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), cryopreserved 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 IC_{50} shift). Based on the K_I and k_{inact} 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 CYP2C19, it does not predict complete inactivation of CYP2C19. Since we also previously showed that omeprazole sulfone is an MDI of CYP2C19 (IC_{50} shift = 3.2 fold), we further investigated the MDI of CYP2C19 by this major metabolite of omeprazole in pooled HLM. Although one major metabolite of omeprazole, namely 5-hydroxyomeprazole, did not inhibit CYP2C19 (IC_{50} > 100 µM), we also investigated the inhibitory potency of another major metabolite of omeprazole, 5-*O*-desmethyl omeprazole, towards CYP2C19 in pooled HLM. As discussed in the recent publication, although lansoprazole is not an MDI of CYP2C19, its 5-hydroxy metabolite is analogous to 5-*O*-desmethyl omeprazole, so its inhibitory potential toward CYP2C19 was also investigated. These studies were undertaken as a step toward accurately predicting the overall effect of omeprazole and its metabolites on active levels of CYP2C19 *in vivo*.

MATERIALS & METHODS

Chemicals: 4′-hydroxy-*S*-mephenytoin was purchased from Sigma Chemical Co. (St. Louis, MO). 5-*O*-desmethyl omeprazole, esomeprazole, 5-hydroxylansoprazole, d_3-4′-hydroxy-*S*-mephenytoin, *S*-mephenytoin, and omeprazole sulfone were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). All other reagents were obtained from commercial sources, as detailed elsewhere (Ogilvie *et al*., 2006; Nassar *et al*., 2009; Parkinson *et al*., 2011).

Incubations with human liver microsomes (HLM) (pooled, n=16), were conducted at approximately 37°C in 200-µL incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (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 without a pre-incubation 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. Marker substrate reactions were initiated by the addition of *S*-mephenytoin (40 µM, ~K_m, or 400 µM, ~10 K_m). In all cases, marker substrate reactions (5 minutes) were terminated by the addition of an equal volume of acetonitrile and internal standard (d_3-4′-hydroxy-*S*-mephenytoin). Metabolite formation (4′-hydroxymephenytoin) was determined by LC-MS/MS as previously described (Parkinson *et al*., 2011).

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, IC_{50} experiments, with and without a 30 minute pre-incubation, were conducted with the inhibitors, (0.1 – 100 µM; 0.1 – 70 µM in the case of 5-*O*-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 (0 – 15 minutes) with HLM (1.0 or 0.1 mg/mL) followed by a 10-fold dilution prior to incubation with marker substrate (400 µM *S*-mephenytoin), for a final HLM concentration of 0.1 or 0.01 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_{inact} data were analyzed with a non-linear regression data analysis program, GraFit 7.0.2 (Erithacus, Surrey, UK).

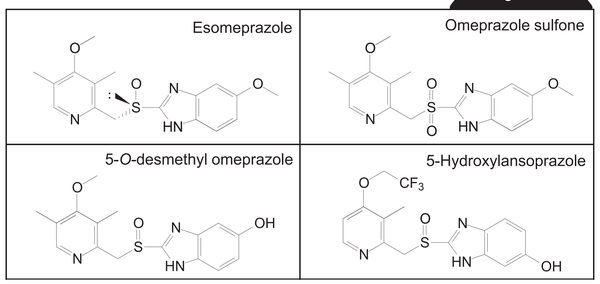
Table 1

Compound	Final [protein] (mg/mL)	K_I (µM)	k_{inact} (min^{-1})	k_{inact} / K_I ($min^{-1} \cdot mM^{-1}$)
Esomeprazole	0.1	17 ± 4	0.072 ± 0.009	4.3
	0.01	5.7 ± 0.6	0.058 ± 0.002	10
Omeprazole sulfone	0.1	4.4 ± 0.6	0.025 ± 0.002	5.7
	0.01	0.80 ± 0.3	0.017 ± 0.001	21

RESULTS

• **Figure 1:** Structures of compounds used in this study.

Figure 1



• **Figure 2** shows the rate of inactivation of human liver microsomal CYP2C19 as a function of esomeprazole concentration. With a starting [HLM] = 1.0 mg/mL and final [HLM] of 0.1 mg/mL, the k_{inact} was 0.072 min^{-1} and the K_I value was 17 µM.

• **Figure 3** shows the rate of inactivation of human liver microsomal CYP2C19 as a function of esomeprazole concentration. With a starting [HLM] = 0.1 mg/mL and final [HLM] of 0.01 mg/mL, the k_{inact} was 0.058 min^{-1} and the K_I value was 5.7 µM.

• **Figure 4** shows the rate of inactivation of human liver microsomal CYP2C19 as a function of omeprazole sulfone concentration. With a starting [HLM] = 1.0 mg/mL and final [HLM] of 0.1 mg/mL, the k_{inact} was 0.025 min^{-1} and the K_I value was 4.4 µM.

• **Figure 5** shows the rate of inactivation of human liver microsomal CYP2C19 as a function of omeprazole sulfone concentration. With a starting [HLM] = 0.1 mg/mL and final [HLM] of 0.01 mg/mL, the k_{inact} was 0.017 min^{-1} and the K_I value was 0.8 µM. **Table 1** summarizes the k_{inact} results from Figs 2-5.

• **Figure 6** shows that the time-dependent inhibition of CYP2C19 in HLM by 5-*O*-desmethyl omeprazole is dependent on the presence of NADPH (and is therefore metabolism-dependent), with a 5.4-fold IC_{50} shift. Because 5-*O*-desmethyl omeprazole has the potential to form a quinoneimine, the effect of glutathione on the MDI was examined. In the presence of glutathione (10 mM), the IC_{50} shift decreased to 1.8-fold.

Figure 2: Normal Protein — Esomeprazole

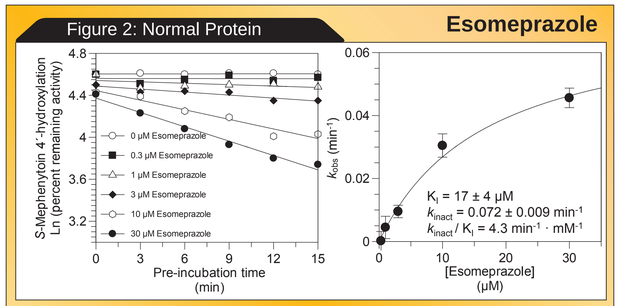


Figure 3: Low Protein

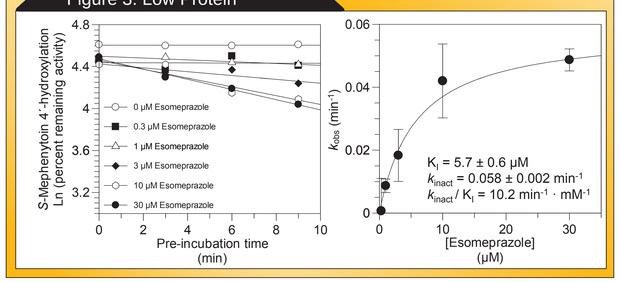


Figure 4: Normal Protein — Omeprazole sulfone

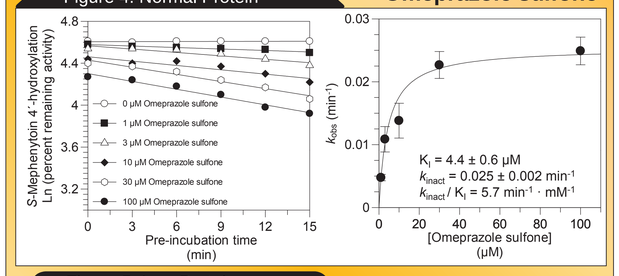


Figure 5: Low Protein

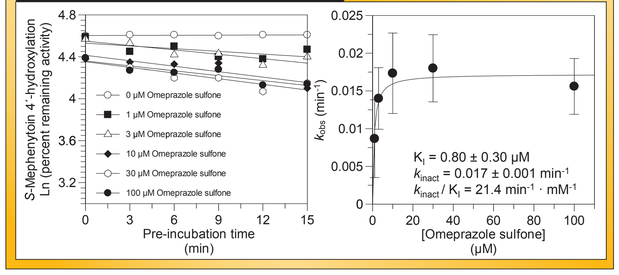
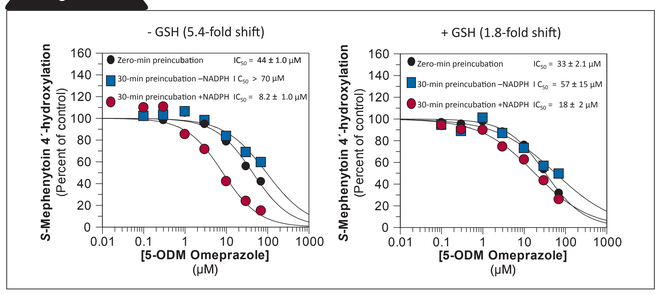


Figure 6



• **Figure 7** shows that the time-dependent inhibition of CYP2C19 in HLM by 5-hydroxylansoprazole is also dependent on the presence of NADPH (and is therefore metabolism-dependent), with a 4.5-fold IC_{50} shift.

• **Figure 8** shows the results of ultracentrifugation experiments which suggest that the MDI of CYP2C19 by omeprazole sulfone and 5-*O*-desmethyl omeprazole is largely irreversible, and unaffected by potassium ferricyanide.

Figure 7

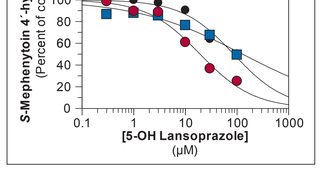
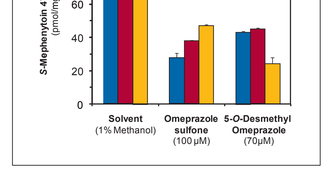


Figure 8



CONCLUSIONS

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_I 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 irreversible. 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*.

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