0022-3565/94/2713-1509$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 271:1509-1513, 1994

Vol. 271, No. 3
Printed in U.S.A.

Enhanced Permeability of Tetragastrin across the Rat Intestinal Membrane and Its Reduced Degradation by Acylation with Various Fatty Acids

ETSUO YODOYA, KAZUYO UEMURA, TAKEHIRO TENMA, TAKUYA FUJITA, MASAHIRO MURAKAMI, AKIRA YAMAMOTO and SHOZO MURANISHI

Department of Biopharmaceutics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto, Japan

Accepted for publication August 9, 1994

ABSTRACT

Three types of chemically modified tetragastrin (TG) with fatty acids such as acetyl-TG, caproyl-TG and lauroyl-TG were synthesized and their *in vitro* intestinal permeability characteristics were estimated by a modified Ussing chamber system using the isolated intestinal membrane of rats. The penetration of TG across the large intestine was increased by conjugation with acetic acid and caproic acid but not lauric acid. Lauroyl-TG, a highly lipophilic derivative, exhibited low permeability across the intestinal membrane. A "bell-shaped" profile was observed between the apparent permeability coefficients and lipophilicity of the acyl-TG derivatives. The stability of acyl-TG derivatives was examined in homogenates of the jejunum, proximal and distal large intestine, liver and plasma. The half-lives for the proteolysis of the TGs were significantly prolonged by chemical modification with fatty acids in each homogenate. Thus, the chemical modification of TG with some fatty acids not only increases the lipophilicity of TG but also reduces its degradation, which resulted in increased intestinal absorption. The extent of the conjugates' hepatic first-pass metabolism was evaluated by gastric acid secretion activities after i.v. and intraportal administration. The amount of gastric acid secretion after intraportal administration of TG was significantly reduced in comparison with that after i.v. administration. On the other hand, conjugation with caproic acid slightly suppressed TG's hepatic first-pass metabolism, which suggests that chemically modified TGs with fatty acids would be more stable than the native TG in the systemic circulation after intestinal absorption.

The bioavailability of peptide and protein drugs after oral administration is poor because they are extensively degraded by proteases in the gastrointestinal tract and are poorly absorbed through the intestinal mucosa (Lee and Yamamoto, 1990). Various approaches have been investigated to overcome these problems, *e.g.*, attempts to improve intestinal absorption include; coadministration with absorption enhancers such as mixed micelles (Muranushi *et al.*, 1980ab), salicylates (Nishihata *et al.*, 1986), bile acids and polyoxyethylene-9-lauryl ether (Yamamoto *et al.*, 1992) and protease inhibitors such as aprotinin (Ziv *et al.*, 1987; Saffran *et al.*, 1988), soybean trypsin inhibitor (Kidron *et al.*, 1982) and FK-448 [4-(4-isopropylpiperazinocarbonyl) phenyl 1, 2, 3, 4-tetrahydro-1-naphthoate methanesultonate] (Fujii *et al.*, 1985). However, additives may cause local irritation of the intestinal mucosa and nonselective absorption of other exogenous compounds. Therefore, alternative methods are required to enhance the selective absorption of peptide and protein drugs from the gastrointestinal tract.

Received for publication March 21, 1994.

Chemical modification of peptides and proteins is a potentially useful approach because this method can alter the various physicochemical properties of peptides, such as an increase in their molecular weight and lipophilicity (Hashimoto *et al.*, 1989; Tenma *et al.*, 1993). Furthermore, it has been reported that chemically modified peptides can exhibit increased resistance to enzymatic degradation (Haga *et al.*, 1990; Walsh and Laster, 1973). To improve the intestinal absorption of peptide drugs, we synthesized novel lipophilic derivatives of TRH (Muranishi *et al.*, 1991; Yamada *et al.*, 1992), insulin (Hashimoto *et al.*, 1989; Hashizume *et al.*, 1992) and TG (Muranishi *et al.*, 1992; Tenma *et al.*, 1993) by covalent attachment with various saturated fatty acids. We previously observed that lauroyl-TRH, a chemically modified TRH derivative to an N-terminal pyroglutamyl group with lauric acid, retained 64% and 81% of the original central nervous system activity and thyrotropin-stimulating hormone releasing activity, respectively, and that the intestinal absorption of TRH was significantly increased by such chemical modification. Furthermore, we found that acylation also improved the intestinal absorption of insulin (Hashizume *et*

ABBREVIATIONS: TG, tetragastrin; Ac-TG, acetyl-TG; Cap-TG, caproyl-TG; Lau-TG, lauroyl-TG; DMAc, dimethylacetoamide; PBS, phosphate-buffered saline; TRH, thyrotropin-releasing hormone; P_{app}, apparent permeability coefficients; HPLC, high-performance liquid chromatography.

al., 1992) and these acyl derivatives of insulin retained their pharmacological activities as assessed by their hypoglycemic effects after i.v. administration (Hashimoto *et al.*, 1989). In addition, such acylated derivatives of peptides were more stable than the parent peptides in plasma and the intestinal mucosa.

TG, the C-terminal tetrapeptide sequence of gastrin, Try-Met-Asp-Phe-NH_2 (fig. 1), possesses the entire range of physiological properties compared with the intact 17-amino acid peptide, although it is not as potent on a molar basis (Tracy and Gregory, 1964; Morley *et al.*, 1965). The intestinal absorption of TG was reported to be relatively poor on account of its extensive enzymatic degradation in the gastrointestinal tract (Jennewein *et al.*, 1974; Laster and Walsh, 1968; Walsh and Laster, 1973). We previously synthesized several novel lipophilic TG derivatives by acylation and confirmed that they were more lipophilic than TG (Tenma *et al.*, 1993). Furthermore, we demonstrated that the pharmacological activities of these lipophilic TG derivatives in rats were higher than that of TG after i.v. injection and that their intestinal absorption, except for Lau-TG was better than that of TG after administration into the large intestine (Tenma *et al.*, 1993). An *in situ* intestinal absorption study of acyl-TG derivatives also showed that they were better absorbed from the large intestine than the small intestine. However, the mechanism whereby the regional differences in their absorption was not fully examined.

To clarify the relationship between the intestinal absorption of TG derivatives and their lipophilicity and stability in the gastrointestinal tract, we examined the intestinal permeability of TG derivatives with a modified Ussing chamber and their stability using intestinal mucosal homogenates. We also investigated their absorption characteristics from the small and large intestine.

Materials and Methods

Chemicals. TG was purchased from Peptide Institute (Osaka, Japan). Three acyl-TG derivatives (fig.1), Ac-TG, Cap-TG and Lau-TG were synthesized according to the method of Tenma *et al.* (1993). DMAc was purchased from Wako Pure Chemical Industries (Osaka, Japan). Trifluoroacetic acid and acetonitrile were obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the finest reagent grade available and were used without further purification.

Determination of lipophilicity of acyl-TG derivatives. The lipophilicity of acyl-TG derivatives was determined with reversed-phase HPLC (Hitachi, Ltd., Tokyo, Japan) using a C_{18} column (4.6 × 150 mm) of YMC-AM302 (YMC, Ltd., Kyoto, Japan). The column was eluted with a linear gradient of acetonitrile (20–100%, 30 min) in 0.1% trifluoroacetic acid at a flow rate of 0.7 ml/min. The eluate was monitored with an ultraviolet detector at a wavelength of 230 nm. Lipophilic index values of acyl-TG derivatives were calculated using the following equation: lipophilic index = $\log(T_R - T_0)/T_0$, where T_R is the retention time of acyl-TG derivatives and T_0 is that of the solvent (acetonitrile/acetic acid = 1:1).

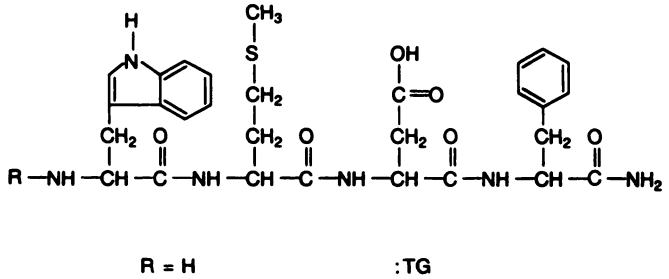

Fig. 1. Structure of TG and its acyl derivatives.

Intestinal penetration of TG derivatives. The penetration of TG derivatives across the jejunal and colonic mucosal membrane was studied according to the methods of Buur *et al.* (1990) with a slight modification. In brief, male Wistar rats (Japan SLC, Shizuoka, Japan; 250–300 g) were fasted for about 24 hr before the experiments but given water *ad libitum*. The rats were anesthetized with sodium pentobarbital (32 mg/kg i.p.) and were sacrificed by exsanguination from the inferior vena cava. The intestine was excised and rinsed in saline. Avoiding Peyer's patches, the experimental segments (jejunum and colon) were obtained and the underlying muscularis was removed before mounting in a modified Ussing chamber. Krebs-Ringer bicarbonate buffer (2.5 ml) containing 5% DMAc (transport buffer) was added to the reservoir bathing the serosal side. An equal volume of transport buffer containing 1 mM of acyl-TG derivatives was then added to the mucosal side. Each compartment of chamber was mixed by bubbling a 95% O_2/5% CO_2 mixture and the temperature within the chamber was maintained at 37°C by a circulating water bath. At predetermined times up to 3 hr, 100 μl of solution was sampled from the serosal side and immediately replaced by an equal volume of transport buffer. The analysis of acyl-TG derivatives was carried out by reversed-phase HPLC. P_{app} were calculated by the relationship $P_{app} = dX_R/dT \cdot 1/A \cdot C_0$, where P_{app} is the apparent permeability coefficient in centimeters per second, X_R is the amount of acyl-TG derivatives in moles in the receptor side, A is the diffusion area (*i.e.*, in square centimeters) and C_0 is the initial concentration of acyl-TG derivatives in the donor side in moles per milliliter.

Stability of acyl-TG derivatives in plasma and various homogenates. Intestinal mucosal homogenates were prepared according to the method of Hayakawa *et al.* (1989) with slight modifications. The isolated rat mucosa was scraped off with a glass slide and pooled in ice-cold isotonic PBS solution, separating each intestinal segment into jejunal, proximal and distal large intestine. Each specimen of the intestinal mucosa was homogenized using a Polytron homogenizer (Kinematica AG, Switzerland). Liver homogenate was also prepared by a similar method after the infusion of 0.15 M NaCl (saline) from the portal vein to avoid blood contamination. Each homogenate was centrifuged at 5000 × *g* in a refrigerated (4°C) centrifuge for 10 min to remove cellular and nuclear debris. The resulting supernatant and plasma were adjusted with PBS to a protein concentration of 10 mg/ml, as determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. The stability of acyl-TG derivatives was determined by incubating 200 μl of a tissue supernatant or diluted plasma with 800 μl of test solution, which had been preincubated at 37°C for 10 min. The test solution were prepared by dissolving the acyl-TG derivative in PBS that contained 0.5% DMAc at a final concentration of 0.1 mM. Aliquots (50 μl) of the incubation mixture were taken at various time intervals and were added to 100 μl of acetonitrile to terminate the reaction. The resulting mixture was centrifuged at 10,000 × *g* for 5 min to remove the precipitated proteins. The supernatant (30 μl) was assayed for the remaining amount of acyl-TG derivatives by HPLC.

Extent of first-pass effect of acyl-TG derivatives by the liver. To evaluate the contribution of the hepatic first-pass metabolism of acyl-TG derivatives, gastric acid secretion after i.v. or intraportal injection was determined by the method of Ghosh and Schild (1958). Briefly, male Wistar rats (230–270 g) were fasted for about 48 hr before the experiment but given water *ad libitum*. The rats were anesthetized with urethane (1.5 g/kg i.p.) and surgically prepared for the gastric perfusion. The stomach was rinsed and continuously perfused at a rate of 1.0 ml/min with saline. Acyl-TG

derivatives were dissolved in 200 μl of PBS (pH 7.4) containing 30% DMAc (at a final concentration of 63.9 μM). The test solution (200 μl) was administered into the femoral or portal vein and the increase in gastric acid secretion was determined. The amount of gastric acid secretion into the perfusate was determined by a pH-stat (titrant; 0.1 M NaOH). A control experiment was performed in which the basal gastric acid secretion was measured for 90 min after intraportal or i.v. injection of saline. To obtain the net effect in gastric acid output, the basal values were subtracted from the acyl-TG *versus* time-effect profiles. The increase in total acid output after intraportal or i.v. administration was integrated by estimating the area under the time-effect curve minus basal secretion.

Statistical analyses. The results are expressed as the mean ± S.E. and statistical significance was assessed with Student's *t* test.

Results

Intestinal penetration of acyl-TG derivatives. Figure 2 shows the time course of jejunal and colonic penetration of acyl-TG derivatives. TG and Lau-TG did not appear across the jejunal mucosa for up to 3 hr; the recovered amounts of Ac-TG and Cap-TG in the receptor side at the end of the experiment were only 0.05% and 0.04% of the applied dose, respectively. On the other hand, the colonic penetration of Ac-TG and Cap-TG was much better than their jejunal penetration and 1.2% and 0.57% of the applied dose were recovered in 3 hr, respectively. However, in the case of Lau-TG, no penetration was seen in either jejunal and colonic regions.

Table 1 summarizes the P_{app} values of the acyl-TG derivatives across the jejunal and colonic mucosal membrane. The intestinal permeability of TG was improved by chemical modification with fatty acids, except for Lau-TG, in both intestinal segments. In particular, the enhanced permeability of these derivatives by acylation was much greater in the colon than in the jejunum (table 1).

Relationship between lipophilicity and intestinal permeability. In the colon, a "bell-shaped profile" was seen in the relationship between the P_{app} permeability coefficient and lipophilicity of acyl-TG derivatives (fig. 3). Thus, there appeared to be an optimal lipophilicity for the intestinal penetration of acyl-TG derivatives and the P_{app} value of Lau-TG was low in spite of its high lipophilicity. A similar tendency was also observed in the jejunum but its extent was much less than in the colon.

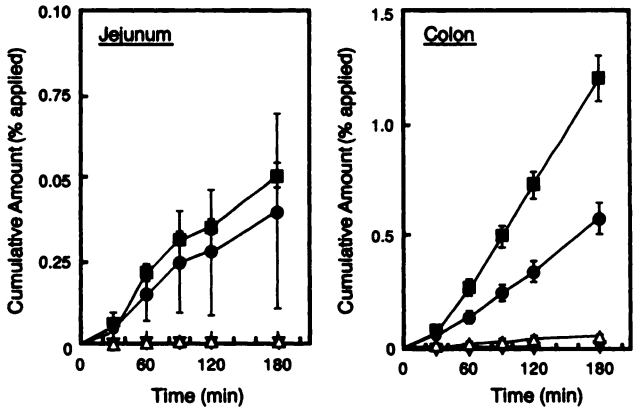

Fig. 2. Time course of penetration of TG and its derivatives across the jejunal and colonic mucous membrane. Results are expressed as the mean ± S.E. of four experiments. TG, △; Ac-TG, ■; Cap-TG, ●; and Lau-TG, ▼.

TABLE 1

P_{app} values for the transport of TG and its derivatives across the jejunal and colonic mucous membranes

	P_{app}[a]	
	Jejunum	Colon
	$\times 10^{-6}$ cm/sec	
TG	ND[b]	0.33 ± 0.04
Ac-TG	0.39 ± 0.05	11.4 ± 0.81*
Cap-TG	0.33 ± 0.25	5.15 ± 0.64*
Lau-TG	ND[b]	ND[b]

[a] Mean ± S.E. (n = 4).
[b] Not detected.
* $P < .01$ compared with TG.

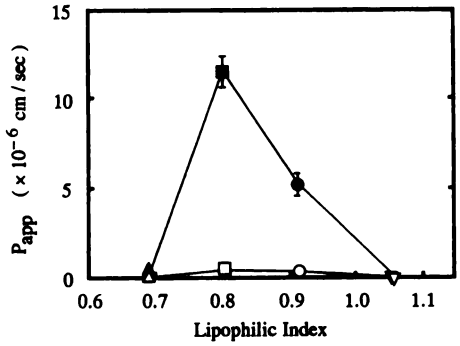

Fig. 3. Relationship between lipophilic index and P_{app} of TG and its derivatives across the jejunal and colonic mucous membrane. Results are expressed as the mean ± S.E. of four experiments. TG, △,▲; Ac-TG, □,■; Cap-TG, ○,●; and Lau-TG, ▽,▼, with open and closed symbols representing jejunal and colonic data, respectively.

Stability of acyl-TG derivatives. The degradation of acyl-TG derivatives in liver and various intestinal mucosal homogenates and plasma followed first-order kinetics (data not shown). Table 2 shows the half-lives for the hydrolysis of acyl-TG derivatives in various homogenates. The half-lives were significantly prolonged by chemical modification with fatty acids, which indicated that such acyl derivatives were more stable than the native TG. In the intestinal mucosal homogenates, the acyl derivatives were much more stable in the colon than in the jejunum. Of these acyl derivatives, Lau-TG was most stable in all homogenates.

Extent of hepatic first-pass metabolism of acyl-TG derivatives. Figure 4 shows the gastric acid output-time curves after i.v. or intraportal administration of TG. When TG was administered into the portal vein, the amount of gastric acid secretion was significantly reduced from 5.46 ± 1.31 to 1.53 ± 0.50 μEq compared with i.v. administration. By contrast, for Cap-TG, the amounts of total acid output after i.v. and intraportal injection were 15.9 ± 1.98 and 7.95 ± 1.10 μEq, respectively. These results suggested that conjugation of fatty acids to TG can avoid the hepatic first-pass metabolism to some extent, although almost one-half of Cap-TG was metabolized in the liver.

Figure 5 shows the correlation between total acid output after large intestinal administration of TG and its analogs and the P_{app} values of these compounds on colonic mucosa. A good positive correlation was obtained between these experimental model systems (r^2 = .987).

TABLE 2

Half-lives for the hydrolysis of TG and its derivatives in plasma and homogenates of liver and intestinal mucosa from various regions

Each value represents mean ± S.E. (n = 3).

	Half-Life				
	Plasma	Liver	Jejunum	Colon	Rectum
			min		
TG	6.48 ± 0.51	0.54 ± 0.07	1.41 ± 0.18	1.16 ± 0.16	0.85 ± 0.04
Ac-TG	—[a]	—[a]	7.43 ± 1.50*	97.6 ± 3.25***	—[a]
Cap-TG	1960 ± 511*	149 ± 38.0*	5.75 ± 0.69**	63.0 ± 11.1**	244 ± 38.3**
Lau-TG	—[a]	—[a]	72.4 ± 0.29***	361 ± 45.3**	—[a]

[a] Not determined.
* $P < .05$.
** $P < .01$.
*** $P < .001$, each compared with TG.

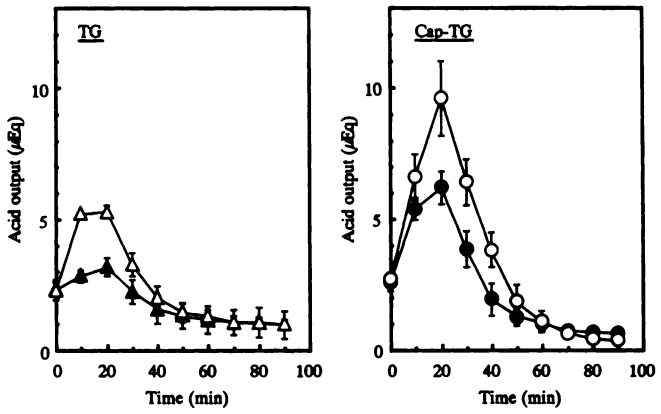

Fig. 4. Contribution of hepatic metabolism on acid output-time curves after i.v. administration of TG and Cap-TG in rats. Results are expressed as the mean ± S.E. of four to six experiments. Femoral vein, △,○; portal vein, ▲,●.

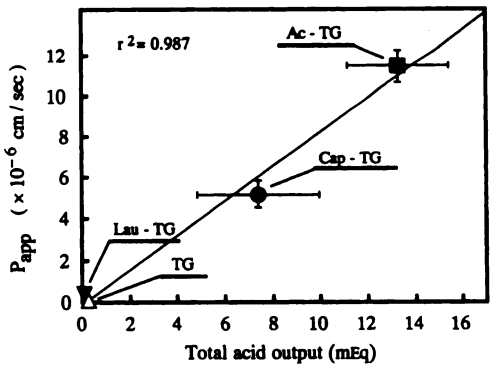

Fig. 5. Correlation of total acid output after administration of TG and its analogs into the large intestine and P_{app} of these compounds across colonic mucosa. Results are expressed as the mean ± S.E. of four to six experiments.

Discussion

Previously, we found that chemically modified TGs with various fatty acids were more potent than native TG, as assessed by gastric acid secretion activity after i.v. administration (Tenma *et al.*, 1993). Accordingly, acyl derivatives of TG maintain their pharmacological activity. Similar results were also obtained in the case of acyl-TRH and insulin derivatives (Hashimoto *et al.*, 1989; Muranishi *et al.*, 1991).

We previously demonstrated that the gastric acid secretion activities of Ac-TG and Cap-TG were much higher than that of the parent TG after administration into the large intestine, although no marked increase in the acid secretion activities occurred after administration into the intestine (Tenma *et al.*, 1993). The high absorption characteristics of acyl-TG *in vivo* were in good agreement with the present results that Ac-TG and Cap-TG were more permeable across the colonic membrane than was native TG with the *in vitro* modified Ussing chamber. Therefore, the improved absorption characteristics of acyl-TGs in the *in vivo* studies may be partly explained by their high permeability characteristics across the intestinal membrane.

In this study, regional differences in the permeability of TG and its acyl derivatives were observed, *i.e.*, Ac-TG and Cap-TG were more permeable across the colon than in the jejunum (fig. 2). This finding is consistent with the results of *in vivo* gastric acid output studies that an increase in gastric acid secretion activity after the administration of TG and its acyl derivatives was more clearly observed after administration into the large intestine than in the small intestine. The reason for the regional different permeability is not clearly understood. Presumably, morphological differences in the thickness of the mucus layer, the unstirred water layer and membrane components between the small or the large intestine may affect the effectiveness of acylation on the intestinal permeability of TG.

A bell-shaped profile was observed between P_{app} values of these derivatives and their lipophilicity. Ac-TG showed the highest permeability across the jejunum and colon; no obvious difference was observed in the intestinal permeability between native TG and Lau-TG, the highest lipophilic derivative. This result was consistent with our previous result of *in vivo* absorption studies, as assessed by gastric acid secretion activities (Tenma *et al.*, 1993).

The higher permeability of Ac-TG and Cap-TG in comparison with native TG might be accounted for by their lipophilicity. This effectiveness of acylation on the intestinal absorption of peptides was also observed in the case of TRH and insulin (Hashizume *et al.*, 1992, Yamada *et al.*, 1992). On the other hand, the lack of permeability of Lau-TG may be explained by its low diffusion process across the unstirred water layer because of its excessive lipophilicity. Alternatively, the diffusion of Lau-TG in the cytosol may be inhibited because of its strong partition and binding to the brush border membranes.

Generally, peptides and proteins are known to be degraded in various intestinal fractions, such as mucosa, brush border membrane surface and cytosol, in which proteases and other metabolic enzymes are ubiquitous (Lee and Yamamoto, 1990). In the stability experiments, the present study demonstrated that the acyl derivatives of TG were more stable

than native TG in plasma, liver and various intestinal homogenates. This finding suggests that chemical modification of TG by various fatty acids might protect the degradation of TG by proteolytic enzymes such as aminopeptidase, carboxypeptidase and amidase (Walsh, and Laster, 1973). A positive effect of acylation on stability was also demonstrated in our previous reports of TRH (Yamada *et al.*, 1992) and insulin (Hashizume *et al.*, 1992; Asada *et al.*, 1994). That is, we observed that Lau-TRH, a lauric acid-TRH conjugate, was much more stable than TRH in rat plasma and monoacyl-insulin derivatives were relatively stable compared with the native insulin in intestinal fluids and the intestinal homogenates (Asada *et al.*, 1994). Similarly, Walsh and Laster (1973) reported that TG was resistant to the aminopeptidase activity by N-acylation with a *t*-butyloxycarbonyl group. These findings suggested that chemical modification of TG might improve the intestinal absorption of TG by not only increasing the lipophilicity and permeability of TG but also inhibiting the degradation of TG by proteolytic enzymes. The good *in vivo* absorption characteristics of acyl-TG derivatives in our previous report might, therefore, be partly explained by the high stability of these compounds.

Regional differences in the intestinal stability of these acyl derivatives were observed, although the reason is unclear. We observed that the acyl derivatives were more stable in the large intestinal homogenate than in the jejunal homogenate. It is possible that the activities of the proteolytic enzymes responsible for TG hydrolysis might be higher in the small intestine than in the large intestine. Alternatively, there may be differences in the types of proteolytic enzymes involved in TG hydrolysis in the mucosa between the small and large intestine.

Our present results indicate that Cap-TG was less hydrolyzed in the liver than the native TG by gastric acid secretion measurement after intraportal or i.v. administration. This result was supported by the result of our stability studies that Cap-TG was more stable than TG in liver homogenate. These results indicate that TG was rapidly degraded by proteolytic enzymes in the liver and chemical modification of TG by caproic acid may inhibit its degradation in the liver, which may also be related to the high activities of gastric acid secretion after the intestinal administration of acyl-TG derivatives in comparison with the native TG.

In conclusion, the present study indicates that, by chemical modification of TG with various fatty acids, it might be feasible to increase its permeability across the intestinal membrane and protect it from degradation in the intestine and liver, hence improving the intestinal absorption of TG. In particular, Ac-TG seems to have the greatest permeability characteristics across the intestinal membrane among the acyl derivatives examined in this study. To improve further intestinal absorption of TG, we are now examining the effects of coadministered absorption enhancers and/or protease inhibitors on the intestinal absorption of acyl-TG derivatives. The synergistic effects of acylation and these adjuvants will be described in a subsequent report.

Acknowledgment

A part of this work was supported by a Grant-in-Aid for Scientific Research from the Uehara Memorial Foundation.

References

Asada, H., Mizokoshi, Y., Douen, T., Yamamoto, A. and Muranishi, S.: Stability of novel acyl derivatives of insulin in the small intestine: Relative importance of insulin association characteristics in aqueous solution. Pharm. Res. (N. Y.) **11:** 1115–1120, 1994.

Buur, A., Yamamoto, A. and Lee, V. H. L.: Penetration of 5-fluorouracil and prodrugs across the intestine of the albino rabbit: Evidence for shift in absorption site from the upper to the lower region of the gastrointestinal tract by prodrugs. J. Controlled Release **14:** 43–51, 1990.

Fujii, S., Yokoyama, T., Ikegaya, K., Sato, F. and Yokoo, N.: Promoting effect of the new chymotrypsin inhibitor FK-448 on the intestinal absorption of insulin in rats and dogs. J. Pharm. Pharmacol. **37:** 545–549, 1985.

Ghosh, M. N. and Schild, H. O.: Continuous recording of gastric secretion in the rat. Br. J. Pharmacol. **13:** 54–61, 1958.

Haga, M., Saito, K., Shimaya, T., Maezawa, Y., Kato, Y. and Kim, S. W.: Hypoglycemic effect of intestinally administered monosaccharide-modified insulin derivatives in rats. Chem. Pharm. Bull. **38:** 1983–1986, 1990.

Hashimoto, M., Takada, K., Kiso, Y. and Muranishi, S.: Synthesis of palmitoyl derivatives of insulin and their biological activities. Pharm. Res. (N. Y.) **6:** 171–176, 1989.

Hashizume, M., Douen, T., Murakami, M., Yamamoto, A. and Muranishi S.: Improvement of large intestinal absorption of insulin by chemical modification with palmitic acid in rats. J. Pharm. Pharmacol. **44:** 555–559, 1992.

Hayakawa, E., Yamamoto, A., Shoji, Y. and Lee, V. H. L.: Effect of sodium glycocholate and polyoxyethylene-9-lauryl ether of the hydrolysis of varying concentrations of insulin in the nasal homogenates of the albino rabbit. Life Sci. **45:** 167–174, 1989.

Jennewein, H. M., Waidech, F. and Konz, W.: The absorption of tetragastrin from different sites in rats and dogs. Arzneim. Forsch. **24:** 1225–1228, 1974.

Kidron, M., Bar-On, H., Berry, E. M. and Ziv, E.: The absorption of insulin from various regions of the rat intestine. Life Sci. **31:** 2837–2841, 1982.

Laster, L. and Walsh, J. H.: Enzymatic degradation of C-terminal tetrapeptide amide of gastrin by mammalian tissue extracts. Fed. Proc. **27:** 1328–1330, 1968.

Lee, V. H. L. and Yamamoto, A.: Penetration and enzymatic barriers to peptide and protein absorption. Adv. Drug Delivery Rev. **4:** 171–207, 1990.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

Morley, J. S., Tracy, H. L. and Gregory, R. A.: Structure function relationships in active C-terminal tetrapeptide sequence of gastrin. Nature (Lond.) **207:** 1356–1359, 1965.

Muranishi, S., Murakami, M., Hashizume, M., Yamada, K., Tajima, S. and Kiso, Y.: Trials of lipid modification of peptide hormones for intestinal delivery. J. Controlled Release **19:** 179–188, 1992.

Muranishi, S., Sakai, A., Yamada, K., Murakami, M., Takada, K. and Kiso, Y.: Lipophilic peptides: Synthesis of lauroyl thyrotropin-releasing hormone and its biological activities. Pharm. Res. **8:** 649–652, 1991.

Muranushi, N., Kinugawa, M., Nakajima, Y., Muranishi, S. and Sezaki, H.: Mechanism for the inducement of the intestinal absorption of poorly absorbed drugs by mixed micelles: I. Effects of various lipid-bile salt mixed micelles on the intestinal absorption of streptomycin in rat. Int. J. Pharm. **4:** 271–279, 1980a.

Muranushi, N., Nakajima, Y., Kinugawa, M., Muranishi, S. and Sezaki, H.: Mechanism for the inducement of the intestinal absorption of poorly absorbed drugs by mixed micelles: II. Effect of the incorporation of various lipids on the permeability of liposomal membranes. Int. J. Pharm. **4:** 281–290, 1980b.

Nishihata, T., Miyake, M., Takahata, H. and Kamada, A.: The effect of adjuvants on the colonic absorption of cefmetazole and [$Asu^{1,7}$]-eel calcitonin in rats: Concentration dependent absorption pathways. Int. J. Pharm. **33:** 89–97, 1986.

Saffran, M., Bedra, C., Kumar, S. and Neckers D.: Vasopressin: A model for the study of effect of additives on the oral and rectal administration of peptide drugs. J. Pharm. Sci. **77:** 33–38, 1988.

Tenma, T., Yodoya, E., Tashima, S., Fujita, T. Murakami, M., Yamamoto, A. and Muranishi, S.: Development of new lipophilic derivatives of tetragastrin: Physicochemical characteristics and intestinal absorption of acyl-tetragastrin derivatives in rats. Pharm. Res. **10:** 1488–1492, 1993.

Tracy, H. J. and Gregory, R. A.: Physiological properties of a series of synthetic peptides structurally related to gastrin I. Nature (Lond.) **204:** 935–938, 1964.

Walsh, J. H. and Laster, L.: Enzymatic deamidation of the C-terminal tetrapeptide amide of gastrin by mammalian tissue. Biochem. Med. **8:** 432–449, 1973.

Yamada, K., Murakami, M., Yamamoto, A., Takada, K. and Muranishi, S.: Improvement of intestinal absorption of thyrotropin-releasing hormone by chemical modification with lauric acid. J. Pharm. Pharmacol. **44:** 717–721, 1992.

Yamamoto, A., Hayakawa, E., Kato, Y., Nishiura, A. and Lee, V. H. L.: A mechanistic study on enhancement of rectal permeability to insulin in the albino rabbit. J. Pharmacol. Exp. Ther. **263:** 25–31, 1992.

Ziv, E., Lior, O. and Kidron, M.: Absorption of protein via the intestinal wall. A quantitative model. Biochem. Pharmacol. **36:** 1035–1039, 1987.

Send reprint requests to: Akira Yamamoto, Ph.D., Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan.