Irreversible Inhibition of CYP2C19 by some but not all proton pump inhibitors and its relevance to the anti-platelet effect of clopidogrel (Plavix)

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Presentation Overview
The clinical interaction between clopidogrel and omeprazole
CYP2C19 inhibition in vitro by omeprazole and other PPIs
Why was the in vitro MDI of CYP2C19 by omeprazole missed?
Why was the in vivo MDI of CYP2C19 by omeprazole missed?
How does omeprazole inactivate CYP2C19?
Recent developments

Clopidogrel and Omeprazole

Clopidogrel (Plavix®)
Anti-platelet drug (thienopyridine P2Y12 platelet inhibitor)
• 2nd most important drug in terms of sales ($9.1 Billion in 2009)
• 11th most prescribed drug in the USA (2008)
• 20% relative risk reduction for cardiovascular death, MI or stroke (versus aspirin alone) – Typically long-term use

Omeprazole (Prilosec-OTC®) and Esomeprazole(Nexium®)
Gastric proton pump inhibitors (PPIs)
• Nexium: 2nd most important drug in terms of sales ($5.9 Billion in 2008)
• Nexium: 7th most prescribed drug in the USA (2008)
• Erosive esophagitis: ~90% healing rate at 8 weeks (long-term use)

Financial perspective

<table>
<thead>
<tr>
<th>Drug</th>
<th>Revenue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>$10 billion per year</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>$6 billion per year</td>
</tr>
<tr>
<td>National debt</td>
<td>$4 billion per DAY</td>
</tr>
<tr>
<td>National debt</td>
<td>$14 thousand billion (14 trillion)</td>
</tr>
</tbody>
</table>

Interaction between clopidogrel and PPIs

Background Information

Clopidogrel increases bleeding risk
PPIs decrease the risk of gastrointestinal bleeding

- Co-prescribed in up to 2/3 of clopidogrel recipients following recommendations by a 2008 multi-society taskforce (Ho et al., 2009)

Numerous clinical publications indicate a loss of clopidogrel efficacy with PPIs, but this is highly controversial:

- "Omeprazole significantly decreased clopidogrel inhibitory effect on platelet[s]"
  The "OCLA study" (Gillard et al., 2008)

- December 6, 2010: "High-quality evidence supporting a clinically significant clopidogrel/PPI interaction is presently lacking" (Paulo de Aquino Lima and Brophy, 2010)

Concern that this is a class effect of PPIs

- May, 2009: European Medicines Agency (EMA) discouraged coadministration of clopidogrel with all PPIs (recently restricted to omeprazole)

- October 27, 2010 FDA "reminder" to avoid coadministration of clopidogrel and omeprazole
  - Not yet a boxed warning
  - "applies only to omeprazole and not to all PPIs. Not all PPIs have the same inhibitory effect on the enzyme (CYP 2C19) that is crucial for conversion of Plavix into its active form."
  - Pantoprazole (Protonix) may be an alternative ... It is a weak inhibitor of CYP2C19."

Clopidogrel is a pro-drug, dependent on CYP2C19


Importance of CYP2C19 for clopidogrel activation

Many (20-40%) patients are classified as "non-responders", "poor responders", or "resistant" to clopidogrel (Brandt JT, et al 2007)

Reduced function CYP2C19 polymorphisms lead to:

- Lower levels of the active metabolite of clopidogrel
- Diminished platelet inhibition
- Higher rate of major adverse cardiovascular events (Mega et al., 2009)

FDA boxed warning: "Reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug" – March 12, 2010

- CYP2C19 poor metabolizers: 2-14% of US population depending on ethnic background

Ho PM, et al., JAMA 301 (2009)
Gillard et al., J Amer Coll Cardiol 51 (2008)
Paulo de Aquino Lima and Brophy, BMC Medicine 8 (2010)
Mega et al., NEJM 360 (2009)
Clopidogrel interaction with omeprazole and other PPIs

Impact of CYP2C19 genetic polymorphisms on clopidogrel pharmacokinetics and pharmacodynamics

Impact of CYP2C19 status on clopidogrel pharmacokinetics and pharmacodynamics

Also controversial - in spite of the Mega and Kim studies:

- October 28, 2010 “... the effect of clopidogrel ... is consistent, irrespective of CYP2C19 loss-of-function carrier status”
  Paré et al., 2010

CYP2C19 status accounts for only 12 percent of the variation in response to clopidogrel

- Other factors (age, disease, cholesterol levels, body mass index etc.), account for another 10 percent
  Shuldiner et al., 2009

Questions arising from the clinical data

Q1 Does omeprazole (but not the other PPIs) cause irreversible inhibition (MDI) of CYP2C19?

Q2 If so, why was this not detected previously in in vitro studies?

Q3 Does if so, why doesn't omeprazole inhibit its own metabolism and cause more DDIs?
Clopidogrel interaction with omeprazole and other PPIs

Determination
When a drug is primarily metabolized by cytochrome P450 its inhibitory potency is generally similar across the three major in vitro test systems (HLM versus rCYP versus hepatocytes).

Example: Omeprazole is a metabolism-dependent inhibitor of CYP2C19 in all three test systems and the final IC50 value is roughly the same (1.3 to 1.7 µM).

Andrew Parkinson, Faraz Kazmi, David B. Buckley, Phyllis Yerino, Brian W. Ogilvie and Brandy L. Paris

http://www.jstage.jst.go.jp/browse/dmpk

System-dependent inhibition
Rule #1: When a drug is primarily metabolized by cytochrome P450, its inhibitory potency is generally similar across the three major in vitro test systems (HLM versus rCYP versus hepatocytes).

Example: Omeprazole is a metabolism-dependent inhibitor of CYP2C19 in all three test systems and the final IC50 value is roughly the same (1.3 to 1.7 µM).

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XenoTech's CYP Inhibition Study Design
Non-dilution method HLM 0.1 mg/mL or less. [S] = Km (5-min incubation)
Three IC50 curves A. Zero-time B. 30-min minus NADPH C. 30-min with NADPH

Time-dependent inhibition (TDI)
A. Zero-time (●)
B. 30-min minus NADPH (△)

Metabolism-dependent inhibition (MDI)
A. Zero-time (●)
B. 30-min minus NADPH (△)

A versus B tests for TDI
A versus C tests for MDI

XenoTech CYP Inhibition Decision Tree
Three IC50 curves: direct (A), time-dependent (B) and metabolism-dependent inhibition (C)
Multiple [inhibitor] × single [substrate] = Km. Low [HLM] (< 0.1 mg protein/mL)

Direct inhibition observed (Low IC50 value)
Determine potency and mechanism of inhibition (competitive, non-competitive, mixed or uncompetitive inhibition)

Kd Determination
Multiple [I] x Multiple [S]
IS THIS NECESSARY?

FDA accepts Ki estimates from well-designed IC50 experiments
Ki = IC50 / 2
Max (>75%) drugs with an IC50 of <1 µM cause clinically significant CYP inhibition. From Scott Obach (Pfizer)

MDI observed (After a 30-min incubation with NADPH. IC50 shift of >1.5 fold or a further 15% inhibition or more when below IC50)
Evaluate slow onset versus non-enzymatic degradation and metabolism by non-NADPH dependent enzymes (e.g., MAO)
Note that Ki (small 'i') for direct inhibition is not the same as Kd (large 'I') for MDI
If IC50 of metabolite
Determine if MDI involves covalent binding
Radiolabeled drug is useful

XTRA – reversibility assay (Ultracentrifugation method to evaluate the mechanism of MDI)
Quasi-reversible or irreversible
No further studies (or determine CI50 of metabolite)

Measure Kd and Kd
Multiple [I] x multiple times
Evaluate MIC complex formation for quasi-reversible inhibition

Andrew Parkinson - XenoTech
Clopidogrel interaction with omeprazole and other PPIs

February 2011

Omeprazole enantiomers

Prilosec = racemic omeprazole
Nexium = esomeprazole (the S-enantiomer)

CYP2C19 inhibition by omeprazole enantiomers

R-Omeprazole and S-omeprazole (esomeprazole) are both MDIs of CYP2C19

CYP2C19 inhibition by omeprazole metabolites

Omeprazole sulfone is an MDI of CYP2C19
Omeprazole sulfide is not

5'-Hydroxyomeprazole is a weak MDI of CYP2C19
Clopidogrel interaction with omeprazole and other PPIs

February 2011

CYP2C19 inhibition by lansoprazole

Lansoprazole is NOT an MDI of CYP2C19

CYP2C19 inhibition by pantoprazole

Pantoprazole is NOT an MDI of CYP2C19

CYP2C19 inhibition by rabeprazole

Rabeprazole MAY BE an MDI of CYP2C19

The absence of a shift in hepatocytes may be misleading. MDI may have occurred in both samples during the incubation with CYP marker substrate (note the IC50 values in hepatocytes equal the shifted IC50 in microsomes)

Summary of PPIs as CYP2C19 in human liver microsomes

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>No preincubation</th>
<th>30 min preincubation NADPH</th>
<th>30 min preincubation (+NADPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>6.9 ± 0.7</td>
<td>8.7 ± 0.6</td>
<td>1.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>15 ± 1</td>
<td>16 ± 1</td>
<td>1.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>R-Omeprazole</td>
<td>8.1 ± 1.2</td>
<td>12 ± 1.1</td>
<td>3.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Omeprazole sulfide</td>
<td>9.7 ± 0.5</td>
<td>8.4 ± 2.7</td>
<td>9.6 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Omeprazole sulfone</td>
<td>18 ± 2</td>
<td>12 ± 3</td>
<td>5.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>5'-Hydroxyomeprazole</td>
<td>160 ± 60</td>
<td>280 ± 100</td>
<td>100 ± 30</td>
<td></td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>50 ± 8</td>
<td>ND</td>
<td>13 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0</td>
<td></td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>93 ± 7</td>
<td>100</td>
<td>65 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Lansoprazole is the most potent direct inhibitor. But it’s not an MDI
Could lansoprazole cause clinically relevant direct inhibition of CYP2C19?

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma protein binding</th>
<th>Plasma half-life</th>
<th>Plasma Cmax</th>
<th>Direct inhibition potential ([I]/Ki)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>95%</td>
<td>0.7 hr</td>
<td>0.7 µM</td>
<td>3.5 µM</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>97%</td>
<td>0.9 hr</td>
<td>1.8 µM</td>
<td>11 µM</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>98%</td>
<td>1.0 hr</td>
<td>3.7 µM</td>
<td>6.5 µM</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>96%</td>
<td>1-2 hr</td>
<td>1.4 µM</td>
<td>2.7 µM</td>
</tr>
</tbody>
</table>

Note: [I]/Ki values are for EMs only because CYP2C19 inhibition cannot occur in PMs.

Lansoprazole may be able to cause direct inhibition of CYP2C19 in vivo, ([I]/Ki > 1.0) but the effect will be short-lived due to its short plasma half-life.

Is the MDI of CYP2C19 by omeprazole reversible?

Three methods were used to assess reversibility:
1. Dialysis
2. Centrifugation assay (XTRA)
3. Microsomes from hepatocytes used in enzyme induction studies with omeprazole as the positive control for CYP1A2 induction

All three methods agree. Only the results of the centrifugation assay are shown.

Irreversible inhibition of CYP2C19 by R-omeprazole, omeprazole, and esomeprazole as determined by ultracentrifugation

PPIs as CYP2C19 inhibitors – A summary

Direct inhibition (non-MDI)
- Pantoprazole
- Lansoprazole (reasonably potent direct-acting inhibitor)

Reversible MDI
- R-Omeprazole

Irreversible MDI
- Esomeprazole (the S-enantiomer)
- Probably rabeprazole too

For omeprazole, pantoprazole and lansoprazole, the pattern of MDI matches the known pattern of interaction with clopidogrel.
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CYP2C19 inhibition in vitro by omeprazole and other PPIs

Why was the in vitro MDI of CYP2C19 by omeprazole missed?

Why was the in vivo MDI of CYP2C19 by omeprazole missed?

How does omeprazole inactivate CYP2C19?

Recent developments

Why wasn’t omeprazole predicted to cause interactions with CYP2C19 substrates?

Metabolism-dependent inhibition was probably missed in vitro

- CYP2C19 activity is usually measured with S-mephenytoin
- This substrate has very low turnover.
- For this reason the assay of CYP2C19 activity is usually performed with high concentrations of HLM and/or long substrate incubation times
- Incubating MDIs with high concentrations of HLM can result in substantially less CYP inactivation
- MDI can also be missed if the incubation time with CYP marker substrate is long (it allows “unintended” MDI to occur)

In vitro inhibition of CYP2C19 by omeprazole in HLM

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ki or IC50 [Protein]</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-mephenytoin</td>
<td>1 µM, 0.5 mg/mL</td>
<td>120 min</td>
</tr>
<tr>
<td>(S)-mephenytoin</td>
<td>4 µM, 0.5 mg/mL</td>
<td>60 min</td>
</tr>
<tr>
<td>(S)-mephenytoin</td>
<td>6.1 µM, 1 mg/mL</td>
<td>30 min</td>
</tr>
<tr>
<td>(S)-mephenytoin</td>
<td>6.2 µM, 0.5 mg/mL</td>
<td>20 min</td>
</tr>
<tr>
<td>(S)-mephenytoin</td>
<td>7.1 µM, 1 mg/mL</td>
<td>60 min</td>
</tr>
<tr>
<td>proguanil</td>
<td>10 µM, 1 mg/mL</td>
<td>40 min</td>
</tr>
<tr>
<td>proguanil</td>
<td>10 µM, 1 mg/mL</td>
<td>40 min</td>
</tr>
<tr>
<td>3-O-methylfluorescein</td>
<td>21 µM, 0.5 mg/mL</td>
<td>30 min</td>
</tr>
<tr>
<td>adinazolam</td>
<td>21 µM, 2 mg/mL</td>
<td>20 min</td>
</tr>
<tr>
<td>diazepam</td>
<td>150 µM, 0.5 mg/mL</td>
<td>10 min</td>
</tr>
</tbody>
</table>

There is a huge range in IC50 values
Notice how the IC50 values decrease with increasing substrate incubation time
MDI of CYP2C19 is occurring during the substrate incubation period
This is how the MDI of CYP2C19 by omeprazole was missed
Irreversible inhibition of CYP2C19 by omeprazole

\[ k_{\text{inact}} / K_i \text{ determination at low concentrations of HLM and substrate concentration} = K_m \]

No dilution; \([S] = K_m\)

Irreversible inhibition of CYP2C19 by omeprazole

\[ k_{\text{inact}} / K_i \text{ determination at low concentrations of HLM and high substrate concentration (to reduce direct inhibition)} \]

No dilution; \([S] = 10 K_m\)

Irreversible inhibition of CYP2C19 by omeprazole

\[ k_{\text{inact}} / K_i \text{ determination with a 10-fold dilution step and high substrate concentration (to reduce direct inhibition)} \]

25-fold dilution; \([S] = 10 K_m\)

Pre-incubation: 0.1 mg/mL protein, no dilution. 5 min substrate incubation

**In vitro inactivation of CYP enzymes by various drugs**

Data from Obach, 2007. DMD 35:246-255, except text in red (XenoTech data)

<table>
<thead>
<tr>
<th>Inactivator (dose)</th>
<th>CYP</th>
<th>(k_{\text{inact}}) (min(^{-1}))</th>
<th>(K_i) (µM)</th>
<th>(k_{\text{inact}}/K_i) (min(^{-1})µM(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritonavir (600 mg b.i.d.)</td>
<td>CYP3A</td>
<td>0.28</td>
<td>0.16</td>
<td>1556</td>
</tr>
<tr>
<td>Ticlopidine (250 mg b.i.d.)</td>
<td>CYP2B6</td>
<td>0.3</td>
<td>0.57</td>
<td>526</td>
</tr>
<tr>
<td>Tienilic acid (250 mg q.d.)</td>
<td>CYP2C9</td>
<td>0.28</td>
<td>1</td>
<td>280</td>
</tr>
<tr>
<td>Paroxetine (20-50 mg q.d.)</td>
<td>CYP2D6</td>
<td>0.17</td>
<td>0.81</td>
<td>210</td>
</tr>
<tr>
<td>Furanil (30 mg q.d.)</td>
<td>CYP1A2</td>
<td>0.19</td>
<td>1.6</td>
<td>119</td>
</tr>
<tr>
<td>MDMA (200 mg ?)</td>
<td>CYP2D6</td>
<td>0.38</td>
<td>6.3</td>
<td>60.3</td>
</tr>
<tr>
<td>Ticlopidine (250 mg b.i.d.)</td>
<td>CYP2C9</td>
<td>0.097</td>
<td>4.3</td>
<td>22.6</td>
</tr>
<tr>
<td>Gemfibrozil (600 mg b.i.d.)</td>
<td>CYP2C8</td>
<td>0.21</td>
<td>20</td>
<td>10.5</td>
</tr>
<tr>
<td>Diltiazem (120 – 320 mg q.d.)</td>
<td>CYP3A</td>
<td>0.015</td>
<td>2.4</td>
<td>6.25</td>
</tr>
<tr>
<td>Desethylamiodarone (200 - 400 mg q.d.)</td>
<td>CYP3A</td>
<td>0.012</td>
<td>4.5</td>
<td>2.67</td>
</tr>
<tr>
<td>Erythromycin (250 – 500 mg b.i.d. to q.i.d)</td>
<td>CYP3A</td>
<td>0.018</td>
<td>4</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Pre-incubation: 2.5 mg/mL protein, diluted to 0.1 mg/mL. 5 min substrate incubation
Clopidogrel interaction with omeprazole and other PPIs

The MDI of CYP2C19 by omeprazole

Omeprazole is an irreversible MDI of CYP2C19 (mainly due to the S-enantiomer)

The $k_{\text{inact}}/K_i$ is in the range of several drugs known to cause clinically significant MDI of CYP enzymes

This property is easily missed by conducting the in vitro experiments with high concentrations of HLM and/or a long substrate incubation time

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Recent developments

In vivo inhibition of CYP2C19 by omeprazole

Omeprazole decreases the clearance of moclobemide, escitalopram, proguanil, etravirine, voriconazole

- Generally low magnitude (< 2-fold increase in AUC)

<table>
<thead>
<tr>
<th>Victim drug</th>
<th>Enzyme</th>
<th>Percent increase in AUC</th>
<th>PMID</th>
<th>Published</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moclobemide</td>
<td>CYP2C19</td>
<td>129</td>
<td>1139596</td>
<td>2001</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>CYP2C19</td>
<td>91</td>
<td>20642586</td>
<td>2010</td>
</tr>
<tr>
<td>Proguanil</td>
<td>CYP2C19</td>
<td>49</td>
<td>5533295</td>
<td>1997</td>
</tr>
<tr>
<td>Etravirine</td>
<td>CYP2C19</td>
<td>42</td>
<td>NDA # 022187</td>
<td>2008</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>CYP2C19/3A4</td>
<td>41</td>
<td>14691412</td>
<td>2003</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>CYP2C19</td>
<td>38</td>
<td>20944489</td>
<td>2011</td>
</tr>
</tbody>
</table>

In vivo evidence for MDI of CYP2C19 by omeprazole – auto-inhibition by esomeprazole

- Other DDIs mentioned in the omeprazole label include diazepam and cilostazol, but these are also of low magnitude
- Interestingly, omeprazole (but not lansoprazole or pantoprazole) has long been known to inhibit the in vivo metabolism of diazepam, and this inhibition occurs in CYP2C19 EMs but not PMs (proving the mechanism involves CY2C19 inhibition by omeprazole)

University of Washington Metabolism and Transport Drug Interaction Database

Andersson and Weidolf, Clin Drug Invest. 28, 263-279, 2008
**In vivo evidence for MDI of CYP2C19 by omeprazole**

*In vivo* magnitude of CYP2C19 inhibition increases with an increasing number of days of dosing with omeprazole

- Moclobemide AUC increases by ~31% after a single 40 mg dose of omeprazole, but increases by 120% after 8 days of dosing with 40 mg omeprazole. (Yu et al., 2001)

**Time-dependent changes in omeprazole pharmacokinetics**

- Omeprazole appears to inhibit its own CYP2C19-mediated metabolism, with a "metabolic shift" to CYP3A4-mediated sulfone formation (Klotz, 2006)
- Esomeprazole inhibits its own metabolism over time (Andersson et al., 2001)

These clinical findings suggest omeprazole causes MDI of CYP2C19 *in vivo*

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The clinical interaction between clopidogrel and omeprazole

CYP2C19 inhibition *in vitro* by omeprazole and other PPIs

Why was the *in vitro* MDI of CYP2C19 by omeprazole missed?

Why was the *in vivo* MDI of CYP2C19 by omeprazole missed?

**How does omeprazole inactivate CYP2C19?**

We don't know, but two mechanisms suggest themselves

- Methyl-hydroxylation with benzylic radical formation
- O-Demethylation to a para-aminophenol followed by quinoneimine formation

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**Proton pump inhibitor metabolism**

5'-Hydroxylation CYP2C19

Omeprazole

Lansoprazole

Reduction to thioether (non-enzymatic)

Pantoprazole

O-Demethylation of thioether CYP2C19

Rabeprazole
Clopidogrel interaction with omeprazole and other PPIs

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Stereoselective metabolism of omeprazole

CYP2C19 preferentially hydroxylates R-omeprazole
But it preferentially O-demethylates S-omeprazole

S-omeprazole

R-omeprazole

\[ \text{Cl}_{\text{int}} = 14.6 \, \text{µL/min/mg protein} \]

\[ \text{Cl}_{\text{int}} = 42.6 \, \text{µL/min/mg protein} \]

Andersson and Wedoff, Clin Drug Invest. 28, 263-279, 2008

How does omeprazole inactivate CYP2C19?

It is possible that methyl-hydroxylation of omeprazole by CYP2C19 involves formation of a radical analogous to the benzylic radical formed during the methyl-hydroxylation of gemfibrozil glucuronide by CYP2C8

But if this is the mechanism, why isn’t R-omeprazole a more effective MDI of CYP2C19 than S-omeprazole?

MDI of CYP2C8 by gemfibrozil glucuronide

Ogilvie…Parkinson. Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8. Implications for drug-drug interactions.

DMD 34:191-197, 2006

Baer BR, DeLisle RK and Allen A. Benzyl oxidation of gemfibrozil-1-O-β-glucuronide by P450 2C8 leads to heme alkylation and irreversible inhibition.


CLINICAL STUDY


But if O-demethylation/quinoneimine formation is responsible for CYP2C19 inactivation by S-omeprazole, why isn’t lansoprazole an MDI of CYP2C19?
Both are converted to para-aminophenols (but not the same one)
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Why was the in vitro MDI of CYP2C19 by omeprazole missed?
Why was the in vivo MDI of CYP2C19 by omeprazole missed?
How does omeprazole inactivate CYP2C19?

Recent developments in the story

Have we got it all wrong?

Q1  Why doesn’t clopidogrel inhibit its own metabolism?
Q2  Is PON1, not CYP2C19, the key enzyme in clopidogrel activation?

Clopidogrel is a structural analog of ticlopidine, a metabolism-dependent inhibitor of CYP2C19

Clopidogrel is an MDI of CYP2C19 (but less effective and administered at a lower dose)

Why doesn’t clopidogrel inhibit its own metabolism?
Like ticlopidine, clopidogrel is an MDI of CYP2C19

<table>
<thead>
<tr>
<th>Inactivator (dose)</th>
<th>CYP</th>
<th>kinact (min⁻¹)</th>
<th>Kᵢ (µM)</th>
<th>kinact/Kᵢ (min⁻¹µM⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole (20 – 40 mg q.d.)</td>
<td>CYP2C19</td>
<td>0.041</td>
<td>1.7</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.044</td>
<td>2.4</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.046</td>
<td>9.1</td>
<td>5.05</td>
</tr>
<tr>
<td>Ticlopidine (250 mg b.i.d.)</td>
<td>CYP2C19</td>
<td>0.097</td>
<td>4.3</td>
<td>22.6</td>
</tr>
<tr>
<td>Clopidogrel (75 mg q.d)</td>
<td>CYP2C19</td>
<td>0.0557</td>
<td>14.3</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Clopidogrel data from:
Why doesn’t clopidogrel inhibit its own metabolism?

It inhibits CYP2C19 in vivo, but only to a small extent.

In healthy subjects, clopidogrel inhibits the 5-hydroxylation of omeprazole (by CYP2C19) but not the sulfoxidation (by CYP3A4).

In CYP2C19 EMs, clopidogrel increases the plasma AUC of omeprazole by ~30% and decreases the plasma AUC of 5-hydroxyomeprazole by ~24%.

Note: Complete loss of CYP2C19 (in PMs) causes a ~5-fold increase in plasma AUC of omeprazole. A 30% increase is modest.

Clopidogrel causes no changes in omeprazole PK in CYP2C19 PMs.

Is CYP2C19 involved in clopidogrel activation?

Paraoxonase-1 is a major determinant of clopidogrel efficacy.

Major finding:

PON1 QQ192 homozygous individuals [PMs] showed a considerably higher risk than RR192 homozygous individuals [EMs] of:

- Stent thrombosis.
- Lower PON1 plasma activity.
- Lower plasma concentrations of active [clopidogrel] metabolite.
- Lower platelet inhibition.

Variations in the gene encoding PON1 “explain more than 70% of the variability in response to clopidogrel. . . . This dwarfs the contribution of the only variant previously identified to account for the heterogeneous response to the antiplatelet agent . . . CYP2C19.”

“PON1 poor metabolizers are 10 to 12 times more likely to suffer a stent thrombosis following PCI [percutaneous coronary intervention] that PON1 extensive metabolizers.”

PON1 status influences the pharmacological effectiveness of clopidogrel.

Dr. Dirk Taubert (senior author) in www.theheart.org/article/1166763.do

“To claim that it represents 70% of the variability in platelet response to clopidogrel is off base and exuberant.”
PON1 status influences the pharmacological effectiveness of clopidogrel

- PON1 “EMs”
- PON1 “IMs”
- PON1 “PMs”

Event-free survival (Fraction without event through time)

The impact seems small (note the y-axis scale) but there is a very large number of individuals with coronary artery disease (CAD) who take clopidogrel.

Would a paraoxonase be expected to hydrolyze 2-oxo-clopidogrel?

Yes it would

Paraoxonases are arguably better named “lactonases” for their role in hydrolyzing various lactone-containing drugs (e.g., statins)

2-Oxo-clopidogrel is a thiolactone (5-membered ring)

PON1 is in microsomes. Why did no one notice this before?

Paraoxonases need Ca²⁺ No one add calcium to study CYP reactions

CYP2C19 not only failed to convert 2-oxo-clopidogrel to the active metabolite (Step 2), but it also failed to oxidize clopidogrel to 2-oxo-clopidogrel (Step 1)

- (S)-Mephenytoin → 4'-hydroxy-mephenytoin
- Clopidogrel → 2-oxo-clopidogrel
- 2-Oxo-clopidogrel → active metabolite
- Clopidogrel → active metabolite
- CYP2C19*2 (truncated protein)
Clopidogrel interaction with omeprazole and other PPIs

Conventional view of clopidogrel activation

Note the prominent role of CYP2C19

Hydrolysis of the methyl-ester by hCE1 and other esterases to inactive metabolites

2-Oxo-clopidogrel

Clopidogrel active metabolite

Modified from Abraham et al., (2010)
J Amer. Coll. Cardiol. 56: 2051-2066

The new view of clopidogrel activation: Bouman et al. (2011)

NO role for CYP2C19
Step 1 = CYP3A4
Step 2 = Paraoxonase

Hydrolysis of the methyl-ester by hCE1 and other esterases to inactive metabolites

2-Oxo-clopidogrel

Clopidogrel active metabolite

Modified from Bouman et al., Nat Med 17 (2011)

Dilemmas
(Yes, there’s more than one)

Bauman identified PON1 but not CYP2C19 to be associated with clopidogrel’s clinical effectiveness

This is the opposite of Shuldiner’s finding (JAMA, 302, 849, 2009)

If CYP2C19 plays no role in the activation of clopidogrel, why is formation of the pharmacologically active metabolite influenced by the CYP2C19 genetic polymorphism?

Why did Bauman observe no role for CYP2C19 in either Step 1 or Step 2 of clopidogrel activation in vitro contrary to several other groups that implicate CYP2C19 in both steps?

And how does omeprazole cause a clinically relevant decrease formation of the active metabolite of clopidogrel if CYP2C19 is not involved its formation?

Stereochemistry - Not all H4 metabolites are equal
This may underlie some of the confusion

$3Z$ isomer = active
$3E$ isomer = inactive
Critical

$7S$ = active
$7R$ = inactive
Critical

$4R$ isomer = active
$4S$ isomer = active
Not critical

Pereillo et al., DMD 30, 1288-1295, 2002

Clopidogrel: $7S$
Active metabolite: $3Z,4R$ and $3Z,4S$
Inactive metabolite: $3E,4R$ and $3E,4S$
Clopidogrel interaction with omeprazole and other PPIs

February 2011

What the . . . . . . Maybe it's not H4 after all!

Omeprazole (especially the S-enantiomer) - but not pantoprazole or lansoprazole - is an irreversible MDI of CYP2C19

The use of high [HLM] and long substrate incubation times masks this effect

Inhibition of CYP2C19 by omeprazole could account for the clinically relevant impairment of clopidogrel's antiplatelet effect

This mechanism of action is tenable provided CYP2C19 plays a role in the conversion of clopidogrel to its active metabolite

The role of CYP2C19 in clopidogrel activation has recently been brought into question, which spoils an otherwise nice story

Summary

Thank you

And special thanks to

Brian Ogilvie
Faraz Kazmi
Phyllis Yerino
Sabrina Mogle
Maggie McMullen