRNA content, which allowed us to decrease the inter-individual variability due to the proportion of enterocytes in the intestinal mucosa samples that have been removed during surgery.

2.5. Proteomic analysis

The protein expression amounts of the target molecules were simultaneously determined by multiplexed multiple reaction monitoring (MRM) in HPLC_MS/MS or nanoLC_MS/MS as described previously^{38,39}. Briefly, quantification of human transporters was based on the MRM conditions previously developed in the Uchida et al. study, whereas quantification of human CYPs and UGTs was based on the MRM conditions developed in the Kawakami et al. and Sakamoto et al. studies, respectively³⁹⁻⁴¹.

Relative protein expression of gene of interest (*UGT2B7*, *MDR1/ABCB1*, *ABCC2*, *ABCC3*) was determined and normalized for each patient with villin protein content, which allowed us to decrease the inter-individual variability due to the proportion of enterocytes in the intestinal mucosa samples that have been removed during surgery.

2.6. Genetic analyses

DNA was extracted from blood cells with a semi-automatic Promega extractor, as recommended by the manufacturer (Promega, France). DNA concentration was determined with a Nanodrop spectrophotometer (NanoDrop®, Wilmington, USA).

Patients were genotyped for single nucleotide polymorphisms (SNP) in *OPRM1* (c.118A>G; rs1799971). This genotyping was based on Taqman real-time PCR assays (-StepOne plus- Applied Biosystems, Foster City, USA) carried out according to the kit manufacturer's instructions. A control (water), previously genotyped samples and a genomic DNA were included in each experiment to verify the accuracy of genotyping.

2.7 Statistical analysis

Statistical analysis was performed using Statview v4.0 (SAS Institute, Cary, N.C., USA). Quantitative data were presented as mean (standard deviation, SD). Quantitative data were compared using Fisher's PLSD test. Chi-square test was performed in order to compare qualitative data. Associations of gene expression and biological data were tested using Spearman rank correlation test, as well as associations between gene transcription and protein content. A p value of 0.05 or less was considered significant.

3. Results

3.1. Characteristics of the population

The characteristics of the population are reported in the Table 2. Jejunal samples of 27 patients were available. Their mean age of 40.1 (10.1) years and mean BMI of 44.4 (5.9) kg/m² did not significantly differ between genders. Only two patients were smokers, eight had a well-controlled apnea syndrome and eight subjects suffered for hypertension. Biological values did not differ between gender except for serum creatinine and GGT that were higher in men than women (83.8 (18.3) mmol/L versus 66.5 (7.7) mmol/L, $p=0.002$ for creatinine; 49.6 (15.5) UI/L versus 31.0 (10.4) UI/L, $p=0.003$ for GGT).

Inflammatory markers were similar between genders and were not correlated with BMI, neither with percentage of FM or TFM (%). Among adipocytokines, only leptin was significantly higher in men (39.3 (13.9) ng/mL versus 21.4 (5.1) ng/mL, $p=0.009$). Leptin, adiponectin and insulin were not correlated with BMI. However leptin and adiponectin were positively correlated with FM (%) ($r=0.5$, $p=0.08$ and $r=0.54$, $p=0.037$ respectively), whereas leptin (and not adiponectin) was inversely correlated with TFM (%) (0.6, $p=0.012$). Serum insulin level was negatively correlated with FM (%) ($r=0.5$, $p=0.01$) but positively correlated with the TFM (%) ($r=0.47$, $p=0.016$).

3.2. Relative mRNA expression of *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4*, *UGT2B7* genes

We found considerable differences in the expression levels of the two enzymes and three transporters in the small intestine. *ABCB1* was the major expressed transporter in the jejunum, whereas *ABCC3* exhibited the lowest expression. *ABCB1* exhibited a 1.8, 4.4, 5 and 36 fold higher expression than *CYP3A4*, *ABCC2*, *UGT2B7* and *ABCC3* respectively. *CYP3A4* expression was 3-fold higher than *UGT2B7*. There was also considerable interindividual variation in the expression of the genes that cannot be attributed to differences in the proportion of enterocytes in the intestinal samples since the gene expression of villin was considerably less variable among the samples. *UGT2B7* showed the highest level of interindividual variation with a fold difference of 14.7 (Table 3 and Figure 1).

3.3. Relative protein expression of *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4*, *UGT2B7* genes

In the present study, the most abundant enzyme was CYP3A4 and the most abundant transporter was MRP3 in human jejunum. MRP2 was detected in only one patient.

Considerable differences in the content of the two enzymes and three transporters in the small intestine were also observed. But UGT2B7 content showed the lower level of interindividual variability (Table 3. and Figure 1.).

3.4. Correlation between ABCB1, ABCC2, ABCC3, CYP3A4, UGT2B7 mRNA transcript and protein expression

We did not find a correlation between the mRNA and protein content.

At the mRNA level, all the gene expressions were correlated except for *CYP3A4* and *ABCC2* (Table 4.). The highest correlation was observed between *UGT2B7* and *ABCC3* mRNA expression ($r^2=0.6$; $p=0.0008$).

At the protein level, a correlation was only observed between UGT2B7 and MRP3 ($r^2=0.3$, $p=0.05$), UGT2B7 and CYP3A4 ($r^2=0.4$, $p=0.01$)

3.5. Correlation between ABCB1, ABCC2, ABCC3, CYP3A4, UGT2B7 mRNA and protein content and clinical and biological data

At the mRNA level:

We found no statistically significant correlation of enzymes expression with age. *ABCB1* mRNA content was significantly higher in men than in women (13.1 (9.5) versus 10.6 (6.2), $p=0.047$), whereas there was a trend toward a higher *ABCC2* expression in men (2.1 (1.3) versus 2.3 (0.5), $p=0.08$).

None of the body composition or anthropometric data, neither leptin, TSHus, T4l, or inflammatory markers (IL6, CRPus, orosomucoid) was correlated with mRNA expression of the genes of interest. Adiponectin was found positively correlated with *ABCC3* and *UGT2B7* ($r^2=0.21$, $p=0.018$ and $r^2=0.14$, $p=0.04$ respectively), while FM did not. Insulin levels were positively correlated with *ABCC2* expression ($r^2=0.38$, $p=0.001$) but inversely correlated with *CYP3A4* expression ($r^2=0.16$, $p=0.04$).

There was an inverse correlation between serum creatinine and both *CYP3A4* and *UGT2B7* expression, that disappeared after adjustment on sex except in women where there was still a trend toward a decrease in UGT2B7 with creatinine levels ($r=0.4$, $p=0.05$).

At the protein level (n=15)

None of the body composition or anthropometric data, neither leptin, TSHus, T4l, or inflammatory markers (IL6, CRPus, orosomucoid) was correlated with DME content, except for a trend toward a inverse correlation between P-gp content and CRPus ($p=0.07$) and insulin

and creatinine levels that were positively correlated with MRP3 content. DME content was not different between men and women.

Subjects with the CC, CT or TT genotype for *ABCB1* had similar *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4*, *UGT2B7* mRNA and protein expression, as well as carriers for the T or C alleles in comparison with those who were not carrier.

Discussion

The drug-metabolizing enzymes play a major role in the elimination of many xenobiotics, including exogenous and endogenous compounds, in particular in intestine where they may represent one of the first protections of the body against xenobiotic toxicity. To our knowledge, our study is the first investigation that describes, in obese patients, the quantitative expression of drug-metabolizing enzymes (DME) at the mRNA and protein level in the jejunum, the primary site of absorption of orally administered drugs. In addition, most of the previous studies about the human intestinal expression of DME did not concern a homogenous sample of subjects and did not study the effect of different biological and clinical parameters, as in our study. Moreover studies comparing DME expression both at the mRNA and at the protein level are rare.

We found considerable differences in the expression levels of the five enzymes in the small intestine.

At the mRNA level.

P-gp was the major expressed transporter in the jejunum. *ABCB1* exhibited a 4.4 and 36 fold higher expression than *ABCC2* and *ABCC3* respectively. A higher content of *ABCB1* mRNA than *ABCC2* mRNA have yet been reported in different studies in duodenal and jejunal samples^{27,29,34}.

However contradictory results on the *ABCC3* level expression relative to other transporters has been reported. Zimmermann showed that the pattern of mRNA expression differed along the intestine and that *ABCC3* was higher expressed than *ABCB1* along the small intestine, except in the terminal ileum³⁴. In contrary, Taipalensuu showed, in human jejunal mucosa obtained using a Watson capsule, that *ABCC2* was the most transporter expressed whereas the *ABCC3* and *ABCB1* mRNA expressions were similar²⁸. Englund et al. also reported that *ABCC2* expression was higher than *ABCC3*^{28,29}. Hilgendorf et al. reported the expression of different efflux transporters, including P-gp and MRP2, in jejunal samples of five healthy patients undergoing bypass surgery and showed a slightly higher *ABCC2* expression than *ABCB1* expression³⁰. But regarding the high interindividual variability in gene expression, the difference with our study may be due to the low number of intestinal samples collected in this study³⁰. In addition, it is known that *ABCC2* mRNA expression vary along the small intestine and if the surgical technique has not been strictly similar with our study, it may result in a description of drug transporter expression at two different jejunum sites^{10,30}.

In our study, *ABCB1* exhibited a 1.8 and 5 fold higher expression than *CYP3A4* and *UGT2B7* respectively. *CYP3A4* and *ABCB1* relative mRNA duodenal expression have been more investigated than jejunal samples, but a higher level of *CYP3A4* transcript and protein than *ABCB1* are usually reported in the small intestine^{9,10,28}. On one hand, as P-gp increases along the small intestine, intestinal samples may have been collected in a segment where *ABCB1* is more expressed than *CYP3A4*⁴². On the other hand, biological factors associated with obesity and discussed below may regulate *ABCB1* and *CYP3A4* expression in the small intestine in another pattern than normal-weight patients.

Despite its important role in glucuronidation, little is known about UGT intestinal expression and variability⁴³. Strassburg et al. demonstrated a polymorphic expression pattern of all the UGT genes in duodenal, jejunal, and ileal mucosa⁴³. We describe for the first time the level of expression of *UGT2B7* in a large sample of patients and we show that this level is three fold lower than *CYP3A4*.

At the protein level

We only detected MRP2 in one patient (on 15 samples). MRP2 is usually low expressed¹². It has yet been reported that *CYP3A4* intestinal content is higher than *ABCB1* content in duodenal samples¹⁴.

We found a large interindividual variation in gene expression both at the mRNA and protein levels, as previously reported. Taipalensuu et al. reported a fold difference of 1.9 for both for *ABCB1* and *ABCC2* and 2.8 for *ABCC3* at the mRNA expression²⁸. Von Richter et al. reported a 8-fold difference in mRNA expression for *CYP3A4* in duodenal and proximal jejunum and a 3-fold difference for P-gp³¹.

At the protein level, interindividual variability was quite similar with mRNA expression, except for *UGT2B7*. The interindividual variability is lower than others studies. Tucker et al reported a fold-difference of 7 and 40.2 respectively in duodenal content of *ABCB1* and MRP2, while Paine et al. demonstrated a fold difference of 8 and 10 in *ABCB1* and *CYP3A4* duodenal content¹². Bruyères et al. reported a lower interindividual variation of 1.4 fold difference in jejunal P-gp expression but only four samples were compared²⁶.

It may be difficult to compare the relative expression of DME with the existing literature, since patients, intestinal samples, sample analysis method and expression of the results differ between studies. However, the lower interindividual variability in our study is probably due to the large sample of patients included.

Factors of variability in gene expression

Except for age, tobacco, and gender, the factors of variability in gene expression in the human intestine have rarely been studied. We found that the mRNA expression of *CYP3A4* in smoker obese patients was two-fold higher than the non-smokers (10.8 (14.1) versus 5.8 (5.6)), in accordance with the inducer effect of tobacco ⁴⁴. *ABCB1* mRNA content was significantly higher in men than in women (13.1 (9.5) versus 10.6 (6.2), $p=0.047$), whereas it did not differ at the protein level. Whereas a 2.4-fold higher liver P-gp content in men compared with women has yet been reported, Paine et al. failed also to demonstrate an effect of gender on duodenal *ABCB1* protein expression ¹⁴.

Adiponectin was found positively correlated with *ABCC3* and *UGT2B7* mRNA expression ($r=0.46$, $p=0.018$ and $r=0.4$, $p=0.04$ respectively), while none of the anthropometric, clinical or biological data correlated with these DME. In addition, the expressions of these DME were highly correlated ($r^2=0.6$, $p=0.0008$). Intestinal glucuronidation is involved in the metabolism and excretion of endogenous or exogenous compounds that may be potentially toxic for the homeostasis ¹⁸. Whereas glucuronidation is catalyzed by a specific set of UDP-glucuronosyl transferases (UGTs), the hydrophilic and usually less toxic metabolites require specific transporters to be transported across both the sinusoidal and canalicular membranes of hepatocytes and across the basolateral membrane of enterocyte ¹⁸. Hence, similar transcriptional and post-transcriptional factors may be involved in their regulation and adiponectin may play a role in their commune regulation as this adipokine was highly correlated with both *ABCC3* and *UGT2B7* mRNA level but not the other DME, although they were highly correlated with each other.

In human tissues, it has been demonstrated that diabetes mellitus is associated with significantly reduced *UGT2B7* mRNA and protein content, and enzymatic activity in human liver and kidney ⁴⁵. As mRNA expression and probe activities for *UGT1A1* or *UGT1A9* are comparable between diabetic and nondiabetic tissues, the effect of diabetes may be specific to *UGT2B7* ⁴⁵. In addition phenobarbital failed to induce morphine glucuronidation in obese Zucker rats that are known to display low adiponectin level, suggesting a defect in the induction of this enzyme and the role of adiponectin in its regulation ^{46,47}.

The fact that we did not observed a correlation between insulin or leptin level with *UGT2B7* mRNA expression is in accordance with the unchanged UDP-glucuronyl transferase expression after insulin treatment in male insulinopenic diabetic rats or leptin treatment in ob/ob mice ^{48,49}.

The effect of obesity on ABCC3 liver content has yet been reported. In a model of obese Zucker rats, Mrp3 protein levels were reduced, whereas in insulinopenic diabetic rats, Mrp3 has been found increased. In ob/ob mice displaying obesity, insulin-resistance and hyperinsulism, it has been shown that Mrp3 content did not differ significantly between ob/ob and wild-type females but increased 1.6-fold in males^{50,51}. With extrapolation to humans, patients presenting insulinopenic diabetes and/or insulin-resistance may have decreased hepatic uptake and increased sinusoidal efflux of compounds transported by MRP3. In the enterocyte, where ABCC3 is expressed at the basolateral membrane ABCC3 may also participate to the efflux of glucuronconjugated compounds into the blood. Adiponectin is an endogenous insulin-sensitizing hormone and is the most abundant adipokine produced by the human adipose tissue⁵². It is well-known that it is linked to obesity, metabolic syndrome, insulin resistance and type 2 diabetes⁵².

Adiponectin plays a key role as a mediator of peroxisome proliferator-activated receptor (PPAR) gamma action. We did not observe a relationship between *UGT2B7* and *ABCC3* mRNA expression and insulin as in animal studies, but adiponectin has never been investigated as a candidate for drug transporter regulation. We may hypothesized that adiponectin is a factor involved in *ABCC3* and *UGT2B7* regulation in a context of insulin-resistance.

Adiponectin, known to be a key regulator for induction of hepatic and intestinal detoxification and antioxidant mechanisms, may enhance the transport of endogenous or exogenous compounds involved in the homeostasis of adipose tissue and in the pathogenesis of the metabolic syndrome, type 2 diabetes, and atherosclerosis, in stimulating *UGT2B7* and *ABCC3* expression⁵³.

However at the protein level, *UGT2B7* and MRP3 were correlated but adiponectine wasnot associated with their content. Only insulin level was still associated with MRP3 content, demonstrating the role of post-transcriptional factors in intestinal protein content.

In our study, *ABCC2* and *CYP3A4* expression are regulated by insulin but in different ways as while insulin is positively associated with *ABCC2* mRNA expression, insulin is negatively associated with *CYP3A4* expression. Interestingly the expressions of these two DME were not correlated, suggesting that they do not share the same regulation pathways. In contrast, insulin does not seem to be not involved in the expression of others DME.

Decrease in Mrp2 expression has been demonstrated in both insulinopenic and insulin-resistant models of rats, suggesting that this down-regulation may be due to a defect in insulin sensitization^{54,55}. Actually, treatment of obese rats with rosiglitazone reverse some features of

insulin resistance, such as hyperlipidaemia and fatty liver, and significantly increased Mrp2 protein mass by twofold with only partial restorations of biliary transport abnormalities⁵⁵. We observe a positive relationship between *ABCC2* expression and insulin, which reflects insulin-resistance in obese patients. Increase in *ABCC2* expression with insulin may aim to decrease insulin-resistance. Our study suggest than regulation of *ABCC2* seems to involve both transcriptional and post-transcriptional mechanisms related to insulin resistance.

Another hypothesis is that in patients displaying high level of insulin and glycaemia, there may be an enhanced *ABCC2* expression in order to increase bile salt synthesis and transport. Actually, it has been recently shown that glucose and insulin are major postprandial factors that induce bile acid synthesis⁵⁶. In addition, Tumor-necrosis factor α is activated by fatty acids in obesity and also regulate bile acid synthesis and excretion of lipids resulting in beneficial effects⁵⁷.

In contrary, insulin levels were inversely correlated with CYP3A4 expression. It has been demonstrated that CYP3A4 activity is reduced in overweight and obese patients^{8,44}. Although the variability in induced CYP3A4 activity is under strong genetic control, Rhamioglu et al. demonstrated that smoking and BMI collectively explained 20% of the variation in CYP3A4 activity. Clearance of cytochrome P450 (CYP) 3A4 substrates are lower in obese as compared with non-obese patients⁸. Hence, insulin may act as a transcriptional factor in inhibiting CYP3A4 expression.

Conclusion

To our knowledge, the results of the present study provide the first quantification of CYP3A, P-gp, MRP2, MRP3 and UGT2B7 in the jejunum, at the mRNA and protein levels, in a large sample of obese subjects. Although these levels of expression cannot be compared to normal-weight jejunum, correlations with biological and anthropometric data suggest than regulation of enzymes and transporter seems to involve both transcriptional and post-transcriptional mechanisms related to insulin resistance.

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Table 1. Sequences for primers and probes used in real-time reverse-transcription polymerase chain reaction.

| Target | Gene | Forward primer (50–30) | Reverse primer (30–50) | Length (bp) |
|--------|---------------|---------------------------|--------------------------|-------------|
| Pgp | <i>ABCB1</i> | CACCCGACTTACAGA TGATG | GTTGCCATTGACTGAAA GAA | 81 |
| MRP2 | <i>ABCC2</i> | CGACCCTTTCAACAA CTACTC | CACCAGCCTCTGTCACT TC | 119 |
| MRP3 | <i>ABCC3</i> | GTGGGGATCAGACA GAGAT | TATCGGCATCACTGTAA ACA | 99 |
| CYP3A4 | <i>CYP3A4</i> | | | |
| UGT2B7 | <i>UGT2B7</i> | | | |
| Villin | | | | |

Table 2.Characteristics of the population

| | All n=27 | Women n=22 | Men n=5 |
|--------------------------|--------------|---------------|---------------|
| Age (years) | 40,1 (10,1) | 38,3 (9,1) | 47,8 (11,6) |
| Weight (Kg) | 121,8 (24,2) | 114,7 (18,5) | 153,4 (22,3)* |
| BMI (kg/m ²) | 44,4 (5,9) | 43,5 (5,9) | 48,1 (4,2) |
| FM (%) | 46,2 (3,6) | 47,1 (9,4) | 42,2 (8,3)* |
| TFM (%) | 52,8 (4,8) | 51,6 (16,5) | 58,3 (18,7)* |
| Creatinine (mmol/L) | 69,7 (12,1) | 66,5 (7,7) | 83,8 (18,3)* |
| AST | 27 (7,1) | 26,3 (7,0) | 29,8 (7,7) |
| ALT | 31,3 (16,7) | 29,3 (15,5) | 40,2 (20,5) |
| GGT | 34,4 (13,4) | 31,0 (10,4) | 49,6 (15,5)* |
| T4L | 14,9 (1,8) | 15,0 (1,8) | 14,4 (1,9) |
| Leptine (ng/mL) | 35,9 (14,5) | 39,3 (13,9) | 21,4 (5,1)* |
| Adiponectine (mg/L) | 4,7 (2,8) | 5,0 (3,0) | 3,1 (0,5) |
| Insulinemia (mUI/L) | 17,0 (7,5) | 15,8 (7,0) | 21,5 (8,5) |
| IL6 (pg/mL) | 4,1 (2,4) | 3,7 (2,3) | 6,0 (2,8) |
| CRPus (mg/L) | 1,1 (0,9) | 1,2 (1,0) | 0,7 (0,2) |

Data are reported as means (with standard deviation SD)

**Significant difference= $p < 0,05$*

BMI: Body mass index; FM: Fat Mass; TFM: Troncular Fat Mass;

AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase; IL: Interleukine; CRPus: Ultra-sensitive C-reactive protein

Results are presented as Mean \pm Standard deviation

Table 3. Relative transcript and protein levels of *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4*, *UGT2B7* gene and correlation between mRNA and transcript expression in the jejunum of obese patients

| | <i>ABCB1</i> | <i>ABCC2</i> | <i>ABCC3</i> | <i>CYP3A4</i> | <i>UGT2B7</i> |
|--|--------------|--------------|-----------------|---------------|-----------------|
| <u>mRNA</u> | | | | | |
| Mean± SD | 10,9 (2,8) | 2,5 (0,8) | 0,3 (0,1) | 6,1 (2,7) | 2,1 (1,1) |
| | 6,3-17,1 | 1,3-4,8 | | 2,8-13,2 | |
| Range (fold diff) | (2,7) | (3,7) | 0,1-0,5 (5) | (4,7) | 0,36-5,3 (14,7) |
| <u>Protein (n=15)</u> | | | | | |
| | N=15 | N=1 | N=13 | N=15 | N=15 |
| Mean ±SD | 0,29 (0,1) | 0,018 | 0,46 (0,2) | 2,64 (0,8) | 0,41 (0,1) |
| Range (fold diff) | 0,2-0,44 (2) | | 0,16-0,89 (5,6) | 1,19-4,69 (4) | 0,25-0,65 (2,6) |
| <u>Correlation between mRNA and protein expression</u> | | | | | |
| r | R=0,15 | | R=0,2 | R=0,1 | R=0, |
| p | 0,2 | | 0,15 | 0,7 | 0,7 |

Data are reported as means (with standard deviation SD).

ABC: ATP-binding cassette; *CYP*: Cytochrome P450; *UGT*: UDP-glucuronosyl transferases

Table 4.

Correlation between *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4*, *UGT2B7* gene expression in the jejunum of obese patients at the mRNA and protein level

| | <i>ABCC2</i> | <i>ABCC3</i> | <i>CYP3A4</i> | <i>UGT2B7</i> |
|---------------|--------------|--------------|---------------|---------------|
| <i>ABCB1</i> | r=0,43* | r=0,54* | r=0,44* | r=0,41* |
| <i>ABCC2</i> | | r=0,49* | r<0,01 | r=0,4* |
| <i>ABCC3</i> | | | r=0,38* | r=0,6* |
| <i>CYP3A4</i> | | | | r=0,56* |

ABC: ATP-binding cassette; CYP: Cytochrome P450; UGT: UDP-glucuronosyl transferase

**Significant difference= $p < 0,05$*

Figure 1. *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4*, *UGT2B7* gene expression and content

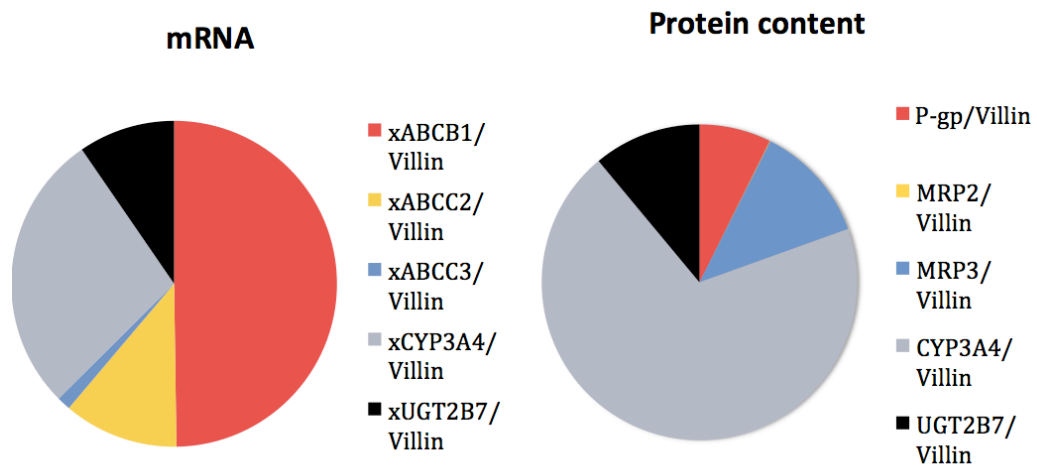
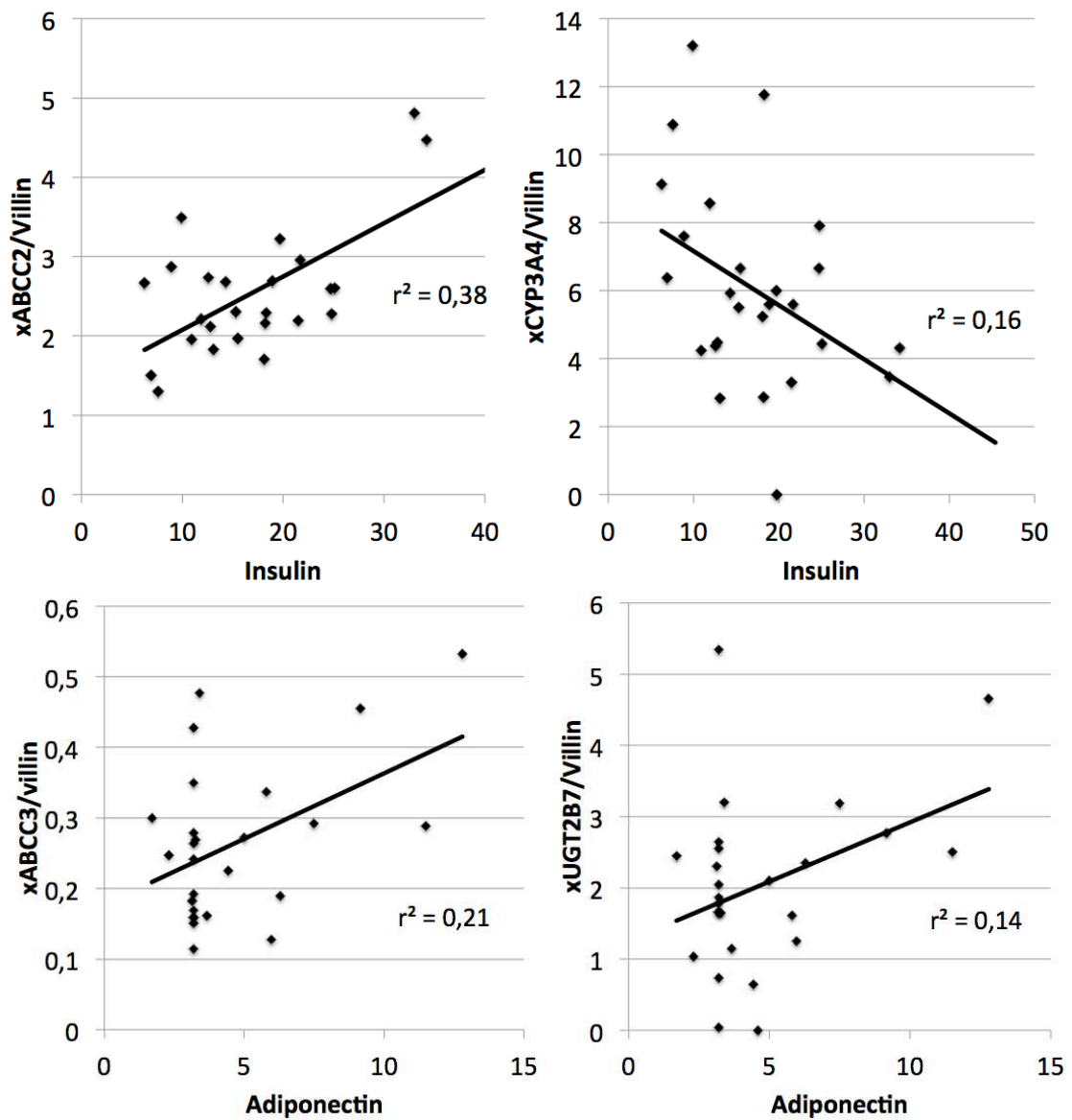


Figure 2. Correlations between *ABCC3* and *UGT2B7* mRNA expression with adiponectin and correlations between *ABCC2* and *CYP3A4* mRNA expression with insulin



Manuscrit 6. Pharmacocinétique de la morphine orale chez les sujets obèses avant et après chirurgie de l'obésité

La chirurgie de type bypass gastrique modifie l'anatomie normale de l'intestin et des études pharmacocinétiques, réalisées sur de faibles échantillons de patients, ont montré qu'elle modifiait le devenir de différents médicaments.

Nous avons comparé les données pharmacocinétiques de la morphine orale, avant chirurgie de l'obésité, à 15 jours et 6 mois après chirurgie de l'obésité.

Nous avons cherché à savoir si le contenu entérocytaire en enzyme UGT2B7 et en transporteur P-gp détermine les modifications de pharmacocinétique de la morphine orale après chirurgie.

Le manuscrit en rapport avec ce travail est en cours de rédaction :

- Les contenus entérocytaires en UGT2B7 et P-gp ont été mesurés chez 15 des 27 sujets
- Les dosages des métabolites de la morphine sont en cours, et nous attendons que l'ensemble des dosages ait été réalisé afin d'interpréter les modifications pharmacocinétiques de la morphine après chirurgie de l'obésité

L'article en cours de rédaction est présenté ici (Article 6.).

The effect of Roux-en-Y gastric Bypass on oral morphine pharmacokinetics

Célia Lloret-Linares, ..., Jean-François Bergmann et Xavier Declèves

Introduction

As 1.5 billion people in the world are considered as being obese (WHO), obesity has become a growing public health concern since it contributes to a substantial increase in morbidity and mortality. Morbidly obese patients who have failed dietary or medical weight loss methods are potential candidates for bariatric surgery to achieve long-term weight reduction. Hence, in the period from 1998 to 2003, the number of bariatric procedures performed increased 10-fold, and in 2009 alone, 220,000 bariatric surgeries were performed in the United States and Canada ^{1,2}. The drastic alteration in gastrointestinal anatomy resulting from Roux-en-Y gastric bypass (RYGB) induces major changes in drug disposition ³. Because metabolizing enzymes are mostly found in the bypassed proximal small intestine, drugs that undergo substantial first-pass metabolism in the intestine may be more bioavailable after RYGB. However the effect of RYGB appears to be drug-specific and controlled studies are needed to evaluate its effect on drug absorption.

Obesity is frequently associated with pain of various origins (e.g. arthritis, fibromyalgia, cancer), which increases the need for analgesic drugs ^{4,5}. Despite the benefits of bariatric surgery on morbidity and mortality in obese patients, acute and chronic pains may occur even after weight loss and require pain killers ⁵⁻⁸. Little is known about the consequences of RYGB on analgesics absorption. To determine the outcome of drugs with a narrow therapeutic index such as morphine pharmacokinetics after RYGB is important ⁸.

After oral administration, morphine is almost completely absorbed by the gastrointestinal tract ⁹. In animals, the fastest absorption of morphine takes place in the medium of the jejunum and duodenum ¹⁰. Morphine is substrate of the drug efflux transporter P-glycoprotein (P-gp) and it has yet been reported that the intestinal barrier is a determinant of morphine pharmacokinetics and pharmacodynamics ¹¹⁻¹⁵.

Oral morphine pharmacokinetics shows a bioavailability of 30-35% approximatively ^{9,16}. Most drug metabolism occurs within the liver, and to a lesser extent the proximal small intestine, where drug metabolizing enzymes are also located ¹⁷. Morphine is primarily metabolized in the liver by UGT (UDP-Glucuronosyltransferase) enzymes, a phase II metabolism enzyme family with several isoforms. Morphine has a specific affinity for the UGT2B7 isoenzyme ¹⁸. Sixty percent of an oral dose of 20 to 30mg morphine is glucuronidated to Morphine-3-glucuronide (M3G), and 6 to 10% to Morphine-6-glucuronide (M6G) ^{19,20}. Among the various members of the MRP (ABCC) transporter family, MRP2 and

MRP3 are respectively encoded by ABCC2 and ABCC3 genes and actively transport morphine-glucuronides, at the apical and basolateral membrane respectively^{21,22}.

In our study, we aimed to determine the effect of RYGB on oral morphine pharmacokinetics.

Materials and methods

Obese Volunteers

Inclusion criteria were obese volunteers candidates for a RYGB. Decision for operation was performed by a multidisciplinary team following guidelines for the management of obese patients²³. Subjects with diabetes, renal or hepatic dysfunction, untreated Obstructive Sleep Apnea Syndrome or usually treated with sedative or analgesic drug were not eligible for this study. None of the subjects had a history of allergic response to morphine or other opioid. They had complete clinical biochemistry, including liver and renal function assessment, nutritional assessment (albumin, prealbumin), thyroid function assessment (serum TSH and free T4) and dosage of inflammatory markers (IL6, CRP, orosomucoid). Dosage of adipocytokines (adiponectin and leptin) and insulin were also performed.

Height was measured to the nearest 5 mm, without shoes, using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg and body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m²).

All subjects gave their written informed consent. The protocol was approved by the regional ethics committee of Paris, France (CPP Ile de France I) and registered at ClinicalTrials.gov, with an EudraCT number 2009-010670-38.

Bariatric procedure

Surgery was performed by the same surgeon in all patients using the same laparoscopic technique²⁴. RYGB is a surgical procedure in which most of the stomach and some of the small intestine is bypassed causing restriction with a gastric pouch of 15–30 ml in volume and malabsorption, as the jejunum is divided 40–50 cm distal to the ligament of Treitz, and the distal end is anastomosed to the pouch. The proximal end is anastomosed 75–150 cm downstream to create a bypass, which induces mild malabsorption.

Pharmacokinetic Study

The subjects were given a single oral dose of 30 mg of morphine sulphate oral solution (Oramorph 5mL, Roxane Laboratories, Inc, Columbus, Ohio), in the morning after an overnight fast on three visits: before surgery (Visit 1: one month to 7 days before surgery),

immediately after (Visit 2: 7-15 days after following surgery) and six months after surgery (Visit 3=6 months). A standard meal schedule was given approximately 4 hours after dosing. No food or beverage was allowed before the meal.

Blood samples were drawn in heparinized tubes. Plasma was separated immediately by centrifugation, frozen, and stored in a labelled container at -20°C pending analysis. Ten blood samples per patient were collected for morphine assay, during a 12 hours period for each patient after morphine administration.

Analytical method

The method utilized reversed phase ion-pairing high performance liquid chromatography (HPLC) coupled with coulometric electrochemical detection.²⁵ All reagents were HPLC grade. Briefly, Liquid-Liquid extraction of morphine was performed as follows: the buffer was made by adding 500 µL of plasma sample and 500 µL of borate buffer adjusted at pH 8.9 and spiked with 40 ng of naloxone (100 µL internal standard). The mixture was extracted with 5 mL of chloroform:isopropanol (95:5, v/v) and vortexed for 20s. The organic phase was collected, then dried with anhydrous sodium sulfate and evaporated under a stream of nitrogen at 35°C. Residue was dissolved in 200 µL of the mobile phase. 50 µL of the solution was injected into the liquid chromatograph Lichrocart using Lichrospher® 60 RP-select B C18 chromatographic column (125 x 4 mm ID, 5 µm). The mobile phase contained tetrahydrofurane-acetonitrile-0.005 M, sodium heptanesulfonate and 0.05 M phosphate buffer (pH 4.8) (1/10/89, vol/vol). The HPLC column was run at a flow rate of 1 mL/min and separations were performed at room temperature. Morphine-3-glucuronide, morphine-6-glucuronide and normorphine were evaluated for interferences and none was observed. The retention times for morphine and internal standard were approximately 4.5 and 9.4 min, respectively. A Coulochem® II 5200A Electrochemical Detector with High Sensitivity Analytical Cell Model 5011 (ESA, Bedford, USA) was used. Oxidative potentials were applied at 100 mV and 600mV for E1 and E2 cell, respectively.

A 10-point calibration curve was built over the range of 1 ng/mL to 100 ng/mL. Based on quality control samples, the within-day and between-days coefficients of variation (CVs) were less than 10%. The lower limit of quantification (LOQ) was 1 ng/mL.

Modelling strategy and population pharmacokinetic model

Morphine dosage was expressed in morphine base from sulfate salt. Data were analyzed

using the nonlinear mixed-effect modeling software program NONMEM (version VI, level 1.0) with the Digital Fortran compiler. The first-order conditional estimation with interaction method was used. The first concentration of the visit below the limit of quantification was fixed to LOQ, the following ones were deleted. A 2-compartment and 3-compartment models with zero-order or first-order absorption and first-order elimination were tested to describe morphine data. Additive, proportional and combined error models were tested. Analytical equations were used in a \$PRED section in NONMEM to estimate PK parameters. The structural model choice was based on diagnostic criterion (objective function value (OFV) for nested models and Schwarz criterion (BIC) for not nested models) and goodness-of-fit plots. An exponential model was used for inter-subject variability (ISV) and for inter-occasion variability (IOV). Each variance of ISV random effect was fixed to zero, if the OFV did not increase by more than 3.84 points, the ISV was deleted. IOV and covariance between the ISV was also tested, they were kept if OFV decreased by more than 3.84 units. The effects of the continuous covariates (CO) as age, bodyweight, height, BMI were evaluated from the basic model, one at time. The latter were centred to the median and log-transformed for model interpretation convenience; using Clearance (CL) for example,

$$CL = \theta_{CL} \times \left(\frac{CO}{\text{median}(CO)} \right)^{\beta_{CO}^{CL}}$$

where θ_{CL} is the typical value of clearance for a patient with the median covariate value and β_{CO}^{CL} is the estimated influential factor for the continuous covariate. The effect of categorical covariates (CA) as operation was tested as follow.

$$CL = \theta_{CL} \times \left(\beta_{CA}^{CL} \right)^{CA}, \text{ CA being 0 before surgery or 1 after surgery.}$$

A covariate was kept if its effect was biologically plausible; it produced a minimum reduction of 6.63 in the OFV and a reduction in the variability of the pharmacokinetic parameter, assessed by the associated ISV. An intermediate model with all significant covariates was obtained. A forward selection of these covariates for the population model was performed by use of the Likelihood Ratio Test (LRT) with a significance threshold at $P < 0.05$. From this ascending method, a backward elimination procedure was performed with a significance threshold at $P < 0.01$.

Evaluation and validation. For evaluation of the goodness of fit, the graphs of the following data were performed: observed and predicted concentrations versus time, observed concentrations versus population predictions, weighted residuals versus time, and weighted residuals versus predictions. Similar graphs using individual predictive post hoc estimation

were displayed. The diagnostic graphs were performed using RfN (S. Urien, RFN-831-20070911[https://sourceforge.net/project/showfiles.php?group_id=29501&package_id=140129&release_id=538680]) with the R program²⁶.

Morphine concentration profiles were simulated and compared with the observed data, thanks to the visual predictive check method, in order to validate the model. More precisely, the vector of pharmacokinetic parameters from 1,000 patients was simulated using the final model. Each parameter vector was drawn in a log-normal distribution, with a variance corresponding to the ISV previously estimated. A simulated residual error was added to each simulated concentration. The simulations were performed using NONMEM. The 5th, 50th, and 95th percentiles of the simulated concentrations at each time were then overlaid on the observed concentration data by using the R program, and a visual inspection was performed.

Individual PK parameters were estimated from the final model: morphine Maximal Concentrations (C_{max}), Time to Maximal Concentrations (T_{max}), Area Under the Curve from 0 to 12 hours (AUC_{0-12h}), half-life for phase α and β .

Intestinal Tissues

Jejunal segments were obtained from 26 patients undergoing RYGB. A fragment of jejunal mucosa located about 40-50 centimeters after the usual gastroduodenal junction (or ligament de Treitz) and considered as a surgical waste was preserved during surgery. Immediately after resection the intestinal segments were snap frozen using liquid nitrogen and stored at -80 °C.

mRNA

RNA extraction

Total RNA (100 µg approximately) was extracted from each mucosal sample using the Rneasy micro kit (Qiagen GmbH, Hilden, Germany) according to the supplier's recommendations. Proteinase K was used to lyse the basement membrane surrounding the enterocytes and samples were treated with Dnase I (Rnase-Free Dnase Set; Qiagen SA) to remove genomic DNA. The concentration and purity of the RNA samples were assessed spectrophotometrically at 260 nm and 280 nm using the Nanodrop ND-1000 instrument (NanoDrop Technologies, Wilmington, DE, USA). Ratio of absorbance at 260 over 280 nm was higher than 1.8 for all the samples. Concentrations of total RNA extracted from intestinal mucosa were in the range 130- 750 ng/µL.

Reverse transcription and qRT-PCR

Reverse transcription (RT) was performed on the RNA extracted from the intestinal mucosa of each patient, using 1 mg total RNA in a final reaction mixture (20 mL) containing 500 mM

of each dNTP, 10 mM DTT, 1.5 mM random hexanucleotides primers (Amersham Biosciences, France), 20 U Rnasin ribonuclease inhibitor (Promega, France) and 100 U superscript II Rnase reverse transcriptase (Invitrogen, France). All samples were incubated at 25 °C for 10 min, then at 42°C for 30 min and at 99°C for 5min on a thermal cycler (PTC-100 programmable thermal controller, MJ research INC, USA). cDNAs were stored at -80 °C.

qPCR was performed in a final reaction mixture of 20 µL containing 8 µL of cDNA (5 µL of cDNA for each patient /95 µL of RNase free), 10µL of SYBR Green, 1µL of Forward Primer, 1µL of Reverse Primer on a Light-Cycler1 instrument (Roche Diagnostics, Meylan, France). All the primers used for pRT-PCR analysis were checked on positive controls (human liver RNA, Clontech Laboratories, USA) and all samples were run in duplicate. The genes of interest were *ABCB1*, *ABCC2*, *ABCC3*, *CYP3A4* and *UGT2B7* and the gene of the reference protein: *villin*. The primer sequences used for the qPCR are given in Table 1. All the primers were tested on an ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA) using SYBR Green fluorescence detection.

Relative expression

The relative transcript levels were determined using the comparative Ct method (the $\Delta\Delta$ Ct method). The background was proportionally adjusted and the cycle at which the log-linear signal was distinguishable from the background was taken as the crossing-threshold value (Ct) for each sample. Villin gene expression was used as a reference transcript for each sample, in accordance with previous studies²⁷⁻²⁹. The expression profiles of the gene were then established using the following formula: $\Delta Ct = (Ct \text{ Target gene} - Ct \text{ the Villin gene})$ and were determined from the $2^{-\Delta Ct}$ values.

Proteomic analysis

The protein expression amounts of the target molecules were simultaneously determined by multiplexed multiple reaction monitoring (MRM) in HPLC_MS/MS or nanoLC_MS/MS as described previously^{30,31}. Briefly, quantification of human transporters was based on the MRM conditions previously developed in the Uchida et al. study, whereas quantification of human CYPs and UGTs was based on the MRM conditions developed in the Kawakami et al. and Sakamoto et al. studies, respectively³¹⁻³³.

Relative protein expression of gene of interest (*UGT2B7*, *MDR1/ABCB1*, *ABCC2*, *ABCC3*) was determined and normalized for each patient with villin protein content, which allowed us

to decrease the inter-individual variability due to the proportion of enterocytes in the intestinal mucosa samples that have been removed during surgery.

Statistics

Statistical analysis was performed using Statview v4.0 (SAS Institute, Cary, N.C., USA). Quantitative data were presented as mean (standard deviation, SD). Quantitative data were compared using Fisher's PLSD test. Chi-square test was performed in order to compare qualitative data. Associations of anthropometric and biological data with detection and pain thresholds were tested using Spearman rank correlation test. A p value of 0.05 or less was considered significant.

Results

Obese volunteers

Thirty-one subjects (24 women and 7 men) with a mean Body Mass Index (BMI) of 44.6 (35.4 – 62.2) kg/m² and a mean age of 41 years participated to the oral morphine PK before surgery (Visit 1). Most patients were receiving chronic medication (Eleven subjects were taking proton pump inhibitor, 10 antihypertensive drug, 11 vitamin supplementation, and 6 oral contraceptive method), but none was known to have a drug-interaction with morphine or to induce the enzymes studied. Among the 31 subjects who participated to Visit 1, 25 subjects with a mean BMI of 41.4 (32.0 – 61.0) kg/m² participated to the PK study immediately after surgery (Visit 2) and twenty-six subjects with a mean BMI of 32.4 (25.4 – 46.0) participated to the PK study 6 months after surgery (Visit 3).

Population pharmacokinetics

A total of 693 concentrations time points in obese subjects were available for the pharmacokinetic analysis: 258 concentrations from 31 patients at Visit 1, 215 concentrations from 25 patients at Visit 2 and 220 concentrations from 26 patients at Visit 3. Fifty-four concentrations (7.8 %) were below the limit of quantification (LOQ) and kept at the LOQ/2 value. A 2-compartment model with first-order absorption and elimination best described morphine data. Parameters of the model were the absorption rate constant (k_a), apparent elimination clearance from the central compartment (CL/F), apparent volume of the central compartment (V_2/F), apparent intercompartmental clearance (Q/F) and apparent volume of the peripheral compartment (V_3/F).

Intersubject Variability and weight loss

The available data were not sufficient to estimate inter-subject variability for Q/F and fixing the variance of this random effect to zero had no influence on the objective function values (OFV). Residual variabilities were best described by proportional error model.

Volumes

During the ascending phase, the most significant covariate was BMI on V_3/F , decreasing the OFV by 39.1 units and the Intersubject Variability (ISV) on V_3 from 49 to 37%, then BMI was significant on V_2/F , decreasing OFV by 38.0 units and ISV on V_2/F from 42 to 28%.

Absorption

Absorption was clearly modified at each visit: two ways were used to modelize it. First, an ioV on k_a was used. Secondly, 2 parameters allowing the estimation of a different k_a at each occasion were used. The second way was the most significant leading to a 26.2 points

decrease in OFV and an ISV on k_a from 131 to 75%. Then an effect of the operation was significant on V3/F (26.6 unit decrease in OFV and a variability on V3 from 42 to 23%).

Clairance

Finally BMI was significant on CL/F with a 19.4 unit decrease in OFV and a reduction of CL ISV from 31 to 27%. A covariance between CL and V was also significant with a 12.4 more decrease in OFV. Finally an iov variability was significant on CL, decreasing residual variability from 38 to 34%. The backward phase confirm the significance of all these covariates with an increase in OFV of 13.5, 26.7, 12.9 units in OFV when BMI was deleted from CL/F, then V2/F and V3/F, an increase of 47.4 and 70.9 units when each parameter coding from each occasion on k_a was deleted, by 15.9 units when the effect of operation was deleted on V3 and finally 12.4 points when the covariance between CL-V was deleted and 81.6 points when the iov on CL was deleted; all the corresponding ISV were also increased.

In the final model, the k_a 6 months after surgery could not be estimated. Indeed, although samples were taken 15 min after morphine administration, all the first concentrations were maximal concentrations, thus we did not have points in the ascending phase allowing estimating correctly the absorption at V3. As the estimations were close to 20 and other values tested did not decrease more the OFV, the estimate of the increase of absorption rate between V1 and V3 was fixed to 20.

Equations for parameters were as follows:

$$k_a = 1.58 \times 4.03^{VISITE\ 2} \times 20^{VISITE\ 3},$$

Visit 2= 1, 15 days after surgery, 0 before and 6 months after surgery

Visit 3= 1, 6 months after surgery, 0 before and 15 days after surgery

$$CL = 423 \times \left(\frac{BMI}{41.7}\right)^{0.641}$$

$$V_2 = 794 \times \left(\frac{BMI}{41.7}\right)^{1.65},$$

$$Q=281$$

$$V_3 = 1580 \times \left(\frac{BMI}{41.7}\right)^{1.41} \times 0.397^{OP}, \text{ OP}=0 \text{ before surgery and } 1 \text{ after.}$$

Table 2 summarizes the final population pharmacokinetic estimates for an obese patient.

Table 3 reports C_{max} and T_{max} , half-life for phase α and β , AUC_{0-12h} .

Evaluation and validation

Satisfactory final model performance can be seen in Figure 1. Concentration – time profile before surgery, 15 days and 6 months after surgery were drawn in Figure 2. Figure 3 (VPC) shows that the average prediction matches the observed concentration time courses and that the variability is reasonably estimated. The percentage of observed points within the 90% prediction interval was 88.9%.

Intestinal samples

mRNA expression: *ABCB1* exhibited a 5 fold higher mRNA expression than *UGT2B7*. There was also considerable interindividual variation in the expression of the genes that cannot be attributed to differences in the proportion of enterocytes in the intestinal samples since the gene expression of villin was considerably less variable among the samples. *UGT2B7* showed the highest level of interindividual variation with a fold difference of 14.7.

Protein content: The most abundant transporter was MRP3 in human jejunum. MRP2 was detected in only one patient. *UGT2B7* content showed the lower level of interindividual variability.

Correlations between Visit 1 pharmacokinetics and drug-metabolizing enzymes or biological values

Individual PK parameters did not correlate with any of the biological data assessed, neither with *UGT2B7* mRNA expression or content. P-gp jejunal content was positively correlated with morphine T_{max} ($r^2=0.26$, $p=0.05$) and negatively with morphine C_{max} ($r^2=0.47$, $p=0.007$) (figure 4.). Morphine AUC did not correlated with any of the biological, clinical, DME intestinal content before surgery and weight loss.

Changes in pharmacokinetics after surgery

C_{max} and T_{max}

An important variability in morphine PK parameters was observed before and after surgery. In comparison with V1, T_{max} was two-fold and 7.5-fold decreased, whereas C_{max} was 1.7 and 3.3-fold increased at Visit 2 and Visit 3 respectively (Table 3). In comparison with Visit 1, morphine absorption was four and twenty-fold increased at Visit 2 and Visit 3 respectively. In comparison with Visit 1, all patients have an increased C_{max} and decreased T_{max} after surgery (at Visit 2 and Visit 3 with significant changes between V2 and V3). Postoperative

C_{max} and T_{max}, at Visit 2 and 3, were highly correlated with their preoperative value (Figure 5).

AUC

Mean morphine AUC significantly increased between Visit 1 and 3 and between Visit 2 and 3. Its increase was not significantly different between Visit 1 and 2. 21/24 and 16/25 patients increased their AUC, at Visit 2 and Visit 3 respectively. The mean (and range) variation in AUC in percentage of the initial AUC ((Visit 2 or 3 AUC- Visit 1 AUC)/ Visit 1 AUC) were +23,4% (-28,3 to 62,1%) at Visit 2 and +55,5% at Visit 3 (-9 to +105%) (Figure 6).

When patients with a mean percentage of variation over the median were compared with those with a mean variation below the median, we did not found biological or clinical differences or difference in DME jejunal content between the two groups, at Visit 2 and 3.

Morphine AUC at Visit 2 was inversely and significantly correlated with P-gp content.

Volume and clearance

At Visit 3, the apparent volume of the peripheral compartment was decreased and represented 60% of the initial volume. A decrease in BMI of 10%, was associated with a decreased morphine clearance of 6.4%, a decreased in V₂ of 16% and a decreased in V₃ of 13.8%.

Discussion

We describe here for the first time the changes in oral morphine PK after RYGB. Few studies have reported the consequences of RYGB on the PK of oral drugs and showed that they appear drug-specific³⁴⁻³⁷. These studies recruited a little number of patients, which limit the analysis of the individual variability in PK outcomes. Moreover, only one study showed the comparison of drug PK at different periods following RYGB, while none reported the role of intestinal enzymes and transporter content in the outcome of drug PK after RYGB.

We first report the role of P-gp content in morphine PK absorption. Indeed, morphine absorption, defined by the Cmax and Tmax parameters, is significantly correlated with P-gp content. Previous studies have suggested the role of intestinal P-gp activity in morphine absorption, pharmacokinetic and pharmacodynamics¹¹⁻¹⁵. We report, for the first time, a significant negative correlation between morphine absorption and jejunal P-gp content, with a decreased Cmax and a increased Tmax while P-gp content increases. The absence of correlation between Cmax or Tmax with UGT2B7 jejunal content suggest that morphine glucuronidation is slightly or not involved in morphine intestinal first-pass. We did not have liver samples to study the role of UGT2B7 liver content in morphine first-pass metabolism.

Oral morphine pharmacokinetics shows a bioavailability of 30-35% approximatively^{9,16}. Most drug metabolism occurs within the liver and may partly explain the interindividual variability in morphine availability, but we show that P-gp content in the proximal small intestine determine morphine absorption in obese patients before surgery and in the postoperative period. However we failed to demonstrate a correlation between P-gp jejunal content and morphine AUC at Visit 1 and 3, suggesting the role of other factors in morphine exposure variability. But the correlation between P-gp content and Morphine AUC at Visit 2 suggest the role of P-gp in morphine exposure, in particular when conditions of absorption are more favourable.

In our study, RYGB induced an increased morphine absorption in all patients immediately after surgery and six months after surgery, with an increased absorption between these two periods. Our results suggest that surgery drastically change oral morphine absorption and that mechanisms different than the new gastrointestinal circuit influence drug absorption following RYGB.

We observed a high and significant correlation between Cmax and Tmax at Visit one, with their values at Visit 2 and 3. These results suggest that factors of morphine absorption variability are similar after RYGB and after weight loss. Actually we found a significant correlation between Tmax and Cmax at the three visits and P-gp content. Hence, patients with

the higher P-gp content are those with the lower morphine absorption, and they display a low increase in absorption after RYGB and surgery. Other factors of variability in morphine absorption may be unchanged as the range of C_{max} and T_{max} were similar whatever the visit.

The increase in C_{max} and T_{max} suggests that the anatomical conditions are more favourable to morphine absorption.

Different mechanisms may explain the increase in morphine absorption. Gastric emptying of liquids increases after RYGB and may affect the bioavailability of oral drugs with a liquid formulation³⁸. The derivation in biliary acid may increase the solubility of morphine. It may be hypothesized that RYGB, weight loss and its associated physiological changes, globally reduce P-gp activity all along the small intestine. This may explain that the absorption is increased between the two post-operative periods.

Hence the fact that the mixture of bile salts and drugs occurs in a more distal part of the intestine, whose functional length is reduced, does not affect morphine absorption. Conversely morphine absorption seems to be facilitated, despite the increase P-gp content and activity from proximal to distal segment of the intestine, that has been previously reported^{39,40}.

Although glucuronidation occurs mainly in the liver, its activity is detected all along the intestinal mucosa with a maximal activity in the jejunum⁴¹. The role of intestine in the morphine first-pass metabolism is unknown, but if present, the bypass of the proximal small intestine may reduce the surface area available for glucuronidation and increase the bioavailability of morphine.

In addition, the intestinal epithelium is a dynamic structure that is entirely renewed every 4-5 days. Various studies suggest that there is a phenomenon of adaptation of the small intestine after intestinal resection, such as hypertrophy of the intestinal mucosa to compensate the decrease of absorption capacity^{42,43}. However, we observe an increased absorption between the two postoperative periods, suggesting that if the hypertrophy exists, it is not accompanied by an increase in intestinal first-pass effect.

RYGB increased morphine exposure.

As morphine exposure at Visit 2 as significantly correlated with P-gp content, we show that an increased absorption or a decrease morphine first-pass effect results in an increase exposure. In addition we show the relationship between BMI and morphine clearance or AUC in our model. An increased and weight-related clearance of drugs metabolized by the UGT

enzymes has been reported in obese patients in comparison with normal-weight patients⁴⁴. Hence as the weight and the liver steatosis are decreasing after RYGB, glucuronidation may decrease, leading to decrease morphine clearance. It has also been suggested that liver steatosis influence liver blood flow and decrease liver metabolism⁴⁴.

The occurrence of somnolence was more frequent in the postoperative period in comparison with the preoperative period, suggesting that the increase in morphine maximal concentration and the slightly increase in morphine exposure is associated with significant changes in pharmacodynamics.

Volume

The change in body composition after RYGB influences morphine volumes of distribution and partly explain the increased morphine maximal concentrations, at visit 2 and 3. Actually, the amount of food and drinks are very limited immediately in the postoperative period as RYGB leads to a marked reduction in the functional volume of the stomach. Weight loss is mainly explained by a loss of water and lean body mass in the immediate post operative period, while fat mass is further decreased continuously and is also involved six months later⁴⁵.

We show a important inter-individual variability in oral morphine absorption and exposure that remained after RYGB. Interdividual variability of intestinal barrier, especially P-gp content, determines the degree of change in morphine absorption after RYGB.

Oral morphine absorption, exposure and concentrations are increased after RYGB, while morphine clearance is decreased. Hence, oral morphine dosage should be reduced after RYGB in comparison with preoperative dosage, especially in patients with a lower first-pass effect and in the case of liquid and immediate release formulations.

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Table 1. Characteristics of the obese subjects enrolled in the pharmacokinetic study

| | Before surgery | After surgery | |
|---|--------------------|--------------------|--------------------|
| Covariates | Visit 1 | Visit 2 | Visit 3 |
| Median (Min-Max) | Before surgery | 15 days after | 6 months after |
| n | 31 | 25 | 26 |
| Age (years) | 41 (18 – 62) | 38 (18 – 55) | 39.5 (18 – 61) |
| Bodyweight (kg) | 116.9 (94.1 – 180) | 107.4 (85 – 173) | 89.4 (67.2 – 150) |
| Body mass index (kg/m ²) | 44.6 (35.4 – 62.2) | 41.4 (32.0 – 61.0) | 32.4 (25.4 – 46.0) |

Table 2. Population pharmacokinetic parameters of morphine for an obese patient with a BMI of 41.7 kg/m² after receiving 30 mg of sulfate morphine (22.6 mg of morphine) oral solution

| Structural model | | Statistical model | |
|--------------------------|------------------|-----------------------|------------------|
| Parameter | Estimate (RSE %) | Parameter | Estimate (RSE %) |
| k_a (h ⁻¹) | 1.58 (18) | ω_{ka} (%) | 52 (46) |
| CL/F (L/h) | 423 (6) | $\omega_{CL/F}$ (%) | 29 (30) |
| V_2/F (L) | 794 (10) | $\omega_{V2/F}$ (%) | 36 (26) |
| Q/F (L/h) | 281 (14) | $\omega_{V3/F}$ (%) | 22 (91) |
| V_3/F (L) | 1580 (80) | ω_{iov} (CL) | 19 (20) |
| CL/F, θ_{BMI} | 0.641 (41) | Corr (CL/F, V_2/F) | 0.75 (36) |
| V_2/F , θ_{BMI} | 1.65 (25) | σ_{OBEMO} (%) | 34 (10) |
| V_3/F , θ_{BMI} | 1.41 (34) | | |
| k_a , θ_{V2} | 4.03 (39) | | |
| k_a , θ_{V3} | 20 FIXED | | |
| OP, θ_{V3} | 0.397 (19) | | |

Key: RSE%, relative standard error; k_a absorption rate constant; CL/F apparent elimination clearance from the central compartment; V_2/F apparent central volume of distribution; Q/F intercompartmental clearance; V_3/F apparent peripheral volume of distribution; CL/F, θ_{BMI} effect of body mass index on CL/F; σ residual variability estimates (proportional error model); ω , between subject variability estimates, iov inter-occasion variability, $corr$: correlation

Table 3. Secondary pharmacokinetic parameters (median, range) of obese patients enrolled in the OBEMO, after receiving 30 mg of sulfate morphine (22.6 mg of morphine) oral solution, before surgery, 15 days after surgery and 6 months after surgery.

| | median (min - max) PK parameters | | |
|------------------------|----------------------------------|-----------------------|------------------------|
| | before surgery | 15 days after surgery | 6 months after surgery |
| t_{\max} (min) | 53 (31 - 84) | 23 (12 - 37) | 7 (3 - 11) |
| C_{\max} (µg/L) | 11.3 (6.4 - 24.0) | 19.1 (9.0 - 38.3) | 38.1 (16.7 - 77.2) |
| $t_{1/2\alpha}$ (h) | 0.8 (0.5 - 1.4) | 0.6 (0.4 - 1.1) | 0.4 (0.3 - 0.8) |
| $t_{1/2\beta}$ (h) | 8.0 (4.9 - 14.0) | 3.3 (2.0 - 4.6) | 2.4 (1.4 - 3.9) |
| AUC_{0-12h} (µg/L.h) | 44.8 (20.1 - 68.5) | 52.5 (27.6 - 77.4) | 54.7 (25.4 - 113) |

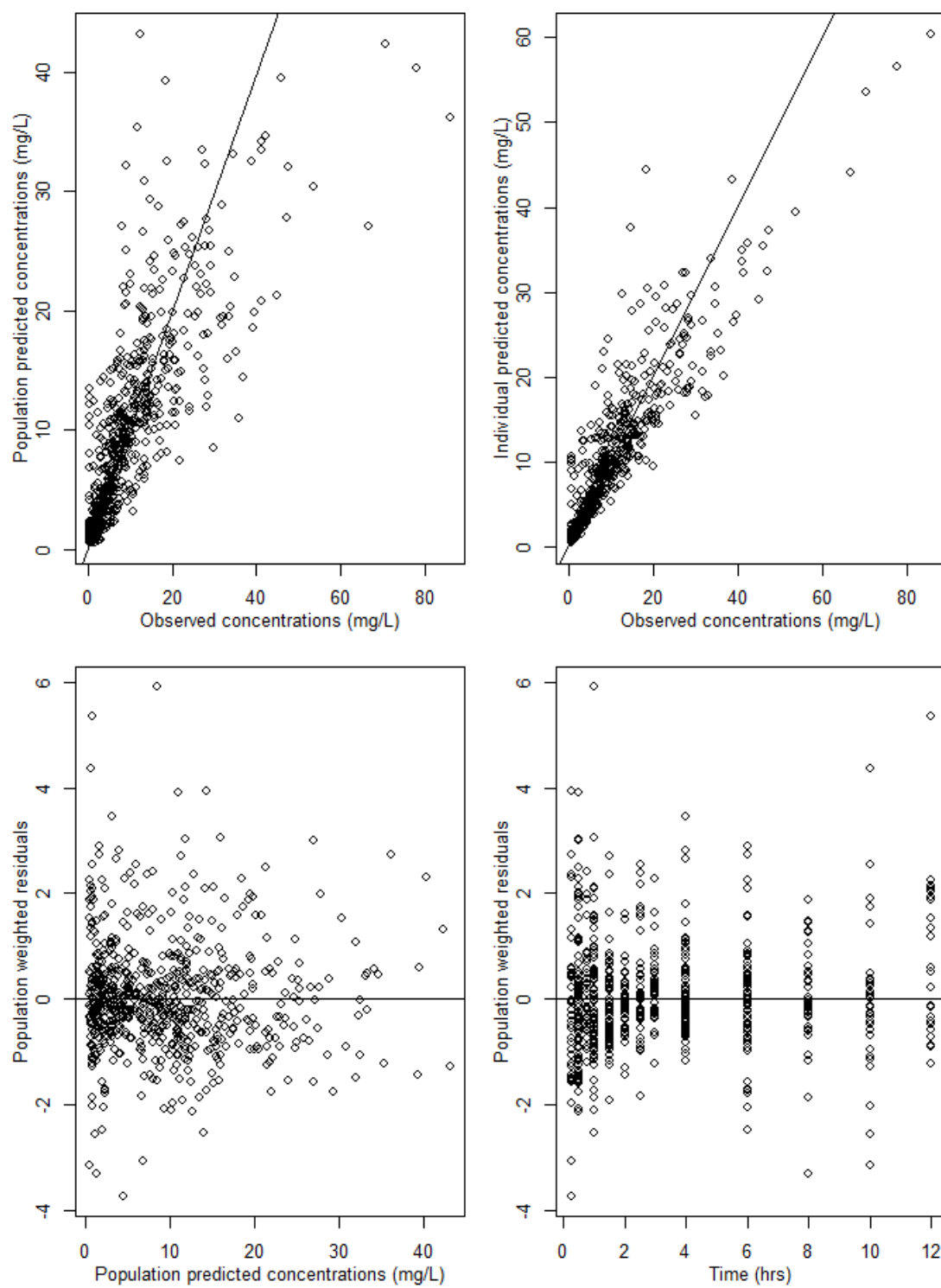


Figure 1

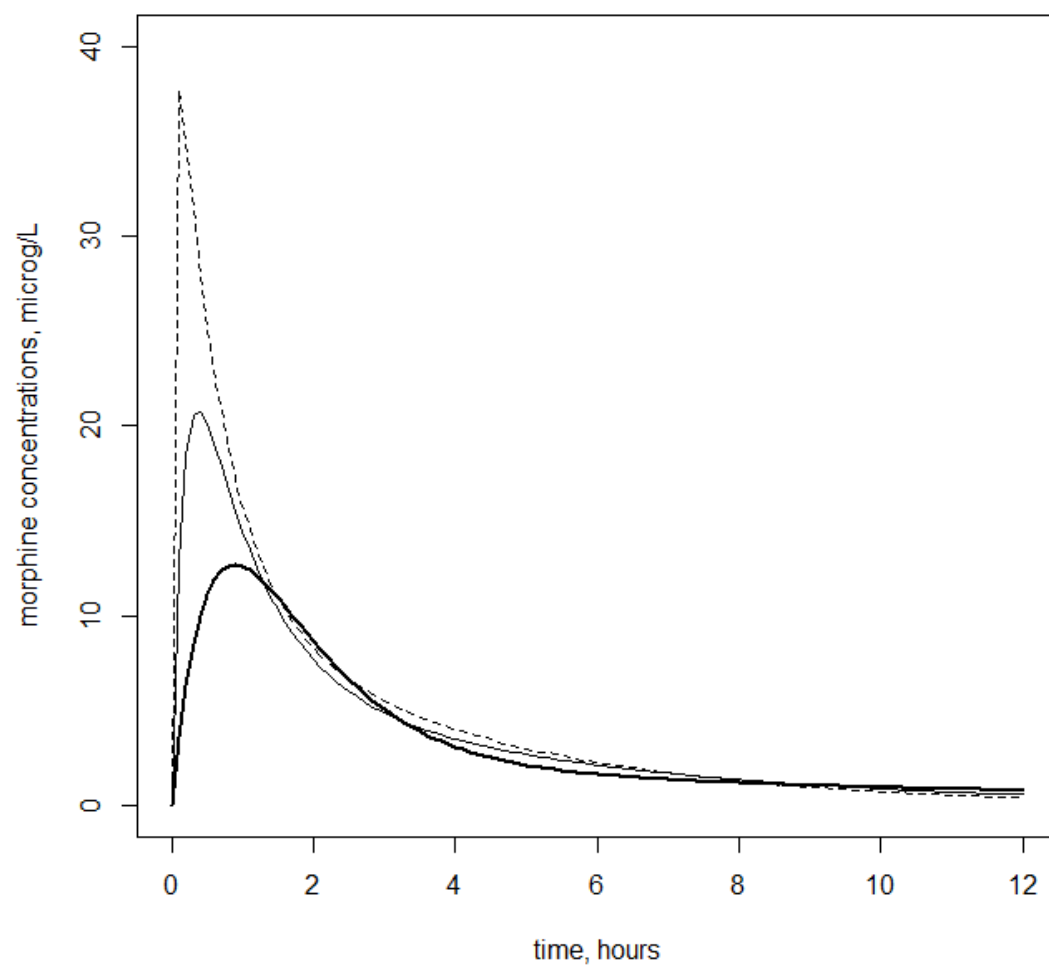


Figure 2

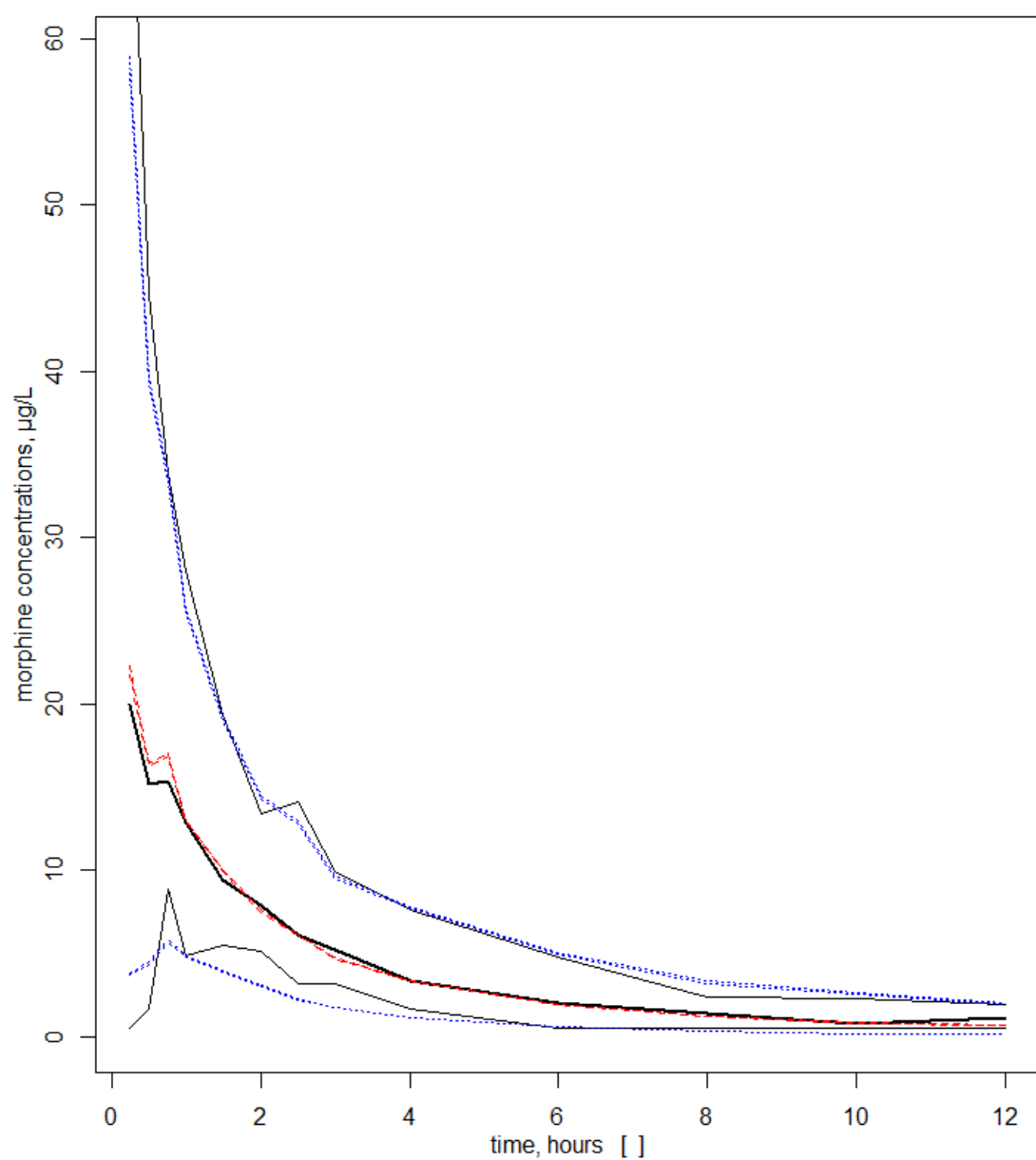


Figure 3

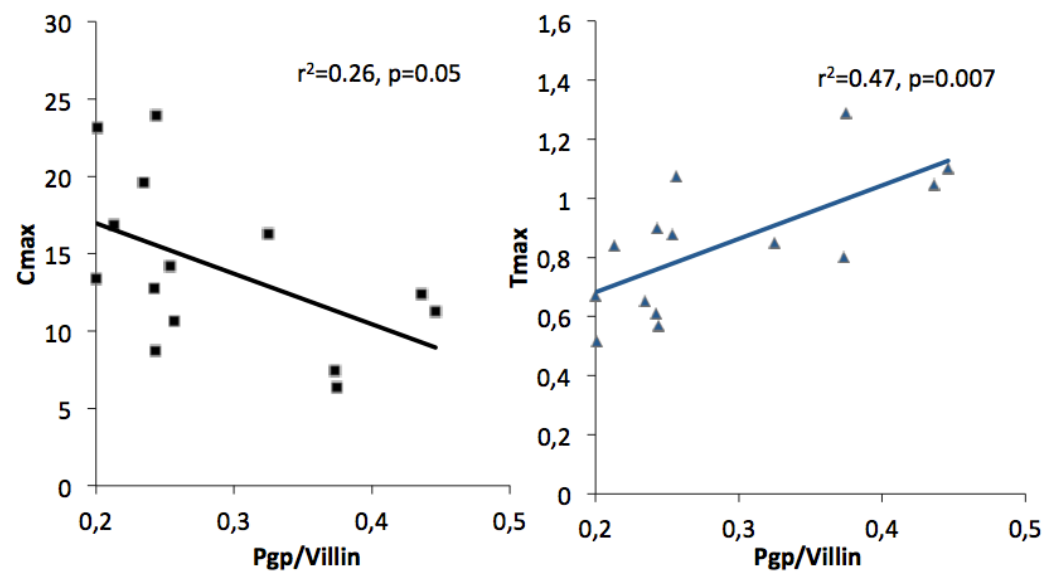


Figure 4

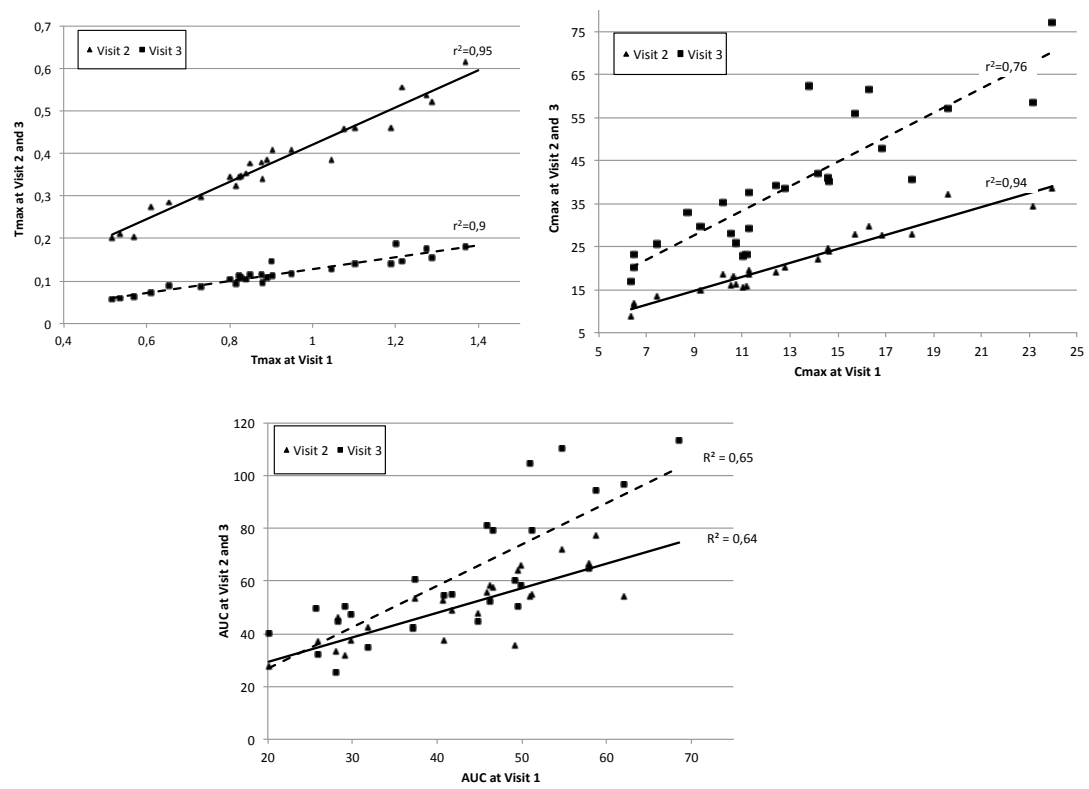


Figure 5

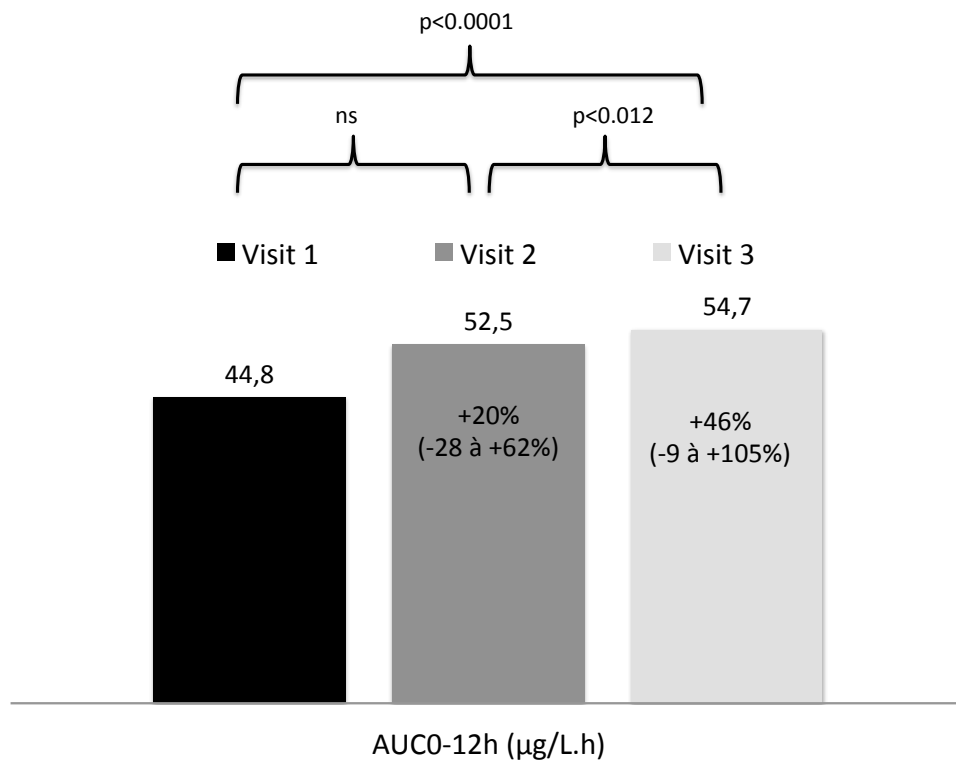


Figure 6.

FIG. 1. Goodness of fit plots based on final model for morphine: population predicted concentrations vs. observed concentrations (top left), individual predicted concentrations vs. observed concentrations (top right), population weighted residuals vs. predicted concentrations (lower left) and population weighted residuals vs. time (lower right).

FIG. 2. Model predicted concentrations for morphine as a function of time for an obese patient with median characteristics before surgery (BMI=44.6 kg/m², bold full line), 15 days after surgery (BMI=41.7 kg/m², thin full line) and 6 months after surgery (BMI=32.5 kg/m², dashed line).

FIG. 3. Evaluation of the final model: comparison between the 5th, 50th, and 95th percentiles obtained from 1,000 simulations (dashed lines) and the 5th, 50th, and 95th percentiles calculated from the observations for morphine concentrations (full line).

FIG 4. Correlations between P-gp content and C_{max} or T_{max}

FIG 5. Correlations between Morphine T_{max}, C_{max} and AUC at Visit 1 and their values at Visit 2 and 3

FIG 6. Morphine exposure at Visit 1, 2 and 3

Manuscrit 7. Analgésie post-opératoire des sujets obèses

La morphine est l'analgésique de choix pour les douleurs post-opératoires.

Les méthodes d'analgésie post-opératoire ont considérablement évolué et leur utilisation a pour objectif de réduire le recours aux morphiniques. Certaines d'entre elles ont spécifiquement fait l'objet de travaux de recherche clinique chez les patients obèses, en particulier en post-opératoire de chirurgie de l'obésité.

Ces travaux cliniques ont fait l'objet d'une revue de la littérature, actuellement soumise à la revue « Obesity Surgery » et under review depuis janvier 2013.

(Article 7.)

Challenges in the Optimisation of Post-operative Pain Management with Opioids in Obese Patients: a Literature Review

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REVIEW

Challenges in the Optimisation of Post-operative Pain Management with Opioids in Obese Patients: a Literature Review

C. Lloret-Linares · A. Lopes · X. Declèves · A. Serrie · S. Mouly · J.-F. Bergmann · S. Perrot

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Abstract An increasing number of obese patients are undergoing surgery, particularly bariatric and orthopaedic surgery. The physiological differences between obese and normal-weight subjects may modify not only anaesthetic requirements during surgery but also post-operative analgesic management, raising a number of challenges in a critical period. In this review, we analyse studies of post-operative pain management with opioids in obese subjects. We discuss the genetic factors common to pain and obesity and the factors potentially modifying opioid pharmacokinetics and pharmacodynamics in obese patients, and we analyse the overall efficacy and safety of opioids for pain management during the post-operative period in obese patients. Both modifications to surgical methods and additional analgesic treatments to decrease the requirement for opioids may

improve early rehabilitation and quality of care and reduce adverse effects in obese patients.

Keywords Morphine · Obesity · Post-operative pain · Surgery · Analgesia

Introduction

With 1.5 billion people worldwide now considered obese (WHO), obesity is a public health problem of growing concern. Obese patients are frequently candidates for bariatric and orthopaedic surgery, and the number of obese patients undergoing surgery is therefore increasing [1–3]. Bariatric surgery appears to be a clinically effective intervention for moderate to severe obesity [4, 5]. The frequency of such surgery is thus steadily increasing, and bariatric procedures are now among the most commonly performed gastrointestinal operations [6]. Obesity also often precedes knee osteoarthritis and is a risk factor for its progression [7–10], so knee surgery is also frequently performed in obese patients [11].

The management of anaesthetic drugs in obese patients has been reviewed elsewhere [12], but few reviews have considered analgesic use in the post-operative period. Indeed the physiological differences between obese and normal-weight subjects are known to modify anaesthetic requirements during surgery, but they may also affect analgesic management after surgery, confronting doctors with major challenges during a critical period.

In this review, we analyse studies of post-operative pain management with opioids in obese subjects. We describe the genetic factors common to pain and obesity and the factors potentially modifying opioid pharmacokinetics and pharmacodynamics in obese patients, and we analyse the overall efficacy and safety of opioids for post-operative pain management in obese patients. An understanding of pain management, particularly with opioids, in obese patients during

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the post-operative period might improve early rehabilitation and quality of care, with a potentially significant impact on the effectiveness of surgery [13].

Does Post-operative Pain in Obese Patients Have Specific Characteristics?

Obesity and Pain: a Common Genetic Background

There are many determinants of human obesity, ranging from purely genetic forms caused by rare mutations (e.g. mutation of the genes encoding leptin or its receptor) to purely behavioural forms. Between these two extremes, there is a whole range of situations, with a frequent interaction of environmental, behavioural and genetic factors. Thus, genetic factors determine susceptibility to the environment, in which behaviour plays a major role in phenotypic expression. Some rare genetic diseases are associated with abnormal pain sensitivity thresholds, abnormal eating behaviour and abnormal energy homeostasis [14, 15]. For example, the Val(66)Met polymorphism of the brain-derived neurotrophic factor (*BDNF*) gene is associated with obesity in various populations and may also modify the pain-modulating signal in patients with chronic pain exposed to an electrical signal [16–18]. Thus, some genetic polymorphisms may be associated with both obesity and effects on analgesic pharmacodynamics in terms of safety and efficacy. We have shown that the G allele of the *OPRM1* gene encoding the mu receptor is more frequent in obese patients than in normal-weight patients [19]. This polymorphism has been associated with a decrease in mechanical pain sensitivity, morphine pain relief, and an increase in the requirement for morphine and fentanyl for pain relief. This polymorphism may also be linked to better tolerance of the active metabolite of morphine, M6G, but no such effect has been demonstrated with morphine [19].

Obesity and Nociceptive Changes

Many studies on pain sensitivity have been performed, on small numbers of patients, with various methods. Miscio et al. explored sensitivity to various experimental pain models in obese subjects without diabetes ($n=21$) and compared the results for these patients with those for normal-weight subjects of similar age [20]. They found a lower sensitivity to vibration, mechanical and heat signals and a lower action potential amplitude in obese subjects than in normal-weight subjects. Furthermore, cold allodynia occurred earlier in obese subjects. We have also reported a significant difference in the sensitivity to and pain thresholds for an electrical signal between obese and normal-weight patients, these differences being unchanged by weight loss due to bariatric

surgery [21]. The pain matcher used in our study produced an electrical signal but, interestingly, its pain thresholds have also been correlated with visceral and joint pains in previous studies [22–24]. Our observations, consistent with those of Miscio et al., suggest that many physiological changes associated with obesity may affect pain pathways. Thus, further studies are required to explore sensory dysfunction in obese patients and its consequences for pain management and analgesic requirements. The use of the pain matcher may be relevant for the prediction of post-operative opioid requirement in obese patients.

Mobilisation and Rehabilitation in Obese Patients: the Importance of Pain Management

Mobilisation and early rehabilitation after surgery are both important and difficult in obese patients. They may help to prevent thromboembolic events, and they also increase the efficacy of orthopaedic and bariatric surgery [25].

Pain is a key issue after orthopaedic and bariatric surgery, and it is, therefore, important to reduce pain intensity in the post-operative period. Opioids have been associated with a significant improvement in rehabilitation after hip replacement and should be used early in the rehabilitation process in obese patients [26].

Obesity is also a risk factor for poor functional outcome after joint replacement surgery [27]. In obese patients, pain should be carefully monitored after surgery to ensure optimal rehabilitation: obesity is a risk factor for longer hospital stay during rehabilitation and physical activity is a predictor of successful weight loss after bariatric surgery [28, 29].

Challenges Relating to Opioid Use in Obese Patients

Is Drug Metabolism Different in Obese Patients?

It is becoming increasingly clear that the pharmacokinetics of analgesics differs between obese and normal-weight patients. Obesity is defined as an excess in body fat, and this excess fat may modify the distribution in the body of highly lipophilic drugs. Other physiological changes are also associated with obesity and may interfere with the pharmacokinetics of analgesics.

Obese patients have a greater muscle mass, total body water and plasma volume than normal-weight patients [30, 31]. Blood volume, cardiac output and kidney and liver size also increase with weight, but not in a linear fashion, suggesting that these physiological changes are less important than the excess body weight itself [32, 33]. The liver and heart increase in size to a greater extent than the kidney,

and these increases in size are due to fat accumulation, as demonstrated by the rapid decrease in the sizes of these organs with weight loss [34]. Severe obesity is associated with increases in systemic blood pressure, renal plasma blood flow, glomerular filtration rate and albumin excretion rate [35, 36]. The glomerular capillary bed is subject to a transcapillary hydrostatic pressure gradient, resulting in high levels of hyperfiltration, potentially causing obesity-related renal failure and nephrotic syndrome [37].

These physiological alterations may modify various pharmacokinetic parameters. Indeed the increases in plasma and water volumes and in fat and muscle mass may modify the distribution volumes of most drugs, with potential consequences for their concentration profiles over time and for their pharmacodynamics. The increase in organ size and cardiac output may influence drug clearance and, thus, exposure.

Obesity is also associated with a higher risk of non-alcoholic fatty liver disease and steatohepatitis, chronic low-grade inflammation, hormonal changes and adipokine production [38–40]. The regulation of enzymes involved in drug metabolism is an important determinant of drug pharmacokinetics and efficacy. The genes encoding these enzymes are induced by exogenous and endogenous compounds through the activation of nuclear receptors, leading to the induction of their targets, including genes encoding drug metabolism enzymes and transporters [41–43].

The effect of obesity on the expression of drug-metabolising enzyme genes has been studied in the livers of animals and humans, mostly in studies focusing on fatty liver diseases [44]. The expression of CYP3A4 and CYP1A2 in the liver may decrease in fatty liver disease in humans, whereas that of CYP2E1, 2C9, 2A6 and enzymes involved in phase 2 glucuronidation may increase. The expression of intestinal and renal drug-metabolising enzymes has also been studied in animals, but with conflicting results [45–47]. Brill et al. recently provided an overview of clinical studies investigating drugs for which clearance is dependent on phase 1 or 2 reactions or liver blood flow, comparing obese and non-obese patients [48]. Clearance levels were lower for cytochrome P450 (CYP) 3A4 substrates but higher for drugs metabolised principally by uridine diphosphate glucuronosyltransferase (UGT), *N*-acetyltransferase or CYP2E1 in obese than in non-obese patients. A trend towards higher levels of clearance in obese patients was observed for drugs metabolised by CYP1A2, CYP2C9, CYP2C19 and CYP2D6.

The pharmacokinetic changes observed in obese patients thus depend on the degree of lipophilicity of the drug and the pathway by which it is metabolised [49]. There is currently no single body size descriptor for the personalisation of drug dosage, and systematic dose adjustment on the basis of total body weight can lead to toxicity [32].

Opioid Pharmacokinetics and Pharmacodynamics in Obese Patients

It is therefore difficult to predict the pharmacokinetics of analgesics. For example, as morphine is extensively metabolised by UGT2B7, higher morphine clearance may be observed in obese patients, resulting in lower levels of pain relief for the same dose as in normal-weight patients [50]. However, morbid obesity had no significant effect on morphine requirement in a study evaluating the doses administered with a patient-controlled analgesia (PCA) system in 46 patients undergoing elective or gastric bypass surgery [51]. By contrast, as CYP3A4 is responsible for sufentanil metabolism, obese patients may have higher concentrations of sufentanil due to a longer elimination half-life, a lower level of hepatic clearance and an increased terminal half-life [52]. However, a higher distribution volume may reduce the concentrations. Schwartz et al. compared sufentanil pharmacokinetics after a 4- μ g/kg single dose in moderately obese (94.1 ± 14 kg) and normal-weight (70 ± 13 kg) patients [53]. The high distribution volume was positively correlated with excess weight in obese patients and persisted even after adjustment for total body weight ($1,254 \pm 326$ versus 745 ± 292 ml/kg). However, this drug had a longer half-life in obese patients (208 ± 82 min versus 135 ± 42 min) but a similar clearance, suggesting that obesity had a much greater impact on the tissue distribution of sufentanil than on its hepatic clearance [53]. Moreover, Slepchenko et al. studied sufentanil pharmacokinetics in obese patients and developed a pharmacokinetic model for dose prediction [54]. This pharmacokinetic model correctly predicted sufentanil pharmacokinetics in obese patients up to a BMI of 40 kg/m². Beyond this BMI, there was a risk of overdose, and the authors concluded that a two-compartment model might better predict the pharmacokinetics of the drug.

Fentanyl is also metabolised by CYP3A4 and is more lipophilic than sufentanil [52]. Phenotypic studies with drugs metabolised by CYP3A4 as probes have demonstrated a decrease in drug metabolism in obese patients that might lead to a lower requirement for fentanyl [48]. Fentanyl titration for analgesia without respiratory side effects in a population of patients of various weights showed that fentanyl requirement increases with weight, but not in a linear manner [55]. For example, the dose for a patient weighing 200 kg should not be adjusted on the patient's actual body weight but on a weight of 109 kg, corresponding to the patient's ideal weight plus some of the excess. Fentanyl may therefore be considered to be preferentially distributed in the fat mass. Adjustments of the dose on the basis of lean mass may therefore result in an underestimation of the dose required, whereas adjustment on total body weight may overestimate the required dose. This study thus highlighted the risk of overdose associated with adjustment

on the basis of weight. Interestingly, the pharmacokinetics of fentanyl is related to its pharmacodynamics, indicating an absence of weight-related resistance to fentanyl.

These examples illustrate the difficulty of predicting the differences in analgesic requirements between lean and obese subjects, even when a pharmacokinetic model is used, and the need to continue clinical investigations for the individualisation of prescriptions for analgesic drugs with a narrow therapeutic index.

Obesity is associated with an increase in the risk of sleep apnoea syndrome and alveolar hypoventilation, which may itself be associated with an increase in the risk of respiratory depression with level III analgesics [56]. This risk is very familiar to anaesthetists, who have reported frequent episodes of desaturation in post-operative surgery and an increase in the risk of difficult tracheal extubation in obese patients [57]. However, Ahmad et al. recently reported that the number of post-operative desaturation episodes was similar in obese patients with and without sleep apnoea syndrome, suggesting that sleep apnoea is not an independent risk factor for desaturation in obese patients, and that the treatment of sleep apnoea syndrome cannot entirely eliminate the risk of post-operative desaturation [58].

Post-operative Pain Management with Opioids in Obese Patients

Future clinical research on post-operative analgesia in obese patients should therefore take into account the factors affecting analgesic drugs as discussed earlier. This literature review aims to guide the clinical research and practice of physicians by identifying research studies investigating post-operative analgesia strategies in obese patients.

Methods

Relevant articles were identified in the Pubmed and EMBASE resources with the following keywords: (("Overweight" [Mesh]) OR "Obesity" [Mesh]) AND "Analgesia"[Mesh].

We limited our search to studies in adults, published in English, in peer-reviewed journals. We retrieved 129 references from Pubmed and 141 from EMBASE. Clinical cases and clinical studies or reviews on anaesthesia during pregnancy were excluded. Other publications were excluded because it was clear from the title that they did not fulfil the selection criteria. Additional publications were identified from review articles, and we selected those in which opioid requirement and pharmacodynamics were evaluated. At the end of this process, 22 references were retained and are described here.

All the work on post-operative analgesia in obese patients concerned subjects undergoing bariatric surgery, in which

the replacement of median incision by the use of laparoscopy has considerably reduced the need for post-operative analgesics [59]. We thought it would be interesting to consider studies written both before and after the advent of laparoscopy as these studies frequently reported and compared the efficacy and safety of methods that may still be relevant, particularly for reducing post-operative opioid requirement. The principal results obtained in studies comparing methods of post-operative analgesia are reported in Table 1, which includes details of the surgical method used and of the efficacy and safety of the analgesic methods. Clinical studies with few data relating to opioid requirement or rehabilitation are not included in this table.

Route of Opioid Administration

A randomised double-blind study in 30 patients undergoing gastroplasty compared the effects of intramuscular and epidural morphine [60]. Patients receiving post-operative epidural morphine were able to sit, stand up and walk sooner than those that did not. They also needed lower doses of ketobemidone, another opioid analgesic. The lower level of sleepiness, more rapid mobilisation and lower frequency of respiratory events in these patients facilitated early physical therapy to decrease post-operative respiratory complications. The early recovery of physical, lung and bowel functions after surgery significantly decreased the length of hospital stay in patients receiving epidural morphine. In addition, a high incidence of acute urinary retention was reported in both groups, but excessive sleepiness was observed only in patients treated with intramuscular morphine. Similar improvements were observed in patients receiving a dose of intrathecal morphine (5 mg) added to parenterally administered morphine, as shown by comparisons with patients who did not receive intrathecal morphine [61].

Kyzer et al. subsequently compared the advantages of morphine PCA ($n=11$) with those of intramuscular morphine ($n=12$) after vertical gastroplasty [62]. Cumulative morphine use in the first day after surgery in the PCA group was twice that in the intramuscular morphine group (52.7 versus 24.5 mg). However, the sedative and analgesic effect was greater in the PCA group. Choi et al. reported their experience in the monitoring of 25 obese patients using intravenous morphine PCA following gastric bypass (GBP) by median laparotomy [63]. The daily use of morphine was 49.1 mg on the first day and 36.6 mg on the second. Twenty-four of the 25 patients were satisfied with pain relief from the first day. The visual analogue scale showed a mean pain score of 5.4 ± 2.1 on the first day after surgery, falling below 4 thereafter. No abnormalities of arterial oxygen saturation or vital signs were reported. Drowsiness was the most common adverse event; only one

Table 1 Opioids for post-operative pain management in obese patients

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety | |
|---------------------------------|---|---|---|---|---|
| Role of route of administration | | | | | |
| 30 | Rawal, 1984 [60] Gastroplasty by laparotomy | | | | |
| 15 | IM morphine plus epidural saline | Morphine=66.1±6.4 mg/36 h Ketobemidone use=23.5±4.0 mg Duration of analgesia=220 min | VAS score<2 after 6 h Stand up: 1,359 min/walk with assistance 1,741 min Walk without assistance 2,049 min Length of hospital stay=7.1±0.3 days | Nausea=20 % | Pulmonary complication=40 % Urinary retention=40 % |
| 15 | Epidural morphine plus IM saline | Morphine=9.3±1.2 mg*/36 h Ketobemidone use=9.7±2.7 mg* Duration of analgesia=750 min* | VAS score<2 after 6 h Stand up: 880 min/walk with assistance 1,116 min* Walk without assistance 1,153 min* Length of hospital stay=9.0±0.6 days* | Nausea=13 % | Pulmonary complication=13 % Urinary retention=53 % |
| 12 | Brodsky, 1984 [61] Gastric banding by laparotomy | Morphine | | | |
| 6 | Preventive epidural morphine + IV morphine | d1=1.8 (0–5) mg d2=5.8 (0–15) mg | Mobilisation=15.5 (5–27) h Length of hospital stay=5.5 (4–7) days | Two pruritus | PaO ₂ d1=67.7/d2=70.1 mmHg PaCO ₂ d1=36.2/d2=37.5 mmHg |
| 6 | IV morphine | d1=19.8 (8–35) mg* d2=40.6 (20–100) mg* | Mobilisation=26.5 (5–46) h Length of hospital stay=6.3 (5–8) days | One pruritus | PaO ₂ d0=64.7/d1=64 mmHg PaCO ₂ d1=40.2/d2=38.4 mmHg |
| 23 | Kyzer, 1995 [62] Gastric banding by laparotomy | Morphine d1 | | | |
| 12 | PCA | Morphine=52.7±1.8 mg | No pain=64 %/moderate=27 %/severe pain=9 % Length of hospital stay=7.2 (4–12) days | Nausea and pruritus frequencies similar | Drowsy=41 % Wound infection=4 |
| 11 | IM pethidine | Morphine=24.5±3.42 mg* | No pain=53 %/moderate=22 %/severe pain=25 %* Length of hospital stay=5 (3–11) days | | Drowsy=24 %* Wound infection=1* |
| 25 | Choi, 2000 [63] GBP by laparotomy | Morphine d1=44.2±28.7 mg | 24 subjects satisfied | One nausea and vomiting | PaO ₂ d0=88.4/d1=76.1 mmHg |
| | IV morphine PCA | d2=49.1±27.4 mg | VAS d0/thereafter=5.4/<4 | Four pruritus | PaCO ₂ d0=39.8/d1=42 mmHg |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|---|---|----------------------------------|---|---|
| | | Duration of PCA use 3.1±0.6 days | Length of hospital stay=5.8±0.9 days | Mild sedation=5 at d1, 3 at d2 |
| Association of different opioids or anaesthetic drugs | | | | |
| 27 | Michaloudis, 2000 [64] Gastric banding by laparotomy Intrathecal (bupivacaine+fentanyl) and IV tenoxicam | | VAS at rest =1–3 VAS on coughing or after mobilization=2–4 All patients mobile in bed 6 h/in a chair at 8 h | Five pruritus 15–36 breaths/min |
| 133 | Charghi, 2003 [65] GBP by laparotomy | | Pain score were similar between groups | Wound infection |
| 40 | PCA morphine IV | | Mobilization=38±17 h Length of hospital=130±46 h | Nausea=40 % Pruritus=20 % |
| 46 | Epidural intermittent doses of morphine | Mobilization=36±14 h | Nausea=26 % | 40 %* |
| 35 | Morphine | | Length of hospital=115±14 h Mobilization=36±17 h Length of hospital=113±17 h | Pruritus=26 % Nausea=29 % Pruritus=26 % |
| 11 | Epidural continuous infusions of bupivacaine/fentanyl | | Mobilization=36±12 h Length of hospital=118±12 h | Nausea=18 % Pruritus=27 % |
| 87 | Pathiraja, 2010 [66] GBP by laparotomy | | | |
| 62 | Thoracic epidural catheter (lidocaine,ropivacaine, 0.125 % bupivacaine or ropivacaine with fentanyl for 96 h) | | Pain score until 96 h were similar | Nausea/vomiting=41.9 % Urinary retention=12.9 % |
| 25 | Bupivacaine pump during 72 h+ morphine PCA | | | Pruritus=14.5 % Nausea/vomiting=60 % Wound infection=9.7 % Urinary retention=4 % |
| 40 | De Baerdemaeker, 2007 [67] Laparoscopic gastric banding | Time to first analgesic request | VAS t0,30,60,120 min after admission to the PACU | Pruritus=8 % Wound infection=4 % |
| 20 | Sufentanil | 70 min (50) | 3.3/4.4/4.0/2.8 | No between-group differences in |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety | |
|------------------------------|---|--|---|---|--|
| 20 | Remifentanyl | 29 min (15)* Cumulative morphine consumption was significantly higher at 30, 60 and 120 min in group R. Thereafter, no significant difference was noted | 4.3/4.5/3.9/2.4* | spirometry measurements | |
| 100 | Bidgoli, 2011 [68] | | | | |
| 50 | Laparoscopic gastric banding Sufentanyl | PCA piritramide Greater piritramide consumption during the first 4 h (quantity not reported, $p<0.001$) than in the remifentanyl group | VAS at awakening=2 ±3 | 19 nausea | |
| 50 | Remifentanyl | | Length of PACU stay=119 ±27 min VAS at awakening=6 ±3 Length of PACU stay=119 ±35 min | Three vomiting 22 nausea Three vomiting | |
| 40 | Cottam, 2007 [69] Laparoscopic GBP | Equivalent use of mepiridine | Overall pain scores were similar | Nausea score and antiemetic use were similar between groups | |
| 20 | Bupivacaine pain pump +Meperidine PCA until 19:00 h on the evening of surgery + oxycodone-paracetamol thereafter/ 4 h | PACU=42±21 mg From leaving the PACU to 06:00 h=129±90 mg* | | | |
| 20 | Mepiridine PCA | PACU=61±35 mg From leaving the PACU to 06:00 h=217±99 mg | | | |
| 40 | Kamal, 2008 [70] Upper abdominal surgery | 48 h morphine PCA | VAS scores were similar | Nausea=8 Pruritus=1 | Episodes of desaturation=12 FVC at 24/48 h =46/58 % FEV ₁ at 24/48 h =60/62 % |
| 20 | Morphine | 84 ±9 mg | | Nausea=2 Pruritus=1 | Episodes of desaturation=5* FVC at 24/48 h =62/77 %* FEV ₁ at 24/48 h =81/87 %* |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|---|--|--|--|--------------------|
| Association with other analgesic procedures | | | | |
| 50 | Sollazzi, 2009 (abstract) [71] Open biliopancreatic diversion | Fentanyl F doses during surgery Tramadol T in PACU | | Time to extubation |
| 23 | Clonidine and ketamine before induction | F=3.8±0.3 g/kg T=138±57 mg | Lower VAS during the first 6 h | 15.1±5 min |
| 27 | Standard anaesthesia | F=5.0±0.2 g/kg* T=252±78 mg* | | 28.2±6 min* |
| 20 | Kamelgard, 2005 (abstract) [73] GBP by laparotomy | | | |
| 10 | Placebo | | | |
| 10 | Preoperative 30 mg IV ketorolac +0.25 % bupivacaine in the rectus fascia before closing | Narcotic use was reduced by 40 % | Length of hospital stay (2.9 days) similar | |
| 20 | Feld, 2006 [74] GBP by laparotomy Intraoperative analgesia | PACU morphine (mg, 2 h) 14.6±5.9 | VAS t1 h and t2 h VAS t1=7 (5.25-8.75)/ VAS t2=6.0 (5.0-7.0) | |
| 10 | Fentanyl 0.5 µg/kg bolus, 0.5 µg/kg/h | 6.1±3.5* | VAS t1=3.5 (0-5.0)* / VAS t2=2.0 (2.0-3.5)* | |
| 10 | Dexmedetomidine 0.5 µg/kg bolus, 0.4 µg/kg/h | | | |
| 80 | Tufanogullari, 2008 [75] Laparoscopic GBP and gastric banding | PCA fentanyl F (µg in PACU) Morphine M d1 | Pain scores in PACU were similar | |
| 20 | Control | F=187±99 mg M d1=49±26 mg M d2=16±14 mg | Length of PACU stay=104 ±33 min Mobilisation=10±7 h Length of hospital stay=1.5 ±3 days | Nausea=65 % |
| 20×3 | Dexmedetomidine 0.2/0.4/0.8 µg/kg/h | F=113±85/ 108±67/ 120±78* M d1=37±26/38±34/39±27 M d2=12±7/24±37/24±28 | Length of PACU stay=81±31 to 87±24* Mobilisation=12±9 h/8±7/9±6 h Length of hospital stay=1±1/1±3/1±1 days | Nausea=25/30/45 % |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety | |
|-------------------------------------|---|--|---|-----------------------------|--------------------------|
| 80 | Cabrera Schulmeyer, 2010 [76] Laparoscopic sleeve gastrectomy Intra-operative and post-operative dose of ketoprofen, intraoperative and post-operative morphine bolus | IV morphine | Mean 24 h-VAS score | Mean ondansetron use | Post-operative shivering |
| 41 | Placebo | 23.07 ±9.57 mg | 5 | <i>n</i> =19, 6.06±4.45 mg | 19 % |
| 39 | Pregabalin (150 mg) 2 hours before surgery | 11.5 ±7.93 mg* | 3* | <i>n</i> =10, 3.07±1.63 mg* | 0 % |
| The role of the surgical procedures | | | | | |
| 53 | El Shobary, 2006 [59] GBP | | | | |
| 33 | Laparoscopic group | d1=22±17 mg d2=8±13 mg* | VAS at rest d1=3±2/d2=2±2 Readiness for discharge=3±1 days Length of hospital stay=4±1 days | Nausea=39 % | |
| 20 | Open group | d1=47±23 mg d2=30±27 mg* | VAS at rest d1=4±2/ d2=3±2* Readiness for discharge=4±2 days* Length of hospital stay=5±2 days* | Nausea=20 % | |
| 50 | Hamza, 2005 (abstract) [80] Laparoscopic GBP | PACU morphine (mg, 2 h) | Higher quality of recovery 48 h after surgery | | |
| 25 | Room temperature insufflation gases | 10±5 | 13/25 | | |
| 25 | Warmed and humidified intraperitoneal gases | 5±4 | 15/25 | | |
| 30 | Savel, 2005 [81] Laparoscopic GBP | Morphine (mg) | VAS score | | |
| 15 | Room temperature insufflation gases | 3 h=12±6/ 6 h=20±11 d1=41±27/d2=44±27 | 3 h=4.3±2.8/ 6 h=3.7±2.1 d1=3.8±1.7/d2=1.6±1.6 Length of hospital stay=4.0±0.9 days | | |
| 15 | Warmed and humidified intraperitoneal gases | 3 h=10±4/6 h=19±6 d1=36±17/d2=43±25 | 3 h=4.3±1.9/ 6 h=4.2±1.9 d1=2.5±2.2/d2=2.3±3.0 Length of hospital stay=3.2±0.4 days* | | |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety | |
|---------------------------------|---|---|---|---|---|
| 48 | Raman, 2011 [82] Laparoscopic gastric banding | PCA morphine use during hospitalisation/ Mean duration of analgesic use | All patients were discharged home the day after surgery | | |
| 24 | Single-incision laparoscopic surgery | 33±19.22 mg/2 d | Mean period to return to work =9.5 days | | |
| 24 | Conventional laparoscopy | 49±23.78 mg*/5 d* | Mean period to return to work=11 days | | |
| Role of route of administration | | | | | |
| 30 | Rawal, 1984 [60] Gastroplasty by laparotomy | | | | |
| 15 | IM morphine plus epidural saline | Morphine=66.1±6.4 mg/36 h Ketobemidone use=23.5±4.0 mg Duration of analgesia=220 min | VAS score<2 after 6 h Stand up 1,359 min/walk with assistance 1,741 min Walk without assistance 2,049 min Length of hospital stay=7.1 ±0.3 days | Nausea=20 % | Pulmonary complication=40 % Urinary retention=40 % |
| 15 | Epidural morphine plus IM saline | Morphine=9.3±1.2 mg*/36 h Ketobemidone use=9.7±2.7 mg* Duration of analgesia=750 min* | VAS score<2 after 6 h Stand up 880 min/walk with assistance 1,116 min* Walk without assistance 1,153 min* Length of hospital stay=9.0 ±0.6 days* | Nausea=13 % | Pulmonary complication=13 % Urinary retention=53 % |
| 12 | Brodsky, 1984 [61] Gastric banding by laparotomy | Morphine | | | |
| 6 | Preventive epidural morphine + IV morphine | d1=1.8 (0–5) mg d2=5.8 (0–15) mg | Mobilisation=15.5(5–27) h Length of hospital stay=5.5 (4–7) days | Two pruritus | PaO2d1=67.7/d2=70.1 mmHg PaCO2d1=36.2/d2=37.5 mmHg |
| 6 | IV morphine | d1=19.8 (8–35) mg* d2=40.6 (20–100) mg* | Mobilisation=26.5 (5–46) h Length of hospital stay=6.3 (5–8) days | One pruritus | PaO2d0=64.7/d1=64 mmHg PaCO2d1=40.2/d2=38.4 mmHg |
| 23 | Kyzer, 1995 [62] Gastric banding by laparotomy | Morphine d1 | | | |
| 12 | PCA | Morphine=52.7±1.8 mg | No pain=64 %/moderate=27 %/severe pain=9 % Length of hospital stay=7.2 (4–12) days | Nausea and pruritus frequencies similar | Drowsy=41 % Wound infection=4 |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|---|--|--|---|---|
| 11 | IM pethidine | Morphine=24.5±3.42 mg* | No pain=53 %/moderate=22 %/severe pain=25 %* Length of hospital stay=5 (3–11) days | Drowsy=24 %* Wound infection=1* |
| 25 | Choi, 2000 [63] GBP by laparotomy IV morphine PCA | Morphine d1=44.2±28.7 mg d2=49.1±27.4 mg Duration of PCA use 3.1±0.6 days | 24 subjects satisfied VAS d0/thereafter=5.4/<4 Length of hospital stay=5.8±0.9 days | One nausea and vomiting Four pruritus PaO ₂ d0=88.4/d1=76.1 mmHg PaCO ₂ d0=39.8/d1=42 mmHg Mild sedation=5 at d1, 3 at d2 |
| Association of different opioids or anaesthetic drugs | | | | |
| 27 | Michaloudis, 2000 [64] Gastric banding by laparotomy Intrathecal (bupivacaine+fentanyl) and IV tenoxicam | | VAS at rest =1–3 VAS on coughing or after mobilization=2–4 All patients mobile in bed 6 h/in a chair at 8 h | Five pruritus 15–36 breaths/min |
| 133 | Charghi, 2003 [65] GBP by laparotomy | | Pain score was similar between groups Mobilization=38±17 h Length of hospital=130±46 h | Wound infection 15 % |
| 40 | PCA morphine IV | | Nausea=40 % Pruritus=20 % | |
| 46 | Epidural intermittent doses of morphine | Mobilization=36±14 h | Nausea=26 % Length of hospital=115±14 h Mobilization=36±17 h Length of hospital=113±17 h | 40 %* Pruritus=26 % Nausea=29 % Pruritus=26 % |
| 35 | Morphine | | Mobilization=36±12 h Length of hospital=118±12 h | Nausea=18 % Pruritus=27 % 36 %* |
| 11 | Epidural continuous infusions of bupivacaine/fentanyl | | | |
| 87 | Pathiraja, 2010 [66] GBP by laparotomy | | | |
| 62 | Thoracic epidural catheter (lidocaine,ropivacaine, 0.125 % bupivacaine or ropivacaine with fentanyl for 96 h) | | Pain score until 96 h was similar | Nausea/vomiting=41.9 % Urinary retention=12.9 % Pruritus=14.5 % Wound infection=9.7 % |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|------------------------------|--|--|---|--|
| 25 | Bupivacaine pump during 72 h+ morphine PCA | | | Nausea/vomiting=60 % Pruritus=8 % Urinary retention=4 % Wound infection=4 % |
| 40 | De Baerdemaeker, 2007 [67] Laparoscopic gastric banding | Time to first analgesic request | VAS t0, 30, 60, 120 min after admission to the PACU | |
| 20 | Sufentanil | 70 min (50) | 3.3/4.4/4.0/2.8 | No between-group differences in spirometry measurements |
| 20 | Remifentanil | 29 min (15)* Cumulative morphine consumption was significantly higher at 30, 60 and 120 min in group R. Thereafter, no significant difference was noted | 4.3/4.5/3.9/2.4* | |
| 100 | Bidgoli, 2011 [68] Laparoscopic gastric banding | PCA piritramide | | |
| 50 | Sufentanil | Greater piritramide consumption during the first 4 h (quantity not reported, $p<0.001$) than in the remifentanil group | VAS at awakening=2 ±3 | 19 nausea |
| 50 | Remifentanil | | Length of PACU stay=119 ±27 min VAS at awakening=6 ±3 Length of PACU stay=119 ±35 min | Three vomiting 22 nausea Three vomiting |
| 40 | Cottam, 2007 [69] Laparoscopic GBP | Equivalent use of mepiridine | Overall pain scores were similar | Nausea score and antiemetic use were similar between groups |
| 20 | Bupivacaine pain pump +Meperidine PCA until 19:00 h on the evening of surgery. + oxycodone-paracetamol thereafter/ 4 h | PACU=42±21 mg From leaving the PACU to 06:00 h= 129±90 mg* | | |
| 20 | Mepiridine PCA | PACU=61±35 mg From leaving the PACU to 06:00 h= 217±99 mg | | |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|---|--|---|---|--|
| 40 | Kamal, 2008 [70] Upper abdominal surgery | 48 h morphine PCA | VAS scores were similar | Nausea=8 Pruritus=1 Episodes of desaturation=12 FVC at 24/48 h =46/58 % FEV ₁ at 24/48 h =60/62 % |
| 20 | Morphine | 84 ±9 mg | | Nausea=2 Pruritus=1 Episodes of desaturation=5* FVC at 24/48 h =62/77 %* FEV ₁ at 24/48 h =81/87 %* |
| Association with other analgesic procedures | | | | |
| 50 | Sollazzi, 2009 (abstract) [71] Open biliopancreatic diversion | Fentanyl F doses during surgery Tramadol T in PACU | | Time to extubation |
| 23 | Clonidine and ketamine before induction | F=3.8±0.3 g/kg T=138±57 mg | Lower VAS during the first 6 h | 15.1±5 min |
| 27 | Standard anaesthesia | F=5.0±0.2 g/kg* T=252±78 mg* | | 28.2±6 min* |
| 20 | Kamelgard, 2005 (abstract) [73] GBP by laparotomy | | | |
| 10 | Placebo | | | |
| 10 | Preoperative 30 mg IV ketorolac | Narcotic use was reduced by 40 % | Length of hospital stay (2.9 days) similar | |
| | +0.25 % bupivacaine in the rectus fascia before closing | | | |
| 20 | Feld, 2006 [74] GBP by laparotomy | | | |
| | Intraoperative analgesia | PACU morphine (mg, 2 h) | VAS t1 h and t2 h | |
| 10 | Fentanyl 0.5 µg/kg bolus, 0.5 µg/kg/h | 14.6±5.9 | VAS t1 =7 (5.25–8.75)/ VAS t2 =6.0 (5.0–7.0) | |
| 10 | Dexmedetomidine 0.5 µg/kg bolus, 0.4 µg/kg/h | 6.1±3.5* | VAS t1 =3.5 (0–5.0)*/ t2 =2.0 (2.0–3.5)* | |
| 80 | Tufanogullari, 2008 [75] Laparoscopic GBP and gastric banding | PCA fentanyl F (µg in PACU) | | |
| | | Morphine M d1 | Pain scores in PACU were similar | |
| 20 | Control | F=187±99 mg | Length of PACU stay=104 ±33 min | Nausea=65 % |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|-------------------------------------|--|--|---|--|
| 20×3 | Dexmedetomidine 0.2/0.4/0.8 µg/kg/h | M d1=49±26 mg M d2=16±14 mg F=113±85/ 108±67/ 120±78* M d1=37±26/38±34/39±27 M d2=12±7/24±37/24±28 | Mobilisation=10±7 h Length of hospital stay=1.5±3 days Length of PACU stay=81±31 to 87±24* Mobilisation=12±9 h/8±7/9±6 h Length of hospital stay=1±1/1±3/1±1 days | Nausea=25/30/45 % |
| 80 | Cabrera Schulmeyer, 2010 [76] Laparoscopic sleeve gastrectomy Intra-operative and post-operative dose of ketoprofen, intraoperative and postoperative morphine bolus | IV morphine | Mean 24 h—VAS score | Mean ondansetron use Post-operative shivering |
| 41 | Placebo | 23.07 ±9.57 mg | 5 | <i>n</i> =19, 6.06±4.45 mg 19 % |
| 39 | Pregabalin (150 mg) 2 h before surgery | 11.5 ±7.93 mg* | 3* | <i>n</i> =10, 3.07±1.63 mg* 0 % |
| The role of the surgical procedures | | | | |
| 53 | El Shobary, 2006 [59] GBP | | | |
| 33 | Laparoscopic group | d1=22±17 mg d2=8±13 mg* | VAS at rest d1=3±2/d2=2±2 Readiness for discharge=3±1 days Length of hospital stay=4±1 days | Nausea=39 % |
| 20 | Open group | d1=47±23 mg d2=30±27 mg* | VAS at rest d1=4±2/ d2=3±2* Readiness for discharge=4±2 days* Length of hospital stay=5±2 days* | Nausea=20 % |
| 50 | Hamza, 2005 (abstract) [80] Laparoscopic GBP | PACU morphine (mg, 2 h) | Higher quality of recovery 48 h after surgery | |
| 25 | Room temperature insufflation gases | 10±5 | 13/25 | |
| 25 | Warmed and humidified intraperitoneal gases | 5±4 | 15/25 | |
| 30 | Savel, 2005 [81] Laparoscopic GBP | Morphine (mg) | VAS score | |

Table 1 (continued)

| Number of patients, <i>n</i> | Author, year Surgical method Analgesic method studied | Opioid requirement | Efficacy | Safety |
|------------------------------|---|--|--|--------|
| 15 | Room temperature insufflation gases | 3 h = 12 ± 6/ 6 h = 20 ± 11 d1 = 41 ± 27/d2 = 44 ± 27 | 3 h = 4.3 ± 2.8/ 6 h = 3.7 ± 2.1 d1 = 3.8 ± 1.7/d2 = 1.6 ± 1.6 Length of hospital stay = 4.0 ± 0.9 days | |
| 15 | Warmed and humidified intraperitoneal gases | 3 h = 10 ± 4/ 6 h = 19 ± 6 d1 = 36 ± 17/d2 = 43 ± 25 | 3 h = 4.3 ± 1.9/ 6 h = 4.2 ± 1.9 d1 = 2.5 ± 2.2/d2 = 2.3 ± 3.0 Length of hospital stay = 3.2 ± 0.4 days* | |

IM intramuscular, *d1* day 1, *d2* day 2, *VAS* visual analogue scale score, *PCA* patient-controlled analgesia, *P4CU* post-operative evaluation in the post-anaesthesia care unit, *GBP* gastric bypass, *FVC* forced vital capacity, *FEV₁* forced expiratory volume in 1 s

patient had nausea and vomiting and four patients had pruritus.

Association of Different Opioids or Anaesthetic Drugs

Michaloudis et al. reported the efficacy of a protocol combining continuous bupivacaine analgesia, intrathecal fentanyl and intravenous itenoxicam in 27 patients undergoing vertical-banded gastroplasty by laparotomy [64]. These patients received post-operative analgesia by intrathecal bolus on request. All patients were able to sit in a chair within 8 h of surgery and to walk the next day.

Post-operative analgesia with intravenous morphine PCA has also been retrospectively compared with epidural morphine (continuous or on request) or continuous bupivacaine plus fentanyl in a sample of 40 patients undergoing laparoscopic GBP [65]. Pain relief at rest, the occurrence of pruritus, nausea and vomiting, time until ambulation and bowel function recovery were similar between groups. By contrast, patients receiving epidural analgesia had a higher frequency of skin infections (epidural group 39 %, PCA group 15 %, $P=0.01$). These observations were recently confirmed by Pathiraja et al., who compared the use of a thoracic epidural catheter with a bupivacaine pump in patients undergoing GBP by laparotomy [66].

De Baerdemaeker et al. compared the intraoperative administration of remifentanyl with that of sufentanil in terms of post-operative morphine requirements. Cumulative morphine consumption during the first 2 h after surgery was found to be higher in the remifentanyl group than in the sufentanil group [67]. Recovery profiles and spirometry showed no significant difference between groups. In a larger comparative study, Bidgoli et al. recently confirmed significantly higher levels of pain and opioid consumption in the first 4 h after awakening in patients receiving remifentanyl [68]. However, recovery time after surgery and length of stay in the intensive care unit were similar between groups.

The use of bupivacaine pumps in a subxiphoid position and radiating in both directions caudally beneath the lowest rib may also help to decrease opioid requirement, as shown in a prospective randomised study in 40 patients undergoing laparoscopic GBP, in which this approach greatly decreased opioid use [69]. The addition of small doses of ketamine to the morphine administered during the post-operative period has been evaluated in a prospective randomised double-blind study of 40 morbidly obese patients undergoing upper abdominal surgery [70]. Cumulative morphine consumption was significantly lower in the morphine/ketamine group despite similar analgesia. A comparison with preoperative values showed, in terms of pulmonary function parameters, a significantly larger change in the group receiving morphine alone than in those receiving morphine/ketamine. PaO_2 , respiratory rate, SPO_2 and morphine side effects were

also significantly better in the morphine/ketamine group for up to 32 h after PCA initiation. Sollazzi et al. showed that a slow injection of clonidine and ketamine before anaesthesia resulted in lower levels of tramadol consumption in the post-operative period following open biliopancreatic diversion in 23 patients than in patients who did not receive this injection [71].

Combination of Opioids with Other Analgesic Approaches

The perioperative use of ketorolac, a non-steroidal anti-inflammatory drug, has been shown to be associated with a shorter stay in the intensive care unit after laparotomy and greater satisfaction with pain relief [72, 73]. In a second double-blind placebo-controlled study, preemptive analgesia with a 30-mg intravenous dose of ketorolac was associated with a subcutaneous injection of bupivacaine and epinephrine during incision [73]. This treatment was associated with lower levels of post-operative pain and a lower opioid requirement (40 % decrease) during the 3 days immediately after surgery.

The beneficial effect of dexmedetomidine was assessed in a placebo-controlled study of 20 patients undergoing GBP by laparotomy [74]. In the group receiving dexmedetomidine, lower concentrations of desflurane were required to maintain the bispectral index between 45 and 50 during surgery, and blood pressure and heart rate were lower. In the recovery room, pain scores and morphine requirements were significantly lower in the dexmedetomidine group. Conversely, Tufanogullari et al. investigated various doses of dexmedetomidine and observed no benefit in terms of pain onset and morphine consumption in patients undergoing laparoscopic GBP [75]. However, dexmedetomidine decreased the duration of the patient's stay in the intensive care unit and the frequency of nausea. Cabrera et al. studied the effect of pregabalin on the prevention of neuropathic pain in 80 subjects undergoing sleeve gastrectomy. The intra-operative sectioning of nerve fibres and the high temperature of the electrical scalpels used in this procedure may contribute to the generation of post-operative neuropathic pain. The use of a single dose of pregabalin decreased morphine consumption in the first 24 h after surgery (11.5 versus $23.7 \pm 7.93 \pm 9.57$ mg, $p < 0.0001$), VAS score and the frequency of nausea and vomiting [76]. However, one of the first studies published showed the use of opioid analgesics and the occurrence of post-operative complications to be similar in patients who underwent gastric bypass surgery with ($n=74$) and without ($n=131$) transcutaneous electrical nerve stimulation (abstract only) [77]. In a letter, Batistich et al. suggested that the use of a rectus sheath and skin infiltration with a local analgesic solution should be considered as a method of reducing morphine requirement after open gastric bypass [78].

The Effect of Surgical Procedures on Opioid Requirement and Efficacy

Lee et al. reported that opioid requirement and length of hospital stay after laparoscopic gastric bypass were double those after laparoscopic vertical banding gastroplasty [79]. As expected, El Shobary et al. reported a lower morphine requirement in patients undergoing laparoscopic surgery than in those undergoing open GBP and significant improvements in terms of rapid mobilisation and length of hospital stay. In addition, post-operative recovery took longer after laparoscopic gastric bypass than after laparoscopic vertical-banded gastroplasty, as shown by the longer hospital stay (5.7 vs 3.5 days, $P < 0.001$) and greater analgesic requirement (2.4 vs 1.4 doses, $P < 0.05$) in patients undergoing laparoscopic GBP [59].

Conflicting results concerning the effect of high-temperature intraperitoneal gas (35 °C) have been reported. Hamza et al. reported that this procedure decreased both post-operative pain and analgesic requirement, but this procedure created a fog, limiting the surgical procedure [80]. By contrast, Savel et al. reported no significant benefit [81].

Conclusion

Despite the increasing diversity of analgesic strategies, opioids remain the gold standard for post-operative pain management. Concerns about the impairment of ventilation are warranted, particularly in the obese population, in which the risk of post-operative desaturation is high and may be worsened by systemic opioids. As the requirement, efficacy and safety of opioids are highly variable, one way to improve medical practice may be to personalise post-operative analgesia, as reported in recent studies [82, 83]. Actually, age, gender, and previous psychiatric hospitalisation are independently associated with greater opioid requirements after bariatric surgery [82]. Acute nicotine abstinence during hospitalisation after knee and hip replacement increases opioid medication seeking but not consumption during post-operative recovery [83]. To identify the risk factors associated with higher opioid use may be useful in designing individual post-operative pain management.

Moreover, published studies have shown that improvements in surgical methods and additional analgesic treatments designed to decrease opioid requirement may help to decrease the adverse effects of opioids in obese patients.

Conflict of Interest None

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Manuscrit 8 et 9. Autres exemples de la pharmacologie de l'obésité

Deux cas cliniques ont permis de compléter la réflexion concernant la prescription de médicaments chez les sujets obèses, l'un en rapport avec la prescription d'etonogestrel par voie sous-cutanée et l'autre sur les antituberculeux par voie orale.

(Articles 8 et 9)

Case report

Etonorgestrel concentrations in morbidly obese women following Roux-en-Y gastric bypass surgery: three case reports^{☆,☆☆}

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Abstract

Background: Pregnancy should be avoided for 12 to 18 months after Roux-en-Y gastric bypass (RYGB) surgery. The etonorgestrel (ENG)-releasing implant (Implanon®) may represent a safe and effective contraceptive method in morbidly obese women who are candidates for bariatric surgery. In addition, the subcutaneous delivery of steroid is unaffected by malabsorptive surgery.

Methods: Three cases of young women with ENG-releasing implant are reported. The device was inserted 1–2 months prior to RYGB.

Results: Their initial weights were 130 to 176 kg, and the mean weight loss was 33.6 kg at 6 months. The concomitant serum ENG concentrations decreased currently with weight loss but remained above the minimum concentration required for effective contraceptive effect of the implant for at least 6 months following RYGB (average, 170 pg/mL). The concentrations observed before weight loss were lower than in normal-weight women, but decreases in ENG concentrations following implant insertion were similar.

Conclusion: These unique data in morbidly obese women highlight the need for further pharmacokinetic studies of contraceptive agents in obese women during weight loss.

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Keywords: Obesity; Bariatric surgery; Gastric bypass; Etonorgestrel; Contraception

1. Introduction

Bariatric surgery is increasingly used in morbidly obese fertile women to obtain substantial weight loss and reduce obesity related comorbidities [1]. Adequate contraception is essential, as pregnancy should be avoided during the period of intensive weight loss, from 12 to 18 months after Roux-en-Y gastric bypass (RYGB) surgery [2,3]. Although obese women frequently suffer anovulation before surgery,

fertility increases after RYGB, and pregnancy occurring after surgery may be associated with an increased risk of both maternal and child complications [3,4]. In addition, progestins and especially microprogestin contraceptive are considered more appropriate in obese patients than estrogen and progestin oral contraceptive, especially in the context of surgery [5,6].

The absorption of oral contraceptive agents may be affected after RYGB, and cases of pregnancy occurring postoperatively have been reported despite adequate oral contraception [3].

The subdermal etonorgestrel (ENG)-releasing implant (Implanon®) is a long-term progestatin contraceptive developed to provide continuous and stable release of the active substance ENG. This active metabolite inhibits ovulation by altering the hypothalamic-pituitary-ovarian

[☆] The authors declare that they have no conflict of interest.

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Table 1
Patient characteristics and the changes in ENG concentration over time

| | | Before surgery | 3 months | 6 months |
|--------------------------------|-----------------------------------|----------------|-------------|-------------|
| Patient 1, 24 years, 163 cm | Weight (kg) | 172 | 143.3 | 130.6 |
| | BMI (kg/m ²) | 64.7 | 53.9 | 49.2 |
| | Fat mass DEXA [kg (%)] | 88.8 (52.5) | 67.1 (47.9) | 55.6 (43.9) |
| | Albumin | 33 | 35 | 36 |
| | Duration of Implanon use (months) | 2 | 5 | 8 |
| | ENG serum concentration (pg/mL) | 236 | 134 | 125 |
| Patient 2, 19 years, 180 cm | Weight (kg) | 176.7 | 157 | 148.8 |
| | BMI (kg/m ²) | 60.4 | 53.7 | 50.9 |
| | Fat mass DEXA [kg (%)] | 86 (49.7) | 78.3 (50.8) | 69.1 (47.2) |
| | Albumin | 38 | 39 | 37 |
| | Duration of Implanon use (months) | 1 | 4 | 7 |
| | ENG serum concentration (pg/mL) | 355 | 230 | 201 |
| Patient 3, 23 years, 159 cm | Weight (kg) | 130.9 | 104.7 | 99.7 |
| | BMI (kg/m ²) | 51.8 | 41.4 | 39.4 |
| | Fat mass DEXA [kg (%)] | 63 (49.1) | 50.1 (48.6) | 44.6 (45.1) |
| | Albumin | 35 | 36 | 38 |
| | Duration of Implanon use (months) | 2 | 5 | 8 |
| | ENG serum concentration (pg/mL) | 326 | 291 | 194 |

control mechanism. The implant also hinders conception by preventing implantation through inhibition of endometrial proliferation [7]. It may represent a safe contraceptive method in women candidate for the bariatric surgery [6]. However, little is known about its pharmacokinetic and safety profile in obese women who are candidates for bariatric surgery.

2. Case reports

Three young women with constitutional obesity [age, 19–24 years; body mass index (BMI), 49.2–64.7 kg/m²], gravida 0 and with an implant in situ, were consecutively admitted to the Department of Nutrition, Pitié-Salpêtrière Hospital, for surgical treatment of their obesity by RYGB (Table 1). An implant (Implanon®; Organon, France), containing 68 mg of ENG had been inserted to the inner side of the nondominant upper arm in anticipation of bariatric surgery, 1 to 2 months before surgery, in discussion with their gynecologist.

3. Methods

Surgery was performed by the same surgeon in all patients, with a loop length of about 1.5 m, using the same laparoscopic technique [8]. The three women were investigated before surgery, 3 and 6 months postoperatively. Anthropometric data with fat mass estimated by whole-body Dual energy X-ray absorptiometry (DEXA) scanning (Discovery W, S/N 80632, France) and ENG concentrations were assessed (Table 1). None of the women were receiving drugs known to induce or inhibit cytochrome P450, or other drug-metabolizing enzymes, the activity of which may modify ENG-releasing implant pharmacokinetics.

ENG serum concentrations were measured as previously described [9]. After centrifugation, serum concentrations of ENG were quantified by means of radioimmunoassay, after C18 solid-phase extraction, using antirabbit antiserum against ENG and titrated ENG as tracer and donkey antirabbit-antibody cellulose to separate free and bound antigen. The sensitivity of the assay was 20.0 pg/mL, and the specificity was higher than 94.5%.

4. Results

The serum ENG concentrations at the above intervals following ENG-releasing implant insertion decreased progressively and were, respectively, 236–134–125, 355–230–201 and 326–291–194 pg/mL in patients 1, 2 and 3 (Table 1, Fig. 1). Demographic characteristics of the three enrolled women as well as changes in ENG serum concentration over time are shown in Table 1.

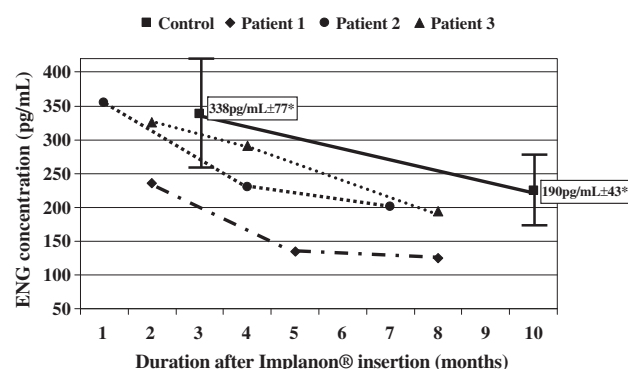


Fig. 1. Individual ENG concentrations versus time after ENG-releasing implant insertion. *Data control pharmacokinetic parameters (seven women; mean BMI±SD, 21.85±2.54 [9]).

5. Discussion

In normal-weight women, the contraceptive efficacy of ENG-releasing implants is higher than that of any other contraceptive method, with a Pearl Index of 0.0 (95% confidence interval, 0.00–0.09) during 53,530 cycles, due to the extensive inhibition of ovulation and their independence of user compliance [7,10]. Ovulation is inhibited, as judged by progesterone levels in pharmacodynamic studies, in 97% of patients with a serum ENG concentration over 90 pg/mL, this being considered the cutoff value observed after 18 months of use [7,10]. Lower concentrations (<90 pg/mL) may be associated with ovulation in as many as 52% of women [7,10]. In these cases, both an increased viscosity of cervical mucus and inhibition of endometrial proliferation may contribute to their contraceptive effect [7].

Longitudinal analysis reports that the maximum serum concentration of ENG of 813 pg/mL are seen on day 4 after implant insertion and are followed by a decline in mean ENG concentrations to 196 pg/mL (range, 150–261 pg/mL) by the end of the first year and 156 pg/mL (range, 111–202 pg/mL) by the end of the third year [11,12]. As previously observed in normal-weight women, ENG serum concentrations decreased gradually over time in subjects using ENG-releasing implant who had undergone RYGB.

The serum ENG concentrations of patients 2 and 3 remained higher than 150 pg/mL 6 months after surgery, corresponding to 8 months after Implanon® insertion, and were consistent with an effective contraceptive effect [7,11].

ENG concentrations in patient 1 were also consistent with a contraceptive effect but were low, especially at 9 months following Implanon® insertion at 125 pg/mL. This concentration corresponds to the lower range of ENG concentrations observed after 3 years of use in normal-weight women [11]. Increased volume of distribution with fat mass in this woman may partly explain this unexpected result. This hypothesis is supported by the fact that the ENG serum concentrations in patients 2 and 3 decreased to close to the concentrations of normal-weight women over time with weight loss and that patient 1's BMI and fat mass are particularly high.

Little is known about the efficacy of the ENG-releasing implant in morbidly obese women with a BMI over 40 kg/m². Huber [11] and Wenzl et al. [9] only reported that the ENG serum concentration at the end of the third year of use among women with body weight over than 70 kg was lower than in normal-weight women. Another progestin-releasing implant using levonorgestrel displayed significant variability in drug concentration, and weight explained an important part of this variability (23% of the variance) [12]. In addition, the variability in ENG clearance is wide and may contribute to these differences, as reported by Wenzl et al. (clearance, 8.46±2.65 L/h) [9], and drug elimination rate can be influenced by a number of covariants, such as drug-metabolizing enzyme activity, alongside genetic and environmental factors [13,14]. Patient 1 probably displayed

many of these covariants responsible for lower ENG concentrations (increase in volume distribution and in drug elimination rate), and further investigations are required to better identify these covariants in clinical practice that may be responsible for a decrease in serum ENG levels and potentially contraceptive efficacy. Due to the variability in ENG concentration, it is possible that some patients may have lower concentrations (<90 pg/mL) after 6 months following insertion.

In conclusion, these cases suggest that ENG-releasing implants may provide ENG concentrations considered sufficient to inhibit ovulation until 8 months after insertion and may therefore represent a safe contraceptive method in women undergoing bariatric surgery. However, the wide variability in ENG concentration suggests that further evaluation in longer large-scale studies is needed to decide when implants should be replaced in obese women having RYGB.

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Inadequate Therapeutic Response to a Recommended Antituberculosis Fixed-Dose Combination Regimen in an Overweight Patient with *Mycobacterium bovis* Infection

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A 6-month combined antibiotic regimen (2 months with isoniazid, rifampin, pyrazinamide, often with ethambutol, then 4 months with isoniazid and rifampin) is currently recommended to treat active tuberculosis in adults (Table 1).¹⁻³ The doses of isoniazid and rifampin should be 5 mg/kg and 10 mg/kg of body weight, with maximal doses of 300 mg and 600 mg, respectively, in accordance with the maximal dosage of the fixed-dose combinations (FDCs).^{2,3} The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease recommend an FDC regimen as first-line treatment of tuberculosis because of its easy administration, minimal risk of prescription errors, improved treatment adherence, and possible limited risk of drug-resistant tuberculosis.^{4,5}

The prevalence of overweight and obesity is increasing worldwide, especially in patients living in poor socioeconomic conditions who are at increased risk for tuberculosis.⁶ Overweight and obesity are associated with changes in drug metabolism and requirement.⁷ The currently recommended treatment of tuberculosis, suitable for adults weighing 70 kg or less, may lead to drug concentrations below the therapeutic ranges in overweight and obese patients, poor clinical outcome, and increased risk of developing resistant strains in this particular population.

OBJECTIVE: To report a case of an overweight man with lymph node tuberculosis due to *Mycobacterium bovis*, a part of the *Mycobacterium tuberculosis* complex, treated with fixed-dose combination (FDC) chemotherapy.

CASE REPORT: Following guidelines, according to the patient's weight (92 kg), we prescribed the maximum recommended doses of isoniazid-rifampin-pyrazinamide FDC. It led initially to underdosing, with a poor clinical outcome, justifying increased doses and a complex regimen using separate drugs (isoniazid 600 mg, rifampin 1200 mg, and levofloxacin 1000 mg) to achieve therapeutic drug concentrations and clinical response.

DISCUSSION: Usually recommended doses of FDC chemotherapies may be inappropriate in overweight patients. We discuss here the different factors that may be involved in poor clinical outcomes, particularly the consequences of excess weight on drug metabolism: drug-drug interaction, FDC use, generic formulation use, intestinal malabsorption, and acetylation profile.

CONCLUSIONS: Therapeutic drug monitoring in overweight patients may be useful in the clinical setting to help clinicians individualize drug therapeutic regimens and optimize drug response, adherence, and safety.

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We report a case of an overweight Algerian man with lymph node tuberculosis treated at the highest recommended dosing regimen of an isoniazid-rifampin-pyrazinamide FDC.

Case Report

A 36-year-old Algerian man (weight 92 kg, height 180 cm, body surface area 2.1 m², body mass index 28.4 kg/m²) who had been living in France for 20 years, with no remarkable medical history, was referred to our internal medicine department for fever, fatigue, and cervical lymph node enlargement (35 mm in diameter). Results of laboratory tests, including serum creatinine, prothrombin time, and liver function, were within normal limits, and HIV infection testing was negative. Chest X-ray was unremarkable and 3 consecutive sputum smear analyses for acid-fast

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bacilli were negative. Culture of a lymph node biopsy returned positive for *Mycobacterium tuberculosis* complex. An FDC of isoniazid-rifampin-pyrazinamide 6 tablets once daily in the morning, fasting, was started at the maximum authorized dose (isoniazid 300 mg, rifampin 600 mg, pyrazinamide 1800 mg daily).² Ethambutol was not added because the incidence of isoniazid resistance is about 6.6% in France and Algeria, where tuberculosis was presumably contracted. Moreover, our patient had no risk factor for isoniazid resistance.⁸ One month later, the *M. tuberculosis* complex strain was identified as *M. bovis*, using the Genotype MTBC Biocentric Kit. The strain showed a wild-type susceptibility pattern to antituberculosis drugs, with minimum inhibitory concentrations (MICs) of rifampin and isoniazid below the critical concentrations of 1 mg/L and 0.1 mg/L, respectively, the median MICs being 0.25 and 0.06 mg/L, respectively.^{9,10} As *M. bovis* displays a natural resistance to pyrazinamide, we switched the patient's regimen to an isoniazid-rifampin FDC excluding pyrazinamide 2 tablets once daily, corresponding to the maximum authorized dose (isoniazid 300 mg, rifampin 600 mg daily).¹¹ After 3 months of treatment, fatigue, fever, and the lymph node enlargement were unchanged. However, neither diarrhea nor vomiting was reported and pill count confirmed the correct treatment adherence. Hence, levofloxacin 1000 mg once daily was added in accordance to the sensitivity of the strain and serum drug concentrations were performed in the Department of Toxicology of Bichat Hospital, Paris, using high-performance liquid chromatography.¹² Isoniazid plasma concentration, measured 2 and 3 hours following oral administration (T2 and T3), was 1.3 mg/L and 0.3 mg/L, respectively (therapeutic concentration range 2.5-5) (Table 2). Plasma drug concentrations were always measured after seeing the patient take the pills in our department. Rifampin T3 plasma concentration was 5.7 mg/L (therapeutic concentration range 6-10) (Table 2). In view of the poor clinical outcome at the end of the fourth month of treatment, and also because drug concentrations were below the therapeutic range at this dosing regi-

men, despite adherence to treatment, isoniazid-rifampin FDC dosage was increased to 4 tablets once daily, while levofloxacin was kept unchanged. Therapeutic drug monitoring and subsequent changes in isoniazid and rifampin doses during the follow-up are detailed in Table 2. After 6 months of treatment, the patient's clinical status was improved and neck and chest computed tomography showed no lymph node enlargement. Target serum drug concentrations for isoniazid and rifampin were reached with the following regimen: isoniazid 600 mg, rifampin 1200 mg, and levofloxacin 1000 mg once daily. At this dosing regimen, drug concentrations were within the reference range and no hepatic adverse effect was noted.

Discussion

We describe a case of an overweight (BMI >25 kg/m²) man with *M. bovis* infection and insufficient clinical response despite the maximal WHO-recommended FDC regimen and appropriate sensitivity to drugs. An increased dosage regimen was needed to achieve therapeutic drug concentrations and clinical response. Information obtained through this study indicates that usually recommended doses of FDC chemotherapies may be inappropriate in overweight patients, and we discuss rifampin and isoniazid dosing considerations in overweight individuals.^{1,2}

With separate drugs and doses adjusted to take body weight into consideration, the dosage regimen would have been isoniazid 450 mg and rifampin 900 mg (higher than the dosing recommendations, requiring therapeutic drug monitoring). When the patient was given isoniazid-rifampin FDC 2 tablets once daily, drug concentrations were below the normal range despite complete treatment adherence as measured by pill count. When the patient was given isoniazid 600 mg and rifampin 1200 mg once daily, target drug concentrations were reached, no hepatic adverse effects were recorded, and the patient's clinical status im-

Table 1. Recommended Daily Doses of First-Line Antituberculosis Drugs for Adults²

| Drug: Recommended Daily Dose, mg/kg (range) | Maximum Dose (mg) | Treatment Phases |
|---|-------------------|------------------------------|
| Isoniazid: 5 (4-6) | 900 | Intensive: 2 months of HRZE |
| Rifampin: 10 (8-12) | 600 | Continuation: 4 months of HR |
| Pyrazinamide: 25 (20-30) | | |
| Ethambutol: 15 (15-20) | | |
| Streptomycin: 15 (12-18) | | |
| HR = isoniazid-rifampin; HRZE = isoniazid-rifampin-pyrazinamide-ethambutol. | | |

Table 2. Patient Dosing Regimen and Drug Concentrations During Follow-Up

| Months | Weight, kg | Rifampin | Isoniazid |
|--|------------|---|---|
| 0-4 | 92 | 600 mg (6.5 mg/kg) T3 = 5.7 mg/L ^a | 300 mg (3.2 mg/kg) T2 = 1.3 mg/L ^a ; T3 = 0.3 mg/L ^a |
| 4-5 | 88 | 1200 mg (13.6 mg/kg) T3 = 10.3 mg/L ^a | 600 mg (6.8 mg/kg) T2 = 8.3 mg/L ^a ; T3 = 5.3 mg/L ^a |
| 5-6 | 86 | 900 mg (10.4 mg/kg) T3 = 10.3 mg/L ^a | 450 mg (5.2 mg/kg) T3 = 0.7 mg/L ^a |
| 6-8 | 86 | 1200 mg (13.9 mg/kg) T3 = 10.4 mg/L ^a | 600 mg (6.9 mg/kg) T3 = 2 mg/L ^a |
| ^a Therapeutic concentration ranges for rifampin (T3 = 6-10 mg/L) and isoniazid (T2 = 2.5-5 mg/L and T3 = 1.5-3 mg/L), respectively. | | | |

proved, confirming the need for and safety of increased doses in this overweight patient. The WHO Treatment of Tuberculosis Guidelines for National Programmes 2003 provided weight-based dosages and recommended a maximal dose of isoniazid 350 mg and rifampin 750 mg for a patient weighing more than 70 kg, which was not reported in the latest 2009 guidelines.^{2,3}

In our patient, no drug-drug interaction was suspected, as the only concomitant medication was levofloxacin. However, a pharmacokinetic study including 8 healthy volunteers showed a reduced bioavailability of rifampin when coadministered with isoniazid.¹³ This interaction has never been investigated in patients receiving long-term antituberculosis treatment, and the effects of isoniazid may be outweighed by the hepatic enzyme induction due to rifampin. In addition, Agrawal et al. showed that all pharmacokinetic parameters of rifampin-isoniazid from FDC were comparable to those of the individual formulations.¹⁴ Hence, using FDC rather than separate drugs at the same dosage is unlikely to explain the low concentrations that we observed.¹⁴ In addition, no generic formulation was used in our patient, as low rifampin dosing has been measured in some generic formulations of FDCs.¹⁵ A recent clinical trial reported a significant difference in terms of clinical efficacy between FDC regimen and separate drugs, presumably because of the difficulty in precisely individualizing a drug-dosing regimen when using FDC.¹⁶ Intestinal malabsorption was unlikely in our patient, as he had no history of diarrhea, weight loss, anemia, or vitamin deficiency, and laboratory values were within reference ranges. Whether our patient was a fast or extremely fast acetylator with high isoniazid clearance is, unfortunately, unknown as the acetylation profile was not available.¹³

In comparison with normal weight patients, overweight and obese patients have larger muscle mass, total body water, and plasma volume.^{17,18} In addition, overweight is associated with higher systemic blood pressure, renal plasma flow, glomerular filtration rate, and albumin excretion rate.^{19,20} As a result, the glomerular capillary bed is subjected to a transcapillary hydrostatic pressure gradient, resulting in high hyperfiltration.²¹ These physiologic changes may modify some pharmacokinetic parameters. Indeed, increased plasma and water volume, fat, and muscle body mass modify the volume of distribution of most drugs, with potential consequences in drug exposure and concentrations over time, whereas the increased size of organs and cardiac output influence drug clearance and exposure. Hence, despite a relatively low lipophilicity of isoniazid (0.6 L/kg) and rifampin (0.8 L/kg), increased volume of distribution and clearance of these drugs are to be expected in overweight patients, as previously reported with other hydrophilic drugs, such as acetaminophen or other antimicrobials.^{22,23} In addition, the activity of drug-metabolizing enzymes is different in obese patients. *N*-acetyltransferase

activity, the primary isoniazid metabolic pathway, is increased in obese patients and may contribute to lower drug concentrations, whereas CYP3A4 activity, involved in rifampin metabolism, is reduced.²⁴ Lower rifampin metabolism may be associated with higher serum concentrations and an increased prevalence of rifampin adverse effects, as previously reported in overweight patients.²⁵ However, Nijland et al.²⁶ showed a strongly reduced exposure to rifampin in patients with type 2 diabetes. They reported a strong effect of body weight on rifampin exposure, despite lower hepatic metabolism, as demonstrated by the exposure of metabolites.

Additional studies are warranted to assess the influence of excess weight on the pharmacokinetics of antituberculosis drugs and tuberculosis treatment outcomes. Limiting the increase in dosage, as currently recommended by several guidelines, may lead to underdosing in therapeutic regimens and low drug concentrations in overweight patients.⁷ Therapeutic drug monitoring in overweight patients may be useful in the clinical setting to help clinicians individualize therapeutic drug regimens and optimize drug response, adherence, and safety.

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CONCLUSIONS ET PERSPECTIVES

Mon travail de thèse montre que l'obésité morbide et les modifications physiologiques qui lui sont associées influencent la variabilité pharmacocinétique et pharmacodynamique de la morphine avant et après RYGB.

Sur le plan pharmacodynamique, il existe une augmentation de la fréquence du polymorphisme c.118G du gène *OPRM1* codant pour le récepteur de la morphine et une augmentation des seuils de sensibilité à une douleur expérimentale chez les sujets obèses. Ces données, confrontées à celles de la littérature, suggèrent que la douleur doit être plus forte pour être perçue, mais qu'une fois la douleur perçue relevant d'antalgiques de type opioïdes, elle nécessite davantage de morphine pour être soulagée par rapport aux sujets non obèses.

Sur le plan pharmacocinétique, et avant chirurgie de l'obésité dans une cohorte de sujets souffrant d'obésité morbide, la composition corporelle et les données anthropométriques ne permettent pas de prédire les paramètres pharmacocinétiques de la morphine.

En revanche, l'étude de l'ensemble des pharmacocinétiques de la morphine orale avant et après chirurgie de l'obésité montre un effet de l'obésité sur l'exposition systémique de la morphine et son volume de distribution, l'exposition étant diminuée et la clairance augmentée lorsque l'indice de masse corporelle augmente. Ces données suggèrent que l'excès pondéral influence la clairance de la morphine.

L'analyse des données pharmacocinétiques avant chirurgie et des données tissulaires de l'expression des enzymes du métabolisme montre que le contenu jéjunal en P-gp influence l'absorption de la morphine (T_{max} et C_{max}), mais n'explique pas l'exposition.

L'absorption de la morphine est augmentée après RYGB avec un raccourcissement du T_{max} et une élévation des C_{max} , de façon significative et constante chez l'ensemble des patients, supposant que le RYGB crée des conditions anatomiques favorables à l'absorption de morphine à libération immédiate, et notamment sous forme liquide. La variabilité interindividuelle post-opératoire des paramètres pharmacocinétiques d'absorption est cependant étroitement liée à la variabilité pré-opératoire et varie en fonction du contenu

initial en P-gp. Ainsi l'augmentation de l'absorption post-opératoire semble d'autant plus importante que l'absorption était importante avant chirurgie.

Nos données préliminaires ne montrent pas de lien entre le contenu en P-gp et l'exposition à la morphine en pré-opératoire mais cependant avec son exposition post opératoire. Ces données suggèrent que l'augmentation de l'absorption et la variabilité individuelle du contenu intestinal en P-gp déterminent l'augmentation de l'exposition en post-opératoire. La diminution de la glucuronidation de la morphine pourrait réduire la clairance de la morphine après chirurgie de l'obésité et contribuer à l'augmentation de l'absorption et de l'exposition entre les périodes post opératoire immédiate et tardive.

Ainsi, la prescription de morphine orale doit être prudente après une chirurgie bariatrique, notamment lorsque la dissolution du principe actif est facilitée par sa forme galénique.

Par ailleurs, le niveau d'expression entérocytaire des gènes des enzymes du métabolisme des médicaments et leur contenu protéique sont hautement variables d'un individu à l'autre. Des facteurs biologiques tels que l'insuline et l'adiponectine pourraient moduler l'expression de différents gènes et expliquer des différences d'activité métabolique entre sujets obèses et non obèses. En revanche nos données préliminaires ne permettent pas de montrer un lien entre le contenu protéique en enzymes du métabolisme des médicaments et ces mêmes marqueurs biologiques.

PERSPECTIVES

Les données concernant le contenu protéique en enzymes du métabolisme des médicaments chez les 12 autres patients du projet OBEMO complèteront notre analyse préalable et permettront potentiellement de rendre plus significatives des associations qui semblent importantes.

Le dosage des métabolites, actuellement en cours, permettra de préciser l'évolution de la glucuronidation après chirurgie de l'obésité et d'étudier ses déterminants. Par ailleurs, compte tenu du caractère actif du métabolite de la morphine, la M6G, il apparaît nécessaire d'étudier l'évolution de ses concentrations afin de mieux discuter l'impact de la chirurgie sur la pharmacodynamique de la morphine.

Il sera intéressant de confronter les données concernant le rôle la P-gp à celles de la pharmacocinétique de la digoxine, substrat spécifique de la P-gp, avant et après chirurgie de l'obésité. Cela nous sera permis grâce au projet SODA, actuellement en cours. Cet essai permettra, par une approche phénotypique d'étudier l'évolution de l'activité de différentes enzymes du métabolisme des médicaments (CYP2D6, CYP1A2, CYP3A4, CYP2C9 et CYP2C19) et du transporteur P-gp, avant et après chirurgie de l'obésité. Nous espérons pouvoir ainsi discuter le devenir de l'absorption et de l'exposition de différents substrats de ces enzymes et transporteur. Par ailleurs les analyses concernant le niveau d'expression des gènes et le contenu protéique concerneront le jéjunum et le foie, et ces données permettront de discuter les rôles respectifs du foie et de l'intestin dans l'évolution des activités métaboliques des enzymes et transporteur étudiés.

Afin que la réflexion concernant l'absorption des médicaments soit complète, il semble nécessaire d'initier une réflexion concernant le rôle de la galénique des médicaments dans le devenir de l'absorption des médicaments après chirurgie de l'obésité.

Enfin, il n'y a pas d'études ayant spécifiquement étudié la variabilité des besoins, de l'efficacité et de la tolérance de la morphine chez les sujets obèses en comparaison aux patients non obèses. Une étude prospective sur les douleurs aiguës, telles que les douleurs post-opératoires nécessitant un traitement par opioïdes, chez les sujets obèses et non-obèses, permettrait de mesurer les différences concernant la survenue de la douleur, le recours à la morphine, et l'intérêt d'identifier des facteurs prédictifs grâce à un test de sensibilité et une exploration génétique.

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RESUME

Au cours de cette thèse, nous montrons que l'obésité est un facteur de variabilité pharmacodynamique et pharmacocinétique de la morphine. En particulier, l'absorption et l'exposition à la morphine orale augmentent de façon significative après chirurgie de type bypass gastrique.

Nous démontrons le rôle du contenu entérocytaire en transporteur d'efflux P-gp, dans la détermination de l'absorption et de l'exposition à la morphine.

Mots clés

Transporteur d'efflux P-gp

Glucuronidation

Morphine

Obésité

Composition corporelle

Chirurgie bariatrique

Premier passage intestinal

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