Pharmacokinetics of Epinastine and a Possible Mechanism for Double Peaks in Oral Plasma Concentration Profiles

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The pharmacokinetics of epinastine (EPN), an anti-allergic agent, was investigated in rats. The plasma concentration–time profile of EPN after intravenous (i.v.) administration was triexponential. After oral administration of EPN (7.5 and 20 mg/kg), the drug was rapidly absorbed, and C_{max} was reached 2 h after dosing. A minor secondary peak was observed in EPN plasma concentration–time profiles at both doses. The bioavailability of EPN after oral dosing was 41 and 40%. The kinetic parameters ($T_{1/2}$, *AUC* and *MRT*) for unlabeled EPN were much smaller than those for ^{14}C-EPN, which has already been reported. The total biliary excretion of EPN at a 7.5 mg/kg dose was 15.5% of the dose, but the percentage of conjugates in bile was extremely low and about 11% of the total biliary excretion. The increase in the plasma concentration in bile duct-linked rats after oral administration of EPN (20 mg/kg) was not observed, indicating that a secondary increase in drug concentration based on enterohepatic circulation was ruled out. When the gastrointestinal (GI)-transit of phenol red (PR) after oral administration of EPN (20 mg/kg) was estimated, the GI-transit of PR was significantly delayed, and at 3—4 h after dosing half of the PR dose reached the jejunum. The remaining EPN in the small intestine after oral administration (7.5 mg/kg) reached peak levels 2 h after dosing, but then partly increased again at 4 h. As a result, it was clarified that the double peaks observed after oral doses are mainly due to the delayed absorption of a part of EPN, based on the reduction in gastric motility caused by the drug.

Key words epinastine pharmacokinetics; biliary excretion; enterohepatic circulation; secondary peak; GI transit

Epinastine (EPN) is a non-sedating, histamine H_1 antagonist that is used as an anti-allergic agent or for the treatment of asthma.[1] This drug is characterized by the following profile: a very high affinity for H_1-receptors, an orally potent antihistamine, and devoid of central sedative and anticholinergic effects.[2] Because of these pharmacodynamic properties of EPN, the drug is a clinically valuable agent.

The pharmacokinetics of EPN have been investigated using a ^{14}C-labeled drug. The blood level of radioactivity decreased triexponentially after a single intravenous (i.v.) administration in rats, with a half-life ($T_{1/2\gamma}$) of 57 h. Within 96 h after a single oral administration, 19—22% and 77—78% of the dose were excreted into the urine and faeces, respectively.[3] In man, this drug is also mainly excreted into the urine and faeces in an unchanged form.[1] However, data on the pharmacokinetics and disposition of unlabeled EPN are lacking. It is of great clinical relevance that the precise pharmacokinetic behaviors of EPN are clarified using unlabeled drug, in comparison with those of the labeled drug.

In this paper, EPN was administered intravenously and orally to rats at two different doses, and the pharmacokinetics were investigated. A possibility of enterohepatic cycling of EPN was examined using an acceptor rat which received the bile of a donor rat dosed orally with this drug. Additionally, the mechanism underlying the small double-peak phenomenon in the concentration–time profiles after oral dosing was estimated by measuring the gastrointestinal (GI)-transit time of phenol red (PR).

MATERIALS AND METHODS

Materials EPN hydrochloride was a generous gift of Japan Boehringer Ingelheim Co. (Kawanishi, Japan). Diphenidol, an internal standard for high-performance liquid chromatography (HPLC), was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals and solvents used were of reagent or HPLC quality.

Animals Male Wistar rats, weighing 240—260 g, were used throughout this experiment. A group of three to seven rats was randomly selected from the population. On the day before the experiment, the rat jugular vein was cannulated with silicon tubing.[4] For oral dosing, rats were fasted for 14 h before the experiment.

Intravenous (I.V.) and Oral Administrations EPN hydrochloride (2 and 5 mg/kg, EPN equivalent) was administered to one group of rats intravenously through the cannula as a solution (0.15 ml/100 g) of saline, followed by flushing with saline. A second group received EPN hydrochloride (7.5 and 20 mg/kg, EPN equivalent) orally in saline solution (0.4 ml/100 g) by gavage *via* a stomach tube. In the multiple oral dose study, animals were treated for 7 d with a daily oral administration of EPN (20 mg/kg). Blood samples (0.2 ml) were taken *via* the jugular vein catheter at predetermined time intervals after dosing. The plasma (100 μl) was separated immediately by centrifugation and stored frozen until the time of assay.

Biliary Excretion Study EPN hydrochloride (7.5 mg/kg, EPN equivalent) was administrated orally. At 2 h after the dosing, the animals were anesthetized with pentobarbital (40 mg/kg, intraperitoneally, i.p.) to allow rapid cannulations. In order to avoid a decrease in body temperature during the experiments, an electric lamp was placed over the animals. After anesthesia, the animal was placed on an operation board on its back, followed by an abdominal incision. The common bile duct was cannulated with polyethylene tubing (PE-10), then bile samples were collected at 1 h intervals for 8 h. The conjugates in the bile samples were determined from the difference in the amounts before and after the hydrolysis with 0.5 N NaOH (0.3 ml, at 80 °C for 1 h; this method was adopted because EPN was stable in alkaline pH).

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Reabsorption of EPN in Bile Under anesthesia (pentobarbital, 40 mg/kg, i.p.), the bile duct of a donor rat was connected with the duodenum of the acceptor rat (bile-linked rat) by polyethylene tubing (PE-10).[5] The bile of the acceptor rat was eliminated through the tubing. EPN (20 mg/kg) was administered orally to the donor rat and then blood samples of the acceptor rat were withdrawn from the jugular vein at 0.5—8 h after dosing.

Measurement of GI-Transit *in Vivo* The GI-transit rate after oral administration of EPN was measured by the method of Kimura *et al*.[6] with slight modifications. Briefly, rats were fasted overnight with free access to water prior to the experiment. EPN hydrochloride (7.5 mg/kg, EPN equivalent) or saline was administered orally to the rat as 1 ml of saline solution. At 30 min after EPN administration, 1 ml PR-saline solution (2.5 mg/ml) was dosed orally. After a fixed time period (10, 30 min, 1, 1.5, 2, 3, and 4 h after PR administration), the rat was sacrificed under anesthesia with pentobarbital (30 mg/kg), and the entire GI tract was excised. The contents of the stomach and small intestine, divided into 3 equal segments (about 20 cm in length each) and called S1, S2 and S3, were washed out with 50 ml of saline, respectively. The washings were centrifuged at 3000 rpm for 10 min. The PR concentration in the supernatant was determined at 560 nm. The GI-transit was estimated by the ratio of the recovery to total recovery at the time.

In this experiment, the concomitant administration of EPN and PR was not performed because of the precipitation of EPN.

Remaining Amount of EPN in Small Intestine EPN hydrochloride (7.5 mg/kg, EPN equivalent) was administered orally in saline solution *via* a stomach tube. After a fixed time period (1, 2, 3 and 4 h after administration), the rat was sacrificed under anesthesia with pentobarbital (30 mg/kg), and the entire small intestine was excised. The contents of the small intestine, divided into 3 equal segments (about 20 cm in length each); S1, S2 and S3, were washed out with 10 ml of cold saline, respectively. EPN in the washings was determined.

Determination of EPN EPN in plasma and bile was determined by the HPLC method,[7] with slight modifications. A 100 μl aliquot of plasma or bile was mixed with 200 μl of diphenidol solution (0.5 μg/ml) as an internal standard. The solution was made alkaline by the addition of 100 μl of 0.1 M NaOH. EPN was extracted by 5 ml dichloromethane and shaking for 30 min. After centrifugation, 4 ml of the organic phase was transferred into another tube and evaporated under reduced pressure. The residue was dissolved in 50 μl of the mobile phase and injected onto a reversed–phase Cosmosil 5C18 column (0.45 μm, 4.6×150 mm, Nacalai Tesque Co., Kyoto, Japan) which was part of a Shimadzu liquid chromatograph (model LC-10A) equipped with a UV spectrophotometer (model SPD-10A). The mobile phase for the determination of EPN was 0.3% (v/v) triethylamine (pH 4.5 with H_3PO_4) : methanol (60 : 40, v/v), which was pumped at a flow rate of 1.0 ml/min at 25 °C. Detection was at 207 nm. The sensitivity of the method was 10 ng/ml. Linearities of the standard curves were found in the range from 10 to 1000 ng/ml ($r^2=0.999$). Intra- and interday variabilities were $<5\%$.

Data Analysis Pharmacokinetic parameters were calculated with the nonlinear least-squares regression program MULTI.[8] The plasma concentration–time data of EPN after i.v. administration were fitted to the following equation using WinNonlin software (Pharsight Corp., Mountain View, CA, U.S.A.).

$$C_t=A\cdot e^{-\alpha t}+B\cdot e^{-\beta t}+C\cdot e^{-\gamma t}$$

where C_t is the drug concentration at time t, and A, α, B, β, C and γ are the triexponential equation constants. The estimation was performed using WinNonlin with a weighting factor equal to the inverse of each concentration.

The *AUC* up to the last sampling point was calculated by the trapezoidal method, and the *AUC* beyond the last observed plasma concentration (C_n) was extrapolated according to C_n/γ. The area under the first moment curve (*AUMC*) and the mean residence time (*MRT*) were calculated by means of moment analysis.[9] The absolute bioavailability (F) was calculated using the i.v. and oral *AUC* values.

The means of all data are presented with their standard deviations (S.D.). Statistical analysis was performed with an unpaired Student's *t*-test, and the significance level adopted was $p<0.05$.

RESULTS AND DISCUSSION

I.V. Administration The plasma concentration–time curves for EPN after i.v. dosing (2 and 5 mg/kg) are shown in Fig. 1. The plasma levels of EPN declined in a triexponential manner. When the plasma concentration–time profile was analyzed by a 3-compartment model, a good fit was obtained between the observed and predicted curves, with a small CV% (<54%). Pharmacokinetic parameters for the i.v. administration are listed in Table 1. The parameters (α, β, γ, $T_{1/2\gamma}$ and *CL*) were not significantly different between the two doses used, although $AUC_{0-\infty}$ at a 5 mg/kg dose was 3.2 times that at a 2 mg/kg dose. Consequently, the linear elimination of EPN was observed at doses used. It was found that there were large differences between parameters such as $T_{1/2\gamma}$, *AUC* and *MRT* for unlabeled and labeled EPN (1 mg/kg),[3] of which the parameters for labeled EPN were much larger than those of an unlabeled drug. A reason for the discrepancy may be due to the metabolites produced from ^{14}C-EPN and to some unknown reason. It is shown that several metabolites were present in the urine,[10] and the radioac-

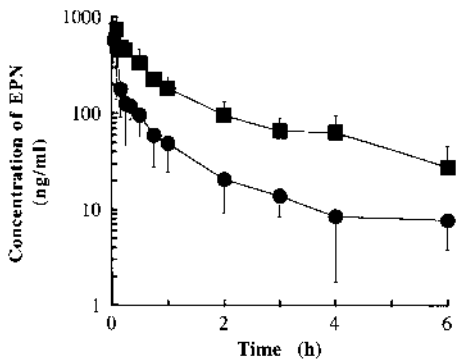

Fig. 1. Plasma Concentration–Time Curves for EPN after I.V. Administration

The does of EPN was 2 mg/kg (●) and 5 mg/kg (■). Points and vertical bars represent the mean±S.D. ($n=4$).

tivity was detected in many tissues such as the liver, thyroid gland and stomach, 48 h after the oral dosing of ^{14}C-EPN to rats.[3)]

Oral Administration A. Single Dosing: The plasma concentration–time curves for EPN after a single oral administration (7.5 and 20 mg/kg) are depicted in Fig. 2A. The plasma EPN concentrations increased rapidly to peak levels at 2 h after oral doses and started to decline as a function of time, although a minor secondary peak 4 h after dosing was observed at both doses. These results indicate the rapid absorption of this drug in rats. The presence of a secondary peak agreed with the data (a secondary peak at 4 h) obtained after the oral dosing of ^{14}C-EPN,[3)] as shown in Fig. 3.

Pharmacokinetic parameters for the oral administration are shown in Table 2, in comparison with the parameters for ^{14}C-EPN. There were no significant differences in the parameters between both doses. Thus, the linear elimination of EPN was also confirmed by the oral dosing. The oral bioavailability (F) of EPN in rats was 41 and 40%. Large differences were also recognized between the parameters for labeled and unlabeled EPN. The $T_{1/2}$, AUC and MRT values after the administration of ^{14}C-EPN were much larger than those after unlabeled EPN.

B. Repeated Dosing: The plasma concentration–time curves for EPN after the final dosing in multiple oral administration schedules (20 mg/kg, 7 d) are shown in Fig. 2B, and the derived pharmacokinetic parameters are listed in Table 2.

Table 1. Pharmacokinetic Parameters of EPN after Intravenous Administration

Parameter	EPN		^{14}C-EPN[a)]
	2 mg/kg	5 mg/kg	1 mg/kg
A (ng/kg)	22318±13020	78646±37171	—
α (h^{-1})	108.5±40.2	75.6±8.4	—
B (ng/kg)	257±140	432±66	—
β (h^{-1})	2.7±2.2	2.3±1.0	—
C (ng/kg)	24.8±1.6	192.4±145.8	—
γ (h^{-1})	0.2±0.1	0.3±0.2	—
$T_{1/2\gamma}$ (h)	4.0±2.5	5.3±4.1	56.8±3.5
K_{10} (h^{-1})	36.5±11.2	39.7±11.8	—
CL_{tot} (ml/h/kg)	3584.9±2108.5	3239.6±823.6	—
$AUC_{0-\infty}$ (ng·h/ml)	439.4±34.9	1435.3±381.7[b)]	1208.7±108.4
MRT (h)	2.4±2.1	2.8±2.2	15.1±0.5

Values are expressed as the mean±S.D. (n=4). *a*) Ref. 3. *b*) $p<0.05$ compared with the value at the 2 mg/kg dose.

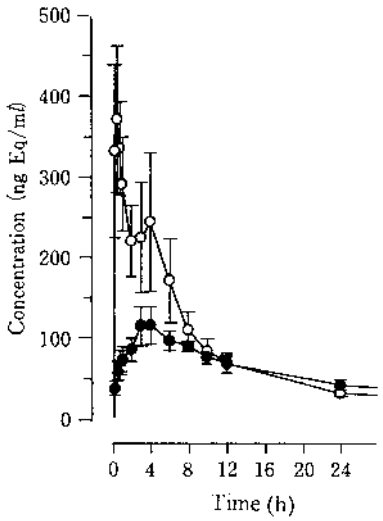

Fig. 3. Blood Concentration of Radioactivity after Oral (5 mg/kg) Administration of ^{14}C-EPN to Male Rats

Points and vertical bars represent the mean±S.D. (n=3−5). ○, fasted rat; ●, fed rat.

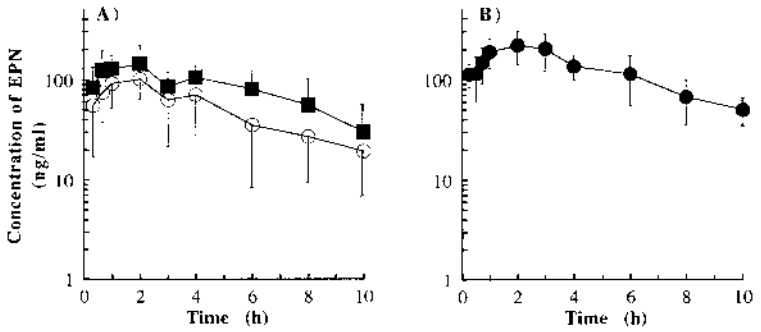

Fig. 2. Plasma Concentration–Time Curves for EPN after Oral Administration

(A) Single dosing (○) 7.5 mg/kg; (■) 20 mg/kg. (B) Multiple dosing (20 mg/kg). Points and vertical bars represent the mean±S.D. (n=6−10).

Table 2. Pharmacokinetic Parameters of EPN after Oral Administration

Parameter	EPN			^{14}C-EPN[a)]
	Single		Repeated	Single
	7.5 mg/kg	20 mg/kg	20 mg/kg	5 mg/kg
$T_{1/2}$ (h)	2.3±1.1	3.7±1.1	—	45.7±2.0
k_e (h^{-1})	0.3±0.1	0.2±0.1	—	—
AUC (ng·h/ml)	679.1±301.0	1755.1±703.0[b)]	2695.7±638.2	3796.0±478.3
MRT (h)	5.9±3.9	6.9±4.3	8.9±2.3	17.9±0.7
F (%)	41.2	39.9	—	62.8

Values are expressed as the mean±S.D. (n=5−7). *a*) Ref. 3. *b*) $p<0.05$ compared with the value at the 7.5 mg/kg dose.

The peak plasma concentrations (C_{max}) occurred later after repeated dosing than that after a single dosing, and no clear secondary peak in the plasma concentration–time profile was observed. Additionally, the C_{max} and *AUC* after repeated dosing were 1.5 times, respectively, higher than those after a single dosing at the same dose (Fig. 2A). This might be due to the delayed elimination or the partial accumulation of the drug in the tissues after the repeated dosing. Thus, these occurrences would obscure the secondary peak. It is reported that the blood concentrations of radioactivity were gradually enhanced by repeated dosing of ^{14}C-EPN at a 5 mg/kg dose to rats, although there was no accumulation of ^{14}C-EPN in any particular tissues.[3]

Biliary Excretion The biliary excretion of EPN at a 7.5 mg/kg dose was measured, and the result is shown in Fig. 4. Total biliary excretion of EPN during 8 h after oral dosing was 15.6±6.6% of the dose. The percentage of conjugates in bile was extremely decreased, about 11% of the total amount excreted. Approximately 60% of total EPN excreted in the bile was secreted within 4 h after administration. These results approximately agreed with the data that 19.5% of ^{14}C-EPN dosed to rat was excreted into the bile.[10] The reason the conjugates of EPN were relatively decreased in bile may be because of minor glucuronidation of the amino group in the molecule. The minor conjugation of EPN was also seen in the interview form of EPN.[1]

Enterohepatic Circulation When EPN in plasma samples from the acceptor rat (bile–linked rat), which received the bile of the donor rat orally given the drug, was determined, an appreciable amount of EPN was not detected by the assay method, suggesting that EPN would hardly be subjected to enterohepatic circulation in rat (data not shown). As a result, the secondary increase in drug concentration based on the enterohepatic recycling was precluded from being the underlying mechanism. This is supported by the fact that the secondary peak was not observed after i.v. administration. Our result, however, did not agree with the data reported by Oiwa *et al.*,[10] in which a 24% of the total radioactivity excreted in bile was reabsorbed. It is not possible at this time to explain the differences in reabsorption, but the lower amount excreted in bile (15.5% of the dose) and the relatively small fraction (41 and 40% of the dose) absorbed after oral dosing are probably associated with negligible reabsorption.

GI-Transit of PR after EPN Administration The GI-transit time of PR after the oral administration of EPN (7.5 mg/kg) was compared with that after saline dosing (control). PR was chosen as a poorly absorbable compound. As shown in Fig. 5, EPN significantly delayed the GI-transit rate of PR. The stomach transit of PR in the control rat was more rapid than that in the EPN-dosed rat, and 0.67—1 h after EPN dosing, the large parts of PR dose were transfered to S1, S2 and S3. However, 85% of the PR dose remained in the stomach of the rat which received EPN, even at 1 h after dosing of the drug, and a half of the dose reached S3 at 3 h after dosing. The delayed time of PR transit to the S3 was approximately consistent with the secondary peak time (4 h) of EPN after oral dosing. The reduction in gastric motility caused by the muscle relaxant effect of EPN might be involved in the delayed transit time. EPN is reported to inhibit a contractile response of isolated guinea-pig ileum to acetylcholine and to inhibit gastric secretion.[11]

Our data provide the first evidence of the reduction in gastric motility by EPN. Thus, the double peaks in the plasma

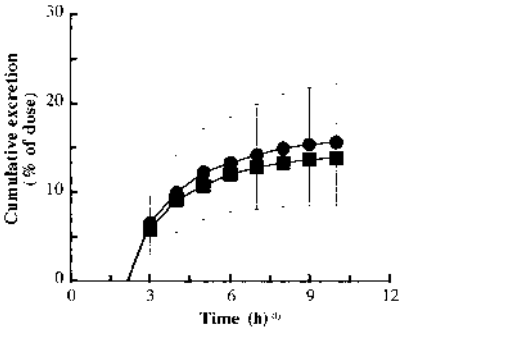

Fig. 4. Cumulative Biliary Excretion of EPN after Oral Administration

The dose of EPN was 7.5 mg/kg. (●) total drug; (■) unchanged drug. Points and vertical bars represent the mean±S.D. (n=3−4). *a*) Time (h) after administration of EPN.

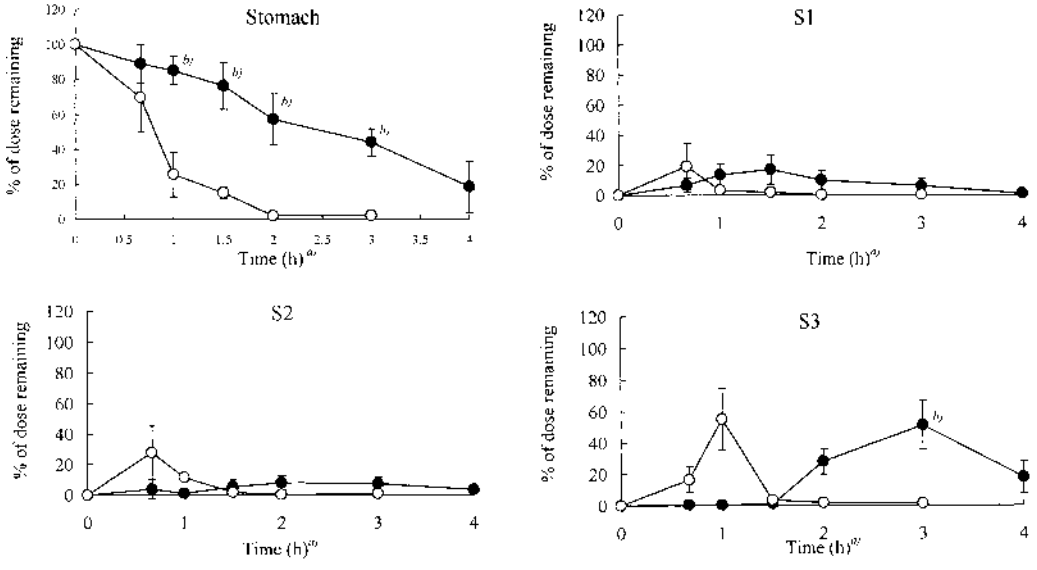

Fig. 5. Gastrointestinal Transit of PR after Oral Administration of EPN or Saline

(○) in control rat; (●) in EPN (20 mg/kg)-dosed Rat. Points and vertical bars represent the mean±S.D. (n=3). *a*) Time after administration of PR. *b*) $p<0.05$ compared with the EPN-dosed rat.

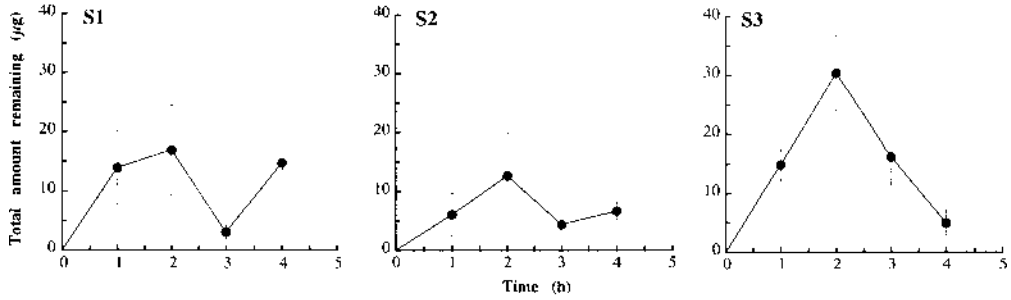

Fig. 6. Remaining Amount of EPN in Small Intestine

(●) Remaining amount after oral dosing (7.5 mg/kg). Points and vertical bars represent the mean±S.D. (n=3).

concentration–time profiles after oral doses could be due to differential rates of absorption along the gastrointestinal tract based on biphasic gastric emptying. Judging from the result of GI-transit, more than 50% of the dose was evacuated from the stomach during the first period and less than 50% was excreted during the second period (Notice: PR was administered 30 min after dosing of EPN in this experiment).

Remaining Amount in Small Intestine The remaining amount of EPN in the small intestine was estimated after oral administration, in relation to the GI-transit time. The remaining amount in S1, S2 and S3 reached peak levels 2 h after oral doses, and the remaining amount in the S1 and S2 increased again 4 h after dosing, as shown in Fig. 6. These indicate that the GI-transit of EPN would consist of large gastric emptying in the initial time stage (at 1—2 h) and another one about 4 h after dosing.

When the total amount remaining in the small intestine was divided into two by time, the remaining amount was 95 mg at 1—2 h and was 50 mg at 3—4 h after dosing. Assuming that the remaining amounts of EPN in the small intestine will probably be proportional to the absorbed amounts in this portion, the ratio of absorbed EPN was two-thirds at the initial time stage and one third at 3—4 h. The data support the appropriateness of the second peak in the plasma-concentration–time curves.

The discrepancy in GI-transit time of PR with the remaining amount of EPN in the small intestine was probably due to that absorption rate. Since PR was administered 30 min after EPN dosing, the extensive reduction in gastric motility induced with EPN increased the residence time of drug in the stomach at the initial time stage after dosing, and the amount of PR that reached the small intestine 1—2 h after dosing was relatively small.

Wang *et al*. have reported a double-peak phenomenon in the pharmacokinetics of alprazolam after oral administration, and they described well the time course of serum concentration data by the first-order absorption model that incorporated a delay site.[12] We also applied their model to analyze the double peak observed after the oral administration of EPN. However, a good fit was not obtained between the observed and predicted curves, because of the small peak and only a few quantified points in the double peak of EPN.

In conclusion, the plasma concentration–time profiles of EPN after i.v. dosing were triexponential. After oral administration, the drug was rapidly absorbed and C_{max} was reached 2 h after dosing, suggesting the rapid absorption of EPN from the intestine. The minor secondary peak was observed in the plasma concentration–time profiles after oral administration. The bioavailability of EPN after oral dosing was 40—41%. The total biliary excretion of EPN at a 7.5 mg/kg dose was 15.5% of the dose, but the percentage of conjugates in bile was extremely low (11%). Enterohepatic circulation was not observed after oral dosing (20 mg/kg dose). Since the GI-transit of PR after EPN administration (7.5 mg/kg) was significantly delayed, the secondary peak observed might mainly be due to the reduction in gastric motility by EPN. The reduction in gastric motility may influence the pharmacokinetics of drugs repeatedly coadministered.

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