EFFECT OF FOOD ON ABSORPTION OF 450191-S, A 1*H*-1,2,4-TRIAZOLYL BENZOPHENONE DERIVATIVE FROM RAT SMALL INTESTINE

MASAHIRO KOIKE, RYO NORIKURA AND KOICHI SUGENO

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553, Japan

(Received September 4, 1985)

5-[(2-Aminoacetamide)methyl]-1-[*p*-chloro-2-(*o*-chlorobenzoyl)phenyl]-*N*, *N*-dimethyl-1*H*-*s*-triazole-3-carboxamide hydrochloride dihydrate (450191-S), a newly synthesized sleep inducer, is a masked compound which is converted to 1,4-benzodiazepine by intestinal aminopeptidases during absorption from the small intestine. This study was an investigation on the effects of food on 450191-S pharmacokinetics. When ¹⁴C-450191-S was administered to non-fasted rats, peak plasma levels of total radioactivity were markedly reduced and its excretion into the bile was more delayed than when it was administered to fasted rats. One of the causes for these phenomena was thought to be a delayed absorption caused by the direct interaction between 450191-S and food in the intestinal lumen as well as the delayed gastric emptying rate. To confirm this interaction, we examined the effect of food on 450191-S uptake by the small intestine using rat everted jejunal sac *in vitro*. Subsequently, we found that the desglycylation of 450191-S was inhibited by rat food and that the uptake of 450191-S was reduced.

In conclusion, 450191-S absorption is delayed by food, which results in the decreased total plasma level of 450191-S metabolites.

Keywords — 1*H*-1,2,4-triazolyl benzophenone derivative; ring-opened 1,4-benzodiazepine derivative; metabolic activation; sleep inducer; decreased absorption rate; food; delayed excretion; bile; rat

INTRODUCTION

5-[(2-Aminoacetamide)methyl]-1-[*p*-chloro-2-(*o*-chlorobenzoyl)phenyl]-*N*, *N*-dimethyl-1*H*-*s*-triazole-3-carboxamide hydrochloride dihydrate (450191-S) is a newly synthesized sleep inducer¹⁻³⁾ which is a water-soluble, ring-opened derivative of 1,4-benzodiazepine. It is metabolized to 191DG by intestinal aminopeptidases during a single passage through the intestinal wall and is spontaneously converted to M-1, an active metabolite.⁴⁾ The carbamoyl side chain of M-1 is successively metabolized to form M-D, M-2, M-A, M-3 and M-4 (Fig. 1).^{5,6)} All of these metabolites have high affinities for benzodiazepine receptors.^{5,7)} In studying the absorption, distribution, metabolism and excretion of 450191-S using a ¹⁴C-labeled compound, we noticed that the plasma levels and the area under the plasma concentration-time curves (*AUC*) of radioactivities were much higher when the compound was orally administered to fasted rats than to non-fasted rats. The purpose of this study was to clarify the effect of food on the pharmacokinetics of 450191-S.

MATERIALS AND METHODS

Animal — Male Jcl-SD rats were purchased from Clea Japan Inc. and were maintained with a standard animal chow (CA-1, Clea Japan Inc., Tokyo, Japan) and water in a room kept at a constant temperature (24 ± 1 °C) and humidity (55 ± 5%) until use. Rats weighing 250–300 g were used. When rats were to be fasted, they were transferred to a cage with a stainless steel net floor (mesh size: 1.5 × 1.5 cm) 24 h prior to drug administration and deprived of chow but not water.

Chemicals — ¹⁴C-450191-S (specific activity: 20.53 μCi/mg) was synthesized by Dr. M. Yoshioka and his co-workers in this laboratory. The purity determined using thin-layer chromatography (TLC) was of more than 97%. The labeled position is denoted in Fig. 2. Other reagents were of reagent grade.

Administration — ¹⁴C-450191-S dissolved in 0.9% saline at 1.5 mg/ml was orally administered to fasted or non-fasted rats at a dose of 3 mg/kg at 12:00.

Determination of Plasma Level — Polyethylene tubing (PE-50, Clay Adams, N.J., USA) was inserted into the right jugular vein of fasted and

FIG. 2. ¹⁴C-Labeled Position of 450191-S

amount.

Determination of Biliary Excretion — Fasted and non-fasted rats were similarly anesthetized. After midline incision of the abdomen, polyethylene tubing (PE-10, Clay Adams, N.J., USA) was inserted into the common bile duct and each rat was placed in a Bollman cage. After at least 2 h, ¹⁴C-450191-S was orally administered and the bile was periodically collected until 48 h. The radioactivity was determined as described above.

In Vitro Everted Sac of Small Intestine — Details for preparing and incubating the everted sac of rat small intestine have been previously described.⁴⁾ A 15-cm length of everted sac containing 3 ml of Krebs-Henseleit buffer (serosal fluid) was incubated in 15 ml of Krebs-Henseleit buffer containing 0.01 mM of 450191-S (mucosal fluid) at 37 °C. The everted jejunal sacs prepared from non-fasted or 24-h fasted rats were incubated with or without food extracts in the mucosal fluid for 1 h. The food extracts were prepared as described below. Rat chow (CA-1, Clea Japan Inc., Tokyo, Japan) was ground with a mortar and suspended in 10 vol of Krebs-Henseleit buffer, the mixture kept at 0 °C overnight under an atmosphere of 5% CO₂-95% O₂ and used after filtration. 450191-S and its metabolites in the mucosal fluid or intestinal tissue were determined using high performance liquid chromatography (HPLC) as described elsewhere.⁴⁾ As 191DG and M-1 were not separately determined, the value of M-1 represented the sum of 191DG and M-1.

RESULTS

The time course of plasma radioactivity level following oral administration of ¹⁴C-450191-S

FIG. 3. Plasma Level of Radioactivity after Oral Administration of ¹⁴C-450191-S (3 mg/kg) in Fasted and Non-fasted Rats

Each value was calculated in term of 450191-S amount and represented as the mean $\pm$ S.E. of fasted ($\circ$, $n = 7$) and non-fasted ($\bullet$, $n = 8$) rats.

(3 mg/kg) to fasted or non-fasted rats is shown in Fig. 3. In fasted rats, the concentration attained its peak within 15 min, amounting to 804 ng/ml (calculated as 450191-S level). In non-fasted rats, the peak of radioactivity appeared at 1 h and its concentration was only 20% of that in fasted rats. The AUC calculated by the trapezoidal rule over 24 h was 2008 ng $\cdot$ h/ml in fasted rats and 1118 ng $\cdot$ h/ml in non-fasted rats.

Biliary excretion of radioactivity (represented as % of dose) following oral administration of ¹⁴C-450191-S to fasted or non-fasted rats is shown in Fig. 4. More than 90% of the radioactivity was excreted in the bile in both groups of rats, while the excretion in urine was very low, amounting to 5.9 and 2.5% in fasted and non-fasted rats, respectively (data not shown). In fasted rats, the biliary excretion rate was more rapid than that in non-fasted rats, namely, in the initial 2 h, 64% of the radioactivity administered was excreted in fasted rats and 22% in non-fasted rats.

Tables I and II show the effects of food on 450191-S metabolism following incubation of 0.01 mM 450191-S with a rat everted intestinal

FIG. 4. Biliary Excretion of Radioactivity after Oral Administration of ^{14}C -450191-S (3 mg/kg) in Fasted Rats and Non-fasted Rats

Each value was calculated as percent of dose and represented as the mean $\pm$ S.D. of fasted (○, $n = 4$) and non-fasted (●, $n = 3$) rats.

sac at 37 °C for 1 h. The total levels (sum of 450191-S, M-1 and M-2) in the intestinal tissue obtained from 24-h fasted rats was the same as that obtained from non-fasted rats (Table II). On the other hand, when 450191-S was incubated with an everted sac prepared from non-fasted rats in the buffer containing food extracts, unchanged 450191-S in the mucosal fluid significantly increased (Table I). When 8 ml of food extract was added to mucosal fluid, the total concentration of uptake of 450191-S and its metabolites significantly decreased.

DISCUSSION

The mechanisms of metabolic activation of 450191-S have been previously reported.^{4,5,7)} As a first step, 450191-S is desglycylated by intestinal aminopeptidases, then spontaneously cyclized to M-1 (Fig. 1). Aminopeptidase hydrolysis occurs during single passage through the intestinal wall. The aminopeptidases also enhances the uptake of 450191-S by the small intestine,

TABLE I. Effect of Food Extracts on Metabolism of 450191-S by Everted Jejunal Sac Obtained from Fasted or Non-fasted Rats

Rat	Food extract (ml) ^{b)}	$n^c)$	Concentration in mucosal fluid (nmol/ml) ^{a)}			
			450191-S	M-1	M-2	Total ^{d)}
Non-fasted	—	9	0.22 $\pm$ 0.06	4.46 $\pm$ 0.35	1.02 $\pm$ 0.20	5.69 $\pm$ 0.41
Non-fasted	2	3	0.34 $\pm$ 0.01 ^{f)}	4.32 $\pm$ 0.33	0.90 $\pm$ 0.08	5.56 $\pm$ 0.31
	4	3	0.84 $\pm$ 0.11 ^{f)}	3.81 $\pm$ 0.20 ^{e)}	0.99 $\pm$ 0.21	5.64 $\pm$ 0.05
	8	3	1.42 $\pm$ 0.19 ^{f)}	3.81 $\pm$ 0.36 ^{e)}	0.69 $\pm$ 0.03 ^{f)}	5.92 $\pm$ 0.20
Fasted	—	3	0.24 $\pm$ 0.10	4.55 $\pm$ 0.24	0.76 $\pm$ 0.09 ^{e)}	5.55 $\pm$ 0.27

a) Mean $\pm$ S.D. b) Volume of food extracts in mucosal fluid (15 ml). c) Number of experiments. d) Sum of 450191-S, M-1 and M-2. e) Significant at $p < 0.05$ compared with the value obtained from the incubation without food extracts in non-fasted rats. f) Significant at $p < 0.01$ compared with the value obtained from the incubation without food extracts in non-fasted rats.

TABLE II. Effect of Food Extracts on Uptake of 450191-S by Everted Jejunal Sac Obtained from Fasted or Non-fasted Rats

Rat	Food extract (ml) ^{b)}	$n^c)$	Concentration in intestinal tissue (nmol/g) ^{a)}			
			450191-S	M-1	M-2	Total ^{d)}
Non-fasted	—	9	1.19 $\pm$ 0.27	15.52 $\pm$ 1.12	5.04 $\pm$ 1.42	21.76 $\pm$ 1.44
Non-fasted	2	3	1.50 $\pm$ 0.03 ^{e)}	17.44 $\pm$ 1.46 ^{e)}	3.80 $\pm$ 0.42	22.74 $\pm$ 1.03
	4	3	1.69 $\pm$ 0.21 ^{e)}	16.27 $\pm$ 1.31	4.39 $\pm$ 1.41	22.35 $\pm$ 1.56
	8	3	1.57 $\pm$ 0.55	15.61 $\pm$ 1.60	2.25 $\pm$ 0.11 ^{f)}	19.43 $\pm$ 1.81 ^{e)}
Fasted	—	3	0.38 $\pm$ 0.66 ^{f)}	17.82 $\pm$ 0.45 ^{e)}	3.47 $\pm$ 0.62	21.67 $\pm$ 1.48

a) Mean $\pm$ S.D. b) Volume of food extracts in mucosal fluid (15 ml). c) Number of experiments. d) Sum of 450191-S, M-1 and M-2. e) Significant at $p < 0.05$ compared with the value obtained from the incubation without food extracts in non-fasted rats. f) Significant at $p < 0.01$ compared with the value obtained from the incubation without food extracts in non-fasted rats.

which was substantiated by the results indicated that the magnitude of the inhibition of aminopeptidases was negatively correlated with the extent of 450191-S uptake using everted sac of rat jejunum.⁸⁾

In general, intestinal aminopeptidases are known to be related with digestion and absorption of proteins and peptides in food.⁹⁾ As shown in a previous paper,⁸⁾ the desglycylation of 450191-S is competitively inhibited by dipeptides such as glycyl-L-leucine or L-leucyl-L-leucine. Accordingly, the protein in food or peptides formed by digestive enzymes may inhibit the aminopeptidases, which may affect 450191-S absorption from the intestinal tract. Thus, the plasma level of radioactivity was compared after ¹⁴C-450191-S was orally administered to fasted or non-fasted rats (Fig. 3). Although, the biliary excretion in fasted rats was more rapid than that in non-fasted rats (Fig. 4), the plasma level in fasted rats was higher than that in non-fasted rats. From these results, we believed that the delayed absorption of 450191-S in small intestine occurred in non-fasted rats. Watanabe *et al.*¹⁰⁾ have reported that the gastric emptying rate is delayed in the non-fasted rat, which may be responsible for the delayed absorption. Another reason for this can be that the direct interaction between 450191-S and food may occur in intestinal lumen. To clarify this, we examined *in vitro* uptake of 450191-S in the presence of food using everted sac of rat jejunum. Since a previous result⁴⁾ revealed that the transport of 450191-S and its metabolites to the serosal fluid in this system was very low, the metabolites there were not determined. As shown in Table I, unchanged 450191-S in mucosal fluid was increased as addition of food extracts to mucosal fluid was increased, which certainly indicated that the aminopeptidases was inhibited by the food extracts. On the other hand, the uptake of 450191-S represented as total concentration in Table II was significantly reduced when 8 ml of food extracts was added. This is direct evidence for reduced 450191-S uptake by food, which led us to expect that food might decrease the absorption rate of 450191-S from the intestinal lumen. In fact, the effect *in vivo* is expected to be more distinct, because the amounts of food in the intestinal tract are greater than those in this everted-sac experiment. Thus, the cause for the decreased absorption rate was

demonstrated, but the cause for the decreased *AUC* of plasma level remained obscure. A previous paper¹¹⁾ has reported that the hepatic first-pass extraction of 450191-S is markedly large and easily saturated. The larger *AUC* in fasted rats might be responsible for the saturated hepatic extraction because of faster absorption. As the metabolites in the plasma was not separately determined, the cause for this remains obscure.

In conclusion, the absorption rate of 450191-S is found to be reduced by food, which results in the decreased peak plasma level and *AUC* in rats.

REFERENCES

- 1) K. Hirai, H. Sugimoto, T. Ishiba, T. Fujishita, Y. Tsukinoki and K. Hirose: Synthesis and biological activity of (3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)benzophenone derivatives, *J. Heterocycl. Chem.*, **19**, 1363–1369 (1982).
- 2) K. Yamamoto, A. Matsushita, T. Sawada, Y. Naito, K. Yoshimura, H. Takesue, S. Utsumi, K. Kawasaki, S. Hirono, H. Koshida and T. Tazaki: Pharmacology of a new sleep inducer, 1*H*-1,2,4-triazolyl benzophenone derivative, 450191-S (II). Sleep-inducing activity and effect on the motor system, *Nippon Yakurigaku Zasshi*, **84**, 25–89 (1984).
- 3) K. Yamamoto, K. Hirose, A. Matsushita, K. Yoshimura, T. Sawada, M. Eigyo, H. Jyoyama, A. Fujita, K. Matsushita, Y. Tsukinoki, H. Hatakeyama, T. Shiomi, N. Ibii, Y. Inoue, S. Hirose and H. Koshida: Pharmacological studies of a new sleep-inducer, 1*H*-1,2,4-triazolyl benzophenone derivatives (450191-S) (I). Behavioral analysis, *Nippon Yakurigaku Zasshi*, **84**, 109–154 (1984).
- 4) M. Koike, R. Norikura and K. Sugeno: Intestinal activation of a new sleep inducer 450191-S, a 1*H*-1,2,4-triazolyl benzophenone derivative in rats, *J. Pharmacobio-Dyn.*, **9**, 315–320 (1986).
- 5) M. Fujimoto, S. Hashimoto, S. Takahashi, K. Hirose, H. Hatakeyama and T. Okabayashi: Detection and determination of active metabolites of 1-(2-*o*-chlorobenzoyl-4-chlorophenyl)-5-glycylaminomethyl-3-dimethylcarbamoyl-1*H*-1,2,4-triazole hydrochloride dihydrate, (450191-S), in rat tissue, using a radioreceptor assay for benzodiazepines, *Biochem. Pharmacol.*, **33**, 1645–1651 (1984).
- 6) M. Koike, R. Norikura, S. Futaguchi, T. Yamaguchi, K. Sugeno, K. Iwatani, Y. Ikenishi and Y. Nakagawa: *N*-Hydroxymethyl metabolites of 450191-S, a 1*H*-1,2,4-triazolyl benzophenone derivative, in dog plasma, *Drug Metab. Dispos.*, in preparation.
- 7) M. Koike, S. Futaguchi, R. Norikura, T. Yoshimori, M. Nakanishi, S. Takahashi and K. Sugeno: Absorption, distribution, metabolism and excretion of a new sleep inducer, 450191-S, a 1*H*-1,2,4-triazolyl benzophenone derivative, *J. Pharmacobio-Dyn.*, **8**, s-147 (1985).
- 8) M. Koike, M. Nakanishi and K. Sugeno: Uptake of 450191-S, a 1*H*-1,2,4-triazolyl benzophenone deriva-

- tive, in the everted sac of rat small intestine: Role of intestinal aminopeptidases, *J. Pharmacobio-Dyn.*, in press.
- 9) D. M. Matthews: Intestinal absorption of peptides, *Pharmacol. Rev.*, **55**, 537–608 (1975).
- 10) J. Watanabe, H. Okabe, T. Ichihashi, K. Mizojiri, H. Yamada and R. Yamamoto: Gastric emptying rate constants after oral administration of drug solution to mice, rats, and rabbits, *Chem. Pharm. Bull.*, **25**, 2147–2155 (1977).
- 11) M. Koike, R. Norikura, T. Yoshimori, S. Futaguchi and K. Sugeno: Biopharmaceutical characterization of 450191-S, a ring-opened 1,4-benzodiazepine derivative. I. Active metabolite levels in rat plasma, *J. Pharmacobio-Dyn.*, in press.