Intérêt des modèles in-vitro dans la prédiction du métabolisme et des interactions médicamenteuses

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Pharmacologie et Toxicologie Cliniques

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12.09.2013
Summary

Introduction

PBPK model for oxycodone metabolism

In-vivo / in-vitro Studies: Antiplatelet drug Clopidogrel

In vitro / in vivo studies: Antiplatelet drug Prasugrel

Conclusion
The Practice of Medicine in 1892

“If it were not for the great variability among individuals, medicine might as well be a science and not an art”

Sir William Osler (1849-1919)
Sources of Inter-individual Variability

Dose  Concentration PK  Effect PD

Environmental factors
• Food
• Co-medications
• Pollution

Genetic factors
• Enzymes (CYP450)
• Transporters
• Receptors
Following nonparenteral administration of a drug, a significant portion of the dose may be metabolically inactivated in either the intestinal endothelium or the liver before it reaches the systemic circulation.

- Limits oral availability of highly metabolized drugs

\[ F = 27\% \]
Métabolisme et transport dans le foie et l’intestin

Métabolisme et transport dans les reins

Metabolism represents the elimination mechanism of approximately 75% of all drugs. 75% of this metabolism as a result of CYP activity. Approximately half of CYP-mediated metabolism is a result of CYP3A.

(Figure from Wienkers and Heath, 2005)
Drug development phases and cost

10,000 candidate drugs
- ~5% advance to preclinical studies
- ~2% advance to clinical trials
- ~20% of compounds advanced at each stage

Cost:
- Early-stage research and discovery: $335 million
- Preclinical studies in animal models: $467 million
- Pharmacology: $95 million

1 drug into the market
Determination of the nature of metabolites:

- Stable metabolites → Good
- Electrophiles → Bad
  - Bind to cellular nucleophile – DNA, RNA and proteins
  - Cause cell death or transformation (cancer)

Determination of the nature of enzymes involved in the metabolism:

- Several enzymes → Good
- Single enzyme → Bad
  - Metabolism dependent on genetic polymorphism and DDI

F. Gonzalez 2009
Metabolism and Structure-Toxicity Relationships

Sudoxicam
Hepatotoxic (acute liver failure)
Withdrawn from Phase III trials

Meloxicam
“Clean” drug

Piroxicam
“Clean” drug

Kalgutkar, Pfizer Global Research and Development, USA
Rationalizing Differences in Toxicological Profile of Sudoxicam Through Differences in Metabolism

Sudoxicam

Methyl group prevents the formation of Epoxide intermediate metabolite

Meloxicam

Thioureas are toxic substances – Can oxidize proteins, glutathione, etc

Kalgutkar, Pfizer Global Research and Development, USA
### Méthodes: les techniques d’étude du métabolisme *in vitro*

<table>
<thead>
<tr>
<th>technique</th>
<th>avantages</th>
<th>désavantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes du foie humain</td>
<td>- simple utilisation&lt;br&gt;- stockés à -80°C pendant plusieurs années</td>
<td>- Préparation longue&lt;br&gt;- Faible activité des enzymes de phase II&lt;br&gt;- Incubations courtes&lt;br&gt;- pas pour les études d’induction</td>
</tr>
<tr>
<td>Cellules recombinantes</td>
<td>- Identification et confirmation de l’implication des isozymes individuels</td>
<td></td>
</tr>
<tr>
<td>Lignées cellulaires</td>
<td>- Culture cellulaire simple&lt;br&gt;- Durée de vie non-limitée&lt;br&gt;- Etudes d’induction possibles</td>
<td>- Onéreux&lt;br&gt;- Temps de préparation relativement long</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>- modèle proche de la situation in vivo&lt;br&gt;- maintenus quelques jours en culture</td>
<td>- croissance inefficace in vitro&lt;br&gt;- préparation à partir du tissu hépatique frais&lt;br&gt;- disponibilité de tissus humains</td>
</tr>
<tr>
<td>Coupes de foie</td>
<td>- bonne représentation de la situation in vivo&lt;br&gt;- enzymes de phase I et phase II</td>
<td>- Diminution rapide des niveaux de CYPs&lt;br&gt;- disponibilité de tissus humains</td>
</tr>
</tbody>
</table>
sandwich-cultured hepatocytes

DDI, metabolism and transport studies

Medium

Hepatocytes

Bile
In-vitro in-vivo models used in drug development and DDI

Brandon et al. 2003

<table>
<thead>
<tr>
<th></th>
<th>Supersomes</th>
<th>Microsomes + Cytosol</th>
<th>S9 Fraction</th>
<th>Transgenic Cell Lines</th>
<th>Liver Slices</th>
<th>Perfused Liver</th>
<th>In Vivo Animal Model</th>
<th>Human</th>
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<td>Complexity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Easy Applicable</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ethically Acceptable</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Resemblance of true in vivo situation</td>
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</table>
Gathering Data (Starting Point Not An End): Communication Tool

PRE-CLINICAL

CLINICAL
Three stage approach for prospective simulations

**Stage 1: Evaluation of Input Parameters**
- **Absorption**
  - Solubility, Permeability
  - Predict $f_a$, $k_a$, $P_{eff}$
  - Preclinical in vivo $f_a$, $k_a$
- **Distribution**
  - Log $P$, pKa, $f_{up}$
  - Tissue: plasma partition Vss
- **Hepatic Clearance**
  - $CL_{int}$, $f_{up}$, $f_{mic}$, B:P ratio
  - Scaled hepatic CL preclinical IVIVE

*in silico / in vitro parameters (in blue), Predicted ADME parameters (in bold)*
*Preclinical observed parameters (normal)*

**Stage 2: Model Selection**
- **Absorption**
  - First-order
  - ADAM
- **Distribution**
  - Mini-PBPK
  - Full PBPK
- **Clearance**
  - Well-stirred parallel tube dispersion

**Stage 3: Simulation**
- Evaluate multiple scenarios and test hypothesis to inform decision-making

*Compare simulations to observed data when available*
Structure of IVIV simulator

- Population Data
- Drug data
- Clinical trial design
- PK/PD Parameters
- PK/PD Profiles
FDA recommends PBPK modeling for DDI studies

II - SUMMARY GUIDANCE
The likelihood of drug interactions in specific populations (e.g., .... and pediatric .... patients) should be considered on a case-by-case basis.

PBPK modeling (if well verified for intended purposes) can be helpful to guide the determination of the need to conduct population-specific studies (see “Populations” in section V.B and “Complex Drug Interactions” section V.C.4).

Age-related changes in physiological processes governing drug disposition and drug effect have been investigated. In some cases, disproportional alterations in binding proteins, drug metabolizing enzymes and/or transporters, and renal filtration/secretion caused by developmental changes have been known to result in different drug disposition characteristics in pediatric and geriatric populations. However, dedicated drug interaction studies in these populations may not be feasible. Simulations using system biology approaches such as PBPK models (see section IV-A) may be helpful to predict drug interaction potential when the model can be constructed based on sufficient in vitro and clinical pharmacology and drug interaction data and incorporates development changes. Population pharmacokinetic approaches with sparse sampling can be used if properly designed (section IV - C).

Regulatory Submissions with PBPK Data

Area of applications in the 33 PBPK submissions in IND/NDA received by FDA’s Office of Clinical Pharmacology from 2008-12

Updated from <Zhao, Rowland and Huang, Clin Pharmacol ther 2012>
Huang, Abernethy, Wang, Zhao, Zineh, J Pharm Sci (submitted)
The Complexity of Covariate Effects

- Genotypes (Distribution in Population)
- Renal Function
  - Serum Creatinine
- Ethnicity
- Disease
- Sex (Distribution in Population)
- Age (Distribution in Population)
- Body Fat
- Plasma Proteins & Haematocrit
- Body Surface Area
- Height
- Weight
- Brain Volume
- Heart Volume
- Cardiac Output
- Cardiac Index
- Liver Volume
- Liver Weight
- MPPGL
- HPGL
- Enzyme & Transporter Abundance
- Serum Creatinine
- Renal Function

(Updated after Jamei et al., 2009)
CYP Abundance & Phenotype Frequency

**CYP2D6**
- Caucasian: PM = 8.2%
- Japanese: PM = 0.4%
- UM = 5.3%

**CYP3A5**
- Caucasian: PM = 83.0%
- Japanese: PM = 58.0%

**CYP2C19**
- Caucasian: PM = 2.4%
- Japanese: PM = 18.0%

**CYP2C9**
- Caucasian: PM = 6%
- Japanese: PM = 0.8%

**CYP2D6**
- Caucasian: PM = 11.0%
- Japanese: PM = 5.0%
### Other Key Changes in Chronic Kidney Disease

#### ESRD Incident Patients (USA, 2002-2004)

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<th>Age (year)</th>
<th>Frequency (×1000)</th>
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<td>31</td>
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<td>43</td>
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<td>67</td>
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<td>73</td>
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<td>79</td>
<td>20</td>
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<tr>
<td>85+</td>
<td>22</td>
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</table>

#### Haematocrit (%)

- **General Population**
- **ESRD Patients**

<table>
<thead>
<tr>
<th>Haematocrit (%)</th>
<th>Male</th>
<th>Female</th>
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</thead>
<tbody>
<tr>
<td>20</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>40</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.05</td>
<td>0.05</td>
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</table>

#### Cardiac Output (L/min)

- **General Population**
- **ESRD Patients**

<table>
<thead>
<tr>
<th>Cardiac Output (L/min)</th>
<th>Male</th>
<th>Female</th>
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<tr>
<td>0</td>
<td>0.05</td>
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<tr>
<td>2</td>
<td>0.05</td>
<td>0.05</td>
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<tr>
<td>4</td>
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<tr>
<td>6</td>
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#### Serum Albumin (gr/L)

- **Control**
- **ESRD Patients**

<table>
<thead>
<tr>
<th>Serum Albumin (gr/L)</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>20</td>
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<td>0.05</td>
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<tr>
<td>40</td>
<td>0.05</td>
<td>0.05</td>
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<tr>
<td>60</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>80</td>
<td>0.05</td>
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#### Half Gastric Emptying Time

- **Control**
- **ESRD Patients**

<table>
<thead>
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<th>Half Gastric Emptying Time</th>
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<th>Female</th>
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<tbody>
<tr>
<td>0</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.05</td>
<td>0.05</td>
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<tr>
<td>120</td>
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<td>0.05</td>
</tr>
<tr>
<td>180</td>
<td>0.05</td>
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</table>
PBPK model for oxycodone
Métabolisme de l’oxycodone et affinité au récepteur aux opoïdes (mu)

Lalovic et al CPT 2006
A Physiologically-based mechanistic pharmacokinetic (PBPK) model to assess the metabolism of oxycodone

<table>
<thead>
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<th>% fm</th>
<th>Cl int (ul/min/pmol)</th>
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<tr>
<td>Oxycodone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A4 (N-demethylation)</td>
<td>50</td>
<td>0.115</td>
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<tr>
<td>1A2/other (N-demethylation)</td>
<td>16</td>
<td>0.097</td>
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<tr>
<td>2D6 (O-demethylation)</td>
<td>1.5</td>
<td>1.22</td>
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<tr>
<td>2C19 (O-demethylation)</td>
<td>0.8</td>
<td>0.06</td>
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<tr>
<td>2D6 (Other mechanism)</td>
<td>26</td>
<td>0.018</td>
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<tr>
<td>Other pathways</td>
<td>5.7</td>
<td>0.22</td>
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<tr>
<td>Noroxycodone</td>
<td></td>
<td></td>
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<tr>
<td>2D6 (O-demethylation)</td>
<td>60</td>
<td>1.08</td>
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<tr>
<td>Other pathways</td>
<td>40</td>
<td>0.11</td>
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<tr>
<td>Oxymorphine</td>
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<tr>
<td>3A4 (N-demethylation)</td>
<td>1</td>
<td>0.0008</td>
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<tr>
<td>Other pathways</td>
<td>99</td>
<td>10.7</td>
</tr>
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</table>

Marsoussi N et al. 2013
## Input parameters for the PBPK model

Marsousi N et al. 2013

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>B/P</td>
<td>1.3</td>
<td>[1], [6]</td>
</tr>
<tr>
<td>Cl po (l/h)</td>
<td>81</td>
<td>[2], [4], [8], [9]</td>
</tr>
<tr>
<td>Cl R (l/h)</td>
<td>8.1</td>
<td>[8]</td>
</tr>
<tr>
<td>fa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fu gut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fu hepatocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fu plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ka (h⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LogP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW (g/mol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pKa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSA (Å²)</td>
<td></td>
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</tr>
<tr>
<td>Vd (l/kg)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>B/P</td>
<td>0.9</td>
<td>Simcyp predicted</td>
</tr>
<tr>
<td>Cl iv (l/h)</td>
<td>52</td>
<td>Estimated</td>
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<tr>
<td>Cl R (l/h)</td>
<td>21</td>
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<tr>
<td>fu gut</td>
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<td>Estimated</td>
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<tr>
<td>HBD</td>
<td>2</td>
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<tr>
<td>LogP</td>
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<tr>
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<td>PSA (Å²)</td>
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<tr>
<td>Vd (l/kg)</td>
<td>1.5</td>
<td>Estimated</td>
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**DDI Clinical study in healthy volunteers: oxycodone / ketoconazole / Quinidine**

Samer C et al. 2010
### DDI: Clinical and simulated AUC and $C_{max}$ ratios

<table>
<thead>
<tr>
<th></th>
<th>AUC$_{24h}$ ratio</th>
<th></th>
<th>$C_{max}$ ratio</th>
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<tr>
<td></td>
<td>Clinical</td>
<td>Simulated</td>
<td>Clinical</td>
<td>Simulated</td>
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<tr>
<td>Quinidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxycodone</td>
<td>1.3</td>
<td>1.3</td>
<td>1.0</td>
<td>1.2</td>
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<tr>
<td>Oxymorphone</td>
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<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
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<tr>
<td>Noroxycodone</td>
<td>1.7</td>
<td>1.7</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxycodone</td>
<td>1.7</td>
<td>1.7</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>1.8</td>
<td>1.9</td>
<td>1.4</td>
<td>1.4</td>
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<tr>
<td>Noroxycodone</td>
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<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Quinidine+Ketoconazole</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Oxycodone</td>
<td>2.7</td>
<td>2.7</td>
<td>1.6</td>
<td>1.8</td>
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<tr>
<td>Oxymorphone</td>
<td>0.9</td>
<td>1.3</td>
<td>0.6</td>
<td>0.8</td>
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<tr>
<td>Noroxycodone</td>
<td>0.8</td>
<td>1.1</td>
<td>0.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Marsousi N et al. 2013
In-vivo / in-vitro Studies
Antiplatelet drug Clopidogrel
Background

• Stent thrombosis is associated with considerable risk of morbidity and mortality.

• Current guidelines recommend a combination of aspirin and an anti P2Y12 drug to prevent recurrent ischemic events in patients with an acute coronary syndrome or patients undergoing percutaneous coronary interventions.

Sabatine M et al. New Engl J Med; 2005
**Clopidogrel response variability**

**Baseline Individual Variability**
- Increased baseline platelet reactivity
- Increased body mass index
- Diabetes or insulin resistance
- Up-regulation of other platelet pathways in setting of stress

**Genetic Variation**
- P2Y12 receptor
- Metabolic enzymes

**Reduced Bioavailability**
- Drug-drug interactions
- Metabolic enzymes

**Accelerated Platelet Turnover**
- Introducing new platelets unexposed to clopidogrel

---


Aleil B et al. J Thromb Haemost 2005
Bioactivation of clopidogrel

Topol EJ Nat Med 2011

Oxidation by cytochromes

Clopidogrel (inactive)

2-oxo-clopidogrel (inactive)

No platelet inhibition

Stent thrombosis

Heart

Coronary artery located on the surface of the heart

Compressed plaque

Widened artery

Stent

Thrombus

Stent thrombus
Pharmacogenomics of Antiplatelet Intervention (PAPI) Study in healthy Amish persons

Response to clopidogrel was highly heritable ($h^2 = 0.73; P < .001$).

- CYP 2C19*2: 12%
- Age
- BMI
- Triglyceride levels: 10%
- HDL cholesterol
- ?

Shuldiner AR et al, *JAMA* 2009;302:849
**PON1 Q192R polymorphism and risk of stent thrombosis**

**PON1 activity and risk of stent thrombosis**

**Variant genotype SNP (star allele or variant protein, if applicable) | Number with stent thrombosis (%) (n = 41) | Number without stent thrombosis (%) (n = 71) | P value**

| CYP2C19 | | |
| G681A (CYP2C19*2) | | |
| GG (*1/*1) | 26 (63.4) | 49 (69.0) |
| GA (*1/*2) | 12 (29.3) | 20 (28.2) |
| AA (*2/*2) | 3 (7.3) | 2 (2.8) |

P value: 0.31 [0.53]

*Bouman H et al. Nature med. 2011; 17(9):1153*
Flow chart of the meta-analysis

### 1.1.1 CASE-CONTROL DESIGN

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>QQ Events</th>
<th>Total Events</th>
<th>QR-RR Events</th>
<th>Total</th>
<th>Weight</th>
<th>Odds Ratio IV, Random, 95% CI</th>
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</thead>
<tbody>
<tr>
<td>BOUMAN 2011a</td>
<td>27</td>
<td>52</td>
<td>14</td>
<td>60</td>
<td>6.9%</td>
<td>3.55 [1.58, 7.97]</td>
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<tr>
<td>CAYLA 2011</td>
<td>61</td>
<td>164</td>
<td>62</td>
<td>204</td>
<td>11.8%</td>
<td>1.36 [0.88, 2.10]</td>
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<tr>
<td>DELANEY 2012</td>
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<td>349</td>
<td>111</td>
<td>344</td>
<td>13.7%</td>
<td>1.02 [0.74, 1.40]</td>
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<tr>
<td>SIBBING 2011b</td>
<td>76</td>
<td>840</td>
<td>51</td>
<td>726</td>
<td>12.9%</td>
<td>1.32 [0.91, 1.91]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>1405</td>
<td>1334</td>
<td></td>
<td></td>
<td>45.2%</td>
<td>1.40 [0.97, 2.01]</td>
</tr>
</tbody>
</table>

Total events: 278 (238)

Heterogeneity: $\chi^2 = 8.18$, df = 3 ($P = 0.04$); $I^2 = 63$

Test for overall effect: $Z = 1.82$ ($P = 0.07$)

### 1.1.2 PROSPECTIVE COHORT DESIGN

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>QQ Events</th>
<th>Total Events</th>
<th>QR-RR Events</th>
<th>Total</th>
<th>Weight</th>
<th>Odds Ratio IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOUMAN 2011b</td>
<td>31</td>
<td>808</td>
<td>13</td>
<td>1174</td>
<td>8.8%</td>
<td>3.56 [1.85, 6.85]</td>
</tr>
<tr>
<td>CAMPO 2011</td>
<td>9</td>
<td>127</td>
<td>12</td>
<td>173</td>
<td>6.1%</td>
<td>1.02 [0.42, 2.51]</td>
</tr>
<tr>
<td>HULOT 2011b</td>
<td>14</td>
<td>168</td>
<td>21</td>
<td>203</td>
<td>7.9%</td>
<td>0.79 [0.39, 1.60]</td>
</tr>
<tr>
<td>LEWIS 2011b</td>
<td>13</td>
<td>92</td>
<td>14</td>
<td>118</td>
<td>6.9%</td>
<td>1.22 [0.54, 2.75]</td>
</tr>
<tr>
<td>RIDEQ 2011</td>
<td>10</td>
<td>92</td>
<td>8</td>
<td>100</td>
<td>5.4%</td>
<td>1.40 [0.53, 3.72]</td>
</tr>
<tr>
<td>SIMON 2011</td>
<td>54</td>
<td>660</td>
<td>87</td>
<td>878</td>
<td>13.1%</td>
<td>0.81 [0.57, 1.16]</td>
</tr>
<tr>
<td>TRENK 2011</td>
<td>11</td>
<td>384</td>
<td>13</td>
<td>376</td>
<td>6.8%</td>
<td>0.82 [0.36, 1.86]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>2331</td>
<td>3022</td>
<td></td>
<td></td>
<td>54.8%</td>
<td>1.17 [0.75, 1.83]</td>
</tr>
</tbody>
</table>

Total events: 142 (168)

Heterogeneity: $\chi^2 = 16.87$, df = 6 ($P = 0.010$); $I^2 = 64$

Test for overall effect: $Z = 0.71$ ($P = 0.48$)

**Total (95% CI)**

- QQ Events: 3736
- QR-RR Events: 4356
- Total: 100.0%
- Odds Ratio: 1.28 [0.97, 1.68]

Total events: 420 (406)

Heterogeneity: $\chi^2 = 26.16$, df = 10 ($P = 0.004$); $I^2 = 62$

Test for overall effect: $Z = 1.73$ ($P = 0.08$)

Test for subgroup differences: $\chi^2 = 0.36$, df = 1 ($P = 0.55$), $I^2 = 0$

### PON1-Q192R and platelet reactivity


<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>QQ</th>
<th>QR-RR</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
</tr>
<tr>
<td>1.2.1 ADP 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOUMAN 2011a</td>
<td>62.05</td>
<td>11.83</td>
<td>52</td>
</tr>
<tr>
<td>LEWIS 2011a</td>
<td>32.4</td>
<td>12.5</td>
<td>261</td>
</tr>
<tr>
<td>LEWIS 2011b</td>
<td>36.6</td>
<td>22.3</td>
<td>77</td>
</tr>
<tr>
<td>TRENK 2011</td>
<td>54.2</td>
<td>17</td>
<td>384</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>774</td>
<td>839</td>
<td>32.5%</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.23; Chi² = 59.73, df = 3 (P < 0.00001); I² = 95%
Test for overall effect: Z = 1.40 (P = 0.15)

1.2.2 VASP

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>QQ</th>
<th>QR-RR</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
</tr>
<tr>
<td>CUISSET 2011</td>
<td>43</td>
<td>21</td>
<td>240</td>
</tr>
<tr>
<td>DERY 2011</td>
<td>44.1</td>
<td>17.9</td>
<td>103</td>
</tr>
<tr>
<td>FONTANA 2011</td>
<td>47.4</td>
<td>18.2</td>
<td>278</td>
</tr>
<tr>
<td>RIDE 2011</td>
<td>48.3</td>
<td>23.2</td>
<td>91</td>
</tr>
<tr>
<td>TSELEFIS 2011</td>
<td>57</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>749</td>
<td>745</td>
<td>39.9%</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.02; Chi² = 9.96, df = 4 (P = 0.04); I² = 60%
Test for overall effect: Z = 0.66 (P = 0.51)

1.2.3 VERIFYNOW/P2Y12

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>QQ</th>
<th>QR-RR</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
</tr>
<tr>
<td>CAMPO 2011</td>
<td>206</td>
<td>103</td>
<td>127</td>
</tr>
<tr>
<td>HULOT 2011b</td>
<td>168</td>
<td>85</td>
<td>168</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>295</td>
<td>376</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.00; Chi² = 0.35, df = 1 (P = 0.55); I² = 0%
Test for overall effect: Z = 1.37 (P = 0.17)

1.2.4 MULTIPLATE

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>QQ</th>
<th>QR-RR</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
</tr>
<tr>
<td>SIBBING 2011a</td>
<td>289.9</td>
<td>215.5</td>
<td>812</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>812</td>
<td>712</td>
<td>10.1%</td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: Z = 0.43 (P = 0.67)

Total (95% CI) 2630 2672 100.0% 0.10 [-0.06, 0.25]

Heterogeneity: Tau² = 0.06; Chi² = 78.18, df = 11 (P < 0.00001); I² = 86%
Test for overall effect: Z = 1.23 (P = 0.22)
Test for subgroup differences: Chi² = 3.62, df = 3 (P = 0.31), P = 17.2%

Graph showing comparison of QQ and QR-RR reactivity with 95% confidence intervals.
Key results from the meta-analysis

• PON1 polymorphism does not alter the biological response to clopidogrel.

• PON1 polymorphism has no impact on the risk of major cardiovascular ischemic events.

• Heterogeneity was mainly driven by one publication (Bouman et al.)
Let’s go back to the bench!

**IN VITRO STUDIES...**
**Clopidogrel metabolism in CYP2C19 genotyped microsomes**

**Clopidogrel → 2-oxo-clopidogrel**

**2-oxo-clopidogrel → active metabolite**

![Graph showing Clopidogrel concentration vs. 2-oxo-clopidogrel production](image1)

![Graph showing 2-oxo-clopidogrel concentration vs. Clopidogrel-AM production](image2)

**CYP2C19 genotypes:**
- CYP2C19 *1/*1
- CYP2C19 *2/*2

**PON1 activities:**
- CYP2C19*1/*1: 1.99 ± 0.04 U/mg
- CYP2C19*2/*2: 2.0 ± 0.04 U/mg

*Ancrenaz V et al. Br J Pharmacol. 2012*
Active metabolite production in serum and HLMs

Ancrenaz V et al., Br J Pharmacol. 2012

PON1 activity in serum = PON1 activity in HLMs

2-oxo-clopidogrel → active metabolite

clopidogrel → active metabolite

Clopidogrel-AM production (AUC m/z: 504-354)

2-oxo-clopidogrel production (AUC m/z: 338-183)

HLM

serum

Ancrenaz V et al., Br J Pharmacol. 2012
Postulated clopidogrel metabolism

Gong I et al., Euro Heart J. 2012
PON1 and CYPs mediated metabolism

Endo metabolite standard

Active metabolite standard

PON1 mediated metabolism

Human plasma sample

Gong I et al., Euro Heart J. 2012
Dr Bouman...

Not

Paraoxonase-1 is not a major determinant of clopidogrel efficacy

Heleen J Bouman1-3, Edgar Schöning4, Jochem W van Werkum1,2, Janna Velder5, Christian M Hackeng1,6, Christoph Hirschhäuser5, Christopher Waldmann7, Hans-Günther Schmalz5, Jurriën M ten Berg1,2 & Dirk Taubert4

Clinical efficacy of the antiplatelet drug clopidogrel is hampered by its variable biotransformation into the active metabolite1,2. The variability in the clinical response to clopidogrel treatment has been attributed to genetic factors, but the specific genes and mechanisms underlying clopidogrel bioactivation remain unclear. Using in vitro metabolomic profiling techniques, we identified paraoxonase-1 (PON1) as the crucial enzyme for clopidogrel bioactivation, with its common Q192R polymorphism determining the rate of active metabolite formation. We validated that this enzyme, with the PON1 Q192R variability in platelet response is explained by the variability in plasma concentrations of the active metabolite8,9.

We postulated that genetic variants of drug-metabolizing enzymes would affect the response to clopidogrel. Using a validated microsomal expression system of metabolizing enzymes, we identified PON1, an esterase synthesized in the liver and associated with HDL in blood, as the rate-determining enzyme for the formation of the thiol active metabolite from clopidogrel. We found that the common functional PON1 Q192R gene polymorphism resulted in a more efficient clopi-
In vitro / in vivo studies
Antiplatelet drug Prasugrel
Prasugrel pharmacokinetics and DDI with ritonavir

Déglon et al. 2011
**Résultats: étude du métabolisme in vitro du prasugrel**

- Implication principale des CYP3A et CYP2B6 avec participation mineure des CYP2C9 et 2C19 dans le métabolisme du prasugrel

*Daali et al., Metabolism, 2011*
Résultats: interaction prasugrel – ritonavir in vitro

Ritonavir
Inhibiteur des protéases du VIH (antirétroviral)

- Le ritonavir est un inhibiteur fort du CYP3A4 \((K_{i} = 0.017 \, \mu M)\) (Kumar et al. JPET, 1996)

- Le ritonavir est un inhibiteur in vitro du CYP2B6 (expérimental)

Daali et al., Metabolism, 2011
Prasugrel-ritonavir interaction in-vitro

---

**Graph Description:**
- Y-axis: Production of active metabolite (% control)
- X-axis: Concentration of prasugrel (µM)
- Legend:
  - Control
  - 0.1 µM
  - 1 µM
  - 5 µM
  - 10 µM
  - 15 µM
  - 30 µM
- The graph shows the percentage production of active metabolite compared to the control at different concentrations of prasugrel.

---

Daali et al., Metabolism, 2011
PK interaction between prasugrel and ritonavir in healthy volunteers (n=10)

Ancrenaz et al., Basic and Clinical Pharmacology and Toxicology, 2012

<table>
<thead>
<tr>
<th>Time</th>
<th>D-1</th>
<th>D0</th>
<th>D1</th>
<th>&gt;1 week</th>
<th>D8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit</td>
<td>Inform</td>
<td>Inclusion</td>
<td>Session 1</td>
<td>Session 2</td>
<td></td>
</tr>
</tbody>
</table>

Study design

Prasugrel 10mg

Ritonavir 100mg

Plasma samples

Breakfast

0' 15' 30' 1H 1H30 2H 3H 4H

Prasugrel 10mg

Ritonavir 100mg

Plasma samples

Breakfast
Prasugrel-ritonavir interaction in-vivo - PK study in healthy volunteers (n=10)

Ancrenaz et al., Basic and Clinical Pharmacology and Toxicology, 2012
Prasugrel pharmacokinetics and DDI with ritonavir

Prasugrel → Oxo-Prasugrel → AM-Prasugrel

Derivatization

R-106583

3A4
2B6

R-119251

Déglon et al. 2011
On-paper derivatization

Analytical strategy

Filter paper without MPBA  Filter paper with MPBA

Déglon et al, 2011
Prasugrel-Ritonavir interaction in healthy volunteers

Ancrenaz et al., Basic and Clinical Pharmacology and Toxicology, 2012

<table>
<thead>
<tr>
<th></th>
<th>Prasugrel alone</th>
<th>Prasugrel + ritonavir</th>
<th>Ratio</th>
<th>CI95</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC, h.ng/ml</td>
<td>339.6 (144.7)</td>
<td>207.5 (91.1)</td>
<td>0.62</td>
<td>0.52-0.71</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>C_max, ng/ml</td>
<td>389.8 (226.2)</td>
<td>185.4 (83.1)</td>
<td>0.55</td>
<td>0.37-0.73</td>
<td>0.008**</td>
</tr>
<tr>
<td>t_max (h)</td>
<td>0.65 (0.24)</td>
<td>0.70 (0.40)</td>
<td>1.20</td>
<td>0.70-1.60</td>
<td>0.73</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>1.62 (0.54)</td>
<td>1.42 (0.66)</td>
<td>0.94</td>
<td>0.59-1.29</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Résultats: pharmacocinétique du métabolite actif du prasugrel

CYP3A4/5 : Midazolam MR 97.8±1.4% (Mean ratio : 0.02, CI95 : 0.01; 0.03, p < 0.001)

CYP2C19 : Omeprazole MR (Mean ratio : 0.92, CI95 : 0.49 ; 1.35, p = 0.59)

CYP2C9 : Flurbiprofen MR (Mean ratio : 1, CI95 : 0.78 ; 1.26, p = 0.58)

Pas d’influence du ritonavir

CYP2B6 : Bupropion MR (Mean ratio : 1.02, CI95 : 0.85 ; 1.19, p = 0.88)

Le ritonavir inhibe la bioactivation du prasugrel via une forte inhibition du CYP3A4/5

Ancrenaz et al., Basic and Clinical Pharmacology and Toxicology, 2012
Conclusions

• In-vitro metabolism is helpful in drug development and clinic

• PBPK models and simulation are increasingly used and encouraged by the authorities (FDA, EMEA, ...)

• Multidisciplinary expertise is needed for conducting drug metabolism and PBPK simulations (Analytical chemistry, Clinical pharmacology, biostatistics and informatics).
Remerciements

• Dre. V. Ancrenaz, Dr. J. Déglon
• N. Marsousi, M. Bosilkovska, Dre. C. Samer
• Dr. C. Staub, Prof. S. Rudaz
• Prof. J. Desmeules, Prof. P. Dayer
• SIMCYP group
CONSEQUENCES OF BIOTRANSFORMATION

Active Drug to Inactive Metabolite
- Phenobarbital: hydroxylation → Hydroxyphenobarbital

Active Drug to Active Metabolite
- Clobazam: demethylation → N-desmethylclobazam

Inactive Drug to Active Metabolite
- Codeine: demethylation → Morphine

Active Drug to Reactive Metabolite
- Acetaminophen: CYP2E1 → Reactive metabolite
Métabolisme et transport dans le foie

**Phase I**: fonctionnalisation

**Phase II**: conjugaison (+ hydrophile)

**Phase III**: expulsion de conjugués ou du produit parent (ex: mdr, MRP)

: expulsion du xénobiotique inchangé ou conjugué