[Chem. Pharm. Bull. 36(7)2703—2707(1988)]

# Enhancement of Small Intestinal Absorption of *N*,*N*′-Dimethylcarbamoylmethyl 4-(4-Guanidinobenzoyloxy) Phenylacetate Methanesulfonate (FOY305®) in Rats

TOSHIAKI NISHIHATA,*[,a,1)] HIRONORI YOSHITOMI,[b] CHIEKO IKAWA,[c] and KIYOSHI SAKAI[c]

*Faculty of Pharmaceutical Sciences, Osaka University,[a] 1–6 Yamadaoka, Suita, Osaka 565, Japan, Faculty of Pharmaceutical Sciences, Fukuyama University,[b] Sanzo, Fukuyama, Hiroshima, Japan, and Osaka University of Pharmaceutical Sciences,[c] 1–10–65 Kawai, Matsubara, Osaka 580, Japan*

(Received October 26, 1987)

In the rat, small intestinal absorption of FOY305® was improved by the addition of citric acid, probably through the reduction of enzymatic degradation of FOY305 by esterase in the intestinal lumen. A solid formulation prepared with trilaurin also increased the intestinal absorption of FOY305, probably through an effect of laurate, which is produced from trilaurin by lipase activity in the small intestinal lumen. The combination of citric acid and trilaurin in the solid formulation resulted in a significant increase of intestinal absorption of FOY305, amounting to 10 times that after administration of a simple FOY305 solution.

**Keywords**——FOY305®; rat intestinal absorption; plasma concentration; citric acid; trilaurin

*N*′,*N*′-Dimethylcarbamoylmethyl 4-(4-guanidinobenzoyloxy) phenylacetate methanesulfonate (FOY305®) was synthesized as a candidate drug to prevent pancreatitis through proteolytic enzyme inhibition.[2)] Although absorption of FOY305 was good after administration in suspension form into rat rectal loops, much lower absorption was observed after administration into small intestinal loops.[3)] It was demonstrated that the apparent small absorption of FOY305 from the small intestine was due to rapid enzymatic degradation in the intestinal lumen, rather than poor absorption.[3)] Thus, it is considered that inhibition of FOY305 degradation in the small intestinal lumen could increase the absorption of FOY305 after oral administration.

In the present study, we investigated the effect of various additives on the apparent absorption of FOY305® from rat small intestine.

## Experimental

**Materials**——FOY305, 4-(4-guanidinobenzoyloxy) phenylacetate (FOY251®) and *p*-guanidino benzoate (GBA) were supplied by Ono Pharmaceutical Co. (Osaka, Japan). Trilaurin was obtained from Sigma Inc. (St. Louis, U.S.A.). Citric acid was obtained from Wako Pure Chemicals Co. (Osaka, Japan). Other reagents used were of analytical grade.

**Animals**——Sprague–Dawley male rats, weighing 350 to 400 g, were fasted for 16 h prior to experiments but water was given freely. During experiments, rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and were kept on a warm surface (electric hot plate) at 38 °C to maintain body temperature.

**Formulation**——Formulations used are listed in Table I. To prepare the solid formulation, FOY305 was suspended in molten trilaurin at 40 °C, and was then poured into Teflon tubing (3 mm i.d. × 100 mm), and allowed to solidify at room temperature. The solid was extruded and kept at 4 °C until use.

**Intestinal Absorption Study**——The absorption study was performed in rats with pyloric ligation. Formulation administration was carried out by a method described previously.[4)] Briefly, the intestine was exposed by ventral midline incision. Another incision was made in the fundus of the stomach, through which the test formulation was

TABLE I. Codes and Constituents of the Administered Formulations

| Code | FOY305 (μmol) | Citric acid (mg) | Water (g) | Trilaurin (mg) |
|---|---|---|---|---|
| Solution I | 40.4 (20 mg) | 0 | 20 | 0 |
| Suspension[a)] | 40.4 | 0 (50 mg of lactose) | 2 | 0 |
| Powder | 40.4 | 0 | 0 | 0 |
| Solution II | 40.4 | 50 | 2 | 0 |
| Solution III | 40.4 | 100 | 2 | 0 |
| Solid I | 40.4 | 0 | 0 | 150 |
| Solid II | 40.4 | 50 | 0 | 150 |

*a*) The suspension contained 50 mg of lactose/2 ml.

introduced into the duodenum. After administration, the pylorus was ligated. For administration, the aqueous solution or suspension was injected through polyethylene tubing (PE 50), and powdered or solid formulations were administered *via* Teflon tubing (3 mm i.d. × 100 mm) through which the formulation was extruded with a stainless steel rod. After administration of FOY305, blood was collected at designated time intervals for 2 h. After all blood samples had been collected, the entire intestinal segment was excised to measure any residual FOY305 and metabolites. The excised intestinal segment was cut to expose the intestinal lumen in the beaker, and any residue remaining in the segment was collected by rinsing with 25 ml of distilled water.

**Assay of FOY305 and Its Metabolite**——Assay of FOY305 and its metabolites was performed by a high-performance liquid chromatographic method as described in a previous paper.[3)] FOY305, FOY251 and GBA in the intestinal lumen could be assayed by high-performance liquid chromatography (HPLC). However, because of interference from a biological component in plasma, GBA was not determined in plasma samples.

**Statistical Analyses**——Statistical analyses were performed by use of Student's *t*-test.

## Results and Discussion

Before investigating the effect of additives on rat small intestinal absorption of FOY305, absorptions from solution, suspension, and powdered formulations were compared. Since it has been reported[5)] that FOY251, a metabolite of FOY305, also inhibits proteolytic enzymes in the same way as FOY305, effective plasma drug concentration was calculated by using the sum of molar concentrations of FOY305 and FOY251. The area under the curve of the sum of molar concentrations of FOY305 and FOY252 (AUC) for 2 h was referred to as "active AUC", as reported previously.[3)] We did not investigate in detail the pharmacokinetics after administration of FOY305, because it was difficult to collect sufficient volume of blood from small experimental animals such as rats. Thus, we could not obtain $[\text{active AUC}]_{0-\infty}$; *i.e.*, the bioavailability was not determined in the present study.

After administration of FOY305 in the form of solution I, only FOY251 was detected in plasma. Administration of FOY305 in the suspension form slightly increased the plasma FOYs concentrations (FOY305 and FOY251), and a small amount of FOY305 was detected in plasma 15 min after the administration (Fig. 1). An increase in the sum of plasma concentrations of FOY305 and FOY251 was observed after administration of FOY305 in powdered form (Fig. 1).

Active AUC of FOYs (FOY305 and FOY251) after administration in powdered form was two twice that after administration as solution I (Table II). To determine the disappearance of FOY305 from the intestine, residual amounts of FOY305 and its metabolites were measured after 2 h (Table II). In all experiments, only GBA was detected in the intestinal lumen.

It has been reported that absorption of FOY305 was the same from duodenum, jejunum

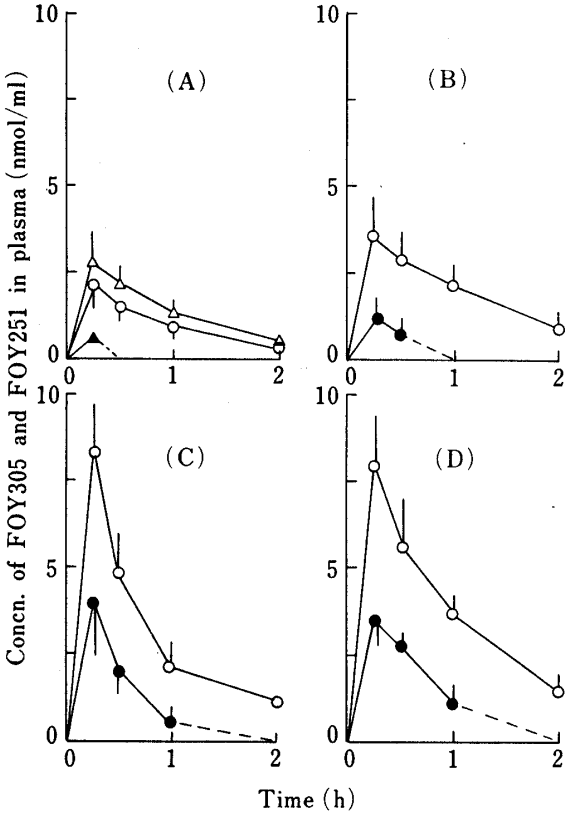


Fig. 1. Plasma Concentration of Total Active FOYs after Administration of FOY305 at a Dose of 20.2 $\mu$mol per Rat

Dosage form: A, solution I (○) and suspension (△); B, powdered form; C, solution II; D, solution III.

Open symbols show the sum of molar concentrations of FOY305 and FOY251. Closed symbols represent the concentration of FOY305. Each value represents the mean ± S.D. ($n$ = 3 to 5).

TABLE II. Active AUC[a] and Percent Remaining[b] of FOY305 after Intestinal Administration

| Code | Dose ($\mu$mol) | AUC[a] (nmol h/ml) | $\frac{[AUC]_x}{[AUC]_{S\text{-}I}}$ | Percent[b] remaining ($R$) | $\frac{(100-R_x)}{(100-R_{S\text{-}I})}$ |
|---|---|---|---|---|---|
| Intestinal administration of FOY305 | | | | | |
| Solution I (S-I) | 20.2 | 1.7±0.3 | 1 | 76.4± 6.9 | 1 |
| Suspension | 20.2 | 2.0±0.4 | 1.2 | 70.6±10.2 | 1.2 |
| Powder | 20.2 | 3.9±1.0[d] | 2.3 | 69.4± 5.7[d] | 1.3 |
| Solution II | 20.2 | 6.3±2.4[d] | 3.7 | 51.4± 7.1[d] | 2.1 |
| Solution III | 20.2 | 9.0±3.7[d] | 5.3 | 52.3± 5.7[d] | 2.0 |
| Solid I | 20.2 | 7.9±2.5[e] | 4.6 | 27.2± 8.1[e] | 3.1 |
| Solid II | 20.2 | 19.2±5.2[e] | 11.3 | 16.9± 4.9[e] | 3.5 |
| Intestinal administration of GBA[c] | | | | | |
| Solution III | 20.2 | — | — | 96.6± 4.2 | |
| Solid I | 20.2 | — | — | 41.7±10.4[f] | |
| Solid II | 20.2 | — | — | 19.6± 4.2[f] | |

*a*) AUC for 2 h after administration was determined by using the sum of molar concentrations of FOY305 and FOY251. *b*) Remaining amount in the intestinal lumen was assessed as the amount of GBA, because only GBA was detected. *c*) These formulations did not contain FOY305. Each value represents the mean ± S.D. ($n$ = 3 to 5). Significant differences: *d*) $p < 0.05$ *versus* solution I; *e*) $p < 0.01$ *versus* solution I; *f*) $p < 0.001$ *versus* solution III.

and ileum.[3] It has also been reported[4] that administration of cefoxitin in powdered form into rat small intestine resulted in increased bioavailability compared to administration as a solution form. It has been further demonstrated[3] that the apparent small absorption of FOY305 after administration in the small intestine was due to rapid metabolism of FOY305

to GBA in the small intestinal lumen. Since FOY305 was detected in plasma after administration as a powder or as a suspension form, but not after administration as a solution, enzymatic degradation of FOY305 may become saturated at high drug concentrations. We previously reported[3] that rectal absorption of FOY305 occurred without significant drug degradation, and that about 90% of FOY305 was degraded to GBA within 1 h in an *in vitro* jejunal sac study. We therefore consider that avoidance of FOY305 metabolism is necessary to increase the absorption of FOY305 from the small intestine.

Addition of citric acid to the administered solution (solutions II and III) lowers the pH value as well as increasing the solubility of FOY305 (FOY305 is a basic compound). Administration of an FOY305 solution containing citric acid significantly increased the sum concentrations of FOY305 and FOY251 in plasma, with high concentrations of FOY305 (Fig. 1). The active AUC from solution III was about 5 times greater than that from solution I.

Since it has been reported[13] that GBA was not absorbed from the small intestine, we investigated the effect of citric acid on GBA absorption. The recovery of GBA was $96.6 \pm 4.2\%$ ($n=4$), 2 h after administration into the duodenum (Table II). This result confirms that the disappearance of total FOYs from the intestine in the presence of citric acid is not due to an enhancement of GBA absorption. Concerning the increase of active AUC from solutions II and III compared to that from solution I, the higher concentration of FOY305 in solutions II and III seems to be the main factor in increasing the intestinal absorption of FOY305. The intestinal absorption of many drugs occurs by passive transport, being dependent on the concentration in the intestinal lumen, and further, the ratio of enzymatic degradation of compounds decreases when their concentrations are increased to levels that saturate the enzyme activity.

To examine further the improvement of intestinal absorption of FOY305 in the presence of citric acid in the administered solution, the ratio of active AUC and the ratio of disappearance of total FOYs from intestinal lumen after administration of each of various formulations against that after administration of solution I, were determined (Table II). The discrepancy between the results obtained by the AUC method and the disappearance method may be due to a first-pass degradation of FOY305 and FOY251 within the intestine and/or liver. It is likely that citric acid decreases the ratio of the enzymatic degradation of FOY305 by increasing the FOY305 concentration in the intestinal lumen as described earlier. It is also likely that citric acid inhibited esterase activity by lowering the pH in the intestinal lumen, thereby allowing absorption of FOY305 to occur (pH values of solutions II and III were less than 2.5). Thus, the reduction of enzymatic degradation of FOY305 by lowering the pH in the intestinal lumen may be one method to increase the intestinal absorption of FOY305. However, since it is well known that the pH of the intestinal luminal fluid is readjusted rapidly to the physiological pH (neutral pH), it seems that the reduction of enzymatic activity of esterase by lowering the pH is effective only for short periods. Therefore, high concentrations of FOYs (FOY305 and FOY251) in plasma were observed at an early stage after administration of solutions II and III, as shown in Figs. 1C and 1D.

It has been reported[4] that a solid formulation of cefoxitin, prepared with triglyceride base, gave increased drug absorption from the small intestine, probably through an effect of fatty acids[6] which are produced from the triglyceride by the action of lipase. Administration of solid I increased the plasma concentration of total active FOYs (FOY305 and FOY251) (Fig. 2). The AUC of the sum of molar concentrations of FOY305 and FOY251 was 4.6 times greater than that after administration of solution I. The disappearance of total FOYs from the intestinal lumen was enhanced after administration of solid I, with about 70% disappearance. Disappearance of GBA when administered as solid form I, was also increased significantly. These results suggest that the increased disappearance of total FOYs from the intestine is due

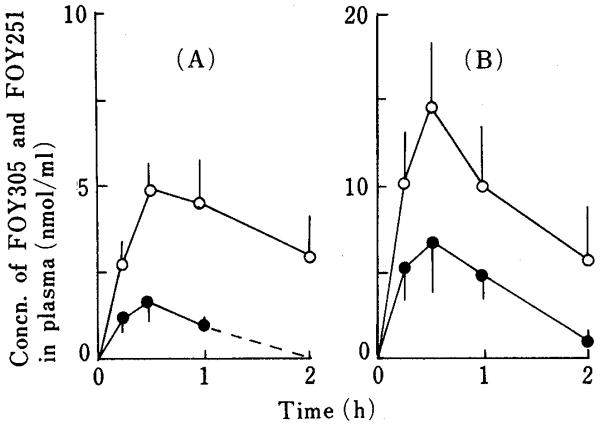


Fig. 2. Plasma Concentration of Total Active FOYs after Administration of FOY305 at a Dose of 20.2 $\mu$mol per Rat

Dosage form: solid I (A) or solid II (B).
Open symbols show the sum of molar concentrations of FOY305 and FOY251. Closed symbols represent the concentration of FOY305. Each value represents the mean $\pm$ S.D. ($n=3$ to 5).

to greater absorption of both FOY305 and FOY251, and also to an enhancement of GBA absorption.

To investigate the combination effect of citric acid and trilaurin on the absorption of FOYs, solid II was administered into rat small intestine. The active FOYs concentration (sum of molar concentrations of FOY305 and FOY251) in plasma was increased significantly (Fig. 2). The active AUC (the sum of molar concentrations of FOY305 and FOY251) was more than 10 times greater than that observed with solution I (Table II). Absorption of FOY305 and FOY251 from rat small intestine was accelerated not only through an apparent reduction of enzymatic degradation, but also through the adjuvant action of laurate.

**Acknowledgement** This work was supported in part by a grant from Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

## References and Notes

1) Present address: *Pharmaceutical Chemistry Department, University of Kansas, Lawrence, Kansas 66046, U.S.A.*
2) Y. Tamura, M. Hirado, K. Okamura, Y. Minate, and S. Fujii, *Biochim. Biophys. Acta*, **484**, 417 (1977).
3) T. Nishihata, Y. Saitoh, and K. Sakai, *Chem. Pharm. Bull.*, **36**, 2544 (1988).
4) T. Nishihata, H. Yoshitomi, and T. Higuchi., *J. Pharm. Pharmacol.*, **38**, 69 (1986).
5) S. Ooki, K. Nishiyama, Y. Ozaki, H. Itoh, and F. Hirata, *Gendai-Rinsho*, **12**, 71 (1980).
6) K. Nishimura, A. Nozaki, A. Yoshimi, N. Kakeya, and K. Kitao, *Jpn. J. Antibiot.*, **36**, 1692 (1983).