Determination of R(+)- and S(−)-Lansoprazole Using Chiral Stationary-Phase Liquid Chromatography and Their Enantioselective Pharmacokinetics in Humans

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Purpose. Stereoselective and sensitive methods employing chiral stationary phase columns for HPLC determination of enantiomers of lansoprazole in the human serum were developed and pharmacokinetic behaviors of the enantiomers were evaluated in seven subjects.

Methods. Five chiral stationary phase columns: Chiralcel OD (cellulose tris(3,5-dimethyl-phenylcarbamate)), OF (cellulose tris(4-chlorophenylcarbamate)), OG (cellulose tris(4-methylphenylcarbamate)) and OJ (cellulose tris(4-methylbenzoate)), and Chiralpak AS (amylose tris ((S)-1-phenylethylcarbamate)) were investigated.

Results. Chiralcel OD and Chiralpak AS columns gave a good resolution of R(+)- and S(−)-enantiomers from racemic lansoprazole, but Chiralcel OF, OG, and OJ did not. The mean C_{max} and the AUC values of R(+)-enantiomer were 3–5 times greater than those of S(−)-enantiomer following oral administration of 30 mg of racemic lansoprazole. The CL_{tot} values of R(+)-enantiomer were significantly smaller than those of S(−)-enantiomer. Binding of R(+)-enantiomer to human serum proteins was significantly greater than that of S(−)-enantiomer. The mean metabolic ratio (metabolites/parent compound) in human liver microsomes of S(−)-enantiomer was significantly greater than that of R(+)-enantiomer.

Conclusions. The stereoselective pharmacokinetics of lansoprazole enantiomers is likely due to its stereoselective protein binding and/or metabolism.

KEY WORDS: lansoprazole; chiral separation; enantiomer; metabolic ratio.

INTRODUCTION

Since macromolecules in the human body have an ability to distinguish enantiomers, the physiological activities frequently differ among enantiomers (1). There are many reports on pharmacokinetic and/or pharmacodynamic differences between enantiomers (2–4) and such differences have been regarded as important in the development of new drugs from the clinical pharmacological point of view. Laboratory evaluations such as pharmacological effect, toxicity and pharmacokinetics for individual enantiomers are necessary to judge whether to develop a drug candidate as an enantiomer or not. For such evaluation, the establishment of a reliable analytical method for determination of enantiomers is in great demand.

Lansoprazole is a benzimidazole derivative which powerfully and continuously inhibits the gastric proton pump (H^+/K^+-ATPase) activity acting in the final step on the gastric acid secretion in the parietal cells. It possesses an asymmetric sulfur in the chemical structure and is clinically administered as a racemic mixture of R(+)- and S(−)-enantiomers. On the stereoselective pharmacokinetics, there is only one paper reporting that the plasma concentrations of the R(+)-enantiomer after oral administration of racemic lansoprazole to rats and dogs were higher than those of the S(−)-enantiomer (5). However, to our knowledge, there is no report on the pharmacokinetics of lansoprazole enantiomers in humans.

The purpose of the present study is to establish a stereoselective and sensitive HPLC method for assaying lansoprazole in the human serum using a chiral column, and to assess the pharmacokinetics of the enantiomers in humans.

MATERIALS AND METHODS

Materials

Lansoprazole was generously supplied from Takeda Chemical Industries, Osaka. Takepron^R capsules containing enteric-coated granules of lansoprazole were purchased from a local wholesaler. All other chemicals used in this study were of analytical grade.

Subjects and Study Design

Human studies were conducted according to the Declaration of Helsinki. Six healthy Japanese volunteers (4 males and 2 females) participated in this study after giving informed consent based on explanation of an objective of the study and possible risks. They had not suffered from any recent illness and were not taking any other medication. Their clinical and biochemical characteristics are given in Table I.

A capsule containing 30 mg racemic lansoplazole was taken around 8 a.m. with 100 ml of water after an overnight fast. They refrained from drinking alcohol and any extreme physical activity for a day and any food for 12 h before the experiments and were served a lunch 4 h after the drug ingestion. Blood samples were collected periodically *via* a cannula in an antecubital vein.

Isolation of Racemic Lansoprazole

Racemic lansoprazole in the serum was isolated using a reversed phase high performance liquid chromatography (HPLC; Apparatus: Shimadzu LC-6A, Kyoto, Japan) as reported by Aoki *et al.* (6). To a 10-ml centrifuge tube were placed 1 ml of serum or incubation sample and 3 ml of a diethyl ether-dichloromethane (7:3, v/v) mixture. The tube was shaken for 30 sec and centrifuged at 1000 × g for 10 min at 4°C. The extraction procedure was repeated twice with the same amount of the diethyl ether-dichloromethane mixture. After the organic

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Table I. Clinical and Biochemical Characteristics of Healthy Volunteers

Subject	Sex (m/f)	Age (y)	Weight (kg)	AST (IU/l)	ALT (IU/l)	LDH (IU/l)	BUN (mg/dl)	CLcr (ml/min)
H.K.	m	25	65	21	15	491	19.0	120
T.U.	m	24	53	11	8	254	10.3	142
Y.K.	m	24	60	15	11	276	7.8	138
M.M.	m	24	52	19	13	306	15.2	105
A.M.	f	25	46	15	7	257	11.5	104
Y.T.	f	30	48	17	10	284	11.9	125
Mean		25.3	54	16.3	10.7	311	12.6	124
±SE		0.95	3.0	1.4	1.2	37	1.6	6.6

Abbreviations: AST = aspartate aminotransferase, ALT = alanine aminotransferase, LDH = lactate dehydrogenase, BUN = blood urea nitrogen, CLcr = creatinine clearance.

phase was evaporated under a reduced pressure, the residue was immediately reconstituted in 200 μl of the mobile phase and 100 μl of the aliquot was injected into the HPLC column for isolation of racemic lansoprazole.

The isolation was performed with a reversed phase column (LiChrospher 100 RP-18(e), 5 μm, 4.0 mm I.D. × 250 mm L.). The mobile phase consisted of acetonitrile and water (35:65) containing 0.1% of n-octylamine. At a flow rate of 1.0 ml/min, the eluate was monitored for absorbance at 285 nm and a portion eluated over 2 min before and after the peak of racemic lansoprazole was collected. The collected eluate was subjected to the same extraction and concentration procedure as described for the serum. Then, the residue was reconstituted in 200 μl of a n-hexane:ethanol (8:2, v/v) mixture and 100 μl of the aliquot was injected into the Chiral HPLC column for separation of the enantiomers.

Chiral Separation

Determination of lansoprazole enantiomers in the serum was performed by normal-phase HPLC systems consisted of a model L-6000 pump (Hitachi, Tokyo, Japan), a model L-4000 UV detector (Hitachi), a model C-R6A Chromatopak integrator (Shimadzu, Kyoto, Japan). Chiral separation was attempted with several chiral stationary phase columns: Chiralcel OD (based on cellulose tris(3,5-dimethylphenylcarbamate)), OF (cellulose tris(4-chlorophenylcarbamate)), OG (cellulose tris(4-methylphenylcarbamate)), OJ (cellulose tris(4-methylbenzoate)), and Chiralpak AS (amylose tris((S)-1-phenylethylcarbamate)); 4.6 mm I.D. × 250 mm L.; Daicel Chemical Industries, Tokyo, Japan. The HPLC mobile phase composed of n-hexane:ethanol (8:2, v/v) was used at a flow rate of 1.0 ml/min. The analytical column was maintained at a temperature of 38°C and the eluate was monitored for absorbance at 285 nm. The resolution factor (R) was calculated from a HPLC chromatogram by the following equation:

$$R = 2(t_{R(-)} - t_{R(+)})/(W_{(+)} + W_{(-)})$$

where $t_{R(-)}$ and $t_{R(+)}$ are the retention times of S(−)- and R(+)-enantiomers and $W_{(+)}$ and $W_{(-)}$ are the peak width of R(+)- and S(−)-enantiomers, respectively.

Determination of Enantiomers

Calibration samples were prepared by adding known amounts of racemic lansoprazole to blank sera. Each analytical run included triplicate calibration standards at five concentrations over the range 0.025–1.5 μg/ml. The peak of R(+)- and S(−)-enantiomers were identified based on the report of Miwa *et al.* (5) after collecting each fraction. The determination of each enantiomer was performed by the absolute calibration method.

Examination of Protein Binding

Protein binding experiments were performed in triplicate with an ultrafiltration technique using Centrifree MPS-3 (Amicon Corp., Danvers, MA). Drug-free serum was obtained from one of volunteers. Briefly, serum aliquots (1 ml) containing added racemic lansoprazole (6.3 μg/ml) were incubated for 30 min at 37°C. After incubation, the aliquots were ultrafiltered under a condition of 1000 × g for 20 min at 4°C. The fraction of drug unbound was determined by the following equation: $f_u = C_u/C_t$ where f_u is the fraction of a drug unbound in serum and C_u and C_t are the unbound and total concentrations of the drug in the serum, respectively.

Preparation of Human Liver Microsomes

A human liver sample was obtained from a patient who underwent a partial hepatectomy for the removal of metastatic tumor at the department of surgery. The liver was subsequently homogenized with 3 volumes of 50 mM phosphate buffer, pH 7.4 containing 0.1 mM EDTA. Microsomes were prepared by differential centrifugation as described (7). After protein concentration was determined by the method of Lowry *et al.* (8), a microsomal suspension prepared at a concentration of 1–2 mg/ml, was kept at −80°C until used.

Determination of Metabolic Ratio

Metabolic ratio is defined as a ratio of the residual amount of each enantiomer to the amount of each enantiomer of lansoprazole added, which was metabolized by microsomal enzymes. The reaction medium contained 100 μl of 0.1 mg/ml of microsomes, 200 μl of 0.3 mM potassium-phosphate buffer, pH 7.4, 100 μl of 0.6 mM EDTA and 5.6 μM of lansoprazole. The mixture was preincubated at 37°C for 5 min and subsequently at 37°C for 30 min after adding 100 μl of NADPH-generating system (3 mM $NADP^+$, 12 mM glucose 6-phosphate, 6 IU/ml of glucose-6-phosphate dehydrogenase, 24 mM $MgCl_2$). The reaction was stopped by adding 3 ml of the diethyl ether-dichloromethane (7:3, v/v) mixture.

Table II. Pharmacokinetic Parameters of R(+)-and S(−)-Lansoprazole in Healthy Volunteers

Enantiomer	$AUC_{0-\infty}$ (μg · h/ml)	CL_{tot}/F (ml/min/kg)	t_{max} (h)	C_{max} (μg/ml)	k_a (h^{-1})	k_{el} (h^{-1})	V_d/F (l)
R(+)-lansoprazole	7.14 ± 2.00**	0.96 ± 0.28**	2.92 ± 0.49	1.04 ± 0.15**	1.30 ± 0.35	0.40 ± 0.16	11.0 ± 2.67
S(−)-lansoprazole	1.56 ± 0.30**	4.04 ± 1.12**	1.91 ± 0.24	0.36 ± 0.09**	1.90 ± 0.50	0.37 ± 0.07	53.5 ± 23.1

Note: Each value represents mean ± SEM of 6 subjects.
**P < 0.01.

Pharmacokinetic Analysis

Serum concentration-time curves were subjected to the least-squares regression analysis (program AUTOAN for a one-compartment model with absorption lag time). The maximum serum concentration (C_{max}) and the time required to reach C_{max} (t_{max}) were obtained graphically. The plasma concentrations of the elimination phase were used to calculate the elimination rate constant (k_{el}) by an exponential regression analysis. The areas under the concentration-time curves ($AUC_{0-\infty}$) were calculated by a trapezoidal rule and by extrapolating time to infinity with use of k_{el} values. The apparent total body clearance (CL/F) was calculated by $CL/F = dose/AUC_{0-\infty}$.

Statistical Analysis

Results are expressed as mean ± SEM. Pharmacokinetic data were analyzed for statistical difference using the Student's *t*-test. Statistical significance is assumed when $P < 0.05$.

RESULTS AND DISCUSSION

Optical Resolution of Lansoprazole in Serum Samples

Of five chiral stationary phases evaluated for optical resolution of lansoprazole, Chiralcel OD and Chiralpak AS columns gave good resolution of R(+)- and S(−)-enantiomers from

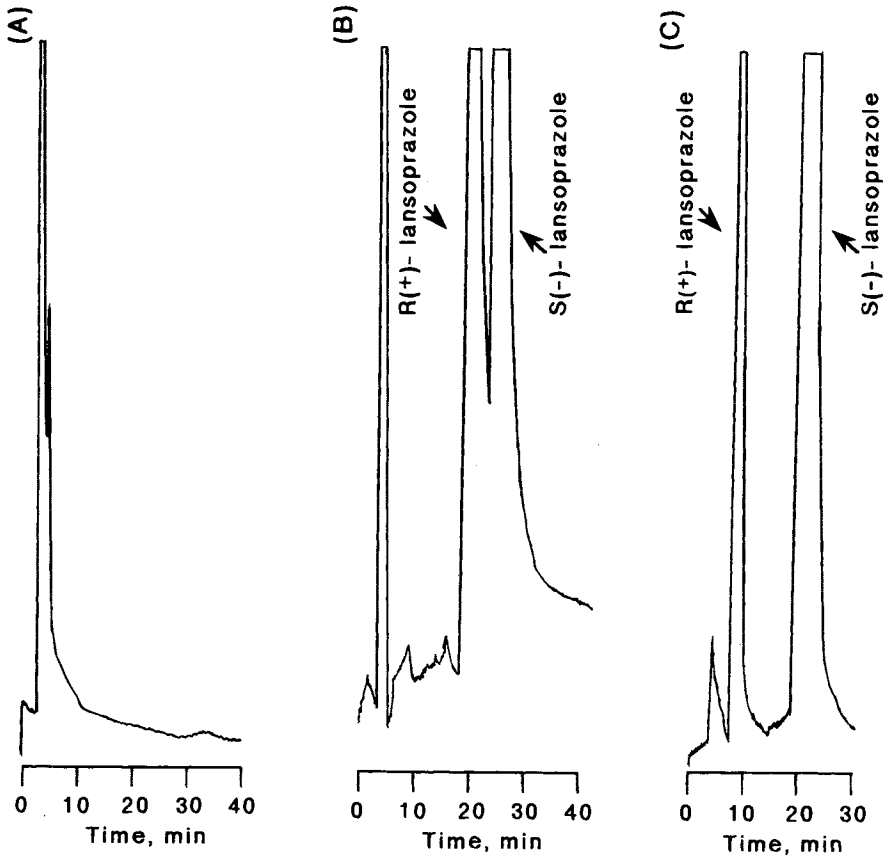

Fig. 1. HPLC chromatogram of a drug-free serum on Chiralpak AS (A) and that for lansoprazole enantiomers when Chiralcel OD (B) or Chiralpak AS (C) was used after isolation of the racemic drug in human sera on LiChrospher 100 RP-18 column. The amount of racemic lansoprazole injected into HPLC column was 0.5 μg.

racemic lansoprazole, but the others did not under the condition of the mobile phase (n-hexane:ethanol = 8:2, v/v, flow rate of 1.0 ml/min and column temperature of 38°C).

Among the two, we selected the Chiralpak AS column since it exhibited a shorter retention time and a better resolution than Chiralcel OD column. The optimum resolutions of both columns achieved with the mobile phase were R = 2.07 for Chiralpak AS and R = 0.442 for Chiralcel OD. Typical chromatograms illustrating the optimum resolution of lansoprazole enantiomers achieved on the Chiralcel OD and the Chiralpak AS column are shown in Figure 1. The retention times of the R(+)- and S(−)-enantiomers of lansoprazole on Chiralpak AS column were approximately 8.8 and 20.9 min, respectively. There was no interfering peaks in the serum blank at the retention times of the enantiomers as demonstrated with the serum obtained before oral doses of racemic lansoprazole in the healthy volunteers. The peak area of each enantiomer showed excellent linear relationships with their corresponding concentrations in the samples in the range of 0.025 to 1.5 μg/ml ($r \geq 0.999$) and the coefficients of variation (CV) in between-day assays were 0.56–8.8 and 0.67–7.4% for the R(+)- and S(−)-enantiomers (n = 6), respectively.

Pharmacokinetics of Enantiomers

Pharmacokinetic behaviors of both enantiomers of lansoprazole in healthy volunteers following the oral administration of 30 mg of the racemic drug as enteric-coated beads in capsule are shown in Figure 2. The mean serum levels of the R(+)-enantiomer were higher at all time points than those of the S(−)-enantiomer. The pharmacokinetic parameters are summarized in Table II. The C_{max} and the AUC values in the R(+)-enantiomer were significantly greater than those in the S(−)-enantiomer ($P < 0.01$). The mean AUC value of the R(+)-enantiomer was approximately 5 times greater than that of the S(−)-enantiomer. In addition, the CL_{tot}/F values of the R(+)-enantiomer were significantly smaller than those of the S(−)-enantiomer ($P < 0.01$). There was no significant difference in the t_{max} between the enantiomers.

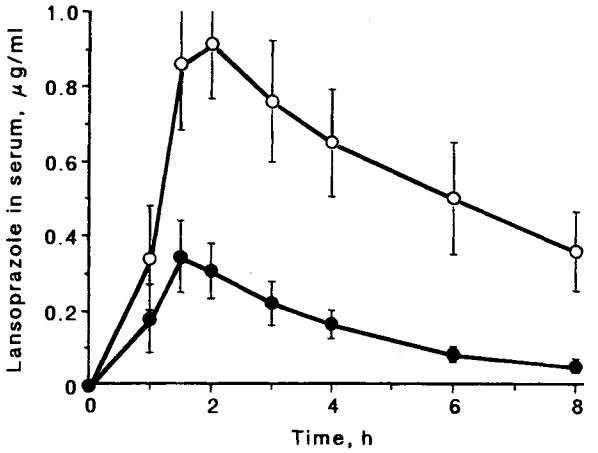

Fig. 2. Serum concentration-time profiles of R(+)-lansoprazole (○) and S(−)-lansoprazole (●) after oral administration of 30 mg racemic lansoprazole to healthy volunteers. Each point represents the mean ± SE of six subjects.

Table III. Stereoselective Protein Binding and Metabolism of R(+)- and S(−)-Lansoprazole in Vitro

Enantiomer	Unbound fraction %	Metabolic ratio %
R(+)-lansoprazole	2.9 ± 0.2*	8.4 ± 1.7
S(−)-lansoprazole	5.8 ± 0.9*	13.4 ± 3.4

Note: Each value represents the mean ± SE of 4 (protein binding study) or 3 (metabolic study) experiments.
*$P < 0.05$.

It is known that enantiomers can bind differently to human plasma proteins (9). The results of studies on the extent of enantioselective binding of lansoprazole to human serum proteins estimated by an ultrafiltration technique are presented in Table III. The binding to human serum proteins was significantly greater for R(+)-enantiomer than for S(−)-enantiomer ($P < 0.05$). Distribution of a drug to compartments other than serum is limited by the drug binding to plasma proteins such as albumin and α_1-acid glycoprotein. The extent of binding has been reported to be 96% to albumin and 2% to α_1-acid glycoprotein (10). Consequently, the R(+)-enantiomer which is extensively bound to albumin may be poorly distributed and slowly eliminated, resulting in the higher serum concentrations than those of the S(−)-enantiomer. Thus, the enantioselective protein binding may have an influence on the enantioselective behavior of lansoprazole in the body following oral administration.

There is a possibility of stereoselective differences in the liver metabolism of lansoprazole enantiomers, as reported for many drugs which are extensively metabolized (11) since lansoprazole is metabolized extensively by the liver and its primary metabolites in serum are hydroxylansoprazole and lansoprazole sulfone with no recovery of the unchanged drug in the urine (12). In our study on the enantioselective metabolism of lansoprazole investigated at a concentration of 5.6 μM in human liver microsomes, the mean metabolic ratio of the S(−)-enantiomer (13.4%) tended to be greater than that of the R(+)-enantiomer (8.4%, Table III). Miwa *et al.* (5) reported that the plasma levels of R(+)-enantiomer were higher than those of S(−)-enantiomer when lansoprazole was intravenously administered, where first pass effect has no relevance, to rats and dogs. They suggest that the differences in plasma levels of both lansoprazole enantiomers could be mainly due to difference in the metabolic rates of the enantiomers.

From the observations mentioned above, it was confirmed that there is stereoselectivity in protein binding and metabolic rates of lansoprazole. Consequently, these stereoselective differences seem to bring about the difference in the pharmacokinetics of lansoprazole enantiomers.

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