Rapid identification of the hepatic cytochrome P450 (CYP) 2C19 activity using a novel and noninvasive $^{13}$C-pantoprazole breath test.

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Running Title Page

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CYP, Cytochrome P450; HPLC, high performance liquid chromatography; CO$_2$, Carbon dioxide; DOB, Delta over baseline; PDR, Percent dose recovered; IR, infra red spectroscopy; BMI, body mass index; EM, extensive metabolizers; IM, intermediate metabolizers; and PM, poor metabolizers of CYP2C19; AUC, area under the concentration-time curve or area under DOB-time curve; Tmax, time to maximum concentration or DOB; Cmax, maximum plasma concentration; Vz/F, apparent volume of distribution; Cl, apparent oral clearance

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Abstract

We tested the hypothesis that stable isotope $^{13}$C-pantoprazole is O-demethylated by CYP2C19 and that the $^{13}$CO$_2$ produced and exhaled in breath as a result can serve as a safe, rapid, and noninvasive phenotyping marker of CYP2C19 activity in vivo. Healthy volunteers who had been genotyped for the CYP2C19*2, CYP219*3 and CYP2C19*17 alleles were administered a single oral dose of $[^{13}$C]-pantoprazole sodium-sesquihydrate (100mg) with 2.1g sodium-bicarbonate. Exhaled $^{13}$CO$_2$ and $^{12}$CO$_2$ were measured by IR spectroscopy before (baseline) and 2.5-120min after dosing. Ratios of $^{13}$CO$_2$/12CO$_2$ after $[^{13}$C]-pantoprazole relative to $^{13}$CO$_2$/12CO$_2$ at baseline were expressed as delta over baseline (DOB). Maximum DOB (DOB$_{max}$), DOB$_{15}$ to DOB$_{120}$, and area under the DOB versus time curve (AUC$_{0-120}$ and AUC$_{0-\infty}$) were significantly different among 3 genotype groups (CYP2C19*1/*1, n=10; CYP2C19*1/*2 or CYP2C19*1/*3, n=10; and CYP2C19*2/*2, n=5) with predicted extensive (EM), intermediate (IM) and poor (PM) metabolizers of CYP2C19 respectively (Kruskal-Wallis test, P<0.01); linear regression analysis indicated a gene dose effect relationship ($r^2$ ranged between 0.236 to 0.522; all p<0.05). These breath test indices were significantly lower in PM than IM (P<0.05) or EM (P<0.01) of CYP2C19. $[^{13}$C]-Pantoprazole plasma exposure showed significant inverse correlation with breath test indices in the respective subjects (Pearson r = -0.74; P=0.038). These feasibility data suggest that the $[^{13}$C]-pantoprazole breath test is a reliable, rapid and non-invasive probe of CYP2C19 and appears to be a useful research tool and to optimize drug therapy metabolized by CYP2C19.
Introduction

Human cytochrome P450 (CYP) 2C19 is important in the metabolism of several drugs including proton pump inhibitors (e.g. omeprazole, lansoprazole and pantoprazole), antidepressants, diazepam, carisoprodol, nelfinavir, clopidogrel, voriconazole, thalidomide, clonazepam and cyclophosphamide (Destá, et al., 2002; Ando, et al., 2002; Takada, et al., 2004; Hulot, et al., 2006). The clearance of drugs metabolized by CYP2C19 varies 5-20-fold among individuals and ethnic groups primarily due to effects of genetic polymorphisms (Goldstein, 2001; Destá, et al., 2002) but also as a result of nongenetic factors (e.g. drug interactions) (Destá, et al., 2002), age (Ishizawa, et al., 2005), pregnancy (McGready, et al., 2003), and disease state (Destá, et al., 2002; Frye, et al., 2006).

S-Mephenytoin hydroxylase, which was later purified as CYP2C19 (Wrighton, et al., 1993), was first reported in 1979 (Kupfer A, et al., 1979). The molecular basis of this polymorphism was identified later with the cloning of the gene (Goldstein and De Morais, 1994; De Morais, et al., 1994). Currently, 21 alleles associated with either complete loss of enzyme activity (e.g. CYP2C19*2 to *8) (Goldstein, 2001; Destá, et al., 2002), decreased activity (e.g. CYP2C19*9, CYP2C19*11, and CYP2C19*13) (Blaisdell, et al., 2002), or increased activity (CYP2C19*17) (Sim, et al., 2006) have been reported (http://www.cypalleles.ki.se/cyp2C19.htm). Based on the ability to metabolize probe drugs, individuals can be categorized as CYP2C19 poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM) (Destá, et al., 2002), (Sim et al., 2006). The most common loss-of-function alleles accounting for the majority of PM are CYP2C19*2 and CYP2C19*3 (Goldstein, et al., 1997; Xie, et al., 1999; Goldstein JA, 2001; Destá, et al., 2002; Hamdy, et al., 2002). The allelic frequency of the CYP2C19*2 allele is 23-39%, 11-16% and 13-25% in Asians, Caucasians and Blacks respectively. The frequency of the CYP2C19*3 allele is 5-12% in Asians and
<2% in Caucasians and Blacks. Thus, considerable interethnic differences in the distribution of PM have been observed: e.g., 2-5% in Caucasians, 4-7.5% in blacks, 13-20% in East Asians, and 38-79% in Pacific Islanders (Xie, et al., 1999; Goldstein, 2001; Desta, et al., 2002). The allelic frequency of the CYP2C19*17 allele (associated with gain in function) ranges from 18% to 32.9% in Caucasians, 4% in Ethiopians and 1.3% in Japanese (Kurzawski, et al., 2006; Rudberg, et al., 2008; Bohanec, et al., 2008; Sugimoto, et al., 2008).

A growing body of evidence suggests that altered CYP2C19 activity is clinically important. When compared to EM, PM of CYP2C19: a) are at increased risk for diazepam and clonazepam adverse effects (Desta, et al., 2002); b) achieve greater exposure of proton pump inhibitors, gastric acid suppression and eradication of H. pylori infection (Furuta, et al., 1998; 1999; 2005; 2007); and c) show markedly less clinical response to prodrugs that requires metabolic activation by CYP2C19 (e.g. clopidogrel, cyclophosphamide and thalidomide) (Takada, et al., 2004; Hulot, et al., 2006; Gilard, et al., 2008; Li, et al., 2007). Thus, knowledge of CYP2C19 activity may help optimize therapy and avoid adverse effects of drugs metabolized by this enzyme.

CYP2C19 metabolic status in vivo can be inferred from genotype or by measuring the metabolism of a probe substrate (Desta, et al., 2002). Reliable genotyping platforms are currently available, although accurate prediction of phenotype from genotype seems difficult in some cases for similar reasons outlined for CYP2D6 recently (Gaedigk, et al., 2008): uncertainty of the functional consequences of certain variants; inability to capture changes in activity caused by nongenetic factors; and the need to genotype for large number of (rare) variants and their combinations. Conventional in vivo CYP2C19 phenotyping tests (e.g. S-mephenytoin 4-hydroxylation or omeprazole 5-hydroxylation) are attractive tools as they can capture changes in CYP2C19 activity caused by both genetic and
nongenetic factors (Destá, et al., 2002). However, their routine clinical use has been limited because these procedures are time and resource intensive and often invasive. A phenotyping test that overcomes deficiencies of existing approaches would be of great value.

Stable isotope $^{13}$C-labeled compounds have been increasingly used as diagnostic probes in a variety of settings (Modak A, 2005) including assessment of drug metabolism (Mattison, et al., 2004; Leeder et al, 2008). The main purpose of the present study was to determine whether stable isotope $[^{13}$C$]$-pantoprazole is $O$-demethylated by CYP2C19 and the $^{13}$CO$_2$ produced and exhaled in breath as a result (Figure 1) can serve as a safe, rapid, and noninvasive phenotyping marker of CYP2C19 activity in vivo. Pantoprazole was selected for study because of its wider clinical use, wide safety margin, extensive metabolism in the liver primarily through CYP2C19-mediated $O$-demethylation (Figure 1) (Andersson, 1996; Tanaka, et al., 1997a; 2001), and a favorable structural feature for $^{13}$C stable-isotope labeling (Figure 1).
Methods

Study subjects: A total of 25 healthy female and male volunteers, mainly of Asian origin, (age 18-49 years old with body weight of at least 110 pounds and body mass index ≤ 30) pregenotyped for CYP2C19*2, *3 and *17 alleles were studied at the out-patient clinic of the Indiana University School of Medicine General Clinical Research Center (GCRC). This study was approved by the Institutional Review Board of the Indiana University. Investigative Device Exemption (IDE) application G070004 to conduct the study was also approved by the FDA. This trial is registered in the ClinicalTrials.gov (identifier: NCT00668902). All study subjects provided written informed consent prior to participation. Subjects were screened for any medical abnormalities within six weeks before initiating the breath test study and judged healthy on the basis of medical history, physical examination, vital signs and standard laboratory tests. Blood samples (about 10 ml) were obtained during the screening for DNA analysis. Subjects were asked to refrain from taking any prescription, over-the-counter medications, any herbal medications, and alcohol consumption one week before the start of the study and during the study period. Excluded from the study were those who: were tobacco smokers; have a history of intolerance or allergy to pantoprazole or sodium bicarbonate; have donated blood within the last 60 days of the screening visit or have plan to donate blood during the course of the study; had treatment with any investigational drug within the past 30 days; have used illegal drugs within three months prior to enrollment; are female pregnant or lactating; are female currently taking oral contraceptive birth control pills and who are unwilling or unable to stop oral contraceptives and use a barrier contraceptive method starting from the time of screening phase to the completion of the study; and are unreliable in the opinion of the study physician.
Study design: This was an open label single dose clinical trial. Eligible subjects were admitted to the GCRC at about 7 a.m. after an overnight fasting. For female subject, a urine pregnancy test was conducted prior to administration of study medications.

A 100 mg of [13C]-pantoprazole sodium-sesquihydrate (4-O-methyl-13C-pantoprazole, 99%; CLM-7831-SP; Lot # PR-17177) that was synthesized and supplied by Cambridge Isotope Laboratories Inc. (Andover, MA) as powder meeting chemical purity specification (>98%) was weighed and placed into a snap seal plastic container provided by Cambridge Isotope Laboratories Inc. As the pharmacokinetics of pantoprazole is linear with dose and pantoprazole has wide safety margin, an oral dose of 100 mg [13C]-pantoprazole that represents higher therapeutic dose range was used in this proof of concept study to maximize production and quantification of 13CO2. Sodium bicarbonate (2.1g) was weighed and transferred into the same snap seal plastic container that contains [13C]-pantoprazole and dissolved with water. As uncoated pantoprazole is acid labile, sodium bicarbonate was concomitantly dispensed with [13C]-pantoprazole to alkalinize the pH and facilitate absorption by preventing its degradation in the gut. This approach has been successfully used previously to prevent degradation of omeprazole (Howden, 2005) and pantoprazole (Ferron GM, 2003) by acid in the stomach. After baseline breath samples were collected in 1.2-liter aluminum-lined bags (Otsuka Pharmaceuticals, Tokushima, Japan), the solution containing [13C]-pantoprazole and 2.1 g sodium bicarbonate was then orally administered to each subject. The caps were rinsed 3 times with water and administered to the subjects. Breath samples were collected at 2.5, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 minutes after dosing. Venous blood samples (10 ml each) were collected before (predose) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 hours after [13C]-pantoprazole administration. Plasma was separated by centrifugation and stored frozen at –80°C until use. All subjects were allowed to eat regular meals after
the last breath test was obtained, which was 120 min (2 hrs) following [13C]-pantoprazole dosing. They were allowed to drink water freely.

**CYP2C19 genotyping:** Genomic deoxyribonucleic acid (DNA) was extracted at the Indiana University GCRC Biochemistry Core laboratory from human whole blood with the Qiagen DNA MiniKit (Qiagen, Valencia, CA). Genotyping for *CYP2C19*<sup>2</sup> (rs4244285), *CYP2C19*<sup>3</sup> (rs4986893) and *CYP2C19*<sup>17</sup> (rs12248560) were performed by use of the predeveloped *Taq*Man Assay-Reagents Allelic Discrimination Kits (Applied Biosystems, Foster City, CA) according to the suppliers instruction. Their assays ID were C___25986767_70, C___27861809_10 and C___469857_10 respectively. Three groups of genotypes were identified: homozygous wild type (*CYP2C19*<sup>1</sup>/<sup>1</sup>), heterozygous (*CYP2C19*<sup>1</sup>/<sup>2</sup> or *CYP2C19*<sup>1</sup>/<sup>3</sup>) and homozygous variant (*CYP2C19*<sup>2</sup>/<sup>2</sup>).

**Quantitation of 13CO<sub>2</sub>:** The [13C]-pantoprazole breath test exploited the use of the 13C-label that is incorporated at the 4-<i>O</i>-methyl site of pantoprazole, which specifically was designed for the hepatic CYP2C19-mediated <i>O</i>-demethylation. The assumption was that, since isotope unlabeled pantoprazole is mainly cleared by CYP2C19-mediated <i>O</i>-demethylation (Tanaka, et al., 1997a; 2001), [13C]-pantoprazole is <i>O</i>-demethylated by the same enzyme resulting in stepwise release of 13CO<sub>2</sub> and ultimately elimination from the body via pulmonary exhalation (Figure 1) and that quantification of 13CO<sub>2</sub> exhaled in breath will serve as a measure of in vivo hepatic CYP2C19 activity. To test this possibility, we measured the concentrations of 13CO<sub>2</sub> and 12CO<sub>2</sub> in exhaled breath samples at base line and after dosing using the UBiT-IR300 infrared spectrometry (Meretek Diagnostics, Rockville, MD) equipped with interference filters that are wavelength-selective for the absorbance of 13CO<sub>2</sub> and 12CO<sub>2</sub> (Figure 2). The assay was conducted within 3 days of sample collection. The 13CO<sub>2</sub> content in breath collection bags stored at room temperature has been shown to be stable up to 210 days (Mattison, et al.,
Enrichment of $^{13}$CO$_2$ in expired air was calculated at each sampling point. The change (delta-over-baseline, DOB) in the $^{13}$CO$_2$ / $^{12}$CO$_2$ ratio after $[^{13}$C]-pantoprazole relative to pre-dose (baseline) $^{13}$CO$_2$ / $^{12}$CO$_2$ ratio was calculated as follows (Mattison, et al., 2004; Leeder et al., 2008):

$$\text{DOB} = 1000 \times \frac{\left(\frac{^{13}\text{CO}_2}{^{12}\text{CO}_2}\right)_{\text{post-dose}} - \left(\frac{^{13}\text{CO}_2}{^{12}\text{CO}_2}\right)_{\text{baseline}}}{R_{\text{PDB}}}$$

where DOB was expressed as delta per ml (0/00), and $R_{\text{PDB}} = 0.0112372 = \frac{^{13}\text{CO}_2}{^{12}\text{CO}_2}$ in PDM (international standard Pee Dee Belemnite).

The relative amount of $[^{13}$C]-pantoprazole metabolized and released into the breath as $^{13}$CO$_2$ at each sampling time was calculated using the equation described elsewhere (Mattison, et al., 2004) and expressed as the cumulative percentage of dose recovered (PDR).

**Measurement of $[^{13}$C]-pantoprazole:** Plasma concentrations of $[^{13}$C]-pantoprazole was measured using a previously described method (Tanaka and Yamazaki, 1996), with slight modification. To 100 µL plasma, internal standard (50µg/ml phenacetin) was added and deproteinized with 200 µL acetonitrile. After centrifugation at 3000g for 10 min, the supernatant was evaporated to dryness and the residue reconstituted in 50 µL of 50 mM sodium perchlorate and acetonitrile (80:20 v/v) (solvent A) and 25 µL was injected into an HPLC system. Separation was performed by a Chiralcel OJ column (5.0 x 150 mm, 5 µm) (Chiral Cell Technologies, Inc., West Chester, PA) and a mobile phase delivered by a gradient pump: 0-10min, 100% solvent A (flow rate 1.0 mL/min); 10-25min, 100% solvent B (50 µL of 50 mM sodium perchlorate and 70:30 v/v acetonitrile); and 25-35min, 00% solvent A. The UV detector was set at 290 nm.
Analysis of breath test indices and pharmacokinetics: Breath test indices and pharmacokinetic parameters were determined by fitting the DOB data or plasma concentration data to a standard non-compartmental analysis using WinNonlin professional software (Version 5.01; Pharsight, Mountain View, CA).

Statistical analysis: Continuous variables were summarized by groups using descriptive statistics. Differences in pharmacokinetic parameters and breath test indices (DOB\textsubscript{max}, T\textsubscript{max}, AUC, DOB\textsubscript{30min} and PDR) among different genotypes of CYP2C19 were analyzed by the non-parametric Kruskal Wallis test with Dunnett’s Multiple Comparison post test. Linear regression analysis was implemented to determine gene-dose effects. Pearson’s correlation analysis was performed to determine relationships between breath indices. All statistical tests were conducted using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). P<0.05 was considered statistically significant.
Results

A total of 25 subjects of mainly Asian origin (8 Chinese, 4 Vietnamese, 3 Taiwanese, 2 Koreans, 2 Philippines, 1 Japanese, 1 Indian, and 4 others (1 Japanese/African, 1 Korean/Caucasian, 1 Chinese/Vietnamese/Caucasian, 1 Philippines/Caucasian) genotyped for the \( CYP2C19^*2 \), \( CYP2C19^*3 \) and \( CYP2C19^*17 \) alleles were studied (Table 1). Ten subjects were carriers of 2 functional alleles (\( CYP2C19^*1/*1 \) genotype, \( n=9 \); and \( CYP2C19^*1/*17 \) genotype, \( n=1 \)); 10 carried one-loss-of-function allele (\( CYP2C19^*1/*2 \) genotype, \( n=9 \); and \( CYP2C19^*1/*3 \) genotype, \( n=1 \)); and 5 carried two-loss-of-function alleles (\( CYP2C19^*2/*2 \) genotype). The \( CYP2C19^*17 \) alleles was rare in this population consistent with the literature (Sim, et al., 2006; Sugimoto, et al., 2008). However, the frequency of the \( CYP2C19^*3 \) allele was lower than what would be expected in an Asian population (Destá, et al., 2002; Hamdy, et al., 2002), probably due to the heterogeneity of the Asian populations studied. A subject with \( CYP2C19^*1/*17 \) genotype was analyzed together with \( CYP2C19^*1/*1 \) genotype based on the inferred phenotype. CYP2C19 extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM) were inferred from \( CYP2C19^*1/*1 \) and \( CYP2C19^*1/*17 \), \( CYP2C19^*1/*2 \) and \( CYP2C19^*1/*3 \), and \( CYP2C19^*2/*2 \) respectively. In the subsequent texts, EM, IM and PM are used to reflect these specific genotype groups. There was no statistically significant difference in the distribution of demographic characteristics among the three genotype groups, except that female subjects dominated over male in EM (6:4) and IM (7:3) of CYP2C19 (Table 1).

Enrichment of \(^{13}\text{CO}_2\) in expired air, expressed as the change or delta-over-baseline (DOB) in the \(^{13}\text{CO}_2 / ^{12}\text{CO}_2\) ratio after \([^{13}\text{C}]-\text{pantoprazole}\) relative to pre-dose (baseline) \(^{13}\text{CO}_2 /\)
\(^{12}\text{CO}_2\) ratio, was determined as a marker of \(^{13}\text{C}\)-pantoprazole O-demethylation. When data from all 25 subjects who completed the study were analyzed together, DOB values progressively increased with time, reached a maximum DOB (DOB\(_{\text{max}}\)) value (3.36±1.86\%) at 33.8±12.36 min after \(^{13}\text{C}\)-pantoprazole dosing, and then declined thereafter (DOB, 2.20±1.2 at t=120 min after dosing) (Figure 3A). The relative amount of \(^{13}\text{C}\)-pantoprazole metabolized and released into the breath as \(^{13}\text{CO}_2\) (expressed as cumulative percent dose recovered, PDR), also progressively increased over time (cumulative PDR at t=120 min: 10.43±5.42) (Figure 3B).

To test the influence of \(\text{CYP2C19}\) genetic polymorphisms on \(^{13}\text{C}\)-pantoprazole O-demethylation, as measured by \(^{13}\text{CO}_2\) in expired air, DOB values were determined in EM, IM and PM of \(\text{CYP2C19}\) at different times (2.5 min to 120 min) following the administration of a fixed dose of \(^{13}\text{C}\)-pantoprazole (Figure 4A). Upon visual inspection, DOB values were lower in PM than IM and EM of \(\text{CYP2C19}\). However, previous studies have suggested that body weight might influence breath test indices when a fixed dose of a phenotyping probes such as \(^{13}\text{C}\)-uracil(Mattison, et al., 2004) and dextromethorphan(Leeder et al, 2008) were administered. To test this possibility, DOB values at each time point were multiplied by body weight and plotted against time (Figure 4B) from which DOB\(_{\text{max}}\) and area under DOB versus time curve (AUC\(_{0-120}\)) were calculated. DOB\(_{\text{max}}\) or AUC\(_{0-120}\) values after correcting for body weight significantly correlated with those without correction for body weight (Pearson r = 0.9; P<0.0001). Therefore, data uncorrected for body weight were used for subsequent analysis. The corresponding breath test parameters are listed in Table 2. DOB\(_{\text{max}}\) and AUC\(_{0-120}\) showed a statistically significant difference among the three groups (\(Ps < 0.01\), Kruskal-Wallis test) (Table 2) and with a gene-dose effect (\(r^2 = 0.455\) to 0.485, P<0.001). The AUC\(_{\infty}\) showed a statistically significant difference
among the three groups (P=0.0028, Kruskal-Wallis test) (Table 2) and with a gene-dose effect \( (r^2 = 0.225, P=0.017) \). \( T_{\text{max}} \) remained comparable between the genotypes (P=0.51). Post-hoc analysis (Dunn's Multiple Comparison Test) revealed that PM had significantly lower \( \text{DOB}_{\text{max}} \) and \( \text{AUC}_{0-120} \) compared to IM (P<0.05) or EM (P<0.01) of CYP2C19 (Table 2); the \( \text{AUC}_{0-120} \) in PM (\( CYP2C19^{*2/*2} \) genotype) was \( \sim 5.3 \)-fold lower than in IM (\( CYP2C19^{*1/*2/*1/*3} \) genotypes) and \( \sim 6.4 \)-fold lower than in EM (\( CYP2C19^{*1/*1} \) genotype) of CYP2C19. The same trend was observed between the three genotype groups with regard to \( \text{AUC}_{0-\infty} \). Although the \( ^{13}\text{CO}_2 \) exhaled in breath was lower in genotypes that are associated with partially reduced function (IM) than those with two fully functional alleles (EM), this difference did not reach a statistically significant level (Table 2).

We also calculated the relative amount of \([^{13}\text{C}]\)-pantoprazole metabolized and recovered in the breath as \( ^{13}\text{CO}_2 \) at each sampling time from which the cumulative PDR in breath could be estimated in the different genotypes (Figure 4C). The cumulative PDR values (up to \( t=120 \) min) are shown in Table 2. Consistent with the changes observed with DOB and AUC values, there was a statistically significant difference (Kruskal-Wallis test) in cumulative PDR values among the three genotype groups (P<0.01). PM had significantly lower cumulative PDR values (post-hoc) when compared with IM (P<0.05) or EM (P<0.01) of CYP2C19 (Table 2) and the effect seen was consistent with a gene-dose effect \( (r^2 = 0.55, P<0.0001) \). Differences in cumulative PDR values among IM and EM did not reach a statistically significant level.

The differences in \( ^{13}\text{CO}_2 \) breath indices among the different genotypes are more apparent when the individual values are displayed (Figure 5A-D). PM of CYP2C19 had not only significantly lower \( ^{13}\text{CO}_2 \) breath indices but the indices in PM did not overlap and were clearly
separated from IM and EM of CYP2C19. Although the mean (Table 2) and median (Figure 5A-D) values were lower in IM than EM of CYP2C19, none of the $^{13}$CO$_2$ breath indices could segregate the two genotype groups with certainty because the values in the two groups show substantial overlap.

To determine whether $[^{13}$C$]$-pantoprazole breath test serves as a rapid phenotype marker of CYP2C19 activity in the different genotypes, DOB values at each sampling point (2.5 to 120 min) were compared among EM, IM, and PM of CYP2C19 by non-parametric ANOVA and for gene-dose effect relationship by linear regression analysis (Table 3). A statistically significant difference among the 3 genotype groups was observed as early as 10 min after $[^{13}$C$]$-pantoprazole dosing, but more robust relationships were seen starting 15 min (peaked at approximately 20 to 25 min) and this robustness continued until the last breath sampling (t=120 min), with a significant gene-dose effect (Table 3). A multiple regression analysis was also performed in an attempt to relate AUC$_{0-\infty}$ with DOB values (10 min to 120 min) in all subjects without considering the genotypes. While a significant association was observed between AUC$_{0-\infty}$ and DOB at the different time points (10-120 min), model derived $r^2$ was improved from $r^2=0.297$ for 20 min or less to $r^2=0.886$ for 120 min or less sampling time, suggesting that the last sampling time is more predictive of AUC$_{0-\infty}$.

The pharmacokinetic profiles of $[^{13}$C$]$-pantoprazole from 9 subjects (n=3 in each genotype) are shown in Figure 6 and the pharmacokinetic parameters derived are listed in Table 4. Significant differences in terminal elimination half-life, AUC$_{0-120}$, AUC$_{0-\infty}$ and apparent oral clearance (body weight adjusted) was observed among the three genotypes (all $P$s < 0.05, Kruskal-Wallis test) (Table 4), with post-hoc analysis showing that PM had significantly
(P<0.05) longer t1/2, higher AUC0-12, AUC0-∞ and lower clearance compared to EM of CYP2C19. The AUC0-∞ of [13C]-pantoprazole was inversely correlated with the AUC0-∞ obtained from the breath test and this was statistically significant (Pearson r=0.74; P=0.038).
Discussion

In the present study, we have shown for the first time that: a) $[^{13}\text{C}]-\text{pantoprazole}$ is effectively O-demethylated in humans, as shown by increase in $^{13}\text{CO}_2$ in breath; b) $^{13}\text{CO}_2$ production was dependent on CYP2C19; and c) enrichment of exhaled $^{13}\text{CO}_2$ through the lung was inversely related to $[^{13}\text{C}]-\text{pantoprazole}$ exposure. These data support the idea that $^{13}\text{CO}_2$ exhaled via the lung as a result of O-demethylation of $^{13}\text{C}$-labeled pantoprazole is a reliable phenotype probe to identify PM individuals from IM and EM of CYP2C19.

Non-isotope labeled pantoprazole is predominantly cleared by hepatic CYP2C19-mediated O-demethylation and to some extent through CYP3A-mediated sulfone formation (Andersson T, 1996; Tanaka, et al., 1997a; 2001; Furuta, et al., 2005). The selection of $^{13}\text{C}$-labeled pantoprazole for study was based on the assumption that incorporation of the $^{13}\text{C}$-label at the 4-O-methyl site of the pyridine ring of pantoprazole would not influence the pattern of metabolism. Consistent with this suggestion, we have shown that $[^{13}\text{C}]-\text{pantoprazole}$ is effectively O-demethylated in humans as shown by substantial increase in the enrichment of $^{13}\text{CO}_2$ in breath over time (Figure 3). We have also shown that $[^{13}\text{C}]-\text{pantoprazole}$ O-demethylation is mainly mediated by CYP2C19: the amount of $^{13}\text{CO}_2$ in breath and the cumulative percent of dose recovered as $^{13}\text{CO}_2$ in breath was significantly lower in subjects being PM than IM or EM with respect to CYP2C19 (Figures 4 and 5; and Tables 2 and 3); and $[^{13}\text{C}]-\text{pantoprazole}$ exposure in plasma (Table 4) was significantly higher in PM than EM subjects. These data suggest that the differences in breath test indices among the genotypes were due to marked reduction in $[^{13}\text{C}]-\text{pantoprazole}$ O-demethylation in PM and are consistent with
previous data that the systemic exposure of non-isotope labeled pantoprazole is approximately 6-
fold higher in PM than in EM subjects (Tanaka, et al., 1997a). Therefore, CYP2C19-mediated O-
demethylation appears to be the major route of metabolism of both $^{13}$C labeled and un-labeled
pantoprazole; incorporation of the $^{13}$C-label at the 4-O-methyl site of the pyridine ring does not
seem to alter the pattern of pantoprazole metabolism.

The time to $\text{DOB}_{\text{max}}$ and $C_{\text{max}}$ of $[^{13}\text{C}]$-pantoprazole was shorter (on the average, 33.8min
and 30min respectively) as opposed to the time to $C_{\text{max}}$ of the unlabeled drug which is $\sim$2.4 h in
pharmacokinetic studies (Tanaka, et al., 1997a; Tanaka, et al., 2001). Like other proton pump
inhibitors, pantoprazole is acid labile and the oral formulation of pantoprazole is often
administered as an enteric-coated tablet to avoid degradation by gastric acid. In the present study,
sodium bicarbonate was used to transiently neutralize the gastric acid and to prevent degradation
of pantoprazole. This and the administration of the test compounds as a solution might have
enhanced its rapid absorption, allowing a rapid and early time point breath test measurement to
effectively distinguish PM from IM and EM of CYP2C19. Indeed, as shown in Table 3, a
statistically significant difference in DOB values among the genotypes was observed as early as
10 min after $[^{13}\text{C}]$-pantoprazole administration and robust difference was seen at 15 min and
thereafter. This test appears to be as reliable as the established phenotyping approaches (Desta, et
al., 2002). Our feasibility study suggests that the $[^{13}\text{C}]$-pantoprazole breath test offers
advantages over existing genotype and phenotype approaches in predicting or assessing
CYP2C19 activity in vivo, particularly in effectively distinguishing PM from IM and EM of
CYP2C19 (Figure 5A-D), because it can be performed rapidly and possibly at a single time point
in a noninvasive manner. However, the optimal time breath test measurement that effectively
distinguishes PM from IM and EM of CYP2C19 awaits further investigation and should take into account factors such as absorption lag time. In addition, a relatively higher dose of $[^{13}\text{C}]$-pantoprazole was used in this feasibility study to maximize the $^{13}\text{CO}_2$ signal in expired air, but the utility of the lowest doses of this probe that is appropriate for the phenotyping purpose and avoids any potential adverse effects associated with the use of high dose should be explored in the future.

Although $[^{13}\text{C}]$-pantoprazole breath test is expected to discriminate PM from UM and probably IM from UM, our sample size (only one subject with $\text{CYP2C19}^{*1/*17}$ genotype) did not allowed us to properly evaluate the performance of this test with respect to UM versus other phenotype groups. The ability of $[^{13}\text{C}]$-pantoprazole breath test to discriminate IM from EM of CYP2C19 with certainty seems to be weak. While a gene-dose effect relationship was noted regarding the influence of CYP2C19 genotype on the inferred phenotypes (Figures 4 and 5; Table 2 and 3), no statistically significant difference in phenotype was observed among those that were predicted to be CYP2C19 IM and EM. Of note, conventional probes such as omeprazole and S-mephenytoin also accurately discriminate PM from IM or EM, but there is often uncertainty in their ability to effectively distinguish subjects with IM from those with EM of CYP2C19 despite the fact that a statistically significantly difference between the two groups have been reported (Yin, et al., 2004; Furuta, et al., 2005). Our findings suggest a better separation of IM from EM when $[^{13}\text{C}]$-pantoprazole exposure is considered relative to the breath test indices. The reasons for this observation are not fully known. The methyl group formed from CYP2C19-mediated $[^{13}\text{C}]$-pantoprazole $O$-demethylation passes through the carbon pool ($^{13}\text{CH}_3$ to $^{13}\text{CHO}$ to $^{13}\text{COO}$ and $^{13}\text{CO}_2$) before it will be eventually traverse to the lung and exhaled.
Differential handlings during these processes might influence the breath test indices. Second, although we attempted to minimize the impact of baseline $^{13}$CO$_2$ on the calculation of enriched $^{13}$CO$_2$ by requesting subjects to refrain from activities that increase $^{13}$C in the body before and during the study (e.g. eating foods enriched with $^{13}$C, exercise, alcohol and cigarette consumption, and nonfasting overnight before the study), the level of compliance was difficult to assess. The influence of baseline $^{13}$CO$_2$ level was partially corrected because the $^{13}$CO$_2$ measured after the administration of $[^{13}$C]-pantoprazole was corrected for baseline values. However, given the small difference and overlapping DOB values among the IM and EM groups, the possibility that baseline $^{13}$CO$_2$ might influence the calculation of $^{13}$CO$_2$ enrichment cannot be ruled out. Third, pantoprazole is also metabolized to pantoprazole sulfone which could be O-demethylated and contribute to $^{13}$CO$_2$ production. While factors independent of hepatic CYP2C19 activity could potentially affect $^{13}$CO$_2$ measurement, this test still appears noninvasive, safe and rapid indirect surrogate marker of CYP2C19 activity \textit{in vivo}.

The $CYP2C19^{*2/*2}$ genotype associated with PM status is expected to produce no $^{13}$CO$_2$ in breath because this genotype is expected to produce nonfunctional CYP2C19 enzymatic activity. Assuming that pantoprazole O-demethylation is exclusively catalyzed by CYP2C19, no production of $^{13}$CO$_2$ should have been expected in PM of CYP2C19. However, as shown in Figures 3 and 4 as well as Table 2, small but appreciable enrichment of $^{13}$CO$_2$ in breath in PM subjects was observed. These data suggest that, although CYP2C19 is the major enzyme catalyzing $O$-demethylation of pantoprazole, the contribution of other enzymes cannot be ruled out. Pantoprazole is a chiral drug that is clinically administered as a racemic mixture. \textit{In vivo}, (+)-pantoprazole has been shown to be more dependent on CYP2C19 than (−)-pantoprazole.
(Tanaka, et al., 1997b; 2001), which appears to be a characteristic of omeprazole and
lansoprazole (Andersson and Weidolf, 2008). It is thus likely that O-demethylation of (-)-
pantoprazole is catalyzed by CYPs other than CYP2C19, particularly when the activity of
CYP2C19 is substantially diminished. The $^{13}$C-pantoprazole used in our study is a racemic
mixture (approximately 50:50). Since the metabolic profiles of stable isotope labeled and
unlabeled $^{13}$C-pantoprazole seems similar, it is logical to suggest that (+)-$^{13}$C-pantoprazole is
more dependent on CYP2C19 and that enzymes other than CYP2C19 that are involved in the O-
demethylation of (-)-$^{13}$C-pantoprazole might have contributed to the residual $^{13}$CO$_2$ in breath of
PM of CYP2C19. The possibility that the sulfone metabolite of pantoprazole might also be O-
demethylated to release $^{13}$CO$_2$ cannot be excluded.

In summary, given the salient features of nonradioactive $^{13}$C-labeling, the wide margin of
pantoprazole safety, and the noninvasive, inexpensive and rapid procedures involved, the $^{13}$C-
pantoprazole breath test appears to offer a useful screening method that can be applied in most
clinical settings (e.g., hospitals and physicians’ offices) to identify CYP2C19 function before
dosing with CYP2C19 substrates. Further, this novel tool should facilitate research and screening
of subjects in clinical trials involving CYP2C19. Emerging evidence suggest that interindividual
and interethnic differences in CYP2C19 activity influence therapeutic response of drugs such as
proton pump inhibitors, clopidogrel, cyclophosphamide and thalidomide) (Desta, et al.,
2002),(Furuta, et al., 1998),(Furuta, et al., 2005;Furuta, et al., 2007),(Hulot, et al., 2006),(Gilard,
et al., 2008),(Gilard, et al., 2006), (Takada, et al., 2004), (Li, et al., 2007). A rapid phenotype test
that captures variability of CYP2C19 enzyme activity due to genetic and nongenetic factors and
potentially offer greater practical clinical utility than the existing approaches, such as the $^{13}$C-

pantoprazole breath test described herein, should be an important step to optimize therapy with CYP2C19 substrates or select alternative drugs for the individual patient.
Acknowledgements

We are grateful to the volunteers who participated in this clinical trial. The nurses and technicians at the Indiana University School of Medicine General Clinical Research Center helped with conducting the healthy volunteers study and processing the samples including DNA extraction.
References


Footnotes

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Drs. Desta, Flockhart, Modak and Kurogi are co-owners of a patent on a clinical test designed to determine CYP2C19 activity using the [13C]-pantoprazole breath test. Dr. Flockhart has served as a paid consultant for Roche Molecular Diagnostics, Indianapolis, IN. The other authors declare no conflict of interest.
Legends for Figures

Figure 1. Proposed human metabolism of $[^{13}\text{C}]-\text{pantoprazole}$ and production of $^{3}\text{CO}_2$ in breath. This metabolic pathways of $^{13}\text{C}$-labeled is inferred from known metabolism of unlabeled pantoprazole (Tanaka, et al., 1997a), assuming that both have similar metabolic pathways.

*Formaldehyde; **Formic acid; ***Carbon dioxide

Figure 2. Quantitation of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ by Infra Red Spectroscopy.

Figure 3. $^{13}\text{CO}_2$ breath patterns (mean± SE) versus time in all healthy volunteers (n=25) after oral administration of a solution consisting of 100 mg $[^{13}\text{C}]-\text{pantoprazole}$ sodium-sesquihydrate and 2.1 g of sodium bicarbonate. Breath samples were collected at baseline (before) and up to 120 min after pantoprazole dosing. The amounts of $^{13}\text{CO}_2$ in breath (expressed as delta over baseline, DOB) versus time (A) and the cumulative percent dose recovered (PDR) versus time (B) are presented as mean ± SE.

Figure 4. $^{13}\text{CO}_2$ breath patterns (mean ± SE) versus time in healthy volunteers with $\text{CYP2C19}^{*1/\ast 1}$ (extensive metabolizers, EM), $\text{CYP2C19}^{*1/\ast 2}$ or $\text{CYP2C19}^{*1/\ast 3}$ (intermediate metabolizers, IM) and $\text{CYP2C19}^{*2/\ast 2}$ (poor metabolizers, PM) genotypes after oral administration of a solution consisting of 100 mg $[^{13}\text{C}]-\text{pantoprazole}$ sodium-sesquihydrate and 2.1 g of sodium bicarbonate. Breath samples were collected at baseline (before drug administration) and up to 120 min after pantoprazole dosing. The amounts of $^{13}\text{CO}_2$ in breath (expressed as delta over baseline, DOB) multiplied by body weight versus time (A), body weight uncorrected DOB versus time (B), and cumulative percent dose recovered (PDR) (C). Breath test indices calculated from the data in Figure B and C are presented in Table 2.
Figure 5. Individual values of $[^{13}\text{C}]-\text{pantoprazole}$ breath test indices in healthy volunteers with $CYP2C19^{*1/*1}$ (extensive metabolizers, EM), $CYP2C19^{*1/*2}$ or $CYP2C19^{*1/*3}$ (intermediate metabolizers, IM) and $CYP2C19^{*2/*2}$ (poor metabolizers, PM) genotypes after oral administration of a solution consisting of 100 mg $[^{13}\text{C}]-\text{pantoprazole}$ sodium-sesquihydrate and 2.1 g of sodium bicarbonate.

*Data from a subject who carried $CYP2C19^{*1/*17}$ genotype.

DOB, delta over baseline; AUC, area under the DOB versus time curve; and PDR, percent dose recovered as $^{13}\text{CO}_2$.

Figure 6. $[^{13}\text{C}]-\text{Pantoprazole}$ plasma concentrations (mean±SD) versus time curves in healthy volunteers with $CYP2C19^{*1/*1}$ (extensive metabolizers, EM), $CYP2C19^{*1/*2}$ or $CYP2C19^{*1/*3}$ (intermediate metabolizers, IM) and $CYP2C19^{*2/*2}$ (poor metabolizers, PM) genotypes after oral administration of a solution consisting of 100 mg $[^{13}\text{C}]-\text{pantoprazole}$ sodium-sesquihydrate and 2.1 g of sodium bicarbonate.
Table 1. Subject demographics in CYP2C19*1/*1 (extensive metabolizers, EM), CYP2C19*1/*2 or CYP2C19*1/*3 (intermediate metabolizers, IM) and CYP2C19*2/*2 (poor metabolizers, PM) genotypes

<table>
<thead>
<tr>
<th>CYP2C19 metabolic status</th>
<th>EM (n=10)</th>
<th>IM (n=10)</th>
<th>PM (n=5)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>26.2±5.3</td>
<td>26.4±6.1</td>
<td>24.3±3.3</td>
<td>0.86</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.4±7.0</td>
<td>165.9±9.9</td>
<td>163.4±7.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.2±11.3</td>
<td>70.0±16.1</td>
<td>61.5±5.3</td>
<td>0.62</td>
</tr>
<tr>
<td>BMI</td>
<td>24.8±3.6</td>
<td>24.8±3.5</td>
<td>23.0±1.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Female/Male</td>
<td>6:4</td>
<td>7:3</td>
<td>2:3</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

BMI: body mass index.

Note: all subjects were Asian

P values were determined by Kruskal-Wallis test among 3 different CYP2C19 genotype groups
Table 2: [\textsuperscript{13}C]-Pantoprazole breath test indices in healthy volunteers with $CYP2C19^{*1/*1}$ (extensive metabolizers, EM), $CYP2C19^{*1/*2}$ or $CYP2C19^{*1/*3}$ (intermediate metabolizers, IM) and $CYP2C19^{*2/*2}$ (poor metabolizers, PM) genotypes after oral administration of a solution consisting of 100 mg [\textsuperscript{13}C]-pantoprazole sodium-sesquihydrate and 2.1 g of sodium bicarbonate.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EM (n=10)</th>
<th>IM (n=10)</th>
<th>PM (n=5)</th>
<th>P value$\S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (min)$¶$</td>
<td>30 (20-50)</td>
<td>30 (25-60)</td>
<td>40 (10-50)</td>
<td>0.51</td>
</tr>
<tr>
<td>DOB$_{\text{max}}$</td>
<td>4.44±1.79**</td>
<td>3.49±1.18*</td>
<td>0.92±0.18</td>
<td>0.0017</td>
</tr>
<tr>
<td>AUC$_{0-120}$</td>
<td>378.9±133.8**</td>
<td>315.8±101.6*</td>
<td>59.6±18.0</td>
<td>0.0019</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$</td>
<td>744.2±212.8*</td>
<td>852.7±378.8**</td>
<td>163.2±75.8</td>
<td>0.0028</td>
</tr>
<tr>
<td>PDR</td>
<td>13.7±4.62**</td>
<td>11.28±2.58*</td>
<td>2.21±0.55</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

Breath test indices are presented as mean ± SD.

$\S$Comparison between the three genotypes was made by Kruskal-Wallis statistic with post-hoc analysis using Dunn's Multiple Comparison Test.

*P<0.05, PM versus IM; **p<0.01, PM versus EM; no statistically significant difference between IM and EM.

$¶$Median (min to max).

DOB, delta over baseline; AUC, area under the DOB versus time curve 0 to 120 min or 0 to infinity; PDR, cumulative percent dose recovered
Table 3. Analysis of DOB values at each sampling time in healthy volunteers with

\textit{CYP2C19*1/*1} (extensive metabolizers, EM), \textit{CYP2C19*1/*2} or \textit{CYP2C19*1/*3} (intermediate metabolizers, IM) and \textit{CYP2C19*2/*2} (poor metabolizers, PM) genotypes following oral administration of a solution consisting of 100 mg [\textsuperscript{13}C]-pantoprazole sodium-sesquihydrate and 2.1 g of sodium bicarbonate.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{DOB values at each sampling time} & \textbf{Differences among genotypes§} & \textbf{Differences between genotypes¶} & \textbf{Gene-dose-effect£} \\
 & (p values) & & & \\
 & & PM versus IM & PM versus EM & \textit{r}\textsuperscript{2} & p-values \\
\hline
DOB\textsubscript{2.5} & 0.0455 & & * & 0.18 & 0.038 \\
DOB\textsubscript{5} & 0.99 & & & 0.01 & 0.72 \\
DOB\textsubscript{10} & 0.017 & & * & 0.236 & 0.014 \\
DOB\textsubscript{15} & 0.0017 & * & ** & 0.299 & 0.0048 \\
DOB\textsubscript{20} & 0.0008 & * & *** & 0.474 & 0.0001 \\
DOB\textsubscript{25} & 0.0016 & * & ** & 0.522 & <0.0001 \\
DOB\textsubscript{30} & 0.0044 & * & ** & 0.459 & 0.0003 \\
DOB\textsubscript{40} & 0.002 & * & ** & 0.430 & 0.0004 \\
DOB\textsubscript{50} & 0.0019 & * & ** & 0.464 & 0.0002 \\
DOB\textsubscript{60} & 0.0023 & * & ** & 0.438 & 0.0003 \\
DOB\textsubscript{90} & 0.0022 & * & ** & 0.478 & 0.0001 \\
DOB\textsubscript{120} & 0.003 & ** & ** & 0.366 & 0.0014 \\
\hline
\end{tabular}
\end{table}

§Comparison of delta over baseline (DOB) values at each sampling time among the 3 genotype groups (EM, IM and PM) was performed by Kruskal-Wallis test

¶ Post-hoc analysis was performed using Dunn's Multiple Comparison Test.

*P<0.05; **p<0.01; ***p<0.001. No statistically significant difference between IM and EM at any of the time points.

£Gene-dose-effect among the 3 genotypes was determined by linear regression.
Table 4: Pharmacokinetic parameters of $[^{13}\text{C}]$-Pantoprazole (Mean±SD) in healthy volunteers with CYP2C19*1/*1 (extensive metabolizers, EM), CYP2C19*1/*2 or CYP2C19*1/*3 (intermediate metabolizers, IM) and CYP2C19*2/*2 (poor metabolizers, PM) genotypes after oral administration of a solution consisting of 100 mg $[^{13}\text{C}]$-pantoprazole sodium-sesquihydrate and 2.1 g of sodium bicarbonate.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EM (n=3)</th>
<th>IM (n=3)</th>
<th>PM (n=3)</th>
<th>P value$§$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Half-life (hr)</td>
<td>0.88±0.14*</td>
<td>1.60±0.61</td>
<td>4.23±0.57</td>
<td>0.0439</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>4.06±1.16</td>
<td>4.39±0.61</td>
<td>4.29±1.88</td>
<td>0.88</td>
</tr>
<tr>
<td>$AUC_{0-120}$ (hr*µg/ml)</td>
<td>4.99±1.08*</td>
<td>9.96±3.43</td>
<td>21.19±4.25</td>
<td>0.0439</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (hr*µg/ml)</td>
<td>5.09±1.08*</td>
<td>10.35±3.69</td>
<td>24.94±4.08</td>
<td>0.0439</td>
</tr>
<tr>
<td>$V_{\text{z/F}}$ (L)</td>
<td>26.20±8.35</td>
<td>22.20±1.48</td>
<td>25.10±7.35</td>
<td>0.88</td>
</tr>
<tr>
<td>$Cl$ (ml/hr/kg)</td>
<td>326.9±97.00*</td>
<td>159.30±52.00</td>
<td>67.78±7.60</td>
<td>0.0439</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters are presented as mean ± SD.

§Comparison between the three genotypes was compared by Kruskal-Wallis statistic with post-hoc analysis using Dunn's Multiple Comparison Test.

*P<0.05, PM versus EM

$T_{\text{max}}$, time to maximum concentration; $C_{\text{max}}$, peak plasma concentration; $AUC$, area under the concentration versus time curve 0 to 120 min or 0 to infinity; $V_{\text{z/F}}$ apparent volume of distribution; $Cl$, apparent oral clearance corrected for body weight.
Figure 2

Absorbance vs. Wavelength (cm$^{-1}$)

- Interference filter for $^{12}$CO$_2$
- Enhancement filter for $^{13}$CO$_2$
Figure 5

A

DOB_{max} (\%)

\begin{itemize}
  \item EM
  \item IM
  \item PM
\end{itemize}

CYP2C19 Genotypes

B

Cumulative PDR

\begin{itemize}
  \item EM
  \item IM
  \item PM
\end{itemize}

CYP2C19 Genotypes

C

AUC_{0-120}

\begin{itemize}
  \item EM
  \item IM
  \item PM
\end{itemize}

CYP2C19 Genotypes

D

AUC_{0-\infty}

\begin{itemize}
  \item EM
  \item IM
  \item PM
\end{itemize}

CYP2C19 Genotypes