Determination of Omeprazole and its Metabolites in Human Plasma as a Probe For CYP2C19 Phenotype

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Omeprazole is a benzimidazole compound that acts as proton pump inhibitor and recently used for the treatment of infection caused by Helicobacter pylori. In the liver, it is metabolized to varying degree by several cytochrome P-450 (CYP) isoenzymes which are further categorized into subfamilies of related polymorphic gene products. The metabolism of omeprazole is to a large extent dependent on CYP3A4 and CYP2C19. Omeprazole is metabolized to two major metabolites, 5-hydroxyomeprazole (CYP2C19) and omeprazole sulfone (CYP3A4). Minor mutations in CYP2C19 affect its activity in the liver and, in turn, the metabolic and pharmacokinetic profiles of omeprazole. The frequency of CYP2C19 poor metabolizers in population of Asian descent has been reported to range from 10% to 20%. This study demonstrates determination of omeprazole in human plasma as a probe drug of CYP2C19 phenotyping. The method allows the quantitation of omeprazole and its metabolite in human plasma after the administration of therapeutic dose of the drug. The present study is useful because that the polymorphism plays in the therapeutic effectiveness of proton pump inhibitors during the treatment of acid-related diseases.

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Experimental

LCMS conditions: The assay was developed using a Model M-8000 LC/MS system (Hitachi, Tokyo, JAPAN). The analytical column was YMC-Pack Pro C18 (50 x 2.0 mmI.D., YMC, JAPAN) and operated at 25 °C. The mobile phase was acetonitrile-ammonium acetate at a flow-rate of 0.2 mL/min. The drift voltage was 30 V. The sampling aperture was heated at 110 °C and shield temperature was 230 °C.

Chiral separation: High-performance liquid chromatography (HPLC) was performed using an L-6200 pump, D-2500 integrators for each detector (Hitachi, Tokyo, JAPAN). The column effluent was introduced to a CD-1595 circular dichroism detector (JASCO Corporation, Tokyo, Japan). The column used for chiral separation was CHIRALPAK AD-RH column (4.6 x 150 mm) using phosphate buffer/acetonitrile as an eluent and operated at 40 °C. The flow rate was 0.5 mL/min and the detection was 302 nm.

Sample Preparation: Venous blood samples were separated by centrifugation for 10 min at 1500 r.p.m. To remove proteins prior to injection, the plasma sample was treated with solid phase extraction.

Results and Discussion

We have been interested in determining the enantiomeric composition of the drug in plasma after racemate administration. Recently, we reported the stereospecific analysis of chiral drugs on a chiral column with UV and circular dichroism (CD) detection. The good resolution of enantiomers was obtained on CHIRALPAK AD-RH column using phosphate buffer (ammonium acetate) and acetonitrile as an eluent.
The metabolism of omeprazole is to a large extent dependent on CYP3A4 and CYP2C19. Omeprazole is metabolized to two major metabolites, 5-hydroxyomeprazole (CYP2C19) and omeprazole sulfone (CYP3A4), as shown in Fig.1. In extensive metabolizers of mephenytoin, hydroxylation by CYP2C19 is the principal route of elimination for omeprazole. Moreover, CYP2C19 also catalyzes the hydroxylation of omeprazole sulfone.

Figure 2 shows the mass spectra of omeprazole and its metabolites under positive ion conditions, respectively, at drift voltage of 30 V. There was no interference from extracted components of the incubation system. The well resolved chromatograms were obtained with acetonitrile-ammonium acetate as the eluent at a flow rate of 0.2 mL/min. The mass spectrum of omeprazole is almost the same as that obtained by direct analysis. The protonated molecular ions [M+H]+ of the mass spectra of omeprazole and its metabolites were clearly observed at m/z 361, 345 and 362, respectively, as base peaks. The fragment ions of 5-hydroxyomeprazole and omeprazole sulfone were observed at m/z 214 and 198, respectively.

The linear relationship calculated between the peak-area and the concentration (µg/mL) of omeprazole up to 20 µg/mL. The lower limit of quantification of omeprazole was 0.5 ng at a signal-to-noise ratio of 3. The present method is sufficiently sensitive and accurate to measure pharmacokinetic parameters.

The allele-specific polymerase chain reaction (PCR) based method allows genetic determination, thus predicting their phenotype. Therefore, we examined the pharmacokinetic profile of omeprazole as a probe drug in relation to genotyping for two known mutations, CYP2C19*2 and CYP2C19*3.

Omeprazole even has some advantages as a probe drug for CYP2C19 because of its more favorable safety profile, higher intrapatient reproducibility of phenotyping, and potential to differentiate between rapid and ultrarapid phenotypes.

In this study, three different human samples who were genotyped for CYP2C19 gene were used for analysis. The significant differences in the metabolism rate of omeprazole among subjects with different genotype are clearly observed. The present method is sufficiently sensitive and accurate for a study of phenotyping of CYP2C19.

References