

## First-Pass Metabolism of ONO-5046 (*N*-[2-[4-(2,2-Dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetic Acid), a Novel Elastase Inhibitor, in Rats

Fumiko WATANABE,<sup>a</sup> Masahiko SATO,<sup>a</sup> Akiko KATO,<sup>a</sup> Teruo MURAKAMI,<sup>\*,a</sup> Yutaka HIGASHI,<sup>a</sup> and Noboru YATA<sup>a,b</sup>

*Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,<sup>a</sup> 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan and Faculty of Pharmaceutical Sciences, Setsunan University,<sup>b</sup> 45-1 Nagaotoge-cho, Hirakata, Osaka 573-01, Japan.* Received September 24, 1996; accepted December 11, 1996

The first-pass metabolism in the intestine and liver of ONO-5046 (*N*-[2-[4-(2,2-dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetic acid), a newly synthesized elastase inhibitor, was separately estimated in rats. When ONO-5046 solution was administered into the whole intestine *via* the bile duct at a dose of 5  $\mu\text{mol}/\text{rat}$ , the extent of bioavailability was only 1.5%. A small but significant increase in the bioavailability with an increase in the dose suggested marked first-pass metabolism with a saturable process. Hepatic first-pass metabolism was estimated by determining the hepatic extraction ratio of ONO-5046 after administration into the portal vein at two different infusion rates (5  $\mu\text{mol}/\text{kg}/9$  min or 5  $\mu\text{mol}/\text{kg}/20$  s). The extraction ratio was relatively small and constant (about 20%) under 2 different infusion rates of the drug. Intestinal first-pass metabolism was estimated by determining the drug recovery in the mesenteric plasma after administering the drug into the intestinal loop *in situ* (mesenteric blood collecting method *in situ*). The recovery percentage of ONO-5046 in the mesenteric plasma was small ( $2.58 \pm 0.04\%$  at a dose of 1  $\mu\text{mol}/\text{rat}$ ), and the remaining ONO-5046 recovered in the mesenteric plasma and in the intestinal loop was a metabolite of ONO-5046 (EI-601, *N*-[2-[(4-hydroxyphenyl)sulfonylamino]benzoyl]aminoacetic acid). Recovery percentage of ONO-5046 in the mesenteric plasma increased significantly with an increase in the dose, although the recovery percentage was still low, even at a higher dose ( $9.55 \pm 1.17\%$  of dose at a dose of 5  $\mu\text{mol}/\text{rat}$ ). These results indicate that the low oral bioavailability of ONO-5046 *in vivo* is mainly due to the marked intestinal first-pass metabolism, including the metabolism in the intestinal fluid, and the dose-dependent oral bioavailability was derived from the saturable intestinal first-pass metabolism.

**Key words** ONO-5046; elastase inhibitor; first-pass metabolism; intestinal first-pass metabolism; hepatic extraction ratio; rat

Human neutrophil elastase is the most destructive protease which hydrolyzes most connective tissue components such as proteoglycan, certain types of collagen, and especially elastine.<sup>1-3)</sup> Although plasma and interstitial fluid contain human leukocyte elastase inhibitors such as  $\alpha_1$ -proteinase inhibitor and  $\alpha_2$ -macroglobulin, these macromolecular inhibitors have been suggested to be circumvented from close contact between migrating neutrophils and substrate.<sup>4,5)</sup> On the other hand, it has been reported that a low molecular weight inhibitor of neutrophil elastase, peptide chloromethyl ketone, completely blocks the neutrophil-mediated tissue destruction by achieving a close contact with neutrophils. Many elastase inhibitors with low molecular weights have been synthesized based on the above considerations.<sup>6-9)</sup>

ONO-5046, (*N*-[2-[4-(2,2-dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetic acid) is a low molecular potent active neutrophil elastase inhibitor, which was newly synthesized by Ono Pharmaceutical Co., Ltd.<sup>10-12)</sup> ONO-5046 selectively inhibits neutrophil elastase and leukocyte elastase obtained from rabbit, rat, hamster and mouse without inhibiting trypsin, thrombin, plasmin, kallikrein, and so on.<sup>10)</sup> Also, in *in vivo* studies, this drug suppressed human neutrophil elastase-induced lung hemorrhage in hamster by intratracheal administration and suppressed an increase in capillary permeability in guinea pig by intravenous administration.<sup>10)</sup> However, it is also known that the oral bioavailability of ONO-5046 is very low, and ONO-5046 is hydrolyzed by esterase to

an inactive metabolite, EI-601 ((*N*-[2-[(4-hydroxyphenyl)sulfonylamino]benzoyl]aminoacetic acid), at a carboxylic ester linkage in the liver, intestinal mucosa, and/or intestinal fluid (unpublished data). Therefore, in the present study we analyzed each contribution of intestinal and hepatic first-pass metabolism of ONO-5046 in rats as a pre-formulation study for the oral dosage form.

### MATERIALS AND METHODS

**Materials** ONO-5046 and its metabolite, EI-601, were kindly supplied from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Other reagents used were of reagent grade.

**Animal Studies** Male Wistar rats weighing 240—300 g were used. Rats were fasted overnight prior to the experiments. Rats were anesthetized with pentobarbital (30 mg/kg, intraperitoneal injection), and were affixed supine on a surface kept at 37 °C to maintain their body temperature above 36 °C.

**Intravenous Administration** Cannulation (polyethylene tubing, PE-50) was made at a femoral vein for the administration of drug and a femoral artery for the sampling of blood, respectively. ONO-5046 was dissolved in a mixture of propylene glycol, ethanol and water (15/15/70, v/v) at a concentration of 1, 2.5 or 5  $\mu\text{mol}/\text{ml}$ . The drug solution was administered intravenously from a femoral vein *via* the cannula at a dosing volume of 1 ml/kg. Blood was taken at designated time intervals *via* the

\* To whom correspondence should be addressed.

cannula at a femoral artery.

**Intraduodenal Administration *in Vivo*** Two polyethylene tubes (PE-10) were inserted into the bile duct toward the duodenum and the liver, respectively. Cannulation was also made at a femoral artery for blood sampling. The bile was eliminated out of the body *via* the cannula inserted toward the liver. ONO-5046 was dissolved in a mixture of propylene glycol, ethanol and water (1/1/2, v/v) at a concentration of 5, 10, 25, or 50  $\mu\text{mol/ml}$ . In this solution, ONO-5046 was completely stable for more than 2 h. After intraduodenal administration of different ONO-5046 solutions at a dosing volume of 1 ml/rat *via* the cannula inserted toward the duodenum, the femoral arterial blood was periodically taken for analyses of ONO-5046 and EI-601 in plasma.

**Intraportal Administration *in Vivo*** Polyethylene tubing (PE-50) was cannulated into a pyloric vein and a femoral artery for the administration of drug solution and for blood sampling, respectively. Drug solution was prepared in the same manner as for the intravenous administration study at a concentration of 5  $\mu\text{mol/ml}$ . After a constant infusion (5  $\mu\text{mol/kg/9 min}$ ) or bolus injection (5  $\mu\text{mol/kg/20 s}$ ) of the drug solution, the femoral arterial blood was sampled periodically.

**Mesenteric Blood Collecting Method *in Situ*** After flushing the small intestine with 50 ml of saline, ligation was made at two sites of the jejunum to make a 10 cm-long loop. The mesenteric blood collecting method *in situ* was carried out as reported by Goon and Klaassen.<sup>13)</sup> Briefly, the mesenteric vein derived from the jejunal loop was cannulated with polyethylene tubing (PE-50), through which all mesenteric venous blood was collected into the heparinized tube. Lost blood was substituted by infusing fresh blood obtained from healthy rats at a flow rate of 0.38 ml/min *via* a cannula at a femoral vein. The dosing solution of ONO-5046 or EI-601 was prepared by dissolving it in distilled water with the aid of a suitable amount of NaOH (1, 2.5, and 5  $\mu\text{mol/ml}$  for ONO-5046, 1  $\mu\text{mol/ml}$  for EI-601). The pH of the solution was 9.0. After administration of ONO-5046 or EI-601 solution into the loop at a dosing volume of 1 ml/loop, all mesenteric blood was serially collected at 3, 10, or 15 min intervals. At 60 min after administration, the luminal fluid in the loop was also collected by washing it with 50 ml of ice cold saline to determine the remaining amount of ONO-5046 and/or EI-601.

**Pharmacokinetic Model Analysis** Results in intravenous and intrajejunal administration studies of ONO-5046 or EI-601 were analyzed by using the MULTI (RUNGE) program for microcomputers.<sup>14)</sup>

**Analysis** The concentrations of ONO-5046 and/or EI-601 in plasma and luminal fluid were analyzed by high performance liquid chromatography (HPLC) employing a reverse phase TSK gel ODS-80TM column (Tosoh, Tokyo, Japan). Briefly, plasma was separated from the blood by centrifugation. One hundred microliters of plasma or luminal fluid sample was mixed with 100  $\mu\text{l}$  of an internal standard (*n*-propyl *p*-hydroxybenzoate for ONO-5046, ethyl *p*-hydroxybenzoate for EI-601) solution and 1 ml of 2 N-HCl. Drugs were extracted with ethyl acetate. The organic solvent was evaporated

under reduced pressure and the residues were dissolved by adding 100  $\mu\text{l}$  of methanol. Mobile phases used were a mixture of H<sub>2</sub>O and CH<sub>3</sub>OH (50/50, v/v) containing 2% CH<sub>3</sub>COOH and 5% tetrahydrofuran for ONO-5046, and a mixture of H<sub>2</sub>O and CH<sub>3</sub>OH (65/35, v/v) containing 0.2% CH<sub>3</sub>COOH and 5% tetrahydrofuran for EI-601. Detection was made at 240 nm.

## RESULTS

**Intravenous Administration** ONO-5046 at a dosing range from 1 to 5  $\mu\text{mol/kg}$  or EI-601 at a dose of 1  $\mu\text{mol/kg}$  was administered intravenously. Both ONO-5046 and EI-601 disappeared biexponentially from plasma (Fig. 1). Some pharmacokinetic parameters were obtained by analyzing the plasma profiles according to a 2-compartment model (Table 1). Total plasma clearance (*CL*), estimated by the dose/(*A/a* + *B/b*) of ONO-5046, was relatively small compared to hepatic plasma flow, and there was no significant difference in *CL* value or distribution volume (*V<sub>d</sub>*), estimated by the dose/(*A* + *B*) of ONO-5046 among three different doses. Thus, the plasma disposition of ONO-5046 showed a linear pharmacokinetic characteristic, at least in the present dosing range. The *CL* value of EI-601 at a dose of 1  $\mu\text{mol/rat}$  ( $6.95 \pm 0.29 \text{ ml/min/kg}$ ) was to the same extent as that of ONO-5046, although the *V<sub>d</sub>* value of EI-601 was greater ( $103.7 \pm 21.7 \text{ ml/kg}$ ) by about double and the elimination rate constants were smaller compared to those of ONO-5046.

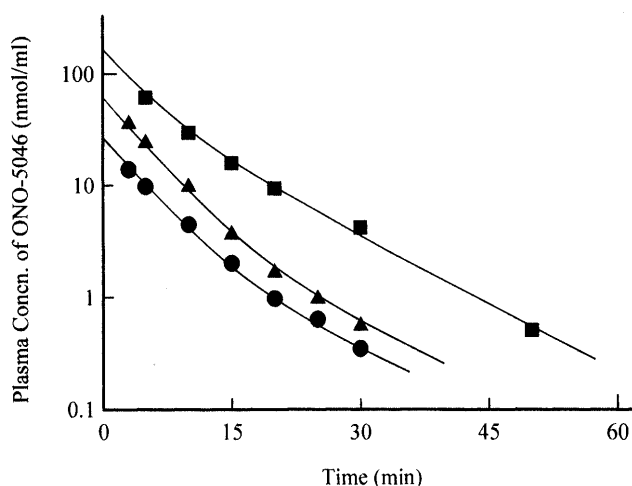


Fig. 1. Plasma Concentration of ONO-5046 after Intravenous Administration at Different Doses in Rats

●, 1; ▲, 2.5; ■, 5  $\mu\text{mol/rat}$ . Each point represents the mean of 4–7 trials.

Table 1. Pharmacokinetic Parameters of ONO-5046 after Intravenous Administration in Rats

	Dose ( $\mu\text{mol/kg}$ )		
	1	2.5	5
<i>AUC</i> (nmol·min/ml)	141.4 ± 11.8	343.5 ± 21.9	788.48 ± 55.26
<i>V<sub>d</sub></i> (ml/kg)	45.99 ± 3.01	40.42 ± 2.76	47.35 ± 2.98
<i>CL</i> (ml/min/kg)	7.345 ± 0.562	7.415 ± 0.419	6.432 ± 0.432

Each value represents the mean ± S.E. (*n* = 4–7).

**Intraduodenal Administration *in Vivo*** Plasma profiles of ONO-5046 after the intraduodenal administration of ONO-5046 at different doses (5–50  $\mu\text{mol}/\text{rat}$ ) are shown in Fig. 2. The bioavailability, estimated by the ratio of the area under the curve (AUC) of ONO-5046 in plasma estimated by a trapezoidal rule after intraduodenal administration against that after intravenous administration, was small at all of the doses, although it was significantly increased with an increase in the dose as follows:  $1.45 \pm 0.15\%$  at a dose of 5  $\mu\text{mol}/\text{rat}$ ;  $1.45 \pm 0.20$  at 10;  $2.01 \pm 0.19$  at 25;  $2.75 \pm 0.11$  at 50 (mean  $\pm$  S.E.,  $n = 3-4$ ). Plasma levels of EI-601 after the administration of ONO-5046 or EI-601 alone at a dose of 5  $\mu\text{mol}/\text{rat}$  are shown in Fig. 3. Supposing that the intestinal membrane permeabilities to ONO-5046 and EI-601 are almost comparable, close EI-601 levels in plasma after ONO-5046 and EI-601 administrations may suggest that most of the ONO-5046 administered into the duodenum is rapidly metabolized to EI-601 in the absorption process.

**Hepatic First-Pass Metabolism** ONO-5046 was administered into the portal vein at two different infusion rates

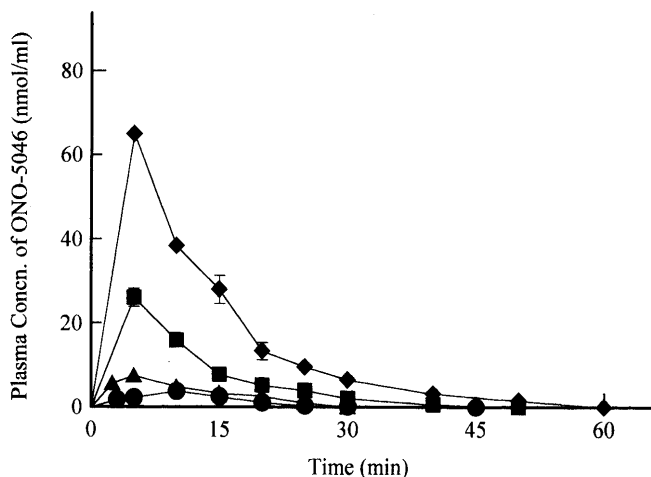


Fig. 2. Plasma Concentration of ONO-5046 after Intraduodenal Administration at Different Doses in Rats

●, 5; ▲, 10; ■, 25; ◆, 50  $\mu\text{mol}/\text{rat}$ . Each point represents the mean  $\pm$  S.E. ( $n = 3-8$ ).

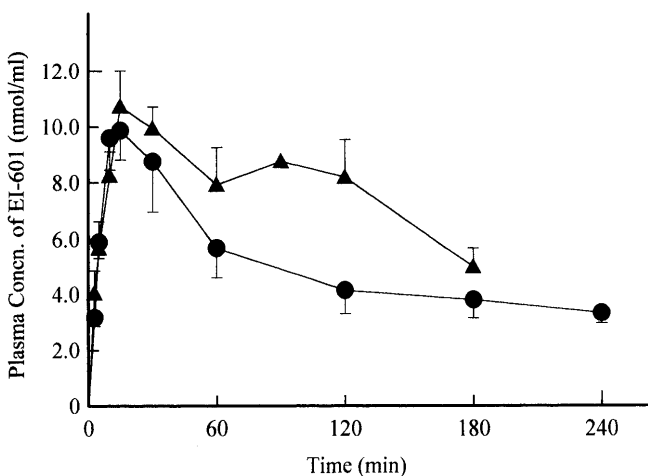


Fig. 3. Plasma Concentration of EI-601 after Intraduodenal Administration of ONO-5046 or EI-601 at a Dose of 5  $\mu\text{mol}$  in Rats

●, administration of ONO-5046; ▲, administration of EI-601. Each point represents the mean  $\pm$  S.E. ( $n = 4-5$ ).

(5  $\mu\text{mol}/\text{kg}/20\text{ s}$  or 5  $\mu\text{mol}/\text{kg}/9\text{ min}$ ). The extent of bioavailability of ONO-5046, estimated by the AUC ratio of ONO-5046 in plasma between the intraportal and intravenous administrations, was  $81.4 \pm 5.8\%$  and  $82.6 \pm 7.4\%$ , respectively. Thus, in spite of markedly different infusion rates, the hepatic extraction ratio of ONO-5046 from plasma was of the same magnitude (about 20%). This finding indicates that the hepatic first-pass metabolism of ONO-5046 is not very significant, and the dose-dependent first-pass metabolism of ONO-5046 observed after intraduodenal administration is not subject to the saturation of hepatic first-pass metabolism.

**Intestinal First-Pass Metabolism** Intestinal first-pass metabolism of ONO-5046 was determined employing a mesenteric blood collecting method *in situ*. The flow rate of mesenteric venous blood was kept constant ( $0.38 \pm 0.01\text{ ml}/\text{min}$ ) by infusing fresh blood for 60 min. Time profiles of the cumulative amount of ONO-5046 and EI-601 recovered in the mesenteric venous plasma after administration of ONO-5046 into the intestinal loop are shown in Fig. 4. As summarized in Table 2, as the dose of ONO-5046 increased (1–5  $\mu\text{mol}/\text{rat}$ ), the percentage of ONO-5046 recovered in mesenteric venous plasma within 60 min was significantly increased, and consequently, the amount of EI-601 was decreased. At any dose, about half the amount of the dose was found to be EI-601 in the intestinal fluid, although a small amount of ONO-5046 was also detected at the highest dose of ONO-5046. The sum of ONO-5046 and EI-601 recovered from the loop and mesenteric plasma was about 65% of the dose. The recovery of EI-601 within 60 min in mesenteric plasma after administration of EI-601 alone (dose 1  $\mu\text{mol}/\text{rat}$ ) was  $29.9 \pm 3.3\%$ , and  $70.0 \pm 3.3\%$  of dose was found in the loop (the mean  $\pm$  S.E.,  $n = 5$ ). Thus, in the case of EI-601, EI-601 was almost completely recovered from the mesenteric plasma and intestinal fluid. The reason for the lower total recovery percentage in the case of ONO-5046 is not clear at present. However, it may be that the unrecovered amount is retained in the intestinal tissue as

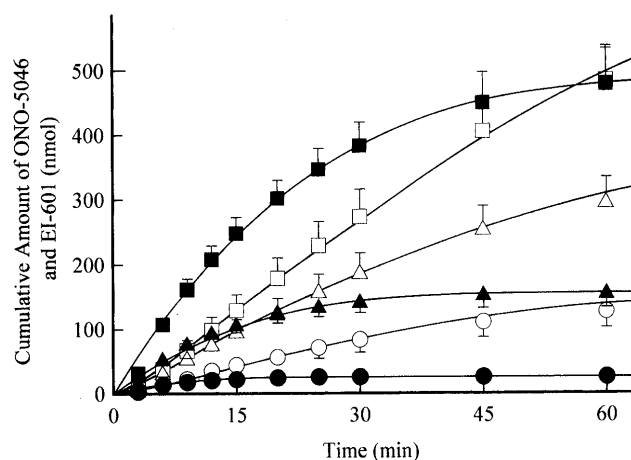


Fig. 4. Cumulative Amount of ONO-5046 and EI-601 Recovered in Mesenteric Venous Plasma after *in Situ* Intraluminal Administration of ONO-5046 at Three Different Doses in Rats

●, ○, 1; ▲, △, 2.5; ■, □, 5  $\mu\text{mol}/\text{rat}$ . Closed symbol, ONO-5046; open symbol, EI-601. Each solid line represents the fitting curve which was calculated using parameters listed in Table 3 by the use of a proposed model (Fig. 5). Each point represents the mean  $\pm$  S.E. ( $n = 3-4$ ).

Table 2. Recovery Percent of ONO-5046 and EI-601 after *in Situ* Intraluminal Administration of ONO-5046 at Various Doses in Rats

	Dose ( $\mu\text{mol}/\text{rat}$ )		
	1	2.5	5
ONO-5046 in plasma (% of dose)	2.58 ± 0.04	6.24 ± 0.89 <sup>b)</sup>	9.55 ± 1.17 <sup>b)</sup>
EI-601 in plasma (% of dose)	12.71 ± 2.51	11.85 ± 1.51	9.67 ± 0.98
ONO-5046 in intestinal loop (% of dose)	ND <sup>a)</sup>	ND <sup>a)</sup>	16.09 ± 6.63
EI-601 intestinal loop (% of dose)	47.54 ± 2.82	47.80 ± 2.80	33.37 ± 3.92 <sup>b,c)</sup>
Amount of recovery (% of dose)	62.83 ± 2.35	65.89 ± 1.88	68.68 ± 4.48

a) Not detected. b) Significantly different from 1  $\mu\text{mol}$  dose,  $p < 0.05$ . c) Significantly different from 2.5  $\mu\text{mol}$  dose,  $p < 0.05$ . Each value represents the mean  $\pm$  S.E. ( $n = 3-5$ ).

ONO-5046 and/or EI-601, since EI-601 is not metabolized further.

**Pharmacokinetic Analysis** We tried to analyze the intestinal and hepatic metabolism of ONO-5046 during the absorption process after intraluminal administration of ONO-5046 pharmacokinetically using some pharmacokinetic models. The model shown in Fig. 5 showed the best fitting on the observed values of ONO-5046 and EI-601. The solid lines in Fig. 4 are the fitting curves as calculated, and the observed values fitted well on the curve.

The differential equations employed for the calculation were as follows:

$$dX_1/dt = k_{51}X_5 \quad (1)$$

$$dX_2/dt = k_{62}X_6 \quad (2)$$

$$dX_3/dt = -(k_{34} + k_{35} + k_{37})X_3 \quad (3)$$

$$dX_4/dt = k_{34}X_3 - k_{46}X_4 \quad (4)$$

$$dX_5/dt = k_{35}X_3 - (k_{51} + k_{56})X_5 \quad (5)$$

$$dX_6/dt = k_{46}X_4 + k_{56}X_5 - k_{62}X_6 \quad (6)$$

$$dX_7/dt = k_{37}X_3 \quad (7)$$

Where,  $X_3$ ,  $X_5$  and  $X_1$  represent the fractions of ONO-5046 in the loop, intestinal tissue and plasma compartments, respectively.  $X_4$ ,  $X_6$  and  $X_2$  denote the fractions of EI-601, respectively, as well. Also, the unrecovered amount of drug which may be trapped in the intestinal tissues was regarded as a lost amount in the loop ( $X_7$ , unknown).  $X_3$  at time 0 is the dose, and others at time 0 are 0. For the calculation of each transfer rate constant between each compartment, simultaneous analysis was made employing a MULTI(RUNGE) program and an algorithm of Simplex followed by Damping Gauss-Newton methods,<sup>14)</sup> based on experimental data after the administration of ONO-5046 or EI-601 (each 1  $\mu\text{mol}$  dose). For calculation of a higher dose of ONO-5046, the transfer rate constant of EI-601 was fixed at the value of 0.00643 for  $k_{46}$  and 0.62627 for  $k_{62}$ , respectively. The transfer rate constants of EI-601,  $k_{46}$  and  $k_{62}$ , were separately calculated using equations (2), (4) and (6), and the curve calculated fitted well on the observed values of EI-601 in the mesenteric venous plasma. As summarized in Table 3, the obtained parameters indicate that 95.3% of the dose of ONO-5046, calculated by  $(k_{34} + k_{37})/(k_{34} + k_{35} + k_{37})$ , is metabolized in the intestinal loop, including an unknown fraction. Also, 45.4% of the absorbed amount, calculated by  $k_{56}/(k_{51} \pm k_{56})$ , is metabolized in

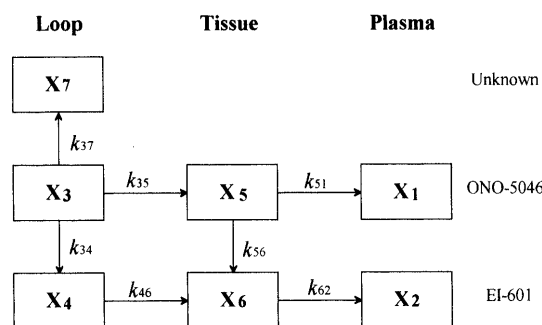


Fig. 5. Pharmacokinetic Model Proposed for the Analysis of the Rate of ONO-5046 after Intraluminal Administration in Rats

Table 3. Pharmacokinetic Parameters of ONO-5046 after *in Situ* Intraluminal Administration at Three Doses in Rats

	Dose ( $\mu\text{mol}$ )		
	1	2.5	5
$k_{35}$ ( $\text{min}^{-1}$ )	0.01202	0.00887	0.00622
$k_{51}$ ( $\text{min}^{-1}$ )	0.15435	0.51971	1.23964
$k_{34}$ ( $\text{min}^{-1}$ )	0.10180	0.03255	0.01337
$k_{56}$ ( $\text{min}^{-1}$ )	0.12849	0.30404	0.49712
$k_{37}$ ( $\text{min}^{-1}$ )	0.14217	0.04928	0.02180

Value of  $k_{46}$  and  $k_{62}$  were fixed at 0.00643 and 0.62627, respectively. These parameters were calculated using an average value of the cumulative amount of ONO-5046 and EI-601 in mesenteric venous blood (see Fig. 4) employing a MULTI (RUNGE) program.

the intestinal tissue (corresponds to 2.1% of initial dose) and 18.0% of ONO-5046 is metabolized in the liver to EI-601 (corresponding to 0.5% of the initial dose). Thus, at a dose of 1  $\mu\text{mol}$  of ONO-5046, only 2.1% of the dose escaped from metabolism in the loop, intestinal tissues and the liver and reached systemic circulation. At a dose of 5  $\mu\text{mol}$  of ONO-5046, the metabolism in the intestinal loop and intestinal tissues decreased as follows: 85.0% of the dose in the intestinal loop, 4.3% of the dose in the intestinal tissues; the percentage of ONO-5046 which reached systemic circulation increased to 8.8% of the dose.

The above pharmacokinetic analysis of the metabolism of ONO-5046 in the absorption process may provide clues for a preformulation study to increase the oral bioavailability of ONO-5046.

## DISCUSSION

In the present study, we estimated the intestinal and hepatic first-pass metabolism of ONO-5046, separately, in rats. The hepatic first-pass metabolism was constant (20% of dose) under two different dosing rates. On the other hand, the intestinal first-pass metabolism, including the metabolism in the intestinal fluid, was considerably high and saturable. Thus, the low and dose-dependent oral bioavailability of ONO-5046 was found to be derived from the marked metabolism in the intestinal lumen and the intestinal tissues in the absorption process.

The metabolism of ONO-5046 in the intestinal fluid and tissues is considered to be due to esterases, since EI-601 alone, which is an inactive metabolite of ONO-5046 cleaved at the ester linkage, was detected as a metabolite. It is well known that esterases have broad substrate specificity, and exist in most organs, including the intestinal membranes.<sup>15,16</sup> However, intravenously administered ONO-5046 showed linear pharmacokinetic characteristics and the *CL* was relatively small (about 6.4–7.4 ml/min/kg), with a constant hepatic extraction ratio of about 20%. The low *CL* of ONO-5046 was considered to be due to the extremely high plasma protein binding of ONO-5046 (the free fraction of ONO-5046 in plasma is less than 0.1%, and its distribution into blood cells is negligible). On the other hand, the reason for the low oral bioavailability was considered to be due to marked intestinal first-pass metabolism including the metabolism in the intestinal fluid in the absorption process. In the present study, bile was discarded and the inner contents of the intestine were washed out with enough saline before the drug administration. Therefore, the degradation of ONO-5046 in the intestinal loop may be due to esterases such as nonspecific carboxylesterase and pseudoacetylcholinesterase which are bound to cell membranes.<sup>16</sup> To overcome the instability of drugs in the intestinal lumen before its absorption, several methods are suggested: stabilization of a drug by the aid of a cholinesterase inhibitor and/or the addition of other ester agents as a dummy substrate, increase of the absorption rate of a drug to saturate the metabolism, and so on. In the present study, a higher dose of ONO-5046 showed higher bioavailability due to the saturation of intestinal metabolism (Table 2). This may indicate that an increase in the absorption rate of ONO-5046 can also decrease the intestinal first-pass metabolism, resulting in an increase of bioavailability. In a preliminary study, ONO-5046 solution containing 3% ethanol was administered at a dose of 1  $\mu$ mol/loop in a mesenteric collecting method *in situ*, and higher bioavailability of ONO-5046 was observed. The recoveries of ONO-5046 and EI-601 were as follows: 7.7  $\pm$  1.0%

ONO-5046 in mesenteric plasma, 23.2  $\pm$  2.9% EI-601 in mesenteric plasma, 2.3  $\pm$  1.0% ONO-5046 in the intestinal loop, and 58.7  $\pm$  3.3% EI-601 in the loop; the total recovery was 91.8  $\pm$  2.5%. Thus, the recovery of ONO-5046 in the mesenteric plasma was significantly increased by administering it as a solution containing ethanol. This may be due to the increase in the absorption rate of ONO-5046, and/or to the inhibiting action of ethanol on the activity of metabolizing enzymes. Further investigation will be necessary.

In conclusion, the extremely low oral bioavailability of ONO-5046 was found to be derived from marked intestinal first-pass metabolism including the metabolism in the intestinal fluid, whereas the total plasma clearance, including hepatic first-pass metabolism of ONO-5046 was relatively small, probably due to the great protein binding of the compound. Based on the above findings, study for the enhancement of the oral bioavailability of ONO-5046 is now underway.

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