Esomeprazole, omeprazole sulfone, 5-O-desmethyl omeprazole and 5-hydroxylansoprazole are in vitro metabolism-dependent inhibitors of CYP2C19

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INTRODUCTION

The intensively researched interaction between clopidogrel and proton pump inhibitors (PPIs) and the impact of CYP2C19 poor metabolizer phenotype on clopidogrel efficacy have prompted warnings from the FDA and EMA in recent years (2010). The FDA specifically warns against coadministration of clopidogrel and omeprazole and further specifically suggests that pantoprazole may be a safer alternative (2010).

Recently, we have shown that omeprazole (but not pantoprazole or lansoprazole) is an MDI of CYP2C19 in human liver microsomes (HLM), cytoreserved human hepatocytes and recombinant human CYP2C19 (Ogilvie et al., 2011). We provided evidence that esomeprazole is more likely to irreversibly inactivate CYP2C19 than is R-omeprazole (4-fold greater IC50 shift). Based on the K_{inact} and K_{IC50} values for the MDI of CYP2C19 by racemic omeprazole in HLM, active levels of hepatic CYP2C19 in the presence of omeprazole (40 mg b.i.d. for 14 days) were also simulated with the Simcyp Simulator V10.2. Although this simulation predicts clinically significant inactivation of CYP2C19, it does not predict complete inactivation of CYP2C19. Since we also previously showed that omeprazole sulfone is an MDI of CYP2C19 (IC50 shift = 3.2 fold), we further investigated the impact of CYP2C19 poor metabolizer phenotype on clopidogrel efficacy have prompted warnings that the effect of glutathione on the MDI was examined. In the presence of glutathione (10 mM), the IC50 shift decreased to 1.8-fold.

MATERIALS & METHODS

Chemicals: 4'-hydroxy-S-mephenytoin was purchased from Sigma Chemical Co. (St. Louis, MO), O-(5-O-desmethyl omeprazole, esomeprazole, 5-hydroxylansoprazole, 4'-hydroxymephenytoin, and omeprazole were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). All other reagents were obtained from commercial sources, as detailed elsewhere (Parkinson et al., 2011). Incubations with human liver microsomes (HLM) (poled, n=16), were conducted at approximately 37°C in 200-µL incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), MgCl2 (3 mM), EDTA (1 mM, pH 7.4) and a NADPH-generating system (1 mM NADP, 5 mM glucose-6-phosphate, and 1 U/mL glucose-6-phosphate dehydrogenase) at the final concentrations indicated. Reactions were initiated by the addition of the NADPH-generating system to the incubation mixture containing HLM, marker substrate (S-mephenytoin), and inhibitor. Reactions with a pre-incubation (3–30 minutes at 37°C) were started by addition of the NADPH-generating system to the incubation mixture containing HLM and inhibitor. Preincubation and substrate reactions were initiated by the addition of S-mephenytoin (40 μM, K_{IC50} = 400 μM, 10 K_{IC50}). Incubations were performed at 34°C in 0.2% methanol and final HLM concentration of 0.1 µg of microsomal protein/mL.

Inhibition experiments were conducted in accordance with previously described methods (Ogilvie et al., 2006; Ogilvie et al., 2008; Nassar et al., 2009; Parkinson et al., 2011). Briefly, K_{IC50} experiments, with and without pre-incubation, were conducted with the inhibitors, (0.1 – 100 μM), 0.1 – 70 μM in the case of S-desmethyl omeprazole) in HLMs (0.1 mg/mL, +/- NADPH). K_{inact} experiments with esomeprazole (0.3-30 μM) or omeprazole sulfone (1 – 100 μM) were pre-incubated (10 – 30 minutes with HLM (100 μg/mL) followed by a 10-fold dilution with the NADPH-generating system to incubation mixture containing HLM and marker substrate (400 μM S-mephenytoin), for a final HLM concentration of 0.1 or 0.5 mg/mL. The assessment of MDI reversibility by ultracentrifugation was performed as described previously (Ogilvie et al., 2011; Parkinson et al., 2011) with concentrations of inhibitors as indicated on graphs. IC_{50} and K_{IC50} data were analyzed with a non-linear regression data analysis program, GraphPad 7.5.2 (Entac rash, Surrey, UK).

RESULTS

Table 1: Summary of K_{inact} and K_{IC50} values for the MDI of CYP2C19 by esomeprazole and 5-O-desmethyl omeprazole in HLMs (0.1 mg/mL, +/- NADPH).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Final [protein] (mg/mL)</th>
<th>K_{IC50} (µM)</th>
<th>K_{inact} (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-O-desmethyl omeprazole</td>
<td>0.1</td>
<td>0.13</td>
<td>0.016</td>
</tr>
<tr>
<td>5-O-desmethyl omeprazole</td>
<td>0.5</td>
<td>0.13</td>
<td>0.016</td>
</tr>
<tr>
<td>5-O-desmethyl omeprazole</td>
<td>1.0</td>
<td>0.13</td>
<td>0.016</td>
</tr>
<tr>
<td>Omeprazole sulfone</td>
<td>0.1</td>
<td>0.13</td>
<td>0.016</td>
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In the present study, metabolites of the proton pump inhibitor omeprazole, namely omeprazole sulfone and 5-O-desmethyl omeprazole, as well as a metabolite of lansoprazole, namely 5-hydroxylansoprazole, were identified as metabolism-dependent inhibitors of CYP2C19 in pooled human liver microsomes. K_{IC50} and K_{inact} values were determined under normal and low protein conditions for esomeprazole and omeprazole sulfone. The MDI of CYP2C19 by omeprazole sulfone and 5-O-desmethyl omeprazole was found to be reversible. In addition, the MDI caused by 5-O-desmethyl omeprazole was found to be somewhat reduced by the presence of 10 mM glutathione. The results indicate that the 5-O-desmethyl and sulfone metabolites of omeprazole may contribute to its overall effect on CYP2C19 in vivo.

CONCLUSIONS

REFERENCES


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