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## Is (+)-[<sup>13</sup>C]-Pantoprazole Better than (±)-[<sup>13</sup>C]-Pantoprazole for the Breath Test to Evaluate CYP2C19 Enzyme Activity?

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### Abstract

**Objective**—Recently, we have shown that the (+)-[<sup>13</sup>C]-pantoprazole is more dependent on CYP2C19 metabolic status than (–)-[<sup>13</sup>C]-pantoprazole. In this study, we tested the hypothesis that (+)-[<sup>13</sup>C]-pantoprazole is a more sensitive and selective probe for evaluating CYP2C19 enzyme activity than the racemic mixture.

**Methods**—(+)-[<sup>13</sup>C]-Pantoprazole (95 mg) was administered orally in a sodium bicarbonate solution to healthy volunteers. Breath and plasma samples were collected before and up to 720 minutes after dosing. The <sup>13</sup>CO<sub>2</sub> in exhaled breath samples was measured by infrared spectrometry. Ratios of <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> after (+)-[<sup>13</sup>C]-pantoprazole relative to <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> at baseline were expressed as delta over baseline (DOB). (+)-[<sup>13</sup>C]-Pantoprazole concentrations were measured by HPLC. Genomic DNA extracted from whole blood was genotyped for *CYP2C19*\*2, \*3 and \*17 using Taqman assays.

**Results**—Statistically significant differences in the area under the plasma concentration time curve (AUC<sub>plasma(0-∞)</sub>) (p<0.001) and oral clearance (<0.01) of (+)-[<sup>13</sup>C]-pantoprazole as well as in the breath test indices [delta over baseline, DOB<sub>30</sub>; and area under the DOB versus time curve, AUC<sub>DOB(0-120)</sub>] (p<0.01) were observed among poor, intermediate and extensive metabolizer of CYP2C19. DOB<sub>30</sub> and AUC<sub>DOB(0-120)</sub> adequately distinguished poor metabolizer from intermediate and extensive metabolizer of CYP2C19. Breath test indices significantly correlated with plasma elimination parameters of (+)-[<sup>13</sup>C]-pantoprazole (Pearson correlations: –0.68 to –0.73). Although relatively higher breath test indices were observed after administration of (+)-[<sup>13</sup>C]-pantoprazole (present study) than after (±)-[<sup>13</sup>C]-pantoprazole (previous study), the performance of the racemic and the enantiomer as marker of CYP2C19 activity remained similar.

**Conclusions**—Our data confirm that the metabolism of (+)-[<sup>13</sup>C]-pantoprazole is highly dependent on CYP2C19 metabolic status, but the breath test derived from it is not superior to the racemic [<sup>13</sup>C]-pantoprazole in evaluating CYP2C19 activity *in vivo*. Thus, racemic [<sup>13</sup>C]-pantoprazole which is relatively easy to synthesis and more stable than (+)-[<sup>13</sup>C]-pantoprazole is adequate as a probe of this enzyme.

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Competing Interest Z.D., A.M., and D.A.F. are co-owners of a patent on a clinical test designed to determine CYP2C19 activity using the [<sup>13</sup>C]-pantoprazole breath test (patent #: US 7,833,744 B2). D.A.F. has served as a paid consultant for Roche Molecular Diagnostics, Indianapolis, IN. A.M. is employed by Cambridge Isotope Laboratories which manufactures the <sup>13</sup>C substrate used in the study for the breath test. Commercialization of the pantoprazole breath test could be financially beneficial to the company. The other authors declare no conflict of interest.

## Introduction

The human cytochrome P450 (CYP) 2C19 is a clearance mechanism for several clinically important drugs, including those with a narrow therapeutic range(1–3). This enzyme is also critical in the metabolism of some prodrugs such as clopidogrel(4) cyclophosphamide(5, 6) and thalidomide(7) to pharmacologically active metabolites. The activity of this enzyme varies widely among individuals, leading to substantial differences in the clearance or metabolic activation of its substrates among patients. This variability is mainly controlled by functionally relevant genetic variations in the *CYP2C19* gene coding for the enzyme(1–3). In addition, nongenetic factors that include exposure to drugs that directly inhibit the CYP2C19 enzyme or enhance its expression contribute to this variability. It follows that knowledge of CYP2C19 metabolic status is important to optimize therapy and avoid adverse effects of drugs metabolized by this enzyme. A current limitation to using pharmacogenetics guided therapy is that it does not capture effects of nongenetic factors on phenotype. In the past, researchers have used phenotypic assays to capture the effect genetic and nongenetic factors have on the metabolism by using enzyme selective probe drug. However, phenotyping enzyme activity using probe drugs has not been practical and unavailable in the clinical setting in the past because of the invasive and time intensive nature of such an approach. . The need for a rapid, point of care phenotyping assay that is safe and reliable would enable clinicians to personalize therapy and avoid costly adverse reactions or sub-optimal therapy.

Recently, we and others have tested and demonstrated that stable isotope labeled racemic ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole breath test is a promising tool to assess CYP2C19 metabolic status(8–11). This test has a number of practical advantages (captures genetic and nongenetic causes of CYP2C19 activity, non-invasive, easy and rapid to perform) and has the potential to offer greater clinical utility than existing approaches (e.g. genetic tests alone).

Studies have shown that the pharmacokinetics of unlabelled pantoprazole is enantioselective and that the (+)-enantiomer is more dependent on CYP2C19 activity than the (–)-enantiomer of pantoprazole(11, 12). In our most recent publication, we confirmed enantioselective elimination of the stable isotope labeled pantoprazole given as a racemic mixture and observed that (+)-[ $^{13}\text{C}$ ]-pantoprazole is preferentially metabolized by CYP2C19 compared to (–)-[ $^{13}\text{C}$ ]-pantoprazole and, based on pharmacokinetic parameters, that this enantiomer can separate extensive metabolizers (EM) or intermediate metabolizers (IM) from poor metabolizers (PM)(11). These data raise the possibility that (+)-[ $^{13}\text{C}$ ]-pantoprazole breath test may be a better marker of CYP2C19 than the racemic mixture.

In the present study, we tested the hypothesis that the rate of O-demethylation of (+)-[ $^{13}\text{C}$ ]-pantoprazole, as measured by exhaled  $^{13}\text{CO}_2$ , is a rapid marker of hepatic CYP2C19 activity *in vivo* in humans and that administering (+)-[ $^{13}\text{C}$ ]-labeled pantoprazole alone may be better at separating the IM subjects from the EM subjects by measuring (+)-[ $^{13}\text{C}$ ]-labeled pantoprazole after administration of the ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole racemate. To test this hypothesis, we determined (+)-[ $^{13}\text{C}$ ]-pantoprazole breath test indices and pharmacokinetics in a separate cohort of healthy volunteers genotyped for common CYP2C19 genetic variants. The (+)-[ $^{13}\text{C}$ ]-pantoprazole breath test results from this study were compared with ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole breath test data obtained from our previous publication (8).

## Methods

### Study Subjects

A total of 15 healthy male (7) and female (8) volunteers of Asian origin (18–49 years old, with body weight of at least 110 pounds and body mass index  $\geq 30$ ) and pre-genotyped for *CYP2C19*\*2, \*3, and \*17 alleles were studied at the outpatient clinic of the Indiana University School of the Medicine Indiana Clinical Research Center (ICRC). We choose to enroll Asian subjects in this study to increase our odds of recruiting PM, since the Asian population has a higher incidence of PM (~30% in Asians versus 10% in Caucasians). This study was approved by the Institutional Review Board of the Indiana University. Investigative Device Exemption application G070004 to conduct the study was also approved by the Food and Drug Administration. This trial was registered at <http://www.ClinicalTrials.gov> (identifier: NCT00668902). All study subjects provided written informed consent before participation. Details of the inclusion and exclusion criteria of the participants were similar to our previous study with ( $\pm$ )-pantoprazole(8, 11).

### Study Design

This was an open-label, single-dose clinical trial. Eligible subjects were administered a single 95mg oral dose of (+)-[<sup>13</sup>C]-pantoprazole sodium- (4-O-[methyl-<sup>13</sup>C]-pantoprazole, 99%; CLM-7831-SP; lot#. PR-17177; Cambridge Isotope Laboratories, Inc., Andover, MA), with 2.1 g sodium bicarbonate to prevent degradation by stomach acid. Breath samples were collected at baseline and at 2.5, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 180, 240, 300, 480, 600 and 720 minutes (+)-[<sup>13</sup>C]-pantoprazole administration. Venous blood samples (10 ml) were collected in parallel at baseline and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours after (+)-[<sup>13</sup>C]-pantoprazole administration. Plasma was separated by centrifugation and stored at  $-80^{\circ}\text{C}$  until use.

### *CYP2C19* Genotyping

Genomic DNA was extracted from human whole blood with the QIAGEN DNA MiniKit (QIAGEN, Valencia, CA) and genotyping for the *CYP2C19*\*2, *CYP2C19*\*3, and *CYP2C19*\*17 alleles was carried out by TaqMan Assay-Reagents Allelic Discrimination Kits (Applied Biosystems, Foster City, CA) as previously described(8, 11).

### Quantitation of <sup>13</sup>CO<sub>2</sub>

The concentrations of <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub> in exhaled breath samples were determined using the UBiT-IR300 IR spectrometry (Meretek Diagnostics, Rockville, MD) equipped with interference filters that are wavelength-selective for the absorbance of <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub>. Enrichment of <sup>13</sup>CO<sub>2</sub> in expired air was calculated at each sampling point. The delta over baseline (DOB) in the <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> ratio after (+)-[<sup>13</sup>C]-pantoprazole relative to predose (baseline) <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> ratio was calculated as described elsewhere by our group(8, 11).

### Measurement of [<sup>13</sup>C]-Pantoprazole

Plasma concentrations of (+)-[<sup>13</sup>C]-pantoprazole were measured using a previously described method, with slight modification(8, 11). Plasma samples were extracted with 6 ml of ethyl acetate, after the addition of 20  $\mu\text{l}$  of internal standard (50  $\mu\text{g}/\text{ml}$  phenacetin) and 250  $\mu\text{l}$  of 2% ammonium hydroxide. The samples were then vortexed and spun and the resultant supernatant was transferred to a new tube to be evaporated. The dried residue was reconstituted with 100  $\mu\text{l}$  of acetonitrile and 50  $\mu\text{l}$  were injected onto the HPLC. Separation was performed using a Chiralcel OJ-RH column (4.6mmx150mm) (Chiral Technologies Inc. West Chester, PA) and a mobile phase consisting of 75% 50mM sodium perchlorate and 25% acetonitrile (flow rate, 0.5 ml/min). Analytes were detected by UV at 290nm.

Quantification of (+)-[<sup>13</sup>C]-pantoprazole was performed using a standard curve generated in blank plasma. The limit of quantification of the assay was 0.025 µg/ml. The inter-day and intraday coefficient of variation (% CV) of the assay was less than 20%.

### Analysis of Breath Test Indices and Pharmacokinetics

(+)-[<sup>13</sup>C]-Pantoprazole breath test indices and pharmacokinetic parameters were determined by fitting the DOB or plasma concentration data to a standard noncompartmental analysis using WinNonlin professional software (version 5.01; Pharsight, Mountain View, CA) as described before(8, 11).

### Statistical Analysis

Continuous variables were summarized by groups using descriptive statistics. Differences in pharmacokinetic parameters and breath test indices among different genotypes of *CYP2C19* were analyzed by the ANOVA test with Dunnett's multiple comparison post-test assuming the subjects were sampled from a Gaussian population. Pearson's correlation analysis was performed to determine correlations between (+)-[<sup>13</sup>C]-Pantoprazole breath indices and pharmacokinetic parameters. In order to assess the performance of (+)-[<sup>13</sup>C]-pantoprazole breath test as marker of *CYP2C19* activity against the racemic preparation, breath test parameters obtained previously in EM, IM and PM of *CYP2C19* after the administration of racemic pantoprazole [(±)-[<sup>13</sup>C]-pantoprazole] (8) were compared with those breath test parameters obtained following the administration of (+)-[<sup>13</sup>C]-pantoprazole (present study). All statistical tests were conducted using PASW Statistics 17.0 (SPSS Software Inc., Chicago, IL). A *p*-value of *p* < 0.05 was considered statistically significant.

### Results

In this study, 14 subjects had both pharmacokinetic and breath test indices and were included for analysis. The genotype predicted *CYP2C19* phenotypes were: 3 PM (\*2/\*2), 6 IM (\*1/\*2, *n*=5; and \*2/\*17, *n*=1) and 5 EM of *CYP2C19* (\*1/\*1, *n*=4; and \*1/\*17, *n*=1). One subject was a no call (genotype indeterminate) and was excluded from the analysis.

The plasma concentration time profile of (+)-[<sup>13</sup>C]-pantoprazole in the three groups of genotypes is shown in Figure 1. The corresponding pharmacokinetic parameters are listed in Table 1. Statistically significant differences in the plasma elimination half-life, area under the plasma concentration time curve [*AUC*<sub>plasma(0-∞)</sub> and *AUC*<sub>plasma(0-720)</sub>] (*p*<0.001 ANOVA), oral clearance (*p*<0.01), *C*<sub>max</sub> and weight adjusted oral clearance (*p*<0.05) of (+)-[<sup>13</sup>C]-pantoprazole were observed among the 3 genotypes [PM (*n*=3), IM (*n*=6), EM (*n*=5)]. Post-hoc analysis showed that PMs had a significantly longer half-life (*p*<0.001), and higher *AUC*<sub>plasma(0-∞)</sub> (*p*<0.001) and *C*<sub>max</sub> (*p*<0.01), and lower oral clearance (*p*<0.01) than EM subjects; a longer half-life (*p*<0.001), and higher *AUC*<sub>(0-∞)</sub> (*p*<0.001), was observed between IMs versus PMs. Only oral clearance (*p*<0.05) was lower in IMs versus EMs (Table 1).

The breath test profile of (+)-[<sup>13</sup>C]-pantoprazole in the three groups of genotypes is shown in Figure 2. The corresponding breath test indices are listed in Table 2. All of the indices were statistically significant, except the area under the Delta Over Baseline (DOB) versus time curve, *AUC*<sub>DOB(0-∞)</sub>, (*p*=0.56). Post-hoc analysis showed that PMs had a significantly longer *T*<sub>max</sub> (*p*<0.01), lower maximum Delta Over Baseline (*DOB*<sub>max</sub>) (*p*<0.01), lower *DOB* at 120 minutes (*DOB*<sub>120</sub>) (*p*<0.001) and lower area under the DOB versus time curve, *AUC*<sub>DOB(0-120)</sub>, (*p*<0.01) than EM subjects. A longer time to maximum DOB (*T*<sub>maxDOB</sub>) (*p*<0.01), a lower DOB at 120 minutes (*DOB*<sub>120</sub>) (*p*<0.01) and a lower *AUC*<sub>DOB(0-120)</sub>,

( $p < 0.05$ ) was observed when IMs were compared to PMs. No difference was observed between IMs versus EMs.

Correlation analyses between (+)-[ $^{13}\text{C}$ ]-pantoprazole pharmacokinetic parameters and breath test indices are listed in Table 3. Statistically significant correlations were observed with the elimination of (+)-[ $^{13}\text{C}$ ]-pantoprazole enantiomer, again pointing towards a much bigger role of CYP2C19 in (+)-[ $^{13}\text{C}$ ]-pantoprazole elimination and breath test indices. For example,  $\text{DOB}_{30}$  and  $\text{AUC}_{\text{DOB}(0-120)}$  were more significantly correlated with  $\text{AUC}_{\text{plasma}(0-\infty)}$  of (+)-[ $^{13}\text{C}$ ]-pantoprazole (Pearson correlations:  $-0.68$  &  $-0.73$ ) (Table 3 & Figure 3).

The two substrates (+)-[ $^{13}\text{C}$ ]-pantoprazole and ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole have identical breath curves for all three genotypes EM, IM and PM. The only difference is higher generation of  $^{13}\text{CO}_2$  by *O*-demethylation with (+)-[ $^{13}\text{C}$ ]-pantoprazole than ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole, but neither substrate can differentiate between EM's and IM's for CYP2C19 enzyme activity (phenotype) (Table 4 and Figure 4). Both substrates reliably identified genetic PM from EM and IM. This was predicted as (+)-[ $^{13}\text{C}$ ]-pantoprazole is preferentially *O*-demethylated over ( $-$ )-[ $^{13}\text{C}$ ]-pantoprazole.

$\text{DOB}_{30}$  values correlated well with  $\text{AUC}_{\text{DOB}(0-120)}$  for both substrates (+)-[ $^{13}\text{C}$ ]-pantoprazole ( $r^2 = 0.99$ ) and ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole ( $r^2 = 0.96$ ) (data not shown) also proving that a single time point breath collection is adequate in evaluating CYP2C19 enzyme activity.

$\text{DOB}_{30}$  values reflecting CYP2C19 enzyme activity (phenotype) correlate significantly ( $p < 0.01$ ) with  $\text{AUC}_{\text{plasma}(0-720)}$  of (+)-[ $^{13}\text{C}$ ]-pantoprazole obtained when (+)-[ $^{13}\text{C}$ ]-pantoprazole ( $r^2 = 0.57$ ) or ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole ( $r^2 = 0.45$ ) was administered.

## Discussion

In previous reports(8, 11), we have shown that the ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole breath test indices and pharmacokinetics were significantly associated with *CYP2C19* genotypes, suggesting a major role of CYP2C19 in the metabolism of ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole. Since pantoprazole is a chiral molecule and its pharmacokinetics (isotope labeled and unlabeled) show enantioselectivity(13), with (+)-[ $^{13}\text{C}$ ]-pantoprazole dependent on CYP2C19 than ( $-$ )-[ $^{13}\text{C}$ ]-pantoprazole, we tested whether (+)-[ $^{13}\text{C}$ ]-pantoprazole metabolism is a more selective probe of CYP2C19 activity *in vivo*. In the present study, we have demonstrated that: a) *CYP2C19* genotypes are significantly associated with (+)-[ $^{13}\text{C}$ ]-pantoprazole elimination and with breath test indices derived from it; and b) the elimination of (+)-[ $^{13}\text{C}$ ]-pantoprazole correlates significantly with breath test indices. These data and our previous report suggest that incorporation of the [ $^{13}\text{C}$ ]-label at the *O*-methyl site of pantoprazole does not alter the metabolic patterns or its stereoselective elimination.

Pantoprazole undergo hepatic clearance via mainly *O*-demethylation and to some extent through sulfoxidation and 6-hydroxylation(14, 15). *In vitro* data indicate that pantoprazole *O*-demethylation is catalyzed predominantly by CYP2C19(16). In humans, the plasma concentrations of *O*-demethylated metabolite of pantoprazole has been shown to be significantly influenced by CYP2C19 genotypes(14). The contribution of these pathways to the elimination of (+)- and ( $-$ )-pantoprazole appears to be different in that (+)-pantoprazole is more efficiently cleared by *O*-demethylation while ( $-$ )-pantoprazole undergo relatively higher sulfoxidation and 6-hydroxylations(15). Using isotope unlabeled pantoprazole, Tanaka et al., (13) have demonstrated marked stereoselective metabolism of pantoprazole in healthy volunteers, with (+)-pantoprazole being more dependent on CYP2C19 metabolic status than ( $-$ )-pantoprazole. Recently, we analyzed the stereoselective metabolism of [ $^{13}\text{C}$ ]-

pantoprazole and have confirmed marked stereoselectivity(11) and, consistent with findings from isotope-unlabeled pantoprazole (13), we have demonstrated that (+)-[<sup>13</sup>C]-pantoprazole was more dependent on CYP2C19 genotype than (-)-[<sup>13</sup>C]-pantoprazole. These data suggest that (+)-[<sup>13</sup>C]-pantoprazole is mainly cleared by O-demethylation and that this pathway is predominantly catalyzed by CYP2C19, while multiple pathways including O-demethylation (CYP2C19), sulfoxidation (CYP3A) and 6-hydroxylation (unknown enzyme) may contribute to the overall elimination of (-)-pantoprazole (14, 15, 17, 18). Consistent with the major role of CYP2C19 in (+)-[<sup>13</sup>C]-pantoprazole elimination compared to that of (-)-[<sup>13</sup>C]-pantoprazole, better correlation of (±)-[<sup>13</sup>C]-pantoprazole breath test indices were observed with the elimination of (+)-[<sup>13</sup>C]-pantoprazole than with that of (-)-[<sup>13</sup>C]-pantoprazole when all data were considered or PMs were excluded(11). Despite this, it is interesting to note that the correlations of breath test indices with the exposure of the (+)-enantiomer (Pearson  $r = -0.68$  &  $-0.73$ ) are not perfect (present study), but nonetheless an improvement over our previous publication (Pearson  $r = -0.46$  and  $-0.68$ ) (11).

One unexpected result was the long tail in the breath test pharmacokinetic data 240 minutes after dosing. Our results suggest that [<sup>13</sup>C] is distributed with time into the carbon pool of the body. It is possible that this observation could involve sequential metabolism (O-demethylation) of pantoprazole sulfone, but this route is highly unlikely to contribute as it accounts for less than 20% of the overall metabolism of pantoprazole(17). However, the possibility of reduced sensitivity of this drug as a CYP2C19 probe due to metabolic switching to the sulfoxidation pathway when CYP3A inducers are co-administered with (+)-[<sup>13</sup>C]-pantoprazole cannot be excluded. The possibility that chiral inversion to (-)-[<sup>13</sup>C]-pantoprazole (less dependent on CYP2C19) may contribute to the delayed peak of the breath test is also unlikely as no (-)-[<sup>13</sup>C]-pantoprazole was detected when plasma samples of (+)-[<sup>13</sup>C]-pantoprazole were analyzed (data not shown).

The optimal clinical utility of the pantoprazole breath test in evaluating CYP2C19 enzyme activity depends on the rapidness and ease of the test. Ideally, this test should allow reliable measurement of CYP2C19 activity during the first pass metabolism of pantoprazole with a single time point breath collection post ingestion of substrate. The DOB<sub>30</sub> time point differentiates PM's from IMs and EMs with both substrates. Although the (+)-[<sup>13</sup>C]-pantoprazole generates higher <sup>13</sup>CO<sub>2</sub> (present study) than when the (±)-[<sup>13</sup>C]-pantoprazole is used(11), it cannot differentiate IMs and EMs any better (Figure 4). Multiple breath sample collections post [<sup>13</sup>C]-pantoprazole ingestion with either substrate is not necessary in evaluating the CYP2C19 enzyme activity. The DOB<sub>30</sub> correlates well with AUC<sub>DOB(0-120)</sub> with either substrate as well as the AUC<sub>plasma(0-720)</sub> of plasma pantoprazole (Table 4). Having a single time point is useful in the clinical setting as it alleviates the need for multiple sampling and 30 minutes is sufficiently long enough to differentiate poor metabolizers but also quick enough to allow timely decision in the clinic.

As described above, the DOB values are higher when (+)-[<sup>13</sup>C]-pantoprazole is used as a substrate compared to that of (±)-[<sup>13</sup>C]-pantoprazole. However, the sodium salt of (+)-[<sup>13</sup>C]-pantoprazole has very poor stability (less than 3 months as an aqueous solution) while the racemic (±)-[<sup>13</sup>C]-pantoprazole sodium salt sesquihydrate is stable as a powder and hence can be formulated into suitable oral preparations (capsules or tablets). Given that breath test from the two substrates adequately separate PM from IM and EM and failed to adequately separate IM from EM, this formulation limitation must also be taken into account when deciding the clinical development or utility of pantoprazole breath test for evaluating CYP2C19 enzyme activity.

## Conclusions

Previously, we have performed a feasibility study showing that ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole breath test is a reliable marker of CYP2C19 activity in healthy volunteers. While this test completely discriminated PM from EM and IM of CYP2C19, there was a significant overlap between EM and IM of CYP2C19, which could be due to differences in the quantitative contribution of CYP2C19 and pathways towards the different enantiomers. The data from the present study indicate that (+)-[ $^{13}\text{C}$ ]-pantoprazole metabolism is highly dependent on CYP2C19 enzyme activity. However, the (+)-[ $^{13}\text{C}$ ]-pantoprazole was not a better marker of CYP2C19 activity than ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole breath test because it did not discriminate EM from IM of CYP2C19. Given these data and challenges for the synthesis, formulation and stability, the (+)-[ $^{13}\text{C}$ ]-enantiomer is not superior to the racemic [ $^{13}\text{C}$ ]-pantoprazole as a diagnostic probe for CYP2C19.

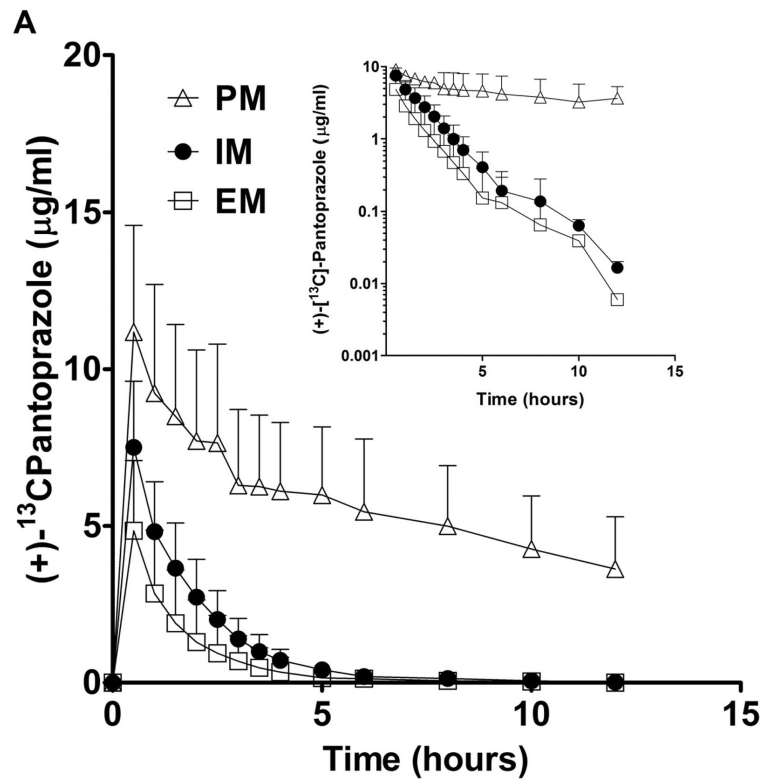
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**Figure 1.** Mean ( $\pm$ S.D.) of the plasma concentration versus time curves of (+)-<sup>13</sup>C-pantoprazole after administration of a single 95 mg oral dose of (+)-<sup>13</sup>C-pantoprazole sodium salt to healthy volunteers with PM (n=3), IM (n=6) and EM (n=5) genotypes of *CYP2C19*.

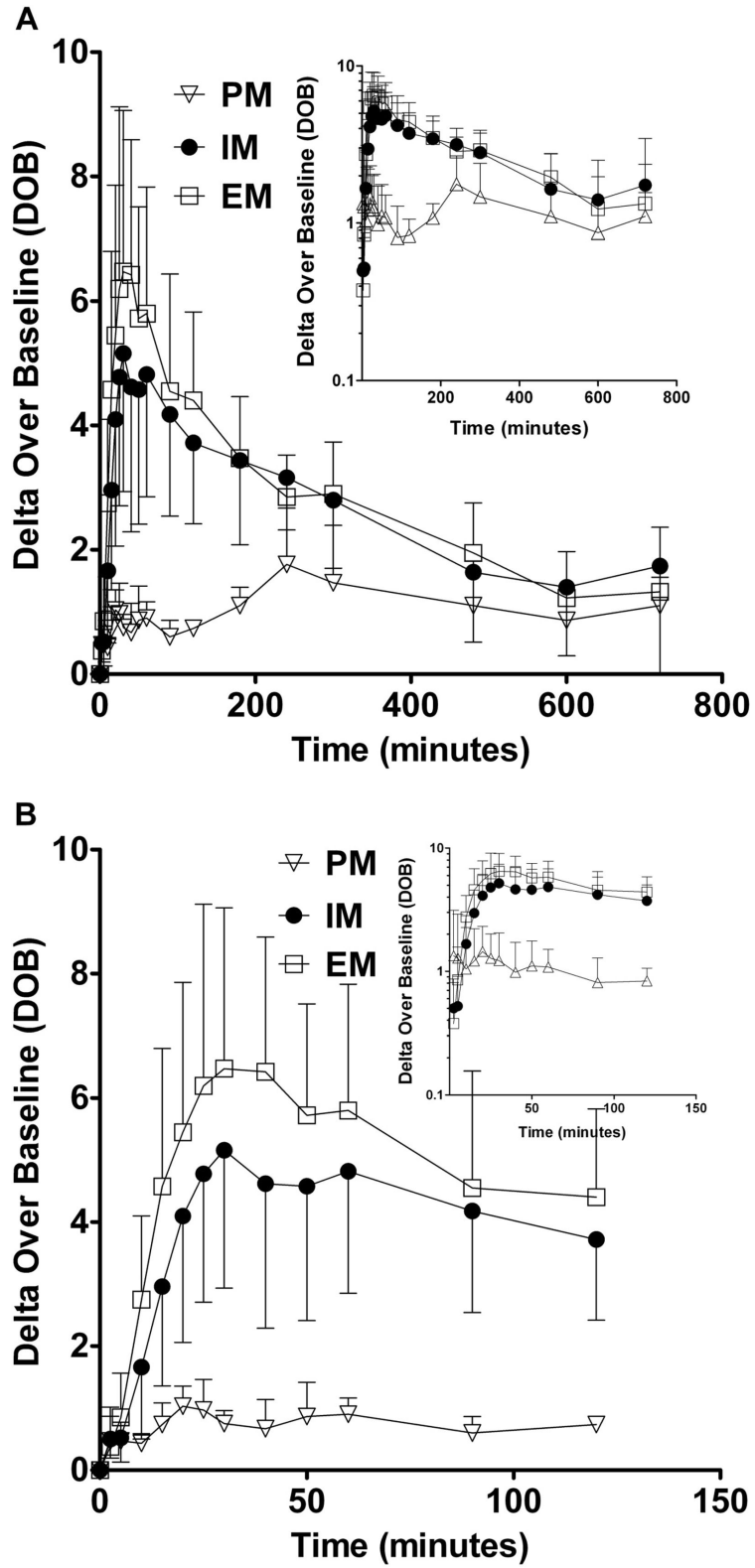
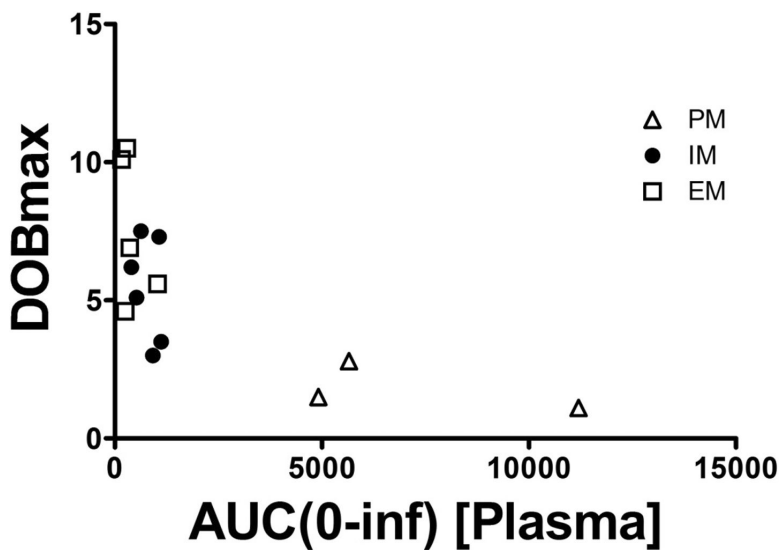
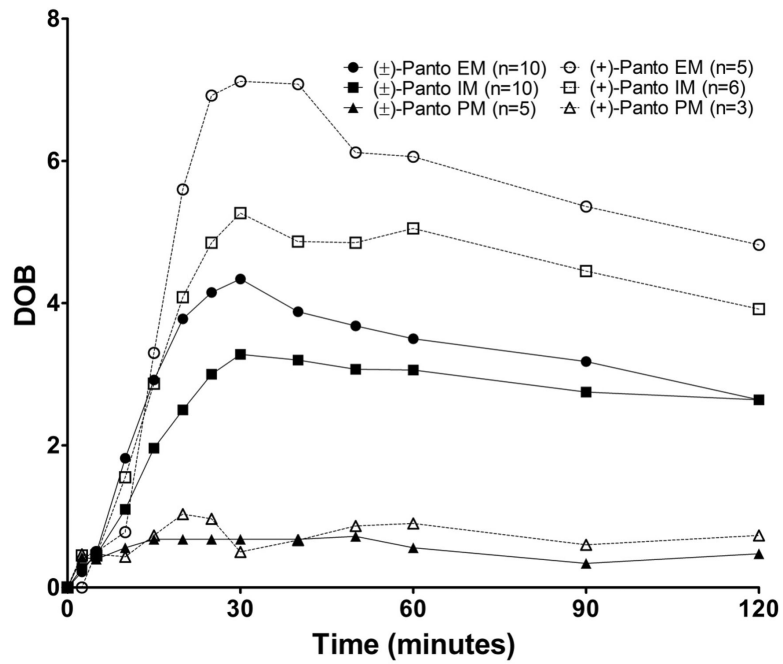


Figure 2.

Mean ( $\pm$ S.D.) of the breath test delta over baseline versus time curves of (+)-[<sup>13</sup>C]-pantoprazole after administration of a single 95 mg oral dose of (+)-[<sup>13</sup>C]-pantoprazole sodium-salt to healthy volunteers with PM (n=3), IM (n=6) and EM (n=5) genotypes of *CYP2C19*.



**Figure 3.** Correlation of Maximal Delta Over Baseline (DOB30) with  $AUC_{(0-720)}$  of (+)-[ $^{13}C$ ]-pantoprazole after administration of a single 95 mg oral dose of (+)-[ $^{13}C$ ]-pantoprazole sodium salt- to healthy volunteers with PM (n=3), IM (n=6) and EM (n=5) genotypes of *CYP2C19*.



**Figure 4.** Average Breath Curves for (±)-[<sup>13</sup>C]-pantoprazole and (+)-[<sup>13</sup>C]-pantoprazole in healthy volunteers with PM, IM and EM genotypes of *CYP2C19*. [data for the racemic pantoprazole is from Desta et al](8)

**Table 1**

Mean (95% C.I.) pharmacokinetic parameters (+)-[<sup>13</sup>C]-pantoprazole after a single 95 mg oral dose of (+)-[<sup>13</sup>C]-pantoprazole in PM, IM and EM genotypes.

	PM (n=3)	IM (n=6)	EM (n=5)	ANOVA <sup>a</sup> (p value)
T <sub>plasmamax</sub> (median) (min) <sup>§</sup>	30 (30,30)	30 (30,30)	30 (30,30)	
C <sub>max</sub> (µg/ml)	11.19 (2.75,19.63)	7.51 (5.29,9.73)	4.85 <sup>##</sup> (2.07,7.63)	<0.05
T <sub>1/2</sub> (min)	564.4 <sup>***</sup> (360.1,768.8)	66.6 (54.9,78.3)	49.0 <sup>###</sup> (28.8,69.3)	<0.001
AUC <sub>plasma0-∞</sub> (µg*min/hr)	7257.1 <sup>***</sup> (-1279.8,15794.0)	774.8 (458.1,1091.5)	417.9 <sup>###</sup> (-17.9,853.6)	<0.001
AUC <sub>plasma0-720</sub> (µg*min/hr)	4154.3 <sup>***</sup> (227.8,8080.8)	770.8 (453.8,1087.9)	416.8 <sup>###</sup> (-18.8,852.4)	<0.001
Vd/F (L)	11.69 (3.36,20.01)	12.98 (8.58,17.38)	20.48 (11.31,29.64)	0.069
CL/F (L/min)	0.892 (0.046,1.738)	8.516 <sup>@</sup> (4.634,12.398)	19.938 <sup>##</sup> (6.333,33.542)	<0.01
CL/F*kg (L/min*kg)	0.0145 (0.0048,0.0243)	0.1413 (0.0671,0.2155)	0.3349 <sup>#</sup> (0.0913,0.5786)	<0.05

PM vs. IM :

IM vs. EM :

PM vs. EM :

Abbreviations: AUC<sub>plasma</sub>, area under the plasma concentration time curve; CL/F, oral clearance, Vd/F, distribution volume, C<sub>max</sub>, maximum concentration; T<sub>plasmamax</sub>, time to C<sub>max</sub>; PM, poor metabolizer; IM, intermediate metabolizer and EM, extensive metabolizer of CYP2C19.

\* p<0.05,

\*\* p<0.01,

\*\*\* p<0.001

@ p<0.05,

@@ p<0.01,

@@@ p<0.001

# p<0.05,

## p<0.01,

### p<0.001

<sup>a</sup>One-Way ANOVA with Dunnett 2-Sided

<sup>§</sup>T<sub>max</sub> [Median (min, max)]

**Table 2**

Mean ( $\pm$ 95% C.I.) breath test parameters after a single 95 mg oral dose of (+)-[<sup>13</sup>C]-pantoprazole in PM, IM and EM genotypes.

	PM (n=3)	IM (n=6)	EM (n=5)	ANOVA <sup>a</sup> (p value)
T <sub>DOBmax</sub> (minutes) <sup>§</sup>	240 <sup>**</sup> (50,240)	30 (25,60)	30 <sup>##</sup> (20,30)	<0.01
Delta Over Baseline at 120 minutes (DOB <sub>120</sub> )	0.73 <sup>**</sup> (0.45,1.02)	3.92 (2.59,5.24)	4.82 <sup>###</sup> (3.33,6.31)	<0.01
Maximum Delta Over Baseline (DOB <sub>max</sub> )	1.80 (-0.41,4.01)	5.43 (3.44,7.43)	7.54 <sup>##</sup> (4.25,10.83)	<0.05
AUC <sub>DOB(0-120)</sub> (minutes*DOB)	88.0 <sup>*</sup> (0.6,175.4)	499.5 (307.4,691.6)	659.9 <sup>##</sup> (402.6,917.2)	<0.01
AUC <sub>DOB(0-∞)</sub> (minutes*DOB)	1768.1 (-1234.5,4770.8)	2167.7 (981.8,3353.6)	2583.4 (1611.0,3555.8)	0.56

PM vs. IM :

IM vs. EM :

PM vs. EM :

Abbreviations: DOB, delta over baseline; AUC<sub>DOB</sub>, area under the DOB versus time curve, DOB<sub>max</sub>, maximum DOB; T<sub>DOBmax</sub>, time to DOB<sub>max</sub>; PM, poor metabolizer; IM, intermediate metabolizer and EM, extensive metabolizer of CYP2C19.

\*  
p<0.05,

\*\*  
p<0.01,

\*\*\*  
p<0.001

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p<0.05,

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p<0.01,

@@@  
p<0.001

#  
p<0.05,

##  
p<0.01,

###  
p<0.001

<sup>a</sup>One-Way ANOVA with Dunnett 2-Sided

<sup>§</sup>T<sub>max</sub> [Median (min,max)]

**Table 3**

Correlations between pharmacokinetic parameters of (+)-[<sup>13</sup>C]-pantoprazole and breath test indices.

Pearson (r) (p value)	T <sub>1/2</sub> Plasma (hr)	AUC <sub>plasma</sub> (0-∞)(μg*hr/ml)	V <sub>d</sub> F Plasma (L)	Cl/F*kg Plasma (L/hr*kg)	DOB <sub>(0-120)</sub> Breath	DOB <sub>max</sub> Breath	AUC <sub>DOB(0-120)</sub> Breath (min*DOB)
C <sub>max</sub> (μg/ml)	0.718 (<0.01)	0.804 (<0.01)	-0.845 (<0.001)	-0.819 (<0.001)	-0.655 (<0.05)	-0.682 (<0.01)	-0.678 (<0.01)
T <sub>1/2</sub> (hr)		0.984 (<0.001)	-0.382 (0.177)	-0.566 (<0.05)	-0.814 (<0.001)	-0.704 (<0.01)	-0.771 (<0.01)
AUC <sub>plasma</sub> (0-∞) (μg*hr/ml)			-0.450 (0.107)	-0.549 (<0.05)	-0.761 (<0.01)	-0.675 (<0.01)	-0.728 (<0.01)
V <sub>d</sub> F (L)				0.902 (<0.001)	0.471 (0.089)	0.614 (<0.05)	0.562 (<0.05)
Cl/F*kg (L/hr*kg)					0.634 (<0.05)	0.779 (<0.01)	0.732 (<0.01)
DOB <sub>(0-120)</sub> Breath						0.919 (<0.001)	0.965 (<0.001)
DOB <sub>max</sub> Breath							0.977 (<0.001)

Data were analyzed using Pearson's Correlation Test. P<0.05 was considered significant.

Abbreviations: DOB, delta over baseline; AUC<sub>DOB</sub>, area under the DOB versus time curve, DOB<sub>max</sub>, maximum DOB; T<sub>max</sub>, time to DOB<sub>max</sub>; AUC<sub>plasma</sub>, area under the plasma concentration time curve; Cl/F, oral clearance, V<sub>d</sub>F, distribution volume, C<sub>max</sub>, maximum concentration; T<sub>plasmamax</sub>, time to C<sub>max</sub>

**Table 4**

Comparison of the breath test parameters obtained after the administration of ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole with those from (+)-[ $^{13}\text{C}$ ]-pantoprazole for EMs, IMs and PMs.

Substrate	Genotype Subjects	DOB <sub>30</sub> Mean ( $\pm$ 95% C.I.)	AUC <sub>DOB0-120</sub> Mean ( $\pm$ 95% C.I.)
( $\pm$ )-[ $^{13}\text{C}$ ]-Pantoprazole	EM (n=10)	4.3 (3.2,5.5)	6.4 (5.0,7.7)
(+)-[ $^{13}\text{C}$ ]-Pantoprazole	EM (n=5)	7.1 (4.8,9.5)	10.4 (7.4,13.4)
( $\pm$ )-[ $^{13}\text{C}$ ]-Pantoprazole	IM (n=10)	3.3 (2.6,3.9)	5.3 (4.2,6.3)
(+)-[ $^{13}\text{C}$ ]-Pantoprazole	IM (n=6)	5.3 (3.7,6.9)	8.2 (5.8,10.6)
( $\pm$ )-[ $^{13}\text{C}$ ]-Pantoprazole	PM (n=4)	0.7 (0.6,0.8)	1.0 (0.8,1.3)
(+)-[ $^{13}\text{C}$ ]-Pantoprazole	PM (n=3)	0.5 (0.0,1.0)	1.5 (0.9,2.1)

Data of (+)-[ $^{13}\text{C}$ ]-pantoprazole were from the present study and those of racemic ( $\pm$ )-[ $^{13}\text{C}$ ]-pantoprazole were derived from Desta et al., (8)

Abbreviations: DOB<sub>30</sub>, delta over baseline after probe administration; AUC<sub>DOB</sub>, area under the DOB versus time curve, DOB<sub>max</sub>, maximum DOB; TDOB<sub>max</sub>, time to DOB<sub>max</sub>; PM, poor metabolizer; IM, intermediate metabolizer and EM, extensive metabolizer of CYP2C19.