Potential of Lansoprazole as a Novel Probe for Cytochrome P450 3A Activity by Measuring Lansoprazole Sulfone in Human Liver Microsomes

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Cytochrome P450 (CYP) 3A enzymes are responsible for the metabolism of many drugs. It is useful to know CYP3A activity in individual patients undergoing drug therapy so as to predict the efficacies or adverse events. Lansoprazole is metabolized to Lansoprazole sulfone (LS) by CYP3A, while to 5-hydroxylansoprazole by CYP2C19. The aim of this study was to evaluate whether lansoprazole can be used to assess CYP 3A activity in human liver. Lansoprazole sulfoxidation activity in 14 human liver microsomes was determined as the ratio of lansoprazole/LS, measuring these parameters by high-performance liquid chromatography. Testosterone 6β-hydroxylation (T6β-OH) activity, a known marker for CYP3A activity was also measured together with lansoprazole sulfoxidation activity. Lansoprazole sulfoxidation activity was also analyzed in microsomes preincubated with anti-CYP2C19 antibody. Interindividual variation was observed in lansoprazole sulfoxidation activity and T6β-OH activities of those microsomes, respectively. Lansoprazole sulfoxidation activity was significantly correlated with T6β-OH activity and CYP3A protein level. Lansoprazole sulfoxidation activity in microsomes with anti-CYP2C19 antibody was closely correlated with T6β-OH activity. In contrast, lansoprazole 5-hydroxylation activity was correlated with the CYP2C19 activity. These results suggest that metabolism of lansoprazole to LS by CYP3A occurs independently of metabolism by CYP2C19. LS can be used as a new marker of CYP3A activity.

Key words cytochrome P450 3A; lansoprazole; lansoprazole sulfone; liver microsome

Cytochrome P450 (CYP) 3A is the primary CYP iso-enzyme subfamily responsible for phase I metabolism of >50% of drug doses administered to humans.1,2) CYP3A4 is the most prominent CYP in humans, comprising about 30% and 70% of total CYP in the liver and intestine, respectively. CYP3A has very broad substrate specificity, encompassing a wide variety of therapeutic agents.3) Many cancer chemotherapeutic agents such as vinca alkaloids, paclitaxel, irinotecan, hydrochloride and other widely used drugs such as amiodarone, diltiazem, lovastatin, erythromycin, and midazolam are substrates for CYP3A.4–7) Recently, individual variations in CYP3A activity in the human liver have been reported.5) Because the pharmacokinetics of various drugs is influenced by individual variations in CYP3A activity, it is important to evaluate those variations in the clinical setting to achieve the optimum therapeutic outcomes.9

Probe drugs including midazolam, erythromycin, nefedipine, lidocaine, and dextromethorphan and endogenous compounds such as cortisol and testosterone have been utilized to evaluate CYP3A activity.10,11) Watkins12) proposed three specific CYP3A probes: erythromycin; midazolam; and cortisol. However, the probe drugs available for accurate, safe, and easy measurement of CYP3A activity remain limited.

Lansoprazole, (+)-2-[[3-methyl-4-(2,2,2-trifluoro-ethoxy)-2-pyridyl][methyl]sulfinyl]benzimidazole, is a proton-pump inhibitor (PPI) that suppresses gastric acid secretion by inhibiting (H+K+)-ATPase and is prescribed commonly for the treatment of gastric ulcer, reflux esophagitis, etc., with few serious side effects reported. Following administration, lansoprazole is extensively metabolized to 5-hydroxylansoprazole and lansoprazole sulfone (LS) by CYP2C19 and CYP3A, respectively.13) Masa et al.14) reported that the metabolism to LS by CYP3A occurs independently of that to 5-hydroxylansoprazole by CYP2C19 in the human liver. Inhibition of one metabolic pathway caused by the genetic polymorphisms of CYPs leads to upregulation of another pathway in vivo. To use lansoprazole as probe drug, these problems should be elucidated in vitro or in vivo. The present study investigated the utility of lansoprazole in assessing CYP3A activity in comparison with previously reported in vitro methods.

MATERIALS AND METHODS

Chemicals Lansoprazole, LS, and 5-hydroxylansoprazole were obtained from Takeda Pharmaceutical Co. (Osaka, Japan). Testosterone was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Diazepam was purchased from Wako Pure Chemical Industries (Osaka, Japan). Nicotinamide adenine dinucleotide phosphate (NADP+), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were from Oriental Yeast Co. (Tokyo, Japan).

Human Liver Microsomes Fourteen microsomes from human liver were obtained from unmatched organs of Caucasian and Hispanic transplantation donors. These microsomes were supplied by the National Disease Research Interchange (Philadelphia, PA, U.S.A.) through the Biochemical Research Institute, Human and Animal Bridging Research Organization (Chiba, Japan). This study was approved by the Ethics Committee of St. Marianna University School of

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Medicine (Kawasaki, Japan).

Measurement of Lansoprazole Sulfoxidation Activity in Human Liver Microsomes  Lansoprazole sulfoxidation activity was analyzed in liver microsomes following a previously reported method.15,16) Fourteen liver microsomes (0.4 mg) were mixed with lansoprazole 5 μM, ethylenediaminetetraacetic acid (EDTA) 0.5 mM, Na-K phosphate 0.33 mM (pH 7.4), and distilled water. The reaction was initiated by addition of an NADPH-generating system (MgCl₂ 5 mM, NADP⁺ 0.5 mM, glucose-6-phosphate 5 mM, and 1 unit of glucose-6-phosphate dehydrogenase). The total volume of 1 ml was incubated at 37 °C and terminated after 15 min by iced. Then, the mixture was centrifuged at 10000×g for 10 min. Diazepam 3.5 μM was added to a 0.9-ml aliquot of the supernatant layer as internal standard and applied to an Oasis HLB extraction cartridge (Waters Co., Milford, MA, U.S.A.) that had previously been activated with 1.0 ml of 100% methanol followed by 1.0 ml of distilled water. The cartridge was then eluted with 1.0 ml of 40% methanol in distilled water. The final extract was eluted from the cartridge with 1.0 ml of 80% methanol in distilled water. The residue was dissolved in 300 μl of mobile phase C with 1 ml of mobile phase D: consisted of acetonitrile and distilled water (1 : 1, v/v). Mobile phase C consisted of acetonitrile and distilled water 0.33 mM (pH 7.4), and distilled water. The reaction was initiated by addition of the fully automated HPLC system. Mobile phase C consisting of acetonitrile and distilled water (1 : 1, v/v). Mobile phase D consisted of acetonitrile and distilled water (3 : 7, v/v).

The HPLC system consisted of a CCPM-II pump (Tosoh Co.), an AS-8020 autosampler (Tosoh Co.), and a MCDP-3600 UV detector (Otsuka Electronics Co., Osaka, Japan). Separation was achieved on a CAPCELL PACK18 column (4.6 mm I.D.×250 mm, Shiseido Co.). The flow rate was 1.0 ml/min over the 35-min run time at 30 °C. The following gradient profile was used, with the percentages indicating the amount of mobile phase C in mobile phase D: t = 0 min, 0% C; t = 10 min, 38% C; t = 17.5 min, 0% C; t = 35 min, 0% C. The eluent was monitored at 254 nm by a MCDP-3600 UV Detector (Otsuka Electronics Co., Osaka, Japan).

Assay for CYP2C19 Activity  CYP2C19 activity was determined as previously described.18,19) Briefly, CYP 2C19 enzyme activity was determined using 100 μM S-mephentoin. The substrate mixture containing 1 mg/ml human liver microsome was incubated at 37°C for 60 min. The reaction was then terminated and subjected to HPLC Gulliver 1500 analysis (Nihonbunko, Tokyo, Japan) with a reversed-phase analytical column.

Immunoblot Analysis of CYP3A  SDS-polyacrylamide gel electrophoresis and Western blot analysis were carried out on human liver microsomes, and the immunoblots were probed with antibody to CYP3A4 (BD Gentist TM, Discovery Labware, Inc., BD Biosciences, MA, U.S.A.).

Statistical Analysis  Statistical analysis was performed using StatView (ver. 4.58, Abacus, Berkeley, CA, U.S.A.). Parts of data are expressed as mean ± S.E.M. The unpaired, Mann–Whitney U-test was used to determine differences between groups for comparisons of human microsomes preincubated with and without anti-CYP2C19 antibody. Correlations between groups were evaluated using regression analysis. Probability values <0.05 were considered to represent a statistically significant difference.

RESULTS

Lansoprazole Sulfoxidation and T6β-OH Activities in Human Liver Microsomes  Lansoprazole sulfoxidation and T6β-OH activities were assessed in 14 human liver microsomes by HPLC. The difference between the highest and lowest lansoprazole sulfoxidation activity ranged from 2.06 to 48.55 nmol/min/mg protein (Fig. 1A). The range of variation in lansoprazole sulfoxidation activity was approximately 23-fold. On the other hand, the difference between the highest and lowest T6β-OH activity ranged from 0.28 to 5.66 nmol/min/mg proteins (Fig. 1B). The range of variation in T6β-OH activity was likewise approximately 20-fold. Our data indicate that the pattern of individual variation in LSx activity was similar to that of T6β-OH activity. Lansoprazole sulfoxidation activity was significantly correlated with T6β-OH activity (r = 0.949, p < 0.0001) (Fig. 1C).

Correlation between LSx Activities and CYP3A Protein Levels in Human Liver Microsomes  We previously reported that CYP3A activity measured with T6β-OH is well correlated with CYP3A protein level.20) In this study, we evaluated the correlation between lansoprazole sulfoxidation activity and CYP3A protein level. Lansoprazole sulfoxidation activity was significantly correlated with CYP3A protein level (r = 0.827, p < 0.001) (Fig. 2A). In addition, T6β-OH activity was reconfirmed to correlate with CYP3A protein
level \( (r=0.804, p<0.001) \) (Fig. 2B).

Correlation between Lansoprazole Sulfoxidation and Lansoprazole 5-Hydroxylation Activities in Human Liver Microsomes

Lansoprazole is metabolized to 5-hydroxylansoprazole by CYP2C19, while to LS by CYP3A4.\(^{13}\) There was no correlation between lansoprazole sulfoxidation activity and lansoprazole 5-hydroxylation activity \( (r=0.088) \) (Fig. 3A). Lansoprazole sulfoxidation activity was not correlated with CYP2C19 activity \( (r=0.280) \) (Fig. 3B). On the other hand, lansoprazole 5-hydroxylation activity was significantly correlated with CYP2C19 activity \( (r=0.706, p<0.05) \) (Fig. 3C). This result suggests that the metabolism of lansoprazole to LS by CYP3A occurs independently of metabolism to 5-hydroxylansoprazole by CYP2C19.

DISCUSSION

In this study, we investigated whether lansoprazole as a new probe drug could be used to assess CYP3A activity. The probe-drug activity of compounds such as erythromycin, midazolam, endogenous cortisol, and testosterone has been already established.\(^{11}\) Although those compounds have high specificity for measurement of CYP3A activity, difficulties in finding an ideal probe remain due to differences in enzyme activity in organs such as the liver, intestines, and kidney; the overlap of substrate specificity with P-glycoprotein and other CYP enzymes; high interindividual proband variability; and
such as lethargy and inhibition of respiratory function. 22) Venously and has central nervous system-inhibitory actions, a single dose of 0.025 mg/kg of midazolam is less than a clinical CYP2C19 antibody. Lansoprazole 5-hydroxylation activity in microsomes with anti-CYP2C19 antibody decreased compared with that without anti-CYP2C19 antibody ($p=0.034$). Data are representative of 14 independent experiments. $p<0.05$ vs. lansoprazole 5-hydroxylation activity in microsomes without anti-CYP2C19 antibody. NS, not significant.

(C) Correlation between Lansoprazole Sulfoxidation Activities in 14 Human Liver Microsomes Preincubated with Anti-CYP2C19 Antibody and T6b-OH Activities in the Microsomes

Lansoprazole sulfoxidation activities in microsomes preincubated with anti-CYP2C19 antibody were significantly correlated with T6b-OH activity ($r=0.919$, $p=0.0000038$).

(D) Correlation between Lansoprazole Sulfoxidation Activities in 14 Microsomes Preincubated with and without Anti-CYP2C19 Antibody

Significant correlation was observed ($r=0.947$, $p=0.0000031$).

Fig. 4. Average of Lansoprazole Sulfoxidation Activities (A) and Lansoprazole 5-Hydroxylation Activities (B) in Human Liver Microsomes Preincubated with and without Anti-CYP2C19 Antibody

Data are presented as mean±S.E.M. from 14 independent experiments. There was no significant difference between lansoprazole sulfoxidation activity with and without anti-CYP2C19 antibody. Lansoprazole 5-hydroxylation activity in microsomes with anti-CYP2C19 antibody decreased compared with that without anti-CYP2C19 antibody ($p=0.034$). Data are representative of 14 independent experiments. $p<0.05$ vs. lansoprazole 5-hydroxylation activity in microsomes without anti-CYP2C19 antibody. NS, not significant.

Possible low affinity of the enzyme for probe drugs. In addition, these probes require invasive methods to administer the drug and collect the samples. The erythromycin breath test has been widely used. 21) However, this method needs intravenous administration of a radioactive isotope. 11) Although a single dose of 0.025 mg/kg of midazolam is less than a clinical dose (0.15 mg/kg), this drug must be administered intravenously and has central nervous system-inhibitory actions, such as lethargy and inhibition of respiratory function. 22) Therefore intravenous administration of midazolam is not sufficient for measuring CYP3A activity in volunteer subjects. Among proposed non-invasive probes, the urinary secretion of cortisol follows a circadian rhythm and testosterone has sex differences. The ideal probes for pharmacokinetic studies should satisfy both specificity for CYP3A activity and high safety probe for patients. The pharmacokinetics and pharmacodynamics of PPIs including omeprazole and lansoprazole were investigated to establish individualized pharmacotherapy. 23) Recently, Böttiger 24) proposed that omeprazole, another PPI, can be used to assess CYP3A activity. Although omeprazole is metabolized by CYP2C19 and CYP3A4, the metabolism of omeprazole to hydroxylation metabolites is 4-fold greater than omeprazole sulfoxidation via the CYP3A pathway. 25) We focused on the metabolic pathway of lansoprazole, which is metabolized almost equally to LS and 5-hydroxylansoprazole by CYP3A and CYP2C19, respectively, which means the contribution of CYP3A4 on lansoprazole metabolism by CYP3A is greater than that of omeprazole. 26) Moreover, it will be important that this metabolic pathway of lansoprazole can be said simple among many PPIs. There are few reports of serious adverse events associated with lansoprazole in various patient populations. Because lansoprazole is both specific and safe, it was adapted as an indicator of CYP3A activity in our study.

To use lansoprazole as a CYP3A probe, the following two points should be clarified. First, does lansoprazole accurately reflect CYP3A activity? Second, is lansoprazole sulfoxidation activity influenced by CYP2C19 activity? We attempted to address these two points. The metabolic pathway of lansoprazole to LS via CYP3A was elucidated. 13) To the best of our knowledge, no experimental lansoprazole sulfoxidation activity data are available comparing the results obtained with typical CYP3A probes. Individual variations of lansoprazole sulfoxidation activity were observed in human liver microsomes. The range of variation in lansoprazole sulfoxidation activity was approximately 20-fold. T6b-OH activity is a typical indicator of CYP3A activity in human liver microsomes. 27) Lansoprazole sulfoxidation activities were compared with T6b-OH activities in 14 human liver microsomes. The pattern of individual variations in T6b-OH activity was similar to that of lansoprazole sulfoxidation activity. Lansoprazole sulfoxidation activity was significantly positively correlated with T6b-OH activity. In addition, lansoprazole sulfoxidation activity was correlated with CYP3A protein level. Our results suggest that lansoprazole sulfoxidation activity accurately reflects CYP3A activity in the human liver.

Furthermore, we investigated whether lansoprazole sulfoxidation activity in human liver microsomes is affected by lansoprazole 5-hydroxylation activity through the CYP2C19 metabolic pathway. Lansoprazole sulfoxidation activity was not correlated with lansoprazole 5-hydroxylation activity and CYP2C19 activity. On the other hand, lansoprazole 5-hydroxylation activity was significantly correlated with CYP2C19 activity.

When human liver microsomes were preincubated with anti-CYP2C19 antibody at a dose of 40 μg so as to block CYP2C19 activity, lansoprazole sulfoxidation was closely correlated with T6b-OH activity in liver microsomes after anti-CYP2C19 antibody treatment. Consequently, we found that there was a significant correlation between lansoprazole sulfoxidation activities with or without anti-CYP2C19 antibody. LS can thus be used as a marker for CYP3A activity, and is not involved in CYP2C19 activity in human liver microsomes in vitro. Measurement of lansoprazole and its metabolites with the method we used is very sensitive and useful both in human liver microsomes and blood. Use of lansoprazole as probe drug of human hepatic CYP3A activity must be readily available in vivo. In our previous study, we demonstrated that plasma concentration of lansoprazole and
its metabolites was measurable oral administration of lansoprazole at a dose of 30 mg in healthy subjects, reflecting human hepatic CYP3A activity.\textsuperscript{23} Furthermore, the plasma concentration of LS was confirmed to correlate with 6β-hydroxycortisol, a known marker of CYP 3A activity in healthy subjects.\textsuperscript{24}

Pauli-Magnus et al.\textsuperscript{25} reported that lansoprazole is a substrate and inhibitor of P-glycoprotein. The pharmacokinetics of lansoprazole may be affected by P-glycoprotein activity or MDR-1 genotypes. We will further elucidate the influence of lansoprazole on CYP 3A metabolism, although lansoprazole is a good indicator for CYP3A activity in studies both in vivo and in vitro. Furthermore, lansoprazole can simultaneously assess CYP3A and CYP2C19.

In conclusion, lansoprazole as a probe drug for CYP3A activity was assessed in vitro. Pending more extensive evaluation, LS has the potential to be utilized as a marker for CYP3A activity, allowing accurate, easy, and safe measurement.

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