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Pharmacokinetic Differences Between Lansoprazole Enantiomers in Rats

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Abstract

Because limited information is available about potential differences between the pharmacokinetics and pharmacodynamics of the enantiomers of lansoprazole, the enantioselective pharmacokinetics of the compound have been investigated in rats.

There was a noticeable difference between the serum levels of the enantiomers of lansoprazole and of their metabolites, 5-hydroxylansoprazole enantiomers, after oral administration of the racemate (50 mg kg^{-1}) to rats. C_{max} (maximum serum concentration) and AUC (area under the serum concentration–time curve) for (+)-lansoprazole were 5–6 times greater than those for (−)-lansoprazole, whereas for (+)-5-hydroxylansoprazole both values were significantly smaller than those for the (−) enantiomer. CL_{tot}/F values (where CL_{tot} is total clearance and F is the fraction of the dose absorbed) for (+)-lansoprazole were significantly smaller than those for the (−) enantiomer. There was no significant difference between the absorption rate constants of the lansoprazole enantiomers in the in-situ absorption study. The in-vitro protein-binding study showed that binding of (+)-lansoprazole to rat serum proteins was significantly greater than for the (−) enantiomer. The in-vitro metabolic study showed that the mean metabolic ratio (45·9%) for (−)-lansoprazole was significantly greater than that (19·8%) for the (+) enantiomer in rat liver microsomes at 5·6 μM lansoprazole.

These results show that the enantioselective disposition of lansoprazole could be a consequence of the enantioselectivity of plasma–protein binding and the hepatic metabolism of the enantiomers.

Lansoprazole is a benzimidazole derivative which powerfully and continuously inhibits gastric proton-pump (H^+/K^+–ATPase) activity in the final step of gastric acid secretion in the parietal cells (Wallmark et al 1983). The drug is extensively metabolized in the liver and the major metabolites present in the plasma are 5-hydroxylansoprazole and lansoprazole sulphone. Formation of the 5-hydroxy metabolite is mediated by cytochrome P450 2C19 (CYP2C19), whereas the formation of the sulphone is mediated by CYP3A4 (Pichard et al 1995; Sohn et al 1997). Lansoprazole has an asymmetric sulphur in the chemical structure (Figure 1) and is administered clinically as a racemic mixture of the (+) and (−) enantiomers. Racemic lansoprazole is metabolized in the liver to (+)- and (−)-5-hydroxylansoprazole; the sulphone metabolite is achiral. Because limited information is available about potential differences between the pharmacokinetics and pharmaco-dynamics of the enantiomers of lansoprazole (Miwa et al 1990; Nagaya et al 1991; Katsuki et al 1996), it is important to evaluate the pharmacokinetics of the individual enantiomers because the pharmacological effects or toxicity, or both, of the enantiomers might be different. On the stereoselective pharmacokinetics of lansoprazole, there is only our report on the pharmacokinetics of the enantiomers in healthy subjects using a chiral stationary phase column, Chiralpak AS (amylose tris(*S*-1-phenylethylcarbamate)) for HPLC determination (Katsuki et al 1996).

This study extends our previous work on stereoselective differences between the pharmacokinetic behaviour, i.e. absorption, metabolism and protein binding, of the enantiomers of lansoprazole in rats.

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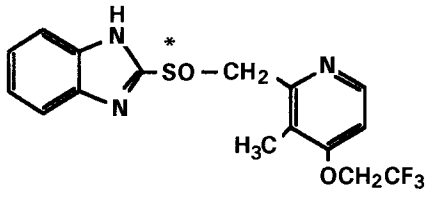
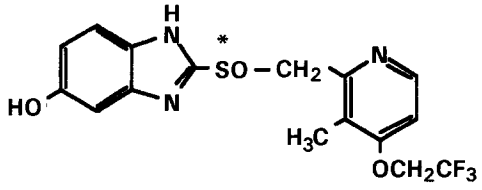
Materials and Methods

Materials

Racemic lansoprazole and its metabolite 5-hydroxylansoprazole were kindly supplied by Takeda



Lansoprazole



5-Hydroxylansoprazole

Figure 1. Chemical structures of lansoprazole and 5-hydroxylansoprazole. *Asymmetric centre.

Chemical Industries (Osaka, Japan). Other chemicals used in this study were of analytical grade.

In-vivo experiments

Male Wistar rats, 250–320 g, were fasted overnight with free access to water. Under light ether anaesthesia, lansoprazole (50 mg kg^{-1}) was administered orally as 1 mL of a suspension in 0·5% methylcellulose containing 0·2% $NaHCO_3$ adjusted to pH 9 with 0·1 N NaOH. Blood samples (300 μL) were collected periodically from a cut at the tip of the tail.

In-situ absorption study

The rats were anaesthetized by intraperitoneal injection of ethyl carbamate (urethane; 1·2 g kg^{-1}). The small intestine was exposed by midline abdominal incision and the upper duodenum and the ileocaecal junction were cannulated with polyethylene tubing. Intestinal absorption experiments were performed by a conventional in-situ recirculation method (Tomimaru et al 1996). Lactated Ringer's solution (pH 6·5, maintained at 37°C; 100 mL) containing lansoprazole (50 μg mL^{-1}) was perfused from the duodenum through the small intestine to the ileocaecal junction at a rate of 5 mL min^{-1}. Perfusates were collected 0 and 60 min after the start of the experiment. The absorption rate constant (k_a) was obtained by use of the equation:

$$k_a = \ln(C_0V_0/C_1V_1)/t \quad (1)$$

where t is the perfusion time, C_0 and C_1 are the concentrations of lansoprazole in the perfusates at $t=0$ and $t=60$ min, respectively, and V_0 and V_1 are the volume of the perfusates at $t=0$ and $t=60$ min, respectively.

Protein-binding study

Protein-binding experiments were performed in triplicate by means of an ultrafiltration technique using Centrifree MPS-3 (Amicon, Danvers, MA) as reported previously (Arimori & Nakano 1987). Briefly, serum samples (1 mL) containing added racemic lansoprazole (6·3 μg mL^{-1}) were incubated for 30 min at 37°C. After incubation, the samples were ultrafiltered at 1000 *g* for 20 min at 4°C. The fraction of unbound drug was determined by use of the equation:

$$f_u = C_u/C_t \quad (2)$$

where f_u is the fraction of unbound drug in the serum and C_u and C_t are, respectively, the concentration of unbound drug and the total concentration of the drug in the serum.

Preparation of rat liver microsomes

The liver microsomes were prepared from male Wistar rats according to a method reported elsewhere (Tsuruta et al 1997). All subsequent procedures were performed at 4°C or lower. After determination of protein concentration by the method of Lowry et al (1951), a microsomal suspension was prepared at a concentration of 1–2 mg mL^{-1} and was kept at −80°C until used.

Determination of metabolic ratio

Metabolic ratio is defined as a ratio of the amount of each enantiomer of lansoprazole eliminated to the amount of each enantiomer added, part of which was metabolized by microsomal enzymes. The reaction medium contained microsomes (0·1 mg mL^{-1}; 100 μL), potassium phosphate buffer (0·3 mM, pH 7·4; 200 μL), EDTA (0·6 mM; 100 μL) and lansoprazole (5·6 μM). The mixture was pre-incubated at 37°C for 5 min and subsequently at 37°C for 30 min after addition of NADPH-generating system ($NADP^+$, 3 mM; glucose 6-phosphate, 12 mM; glucose-6-phosphate dehydrogenase, 6 international units mL^{-1}; $MgCl_2$, 24 mM; 100 μL). The reaction was stopped by adding 3 mL of 7 : 3 (v/v) diethyl ether–dichloromethane.

Isolation and determination of lansoprazole

Isolation of racemic lansoprazole and determination of lansoprazole enantiomers in serum were performed by high-performance liquid chromatography (HPLC) as reported elsewhere (Katsuki et al 1996). The isolation was performed on a 250 mm × 4·0 mm i.d., 5 μm particle LiChrospher

100 RP-18(e) reversed-phase column. The mobile phase was 35 : 65 acetonitrile–water adjusted to pH 7·0 with phosphoric acid and containing 0·1% *n*-octylamine. The eluate, flow rate 1·0 mL min^{-1}, was monitored for absorbance at 285 nm and the portion eluting between 2 min before and 2 min after the peak of racemic lansoprazole was collected. The residue from this fraction was reconstituted in 8 : 2 (v/v) *n*-hexane–ethanol (200 μL) and 100 μL of this solution was injected on to a chiral HPLC column for separation of the enantiomers.

Determination of lansoprazole enantiomers in serum was performed by normal-phase HPLC on a 25 cm × 4·6 mm i.d. Chiralpak AS column (Daicel, Tokyo, Japan). The HPLC mobile phase was 8 : 2 (v/v) *n*-hexane–ethanol; the flow rate was 1·0 mL min^{-1}. The analytical column was maintained at 38°C.

Pharmacokinetic analysis

Serum concentration–time curves were analysed by non-linear regression analysis using a one-compartment model. 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 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 by use of k_{el} values. The terminal half-life ($t_{\frac{1}{2}}$) was calculated by dividing 0·693 by k_{el}. The apparent total body clearance (CL_{tot}/F) was calculated from $CL_{tot}/F = dose/AUC_{0-\infty}$, F being the fraction of the dose absorbed.

Statistical analysis

Results are expressed as means ± s.e.m. Differences between pharmacokinetic data were analysed for statistical significance by use of Student's *t*-test. A probability level of $P < 0.05$ was considered to be indicative of significance.

Results

In-vivo study

Figure 2 shows the serum concentration–time profiles of the enantiomers of lansoprazole and its metabolite 5-hydroxylansoprazole after oral administration of racemic lansoprazole (50 mg kg^{-1}) to rats. There was a noticeable difference between the serum levels of the enantiomers both of lansoprazole and of 5-hydroxylansoprazole. The mean serum levels of (+)-lansoprazole were higher at all time-points than those of (−)-lansoprazole during the experimental period and the mean serum levels of the metabolite, (+)-5-hydroxylansoprazole were lower than those of (−)-5-hydroxylansoprazole. The pharmacokinetic parameters are summarized in Table 1. C_{max} and AUC_{0-6} values for (+)-lansoprazole were 5–6 times greater than those for the (−) enantiomer ($P < 0.01$) and CL_{tot}/F values for (+)-lansoprazole were significantly smaller than those for the (−) enantiomer ($P < 0.01$). C_{max} and AUC_{0-6} for (+)-5-hydroxylansoprazole were significantly smaller than those for (−)-5-hydroxylansoprazole. There was no significant difference between the t_{max} values of the enantiomers.

In-situ absorption study

We investigated enantioselectivity in the intestinal absorption of lansoprazole by the in-situ recirculation

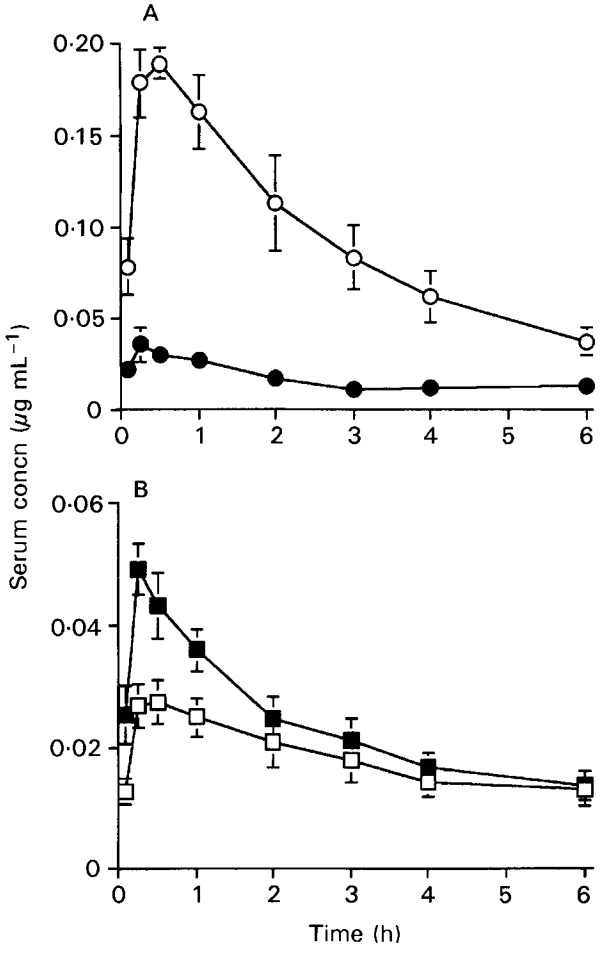


Figure 2. Serum concentration–time profiles of the enantiomers of lansoprazole (A) and 5-hydroxylansoprazole (B) after oral administration of racemic lansoprazole (50 mg kg^{-1}) to rats. ○, (+)-lansoprazole; ●, (−) lansoprazole; □, (+)-5-hydroxylansoprazole; ■, (−)-5-hydroxylansoprazole. Each point and bar represent the mean ± s.e.m. of results from six rats.

Table 1. Pharmacokinetic parameters of lansoprazole and 5-hydroxylansoprazole enantiomers in rats.

	AUC_{0-6} ($\mu g\ h\ mL^{-1}$)	C_{max} ($\mu g\ mL^{-1}$)	t_{max} (h)	$t_{\frac{1}{2}}$ (h)	CL_{tot}/F ($L\ h^{-1}\ kg^{-1}$)
(+)-Lansoprazole	0·549 ± 0·087	0·194 ± 0·012	0·458 ± 0·119	1·88 ± 0·32	90·7 ± 18·8
(−)-Lansoprazole	0·088 ± 0·015†	0·037 ± 0·008†	0·500 ± 0·112	2·62 ± 0·97	437·0 ± 62·5†
(+)-5-Hydroxylansoprazole	0·105 ± 0·019	0·029 ± 0·035	0·375 ± 0·056	3·79 ± 0·52	
(−)-5-Hydroxylansoprazole	0·140 ± 0·017*	0·049 ± 0·004†	0·417 ± 0·124	2·56 ± 0·33	

Each value is the mean ± s.e.m. of results from six rats. *$P < 0·05$, †$P < 0·01$. AUC_{0-6}, area under the serum concentration–time curve between 0 and 6 h; C_{max}, maximum serum concentration; t_{max}, time of maximum serum concentration; $t_{\frac{1}{2}}$, terminal half-life; CL_{tot}, total clearance; F, fraction of the dose absorbed; CL_{tot}/F, apparent total body clearance.

Table 2. Stereoselective protein binding and metabolism of (+)- and (−)-lansoprazole in-vitro.

	Unbound fraction (%)	Metabolic ratio (%)
(+)-Lansoprazole	1·2 ± 0·7	19·8 ± 3·4
(−)-Lansoprazole	4·8 ± 0·3*	45·9 ± 2·5†

Each value is the mean ± s.e.m. of results from four (protein-binding study) or three (metabolism study) rats. *$P < 0·05$, †$P < 0·01$.

method. There was no significant difference between the absorption of the lansoprazole enantiomers. The average absorption rate constants (k_a) of (+)- and (−)-lansoprazole were 0·64 and 0·69 h^{-1} (data not shown).

In-vitro protein-binding study

The extent of enantioselective binding of lansoprazole to rat serum was estimated by an ultrafiltration technique. The binding of (+)-lansoprazole to rat serum was significantly greater than that of the (−) enantiomer (Table 2). The mean unbound fractions of the (+) and (−) enantiomers were 1·2 and 4·8%, respectively.

In-vitro metabolic study

The enantioselective metabolism of lansoprazole by rat-liver microsomes was investigated at a concentration of 5·6 μM. The mean metabolic ratio (45·9%) of the (−) enantiomer was significantly greater than that (19·8%) of the (+) enantiomer in rat liver microsomes (Table 2).

Discussion

We have investigated the stereoselectivity of the pharmacokinetics of lansoprazole in rats after oral administration of racemic lansoprazole as a suspension (pH 9). The C_{max} and AUC_{0-6} values of (+)-lansoprazole were markedly greater than those of (−)-lansoprazole (Figure 2 and Table 1), implying stereoselective absorption, distribution, metabolism or excretion of the drug.

Although absorption of both enantiomers from the small intestine was relatively rapid and their t_{max} values were close there was a pronounced difference between the C_{max} values of the enantiomers (the ratio C_{max} (+)/C_{max} (−) was 5·3). We examined whether or not lansoprazole enantiomers are absorbed enantioselectively. The in-situ absorption study showed no evidence of stereoselective absorption of lansoprazole from the intestine.

The extent of binding of enantiomers to plasma proteins is an important factor in tissue distribution because only unbound drugs can permeate biomembranes. In-vitro and in-vivo experiments have shown that lansoprazole is 91–96% bound to albumin in the rat (Miwa et al 1990). In the current study the extent of protein binding of (+)-lansoprazole was significantly greater than that of (−)-lansoprazole (Table 2). Therefore, (+)-lansoprazole which is more extensively bound to serum proteins could be poorly distributed and slowly metabolized, resulting in the serum concentrations higher than those of (−)-lansoprazole. Consequently, enantioselective protein binding might influence the enantioselective disposition of lansoprazole after oral administration.

There is a possibility of stereoselectivity in the liver metabolism of lansoprazole enantiomers as reported for other proton-pump inhibitors (Uematsu et al 1994; Tybring et al 1997). Lansoprazole is metabolized extensively by the liver and its primary metabolite in the serum is 5-hydroxy–lansoprazole, which is also chiral, and lansoprazole sulphone, with no recovery of the unchanged drug in the urine (Tateno & Nakamura 1991). Our results showed that the C_{max} value of (−)-5-hydroxylansoprazole was significantly greater than that of (+)-5-hydroxylansoprazole (Figure 2). In addition, the metabolic ratio of (−)-lansoprazole was 2·3 times greater than that of (+)-lansoprazole in rat liver microsomes (Table 2). These results

confirm that metabolism of lansoprazole enantiomers in the liver is enantioselective. Possible chiral inversion between lansoprazole enantiomers in the liver and intestine has not yet been studied. Jeffrey et al (1991) reported that inversion of *R*-(−)-ibuprofen to the *S*-(−) antipode occurred in the rat liver. In the current study we did not examine possible inversion because of the unavailability of adequate amounts of the enantiomers.

The metabolism of lansoprazole has been reported to be dependent on CYP2C19 activity for 5-hydroxylation of the drug (Pichard et al 1995). We also reported that subjects with genetic defects of CYP2C19 (m1/m1 and m1/m2) had lower C_{max} values for 5-hydroxylansoprazole than subjects with homozygous wild-type (wt/wt) and heterozygote (wt/m1 and wt/m2) (Katsuki et al 1997). Thus the pronounced enantioselective metabolism of the enantiomers of lansoprazole could be a consequence of different affinities for CYP2C19 isozyme. Omeprazole, inhibitor of the same proton pump as lansoprazole, is also metabolized to 5-hydroxyomeprazole mainly by CYP2C19 (Andersson et al 1990). Tybring et al (1997) reported that AUC_{0-8} values for (+)-omeprazole in the poor metabolizers phenotyped for CYP2C19 were 7·5-fold those for the extensive metabolizers, whereas the AUC_{0-8} values for (−)-omeprazole in the poor metabolizers were only 3·1-fold those in the extensive metabolizers. They suggested that (+)-omeprazole is to a major extent hydroxylated by CYP2C19, whereas (−)-omeprazole might be metabolized partly by this enzyme but mainly by another, presumably CYP3A4, to the achiral sulphone metabolite. This might support the hypothesis that the affinities of the lansoprazole enantiomers for CYP2C19 isozyme are different. Further studies are needed to elucidate to what extent each enantiomer is catalysed by other isozymes in addition to CYP2C19.

In conclusion, it was confirmed that protein binding and metabolism of lansoprazole in the liver are enantioselective and that this is responsible for the different pharmacokinetics of the enantiomers of lansoprazole.

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