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COMPARISON OF PLASMA LEVELS AND EXCRETORY ROUTES BETWEEN NO. 189 AND NO. 407, POTENT THROMBIN INHIBITORS#

Koichiro ODA*, Kunimiki OHTSU*, Yoshikuni TAMAO*,
Ryoji KIKUMOTO*, Akiko HIJIKATA**, Kiyokatsu KINJO**
and Shosuke OKAMOTO**

*Central Research Laboratories
Mistubishi Chemical Industries Ltd.

**1st Department of Physiology
Kobe University School of Medicine

INDEXING WORDS

synthetic thrombin-inhibitor; arginine derivative; absorption; excretion; plasma level

SYNOPSIS

Two potent thrombin inhibitors, No. 189 and No. 407, have equivalent activities in vitro. However, No. 407 offered a greater margin of safety in toxicity test as compared with No. 189. In general, the pharmacokinetic characteristics of a drug, especially plasma levels and distribution, are responsible for the toxicity and moreover for the principal therapeutic efficacy. For the purpose of estimating the difference in pharmacological activity between No. 189 and No. 407, plasma levels after various routes of administration (intravenous injection (i.v.), continuous intravenous infusion (constant infusion), subcutaneous injection (s.c.) and oral administration (p.o.)) were investigated.

The administration of No. 407 to rabbits resulted in high and

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Authors' names in Japanese: 小田晃一郎　大津国幹　玉尾嘉邦　菊本亮二　土方明子　金城清勝　岡本彰祐

sustained plasma levels exceeding markedly that of No. 189. The relative bioavailability was estimated by comparing the Areas Under the plasma concentration - time Curves (AUC). The % ratio of AUC of No. 407 to No. 189 was 4.9 by i.v., 8.5 by s.c. and 72.5 by p.o. The ratio of AUC of p.o. to i.v. was 3.4% in No. 407 and 0.23% in No. 189. In the experiments of constant infusion of No. 407, lower plasma levels were obtained by the infusion into the hepatic portal vein as compared with those by the infusion into the femoral vein.

Subsequently, in order to clarify the difference in the plasma concentrations between No. 189 and No. 407, *in situ* intestinal absorption, excretion and binding to liver homogenate were investigated. In absorption experiments, no significant difference was observed between No. 189 and No. 407. The values of percent absorbed in 1 hour were 8.7% with No. 189 and 12.5% with No. 407. These two inhibitors would be ranked among poorly absorbable compounds. In excretion experiments, 52.5% of No. 189 and 67.0% of No. 407 were excreted within 3 hours after constant infusion in rabbits. In that case, the renal recovery was 7.5% in No. 189 and 32.5% in No. 407. In binding experiments, 50-60% of No. 189 was bound to liver homogenate (mainly composed of the membrane and nuclei fraction of liver) but only 10% was bound in case of No. 407.

From the results of these experiments, it is suggested that hepatic first-pass uptake and biliary excretion were the most important factors by which the anti-thrombin activity of these inhibitors in plasma was mainly determined. In addition, the marked difference in the distribution volume seems to be one of the factors by which toxicity of a drug is influenced.

Furthermore, species difference as to plasma levels and excretory routes was examined using No. 407. Lesser plasma levels were obtained by the administration to beagle dogs than to rabbits after i.v. and p.o. Especially by p.o., these differences were markedly great. Excretion in the rabbits was predominantly renal (67% total recovery) whereas in the dogs predominant biliary excretion (59% of total recovery) was observed.

COMPARISON BETWEEN TWO POTENT THROMBIN INHIBITORS

INTRODUCTION

Thrombin acts not only in converting fibrinogen to fibrin monomers but also in inducing the aggregation of platelets and triggering their release reaction. Therefore, thrombin is considered to play important roles in generating thrombosis. For the purpose of preventing the generation of thrombosis, a potent thrombin-inhibitor has been seriously desired. Heparin is the one that inhibits the action of thrombin potently and is widely used for the prophylaxis of thrombosis. However, it gives no effect by oral administration, because high molecular weight compounds are hardly absorbed from the gastrointestinal tract.[10,20]

For the purpose of searching for orally active thrombin-inhibitors, we have synthesized many chemical compounds which belong to arginine derivatives. Among these compounds, numerous compounds with a potent thrombin inhibitory effect were found by in vitro test. No. 189 and No. 407 are the representative compounds in this series of the synthetic thrombin-inhibitors. Although they have equivalent activities in in vitro test (I_{50} in fibrinogen clotting: 0.3 μM), No. 407 offered a greater margin of safety in toxicity test as compared with No. 189.[13,14] Considering these factors, it would be suspected that pharmacokinetically they behave differently in the body. Many factors have been reported to influence the pharmacokinetic properties of drugs. The degree of intestinal absorption,[17] the binding to plasma protein,[12] apparent volume of distribution[3] and excretory routes[16] are regarded as most influential factors.

The aim of the present investigation was to compare the plasma levels and the pharmacokinetic properties between No. 189 and No. 407.

MATERIALS AND METHODS

Matetials.

Inhibitors, No. 189 (N-dansyl-L-arginine-4-methylpiperidine amide · $2CH_3COOH$) and No. 407 (N-6,7 dimethoxynaphthalene-2-sulfonyl-L-arginyl-N-methoxyethylglycine), were synthesized by authors. All other chemicals and solvents were reagents grade unless otherwise indicated.

Experimental Animals.

Animals used were male Wistar albino rats weighing 180 to 220 g, male albino rabbits weighing 1.8 to 2.1 kg and male beagle dogs weighing 10 to 12 kg.

Methods and Dosages of Administration of Drugs.

For intravenous and subcutaneous injection, the drugs were dissolved in physiological saline and administered through either the femoral vein or the hypoderm of the back. On the other hand, the dosage form for oral administration was either diluted HCl solution (pH 2) or capsules filled with powder. Before oral administration, rabbits and beagle dogs were fasted for 24 hours except for tap water ad libitum.

Continuous Intravenous Infusion in Rabbits.

After held on their back, rabbits were given drugs by constant infusion from the femoral vein or the portal vein. In the case of portal infusion, the abdominal cavity was kept opened during the experiments while preventing the decline of body temperature.

Sampling of Blood.

Blood samples were collected as citrated blood from the foreleg vein in beagle dogs, and from the earlobe and carotid artery in rabbits. After centrifugation and isolation of plasma, anti-thrombin activities in plasma were measured by clotting time method.

Biliary and Renal Excretion.

The abdominal cavity was opened under anesthesia by ethyl-ether, subcutaneous urethane or intraperitoneal pentobarbital, and a thin polyethylene catheter was introduced into the bile duct to collect the bile in test tubes. For urine collection, the cannulation of a polyethylene catheter into the ureter[1] or the direct sampling from urinary bladder by ligating urinary tract was conducted according to circumstances.

Absorption through Rat Small Intestine.

According to the recirculating perfusion method which was derived by Schanker et al.,[6,15] the amount of the drug which disappeared in the perfusate was regarded as the amount absorbed through the site of the lumen which was exposed to the perfusate.

Rats were anesthetized by an intraperitoneal injection of 5 mg pentobarbital per 100 g body weight. The small intestine was exposed by a midline abdominal incision and cannulated at the both ends of the proximal duodenal and distal ileal opening with silicon tubings. The bile duct was ligated to prevent any influx of fluid into the small intestine during the experiments. These ligatures were made carefully not to occlude any blood vessels. The intestine was stored in the abdomen and incision was closed and the cannula was connected to a roller pump (FURUE SCIENCE, CO. LTD.). Thirty ml of the perfusate were recirculated from duodenum to ileum at a rate of 4 ml per minute. After 1 hour, the perfused solution in the small intestine was withdrawn as completely as possible, and the intestine was washed with physiological saline. From the difference in the amounts of drugs between the initial perfusion solution and the combined effluent, the eliminated amount of the drug from the perfused solution was calculated. In these experiments, drug perfused solution was prepared with isotonic buffer solution of pH 6.5 (0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4).

Binding to the Liver Homogenate.

Male albino rabbits, weighing 1.8 to 2.1 kg, were used in this experiment. After the abdominal cavity was opened under ether anesthesia, the liver was removed, homogenized with 3-fold 0.15 M tris-HCl buffer (pH 7.4) and filtrated through a gauze. Five ml of rabbit liver homogenate was added to 5 ml of the drug solution (No. 189 or No. 407), whose concentration was 5 μM or 50 μM, and incubated at 37°C. After 10-20 minute incubation, the solution of the mixture was centrifuged at 10,000 g for 15 minutes and the amount of inhibitors in supernatant fluid was determined by the clotting time method.

Determination of Inhibitor's Concentration.

Thrombin clotting time method was used to determine the amounts of inhibitors in plasma, bile, urine and supernatant fluid of liver homogenate.

In absorption experiments, the amounts of inhibitors in perfused solution were determined by fluorometric method. After diluted by 60% ethanol solution to 25-50 times, the perfused samples were measured fluorometrically by HITACHI spectrofluorometer

of model 512. The optimum excitation and emission wave lengths were 342 nm and 530 nm respectively in No. 189 and 292 nm and 355 nm in No. 407.

Analytical Methods of Other Drugs.

Phenol red: Five ml of 1 N NaOH was added to 1 ml of sample and the mixture was analyzed spectrophotometrically at the wave length of 550 nm using HITACHI spectrophotometer of model 124.

Bromosulfophthalein: Five ml of 0.2 M Na_2CO_3 solution was added to 1 ml of sample and the mixture was analyzed spectrophotometrically at 580 nm.

Caffein: Six ml of chloroform was added to 1 ml of sample and the mixture was shaken for 30 minutes. After centrifugation, the chloroform phase was analyzed spectrophotometrically at 280 nm.

Salicylic acid: Four ml of 4 N HCl and 5 ml of chloroform were added to 4 ml sample and the mixture was shaken for 15 minutes and centrifuged. The chloroform phase was analyzed spectrophotometrically at 310 nm.

RESULTS

Plasma Concentrations after Administration through Various Routes.

The plasma concentrations of No. 189 and No. 407 after intravenous injection (i.v.) of 5 mg/kg each to rabbits are shown in Fig. 1. It can be seen from these curves that administration of No. 407 to rabbits showed higher and longer sustained plasma levels than that of No. 189. Nine minutes after administration the plasma concentrations reduced to 20 μM in No. 407 and 1.9 μM in No. 189. The plasma concentrations of No. 407 was still 0.85 μM at 72 minutes after administration, whereas the concentration of No. 189 was below 0.1 μM.

Biological half-lives and volumes of distribution for No. 189 and No. 407 were determined graphically. They are listed in Table 1. Although little difference was detected as to the biological half-lives of them, marked difference was detected as to the volume of distribution. The volume of distribution of No. 189 (21 ℓ/kg) is 16 times as large as that of No. 407 (1.3 ℓ/kg).

The plasma concentrations after subcutaneous injection of 50 mg/kg are shown in Fig. 2. Thirty minutes after administration the plasma concentration reached 47 μM in No. 407 and 11 μM in

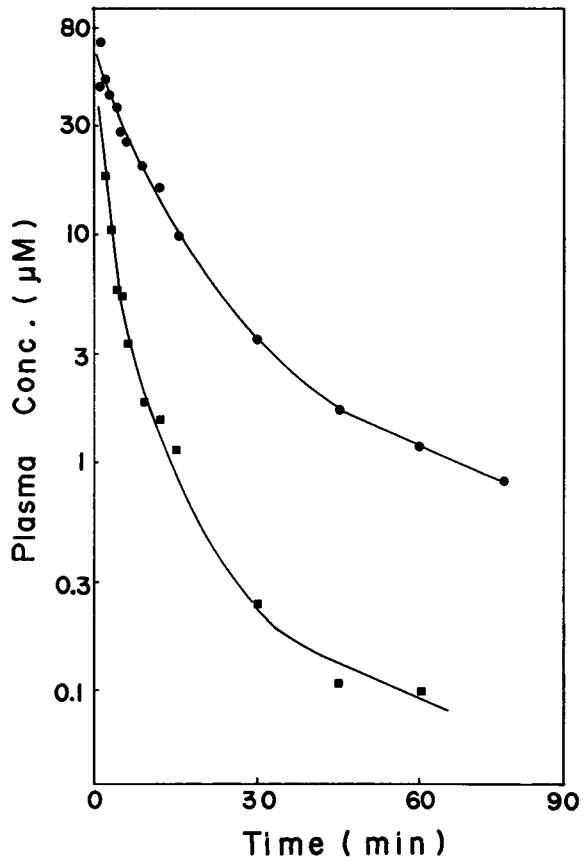

Fig. 1 Plasma levels in rabbits after i.v. injection of 5 mg/kg each of No. 189 and No. 407.

-●- No. 407
-■- No. 189

Table 1 Comparative pharmacokinetic data for No. 189 and No. 407 in rabbits after i.v. injection of 5 mg/kg dose.

inhibitor	*peak plasma level (μM)	biological half-life (min.)	distribution volume (ℓ/kg)
No. 189	44	26	21
No. 407	70	23	1.3

* 1 minute after i.v. injection

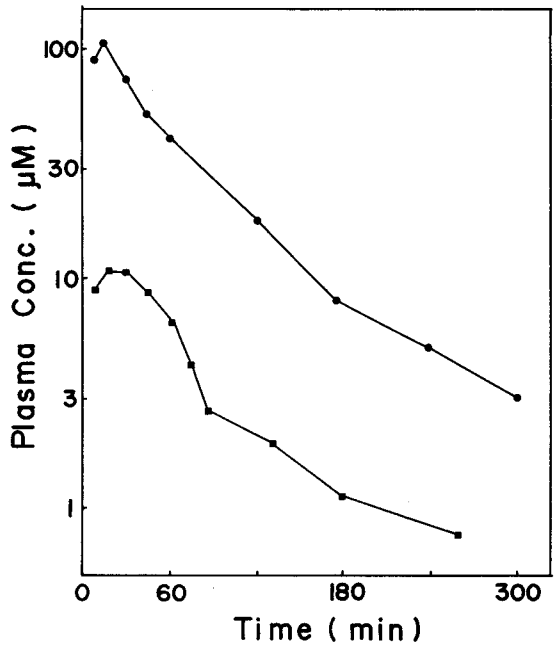

Fig. 2 Plasma levels in rabbits after s.c. injection of 50 mg/kg each of No. 189 and No. 407.

-●- No. 407
-■- No. 189

No. 189. As in the case of intravenous injection, the plasma levels of No. 189 were lower and lasted shorter than those of No. 407.

Fig. 3 shows the plasma levels after oral administration of No. 189 and No. 407. In No. 189, the peak plasma concentration of 0.15 μM was attained at 44 minutes after administration of 240 mg/kg. In No. 407, each plasma concentration was compared at two different dose levels (80 mg/kg and 240 mg/kg). Each of the peak plasma concentrations was 5.3 μM at 136 minutes after 240 mg/kg administration and 2.0 μM at 120 minutes after 80 mg/kg administration. From these data, the oral administration of No. 407 to rabbits resulted in high and sustained plasma levels exceeding markedly that of No. 189 even in the comparison of 80 mg/kg dose of No. 407 with 240 mg/kg dose of No. 189. In addition, the peak plasma

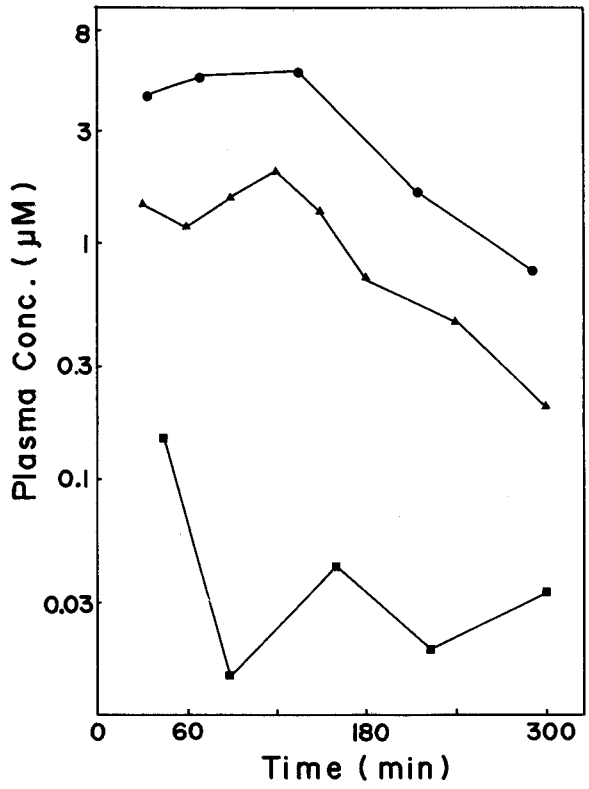

Fig. 3 Plasma levels in rabbits after p.o. administration of No. 189 or No. 407. Drugs were dissolved in diluted HCl solution.

-●- No. 407 (240 mg/kg)
-▲- No. 407 (80 mg/kg)
-■- No. 189 (240 mg/kg)

concentration of No. 189 was lower than I_{50} value of No. 189 in fibrinogen clotting. From this fact, No. 189 would be ineffective to the animal thrombosis by oral administration.

The plasma levels during constant infusion are shown in Fig. 4 (No. 189) and in Fig. 5 (No. 407). In No. 189, plateau levels, 1.0 - 1.2 and 4.0 - 4.2 μM, were established at rates of 49 μg/kg/min. and 103 μg/kg/min. respectively in infusion into the femoral vein. But no steady-state levels were attained at the infusion rate of 224 μg/kg/min. In No. 407, plateau levels, 0.69 - 0.70 and 8 - 9 μM, were established at rates of 7.5 μg/kg/min.

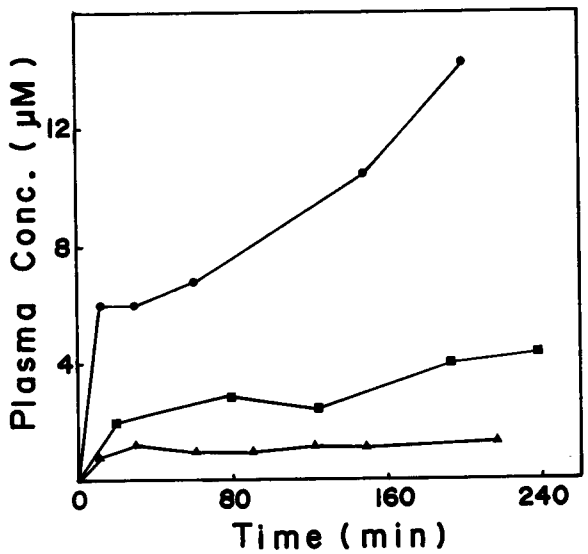

Fig. 4 Plasma levels in rabbits during continuous i.v. infusion of No. 189 into the femoral vein.

-●- 224 μg/kg/min.
-■- 103 μg/kg/min.
-▲- 49 μg/kg/min.

and 75 μg/kg/min. respectively in infusion into the femoral vein. It can be seen from these data that twice higher plateau levels were attained even by a lower dose (75 μg/kg/min.) of No. 407 in comparison with the dose of 103 μg/kg/min. of No. 189. Furthermore, the infusion into the hepatic portal vein led to lower plasma concentrations than the infusion into the femoral vein, which indicates that liver is a significant site of the elimination of No. 407.

In the experiments of thrombin infusion-induced DIC model in rabbits, the decrease in platelet counts caused by thrombin was prevented almost completely at doses of 75 μg/kg/min. and 15 μg/kg/min. At 3.75 μg/kg/min., the inhibition of decrease in platelet count was about 50%.[4] In these experiments, No. 407 was infused from 5 minutes before the thrombin infusion. The plasma concentration at 10 minutes after constant infusion was 4 μM at a dose of 75 μg/kg/min. and 0.4 μM at a dose of 7.5 μg/kg/min. According

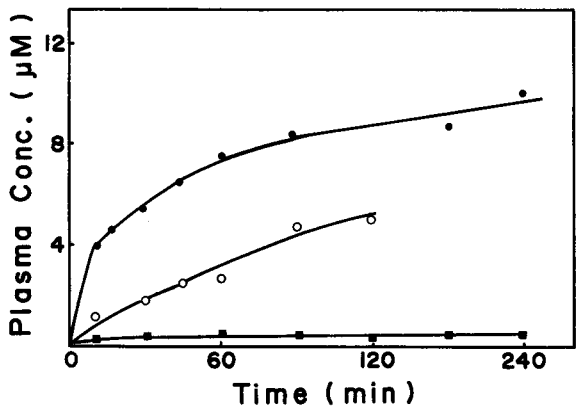

Fig. 5 Plasma levels in rabbits during continuous i.v. infusion of No. 407 into the femoral vein or the portal vein.

-●- 75 μg/kg/min. (femoral vein)
-○- 75 μg/kg/min. (portal vein)
-■- 7.5 μg/kg/min. (femoral vein)

to these linear relations between plasma concentration and infusion dose, the plasma concentrations at 10 minutes after infusion of several doses were estimated. Each of the estimated concentrations were 0.8 μM at 15 μg/kg/min. and 0.2 μM at 7.5 μg/kg/min. From these values the minimal effective plasma concentration for this DIC model was found to be 0.2 μM.

Furthermore, in the experiments of the animal thrombosis induced by acetic acid, the formation of thrombi was markedly prevented at doses of 94 μg/kg/min. and 188 μg/kg/min.[7] In these experiments, No. 407 was infused from 5 minutes before the treatment with acetic acid and the infusion was continued for 180 minutes. The plasma concentrations at 5 minutes and 180 minutes after infusion were shown in this study to be 5 μM and 10 μM respectively at a dose of 94 μg/kg/min. Thus, the minimal effective plasma concentration for this thrombosis model seemed to be 5 - 10 μM.

Fig. 6 shows comparative plasma levels of No. 407 between

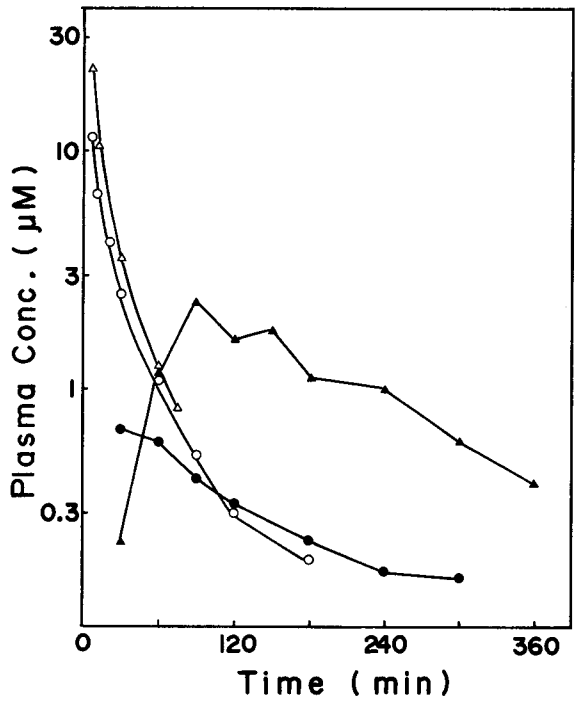

Fig. 6 Comparative plasma levels of No. 407 between rabbits and beagle dogs after i.v. injection (5 mg/kg) and oral administration (80 mg/kg). The dosage form for p.o. was capsules filled with powder.

-△- i.v. (rabbit)
-▲- p.o. (rabbit)
-○- i.v. (dog)
-●- p.o. (dog)

rabbits and beagle dogs after i.v. injection (5 mg/kg) and oral administration (80 mg/kg). In oral administration, the powder of No. 407 was capsulated with soluble starch in each hard gelatin capsule of size No. 3 for rabbits and No. 0 for dogs. Although little difference was detected about the plasma levels after i.v. injection between them, marked difference was detected about the plasma levels after oral administration. The peak plasma concentration of 0.67 μM was obtained at 30 minutes after administration in case of dogs, and at 90 minutes after administration the peak plasma concentration of 2.3 μM was obtained in case of rabbits.

It can be seen from this data that oral administration to rabbits resulted in high and sustained plasma levels exceeding markedly that to beagle dogs.

The bioavailabilities of No. 189 and No. 407 after oral administration to rabbits and beagle dogs are shown in Table 2. Each AUC value was calculated by the trapezoidal rule. The % bioavailability was obtained by dividing AUC values of oral administration by AUC values of the corresponding dose of i.v. injection. In No. 189, the bioavailability of oral administration was only 0.2%, whereas No. 407 showed 15-20 times higher bioavailability of oral administration than No. 189, which was not affected by dosage forms or dose levels. The bioavailability in beagle dogs was somewhat lower than that in rabbits. However, even the bioavailability of 3.5-4.2% in rabbits would be ranked among the most poorly available drugs. In order to elucidate the difference in availability between No. 189 and No. 407, their intestinal absorbabilities are compared with other well-known drugs in next section.

Table 2 Comparative bioavailability of oral administration of No. 189 and No. 407 in rabbits and dogs.

species	dosage form	dose (mg/kg)	A U C (μM·min.)	*bioavailability (%)
rabbit	No. 189 soln**	240	13	0.2
	No. 407 soln**	240	930	3.6
	No. 407 soln**	80	310	3.5
	No. 407 capl***	80	370	4.2
dog	No. 407 capl***	80	110	2.3

* % bioavailability = AUC of p.o./AUC of i.v.

** soln: diluted HCl solution (pH 2)

*** capl: capsule (No. 407 powder with soluble starch)

Absorption from the Rat Small Intestine.

The absorbabilities of No. 189 and No. 407 in the rat small intestine were examined by *in situ* recirculation technique of Kakemi and others.[9] Table 3 shows the values of the percent

Table 3 *In situ* intestinal absorption of drugs from isotonic solution of pH 6.5 in the rat.

drug	concentration	absorption in 1 hr. (%)
phenol red	10 μg/ml	2.1
bromsulfophthalein	0.1 mM	19.2
caffeine	1.0 mM	64.4
salicylic acid	1.0 mM	71.8
No. 189	0.5 mM	8.7
No. 407	0.5 mM	12.5

The absorption in percent in 1 hr. is expressed as the mean of 4-5 animals.

absorbed in 1 hour. The reference drugs were chosen as the standard compounds referring to intestinal absorbability. The standard of poorly absorbable compounds were phenol red[9] and bromosulfophthalein,[19] and the standard of highly absorbable compounds were caffeine[8] and salicylic acid.[8] The absorption of No. 189 and No. 407 in percent were 8.7% and 12.5%, respectively, and no significant difference was detected between them. In addition, these values were ranked between the values of phenol red and bromosulfophthalein. From these facts, No. 189 and No. 407 would be estimated as poorly absorbable compounds.

In this method, no influence was suffered as to species difference, because the structure of biological membrane seems to be analogous in every animal.[18] Consequently, the difference in bioavailability between No. 189 and No. 407 would be caused by the factors except absorbability.

Biliary and Renal Excretion.

In order to elucidate the excretory mechanisms about No. 189 and No. 407, modified clearance techniques were employed.[1] In this method, urine and bile are simultaneously collected in course of time and the drug concentrations in plasma are kept constant by continuous intravenous injection. Fig. 7 shows the cumulative excretion profile of No. 189. In this case, the biliary route was the major route of excretion with little amounts excreted into renal route. In case of No. 407, as shown in Fig. 8, almost equal amounts were excreted via both routes. Total recoveries in

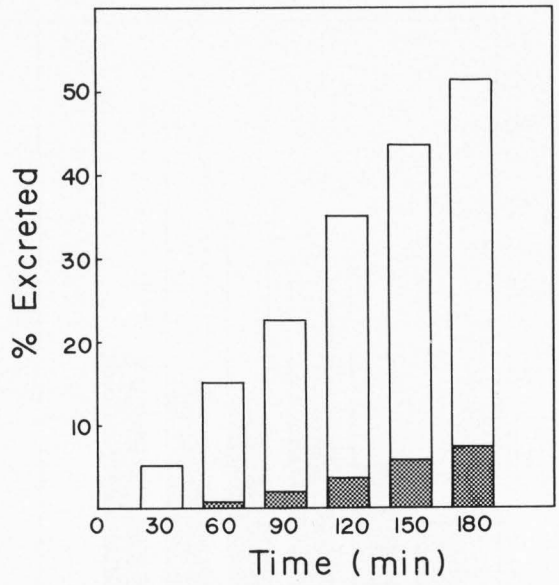

Fig. 7 Cumulative excretion of No. 189 in bile and urine after continuous i.v. infusion (75 μg/kg/min.) in rabbits.

□ % excreted in bile

▨ % excreted in urine

180 minutes were 51% with No. 189 and 66% with No. 407. In Table 4, the bile/plasma concentration ratios (B/P ratios) are compared between No. 189 and No. 407. The B/P ratios of No. 189 and No. 407 were identical. The major difference in excretory behaviour was detected in the route of excretion, namely extremely low excretion to urine in No. 189.

The excretory routes in beagle dogs were compared with the routes in rabbits in i.v. administration (Table 5). In dogs, the biliary excretion was higher than in rabbits, but the renal excretion held 42% of total excretion which was much higher than that found in No. 189 in rabbits (14%). Thus, the species difference in the excretory routes was observed but the difference was not so great as the difference between No. 189 and No. 407.

In case of rabbits, larger amounts were excreted via renal route by bolus injection compared with constant infusion. In general, the excretion via gromerular filtration in kidney is

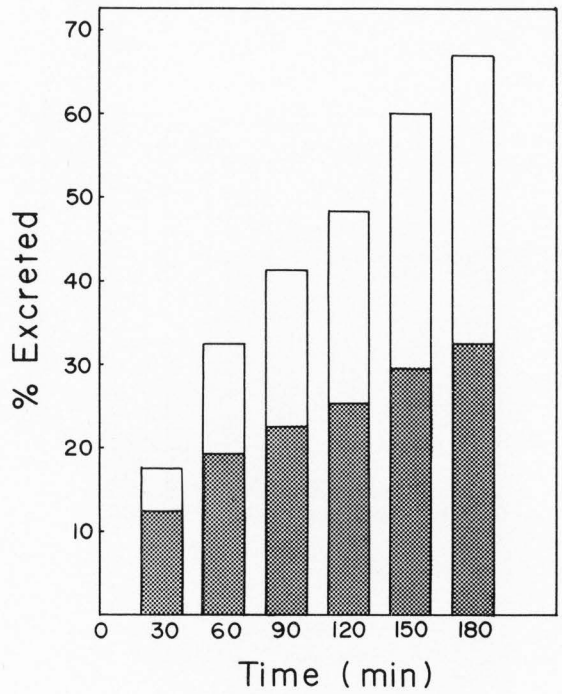

Fig. 8 Cumulative excretion of No. 407 in bile and urine after continuous i.v. infusion (75 μg/kg/min.) in rabbits.

□ % excreted in bile

▨ % excreted in urine

Table 4 Comparative B/P ratios between No. 189 and No. 407 during continuous i.v. infusion at rate of 75 μg/kg/min.

inhibitor	*plasma level (μM)	*bile level (μM)	B/P ratio**
No. 189	7.2	922	128
No. 407	13.8	1670	120

* Each value is the mean of the values from 60 minutes to 120 minutes after the start of infusion.
** bile/plasma concentration ratio

Table 5 Biliary and renal excretion after a bolus i.v. injection of No. 407 in rabbits and beagle dogs.

species	cumulative excretion (%)		
	urine	bile	total
rabbit	59.9	29.8	89.7
dog	35.0	49.4	84.4

dose: 20 mg/kg
collection period: 3 hours

considered to be in proportion to the plasma concentration. The higher renal excretion in bolus injection would result from the higher plasma concentration.

Binding to the Liver Homogenate.

The binding to rabbit liver homogenate was compared between No. 189 and No. 407 (Table 6). The fraction to which No. 189 and No. 407 are bound is the precipitate after the centrifugation at 10,000 g. This fraction is mainly composed of the membrane and nuclei fraction of liver. The percent of bound No. 189 and No. 407 were 64% and 14% respectively at the concentration of 5 μM and 50% and 8% respectively at the concentration of 50 μM. The marked difference in the binding to liver homogenate was detected between No. 189 and No. 407.

This result suggests that the drug concentrations in plasma after oral administration would be greatly influenced by the degree of affinity to the liver.

Table 6 Binding of No. 189 and No. 407 to rabbit liver homogenate.

inhibitor	concentration (μM)	% bound
No. 189	5	64
	50	50
No. 407	5	14
	50	8

DISCUSSION

The therapeutic efficacy of thrombin-inhibitor is determined by plasma concentration. In the experiments of thrombin infusion-induced DIC model in rabbits, No. 189 and No. 407 were effective at the plasma concentration of 0.2 μM. Although the plasma concentrations above 0.2 μM were easily achieved by the oral administration of No. 407 in a dose of 80 mg/kg, oral administration of No. 189 in a dose of 240 mg/kg gave the peak plasma concentration of only 0.14 μM. Because of this low plasma concentration of No. 189 after oral administration, No. 189 would not be so effective as No. 407 by oral administration. What is the mechanism to discriminate the plasma levels between No. 189 and No. 407 ?

We concluded from the present experiments that this difference resulted mainly from the difference in the affinity to liver. The shorter half-life in distribution phase in No. 189 would be explained by the fast distribution to liver and presumably to other tissues. The difference of the plasma levels after oral administration would be also explained by the difference in the affinity to liver, namely the hepatic first-pass effect. In No. 407, 2-3 times higher plasma levels were obtained by the femoral vein infusion compared with the portal vein infusion. This fact suggests that more remarkable decrease of plasma levels would be obtained by the portal infusion of No. 189, because No. 189 has a higher affinity to liver than No. 407. All of the orally administered and portally infused drugs must pass through the liver before reaching the systemic circulation. On the other hand, less than 30% of the drug administered via other routes passes through the liver in first circulatory pass.[5] Thus, the orally administered drug is removed by the liver before entering the systemic circulation in larger amount than the parenterally administered drug. In addition, the species difference in the bioavailability between rabbits and beagle dogs would result from the difference in the extent of the hepatic first-pass effect, because no significant difference was considered as to the intestinal absorbability.[11] Although these drugs showed the poor bioavailability owing to the incomplete absorption from the digestive tract, which was supported by the poor absorbability in *in situ* absorption experiments, their true absorbability is larger than the bio-

availability of No. 407 in rabbits (3-4%), since the first-pass effect was observed even in No. 407.

Brauer originally divided the substances which were excreted into bile into three classes according to their bile/plasma concentration ratios (B/P ratios) : Substances of class A are those whose ratios are nearly one (glucose, Na^+, K^+ and Cl^-), class B includes substances whose B/P ratios usually range from 10 to 1,000 (bile salt bromosulfophthalein, fluorescein and rose bengal) and class C compounds consist of those whose ratios are less than one (inulin, sucrose, phosphate and mucoproteins).[2] And he suggests that most of class B compounds are actively excreted into bile. The B/P ratios of No. 189 and No. 407 are 128 and 120, respectively. According to his classification, both No. 189 and No. 407 belong to class B compounds. Therefore, No. 189 and No. 407 are supposed to be actively excreted into bile. For the purpose of searching for a highly bioavailable drug by oral administration, the selection of the compound which suffers less from the hepatic first-pass effect is one of the points to be considered.

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