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Pharmacokinetic Study of Loprinone Hydrochloride, a New Cardiotonic Agent, in Beagle Dogs

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Abstract—Pharmacokinetic parameters and bioavailability of a new cardiotonic agent, loprinone hydrochloride, in beagle dogs were determined by measuring plasma levels of loprinone after intravenous bolus and oral administration. The plasma half-life after intravenous administration of loprinone varied among individuals over the range 2·65–15·40 h. The bioavailability after oral administration of loprinone as a solution was 37·1%. Effects of enterohepatic circulation on the time-course of plasma levels after intravenous administration of the drug were also studied in bile-duct-cannulated beagle dogs. The amounts of loprinone and its glucuronide excreted in bile during 8 h after administration were 25·4 and 8·6% of the dose, respectively, indicating the possibility of enterohepatic circulation. The plasma half-life in bile-duct-cannulated beagle dogs was 1·98 h. These results indicate that the variation in the half-life in beagle dogs resulted from enterohepatic circulation and that the true half-life is about 2 h. The relative bioavailability after oral administration of the drug as a powder in a capsule compared with a solution was 95·7%. In addition, the amounts of loprinone and its glucuronide excreted in bile, and the AUC of plasma level after infusion for 30 min into the portal vein in bile-duct-cannulated beagle dogs were similar to those after bolus intravenous administration. These results show that the low bioavailability reflects incomplete absorption.

Loprinone hydrochloride, 1,2-dihydro-5-(imidazo[1,2-a]-pyridin-6-yl)-6-methyl-2-oxo-3-pyridinecarbonitrile hydrochloride monohydrate, is a new potent cardiotonic agent with vasodilator activity, and is one of about 200 imidazopyridinylpyridine derivatives synthesized and pharmacologically screened in our laboratories (Yamanaka et al 1991). Loprinone specifically inhibited the cyclic (c)AMP-specific iso-enzyme (fraction III) of phosphodiesterase and caused an elevation of cAMP content in guinea-pig heart (Ogawa et al 1989). In anaesthetized dogs, loprinone (10–100 $\mu g\ kg^{-1}$, i.v.) also increased cardiac contractility, enhanced cardiac index and decreased systemic vascular resistance in a dose-dependent manner (Ohhara et al 1989).

The present study was to ascertain the pharmacokinetic profile of loprinone in beagle dogs.

Materials and Methods

Materials

Loprinone hydrochloride and internal standard, 1,2-dihydro-5-(2-methylimidazo[1,2-a]pyridin-6-yl)-8-methyl-2-oxo-3-pyridinecarbonitrile, were synthesized at Eisai Co. Ltd, Japan. All other reagents and solvents were commercial products of analytical grade.

Intravenous and oral administration—10 mg

Six beagle dogs (31 months old, 10·5–13·7 kg) were divided into two groups of three animals for intravenous and oral administration of 10 mg loprinone using a cross-over design. For intravenous bolus administration, 5 mL of 2 mg mL^{-1} loprinone in 5% D-sorbitol solution was administered into the cephalic vein. For oral administration, 2 mL of solution (5 mg mL^{-1} loprinone in distilled water) or powder enclosed in a gelatin capsule was administered. Beagle dogs were fasted from the day before administration until 6 h after administration. The dogs received 30 mL water immediately following administration. Blood samples were collected from the cephalic vein using a heparinized syringe at predetermined time points for 24 h.

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Intravenous administration—30 mg

Four beagle dogs (8 months old, 10·5–11.5 kg) were intravenously administered 30 mg loprinone by bolus injection of 5 mL of 6 mg mL^{-1} loprinone in 5% glucose solution into the cephalic vein. Beagle dogs were fasted from the day before administration until 10 h after administration. Blood samples were collected from the cephalic vein using a heparinized syringe at predetermined time points for 10 h.

Intravenous and intraportovenous administration to bile-duct-cannulated beagle dogs—30 mg

Six beagle dogs, previously used in the 10 mg study, were divided into two groups of three animals, and 30 mg loprinone was intravenously and intraportovenously administered following bile duct cannulation. Beagle dogs fasted from the previous day were administered 1 mL kg^{-1} thiopentone sodium (2·5%, w/v) to induce anaesthesia. They were then anaesthetized by halothane inhalation, and median laparotomy was performed. The cystic duct was ligated and the common bile duct was cannulated. The mesenteric vein was cannulated at the upper part of the jejunum for intraportovenous administration. After surgery the animals received a 1 : 1 mixture of 0·9% NaCl (saline) and 5% glucose solution via the cephalic vein at an infusion rate of 200 mL/6 h. For intravenous bolus administration, animals received 10 mL of 3 mg mL^{-1} loprinone in 5% glucose solution via the cephalic vein. For intraportovenous administration, animals received 10 mL of 3 mg mL^{-1} loprinone in 5% glucose solution via the tube cannulating the mesenteric vein by infusion at a constant rate for 30 min. Blood samples were collected from the cephalic vein using a heparinized syringe at predetermined time points for 8 h. In

addition, bile samples were collected from the tube cannulating the bile duct into test tubes for predetermined consecutive time periods up to 8 h.

Analysis of loprinone in plasma

The plasma level of loprinone was determined by HPLC using an internal standard. The HPLC system consisted of a Beckman 100A pump, a Waters WISP 710 automatic sample injector and a Jasko UVIDEC 100-II UV detector. The HPLC conditions were as follows: the column was Unisil Q C_{18} 15 μm (4·6 mm i.d. × 150 mm length, GL Sciences Inc., Tokyo, Japan), the mobile phase was 0·1 M phosphate buffer (pH 7·5)/acetonitrile (80:20, v/v), the flow rate was 1·0 mL min^{-1} and detection was at 342 nm. To each 1·0 mL plasma sample, 0·1 mL of the internal standard solution (10 $\mu g\ mL^{-1}$ methanol solution) and 1 mL of 0·1 M phosphate buffer (pH 7·5) saturated with ammonium sulphate were added, and extraction was performed twice with 4 mL ethyl acetate. To the combined organic phase, 1 mL 0·1 M HCl was added for reverse extraction into the aqueous phase. After removal of the organic phase, 1 mL 1·0 M phosphate buffer (pH 7·5) saturated with ammonium sulphate was added to the aqueous phase, and extraction was again performed twice with 4 mL ethyl acetate. The combined organic phase was then evaporated at 50°C under nitrogen. The residue was dissolved in 0·1 mL methanol, and 40 μL was subjected to HPLC. The assay limit was 4·9 ng mL^{-1}.

Analysis of loprinone and its glucuronide in bile

Loprinone in bile was also analysed by HPLC. From each collected sample, 100 μL bile was diluted with 1·0 mL distilled water, and loprinone in 100 μL diluted bile was measured in the same manner as used to measure plasma loprinone. To measure the glucuronide in a 100 μL bile sample, it was diluted with 1·0 mL distilled water. One millilitre of pH 5·0 acetate buffer containing β-glucuronidase (100 units mL^{-1}) (Sigma, G-0751) was then added to 100 μL diluted bile, and the mixture was incubated at 37°C for 3 h for hydrolysis of glucuronide before measuring loprinone as before. The value thus obtained was assumed to be the total concentration of loprinone and its glucuronide in bile, and the level of glucuronide in bile was obtained as the difference between the total and loprinone measured separately.

Data analysis

The value of $AUC_{0\ 10\,h}$ was determined from the plasma level of loprinone at time points up to 10 h after administration by applying the trapezoidal rule. For experiments using bile-duct-cannulated dogs, AUC up to 8 h after administration was determined by applying the trapezoidal rule, and the value from 8 h to infinity was determined by extrapolation using the elimination rate constant. Pharmacokinetic parameters were calculated according to a three-compartment model using MULTI, a nonlinear least-squares program (Yamaoka et al 1981).

Results

Bioavailability after oral administration

Time-courses of plasma level of loprinone in beagle dogs after administration of 10 mg of the drug as a bolus intravenous injection, an oral solution and as powder in a capsule are shown in Fig. 1. Bioavailability parameters are shown in Table 1. Analysis of plasma was performed using

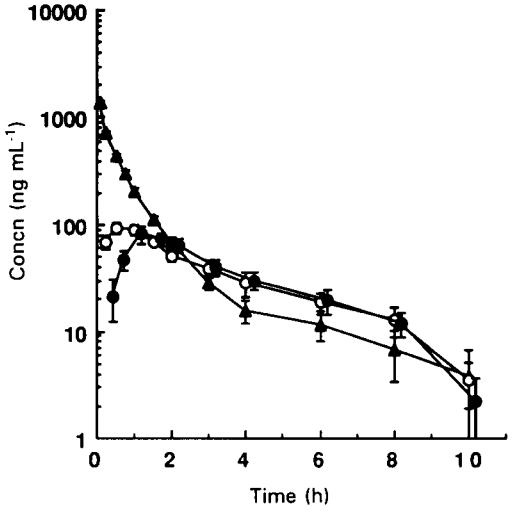

FIG. 1. Plasma levels of loprinone after intravenous bolus (▲) and oral (solution (○) and powder (●)) administration (10 mg) in beagle dogs. Each point and vertical bar represent mean and s.e.m. of six dogs.

values up to 10 h after administration, because the plasma levels of all cases at 24 h after administration, with the exception of two cases of intravenous administration, were below the detection limit (4·9 ng mL^{-1}). When the values of t_{max} and C_{max} for oral administration by solution and by powder are compared, the solution had a shorter median value of t_{max} and a higher mean C_{max}. Values of $AUC_{0\ 10\,h}$, however, were mostly the same. The bioavailability of orally administered loprinone in the form of a solution was calculated to be 37·1% compared with $AUC_{0\ 10\,h}$ after bolus intravenous administration, and the relative bioavailability

Table 1. Bioavailability parameters after intravenous bolus and oral administration of loprinone (10 mg) in beagle dogs.

Parameter	Intravenous bolus	Oral solution	Oral powder
t_{max} (h)	—	0·75 (0·5 1·5)	1·0 (0·5–2·0)
C_{max} (ng mL^{-1})	—	103·4 ± 10·4	86·0 ± 14·5
$AUC_{0\ 10\,h}$ (ng h mL^{-1})	839·7 ± 70·5	317·4 ± 44·6	296·4 ± 39·5
Bioavailability (%)	—	37·1 ± 2·2[a]	95·7 ± 9·2[b]

t_{max} values are reported as median and range; C_{max}, AUC and bioavailability as mean ± s.e.m. of six dogs. [a] Absolute bioavailability $(AUC_{p.o.}/AUC_{i.v.}) \times 100$. [b] Relative bioavailability $(AUC_{powder}/AUC_{solution}) \times 100$.

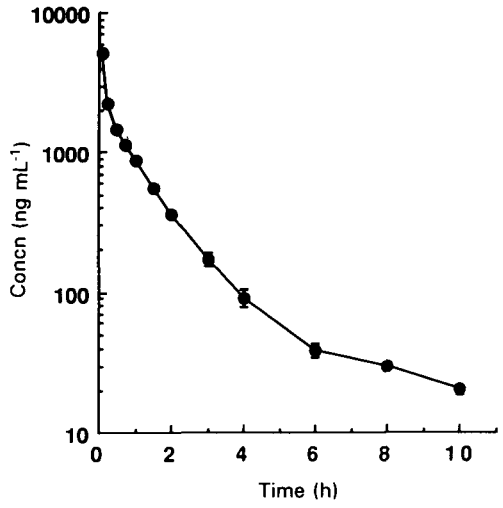

FIG. 2. Plasma levels of loprinone after intravenous bolus administration (30 mg) in beagle dogs. Each point and vertical bar represent mean and s.e.m. of four dogs.

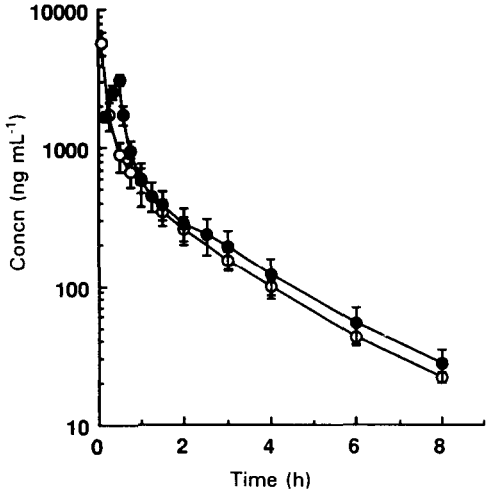

FIG. 3. Plasma levels of loprinone after intravenous bolus administration (○) and intraportovenous infusion (●) for 30 min in bile-duct-cannulated beagle dogs. Each point and vertical bar represent mean and s.e.m. of three dogs.

of orally administered loprinone as a powder compared with the solution was 95·7%.

Pharmacokinetic parameters after bolus intravenous administration

The 10 mg loprinone bolus intravenous dose was increased to 30 mg because the plasma level of loprinone was near the detection limit in the elimination phase after administration of the lower dose. Time-courses of plasma loprinone after administration of the higher dose are shown in Fig. 2. The pharmacokinetic parameters, determined by fitting a three-compartment model are shown in Table 2. The plasma level after intravenous administration of 30 mg loprinone changed in parallel to that after administration of the 10 mg dose. Time-courses were triphasic with respective half-lives of 3·7 min, 41·3 min and 7·55 h. The half-life of the terminal phase varied among individual dogs over the range 2·65–15·40 h. The total body clearance was 140 mL min^{-1}.

Pharmacokinetics using bile-duct-cannulated beagle dogs

Time-courses of plasma levels after bolus intravenous administration and intraportovenous infusion for 30 min of 30 mg loprinone to bile-duct-cannulated beagle dogs are shown in Fig. 3. The pharmacokinetic parameters calculated by applying plasma levels after bolus intravenous administration to the 3-compartment model, and AUC are shown in Table 3. Time-courses after bolus intravenous administration of 30 mg loprinone, were triphasic with respective half-lives of 3·6 min, 30·0 min and 1·98 h. When compared with those obtained in non-cannulated dogs, the half-life of the initial phase was similar, but those of the other phases were shorter, and it should be noted that the inter-individual variation of the terminal half-life was very small. The total body clearance did not significantly differ from that in non-cannulated dogs. Time-courses of plasma levels after intraportovenous infusion for 30 min showed a maximum level, 30 min after the start of administration, and changed in parallel with those observed after intravenous bolus administration. AUC was 2721·6 ± 471·4 ng h mL^{-1}, which is equal to that for intravenous bolus administration (2722·1 ± 592·6 ng h mL^{-1}).

Cumulative excretion rates of loprinone and its glucuronide in bile after bolus intravenous and intraportovenous infusion for 30 min are shown in Fig. 4. In the case of bolus intravenous administration, 25·36 and 8·59% of the dose was excreted as loprinone and its glucuronide, respectively, during 8 h after administration. In the case of intraportovenous infusion, initial excretions were delayed slightly in comparison with those from intravenous bolus administration, but the amounts of loprinone and glucuronide excreted during 8 h after administration were similar.

Table 2. Pharmacokinetic parameters after intravenous bolus administration of loprinone (30 mg) in beagle dogs.

Dog no.	C_1 (μg mL^{-1})	λ_1 (h^{-1})	C_2 (μg mL^{-1})	λ_2 (h^{-1})	C_z (ng mL^{-1})	λ_z (h^{-1})	$t_{\frac{1}{2}}$ (h)	CL (mL min^{-1})
7	7·32	10·44	1·82	0·91	52	0·088	7·88	152
8	7·14	8·89	1·95	0·96	226	0·262	2·65	135
9	8·10	13·09	2·52	1·16	109	0·162	4·28	144
10	8·95	14·05	2·53	1·03	34	0·045	15·40	130
Mean	7·88	11·62	2·21	1·02	105	0·139	7·55	140
±s.e.m.	0·41	1·19	0·19	0·05	43	0·048	2·31	5

Table 3. Pharmacokinetic parameters after intravenous bolus administration of loprinone (30 mg) in bile-duct-cannulated beagle dogs.

Dog no.	C_1 ($\mu g\ mL^{-1}$)	λ_1 (h^{-1})	C_2 ($\mu g\ mL^{-1}$)	λ_2 (h^{-1})	C_z ($ng\ mL^{-1}$)	λ_z (h^{-1})	$t_{\frac{1}{2}}$ (h)	CL ($mL\ min^{-1}$)
2	10·89	12·89	1·06	2·16	403	0·357	1·94	203
5	15·39	11·47	1·71	1·20	435	0·357	1·94	125
6	8·79	10·72	0·66	1·16	252	0·338	2·05	234
Mean	11·69	11·69	1·14	1·51	363	0·351	1·98	187
±s.e.m.	1·95	0·64	0·31	0·33	56	0·006	0·04	32

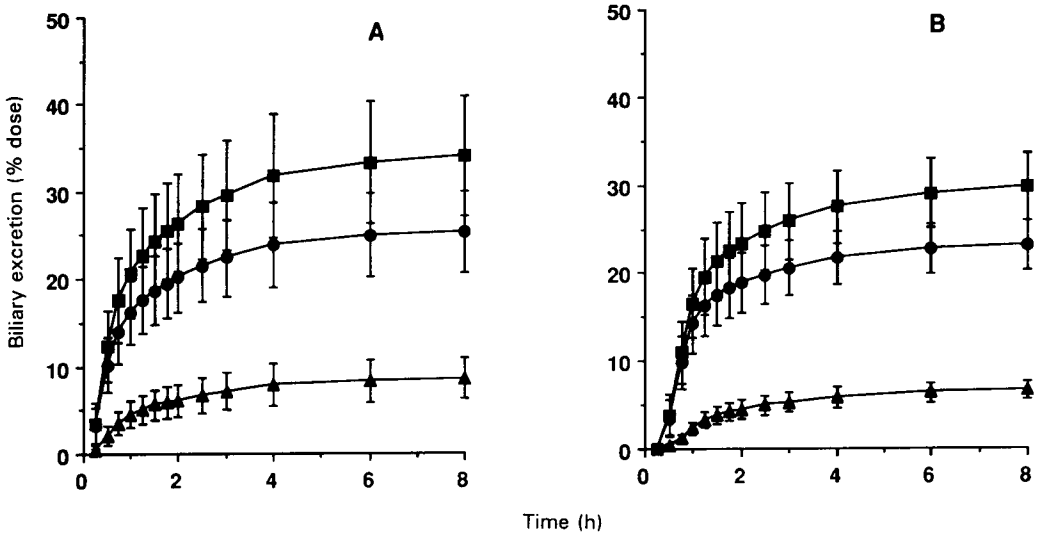

FIG. 4. Cumulative biliary excretion of loprinone and its glucuronide after intravenous bolus administration (A) and intraportovenous infusion for 30 min (B) of loprinone in bile-duct-cannulated beagle dogs. Each point and vertical bar represent mean and s.e.m. of three dogs. (●) Loprinone; (▲) glucuronide; (■) total.

Discussion

Many compounds showing enterohepatic circulation have been reported (Klaassen & Watkins 1984). There have also been many reports that kinetically discuss the phenomenon of enterohepatic circulation, but kinetic analysis of plasma levels of compounds showing enterohepatic circulation is difficult, in many cases requiring some theoretical manipulations such as the use of a lag-time (Steimer et al 1982) or a greater number of compartments (Shepard et al 1985; Pollack & Brouwer 1991) in the model. In addition, when the rate of re-absorption in the enterohepatic circulation is slow, the time-course of plasma level in some cases may appear as a longer elimination half-life.

Intravenous bolus administration of loprinone to bile-duct-cannulated beagle dogs was performed to ascertain whether the variable elimination half-life resulted from enterohepatic circulation. According to the results of this experiment, 25·36 and 8·59% of the dose was excreted in bile in 8 h as loprinone and its glucuronide, respectively. The structure of the latter was confirmed by spectroscopic analysis (Kaneko et al 1993). According to the time-courses of plasma levels obtained in the experiment, the elimination half-life was 1·98 h and did not show such variation among individual dogs as observed in non-cannulated beagle dogs. All experiments on bile-duct-cannulated beagle dogs were performed under anaesthesia, but it is considered that the effects of anaesthesia and surgery were slight since the total body clearance did not decrease in comparison with that obtained in non-cannulated dogs after intravenous bolus administration. In addition, although no data of an individual animal are shown in this study, the elimination half-life determined from the amount of loprinone excreted into bile (2·07±0·42 h) agreed with that obtained from the plasma, and it is therefore considered that the clearance did not decrease with time. It is considered from our results that variation in the elimination half-life of the terminal phase observed among individual dogs after intravenous bolus administration to non-cannulated dogs was due to enterohepatic circulation, and that the true elimination half-life was about 2 h as obtained by the experiment in bile-duct-cannulated dogs.

The bioavailability of loprinone after oral administration in the form of a solution was 37·1%, indicating that more than 60% did not enter the systemic circulation. When loprinone was administered as a powder, it took longer to reach t_{max} and the C_{max} value was lower compared with that of the oral solution, but the relative bioavailability was as high as 95·7%. These results indicate that the dissolving process affected the absorption rate but not the bioavailability. It also became clear that loprinone was not affected by first pass effects in the liver, as there was no significant difference in the amounts of loprinone and glucuronide excreted into bile and no significant difference in the AUC after intraportovenous infusion of bile-duct-cannulated dogs.

One compound which is similar in structure to loprinone,

is milrinone, 1,6-dihydro-2-methyl-6-oxo(3,4′-bipyridine)-5-carbonitrile. According to Alous et al (1985), however, the bioavailability of milrinone in beagle dogs is 92%, which is considerably higher than that of loprinone. When the physicochemical characteristics of both drugs are compared, their pK_a values are 6·0 and 9·0, and 4·5 and 8·6, respectively. Although both are acid-base amphoteric compounds, milrinone exists in a non-dissociated state over a wider pH range than loprinone. In addition, the molecular weight of free milrinone is lower than that of loprinone, with respective values of 211 and 250, and milrinone is also smaller in molecular size. When membrane transport of drug in gastrointestinal tract is considered, two routes, transcellular and paracellular, are possible (Powell 1981; Hayashi 1987). Milrinone is likely to be transported by both routes because of its physicochemical characteristics. A preliminary study demonstrated greater absorption of milrinone than loprinone from an in-situ loop of rat intestine. It is therefore considered that the low bioavailability of loprinone reflects the low membrane transport of loprinone.

In conclusion, the results of this study indicate that the elimination half-life of loprinone varies among individual beagle dogs after intravenous bolus administration, and that this variation is due to enterohepatic circulation, with a true elimination half-life of about 2 h. The bioavailability after oral administration of loprinone was as low as 37·1%, and it is considered that this is not due to the effects of the dissolving process or first-pass effects in the liver, but reflects the low membrane transport of loprinone.

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