CYP2C19 Phenoconversion by Routinely Prescribed Proton Pump Inhibitors Omeprazole and Esomeprazole: Clinical Implications for Personalized Medicine

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ABSTRACT

The phenotype pantoprazole-\(^{13}\)C breath test (Ptz-BT) was used to evaluate the extent of phenoconversion of CYP2C19 enzyme activity caused by commonly prescribed proton pump inhibitors (PPI) omeprazole and esomprazole. The Ptz-BT was administered to 26 healthy volunteers and 8 stable cardiovascular patients twice at baseline and after 28 days of PPI therapy to evaluate reproducibility of the Ptz-BT and changes in CYP2C19 enzyme activity (phenoconversion) after PPI therapy. The average intrapatient interday variability in CYP2C19 phenotype (\(n = 31\)) determined by Ptz-BT was considerably low (coefficient of variation, 17%). Phenotype conversion resulted in 25 of 26 (96%) nonpoor metabolizer (non-PM) volunteers/patients as measured by the Ptz-BT at baseline and after PPI therapy. The incidence of PM status by phenotype following administration of omeprazole/esomprazole (known inhibitors of CYP2C19) was 10-fold higher than those who are genetically PMs in the general population, which could have critical clinical implications for personalizing medications primarily metabolized by CYP2C19, such as clopidogrel, PPI, cyclophosphamide, thiadomide, clariopram, clonazepam, diazepam, phencytoin, etc. The Ptz-BT can rapidly (30 minutes) evaluate CYP2C19 phenotype and, more importantly, can identify patients with phenoconversion in CYP2C19 enzyme activity caused by non-genetic factors such as concomitant drugs.

Introduction

Human CYP2C19 enzyme is critical in the metabolism of several drugs, including proton pump inhibitors (PPIs; omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole), antidepressants, diazepam, carisoprodol, nelfinavir, clopidogrel, voriconazole, thalidomide, clonazepam, and cyclophosphamide (Ando et al., 2002; Desta et al., 2002; Takada et al., 2004; Hulot et al., 2006). The clearance of drugs metabolized by CYP2C19 varies 5- to 20-fold among individuals and ethnic groups primarily because of effects of genetic polymorphisms (Goldstein, 2001; Desta et al., 2002), but also as a result of nongenetic factors, such as drug interactions (Desta et al., 2002), age (Ishizawa et al., 2005), pregnancy (McGreedy et al., 2003), and disease state (Desta et al., 2002; Frye et al., 2006).

CYP2C19 is a clinically relevant drug-metabolizing enzyme for which genotyping and phenotyping information has the potential to improve drug safety and efficacy (Scott, 2011; Ventola, 2013a). At least 27 variant alleles for CYP2C19 have been identified, with the most extensively described being CYP2C19*2, CYP2C19*3, and CYP2C19*17 (Ma et al., 2012). CYP2C19 metabolic status in vivo can be inferred from genotype or determined via therapeutic drug monitoring by measuring the metabolism of a probe substrate (Desta et al., 2002). Reliable genotyping platforms are currently available for both CYP2D6 and CYP2C19 (AmpliChip; Roche, Basel, Switzerland); however, accurate prediction of phenotype from genotype is impossible as phenoconversions due to nongenetic factors, such as drug-drug interactions for CYP2D6, are common (Preskorn et al., 2013). The uncertainty of the functional consequences of certain variant alleles in each individual, the inability to capture changes in activity caused by nongenetic factors, and the need to genotype for a large number of (rare or yet unknown) variant alleles and their combinations make the genotype test clinically and practically impractical.
insufficient for identifying all poor metabolizers (PMs) of CYP2C19 in the general population. Conventional in vivo CYP2C19 phenotype tests (e.g., S-mephenytoin 4-hydroxylation or omeprazole 5-hydroxylation) are attractive tools because they can capture changes in CYP2C19 activity caused by both genetic and nongenetic factors (Destea et al., 2002). However, their routine clinical use has been limited because these procedures are time and resource intensive and invasive. The in vivo phenotype pantoprazole, 13C breath test (Ptz-BT) has a number of practical advantages—it captures genetic and nongenetic factors that can alter CYP2C19 enzyme activity, and it is safe, easy, noninvasive, and rapid (30 minutes) to perform in a point-of-care setting (Destea et al., 2009; Furuta et al., 2009, 2010; Thacker et al., 2011, 2013; Tazaki et al., 2012). It has the potential to offer greater clinical utility compared with the existing genetic test for personalizing medicine for gastroesophageal reflux disease, antiplatelet therapy (Kushner et al., 2009), and Helicobacter pylori eradication therapy (Kuo et al., 2014).

In the present study, we investigated the effect of administration of two CYP2C19 inhibitors (omeprazole and esomeprazole) on the CYP2C19 phenotype using the noninvasive Ptz-BT. We examined the genotype-phenotype discordance and the lowering in CYP2C19 enzyme activity (phenoconversion) resulting from administration of omeprazole and esomeprazole for 28 days, recruiting healthy volunteers and coronary artery disease (CAD) patients with no concomitant medications influencing CYP2C19 enzyme activity.

Materials and Methods

Study Subjects. A total of 26 healthy male (14) and female (12) volunteers (age 18–40 years) and 8 cardiovascular patients (all male of Caucasian origin age 40–54 years) with body weight of at least 50 kg and body mass index 19–39 kg/m2 were recruited at the outpatient clinic of the Innsbruck Medical University (Supplemental Table 1). One volunteer and one cardiovascular patient dropped out of the study after their first visit, whereas one cardiovascular patient was excluded because he was underage and not eligible for inclusion in the study. Of the 6 eligible cardiovascular patients, 3 were on ticagrelor (90 mg) and 3 on prasugrel (10 mg). The study was approved by the Institutional Review Board of the Innsbruck Medical University. All study subjects signed a written informed consent before participation.

Inclusion Criteria. Following are inclusion criteria: volunteers age > 18 years; CAD patients age > 40 years; Eastern Cooperative Oncology Group performance status 0–2; willing to sign informed consent form; willing to give consent for drawing blood samples and/or mouth swabs for genotype; willing to perform overnight fasting of 8 hours and 24-hour alcohol abstinence prior to Ptz-BT; and willing to perform three visits for the study.

Exclusion Criteria. Exclusion criteria are as follows: prior adverse events from taking pantoprazole, sodium bicarbonate, omeprazole, esomeprazole, pregnant and breast-feeding women or not having performed a pregnancy test within last week; volunteers or CAD patients with gastroesophageal reflux disease; abnormal liver and kidney parameters exceeding 2.5 times the normal range; and not willing to stop intake of PPI for at least 2 weeks prior to visits 1 and 2.

Study Design. This was an open label, 3-visit Ptz-BT study recruiting healthy volunteers and CAD patients on antiplatelet therapy. A blood sample/mouth saliva swab was collected at the first visit for genotype (CYP2C19*2, *3, and *17 alleles). On visits 1 and 2, 2 weeks apart, eligible volunteers and cardiovascular patients were administered a single 100 mg oral dose of (±)-pantoprazole, sodium salt: sesquihydrate (4-O-methyl-13C, 98%); CLM-7831-CTM; lot C-7831-RR-G1; Cambridge Isotope Laboratories, Andover, MA), after a minimum 8-hour fast and 24-hour alcohol abstinence with 2.1 g sodium bicarbonate to prevent degradation by stomach acid. Breath samples were collected using breath collection bags (Otsuka Pharmaceutical, Tokyo, Japan) at baseline and at 20, 30, and 40 minutes postdose of (±)-[13C]pantoprazole. The Ptz-BT was performed twice prior to PPI therapy to test for interday reproducibility. The volunteers and CAD patients were then randomly assigned to take either omeprazole 40 mg per day (n = 16) or esomeprazole 40 mg per day (n = 15) for 28 days. Volunteers and CAD patients were administered the Ptz-BT again on visit 3 after 28 days of PPI therapy, with the last dose of the PPI taken 12 hours prior to the Ptz-BT. Patients/volunteers were informed when to take the PPI tablet each day, not to throw out any tablets from the bottle if they missed a dose, to be compliant, and to bring the tablet container at visit 3 to determine compliance. All 31 volunteers/patients included in the analysis were compliant.

CYP2C19 Genotype. DNA was extracted from saliva swabs or blood using the Chelex method (Walsh et al., 1991). Genotyping of CYP2C19 alleles *2 (rs4244285), *3 (rs4986893), and *17 (rs12248560) was accomplished by analyzing polymerease chain reaction products with ion-pair reversed-phase high-performance liquid chromatography–electrospray ionization mass spectrometry (Oberacher et al., 2005; Oberacher, 2008; Beer et al., 2011). A detailed description of the genotyping procedure can be found in the Supplemental Methods.

Quantitation of 13CO2. The concentrations of 13CO2 and 12CO2 in inhaled breath samples were determined using the POCONe infrared spectrometer (Photal Electronics, Tokyo, Japan) equipped with interference filters that are wavelength-selective for the absorbance of 13CO2 and 12CO2. Enrichment of 13CO2 in expired air was calculated at each sampling point. The delta over baseline (DOB) after (±)-[13C] pantoprazole administration relative to predose (baseline) was calculated, as described below (Destea et al., 2009; Thacker et al., 2011).

\[
\text{DOB} = \frac{\left[13\text{CO}_2\right]_{\text{postdose}} - \left[13\text{CO}_2\right]_{\text{predose}}}{\left[12\text{CO}_2\right]_{\text{predose}}} \times 100\%.
\]

where DOB was expressed as change per mille (%).

Statistical Analysis. All statistical tests for lowering of CYP2C19 enzyme activity as measured by the DOB16 values [%] at baseline prior to PPI therapy and after administration of omeprazole and esomeprazole were evaluated using the two-tailed P values. A P value < 0.05 was considered statistically significant. The DOB30 differences between various genotypes and phenotypes have been reported as mean ± S.D. The coefficient of variation for reproducibility of the Ptz-BT in 31 volunteers/patients at baseline (n = 2) was calculated as the ratio of the S.D. to the mean and reported as a percentage.

Results

Reproducibility of Ptz-BT. Thirty-one of 34 volunteers/CAD patients enrolled in the study were eligible for data analysis. The DOB30 values for the Ptz-BT administered at visits 1 and 2 for 31 volunteers/CAD patients for the in vivo phenotype test, which is subject to interday variability in gastrointestinal absorption, varied by an average of 0.7%. The average intrapatient interday variability in CYP2C19 phenotype (n = 31) determined by Ptz-BT was considerably low (coefficient of variation, 17%) (Supplemental Table 2). From the average DOB16max and DOB30max of breath samples collected at 20, 30, and 40 minutes for all 31 volunteers/CAD patients, it was determined that the DOB at 30 minutes (DOB30) reflected the CYP2C19 phenotype (Supplemental Table 3), which is consistent with all previous studies (Destea et al., 2009; Furuta et al., 2009, 2010; Thacker et al., 2011, 2013; Tazaki et al., 2012).
Genotype–Phenotype Discordance. From previous studies (Desta et al., 2009; Furuta et al., 2009, 2010; Thacker et al., 2011, 2013; Tazaki et al., 2012), correlating CYP219 genotype and phenotype using pantoprazole metabolites in plasma, the DOB30 cutoff values for the Ptz-BT were set at <1.2% for PMs, 1.2–3.4% for intermediate metabolizers (IMs), ≥3.5 to 7% for extensive metabolizers (EMs), and >7% for ultrarapid metabolizers (UMs). There was a genotype-phenotype discordance in 19 of 31 subjects at baseline prior to PPI therapy (61%); all 7 UM subjects by genotype were EMs by phenotype, 5 of 12 EMs by genotype were IMs by phenotype, 4 of 10 IMs by genotype were EMs by phenotype, and 3 of 10 IMs by genotype were PMs by phenotype.

After 28 days of PPI therapy, there was a genotype-phenotype discordance in 27 of 29 non-PM subjects (93%), all 7 UM subjects by phenotype were IMs by genotype that did not phenoconvert had a significant change in DOB30 from 4.9‰ to 1.2‰ and 3.7‰ to 1.2‰. After 28 days of PPI therapy, there was genotype-phenotype discordance in 27 of 29 non-PM subjects (93%), all 7 UM subjects by genotype were IMs by phenotype, 5 of 12 EMs by genotype were IMs by phenotype, and 3 of 10 IMs by genotype were PMs by phenotype. We observe almost 61% genotype-phenotype discordance at baseline even prior to initiating PPI therapy. There is wide interindividual variability in the CYP2C19 phenotype in individuals with the same CYP2C19 genotype due to non-genetic reasons, such as age, diet, environment, liver disease, etc. This clearly demonstrates the clinical utility of the Ptz-BT for evaluation of CYP2C19 enzyme activity.

The use of commonly and widely prescribed CYP2C19 inhibitors PPI—omeprazole and esomprazole—could lead to drug-induced phenoconversion of CYP2C19 enzyme activity. In the current study, using the in vivo phenotype Ptz-BT, we illustrate for the first time phenoconversion in CYP2C19 enzyme activity after 28 days of omeprazole/esomprazole administration. On an average, the lowering in CYP2C19 enzyme activity was 80% in EM and IM patients. There was genotype-phenotype discordance in 27 of 29 non-PM patients after 28 days of PPI therapy (93%). Not every individual with the same genotype had the same/identical lowering in CYP2C19 enzyme activity (Fig. 2), which clearly accentuates the need for a diagnostic test that evaluates CYP2C19 enzyme activity (phenotype) for a physician to personalize medications instead of predicting it from the genotype. Clopidogrel needs metabolic activation predominantly by CYP2C19, and there has been conflicting data on the involvement of CYP2C19 enzymatic activity and a possible drug/drug interaction between clopidogrel and PPI affecting clopidogrel efficacy (Drepper et al., 2012; Ventola, 2013b). Regulatory boards in the United States (Food and Drug Administration) and Europe

![Fig. 1. DOB30 values (phenotype) versus genotype (activity scores) (A) at baseline before PPI therapy and (B) after PPI therapy.](image-url)
CYP2C19 enzyme activity as measured by the DOB30 values of ingestion for just 4 weeks leads to significant lowering of the patients, we have shown that bothomeprazole and esomeprazole With our current pilot study with31 volunteers/cardiovascular treatment platelet reactivity. This finding will be tested in conjunction with clopidogrel to reduce the risk of gastrointestinal bleeding, the lowering of CYP2C19 enzyme activity (Abraham et al., 2010; Hamm et al., 2011). Since the first other risk factors who require dual-antiplatelet treatment bleeding and considered appropriate in patients with multiple Risk factors who require dual-antiplatelet treatment

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(Potential inhibitors and weak inhibitor pantoprazole). A number of commonly prescribed medications for treatment of different ailments are CYP2C19 inhibitors (http://medicine.iupui.edu/clinpharm/ddis/main-table/) capable of causing phenoconversion. These inhibitors include moclobemide, fluvoxamine, chloramphenicol, cimetidine, fluoxetine, indomethacin, isoniazid, fluconazole and ketoconazole, modafinil, oral contraceptives, probenecid, ticlopidine, toparimate, vericinazonale, and PPI. The genetic test will be inadequate in predicting patients who would have the PM CYP2C19 phenotype, especially when nongenetic factors are involved in lowering the CYP2C19 enzyme activity. In the present study, the DOB30 (phenotype) and genotype discordance at baseline before PPI therapy was 65% (19 of 29 non-PM patients), whereas the genotype-phenotype discordance after PPI therapy was 93% (27 of 29 non-PM subjects).

Our results clearly prove that the genotype test will underestimate the phenoconversion in volunteers/CAD patients taking CYP2C19 inhibitors omeprazole and esomper-azole. In clinical practice, it would be ideal for the physician to evaluate the CYP2C19 enzyme activity with the Ptz-BT prior to personalizing medications primarily metabolized by CYP2C19 instead of trying to predict it with the genotype test.

Limitations. Because the Ptz-BT is an in vivo phenotype test, there will be differences in gastrointestinal absorption (compliance to 8-hour fasting and 24-hour abstention from alcohol) over the course of time. We intentionally administered the Ptz-BT to volunteers/patients enrolled in the study on two separate visits 2 weeks apart to evaluate interday variability.

Conclusion. It is impossible to predict the phenotype for CYP2C19 enzyme only from the genetic test as shown by genotype-phenotype discordance even prior to initiating PPI therapy. Individuals with the same genotype do not have the same phenotype (large interindividual variability) due to nongenetic factors, such as age, diet, environment, liver disease, and, most importantly, drug-drug interactions. Predicting phenotype from genotype in clinical practice for individualizing therapy gets virtually impossible when individuals start taking commonly prescribed and widely used CYP2C19 inhibitors such as PPI and cimetidine. Subjects with the same phenotype do not drop in CYP2C19 enzyme activity to the same extent with omeprazole/esomeprazole administration, which is critical information for a physician and eliminates the prediction of phenotype. There is considerable genotype-phenotype discordance (93%) due to phenoconversion by administration of CYP2C19 inhibitors. The Ptz-BT is capable of rapidly evaluating CYP2C19 enzyme activity (phenotype), as well as identifying phenoconversion due to administration of CYP2C19 inhibitors both essential in personalizing medications.

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Authorship Contributions

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References


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