Impact of the CYP2C19*17 Allele on the Pharmacokinetics of Omeprazole and Pantoprazole in Children: Evidence for a Differential Effect

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Running title:
Impact of CYP2C19*17 on pantoprazole PK in children

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Abbreviations:
CYP, cytochrome P450; PPI, proton pump inhibitor;
Abstract

The impact of the CYP2C19*17 allele on the pharmacokinetics of pantoprazole and omeprazole in previously studied children (n=40) was explored. When pantoprazole AUC was examined as a function of CYP2C19 genotype, a significantly lower AUC was observed for subjects identified as CYP2C19*1/*1 and *1/*17. For pantoprazole, a statistically significant relationship was observed between CYP2C19 genotype and both dose-corrected AUC (p<0.0001) and the apparent elimination rate constant (K_{el}; p=0.0012); no significant genotype-phenotype relationships were observed for omeprazole.
Introduction

*CYP2C19*17 is characterized by -806C>T (rs12248560) in the regulatory gene region and increases transcription levels. Subjects carrying *CYP2C19*17 have higher CYP2C19 activity towards mephenytoin and omeprazole (Sim et al., 2006). There is limited information regarding the effect of *CYP2C19*17 on the pharmacokinetics of CYP2C19 substrates in adults (Rudberg et al., 2008) and only a single study on clinical outcome (Kurzawski et al., 2006). The goals of this exploratory study were to: 1) characterize the effect of *CYP2C19* genotype, especially the *CYP2C19*17 allele, on the pharmacokinetics of two proton pump inhibitors, omeprazole and pantoprazole, in a pediatric cohort and 2) determine the frequency of *CYP2C19*17 in population samples representing different ethnic backgrounds.
Methods

Clinical Trials: The current investigation was enabled by a re-assessment of data and samples available from previous pharmacokinetic studies of omeprazole (Kearns et al., 2003b) and pantoprazole (Kearns et al., 2008) conducted in pediatric populations for the purpose of product labeling. Both investigations were approved by Institutional Review Boards at participating institutions where subjects were enrolled by parental permission and subject assent as appropriate. The approvals contained provisions for data re-analysis and expanded genotype analysis of stored DNA specimens. Study designs, complete methods and results, were previously described in detail (Kearns et al., 2003b; Kearns et al., 2008) and hence, only pertinent information is recapitulated in this brief communication.

The omeprazole study comprised 37 subjects of which 23 yielded evaluable pharmacokinetic data (Kearns et al., 2003b); these subjects were further investigated for CYP2C19*17. Omeprazole was administered as a single solid oral dose of 10 mg or 20 mg to subjects aged 9.5±3.8 yr (mean±SD; range 2 to 16 years) and weighing 26.2±15.1 kg (range 11 to 75 kg), 10 participants were male.

The pantoprazole study (Kearns et al., 2008) reported two cohorts of children, an oral and an intravenous study arm. The orally dosed cohort consisted of 24 subjects (16 male) aged 10.8±3.4 years (range 5 to 16 years), and weighing 45.0 ± 19.9 kg (range 20 to 90 kg). Subjects received a single oral dose of 20 or 40 mg pantoprazole. DNA was available for all 24 subjects. The intravenous cohort comprised 16 of the originally enrolled 19 children (three subjects were excluded from pharmacokinetic analysis due to incomplete data). Study participants received a single 0.8 or 1.6 mg/kg intravenous dose of pantoprazole. Subjects were 8.1 ± 4.4 years of age.
(range 2 to 14 years) and weighed 37.1 ± 27.0 kg (range 11 to 109 kg). It is important to note that subjects participating in the omeprazole and pantoprazole studies were not related, and participated in only one of the investigations.

**Subjects for allele frequency determination:** The DNA samples of the ethnic panels (Caucasians, n=107; African Americans, n=114; Hispanics n=107) were isolated from discarded, anticoagulated blood obtained for routine clinical management of hospitalized patients after all identifiers have been removed as required by our Institutional Review Board exempt protocol.

**Genotyping:** Genomic DNA was isolated with the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). Genotyping of CYP2C19*2-*8, *10, *12 and *17 and was performed on the PPI study subjects with PCR-RFLP procedures as detailed in the supplemental Table. CYP2C19*17 testing comprised two SNPs, -806C>T and -3402C>T, which reportedly are in complete linkage (Sim et al., 2006). Subjects were grouped according to their genotype into six groups: CYP2C19*1/*1, *1/*17, *2/*17, *2/*2, *17/*17, and *1/*2 and *1/*4 were combined into a single group. The DNA samples of the ethnic panels were only genotyped for CYP2C19*17.

**Statistical analyses:** Associations between CYP2C19 genotype and dose-corrected AUC and K\( \text{el} \) for both omeprazole and pantoprazole were determined by ANOVA. Statistically significant differences (\( \alpha = 0.05 \)) were further investigated by post hoc analysis using Tukey’s Honestly Significant Difference. All statistical analyses were conducted using JMP 8.0.2 (SAS Institute, Inc., Cary, NC).
Results

The allele frequencies for the pantoprazole study cohorts (oral and intravenous combined, n=40) were as follows: \textit{CYP2C19}\textsubscript{*1}, 0.59; \textit{CYP2C19}\textsubscript{*2}, 0.20; \textit{CYP2C19}\textsubscript{*4}, 0.01 and \textit{CYP2C19}\textsubscript{*17}, 0.23. No homozygous \textit{CYP2C19}\textsubscript{*17}/*17 individuals were observed. In the omeprazole cohort (n=23) allele frequencies were 0.52, 0.26 and 0.22 for \textit{CYP2C19}\textsubscript{*1}, *2 and *17, respectively. Frequencies for all subjects enrolled in the original omeprazole study (n=37) are given in Table 1. The frequency of the \textit{CYP2C19}\textsubscript{*17} allele was also determined in DNA samples from three ethnic populations, and was comparable to previously published data (Table 1). Allele frequencies were in Hardy-Weinberg disequilibrium. These observed \textit{CYP2C19}\textsubscript{*17} allele frequencies predict that 4.8%, 4.4% and 1.4% of Caucasians, African Americans and Hispanics are ultrarapid metabolizers with a homozygous \textit{CYP2C19}\textsubscript{*17}/*17 genotype. The two \textit{CYP2C19}\textsubscript{*17}-defining SNPs were linked in all subjects, except one African American. This subject carried -806C>T, the SNP believed to increase \textit{CYP2C19} expression levels (Sim et al., 2006), but lacked -3402C>T.

Selected pharmacokinetic parameters, \textit{i.e.} apparent terminal elimination rate constant (\(K_{el}\)) and area under the plasma concentration vs. time curve (AUC) normalized for drug dose (mg\*hr/L per 1 mg/kg dose) from the omeprazole and pantoprazole studies (Kearns et al., 2003b; Kearns et al., 2008) were examined for their association with \textit{CYP2C19} genotype. The relationships for \(K_{el}\) and AUC for pantoprazole are shown in Figure 1A and B, respectively, and for omeprazole \(K_{el}\) or AUC in Figures 1C and 1D, respectively.

ANOVA revealed a statistically significant relationship between \textit{CYP2C19} genotype and both dose-corrected AUC (\(p<0.0001\)) and \(K_{el}\) (\(p=0.0012\)) for pantoprazole, but not for omeprazole. In
the case of pantoprazole, neither of the pharmacokinetic parameters were different between the
*CYP2C19*/*1 and *CYP2C19*/*1/*17 groups, but in both cases, groups with two functional alleles were statistically significantly different from groups containing only one functional allele, e.g., *CYP2C19*/*1/*2 and *CYP2C19*/*2/*17 groups. In contrast, no statistically significant relationships were observed for omeprazole. Differences between pantoprazole and omeprazole with respect to *CYP2C19* genotype-phenotype relationships were most evident for *K_{el}*, as can be seen in Fig. 1B vs Fig. 1D.
Discussion

Proton pump inhibitors (PPIs) have been used extensively in both adult (Bardou and Martin, 2008) and pediatric (Tafuri et al., 2009) patients to treat a variety of conditions (e.g., gastroesophageal reflux disease, ulcer disease, Zollinger-Ellison syndrome, H. pylori infection, non-ulcer related dyspepsia, drug-associated gastritis) where increasing intragastric pH is considered to be of therapeutic benefit. Recent data generated from a pediatric cohort suggest that long-term PPI use for periods of up to 11 years duration is safe, well tolerated and produces few adverse reactions (Hassall et al., 2007). As previously reviewed (Klotz, 2006; Bardou and Martin, 2008) and reported by others (Hunfeld et al., 2008; Rocha et al., 2008), the pharmacokinetics and pharmacodynamics of the PPIs are primarily dependent upon the activity of the polymorphically expressed CYP2C19 and to some degree, on CYP3A4.

The quantitative significance of the CYP2C19*17 allele with respect to the biotransformation of the PPIs has been demonstrated (Kurzawski et al., 2006; Sim et al., 2006; Baldwin et al., 2008; Hunfeld et al., 2008) in adults. Our data from a single-dose pharmacokinetic study of pantoprazole, given as a racemic mixture (Kearns et al., 2008), also illustrates this dependence in a population of pediatric patients (Fig. 1A and 1B). This finding was not unexpected given the comparable frequency of the CYP2C19*17 allelic variant between pediatric and adult populations (Table) and the known ontogenic pattern for CYP2C19 gene expression (Hines, 2008). However, the data presented in this brief report are not sufficient to attribute a higher level of functional activity in vivo to the CYP2C19*17 allele relative to the reference CYP2C19*1 allele.
The apparent absence of a genotype-phenotype relationship for dose-corrected AUC and $K_{el}$ for omeprazole was totally unexpected given previously published data from adults suggesting (despite a very small (n=16) subject cohort) that the impact of CYP2C19 allelic variants on the systemic exposure (AUC) of omeprazole and pantoprazole was comparable (Hunfeld et al., 2008). The reasons underlying the discrepant findings between omeprazole and pantoprazole in pediatric patients as presented in the current study are not entirely clear. Variability associated with pharmacokinetic parameters within a genotype group composed of relatively small number of subjects (a common feature for pediatric pharmacokinetic studies conducted to support product labeling; Abdel-Rahman et al., 2007) may be one factor contributing to the lack of association between genotype and omeprazole AUC (Fig. 1C). However, values for omeprazole $K_{el}$ appeared to be completely independent of CYP2C19 genotype. As well, our previous study of pantoprazole [Kearns et al., 2008] demonstrated that the pharmacokinetic parameters were not associated with route of administration.

Another possibility relates to differences in the relative contributions of CYP2C19 and CYP3A4 to the overall biotransformation of omeprazole compared to pantoprazole (Savarino et al., 2009). The observed genotype-phenotype relationships presented in this report imply that CYP2C19 is quantitatively more important to pantoprazole elimination than it is to omeprazole elimination, at least within the age range of children included in the original studies. Given that CYP2C19 gene-dose effects have been consistently observed in adults, this observation implies that developmental changes in non-CYP2C19-mediated pathways (i.e. CYP3A4) may result in those alternative pathways being quantitatively more important to omeprazole elimination than CYP2C19, and thereby obscuring the CYP2C19 genotype-phenotype relationship. Although speculative at this point in time, such a hypothesis could be tested by comparing the relative
amounts of hydroxylated CYP2C19-generated metabolites to CYP3A4-mediated sulfone metabolites recovered in the urine of children compared to adults. The potential impact of intestinal CYP2C19 and CYP3A4 on the oral bioavailability of omeprazole (Hosohata et al., 2009); the potential impact of multiple (vs. single) dosing protocols on PPI biotransformation (Schwab et al., 2005) and finally, potential, age-associated differences in the relative activity of the enzymes responsible for the clearance of the two drugs studied (Kearns et al., 2003a) may also be considered. However, the pharmacokinetic data on which the current analysis is based does not allow us to address these issues at the present time.

Despite these potential limitations, our data illustrate that inclusion of the CYP2C19*17 allele in assessing pharmacokinetic data from a cohort of pediatric patients receiving either omeprazole or pantoprazole revealed apparent agent-specific differences in the genotype-phenotype association. These results do not infer information of therapeutic significance. Finally, our findings emphasize the importance of considering multiple routes of drug biotransformation and their relative quantitative importance when using drug metabolizing enzyme genotype to infer information about the pharmacokinetics of drugs within a given pharmacologic class.
Acknowledgements

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References


Footnotes

Financial support:

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Conflict of Interest Declaration:

Dr. Kearns has served as a paid consultant for AstraZeneca, L.P. and Wyeth regarding the development of the pediatric programs for omeprazole and pantoprazole.
Figure Legends

Figure 1

Relationship between *CYP2C19* genotype and the area under the plasma concentration-time curve (AUC, mg*hr/L per 1 mg/kg; panels A and C) and the apparent terminal elimination rate constant (K_{el}; panels B and D) for pantoprazole and omeprazole, respectively. Boxes reflect the interquartile range while the lines in the boxes depict the mean values; whiskers indicate the 10^{th} and 90^{th} percentiles, respectively. Open and closed symbols in A and B represent subjects who received oral and intravenous pantoprazole, respectively. *CYP2C19* genotypes and number of subjects in each genotype group are given at the bottom of the graphs. Horizontal lines above the boxes in panels A and B join genotype groups that are not significantly different from each other as determined by Tukey’s Honestly Significant Difference following an initial ANOVA. Genotype groups not connected by a line in A and B (pantoprazole) are significantly different from each other (AUC, *p*<0.0001; K_{el}, *p*=0.0012). For omeprazole (C, D), no statistically significant differences were detected (*p*=0.099) Statistical comparisons were conducted using JMP 8.0.2 (SAS Institute Inc., Cary, NC).
### Table 1

*CYP2C19*17 allele frequencies in different ethnic groups and patient populations

<table>
<thead>
<tr>
<th>Population</th>
<th>n subjects</th>
<th>frequency CYP2C19*17</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole cohort</td>
<td>40</td>
<td>0.23</td>
<td>This study</td>
</tr>
<tr>
<td>Omeprazol cohort</td>
<td>37 (23)</td>
<td>0.19 (21.7)</td>
<td>This study &lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caucasian</td>
<td>107</td>
<td>0.22</td>
<td>This study; DNA repository &lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>African American</td>
<td>114</td>
<td>0.21</td>
<td>This study; DNA repository &lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hispanic</td>
<td>108</td>
<td>0.12</td>
<td>This study; DNA repository &lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Norwegians</td>
<td>332</td>
<td>0.22</td>
<td>(Rudberg et al., 2008)</td>
</tr>
<tr>
<td>Swedish</td>
<td>314</td>
<td>0.18</td>
<td>(Sim et al., 2006)</td>
</tr>
<tr>
<td>Polish</td>
<td>125</td>
<td>0.27</td>
<td>(Kurzawski et al., 2006)</td>
</tr>
<tr>
<td>Greek</td>
<td>283</td>
<td>0.20</td>
<td>(Ragia et al., 2009)</td>
</tr>
<tr>
<td>Ethiopian</td>
<td>190</td>
<td>0.18</td>
<td>(Sim et al., 2006)</td>
</tr>
<tr>
<td>Chinese</td>
<td>384</td>
<td>0.04</td>
<td>(Chen et al., 2008)</td>
</tr>
<tr>
<td>Japanese</td>
<td>265</td>
<td>0.013</td>
<td>(Sugimoto et al., 2008)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Frequency for all subjects initially enrolled in the omeprazole study. Kinetic data were obtained on a subset of 23 children.

<sup>2</sup> DNA repository refers to samples maintained in the laboratory of the authors; ethnicity determined by self-report.