The Proton Pump Inhibitor, Omeprazole, but not Lansoprazole or Pantoprazole, is a Metabolism-Dependent Inhibitor of CYP2C19:

Implications for Coadministration with Clopidogrel


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Running Title: Omeprazole is a Metabolism-Dependent Inhibitor of CYP2C19

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Abbreviations used are: AhR, aryl hydrocarbon receptor; AUC, area under the plasma concentration vs. time curve; CL_{int}, in vitro intrinsic clearance (i.e., Vmax/Km); C_{max}, maximum plasma concentration; CYP, Cytochrome P450; DDI, drug-drug interaction; EM, extensive metabolizer; HLM, human liver microsomes; IC_{50}, inhibitor concentration that causes 50% inhibition; IM, intermediate metabolizer; KHB, Krebs-Henseleit buffer; K_{i}, inhibitor concentration that supports half the maximal rate of inactivation; k_{inact}, maximal rate of enzyme inactivation; LC/MS-MS, liquid chromatography/tandem mass spectrometry; MTDI, University of Washington Metabolism and Transport Drug Interaction Database™.
http://www.druginteractioninfo.org; MDI, metabolism-dependent inhibitor; OATP, organic anion transportating polypeptide; PM, poor metabolizer; PPI, proton pump inhibitor; rCYP, recombinant cytochrome P450; UGT, UDP-glucuronosyltransferase.
ABSTRACT

As a direct-acting inhibitor of CYP2C19 in vitro, lansoprazole is more potent than omeprazole and other proton pump inhibitors (PPIs), and yet lansoprazole does not cause clinically significant inhibition of CYP2C19 whereas omeprazole does. To investigate this apparent paradox, we evaluated omeprazole, esomeprazole, R-omeprazole, lansoprazole and pantoprazole for their ability to function as direct-acting and metabolism-dependent inhibitors (MDIs) of CYP2C19 in pooled human liver microsomes (HLM), as well as in cryopreserved hepatocytes and recombinant CYP2C19. In HLM, all PPIs were found to be direct-acting inhibitors of CYP2C19 with IC_{50} values varying from 1.2 µM (lansoprazole; C_{max} = 2.2 µM) to 93 µM (pantoprazole; C_{max} = 6.5 µM). In addition, we identified omeprazole, esomeprazole, R-omeprazole, and omeprazole sulfone as MDIs of CYP2C19 (they caused IC_{50} shifts after a 30-min preincubation with NADPH-fortified HLM of 4.2-, 10-, 2.5-, and 3.2-fold, respectively), whereas lansoprazole and pantoprazole were not MDIs (IC_{50} shifts <1.5-fold). The MDI of CYP2C19 by omeprazole and esomeprazole was not reversed by ultracentrifugation, suggesting the inhibition was irreversible (or quasi-irreversible), whereas ultracentrifugation largely reversed such effects of R-omeprazole. Under various conditions, omeprazole inactivated CYP2C19 with K_{i} values of 1.7 – 9.1 µM and k_{inact} values (maximal rate of inactivation) of 0.041 – 0.046 min^{-1}. This study identified omeprazole, and esomeprazole, but not R-omeprazole, lansoprazole or pantoprazole, as irreversible (or quasi-irreversible) MDIs of CYP2C19. These results have important implications for the mechanism of the clinical interaction reported between omeprazole and clopidogrel, as well as other CYP2C19 substrates.
Introduction

Omeprazole and other proton pump inhibitors (PPIs, i.e., esomeprazole, lansoprazole, dexlansoprazole, rabeprazole, and pantoprazole) are well known for a relatively low incidence of adverse events and pharmacokinetic drug-drug interactions (DDIs). Nevertheless, PPIs are the perpetrators of interactions with cyclosporine (omeprazole and rabeprazole), diazepam (esomeprazole and omeprazole) and warfarin (esomeprazole, lansoprazole, omeprazole, and rabeprazole) (Wallace and Sharkey, 2011). In addition, all drugs that increase gastric pH can affect the bioavailability of drugs such as ampicillin esters, iron salts and ketoconazole, among others (Wallace and Sharkey, 2011). Omeprazole, the first approved PPI, decreases the clearance of drugs such as diazepam, moclobemide, escitalopram, carbamazepine, saquinavir, sibutramine, proguanil, etravirine, disulfiram, phenytoin, voriconazole, and clopidogrel, and, by inducing CYP1A2, increases the clearance of several antipsychotic drugs, such as imipramine, theophylline, and tacrine (Angiolillo et al., 2011; Wallace and Sharkey, 2011); MTDI database: http://www.druginteractioninfo.org).

The inhibitory DDIs listed above have been attributed, at least in part, to inhibition of CYP2C19 by omeprazole. However, clinically relevant DDIs with omeprazole are generally of low magnitude (≤120% [2.2-fold] increase in plasma AUC of CYP2C19 substrates) (MTDI database). By way of comparison, there is up to a 14.6-fold increase in the AUC of omeprazole when CYP2C19 is completely absent as occurs in genetically determined CYP2C19 poor metabolizers (Furuta et al., 1999). Nearly 40 in vitro studies examining PPIs as CYP inhibitors have been published to date (MTDI database). Only omeprazole, lansoprazole, (and their S-enantiomers) have been shown to inhibit CYP2C19 with IC_{50} or Ki values ≤ 1.0 μM. Of particular interest is that none of these in vitro studies examined the PPIs as MDIs (a.k.a time-dependent inhibitors) of P450 enzymes. Based on the lowest reported Ki values for CYP2C19 inhibition in the MTDI database (i.e., 0.45 μM for lansoprazole and 1 μM for omeprazole), and the reported plasma C_{max} for lansoprazole and omeprazole in CYP2C19 extensive metabolizers (2.2 and 3.9 μM, respectively; (Li et al., 2004; Hassan-Alin et al., 2005), the [I]_{total}/Ki values for lansoprazole and
omeprazole would be 4.9 and 3.9, respectively (calculated as recommended in the Draft FDA Guidance for Industry, 2006; http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm), which means that both drugs would be expected to cause clinically relevant direct inhibition of CYP2C19, but that lansoprazole would have a higher likelihood of doing so than would omeprazole. However, lansoprazole has been reported to cause no interaction with the CYP2C19 substrates diazepam, and phenytoin (MTDI database, Wallace and Sharkey, 2011). The lack of clinically relevant direct inhibition of CYP2C19 by lansoprazole is likely explained by its relatively short half-life (1.1 hr; Li et al., 2004) and high plasma protein binding (~97%; Prevacid prescribing information, 2010, http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020406s074,021428s021lbl.pdf). However, omeprazole also has a short half-life (0.7 hr; Hassan-Alin et al., 2005) and high plasma protein binding (~95%; Prilosec prescribing information, 2011, http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/019810s092,022056s008lbl.pdf), and yet, as described above, omeprazole does cause clinically significant inhibition of CYP2C19. This apparent discrepancy could be explained if omeprazole, but not lansoprazole, were an irreversible inhibitor of CYP2C19.

In support of this possibility, we presented preliminary in vitro evidence for MDI of CYP2C19 by omeprazole (Paris et al., 2008). More recently, a large clinical study in 282 healthy subjects (Angiolillo et al., 2011) demonstrated that omeprazole inhibited the CYP2C19-dependent activation of clopidogrel as evidenced by a 40-47% decrease in the formation of H4, the purported active antiplatelet metabolite of clopidogrel. Inhibition of clopidogrel activation was observed even when the two drugs were administered 12 hours apart. In the same subjects, no such interaction was observed between pantoprazole and clopidogrel.

The interaction between clopidogrel and PPIs and the impact of the CYP2C19 poor metabolizer phenotype have prompted warnings from the FDA and EMA (2010, http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm20
although the FDA specifically warns against coadministration of clopidogrel and omeprazole and further

The identity of the enzyme(s) responsible for the multi-step activation of clopidogrel is controversial
(Savi et al., 1994; Clarke and Waskell, 2003; Abraham et al., 2010; Kazui et al., 2010; Zahno et al.,
2010). Much of the pharmacogenomic data strongly implicate CYP2C19 as the most important enzyme
for clopidogrel activation, based on poor response to clopidogrel in carriers of reduced function
CYP2C19 alleles (e.g., the *2 and *3 alleles), and also increased bleeding in carriers of the increased
function CYP2C19*17 allele (Sibbing et al., 2010). The DDI between PPIs and clopidogrel is of
particular importance because PPIs are co-prescribed in up to ~2/3 of patients after discharge from
hospital because PPIs lessen the severity of the gastrointestinal hemorrhage associated with clopidogrel
treatment (Ho et al., 2009).

In the present study we examined the in vitro inhibitory potential of omeprazole, its individual
enantiomers and selected metabolites, as well as lansoprazole and pantoprazole (Fig. 1) in pooled HLM,
pooled cryopreserved hepatocytes and rCYP2C19, with a special emphasis on the potential for these
drugs to cause MDI of CYP2C19. The implications of our results were explored by dynamic simulations
assessing the level of active CYP2C19 under multiple doses of omeprazole. This PBPK modeling and
simulation provided additional insight into the ongoing debate surrounding the interaction between PPIs
and clopidogrel.
MATERIALS AND METHODS

Chemicals and Reagents

Omeprazole and 4′-hydroxymephenytoin were purchased from Sigma-Aldrich (St. Louis, MO). Esomeprazole, 5′-hydroxyomeprazole, S-mephenytoin, lansoprazole, omeprazole sulfide and pantoprazole were purchased from Toronto Research Chemicals (North York, ON, Canada). R-Omeprazole and omeprazole sulfone were purchased from SynFine Research (Richmond Hill, ON, Canada) or Toronto Research Chemicals (North York, ON, Canada). Human liver microsomes (pooled from 16 donors) and human hepatocytes were prepared from non-transplantable livers and characterized at XenoTech LLC (Lenexa, KS) as described previously (Parkinson et al., 2011), and recombinant CYP2C19 (Bactosomes) was obtained from Cypex (Dundee, Scotland). All other reagents were obtained from commercial sources, as detailed elsewhere (Ogilvie et al., 2006; Nassar et al., 2009; Parkinson et al., 2011).

CYP2C19 Inhibition: IC₅₀ determinations

CYP2C19 activity in human liver microsomes (HLM) was determined according to previously published procedures (Ogilvie et al., 2006; Nassar et al., 2009; Parkinson et al., 2011). Briefly, incubations were conducted in 200-μL incubation mixtures (pH 7.4) containing high purity water, potassium phosphate buffer (50 mM), MgCl₂ (3 mM), EDTA (1 mM), NADP (1 mM), glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 Unit/mL), S-mephenytoin (approximately equal to Km, i.e., 40 µM, final) and HLM (0.1 mg protein/mL, except where otherwise noted). All incubations were conducted in duplicate at 37°C for 5 min (except where otherwise noted) and were terminated by the addition of 200 µL acetonitrile containing an internal standard (d₃-4′-hydroxymephenytoin). Aliquots of the stock and/or working solutions of the inhibitors (i.e., omeprazole, esomeprazole, R-omeprazole, lansoprazole or pantoprazole; final concentrations ranging from 0.1 to 100 µM in methanol) were added to buffer mixtures containing the components described above, but prior to addition of the NADPH-generating system. To evaluate the potential for MDI, the inhibitors (at the same concentrations used to evaluate
direct inhibition) were incubated at 37°C with NADPH-fortified HLM for approximately 30 min. After the 30-min preincubation, S-mephenytoin (40 µM, final) was added, and the incubation was continued for 5 min to measure residual CYP activity. Precipitated protein was pelleted by centrifugation (920 × g for 10 min at 10°C). Calibration and quality control (QC) standards (4'-hydroxymephenytoin) were prepared in zero-time incubations. IC₅₀ determinations with recombinant human CYP2C19 (15 pmol/mL) were conducted in the same manner except that the incubation time was only 2 min to prevent over-metabolism of omeprazole. Metabolite formation (4'-hydroxymephenytoin) was analyzed by LC-MS/MS as described previously (Ogilvie et al., 2006; Parkinson et al., 2011).

Incubations with cryopreserved human hepatocytes (pooled, n=3, 10⁶ cells/mL) were conducted in 200-µL incubation mixtures at approximately 37°C in Krebs Henseleit Buffer (KHB), in triplicate. In all cases, the solvent or omeprazole was allowed to equilibrate for 10 min with hepatocytes prior to incubations. For samples with no pre-incubation with inhibitor, reactions were started by the addition of hepatocytes to pre-warmed KHB containing inhibitor and S-mephenytoin (40 µM, final concentration). For reactions with a pre-incubation, hepatocytes and inhibitor were incubated at 37°C for 30 minutes and reactions were started by addition of S-mephenytoin (40 µM, final). In all cases, marker substrate reactions were conducted for 60 minutes and terminated by the addition of an equal volume of acetonitrile and internal standard (d₃-4'-hydroxymephenytoin). Metabolite formation (4'-hydroxymephenytoin) was analyzed by LC-MS/MS as described previously (Ogilvie et al., 2006; Parkinson et al., 2011).

Metabolic stability of omeprazole. The metabolic stability of omeprazole (10 µM), was determined at three concentrations of HLM (0.1, 1.0, and 2.5 mg/mL) under conditions similar to those described above for CYP2C19 inhibition experiments. Omeprazole was incubated for zero, 5, 10, 20, 30, 45, and 60 min, in triplicate. Reactions were terminated by the addition of an equal volume of acetonitrile and internal standard (pantoprazole). Precipitated protein was removed by centrifugation (920 × g for 10 min at
10°C). Omeprazole disappearance was monitored by LC-MS/MS. Calibration standards were prepared in zero-time incubations.

**Microsomal binding of omeprazole.** The binding of omeprazole to microsomal protein was determined by ultrafiltration with Millipore Amicon Centriplus centrifugal filter devices (15 mL, 30 kDa membrane) obtained from Fisher Scientific (Pittsburgh, PA). Omeprazole (2 and 10 µM), was incubated with pooled HLM (zero, 0.1, 1.0, or 2.5 mg/mL), as described above, but in the absence of an NADPH-generating system at 37°C for 10 min. Aliquots (1.1 mL) were then removed and added to the ultrafiltration devices, and centrifuged at 1900 × g in a Sorvall RC 5C centrifuge with a Sorvall SS-34 rotor at room temperature for 5 min. Aliquots of the ultrafiltrate (100 µL) were transferred to glass tubes and an equal volume of acetonitrile added and vortexed. Precipitated protein was removed by centrifugation (920 × g for 10 min at 10°C). Following centrifugation, an aliquot (100 µL) was transferred to an equal volume of acetonitrile (with pantoprazole as the internal standard) and analyzed for omeprazole concentration by LC-MS/MS.

**K_i and k_inact determinations.** To determine the K_i and k_inact values for the inactivation of CYP2C19, various concentrations of omeprazole (1 to 60 µM) were incubated for 2.5 to 15 min with pooled human liver microsomes (0.1 and 2.5 mg/mL) at 37°C. For experiments conducted at 0.1 mg/mL HLM, after the preincubations, S-mephenytoin (40 or 400 µM final concentration) was added and residual CYP2C19 activity determined as described above. For experiments conducted at 2.5 mg/mL, after the preincubation, an aliquot (8 µL) was transferred to another incubation tube (final volume 200 µL) containing S-mephenytoin (400 µM) and an NADPH-generating system in order to measure residual CYP2C19 activity as described above. This procedure diluted the microsomes to 0.1 mg/mL and diluted omeprazole to 1/25th its original concentration. Reactions were carried out in triplicate.

**CYP2C19 activity in cultured human hepatocytes.** Cultured human hepatocytes were prepared, treated, and microsomes (0.02 mg/mL) isolated as described previously (Nassar et al., 2009). Briefly, cultured
human hepatocytes were treated for 72 hrs with 0.1% (v/v) DMSO (control) or 100 µM omeprazole. Isolated microsomes were washed and incubated for 30 minutes with marker substrate (40 µM S-mephenytoin) to determine CYP2C19 activity as described above.

Assessment of MDI reversibility by ultracentrifugation. Omeprazole (100 µM), R-omeprazole (100 µM) and esomeprazole (100 µM) were incubated in triplicate with NADPH-fortified pooled HLM (0.1 mg/mL) at 37°C for 30 min in potassium phosphate buffer (50 mM, pH 7.4), MgCl₂ (3 mM), EDTA (1 mM, pH 7.4) and chemically reduced NADPH (1 mM), as described previously (Parkinson et al., 2011). Incubations (n=3) with solvent alone (1% methanol v/v, final) served as controls. Following the 30-min incubation, HLMs were (1) assayed directly for residual CYP2C19 activity, (2) re-isolated by ultracentrifugation and then assayed for residual CYP2C19 activity, or (3) treated with potassium ferricyanide, re-isolated by ultracentrifugation and then assayed for residual CYP2C19 activity. For samples in groups (2) and (3), microsomal protein was re-isolated by ultracentrifugation (100,000 × g for 30 min at 4°C in a Beckman ultracentrifuge with a 70Ti rotor). The supernatant fraction was discarded and the resultant microsomal pellets were rinsed three times with wash buffer (150 mM potassium chloride and 10 mM EDTA, pH 7.4) to remove residual omeprazole and/or any reversible inhibitory metabolites. Microsomal pellets were resuspended in 250 mM sucrose and the microsomal protein concentration was determined by the Pierce BCA Protein Assay (Pierce, Rockford, IL). For samples in group (3), HLMs were incubated with potassium ferricyanide (2 mM) for 10 min at 37°C prior to re-isolation of microsomal protein by ultracentrifugation to disrupt nitrogen-based metabolite inhibitory complexes (MICs) (Franklin, 1977). Residual CYP2C19 activity was assessed at a final concentration of 0.1 mg/mL HLM (supplemented with an NADPH regenerating system) with S-mephenytoin (400 µM, i.e., 10 × Kₘ) to reduce the inhibitory effects of any residual competitive inhibition.
Analytical methods

LC-MS/MS methods were carried out as described previously (Ogilvie et al., 2006; Parkinson et al., 2011). Omeprazole analysis was performed with an Applied Biosystems/Sciex API2000 HPLC-MS/MS system equipped with an electrospray (TurboIonSpray) ionization source (Applied Biosystems, Foster City, CA), two LC-10ADvp pumps with an SCL-10Advp controller, SIL-HTA autosampler, and DGU-14 solvent degasser (Shimadzu, Columbia, MD). The HPLC column used was an Atlantis dC18, 5 µm, 100 x 2.0 mm column (Waters), which was preceded by a direct connection guard column with a C8, 4.0 mm _ 2.0 mm cartridge (Waters). Masses were monitored in the multiple reaction monitoring (MRM) mode: omeprazole, 345.9 → 197.9 and internal standard, pantoprazole, 383.9 → 199.9. Mobile phases were: A=0.2% formic acid in water, B=0.2% formic acid in methanol and omeprazole and pantoprazole were eluted with a linear gradient (25%B to 75%B) over 2.5 min.

Data Analyses and Simulations

All IC_{50}, half-life, K_t and k_{inact} values were determined by nonlinear regression with XLfit3 (version 3.0.5; ID Business Solutions Ltd., Guildford, Surrey, UK, which is an add-in for the computer program Microsoft Excel, [Office 2003; Microsoft Inc., Redmond, WA]) or with GraFit (version 4.0.21; Erithracus Software Ltd., Horley, Surrey, UK), as detailed previously (Ogilvie et al., 2006; Nassar et al., 2009; Parkinson et al., 2011).

In vitro-to-in vivo extrapolation of CYP2C19 inactivation was performed using both a mechanistic static model (MSM) (Grimm et al., 2009) and a mechanistic dynamic model (MDM) (Rowland Yeo et al., 2010). The MSM was implemented based on the following equation:
\[
\frac{AUC_i}{AUC} = \frac{1}{\left( \frac{f_{m,CYP}}{1 + \left( \frac{k_{\text{inact}} \cdot [I]}{k_{\text{deg}} \cdot (K_I + [I])} \right)} \right) + (1 - f_{m,CYP})} 
\]

- equation 1

where \( f_{m,CYP} \) represents the fraction of a hypothetical concomitantly administered drug metabolized by a given P450 enzyme, \([I]\) is the inactivator concentration, and \( k_{\text{inact}} \) and \( K_I \) are the in vitro inactivation parameters, and \( k_{\text{deg}} \) is the rate constant for enzyme degradation (which has not been determined experimentally in vivo for CYP2C19, but for which the average value was reported to be 0.000445 min\(^{-1}\) based on in vitro data (Renwick et al., 2000; Yang et al., 2008). Given that the unbound plasma C\(_{\text{max}}\) was previously found to be more predictive than total C\(_{\text{max}}\) for MDIs (Obach et al., 2007; Grimm et al., 2009), this value was used in the MSM (equation 1), and was based on the total omeprazole plasma C\(_{\text{max}}\) of 3.87 µM after 5 days of dosing (40 mg q.d.) in CYP2C19 EMs (Hassan-Alin et al., 2005), coupled with its plasma protein binding of 95%, for an unbound plasma C\(_{\text{max}}\) of 0.19 µM.

The MDM simulations were carried out using Simcyp Population-Based Simulator (V10.2; Simcyp Limited, UK). The differential equations which make the basis of these simulations are described in detail elsewhere (Rowland Yeo et al., 2010). Input parameters can be found in the supplemental data.

The purpose of simulations was to assess the time varying effect of repeated omeprazole administration (40 mg every 12 h) on the level of active CYP2C19 in liver. This included the self-inhibition effect as the deactivation of CYP2C19 led to lower clearance and higher concentrations of omeprazole itself. The simulations were carried out twice using the lower (1.7 µM) and upper (9.1 µM) boundaries of observed \( K_I \) values and taking into account an unbound fraction of 0.75 (i.e., the free fraction of omeprazole in HLM at 1-2.5 mg/mL). \( k_{\text{inact}} \) was assumed to be 0.045 min\(^{-1}\) and turn-over of CYP2C19 was the same as that assumed for the MSM.
Although the main purpose of the simulations was to assess the level of active enzyme, S-mephenytoin was used as a substrate on day 14 following administration of omeprazole and the effect on AUC was simulated on day 7 following administration of omeprazole. The omeprazole dose was continued until day 14 and the AUC for S-mephenytoin was assessed from day 7 until the end of study (day 14) and compared with AUC in the absence of an inactivator.
RESULTS

Inhibitory effect of omeprazole on S-mephenytoin 4´-hydroxylation in multiple test systems: IC₅₀ determinations, metabolic stability and microsomal protein binding. Omeprazole was evaluated as a direct-acting and MDI of CYP2C19 activity (S-mephenytoin 4´-hydroxylation) in pooled human liver microsomes (n = 16), recombinant CYP2C19 (Bactosomes®), and pooled, cryopreserved human hepatocytes (n = 3), at a substrate concentration approximately equal to Km (40 µM). The results are summarized in Fig. 2A-D and Table 1. The results show that omeprazole caused MDI of CYP2C19 as evidenced by a left shift in IC₅₀ curves following a 30-min pre-incubation with NADPH-fortified HLM, recombinant CYP2C19 and human hepatocytes (IC₅₀ shifts of 4.1, 6.9 and 3.6-fold, respectively). A left shift of ≥ 1.5 fold is considered indicative of MDI (Parkinson et al., 2011). With no pre-incubation, omeprazole inhibited CYP2C19 in all three systems with similar IC₅₀ values ranging from 4.7 to 9.6 µM, which decreased to approximately 1.5 µM in all three test systems after pre-incubation with NADPH. The experiment presented in Fig. 2A also included an IC₅₀ determination with a 30-min pre-incubation step without NADPH, to confirm that the IC₅₀ shift was NADPH-dependent (Table 1 [for clarity, these data are not presented in Fig. 2A]). Omeprazole was also examined as an inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2D6, 3A4 and 2J2 and was found to be a weak direct inhibitor of these enzymes (IC₅₀ values > approximately 100 µM), with no NADPH-dependent IC₅₀ shift > 1.5 fold (see supplemental data).

The data presented in Fig. 2A were obtained under “low microsomal protein−short incubation time” conditions (i.e., 0.1 mg/mL HLM for 5 minutes). However, S-mephenytoin is a low turnover substrate in HLM, for which reason S-mephenytoin 4´-hydroxylation activity is frequently assessed by incubating S-mephenytoin with high concentrations of HLM (i.e., ≥ 0.2 mg/mL) for 20 - 40 min (e.g., Walsky and Obach, 2004; Nishiya et al., 2009; Parkinson et al., 2011). Since MDI of CYP2C19 by omeprazole had not been previously described, we also examined the ability of omeprazole to inhibit CYP2C19 under “high microsomal protein−long incubation time” conditions (i.e., 1 mg/mL HLM for 30 minutes). Under such conditions we detected no MDI of CYP2C19 by omeprazole (i.e., IC₅₀ shift <1.5; Fig. 2B).
Because of the difference in results between “low microsomal protein–short incubation time” and “high microsomal protein–long incubation time” conditions, we examined the metabolic stability and non-specific binding of omeprazole in HLM. In NADPH-fortified HLM at 0.1 mg/mL, omeprazole was relatively stable with a half-life of 98 min (Fig. 2E). However, at 1.0 mg/mL and 2.5 mg/mL HLM (i.e., the concentration utilized in one of the $k_{\text{inact}}$ determinations, see below) omeprazole rapidly disappeared from incubations, with half-life values of only 14 and 5.7 min, respectively. Non-specific binding of omeprazole to HLM was also examined (by ultrafiltration) as a possible cause of the discrepancy in results between “low microsomal protein–short incubation time” and “high microsomal protein–long incubation time” conditions. As shown in Fig. 2F, however, > 75% of omeprazole (2 and 10 µM) remained free in the incubation from 0.1 to 2.5 mg/mL.

Inhibitory effects of the major omeprazole metabolites and enantiomers on S-mephenytoin 4´-hydroxylation in human liver microsomes: IC$_{50}$ determinations. Esomeprazole, R-omeprazole, omeprazole sulfide, omeprazole sulfone, and 5´-hydroxyomeprazole were evaluated as direct-acting and MDIs of CYP2C19 activity (S-mephenytoin 4´-hydroxylation) in pooled human liver microsomes (n = 16, 0.1 mg/mL) at a substrate concentration approximately equal to the Km (40 µM). The results are summarized in Table 1 and Fig. 3. The results show that esomeprazole (Fig. 3A), R-omeprazole (Fig. 3B), and omeprazole sulfone (Fig. 3D) caused MDI of CYP2C19 as evidenced by a left shift in IC$_{50}$ curves (>1.5-fold) following a 30-min pre-incubation with NADPH-fortified HLM (10, 2.5 and 3.2-fold IC$_{50}$ shifts, respectively), with similar “shifted” IC$_{50}$ values ranging from 1.5 to 5.6 µM. The IC$_{50}$ values following a 30-min pre-incubation in the absence of NADPH were higher than those in the presence of NADPH, suggesting that the time-dependent inhibition of CYP2C19 by these compounds was in fact metabolism-dependent (Table 1). The IC$_{50}$ values for omeprazole sulfide after 30 min pre-incubation with or without NADPH remained similar to the value without pre-incubation (IC$_{50}$ shift <1.5-fold), suggesting that omeprazole sulfide is only a direct-acting inhibitor of CYP2C19 (Fig. 3C, Table 1). 5´-Hydroxyomeprazole, one of the major metabolites formed by CYP2C19, was a weak inhibitor of
CYP2C19 (IC$_{50}$ values > 100 µM) (Fig. 3E). The experiments presented in Fig. 3 also included an IC$_{50}$ determination with a 30-min pre-incubation step without NADPH, to confirm that the IC$_{50}$ shift was NADPH-dependent (Table 1 [for clarity, these data are not presented in Fig. 3]).

Inhibitory effects of lansoprazole and pantoprazole on S-mephenytoin 4´-hydroxylation in human liver microsomes and cryopreserved human hepatocytes: IC$_{50}$ determinations. Lansoprazole and pantoprazole were evaluated as direct-acting and MDIs of CYP2C19 activity (S-mephenytoin 4´-hydroxylation) in pooled human liver microsomes (n = 16) and pooled, cryopreserved human hepatocytes (n = 3), at a substrate concentration approximately equal to the Km (40 µM). The results are summarized in Table 1 and Fig. 4. Neither lansoprazole nor pantoprazole caused MDI of CYP2C19 in either HLM or cryopreserved human hepatocytes (i.e., IC$_{50}$ shifts < 1.5-fold). Lansoprazole was a relatively potent (i.e., IC$_{50}$ ≈ 1.0 µM), direct-acting inhibitor of CYP2C19 in both HLM and cryopreserved human hepatocytes (Fig. 4A and B), whereas pantoprazole was a relatively weak inhibitor (Fig. 4C and D). The experiments presented in Fig. 4A and C also included an IC$_{50}$ determination with a 30-min pre-incubation step without NADPH, to confirm that the IC$_{50}$ shift was NADPH-dependent (Table 1 [for clarity, these data are not presented in Fig. 4]).

Inactivation of CYP2C19 by omeprazole: $K_I$ and $k_{\text{inact}}$ determinations. The results in Fig. 2 suggest that omeprazole is an MDI of CYP2C19. Accordingly, experiments were performed to determine $K_I$ (the concentration of omeprazole supporting half maximal rate of CYP2C19 inactivation) and $k_{\text{inact}}$ (the first order rate constant for CYP2C19 inactivation). The results from three different experiments are summarized in Fig. 5. The three experimental conditions were (1) low HLM concentration with $[S] = K_m$ with no dilution step (Fig. 5A-B), (2) low HLM concentration with $[S] = 10 \times K_m$ (Fig. 5C-D), and (3) high [HLM] with $[S] = 10 \times K_m$ with a 25-fold dilution step (Fig. 5E-F). Under all three conditions, the inactivation of CYP2C19 was dependent on the concentration of omeprazole (over the full ranges examined) and the time course conformed to a first-order inactivation process (as indicated by the
linearity of plots of the log of the residual enzyme activity against time; Figs. 5A, C, and E). The $K_I$ values differed depending on whether omeprazole was preincubated with human liver microsomes at 2.5 mg/mL (and subsequently diluted 25 fold to determine residual CYP2C19 activity with substrate = 10Km; Fig. 5E-F) or 0.1 mg/mL (and not diluted to determine residual CYP2C19 activity, with substrate = 10Km or Km; Fig. 5A-D). The $K_I$ value was approximately 9 $\mu$M in the former case, and approximately 2 $\mu$M in the latter cases. In all three cases, the $k_{\text{inact}}$ values were approximately 0.04 min$^{-1}$, which means that, in the presence of saturating concentrations of omeprazole, 4% of CYP2C19 was inactivated every minute. The efficiency of CYP2C19 inactivation ($k_{\text{inact}}/K_I$) decreased by a factor of approximately five when the concentration of HLM was increased and a dilution step was used. Taken together, these data suggest that omeprazole is an irreversible inactivator of CYP2C19.

**Effects of omeprazole on CYP2C19 activity in microsomes prepared from hepatocytes after three days of treatment.** CYP2C19 activity (S-mephenytoin 4'-hydroxylation) was measured in microsomes isolated from fresh primary cultures of human hepatocytes treated with omeprazole (100 $\mu$M; 3 days). It should be noted that this concentration is approximately 19-times greater than the $C_{\text{max}}$ in PMs (5.3 $\mu$M [Chen et al., 2009]; who can be neither induced nor inhibited with respect to CYP2C19), and 26-times greater than the $C_{\text{max}}$ in EMs (Hassan-Alin et al., 2005) after a 40 mg oral dose of omeprazole. Omeprazole treatment decreased microsomal CYP2C19 activity in hepatocytes that initially expressed high levels of CYP2C19, but increased microsomal CYP2C19 activity in hepatocytes that initially expressed low levels of CYP2C19 (Fig. 6). This dual effect likely reflects the overall effect of two opposing actions of omeprazole; in hepatocytes with high CYP2C19 activity, the predominant effect of omeprazole was irreversible inactivation, but in hepatocytes with low CYP2C19 activity the predominant effect was induction via PXR activation (Yueh et al., 2005). These data were obtained from many studies conducted to examine the potential for drug candidates to induce P450 enzymes, in which primary cultures of human hepatocytes were treated with 100 $\mu$M omeprazole, a well known AhR activator and
therefore positive control for CYP1A2 induction. Because the microsomes are obtained from these studies in a way that washes out residual inhibitor, these data provided additional evidence that omeprazole is an irreversible inactivator of CYP2C19.

**Irreversible or quasi-irreversible inhibition of CYP2C19 by omeprazole and esomeprazole, but not R-omeprazole: ultracentrifugation.** Because esomeprazole was approximately 4-fold more effective as a MDI of CYP2C19 than R-omeprazole (Table 1, Fig. 3A-B) with a 10-fold shift in IC$_{50}$ value for the former and a 2.5-fold shift for the latter, omeprazole and its individual enantiomers were further examined to determine whether the inactivation of CYP2C19 involved irreversible or quasi-irreversible inhibition based on an ultracentrifugation method described herein and previously (Parkinson et al., 2011). A concentration of inhibitor that caused nearly complete (i.e., > ~95% inhibition) after 30-min preincubation with NADPH, but incomplete (< ~85%) inhibition after 30-min preincubation in the absence of NADPH was chosen (i.e., 100 µM for all). Pooled HLM were treated with inhibitor or solvent (methanol, 0.1% v/v) for 30 min in the presence of NADPH, and in the presence or absence of potassium ferricyanide (to reverse the formation of metabolite inhibitory complex associated with quasi-irreversible inactivation) (Fig. 7). Following the 30-min incubation, pooled HLM samples were either (1) assayed directly for residual CYP activity (“Pre-spin”), (2) re-isolated by ultracentrifugation and then assayed for residual CYP activity (“Post-spin”), or (3) treated with potassium ferricyanide, re-isolated by ultracentrifugation and then assayed for residual CYP activity (“Post-spin + K$_3$[Fe(CN)$_6$]”). As expected, substantial inhibition was observed after treatment with omeprazole, esomeprazole and R-omeprazole (Fig. 7, “pre-spin”), in a rank-order consistent with the IC$_{50}$ shifts: esomeprazole ≈ omeprazole > R-omeprazole. Surprisingly, centrifugation and re-isolation of HLM after pre-incubation largely reversed the inhibition caused by R-omeprazole (Fig. 7, “Post-spin” and “Post spin + K$_3$[Fe(CN)$_6$]”). In contrast, centrifugation did not fully restore CYP2C19 activity after treatment with omeprazole or esomeprazole, which suggests that esomeprazole is the major contributor to the inactivation of CYP2C19 by omeprazole. Potassium ferricyanide treatment of the samples caused some additional restoration of
activity after treatment with omeprazole and esomeprazole, but did not fully restore CYP2C19 activity. Taken together, these results suggest that the MDI of CYP2C19 observed with racemic omeprazole is largely irreversible (due to covalent binding to the apo-protein, heme moiety or both). However, given the partial restoration of CYP2C19 activity with potassium ferricyanide, contribution of a quasi-irreversible mechanism (due to metabolite inhibitory complex [MIC] formation) cannot be completely ruled out. The results of a previous experiment based on overnight dialysis are also consistent with an irreversible or quasi-irreversible mechanism of MDI of CYP2C19 with racemic omeprazole (Paris et al., 2008). However, the results presented in Fig. 7 show that the MDI of CYP2C19 caused by R-omeprazole is largely reversible after re-isolation of HLM, suggesting that one or more metabolites of this enantiomer is simply a more potent inhibitor of CYP2C19 than the parent, whereas metabolism of esomeprazole largely leads to irreversible inactivation of CYP2C19.

**Simulation of time-dependent changes in active CYP2C19.** Active levels of hepatic CYP2C19 in the presence of omeprazole (40 mg b.i.d. for 14 days) were simulated with Simcyp V10.2, as described in Materials and Methods. Fig. 8 shows the active CYP2C19 levels with time. Depending on the assumption regarding $K_I$ (1.7 or 9.1 µM) the level of active enzyme could decrease to approximately 10 to 60% of the baseline which would be associated with 1.4 to 10 fold increase in AUC of compounds mainly metabolized by CYP2C19. However, the simulated effect of omeprazole on S-mephenytoin AUC showed only a 1.45-fold increase when the $K_I$ value was 9.1 µM, and a 5.46-fold increase when the $K_I$ value was 1.7 µM, rather than a 10-fold increase, partly due to the contribution of other enzymes in its metabolism (e.g. CYP2B6).
DISCUSSION

Given the relatively few drugs that are metabolized extensively by CYP2C19, it is not surprising that few DDIs have been attributed to clinically relevant inhibition of CYP2C19. As noted in the Introduction, there is evidence of clinically relevant inhibition of CYP2C19 by omeprazole, but direct competitive inhibition of CYP2C19 is an unlikely cause; if it were, lansoprazole would be expected to inhibit CYP2C19 more than omeprazole whereas the converse is observed clinically. It should be noted that the dose of lansoprazole versus omeprazole (which depends on indication) could partly explain this difference. For the lowest dose indications (i.e., GERD or maintenance of healing of erosive esophagitis), it is 20 mg q.d. for omeprazole (Prilosec prescribing information, 2011, http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/019810s092,022056s008lbl.pdf), and 15 mg q.d. for lansoprazole (Prevacid prescribing information, 2010, http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020406s074,021428s021lbl.pdf). For the highest dose indication (i.e., Zollinger–Ellison or other hypersecretory syndromes), the doses are equal (60 mg q.d.). Based on this comparison alone, it seems unlikely that the slight difference in the low dose explains the differences in clinical DDIs. This supposition is borne out by clinical data in the MTDI: negative interactions were reported between lansoprazole (60 mg q.d., 10 days) and the CYP2C19 substrates diazepam and phenytoin (lansoprazole dose 60 mg q.d., 9 days with the latter drug). No positive DDIs with CYP2C19 substrates have been reported in the MTDI with lansoprazole at any dose. On the other hand, with a low dose of omeprazole (20 mg q.d.) in various studies (8 to 23 days), the exposure (AUC) of the CYP2C19 substrates diazepam and escitalopram increased from 26 to 91%.

To our knowledge, with the exception of our preliminary findings (Paris et al., 2008; Parkinson et al., 2010), in vitro evidence for MDI of CYP2C19 has not been previously described for any PPI. In the current study, omeprazole, esomeprazole, R-omeprazole, and omeprazole sulfone were identified as MDIs of CYP2C19 (IC₅₀ shifts after a 30-min preincubation with NADPH of 4.2, 10, 2.5, and 3.2, respectively), whereas lansoprazole and pantoprazole were not MDIs (IC₅₀ shifts <1.5). Furthermore, the MDI of CYP2C19 by omeprazole and esomeprazole was not reversed by ultracentrifugation, suggesting the
inhibition was irreversible, whereas ultracentrifugation largely reversed such effects of R-omeprazole.

Under various conditions, omeprazole inactivated CYP2C19 with \( K_1 \) values of 1.7 – 9.1 \( \mu M \) and \( k_{\text{inact}} \) values (maximal rate of inactivation) of 0.041 – 0.046 \( \text{min}^{-1} \) (corresponding to \( k_{\text{inact}}/K_1 \) values ranging from 5.1 to 24 \( \text{min}^{-1} \cdot \text{mM}^{-1} \) depending on the experimental conditions used (Fig. 5). The variation in \( K_1 \) values, but not \( k_{\text{inact}} \) values, is generally consistent with previous reports investigating the impact of dilution (Van et al., 2006; Parkinson et al., 2011). We propose that the quasi-irreversible or irreversible MDI of CYP2C19 by omeprazole (rather than reversible inhibition) explains, at least in part, the observed clinical interactions between omeprazole (and esomeprazole) and CYP2C19 substrates, including clopidogrel, notwithstanding the considerable debate surrounding the role of CYP2C19 in the activation of the latter drug (Bouman et al., 2011; Sibbing et al., 2011).

**Why did previous in vitro studies miss the MDI of CYP2C19 by omeprazole?**

The IC\(_{50}\) or Ki values for inhibition of CYP2C19 in HLM by omeprazole reported in the literature range from 150 \( \mu M \) to 1 \( \mu M \) in nearly 40 studies (MTDI database). The lowest values (1 and 4 \( \mu M \)) were reported with some of the longest substrate incubation periods (60 – 120 min; presumably used due to the low turnover of S-mephenytoin), and few (if any) studies specifically included a preincubation with NADPH-fortified HLM to examine the possibility of MDI of CYP2C19. Based on our data (Fig. 5), 60 – 120 min would provide ample time for inactivation of CYP2C19 by omeprazole during the substrate incubation, therefore leading to “unintentional” MDI and artificially low IC\(_{50}\) or Ki values, as recently discussed (Parkinson et al., 2011). Our data also show that the IC\(_{50}\) shift diminishes as higher protein concentrations and longer substrate incubation times are utilized (Fig. 2B), likely due to the combination of inhibitor depletion (Fig. 2E) and the “unintentional” MDI during the long substrate incubation (Parkinson, et al., 2011).
Would we predict clinically relevant CYP2C19 inhibition?

As noted in the Introduction, we would not predict clinically significant inhibition of CYP2C19 by omeprazole based on competitive inhibition alone. A thorough prediction of the impact of omeprazole on CYP2C19 substrates based on the experimentally determined $K_i$ and $k_{inact}$ values necessitated the use of physiologically based pharmacokinetic (PBPK) modeling to allow dynamic simulation of changes to both omeprazole (including self inhibition), substrate concentrations as well as enzyme turnover (i.e., a so-called mechanistic dynamic model [MDM]), with the mechanistic static model (MSM, equation 1) used as a comparator.

In the MDM under conditions where in vitro inhibitor depletion and microsomal protein binding of omeprazole were minimal (Fig. 2E-F; i.e., $K_i = 1.7$ µM, Fig. 5d) the level of active CYP2C19 is predicted to decrease to ~10% of baseline, after approximately 7 days of omeprazole administration (Fig. 8A) which could cause up to a 10-fold increase in the AUC of compounds predominantly metabolized by CYP2C19. When the higher estimate of $K_i$ is used in the MDM (obtained with a 25-fold dilution, under conditions in which significant inhibitor depletion occurs, Fig. 2E), the level of active CYP2C19 is predicted to decrease to ~60% of baseline, after approximately 12 days of omeprazole administration (Fig. 8B). Although S-mephenytoin is a model CYP2C19 probe substrate, only a 1.45 - 5.46-fold increase in its AUC (depending on $K_i$) is predicted by the MDM after 14 days of omeprazole administration (40 mg b.i.d.), partly because of the contribution of other enzymes to its metabolism (e.g., CYP2B6), and possibly due to less than complete inactivation of CYP2C19.

In the MSM, when the lowest value of $K_i$ is used, omeprazole is predicted to cause a 2.85-fold increase in the AUC of a drug such as moclobemide which has an $f_m$ of 0.72 (Yu et al., 2001). The predicted AUC increase with moclobemide falls to 1.96-fold with the $K_i$ value of 9.1 µM. If the $f_m$ were equal to 1.0, and the lowest $K_i$ value used, omeprazole is predicted to cause up to a 10.4-fold increase in the AUC of such a hypothetical “perfect” CYP2C19 substrate. However, since there is up to a 14.6-fold increase in the AUC of omeprazole in CYP2C19 PMs relative to EMs, (Furuta et al., 1999), this...
prediction suggests that omeprazole does not completely inactivate CYP2C19, which is consistent with either scenario in the MDM after several days of administration of omeprazole (Fig. 8). The clinical example of moclobemide ($f_{\text{MCYP2C19}} = 0.72$) is generally in agreement with these predictions, with $\text{AUC}_\infty$ increases averaging 2.21-fold (1.03 to 3.39-fold) in CYP2C19 EMs with omeprazole coadministration (Yu et al., 2001). The AUC increases predicted with the lowest $K_I$ in the MSM value fall within this observed range. In addition, in order to achieve the recently reported 1.39-fold increase in AUC of clopidogrel with concomitant dosing of omeprazole (Angiolillo et al., 2011), the $f_{\text{MCYP2C19}}$ for clopidogrel would need only to be approximately 0.31 according to the MSM. Given the large difference in predicted active CYP2C19 after several days of omeprazole administration in the MDM depending on which $K_I$ value is used (Fig. 8), the importance of determining the $K_I$ value under conditions where microsomal protein binding and inhibitor depletion are minimized is underscored, similar to the case with IC$_{50}$ values (Parkinson et al., 2011).

**Is there clinical evidence of CYP2C19 inhibition by omeprazole?**

In vivo evidence for MDI of CYP2C19 by omeprazole has already been reported. For instance, Klotz reported that healing rates of GERD after 4 weeks’ therapy with esomeprazole were not dependent on CYP2C19 status, as they are for lansoprazole (2006). Based on the metabolite ratios of 5’-hydroxyomeprazole (formed by CYP2C19) to omeprazole sulfone (formed by CYP3A4), it was concluded that CYP3A4 plays the major role in the metabolism of esomeprazole after multiple dosing, consistent with autoinhibition of CYP2C19 and conversion of CYP2C19 EMs to IMs or PMs after repeat dosing (Klotz, 2006). The changes in metabolite ratio suggests that the impact of multiple dosing on omeprazole reflects autoinhibition of CYP2C19, rather than increased stability due to higher gastric pH as originally suspected, especially because such time-dependent changes do not occur with other PPIs: On the other hand, the prescribing information for Nexium states “at repeated once-daily dosing with 40 mg, the systemic bioavailability is approximately 90% compared to 64% after a single dose of 40 mg” (Nexium prescribing information, 2010,
In addition, Andersson and Weidolf (2008) reported that when 15 mg of either omeprazole (i.e., the racemate), esomeprazole or R-omeprazole was administered orally for seven days, exposure to esomeprazole (plasma AUC) increased by approximately twofold over 7 days, whereas exposure to omeprazole increased by only 52%, and exposure to R-omeprazole actually decreased by 9%. These results are consistent with our in vitro results showing that the inhibition of CYP2C19 by R-omeprazole appears to be largely reversible, whereas that of the racemate and esomeprazole are largely irreversible (Fig. 7).

In vivo evidence for MDI of CYP2C19 by omeprazole also comes from clinical DDI studies with omeprazole as the perpetrator. For instance, in CYP2C19 extensive metabolizers (but not in poor metabolizers), the AUC of moclobemide increased by ~31% after a single 40 mg dose of omeprazole, but increased by 121% after 8 days of dosing with 40 mg omeprazole (Yu et al., 2001). Such an apparent increase in the exposure of a victim drug with repeated dosing of the perpetrator drug is often apparent with MDIs. In addition, omeprazole (but not lansoprazole or pantoprazole) has long been known to inhibit the metabolism of diazepam in vivo, and this inhibition occurs in CYP2C19 EMs but not PMs, further suggesting the mechanism involves CY2C19 inhibition by omeprazole (Andersson et al., 1990; Lefebvre et al., 1992; Gugler et al., 1996).

**Does MDI of CYP2C19 by omeprazole explain the PPI-clopidogrel interaction?**

Our data and the predictions detailed above may explain, at least in part, the interaction between omeprazole (or esomeprazole) and clopidogrel. As noted in the Introduction, the FDA specifically warns against coadministration of clopidogrel and omeprazole (2010, http://www.fda.gov/Drugs/DrugSafety/ucm231161.htm). Given that the in vivo half-life of omeprazole (and other PPIs) is short, and plasma protein binding is high, it is remarkable that many recent publications attribute the clopidogrel-omeprazole interaction to competitive (reversible) inhibition of CYP2C19 by omeprazole, with some suggestion that separation of dosing can prevent the interaction.
(Abraham et al., 2010; Tran et al., 2010; Bates et al., 2011). However, it should be noted that for
clopidogrel, genetic differences in both the metabolism or transport of the drug and in the therapeutic
target (the P2Y₁₂ receptor on platelets), as well as environmental factors (e.g., diet, disease,
coadministered drugs) have been implicated in the variation in its clinical effect (for recent reviews see:
Abraham et al., 2010; Holmes et al., 2010; Tran et al., 2010; Bates et al., 2011).

In addition, the interaction between omeprazole (or esomeprazole) and clopidogrel is particularly
complex, as noted by Zhang et al. (2009). These authors note that clopidogrel itself is an MDI of
CYP2C19, increasing the ratio of 5’-hydroxyomeprazole to omeprazole by ~75% in CYP2C19 EMs
(Chen et al., 2009; Zhang et al., 2009) and that both clopidogrel and its 2-oxo metabolite (the precursor to
the active metabolite) also directly inhibit CYP2C19 with IC₅₀ values ≤1 µM. The authors suggested that
the “stronger” effect of omeprazole on CYP2C19 may be due to the “time-dependent” inhibition we
reported in our preliminary work (Paris et al., 2008; Zhang et al., 2009). Our finding of irreversible or
quasi-irreversible inactivation of CYP2C19 by omeprazole, with up to a predicted 90% decrement in
active CYP2C19 after approximately 7 days of dosing (Fig. 8A) is consistent with this hypothesis. The
fact that the FDA warning applies only to omeprazole
(http://www.fda.gov/Drugs/DrugSafety/ucm231161.htm) and not the other PPIs is consistent with a lack
of MDI by the other PPIs examined in this study.

Given the minor inhibitory effects of omeprazole on other P450 enzymes (see data supplement), the MDI
of CYP2C19 by omeprazole and the reports of clinical interactions are consistent with a significant role
for CYP2C19 in the metabolism of clopidogrel. In addition, as described in the Introduction, direct
inhibition of CYP2C19 by other PPIs is not likely to be the cause of a clinically significant interaction
with clopidogrel, which is consistent with the lack of in vitro MDI of CYP2C19 by lansoprazole and
pantoprazole reported here, and a lack of clinically significant pharmacokinetic interactions between
either lansoprazole or pantoprazole and clopidogrel (MTDI database).
Potential mechanisms of inactivation of CYP2C19 by omeprazole

Follow up studies will be needed to further elucidate the mechanism of inactivation of CYP2C19 by omeprazole (or esomeprazole). However, a few possibilities for the mechanism of CYP2C19 inactivation by omeprazole (or esomeprazole) are suggested by the reported metabolism of the individual enantiomers of omeprazole (Fig. 9, adapted from (Abelö et al., 2000; Andersson and Weidolf, 2008). Methylhydroxylation of omeprazole to form 5'-hydroxyomeprazole could involve the intermediacy of a benzylic radical and heme alkylation, analogous to the inactivation of CYP2C8 by gemfibrozil glucuronide (Ogilvie et al., 2006; Baer et al., 2009). The formation of 5-O-desmethylomeprazole (a para-aminophenol) could lead to a reactive quinoneimine that could inactivate CYP2C19 if formed in its active site. The latter possibility may be unlikely given that the 5-hydroxylation of lansoprazole also leads to para-aminophenol formation (Fig. 10; admittedly a tautomer of the analogous 5-O-desmethylomeprazole para-aminophenol), and yet lansoprazole is not an MDI of CYP2C19 (Fig. 4A). However, the data presented in Fig. 7 suggest that esomeprazole has a greater MDI effect on CYP2C19 than does R-omeprazole. These data are in fact consistent with clinical observations in which the plasma C_{max} of esomeprazole (40 mg q.d., oral solution) increases from 3.07 to 4.86 µM from day 1 to day 5, and that of omeprazole (40 mg q.d., oral solution) increases from 2.32 to 3.87 µM, while that of R-omeprazole (40 mg q.d., oral solution) only increases from 1.62 to 1.98 µM (Hassan-Alin et al., 2005). In addition, published in vitro results in HLM suggest that the CL_{int} for esomeprazole sulfoxidation (catalyzed by CYP3A4) is 4.6-fold greater than that for R-omeprazole (Abelö et al., 2000), even though the total CL_{int} for R-omeprazole is approximately threefold higher than for esomeprazole. This finding is even more apparent in the clinical data, which show an approximately 14-fold higher AUC for the sulfone when esomeprazole is administered for one day (20 or 40 mg) than for R-omeprazole; this ratio increases to nearly 40-fold after 5 days’ dosing (Hassan-Alin et al., 2005) because CYP3A4 plays a more important role in esomeprazole metabolism after multiple dosing (Klotz, 2006). Because we found that omeprazole sulfone is also an MDI of CYP2C19, it is possible that the combination of effects from esomeprazole and
its sulfone explain the much greater inactivation of CYP2C19 by esomeprazole than R-omeprazole, and this possibility needs to be followed up.

At the same time, the CL<sub>int</sub> for the 5′-hydroxylation of R-omeprazole is approximately 10-fold higher than for esomeprazole, which, along with our results (Fig. 7) suggest that, unless there is also a difference in the ultimate fate of R- vs. S-5′-hydroxyomeprazole (e.g., partition ratio, benzylic radical formation and oxygen rebound or other inactivating pathways), this pathway may not explain the formation of a reactive metabolite that inactivates CYP2C19. Enantiomer-enantiomer interactions at the active site of CYP2C19 when the racemate is used (as in some experiments in this study) could complicate the interpretation of results with the single enantiomers as previously described (Li et al., 2005), especially considering that the presence of R-omeprazole acts as an alternative substrate and offers substrate-protection for the irreversible inactivation by esomeprazole or its sulfone.

In conclusion, we have shown that omeprazole (but not pantoprazole or lansoprazole) is an MDI of CYP2C19 in HLM, cryopreserved human hepatocytes and recombinant human CYP2C19. Based on the <i>K</i><sub>1</sub> and <i>k</i><sub>inact</sub> values for the MDI of CYP2C19 by omeprazole in HLM, we predict that this inactivation is clinically significant. Furthermore, we provided evidence that esomeprazole is more likely to irreversibly inactivate CYP2C19 than is R-omeprazole. These findings have implications for the ongoing debate surrounding the interaction between clopidogrel (as well as other CYP2C19 substrates) and omeprazole and, in particular, esomeprazole.
AUTHORSHIP CONTRIBUTIONS

Participated in research design: Parkinson, Kazmi, Buckley, Paris and Ogilvie

Conducted experiments: Yerino, Ogilvie and Kazmi

Contributed new reagents or analytic tools: Toren, Rostami-Hodjegan

Performed data analysis: Yerino, Ogilvie, Kazmi, Toren, Buckley and Rostami-Hodjegan

Wrote or contributed to the writing of the manuscript: Ogilvie, Parkinson, Kazmi, Yerino, Rostami-Hodjegan, Toren, and Buckley

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REFERENCES


FOOTNOTES:

Preliminary accounts of this work were previously presented as follows:


FIGURE LEGENDS

**Fig. 1:** Structures of the PPIs and omeprazole metabolites examined.

**Fig. 2:** Evaluation of omeprazole as a direct-acting and MDI of CYP2C19. Each symbol represents the average of duplicate determinations unless otherwise indicated. (a) Omeprazole inhibited CYP2C19 in pooled HLM with IC₅₀ values as shown (S-mephenytoin 4´-hydroxylation control rates = 97.3 and 95.0 pmol/mg/min with and without preincubation, respectively). (b) Omeprazole inhibited CYP2C19 in pooled HLM with IC₅₀ values as shown (S-mephenytoin 4´-hydroxylation control rates = 97.3 and 74.3 pmol/mg/min with and without preincubation, respectively). (c) Omeprazole inhibited recombinant human CYP2C19 with IC₅₀ values as shown (S-mephenytoin 4´-hydroxylation control rates = 0.690 and 0.828 min⁻¹ with and without preincubation, respectively). (d) Omeprazole inhibited CYP2C19 cryopreserved human hepatocytes with IC₅₀ values as shown (S-mephenytoin 4´-hydroxylation control rates = 24.7 and 24.8 pmol/million cells/min with and without preincubation, respectively). Each symbol represents the average of triplicate determinations and error bars represent the standard deviations. (e) The metabolic stability of omeprazole (10 µM) was evaluated in HLM at the protein concentrations utilized in this study (0.1, 1.0, and 2.5 mg/mL), as described in Materials and Methods. Half-life values for the disappearance of omeprazole are as indicated. Each symbol represents the average of triplicate determinations and error bars represent the standard deviations; (f) The microsomal binding of omeprazole (2 and 10 µM) was evaluated as described in Materials and Methods.

**Fig. 3:** Evaluation of esomeprazole, R-omeprazole, omeprazole sulfide, omeprazole sulfone, and 5´-hydroxyomeprazole as direct-acting and MDIs of CYP2C19. Each symbol represents the average of duplicate determinations unless otherwise indicated. (a) Esomeprazole inhibited CYP2C19 in pooled HLM with IC₅₀ values as shown (S-mephenytoin 4´-hydroxylation control rates = 80.9 pmol/mg/min and 88.1 pmol/mg/min with preincubation). (b) R-Omeprazole inhibited CYP2C19 in pooled HLM with IC₅₀
values as shown (S-mephenytoin 4′-hydroxylation control rates = 106 and 108 pmol/mg/min, with and without preincubation, respectively). (c) Omeprazole sulfide inhibited CYP2C19 in pooled HLM with IC<sub>50</sub> values as shown (S-mephenytoin 4′-hydroxylation control rates = 89.8 and 82.2 pmol/mg/min, with and without preincubation, respectively). (d) Omeprazole sulfone inhibited CYP2C19 in pooled HLM with IC<sub>50</sub> values as shown (S-mephenytoin 4′-hydroxylation control rates = 85.4 and 84.7 pmol/mg/min, with and without preincubation, respectively). (e) 5′-Hydroxyomeprazole weakly inhibited CYP2C19 in HLM (S-mephenytoin 4′-hydroxylation control rates = 108 and 97.0 pmol/mg/min, with and without preincubation, respectively).

Fig. 4: Evaluation of lansoprazole and pantoprazole as direct-acting and MDIs of CYP2C19. Each symbol represents the average of duplicate determinations unless otherwise indicated. (a) Lansoprazole inhibited CYP2C19 in HLM with IC<sub>50</sub> values as shown (S-mephenytoin 4′-hydroxylation control rates = 83.8 and 79.6 pmol/mg/min, with and without preincubation, respectively). (b) Lansoprazole inhibited CYP2C19 in cryopreserved human hepatocytes with IC<sub>50</sub> values as shown (S-mephenytoin 4′-hydroxylation control rates = 30.4 and 26.6 pmol/million cells/min, with and without preincubation, respectively). Each symbol represents the average of triplicate determinations and error bars represent the standard deviations; (c) Pantoprazole inhibited CYP2C19 in pooled HLM with IC<sub>50</sub> values as shown (S-mephenytoin 4′-hydroxylation control rates = 61.7 and 71.0 pmol/mg/min, with and without preincubation, respectively). (d) Pantoprazole inhibited CYP2C19 in cryopreserved human hepatocytes with IC<sub>50</sub> values as shown (S-mephenytoin 4′-hydroxylation control rates = 24.5 and 14.2 pmol/million cells/min, with and without preincubation, respectively). Each symbol represents the average of triplicate determinations and error bars represent the standard deviation.

Fig. 5: Determination of K<sub>i</sub> and k<sub>inact</sub> for the MDI of CYP2C19 by omeprazole. Individual points represent the average of triplicate determinations ± standard deviation, unless otherwise noted. For graphs in (a) and (c), omeprazole was preincubated (at concentrations indicated in the legend of panel c)
and residual CYP2C19 activity determined as described in Materials and Methods. For the graph in (e), omeprazole was preincubated (at concentrations indicated) and residual CYP2C19 activity determined as described in Materials and Methods (after a 25-fold dilution). The graphs in (b), (d), and (f) represent the direct plots of the initial rates of inactivation of CYP2C19. Values are the slopes of the initial rates of inactivation (k_{obs}) at each concentration of omeprazole, shown ± standard error.

**Fig. 6:** CYP2C19 activity (S-Mephenytoin 4'-hydroxylation) in microsomes isolated from fresh-plated hepatocytes treated with DMSO (control) or omeprazole. Primary cultures of human hepatocytes were treated for three consecutive days with DMSO or omeprazole (100 µM) and microsomes prepared from the hepatocytes 24-h after the last treatment. CYP2C19 activity was determined as described in Materials and Methods. Activities were sorted in rank order from highest to lowest control rates (a) and the corresponding omeprazole-treated samples are displayed in panel (b) in terms of percent of control activity.

**Fig. 7:** Reversibility assessment of the MDI of CYP2C19 by omeprazole and its enantiomers with the ultracentrifugation method. The potential reversibility of the MDI of CYP2C19 NADPH-fortified HLM (0.1 mg/mL) by omeprazole and its individual enantiomers (100 µM) was evaluated with the ultracentrifugation method, as described in Materials and Methods. Incubations labeled “Pre-spin” were conducted similarly to analogous samples in the IC_{50} determinations (conducted in triplicate and displayed as the average rates of S-mephenytoin 4'-hydroxylation ± standard deviation). For incubations labeled “Post-spin” (conducted in triplicate and analyzed in triplicate), microsomal protein was isolated by ultracentrifugation after 30 min incubations with the inhibitor and NADPH, and analyzed in triplicate for residual CYP2C19 activity (S-mephenytoin 4'-hydroxylation) displayed as the average rates ± standard error, as described in Materials and Methods. Half of the incubations from the “Post-spin” samples included potassium ferricyanide (2 mM, final concentration) as indicated. Samples treated with methanol (1% v/v, final) served as controls. All rates were normalized to final microsomal protein concentrations,
as described in *Materials and Methods*.

**Fig. 8:** Simulation of time-dependent changes in active CYP2C19. Active levels of hepatic CYP2C19 in the presence of omeprazole (40 mg b.i.d. for 14 days) were simulated with the Simcyp Simulator V10.2, as described in *Materials and Methods*. Panel (a) is based on a $K_I$ value of 1.7 µM; panel (b) is based on a $K_I$ value of 9.1 µM.

**Fig. 9:** Metabolic scheme for omeprazole enantiomers. The conversion of each enantiomer of omeprazole to the major metabolites and enzymes responsible for each is shown. The scheme is adapted from that published by (Andersson and Weidolf, 2008), which is in turn based on in vitro data published by (Abelö et al., 2000), which is the source of the CL$_{int}$ values.

**Fig. 10:** Para-aminophenol formation from metabolites of omeprazole and lansoprazole. Major metabolites of omeprazole (*O*-desmethylomeprazole) and lansoprazole (5-hydroxylansoprazole) are shown. The highlighted area indicates the part of the molecules that are potentially reactive para-aminophenols.
Table 1. Inhibition of CYP2C19 in human liver microsomes by omeprazole, its enantiomers or its major metabolites and lansoprazole and pantoprazole

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; shift (fold)&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No preincubation</td>
<td>30 min preincubation (-NADPH)</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>6.9 ± 0.7</td>
<td>8.7 ± 0.6</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>15 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>R-Omeprazole</td>
<td>8.1 ± 1.2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Omeprazole sulfide</td>
<td>9.7 ± 0.5</td>
<td>8.4 ± 2.7</td>
</tr>
<tr>
<td>Omeprazole sulfone</td>
<td>18 ± 2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>5-Hydroxyomeprazole</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>93 ± 7</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are displayed to two significant figures, ± standard error of the measurement.

<sup>b</sup> Calculated from full precision values as (IC<sub>50</sub> with no pre-incubation) ÷ (IC<sub>50</sub> with 30 min pre-incubation + NADPH) and rounded to two significant figures. IC<sub>50</sub> shifts > 1.5-fold appear in bold.

NA: Not applicable (i.e., IC<sub>50</sub> values greater than the highest concentration examined)
<table>
<thead>
<tr>
<th></th>
<th>Omeprazole</th>
<th>Esomeprazole</th>
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<td><img src="structure2.png" alt="Structure" /></td>
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<td>Omeprazole sulfone</td>
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<td>5´-Hydroxyomeprazole</td>
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<td><img src="structure6.png" alt="Structure" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lansoprazole</td>
<td>Pantoprazole</td>
</tr>
<tr>
<td><img src="structure7.png" alt="Structure" /></td>
<td><img src="structure8.png" alt="Structure" /></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1**
Figure 2

(a) 0.1 mg/mL HLM, 5 min incubation
(b) 1.0 mg/mL HLM, 30 min incubation

(c) Recombinant CYP2C19
(d) Cryopreserved human hepatocytes

(e) Omeprazole metabolic stability (HLM)
(f) Omeprazole free fraction in HLM

Zero-min IC\textsubscript{50} = 6.9 ± 0.7 µM
30-min IC\textsubscript{50} = 1.7 ± 0.0 µM

Zero-min IC\textsubscript{50} = 8.3 ± 0.3 µM
30-min IC\textsubscript{50} = 6.3 ± 0.3 µM

Zero-min IC\textsubscript{50} = 9.6 ± 2.1 µM
30-min IC\textsubscript{50} = 1.4 ± 0.2 µM

Zero-min IC\textsubscript{50} = 4.7 ± 0.3 µM
30-min IC\textsubscript{50} = 1.3 ± 0.2 µM

0.1 mg/mL HLM, 5 min incubation
1.0 mg/mL HLM, 30 min incubation

Omeprazole remaining (Percent of control)

0 1 2 0 4 0 6 0 8 0 1 0 0 1 2 0

Zero protein control
0.1 mg/mL HLM
1 mg/mL HLM
2.5 mg/mL HLM

Omeprazole (Percent unbound)
Figure 3

(a) Esomeprazole

- Zero-min IC<sub>50</sub> = 15 ± 1 µM
- 30-min IC<sub>50</sub> = 1.5 ± 0.1 µM

(b) R-Omeprazole

- Zero-min IC<sub>50</sub> = 8.1 ± 1.2 µM
- 30-min IC<sub>50</sub> = 3.3 ± 0.4 µM

(c) Omeprazole Sulfide

- Zero-min IC<sub>50</sub> = 9.7 ± 0.5 µM
- 30-min IC<sub>50</sub> = 9.6 ± 1.0 µM

(d) Omeprazole Sulfone

- Zero-min IC<sub>50</sub> = 18 ± 2 µM
- 30-min IC<sub>50</sub> = 5.6 ± 0.5 µM

(e) 5'-Hydroxyomeprazole

- Zero-min IC<sub>50</sub> > 100 µM
- 30-min IC<sub>50</sub> > 100 µM
Figure 4
Figure 5

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

(a) Pre-incubation time vs. S-mephenytoin 4’-hydroxylation (percent remaining activity) for different concentrations of omeprazole:

- 0 µM Omeprazole
- 1 µM Omeprazole
- 3 µM Omeprazole
- 10 µM Omeprazole
- 30 µM Omeprazole

(b) Kinetics of S-mephenytoin 4’-hydroxylation with 25-fold dilution, \([S] = 10K_m\):

- \(K_I = 2.4 \pm 0.3 \mu M\)
- \(k_{inact} = 0.044 \pm 0.002 \text{ min}^{-1}\)

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

(c) Pre-incubation time vs. S-mephenytoin 4’-hydroxylation (percent remaining activity) for different concentrations of omeprazole:

(d) Kinetics of S-mephenytoin 4’-hydroxylation with no dilution, \([S] = 10K_m\):

- \(K_I = 1.7 \pm 0.3 \mu M\)
- \(k_{inact} = 0.041 \pm 0.003 \text{ min}^{-1}\)

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

(e) Pre-incubation time vs. S-mephenytoin 4’-hydroxylation (percent remaining activity) for different concentrations of omeprazole:

(f) Kinetics of S-mephenytoin 4’-hydroxylation with 25-fold dilution, \([S] = 10K_m\):

- \(K_I = 9.1 \pm 1.7 \mu M\)
- \(k_{inact} = 0.046 \pm 0.002 \text{ min}^{-1}\)
Figure 6
Figure 7

S-Mephenytoin 4-\(^\prime\)-hydroxylation
(pmol/mg/min)

- Solvent (1% Methanol)
- R-Omeprazole (100 µM)
- Omeprazole (100 µM)
- Esomeprazole (100 µM)

Pre-spin
Post-spin
Post-spin + K\(_3\)Fe(CN)\(_6\)

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Figure 8

(a) $K_i = 1.7 \, \mu M$

(b) $K_i = 9.1 \, \mu M$
Figure 9
**Figure 10**

- O-Desmethylomeprazole
- 5-Hydroxylansoprazole