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Role of intestinal transport and first pass liver extraction on oral delivery of renin inhibitor compounds

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Summary

The absolute bioavailabilities of three renin inhibitor compounds, one uncharged (compound I) and two positively charged (compounds II and III), were found to be comparable (1–3%). To determine the role of intestinal transport and first pass liver extraction (FPLE) in the oral delivery of these compounds intravenous, intraportal, intraduodenal and intraperitoneal studies were performed in the rat. In the intraduodenal studies, drug solutions were injected into the duodenum of anesthetized rats and portal and systemic blood was collected. In the intraportal studies, the drug solutions were injected into the portal vein and systemic blood was collected. From the ratio of the area under the drug concentration-time curves (tAUC) for the oral and intraportal studies, the extent of intestinal transport of compounds I–III was estimated as 9.7, 2.2 and 2.2%, respectively. In the intraduodenal studies the maximum portal plasma concentrations of compounds I–III were 2.8, 0.5 and 0.2 μg/ml, respectively. The tAUC of compound I in portal plasma was 8–26-times higher than those for compounds II and III. From comparison of the intraportal and intravenous tAUC values, the FPLE of compounds I–III was estimated as 76 ± 4, 61 ± 3 and 8 ± 23% (mean $\pm$ SE), respectively. Overall, the results indicated that the intestinal transport and FPLE of compound I was the highest among the three analogs. Compound II showed low intestinal transport and high FPLE and compound III showed low intestinal transport and low but variable FPLE.

Introduction

There have been significant efforts in recent years to discover renin inhibitor compounds that are not only orally active but also have significant bioavailability in various species (Buhlmayer et al., 1988; De Gasparo et al., 1989; Hanson et al., 1989; Kramer et al., 1990; Morishima et al., 1989; Cumin et al., 1990; Rosenberg et al., 1991; Rush et al., 1991; Kleinert et al., 1992).

in the oral absorption screening for poorly available drugs, in vitro models such as the everted gut (Wilson and Wiseman, 1954), brush border membranes (Kessler et al., 1978), the Caco2 cell line (Hidalgo et al., 1989), hepatocytes and liver microsomal preparations (Mazel, 1971)

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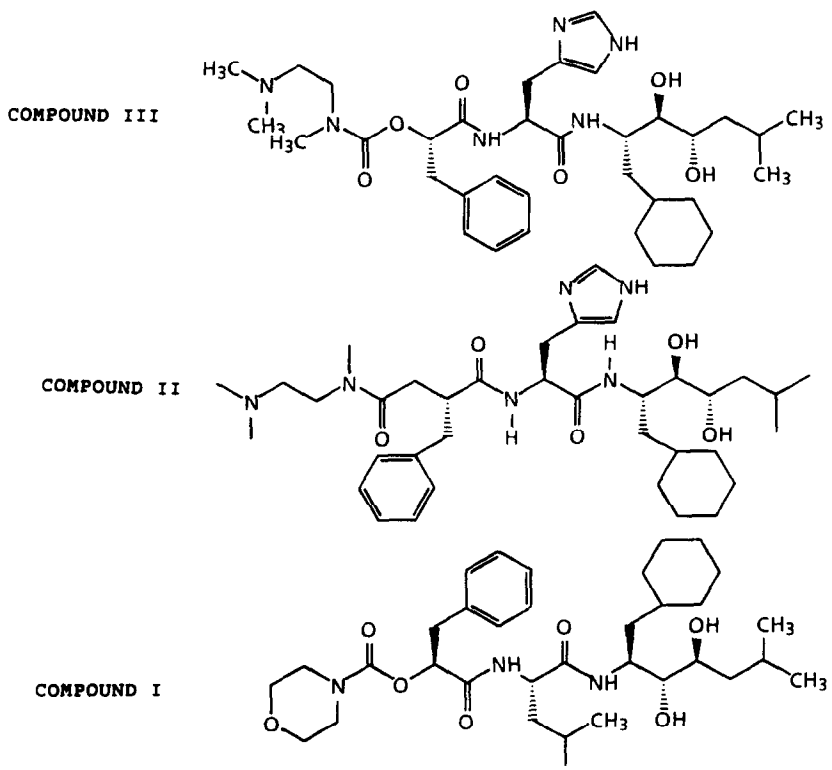
are valuable in solving transport and metabolism related problems. However, the results of the in vitro studies must further be correlated with in situ or in vivo data. The role of intestinal transport and first pass liver extraction (FPLE) in the oral delivery of compounds can be determined from comparison of the drug concentration-time profiles resulting from in situ and in vivo absorption models. The ratio of oral to intraportal total area under the drug concentration-time curve (tAUC) values shows the extent of intestinal transport into the portal vein and the ratio of the intraportal to intravenous tAUC values indicates the extent of FPLE. Further, sampling of the portal plasma after injection of a drug solution into the duodenum supplies direct information about the intestinal transport.

In this study, the role of intestinal transport and FPLE in the oral absorption of three renin inhibitor compounds was determined after administering the compounds by the intravenous, oral, intraduodenal, intraportal, and intraperitoneal routes.

Materials and Methods

Materials

Compound I (*O*-(*N*-morpholinocarbonyl-3-L-phenylaspartyl-L-leucinamide of (2*S*, 3*R*, 4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane) (Mol. Wt 617.8), compound II 2-[[2-[[1*R**-(cyclohexylmethyl)-2*S**,3*R**-dihydroxy-5-methylhexylamino]-1*R**-(1H-imidazol-4-ylmethyl)-2oxoethyl]amino]-2-oxo-1*S*,1*R**-(phenylmethyl)ethyl-[2-(dimethylamino)ethyl]methylcarbamate (Mol. Wt 654.9) and compound III (*N*1-[2-[[1*S**-(cyclohexylmethyl)-2*R**,3*S**-dihydroxy-5-methylhexyl)amino]-1*S**-(1H-imidazol-4-ylmethyl)-2-oxoethyl]-*N*4-[2-(dimethylamino)ethyl]-*N*4-meth-



Scheme 1.

yl-2*R*,2*R**-(phenylmethyl)butanediamide) (Mol. Wt 701.9) (Scheme 1) were synthesized at G.D. Searle and Co. Bovine serum albumin, neomycin, EDTA and Tes were obtained from Sigma Chemical Co. (St. Louis, MO). Phenylmethylsulfonyl fluoride was obtained from Calbiochem (La Jolla, CA), 8-hydroxyquinoline was purchased from Aldrich Chemical Co. (Milwaukee, WI) and recombinant human renin was prepared at Monsanto Co. (St. Louis, MO). Angiotensin 1 radioimmunoassay was obtained from New England Nuclear Corp. (Boston, MA).

Animal studies

Male Sprague Dawley rats (400–550 g) (4–6 animals per study per compound, see figure legends) were fasted overnight prior to all studies. At the end of the studies, the animals were euthanized by cardiac injection of 100 mg/ml sodium pentobarbital solution (1 ml/kg).

Intravenous (i.v.) studies The animals were anesthetized with an isofluorene/nitrous oxide mixture. The femoral vein was exposed through a small incision. Compounds I and II, as a solution in PEG 400, were injected into the femoral vein of the rats at a 0.5 ml/kg volume. Compound III was injected as a solution in pH 3.5 citrate buffer at the same volume. The dose of the compounds in these studies was 1 mg/kg. The incision site was stapled closed and systemic blood was sampled at 2 (or 1), 5, 10 (or 15), 30, 60, 120, 240 and 360 min by cardiac puncture (1 ml). The blood was withdrawn into syringes containing EDTA as an anticoagulant. Plasma was separated by centrifugation and stored at −20°C for later analysis. Animals were kept under general anesthesia during blood collection and drug administration.

In vivo oral delivery Compounds I (20 mg/kg) and II (10 mg/kg) in PEG 400 and Compound III (10 mg/kg) in pH 3.5 citrate buffer were delivered orally at a 1 ml/kg volume through a stomach intubation tube. Systemic blood (1 ml) was collected at 0.25, 0.5, 1, 2, 4, 6 and 8 h and treated the same as in the i.v. studies.

Intraduodenal rat model (i.d.) After the animals were anesthetized with pentobarbital (55 mg/kg), a midline incision was made to expose the small intestine and the portal vein. Drug solutions (10 mg/kg) in PEG 400 (1 ml/kg) were injected into the duodenum at a site posterior to the pyloric sphincter. Blood was collected at 5, 15, 30 and 60 min through either a polyethylene cannula (PE 10) in the portal vein near the liver or by a 26 G bent needle. When a needle was used, pressure was immediately applied to the needle puncture hole for about 30 s to prevent bleeding. Systemic blood was sampled once at the end of the experiment by cardiac puncture. The temperature of the animals was monitored with a rectal probe while the animals were kept warm with a heating pad or heating lamp. The intestines were covered with cotton gauze moistened with saline to prevent dehydration. The portal and systemic blood was treated as in the i.v. studies.

Intraportal rat model (port) Rats were anesthetized with isofluorene/nitrous oxide and a midline incision was made to expose the portal vein. Solutions of drug in PEG 400 or PEG 200/water (50:50) (0.5 ml/kg) were injected into the portal vein at time zero. The dose for all the compounds was 1 mg/kg. Compound I was also dosed at 0.24 mg/kg. To prevent bleeding, pressure was applied to the needle entry point after injection. The abdominal wall was then sutured. Systemic blood was collected at 2, 5, 10 (or 15), 30, 60, 120, 240 and 360 min and treated as in the i.v. studies.

Intraperitoneal delivery (i.p.) Compound I (10 mg/kg) in PEG 400 (0.5 ml/kg) was injected into the peritoneal cavity of the rats. Systemic blood was collected at 0.25, 0.5, 1, 2, 4, 8 and 24 h and treated as in the i.v. studies.

Partition coefficient and solubility determinations

The partition coefficients between octanol and water of compounds I–III were determined in duplicate. Samples were prepared by mixing 2 ml aliquots of octanol stock solutions of the compounds (0.5 mg/ml) and 2 ml octanol-saturated water. The solutions were shaken for 2 h at room temperature. The concentrations of the compounds in the aqueous phases were measured either by HPLC (compound I) (Kararli et al., 1992) or by UV-visible spectrophotometry at 263

nm (compounds II and III). Solubility limit determinations for compounds II and III were estimated in pH 3.0 acetate buffer (10 mM), and pH 7.0 phosphate buffer (10 mM) by adding increasing amounts of solid drug into aqueous solutions and observing whether the amount added completely dissolved.

Renin bioassay for plasma analysis

Plasma samples were analyzed for renin inhibitor content with a bioassay. The samples (0.1 ml) were extracted with acetonitrile (0.3 ml) and the extract was evaporated to dryness under nitrogen. The residue was dissolved in 0.1 ml 4% bovine serum albumin containing 0.9% NaCl and 5% EDTA. The dissolved residue (0.1 ml) was incubated with a reaction mixture containing human plasma (0.12 ml), 5% phenylmethylsulfonyl fluoride (1.2 μl), 10% neomycin (2.4 μl), 0.5 M 8-hydroxyquinoline (2.4 μl), 0.5 M Tes buffer (pH 7.4, 24 μl), and 0.6 mU/ml recombinant human renin (1000 U/mg, 50 μl) at 37°C for 90 min. The renin activity (angiotensin I produced/ml reaction mixture per h) was determined by a standard angiotensin 1 radioimmunoassay. The amount of renin inhibitor in the plasma sample was determined by comparing the extent of inhibition of renin activity with the activity produced by known amounts of renin inhibitor added to plasma and analyzed as above. The sensitivities of the assay for compounds I–III were 1, 2 and 2 ng/ml, respectively.

Analysis of the data

FPLE values were calculated using Eqn 1. The percent absolute bioavailability (A.B.) and % intestinal transport (Intes. Trans.) values were calculated using Eqns 2 and 3:

$$\%\text{FPLE} = 100 - \frac{\text{tAUC}^{\text{port}}}{\text{tAUC}^{\text{iv}}} \times \frac{\text{Dose}^{\text{iv}}}{\text{Dose}^{\text{port}}} \times 100 \quad (1)$$

$$\%\text{A.B.} = \frac{\text{tAUC}^{\text{oral}}}{\text{tAUC}^{\text{iv}}} \times \frac{\text{Dose}^{\text{iv}}}{\text{Dose}^{\text{oral}}} \times 100 \quad (2)$$

$$\%\text{Intes. Trans.} = \frac{\text{tAUC}^{\text{oral}}}{\text{tAUC}^{\text{port}}} \times \frac{\text{Dose}^{\text{port}}}{\text{Dose}^{\text{oral}}} \times 100 \quad (3)$$

where tAUC^{iv}, $\text{tAUC}^{\text{port}}$ and $\text{tAUC}^{\text{oral}}$ are the total area under the plasma concentration-time values for the intravenous, intraportal and oral studies. Dose^{iv}, $\text{Dose}^{\text{port}}$ and $\text{Dose}^{\text{oral}}$ are the doses administered in intravenous, intraportal and oral studies. The AUC values were calculated by the linear trapezoidal rule and were extended to infinite time by using the biological half-life of elimination of the compounds estimated from the i.v. data.

Results and Discussion

The plasma levels of compounds I–III after the oral administration of 10–20 mg/kg doses are given in Fig. 1. The tAUC and absolute bioavailability values are tabulated in Tables 1 and 2. The results indicate that the absolute bioavailabilities of the compounds are similar and all are less than 3%. In Fig. 1, the plasma levels of compounds reached maximum at 0.25 h and then sustained at low levels. This may indicate continuous but low absorption of the compounds throughout the G.I.

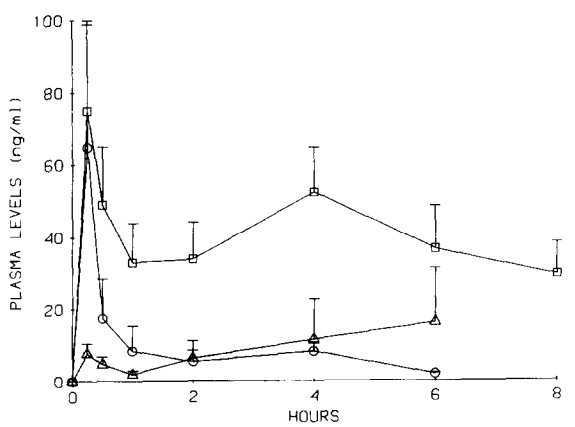


Fig. 1. The systemic plasma concentrations (mean ± SE) of compound I (20 mg/kg, (□), $n = 4$), compound II (10 mg/kg (△), $n = 6$) and compound III (10 mg/kg (○), $n = 6$) following oral delivery.

TABLE 1

The total AUC values for the i.v., intraportal (port.), and oral studies

Compound	tAUC (μg ml^{-1} min) (mean $\pm$ SE)		
	i.v. [a]	port. [a]	Oral [a]
Compound I	39.2 $\pm$ 3	9.5 $\pm$ 1.7 2.0 $\pm$ 0.3 [b]	18.5 $\pm$ 4.8
Compound II	46.7 $\pm$ 4.5	18.4 $\pm$ 1.5	4.0 $\pm$ 3.4
Compound III	17.0 [c]	15.6 $\pm$ 3.9	3.4 $\pm$ 1.1

[a] From 0 to infinity.

[b] Dose is 0.24 mg/kg.

[c] Calculated using average plasma levels. Note that the drug doses in the i.v. studies was 1 mg/kg, and in the intraportal studies, 1 mg/kg (unless otherwise indicated).

tract. Also, the possibility of an active metabolite formation cannot be ruled out based on the results in Fig. 1. In this study, the plasma levels of the compounds were analyzed using a renin bioassay similar to those used in the literature (Morishima et al., 1989; Rosenberg et al., 1991; Rush et al., 1991; Kleinert et al., 1992). Although a bioassay can speed up the drug screening process, it can lead to an overestimation of the plasma levels of drugs if the metabolites have activities similar in potency to the parent compound.

The oral bioavailability of compounds can be limited by low solubility and dissolution rate in the intestinal lumen, low intrinsic membrane permeability, luminal and intestinal tissue binding/metabolism and FPLE (metabolism/bile secretion). The extent of intestinal transport calculated from the intraduodenal (relative transport) and intraportal/oral studies reflects these events. For the uncharged renin inhibitor compound (I), low solubility and dissolution rate can certainly limit oral absorption. The aqueous solubility of compound I is only 1 μg/ml (Kararli et al., 1992). In previous studies, the water suspension of this compound resulted in only 0.1% A.B. while various oil and emulsion formulations produced up to 5% A.B. values (Kararli et al., 1992). For compound I, the A.B. of the PEG solution formulation is 2.4% (Table 1), suggesting that the solubility and dissolution rate of compound 1 may not be rate limiting with this formulation. The aqueous solubilities of compounds II and III in pH 3.0 and 7.0 media are more than 14 and 1 mg/ml, respectively. It is unlikely that the solubility and dissolution rate are the major rate-limiting steps in the intestinal transport of these analogs.

TABLE 2

Summary of the studies for the renin inhibitor compounds

Compound	% A.B. (mean $\pm$ SE)	% intestinal transport	% FPLE (mean $\pm$ SE)	Octanol/water partition
Compound I	2.4 $\pm$ 0.6	9.7	76 $\pm$ 4 79 $\pm$ 9 [a]	$>1\times10^5$
Compound II	0.9 $\pm$ 0.7	2.2	61 $\pm$ 3	2.1
Compound III	2.0 $\pm$ 0.7	2.2	8 $\pm$ 23	1.9

[a] 0.24 mg/kg dose.

The modified peptide structure of some recent renin inhibitors appears to be resistant to the luminal and intestinal tissue degradation (Kleinert et al., 1992). For compound I, the intestinal metabolism was also found to be minimal (Hauswold et al., 1990). For the majority of the renin inhibitor compounds, low intrinsic membrane permeability and high FPLE appear to limit their systemic absorption (Cumin et al., 1990; Rosenberg et al., 1991; Rush et al., 1991; Kleinert et al., 1992).

In order to determine the role of intestinal transport and FPLE in the oral delivery of the renin inhibitor compounds, the intraduodenal and intraportal rat in situ models were used along with the oral and i.v. in vivo models.

In Fig. 2a–c, the systemic plasma levels of compounds I–III in the rat, following intraportal delivery of 1 mg/kg doses are shown. in the same graphs the results of the i.v. studies are given. In Fig. 2a, the results of the intraportal delivery experiments for compound I with a 0.24 mg/kg dose are also shown. The extent of intestinal transport and FPLE values that are calculated from the oral, i.v. and intraportal studies are tabulated in Table 2. These results indicate that the intestinal transport and FPLE of compound I are the highest among the three analogs. Compound II shows low intestinal transport and high

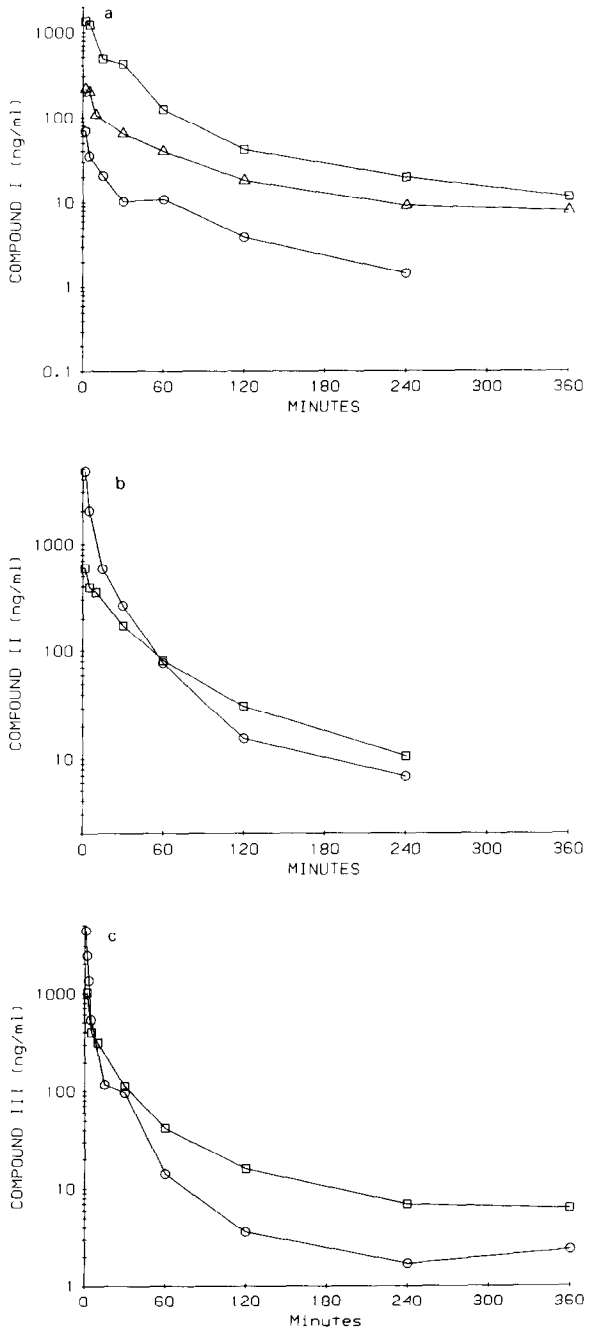


Fig. 2. (a) The systemic plasma concentrations (mean ± SE) of compound I following i.v. ((□), $n = 4$) and intraportal ((△), $n = 6$) delivery at 1 mg/kg. (○) Intraportal delivery results at 0.24 mg/kg dose ($n = 6$). (b) The systemic plasma concentrations (mean ± SE) of compound II following i.v. ((○), $n = 6$) and intraportal ((□), $n = 5$) delivery of 1 mg/kg doses. (c) The systemic plasma concentrations (mean ± SE) of compound III following i.v. ((○), $n = 4$) and intraportal ((□), $n = 4$) delivery of 1 mg/kg doses.

FPLE and compound III displays low intestinal transport and low but variable FPLE. In the present studies with bolus injections, the FPLE of compound I is dose independent at dose levels between 0.24 and 1 mg/kg. At these doses, saturation of the liver extraction does not occur for compound I. The high extraction of compound I found in this study using the bioassay for plasma analysis is consistent with the results of Hauswold et al. (1990) who used a HPLC method for plasma analysis. In their study, it was found that for compound I, both bile secretion and microsomal degradation contributed to high FPLE in the rat. Experiments in laboratory animals with many compounds over a wide range of molecular weights revealed a substantial increase in the percentage of compound excreted in the bile as the molecular weight of the compounds increased (Abou-El-Makarem et al., 1967). The molecular weight of the compounds used in this study ranges from 617 to 701 and they are therefore highly susceptible to biliary secretion The high FPLE of hydrophobic compound I is also consistent with the results of Hunter et al. (1990) who found that hydrophobic tetrapeptides regardless of their charge were extracted 30–86% by rat liver. The extraction of the charged hydrophilic tetrapeptides was negligible. Compounds II and III are hydrophilic and have similar structures and partition coefficients (Scheme 1 and Table 2). The FPLE value of compound III is highly variable. The reason for the higher FPLE value of compound II relative to compound III is not understood.

In the intraduodenal in situ rat model, the drug solutions were injected directly into the duodenum and portal and systemic blood was sampled as a function of time. The use of the same drug solutions in the in vivo and in situ studies (except for compound III), and the injection of the drug solution into the duodenum rather than perfusion were expected to improve the correlation of the in situ and in vivo results. The intraduodenal method has also been used successfully in dogs, monkeys, ferrets and rats by Rosenberg et al. (1991) and Kleinert et al. (1992) in the discovery of orally active renin inhibitors.

In Fig. 3, the portal plasma concentrations of compounds I–III following intraduodenal instilla-

tion of 10 mg/kg doses are shown. The maximum portal plasma concentrations of compounds I–III were 2.8 ± 0.5, 0.5 ± 0.2 and 0.2 ± 0.1 (mean ± SE) μg/ml, respectively. In the same studies, the systemic plasma concentrations of compounds I–III at 60 min were found to be 70 ± 20, 57 ± 29 and 18 ± 8 (mean ± SE) ng/ml, respectively. The ratio of portal to systemic drug plasma levels confirms that the FPLE of compound I is the highest. The intraduodenal tAUC value of compound I (132 ± 17 μg ml^{-1} min) is 8–26-fold higher than those for compounds II (16.2 ± 7.3 μg ml^{-1} min) and III (5 ± 1.2 μg ml^{-1} min), confirming that the intestinal transport of this compound is the highest among the three analogs. In the intraportal and oral delivery studies, the intestinal transport of compound I relative to compounds II and III was estimated as 4.4-fold higher, while the intestinal transport of compounds II and III was about equal (Table 2). The discrepancy in the relative amount of compounds transported in the two studies may be due to different experimental conditions (surgery, anesthesia, duration of the study and formulations). The octanol/water partition coefficient of compound I is greater than 1×10^5 compared to only 2 for the charged analogs (Table 2). The higher transport of compound I thus correlates well with its higher lipophilicity. Kleinert et al. (1992) also found higher intestinal transport for more lipophilic renin inhibitor compounds.

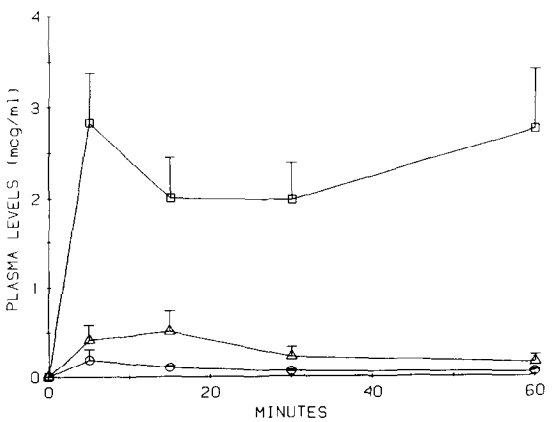


Fig. 3. The portal plasma concentrations (mean ± SE) of compound I ((□), $n = 4$), compound II ((△), $n = 5$) and compound III ((○), $n = 5$) following intraduodenal administration of 10 mg/kg doses.

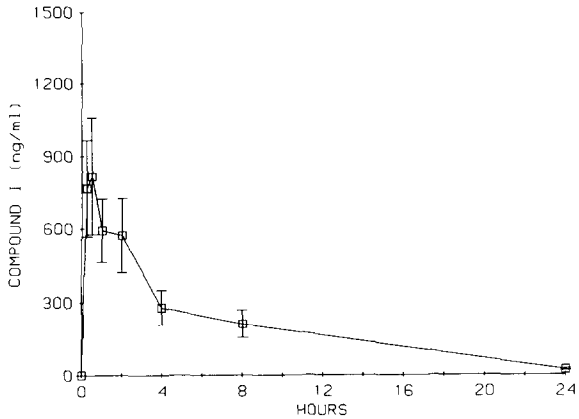


Fig. 4. The systemic plasma concentrations (mean ± SE) of compound I following intraperitoneal delivery of 10 mg/kg dose; $n = 6$.

Intraperitoneal administration can also be used to determine the extent of FPLE for compounds (Lucas et al., 1971). The systemic plasma levels of Compound I following intraperitoneal administration of a 10 mg/kg dose are given in Fig. 4. From the ratio of the tAUC values for the intraperitoneal (263 ± 63 μg ml^{-1} min) and i.v. experiments, FPLE of compound I was estimated as $25 \pm 16\%$ (mean ± SE). After comparison with the intraportal delivery results, it is apparent that intraperitoneal delivery may not be reliable in determining the FPLE values at least for compound I. The systemic absorption of drugs from intraperitoneal administration can be complicated by non-portal absorption, precipitation and incomplete absorption of drugs in the peritoneal cavity.

Overall, the results indicated that the intestinal transport and FPLE of compound I was the highest among the three analogs. Compound II showed low intestinal transport and high FPLE and compound III showed low intestinal transport and low but variable FPLE.

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