Effects of Physiological Factors on the Bioavailability of Ethyl 2-Chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate in an Emulsion in Rats

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The main absorption site of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate (AL-294) in rats was the upper portion of the small intestine. Both AL-294 and AL-294 acid (2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionic acid), a hydrolyzed form of AL-294, were absorbed in a smaller quantity under the bile fistula condition (pancretic juice and bile were excluded). Compared with the absorption of AL-294 as an emulsion under the sham operation condition, the absorption of AL-294 as the emulsion decreased under the condition where only pancreatic juice was excluded. The bioavailability under this condition was very similar to that under the bile fistula condition, whereas the absorption of AL-294 acid did not decrease when the pancreatic juice was excluded. From these results, the absorption mechanism of AL-294 is considered as follows: AL-294 was hydrolyzed to AL-294 acid by lipase in pancreatic juice, then AL-294 acid was solubilized with bile salts to form mixed micelles in the intestinal lumen. AL-294 acid from this form was easily absorbed into the systemic circulation. Absorption of AL-294 increased when the particle size of the emulsion was smaller. The reason was assumed to be that the smaller particle size offered the greater oil–water interface for lipase activity against AL-294.

Keywords oral absorption; oily drug; emulsion; mixed micelle; enzymatic hydrolysis; lipase

In the previous reports, the absorption of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate (AL-294) after orally administering an emulsion dosage increased as the particle size of the emulsion decreased.[1] This increase was partly due to the higher dissolution rate of the drug from the emulsion with a smaller particle size. But this increase was too large to solely attribute it to the change in the dissolution rate of the drug. To elucidate the involvement of other factors than the dissolution rate, the effect of bile secretion and the rate of AL-294 hydrolysis to AL-294 acid (2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionic acid) by lipase was investigated.

Experimental

Materials AL-294, AL-294 acid, Tween-80, and testosterone were the same as described previously.[2] All other chemicals were of reagent grade.

Animal Experiments Male Sprague-Dawley rats weighing 250—300 g (aged 8 weeks) were fasted for 15—18 h and were used under intraperitoneal anesthetization with a mixture of sodium pentobarbital and sodium phenobarbital at a dose of 100 and 50 mg/kg, respectively. The formulations used in the experiments are shown in Table I.

(1) Absorption Site: The stomach, the upper part (duodenum), the middle part (jejunum), and the lower part (ileum) of the small intestine from different rats were used. After cannulating the bile duct to exclude bile and pancreatic juice, a site on the small intestine was ligated at the proximal and distal ends to make a loop of 10 cm long. The formula 1 emulsion (Table 1) was administered into the gastro-intestinal loop at a dose of 10 mg/kg of AL-294. Blood samples were withdrawn from the tail vein periodically.

(2) Effect of Bile on the Absorption: A fine polyethylene tube (PE-10) was cannulated into the bile duct of a rat, and bile was drained out of the body through the cannula. Subsequently the small intestine was washed with 10 ml saline at 37 °C. Half an hour later, formula 1 or 2 emulsion was administered into the duodenum at a dose of 50 mg/kg of AL-294. Blood samples were withdrawn from the cannulated carotid artery because blood was hardly withdrawn from the tail vein in this condition.

(3) Effect of Pancreatic Juice on the Absorption: Two fine polyethylene tubes (PE-10) were cannulated into the proximal (*ca.* 1.5 cm from the liver) and distal ends (*ca.* 1 cm from the duodenum) of the bile duct of a rat, respectively. The distal cannula was for draining pancreatic secretion,[3] and the proximal one was inserted into the duodenum to drain bile by bypassing. After this surgical operation, the same procedure as described in (2) was followed.

(4) Hydrolysis Rate of AL-294 in the Intestinal Lumen: An upper portion of the jejunum was ligated to make a loop of 5 cm long, and the mesenteric veins corresponding to the ligated jejunum were tied up to prevent the drug transfer to the circulation. The formula 1 emulsion (8.3 mg of AL-294) was administered into the loop of the intestine. The rat was sacrificed at a specified time and the contents of the loop were washed out sufficiently to determine the AL-294 and AL-294 acid contents.

(5) Hydrolysis Rate of AL-294 by Pancreatic Juice of Rat: Using the technique described in (3), pancreatic juice from the rat was collected in a cooled microtube. One ml of the juice and 0.1 ml of the test emulsion were mixed and incubated at 37 °C. Periodically 10 μl of the mixture was sampled to determine the AL-294 and AL-294 acid contents.

TABLE I. Composition of Test Samples

	Formula			
	1	2	3	4
AL-294 (mg)	333	—	25	—
AL-294 acid (mg)	—	333	—	25
Tween-80 (mg)	167	167	—	—
Distilled water (ml)	19.5	19.5	—	—
Corn oil (ml)	—	—	2.5	2.5

Results

Absorption Site of AL-294 in Rat There are reports that the absorption of drugs were influenced by food-intake when the drug was absorbed from a restricted site of the gastro-intestinal tract. For example; the absorption of chlorothiazide was doubled in the postprandial condition,[3a] and drug absorption from the intestine was site specific. Phenitoin absorption was enhanced in the presence of bile.[3b] As the absorption rate of AL-294 was influenced by food,[2] the absorption site in the gastro-intestinal tract was studied in rats (three rats for each site). The average areas under the curves within 6 h ($AUC(0—6)$) for AL-294 acid after administration of AL-294 emulsion (formula 1, 10 mg/kg) into each segment were 134 μg·h/ml for duodenum, 84 μg·h/ml for jejunum, 23 μg·h/ml for ileum, and 2 μg·h/ml for stomach. AL-294 was absorbed mainly from the upper part of the small intestine, but was hardly absorbed from the ileum and the stomach.

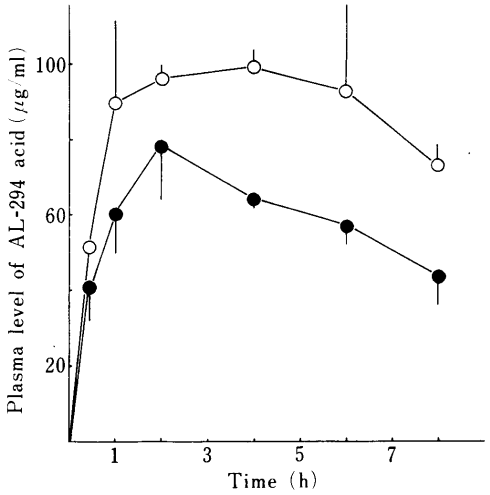

Fig. 1. Effect of Bile Fistula on the Absorption of AL-294 after Intraduodenal Administration of AL-294 Emulsion in Rats at a Dose of 50 mg/kg

○, sham operation; ●, under the condition of bile fistula. Each point represents the mean of three rats with a standard error.

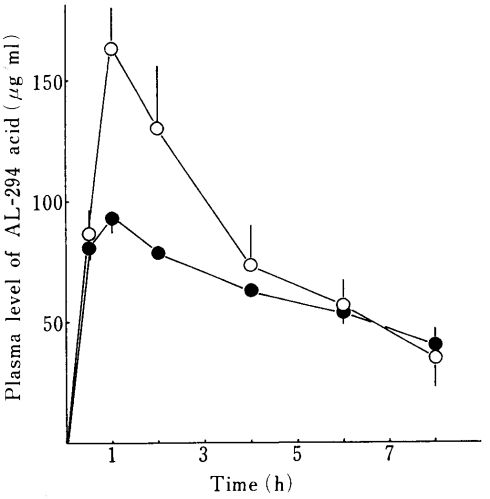

Fig. 2. Effect of Bile Fistula on the Absorption of AL-294 Acid after Intraduodenal Administration of AL-294 Acid Emulsion at a Dose of 50 mg/kg

○, sham operation; ●, under the condition of bile fistula. Each point represents the mean of three rats with a standard error.

Effect of Bile on the Absorption As the absorption of hydrophobic compounds such as oleic acid and indomethacin was influenced by bile salts,[4] the effect of bile on the absorption of AL-294 was investigated. The plasma levels of AL-294 acid after the duodenal administration of AL-294 (formula 1 or 2) to rats with a bile fistula (excluded bile salts and pancreatic juice) are shown in Figs. 1 and 2. Compared with the control rats (without bile fistula), the absorption of both AL-294 and AL-294 acid from the emulsion was decreased in these rats. *AUC*s (0—8) obtained after administration of AL-294 and AL-294 acid orally or intraduodenally are shown in Table II. The *AUC*s of AL-294 and AL-294 acid after intraduodenal administration to rats with the bile fistula decreased to 67% and 83% of the control, respectively. When absorption is dependent on the presence of bile which contains pancreatic juice, two possibilities can be considered. The first is that this oily drug as an emulsion largely is solubilized in the intestinal

TABLE II. *AUC*s of AL-294 Acid and Their Bioavailabilities after Administering AL-294 and AL-294 Acid Orally or Intraduodenally to Rats at a Dose of 50 mg/kg ($n=3$)

Sample	Administration route	Average (S.E.) *AUC*(0—8) (μg·h/ml)	Relative bioavailability (%)
Formula 1	Oral	529.2 (46.5)	—
Formula 2	Oral	763.5 (121.7)	—
Formula 3	Oral	304.9 (14.8)	—
Formula 4	Oral	614.3 (7.7)	—
Formula 1	Intraduodenal	693.8 (17.8)	100.0
	with bile fistula	468.8 (18.9)	67.6
	without pancreatic juice	427.2 (5.5)	61.9
Formula 2	Intraduodenal	657.0 (129.8)	100.0
	with bile fistula	546.5 (30.9)	83.2
	without pancreatic juice	658.7 (31.7)	100.3

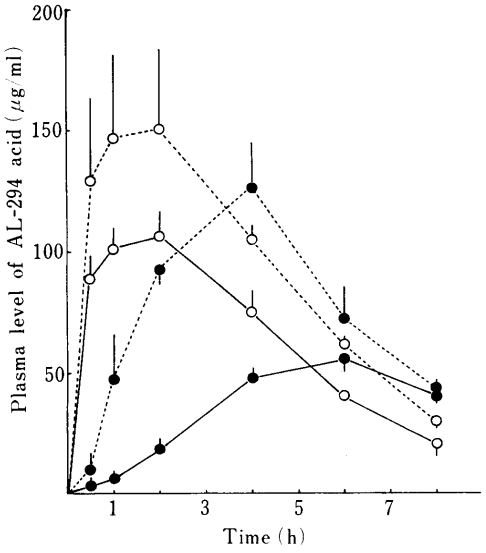

Fig. 3. Plasma Levels of AL-294 Acid in Rats after Oral Administration of AL-294 or AL-294 Acid in Emulsion and in Corn Oil Solution at a Dose of 50 mg/kg

Solid line: AL-294, ○, emulsion; ●, in corn oil solution; broken line: AL-294 acid, ○. emulsion; ●, in corn oil solution. Each point represents the mean of three rats with a standard error.

fluid to form mixed micelles with bile salts, and then the drug in the mixed micelles is absorbed. The second is that AL-294 is metabolized by pancreatic enzymes (mainly lipase) in bile to form AL-294 acid which will be more absorbable. Comparison of Figs. 1 and 2 shows that AL-294 acid was absorbed more easily than AL-294. To confirm this further, formulas 1 and 4 were administered orally to rats at a dose of 50 mg/kg. Figure 3 shows plasma level-time curves after administration of AL-294 and AL-294 acid to rats, and Table II shows their *AUC*s (0—8). When a drug and bile salts form a mixed micelle, the amount of the drug solubilized increases in the intestinal lumen and this increase enhances the drug absorption. The possibility of a mixed micelle formation of AL-294 or AL-294 acid with bile salts was studied. A substance that has a polar head such as decanol or oleic acid was reported to form mixed micelles with bile salts, resulting in better absorption.[5] AL-294 was mixed in a ratio of 1% with artificial bile consisting of sodium cholate, lecithin, and water (7:2:90).[6] AL-294,

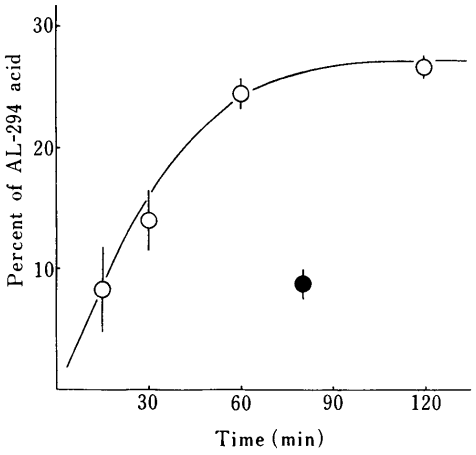

Fig. 4. Hydrolysis Rate of AL-294 after Administration of AL-294 Emulsion in a Segment of Jejunum of Rats

○, sham operation; ●, under the condition of bile fistula. Each point represents the mean of three rats with a standard error.

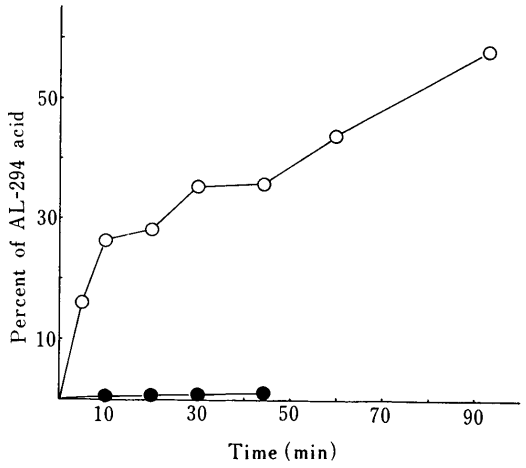

Fig. 5. Hydrolysis Rate of AL-294 *in Vitro* in Pancreatic Juice of Rats at 37 °C

○, in pancreatic juice; ●, in the buffer solution.

however, was not solubilized at all because of its low polarity. Whereas AL-294 acid, that has a polar head, showed an opaque appearance when mixed with the same amount of the artificial bile. That indicated the mixed micelle formation. From these results, the second possibility seems to be reasonable.

Hydrolysis of AL-294 in the Rat Intestinal Lumen There are some reports describing that drugs metabolized by an enzyme in the intestinal lumen to an active metabolite were better absorbed.[7] To confirm that, AL-294 was metabolized to AL-294 acid in the rat intestinal lumen, AL-294 emulsion (formula 1, 8.3 mg of AL-294) was administered into a segment of the rat jejunum and AL-294 and AL-294 acid content in the lumen were determined periodically. As shown in Fig. 4, the concentration of AL-294 acid increased with elapsed time. However, in this experiment, the intestinal wall showed necrosis within one hour because all mesenteric blood vessels corresponding to the site of administration were ligated. Furthermore, bile flow into the segment was stopped. The rate of hydrolysis in this condition is probably slower than that in the intact intestinal lumen. A similar experiment as described above was carried out after

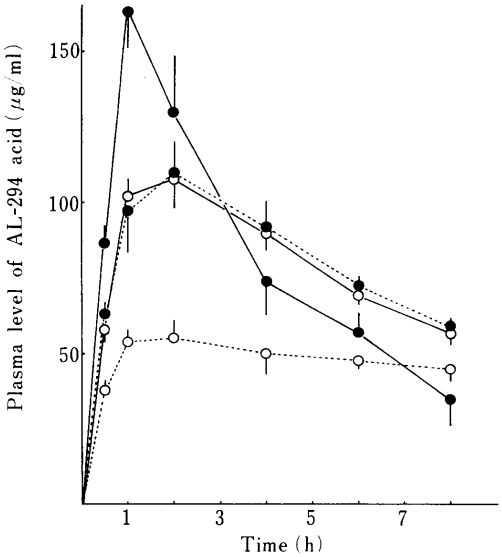

Fig. 6. Effect of Pancreatic Juice on the Absorption of AL-294 or AL-294 Acid after Intraduodenal Administration of AL-294 or AL-294 Acid Emulsions at a Dose of 50 mg/kg

Solid line: in the presence of pancreatic juice, ○, AL-294; ●, AL-294 acid; broken line: in the absence of pancreatic juice, ○, AL-294; ●, AL-294 acid. Each point represents the mean of three rats with a standard error.

cannulating the bile duct to exclude bile and pancreatic juice and washing the intestine with 10 ml of saline, *i.e.*, AL-294 emulsion (formula 1, 8.3 mg) was administered into a segment of the rat jejunum and AL-294 and AL-294 acid content in the lumen were determined periodically. The rate of hydrolysis of AL-294 was only one-fourth that of the former. The results indicated that AL-294 was hydrolyzed by lipase in pancreatic juice in the intestinal lumen.

The Rate of Hydrolysis of AL-294 by Pancreatic Juice of Rats In order to directly prove the hydrolysis of AL-294 in pancreatic juice, 0.1 ml of AL-294 emulsion (formula 1) was incubated at 37 °C with 1 ml of pancreatic juice collected from rats. AL-294 and AL-294 acid content in the mixture were determined periodically. As shown in Fig. 5, AL-294 was hydrolyzed in pancreatic juice to AL-294 acid, while it was not hydrolyzed in the buffer solution, pH 7.0, as seen in the control sample.

Effect of Pancreatic Juice on the Absorption of AL-294 or AL-294 Acid After pancreatic juice was excluded from the rat intestine, AL-294 emulsion (formula 1) or AL-294 acid in an oil solution (formula 4) was administered into the duodenum of rats at a dose of 50 mg/kg, and plasma levels of AL-294 acid were measured. As shown in Fig. 6 and Table II, the absorption of AL-294 decreased in the absence of pancreatic juice. Its $AUC(0—8)$ was only about 60% of the control, that was close to the 64% obtained under the condition of a bile fistula. On the other hand, the $AUC(0—8)$ of AL-294 acid was the same as in the control. These and the results in Fig. 3 indicated that absorption of AL-294 depended mainly on the presence of pancreatic juice, while the absorption of AL-294 acid depended mainly on the presence of bile salts.

Discussion

In the previous report,[2] it was demonstrated that after an oral or intrajejunal administration of AL-294, AL-294 acid appeared in the portal vein of rats. In the present

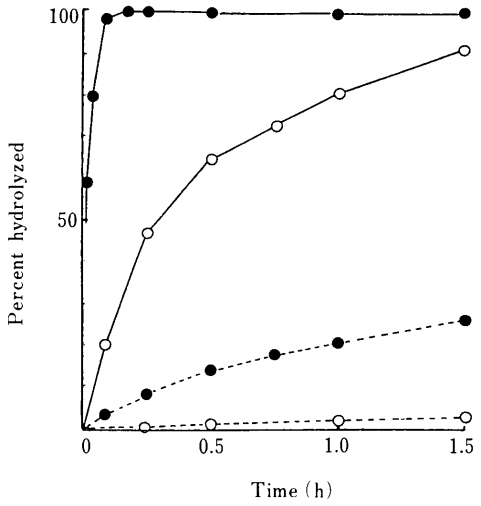

Fig. 7. Effect of Sodium Taurocholate on the Hydrolysis Rate of AL-294 to AL-294 acid in 1 N Sodium Hydroxide Solution

Solid line: emulsion, ●, 1 N sodium hydroxide plus 5% sodium taurocholate; ○, 1 N sodium hydroxide; broken line: drug itself, ●, 1 N sodium hydroxide plus 5% sodium taurocholate; ○, 1 N sodium hydroxide.

report, the following results were obtained: (1) AL-294 was absorbed from the upper part of the small intestine. (2) AL-294 was hydrolyzed to AL-294 acid by an enzyme in pancreatic juice, presumably by lipase, at a comparatively fast rate. (3) AL-294 acid was solubilized by bile salts and formed mixed micelles with bile salts. (4) AL-294 acid was absorbed more easily than AL-294. From these results, it is thought that AL-294 is absorbed through the following three routes. The first: AL-294 dissolved in the intestinal fluid reaches the gut wall and is hydrolyzed there. The presence of this route was confirmed by the fact that even in the absence of both bile salts and pancreatic juice, the absorption of AL-294 was as much as 60% of the control. The second: AL-294 acid, which is formed by hydrolysis of AL-294 by enzyme in pancreatic juice dissolves in the intestinal fluid and reaches the gut wall. The third: AL-294 acid formed by the hydrolysis of AL-294 in the lumen is immediately solubilized by bile salts and reaches the gut wall to be absorbed in the systemic circulation. The contribution of each route in the absorption could be estimated from a detailed pharmacokinetic study. The absorption of AL-294 was influenced by the particle size of the emulsion as described in the previous report.[1] AL-294 acid was more absorbable than AL-294. Therefore, the effects of the particle size of AL-294 emulsion and the presence of bile on the hydrolysis rate of AL-294 were investigated. Sodium taurocholate was used as a component of the bile. As shown in Fig. 7, the hydrolysis rate of AL-294 was faster in the emulsion than that in physiological dispersion. And the hydrolysis rates of both forms became much faster by the addition of 5% sodium taurocholate into the medium. The following were estimated: the hydrolyzed AL-294 acid on the emulsion surface was removed by solubilization with sodium taurocholate to the aqueous bulk and AL-294 continued to appear on the emulsion surface. AL-294 should be almost completely hydrolyzed quickly by the repetition of this process. Further, the smaller the size of the emulsion, the larger the surface area and the faster the hydrolysis of AL-294. The results suggested that the third route was the main route. The same process is considered to occur *in vivo*. The reason for the apparent enhancing of the absorption of AL-294 by the reduction in the particle size of the emulsion is assumed to be as follows. Usually pancreatic lipase activity takes place at the oil–water interface of an emulsion.[8] The smaller is the particle size, the greater is its oil–water interface, and AL-294 is more rapidly converted to AL-294 acid and is easily solubilized. On the other hand, since a corn oil solution of AL-294 has a very small oil–water interface, it is difficult for pancreatic lipase to fully exert its activity. The absorption occurring under the condition simulates the absorption in the case without bile. Following oral administration, the relative bioavailability of the drug in the corn oil solution compared to the emulsion is 58%. It agrees with the 60% obtained following the intraduodenal administration under the condition of bile fistula. Thus, the smaller size emulsion of AL-294 showed the greater bioavailability. The increased absorption of AL-294 seen in dogs after postprandial oral-administration of AL-294 in hard gelatin capsules[2] is assumed to be due to the easier hydrolysis of AL-294 to AL-294 acid because of the enhanced secretion of bile caused by feeding.

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