CYP2C19 Genotype and Pharmacokinetics of Three Proton Pump Inhibitors in Healthy Subjects

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Pharmacokinetics of Three Proton Pump Inhibitors—omeprazole, lansoprazole, and sodium rabeprazole—are now commercially used in Japan. They are structurally similar benzimidazole derivatives, and in vitro human liver microsomal studies have demonstrated that cytochrome P450 2C19 (CYP2C19) is responsible for 5-hydroxylation of omeprazole and lansoprazole and demethylation of rabeprazole, and CYP3A4 is for sulfoxidation of the three PPIs (7–10).

CYP2C19, often referred to the S-mephenytoin 4′-hydroxylase, shows genetically determined polymorphism, which is expected to affect the pharmacokinetics of these PPIs, and the subsequent efficacy and toxicity in anti-H. pylori therapy. The pharmacokinetic profiles of omeprazole and lansoprazole have been found to correlate with the S-mephenytoin 4′-hydroxylator phenotype (11–16). The CYP2C19 gene is located on chromosome 10p, and in addition to the wild-type allele CYP2C19*1, two mutant alleles CYP2C19*2 and CYP2C19*3 were recently found possibly responsible for genetically deficient metabolic activity (17–19). The CYP2C19 genotype is well correlated with the pharmacokinetics of omeprazole (20,21) and the eradication of H. pylori after anti-H. pylori therapy using omeprazole (22,23). In these studies, there was no manifested conclusion concerning the classification of the subjects based on the CYP2C19 genotype. The subjects could be reasonably classified into three groups consisting of the homozygos of CYP2C19*1, the heterozygous of CYP2C19*1, and the combination of mutant alleles, because the metabolic activity from CYP2C19*2 and CYP2C19*3 was almost perfectly deficient. However, the heterozygous CYP2C19*1 is sometimes included with the homozygous CYP2C19*1 as an extensive metabolizer without any rational evidence. The aims of the present study were 1) to predict the CYP2C19 genotype-dependence in anti-H. pylori therapy when lansoprazole or rabeprazole was used instead of omeprazole as a proton pump inhibitor (PPI), and 2) to elucidate the necessity to discriminate the homozygous CYP2C19*1 and its heterozygote in the anti-H. pylori therapy using PPI. A comparative pharmacokinetic study with omeprazole, lansoprazole, and rabeprazole was designed as an open, randomized, and crossover study of 18 Japanese healthy volunteers who were classified into the homozygous, heterozygous extensive metabolizer and the poor metabolizer based on the genotype determined by PCR-RFLP method.

INTRODUCTION

Proton pump inhibitors (PPIs) are superior to H2-receptor antagonists as acid inhibitory agents and are used in the treatment of upper gastrointestinal diseases such as peptic ulcer, gastroesophageal reflux disease, and Zollinger-Ellison syndrome (1). Recently, Helicobacter pylori (H. pylori) was demonstrated to possibly cause peptic ulcer (2,3) and gastric cancer (4,5), and triple therapy with PPI, amoxicillin, and clarithromycin/metronidazole is now recommended because of the high eradication rate (greater than 80%) (6). There still remains the incomplete eradication in the therapy, even though considerable dosage of antimicrobials are used. Three PPIs—omeprazole, lansoprazole, and sodium rabeprazole—are now commercially used in Japan. They are structurally similar benzimidazole derivatives, and in vitro human liver microsomal studies have demonstrated that cytochrome P450 2C19 (CYP2C19) is responsible for 5-hydroxylation of omeprazole and lansoprazole and demethylation of rabeprazole, and CYP3A4 is for sulfoxidation of the three PPIs (7–10).

MATERIALS AND METHODS

Chemicals

Omeprazole and its two primary metabolites, 5-hydroxymephenytoin and omeprazole sulfone, were obtained from AstraZeneca Ltd. (Osaka, Japan). Lansoprazole and its two primary metabolites, 5-hydroxylansoprazole and lansoprazole sulfone, were obtained from Takeda Pharmaceutical Company. Rabeprazole and its two primary metabolites, 5-hydroxrabeprazole and rabeprazole sulfone, were obtained from Merck & Co., Inc. (Rahway, NJ). Omeprazole sulfone, lansoprazole, and sodium rabeprazole were prepared from their corresponding acid form by addition of sodium hydroxide. Clarithromycin, amoxicillin, and metronidazole were acquired from the National Health Insurance Research Database, Japanese Ministry of Health, Labour, and Welfare (Chiba, Japan). The chemicals were stored at −20°C until use.
Co. (Osaka, Japan). Sodium rabeprazole and its two primary metabolites, thioether rabeprazole and rabeprazole sulfone, were obtained from Eisai Co. (Tokyo, Japan). All other chemicals were of reagent grade and obtained commercially.

Subjects and Study Protocol

CYP2C19 genotype was determined by the PCR-RFLP method (22,23). From 90 Japanese healthy volunteers, 18 selected subjects who agreed to participate in the following study were classified into three groups by the CYP2C19 genotype; that is, the homozygous extensive metabolizers (n = 6; CYP2C19*1/CYP2C19*1), the heterozygous extensive metabolizers (n = 6; CYP2C19*1/CYP2C19*2, CYP2C19*1/CYP2C19*3), and the poor metabolizers (n = 6; CYP2C19*2/CYP2C19*2, CYP2C19*2/CYP2C19*3, CYP2C19*3/CYP2C19*3). The demographics were very similar among the three groups (Table I). None of the subjects had hepatic or renal dysfunction or had taken any medication, including alcohol and over-the-counter drugs, for at least 1 week before and during the study. Written informed consent was obtained from all subjects before the study commenced. The protocol was approved by the Institutional Review Board of Kobe University School of Medicine in advance.

Each subject received a single oral dose of 20 mg omeprazole (Omepral® tablet, AstraZeneca Ltd.), 30 mg lansoprazole (Takepron® capsule, Takeda Pharmaceutical Co.), or 20 mg sodium rabeprazole (Pariet® tablet, Eisai Co.) as the respective enteric-coated formulation with 100 ml water at 9:00 a.m. in a crossover manner, with at least 1 week washout period between treatment periods. Each drug was taken after at least a 10 h fasting, and a lunch was served 3 h after drug ingestion. Venous blood samples were collected prior to and at 1, 2, 3, 4, 6, and 12 h after medication. The plasma samples separated after centrifugation at 1500 × g for 10 min were stored frozen at −20°C until analyzed. It was confirmed that there was no alteration in the concentrations of any PPIs or their primary metabolites during storage.

HPLC Assay

The HPLC system consisted of an LC-10AT pump, a SIL-10A auto injector, a SPD-10A detector, a CTO-10A column oven (at 40°C), a SCL-10A system controller, and a C-R7A chromatopack (Shimadzu Co., Kyoto, Japan) and was used for the measurement of plasma concentrations of all PPIs and their primary metabolites.

Plasma concentrations of omeprazole and its two primary metabolites, 5-hydroxylansoprazole and lansoprazole sulfone, were measured according to the method reported by Kobayashi et al. with slight modification concerning the extraction from the serum sample (24). Briefly, 100 μl of 0.1 mg phenacetin/ml methanol (internal standard) and 2 ml of diethyl ether-dichloromethane (7.3, v/v) were added to 0.5 ml of each plasma. They were extracted twice by shaking for 10 min, and the mixture was centrifuged at 1500 × g for 10 min. Then, 0.5 ml of propylene glycol was added to the supernatant, and the solvent was evaporated under a nitrogen stream at 40°C. Plasma concentrations of lansoprazole and its two primary metabolites, 5-hydroxylansoprazole and lansoprazole sulfone, were followed by the previously described HPLC method (25,26). Plasma concentrations of rabeprazole and its two primary metabolites, thioether rabeprazole and rabeprazole sulfone, were also measured according to the HPLC method by Nakai et al (27).

Pharmacokinetic Analysis

The maximum plasma concentrations (C max) were obtained graphically. The area under the plasma concentration-time curve of each PPI and its primary metabolites was calculated using the linear trapezoidal rule from 0 to infinity (AUC). The first-order elimination rate constant (K) was calculated by the linear least-squares regression analysis of the respective terminal log-linear portion of plasma concentration-time profile. It is noted that this linear portion is determined visually, and the calculated value of K depended on the sampling schedule. The elimination half-life (t1/2) was calculated as 0.693/K.

According to rational pharmacokinetic notation, the AUC of PPI metabolites depends on the parent PPI, and the AUC of PPI metabolites was corrected by dividing by that of the parent PPI to consider the metabolic processes more appropriately.

Statistical Analysis

The values are expressed as the mean value ± SE. The statistical differences in pharmacokinetic findings among the three groups were evaluated using one-way analysis of variance with a Scheffe-type multiple comparison test. P values less than 0.05 were considered significant.

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### Table I. CYP2C19 Genotype and Characteristics of Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Homozygous extensive metabolizers (n = 6)</th>
<th>Heterozygous extensive metabolizers (n = 6)</th>
<th>Poor metabolizers (n = 6)</th>
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<tbody>
<tr>
<td>CYP2C19 genotype</td>
<td>CYP2C19<em>1/CYP2C19</em>1 (n = 6)</td>
<td>CYP2C19<em>1/CYP2C19</em>2 (n = 4)</td>
<td>CYP2C19<em>2/CYP2C19</em>2 (n = 2)</td>
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<tr>
<td></td>
<td></td>
<td>CYP2C19<em>1/CYP2C19</em>3 (n = 2)</td>
<td>CYP2C19<em>2/CYP2C19</em>3 (n = 3)</td>
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<td></td>
<td>CYP2C19<em>3/CYP2C19</em>3 (n = 1)</td>
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<tr>
<td>Male:Female ratio</td>
<td>5:1</td>
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<td>Age (years)</td>
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<td>26.8 ± 3.1</td>
<td>25.8 ± 3.1</td>
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<td>Weight (kg)</td>
<td>60.2 ± 6.3</td>
<td>58.5 ± 7.1</td>
<td>59.7 ± 5.9</td>
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