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Improvement of Transdermal Delivery of Tetragastrin by Lipophilic Modification with Fatty Acids

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Abstract

The in-vitro permeability of chemically modified tetragastrin with fatty acids through the rat skin was studied. The permeability of these compounds through intact skin and stripped skin of rat was determined with a Franz-type diffusion cell. The permeation of tetragastrin across the intact skin was improved by chemical modification with acetic acid and butyric acid. However, tetragastrin and caproyl-tetragastrin did not permeate across the intact skin up to the end of experiment. The permeation of tetragastrin across the stripped skin was improved by chemical modification, the skin flux of these acyl derivatives being in the order: acetyl > butyroyl > caproyl. The stability of tetragastrin in skin homogenate was also significantly improved by chemical modification with fatty acids.

These results suggest that chemical modification of tetragastrin with fatty acids increases its lipophilicity, which makes it permeable across the stratum corneum. Moreover, the chemical modification reduced the degradation of tetragastrin in the viable skin, resulting an increase in permeation of tetragastrin across the skin.

The oral administration of peptides and proteins is often not practical or reliable since many peptides are either extensively degraded by proteases in the gut lumen or exhibit low permeability because of their large molecular sizes and hydrophilicity (Lee & Yamamoto 1990). Alternative routes including rectal (Yamamoto et al 1992), vaginal (Okada et al 1983), nasal (Hirai et al 1981) and pulmonary (Yamamoto et al 1994), have been investigated for peptide delivery.

Transdermal delivery of peptides is an attractive route due to the painless and controlled input of these agents and avoidance of the hepatic first-pass effect. However, it is unlikely that hydrophilic and high molecular weight compounds such as peptides will easily permeate across the skin, especially the stratum corneum. In addition, recent studies suggest that peptide drugs may also undergo an extensive enzymatic degradation in the viable skin (Banerjee & Ritschel 1989; Choi et al 1990; Shah & Borchardt 1991). It is, therefore, desirable to increase the transport of peptides across the lipoidal barrier membranes by overcoming the problems of their permeability and stability. One approach is to employ an active driving force such as iontophoresis or sonophoresis (Chien et al 1989; Tachibana 1992). Another approach is to utilize certain adjuvants including absorption enhancers such as oleic acid, azone and metabolic inhibitors (Ruland et al 1994; Choi et al 1990). These invasive approaches might be useful, but there are still some problems in their safety and with skin irritation.

We have previously shown that new acyl derivatives of peptide drugs such as tetragastrin, thyrotropin releasing hormone and insulin could be synthesized, while retaining their pharmacological activities and that the lipophilic modification of these peptides resulted in a significant increase in their enteral absorption by overcoming the poor permeability and enzymatic instability of the native agents (Muranishi et al 1991; Hashimoto et al 1992; Tenma et al 1993).

The purpose of the present study was to evaluate the effects of the chemical modification with fatty acids on transdermal delivery of peptides. Tetragastrin was selected as a model drug, and the transdermal permeability and degradation of tetragastrin and its acyl derivatives was examined using rat skin.

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Materials and Methods

Chemicals

Tetragastrin was purchased from Peptide Institute, Inc (Osaka, Japan). Three kinds of acyl derivatives (acetyl, butyroyl and caproyl) were synthesized as described previously (Tenma et al 1993). Dimethylacetoamide was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Trifluoroacetic acid, ethanol and acetonitrile were obtained from Nacalai Tesque, Inc (Kyoto, Japan). All other chemicals were of the finest reagent grade available and were used without further purification.

Analysis

The concentrations of acyl derivatives were assayed by reversed phase HPLC on a Cosmosil 5C18 packed column (250 × 4·6 mm). The HPLC consisted of a Shimadzu LC-10A pump, Shimadzu SPD-10A UV detector operated at 230 nm, and a Shimadzu SIL-10B auto injector. The mobile phases for tetragastrin and its derivatives were mixtures of methanol and water flowing at 1·0 mL min^{-1}.

Determination of lipophilic indexes of acyl derivatives

The lipophilic index (log k′) was determined by HPLC (Yamana et al 1977). A mixture (pH 7·4) of methanol

(70–40%) and phosphate-buffered saline (PBS) (30–60%) was employed as mobile phase. The elution time of a solvent (t_0) and the retention time of acyl derivatives (t_R) were determined at several mobile phase compositions. The log k′ value, defined by equation 1, was plotted against the methanol concentration in the mobile phase and the extrapolated log k′ value to 0% methanol was obtained as an index of lipophilicity of the acyl derivatives (log k'_0):

$$\log k' = \log (t_R - t_0) / t_0 \quad (1)$$

In-vitro skin permeation of acyl derivatives

Full-thickness abdominal skin excised from a male Wistar rat (Japan SLC, Shizuoka, Japan) (about 250 g) was used. Franz-type diffusion cells with an effective diffusional area of 0·95 cm^2 and receiver volume of 14·47 mL were used. After removal of hair with a hair clipper and of subcutaneous fat, the skin was mounted on a Franz-type diffusion cell with the epidermal side facing the donor cell. To evaluate the contribution of the stratum corneum, permeation through tape-stripped skin was also evaluated. After removal of hair with clippers, the abdominal skin was stripped 15 times with adhesive tape (Scotch tape, Sumitomo 3M Co., Japan). The receiver compartment was filled with 14·47 mL of PBS containing streptomycin sulphate (50 mg L^{-1}, Nacalai Tesque, Inc) and penicillin G potassium salt (30 mg L^{-1}, Nacalai Tesque, Inc) at pH 7·4. The apparatus was maintained at 37°C in a water bath throughout the experiment. The mounted skin was pretreated with PBS for 12 h. After 200 μL test solution containing 10 mM of acyl derivatives was administered to the donor compartment (the epidermis side), 100 μL reservoir solution was periodically collected. Drug concentrations of the sample solution were determined by reversed phase HPLC.

In-vitro stability of acyl derivatives in rat skin homogenate

Preparation of skin homogenate. After removing subcutaneous fat adhering to the underside of full-thickness hair clipped rat skin, the skin was cut into small pieces. Ten percent homogenates were made with PBS by homogenizing the skin, using a homogenizer (Kinematica, GmbH, Switzerland). The homogenate was centrifuged for 5 min at 3000 *g* at 4°C and supernatant was recovered and centrifuged for 30 min at 20 000 *g* at 4°C. The supernatant was collected and adjusted with PBS to a protein concentration of 5 mg mL^{-1}. The protein concentration in the tissue supernatant was determined by the method of Lowry et al (1951), using bovine serum albumin as the standard.

Stability of acyl derivatives in rat skin homogenate. The stability of acyl derivatives was determined by incubating tissue supernatant (final protein concn, 2 mg mL^{-1}) with test solution (final concn, 0·4 mM). Test solution and skin homogenate had been preincubated at 37°C for 10 min. Fifty microlitres of the incubation mixture was taken at various time intervals, and was added to 100 μL acetonitrile to terminate the reaction. The resulting mixture was centrifuged at 10 000 *g* for 5 min to remove the precipitated proteins. Supernatants were assayed for the remaining tetragastrin and its acyl derivatives by HPLC.

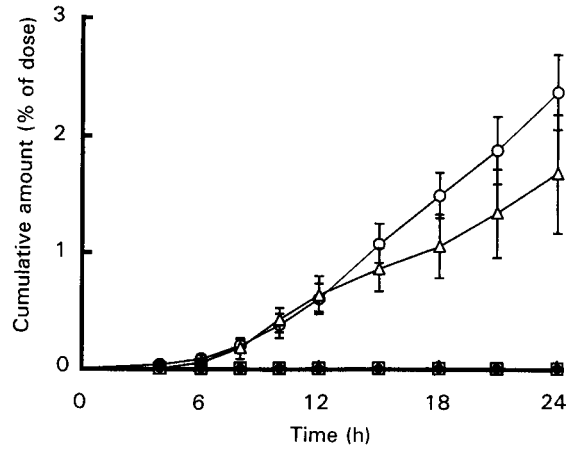

FIG. 1. Permeation profiles of tetragastrin (●) and its acetyl (○), butyroyl (△) and caproyl (□) derivatives across the rat intact skin. Each value represents the mean ± s.e.m. of 3 to 6 experiments.

Statistical analysis

Result are expressed as the mean ± s.e.m. and statistical significance was assessed using Student's *t*-test.

Results

In-vitro skin permeability of tetragastrin and its acyl derivatives

Figs 1 and 2 show the cumulative amount of tetragastrin and its acyl derivatives permeating. The flux of native tetragastrin and caproyl-tetragastrin across intact skin was not detected during the period of observation. In contrast, acetyl- and butyroyl-tetragastrin apparently permeated across the intact skin after a lag time of about 5–7 h; the cumulative amounts transported over 24 h were 2·4 and 1·7%, respectively. The permeability of these drugs through the rat stripped skin is shown in Fig. 2. All three derivatives extensively permeated through the stripped skin, while native tetragastrin did not. The cumulative amounts of acetyl-, butyroyl- and caproyl-tetragastrin transported in 6 h were 24, 16 and 8%, respectively. No lag time for these derivatives was detected in the stripped skin. The skin flux

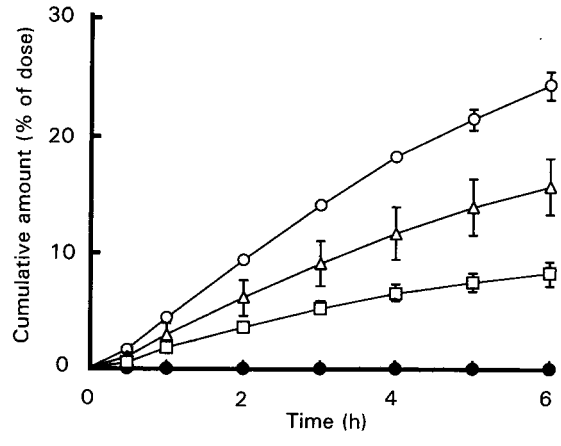

FIG. 2. Permeation profiles of tetragastrin (●) and its acetyl (○), butyroyl (△) and caproyl (□) derivatives across the rat stripped skin. Each value represents the mean ± s.e.m. of 3 to 6 experiments.

Table 1. Lipophilicity and in-vitro skin permeability of tetragastrin and its acyl derivatives across the skin.

	Lipophilic index	Intact skin Permeation (% cm^{-2} in 24 h)	Flux (% h^{-1})	Stripped skin Permeation (% cm^{-2} in 6 h)	Flux (% h^{-1})
Tetragastrin	3·13	ND	ND	ND	ND
Acetyl tetragastrin	3·24	2·36 ± 0·32	0·141 ± 0·017	24·2 ± 2·4	0·414 ± 0·021
Butyroyl tetragastrin	3·62	1·66 ± 0·51	0·085 ± 0·030	15·2 ± 2·4	0·269 ± 0·042
Caproyl tetragastrin	4·33	ND	ND	6·16 ± 1·0	0·143 ± 0·018

Mean ± s.e. (n = 3–4). ND = not detected.

and cumulative amount of compound across intact and stripped skin are summarized in Table 1.

Relationship between lipophilicity of tetragastrin and its acyl derivatives and their flux across the rat skin

Fig. 3 shows the relationship between lipophilicity and flux across rat skin. The lipophilic indexes are summarized in Table 1. The flux of the acyl derivatives decreased as the acyl chain-length increased.

Stability of tetragastrin and its acyl derivatives

Fig. 4 shows the degradation of tetragastrin and its acyl derivatives in rat skin homogenate. Tetragastrin was very rapidly degraded in the skin homogenate, but acyl derivatives were more stable.

Table 2 shows the half-lives for the hydrolysis of acyl derivatives in skin homogenate. The degradation of acyl derivatives in skin homogenate followed first-order kinetics (data not shown). The half-life for the degradation of tetragastrin was significantly prolonged by chemical modification with fatty acids, indicating that these acyl derivatives were more stable than the native compound in rat skin.

Discussion

The present study has demonstrated that the acyl derivatives exhibited improved permeability across the skin. This result was in good agreement with our previous findings that the intestinal absorption of tetragastrin was enhanced by its chemical modification with fatty acids (Tenma et al 1993; Yodoya et al 1994). Thus, acylation is useful for improving both transdermal and intestinal absorption of tetragastrin. A similar result was also observed by Möss & Bundgaard (1990), who demonstrated the enhanced transdermal permeation of TRH using the prodrug approach.

In general, one problem of this chemical modification approach is that the pharmacological activities of peptides should be maintained during the synthetic procedures. However, the pharmacological activities of acyl tetragastrin derivatives, as assessed by gastric acid secretion were similar to those of the native compound (Tenma et al 1993). Thus, it is suggested that the potency of tetragastrin was not affected by the acylation of its N-terminal amino acid residue.

In this study, the permeability of acyl derivatives across the stripped skin was higher than that across the intact skin. In particular, we found a remarkable permeability of caproyl-tetragastrin across the stripped skin, while its transport across the intact skin was not detectable. These findings suggest that like other drugs, stratum corneum acts as a major transport barrier for the acyl derivatives of tetragastrin. However, no significant difference was observed in the transdermal transport of tetragastrin between the intact and the stripped skin. This lack of permeability across these skins may be partly attributed to the low stability of tetragastrin, as discussed below.

The permeability of the acyl derivatives decreased with increasing lipophilicity. The low permeability of caproyl-tetragastrin may be due to its strong partition and binding

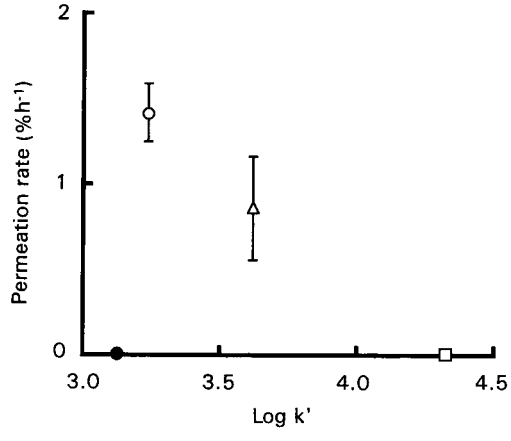

FIG. 3. Relationship between lipophilic index and flux of tetragastrin (●) and its acetyl (○), butyroyl (△) and caproyl (□) derivatives across the rat skin. Results are expressed as the mean ± s.e.m. of 3–5 experiments.

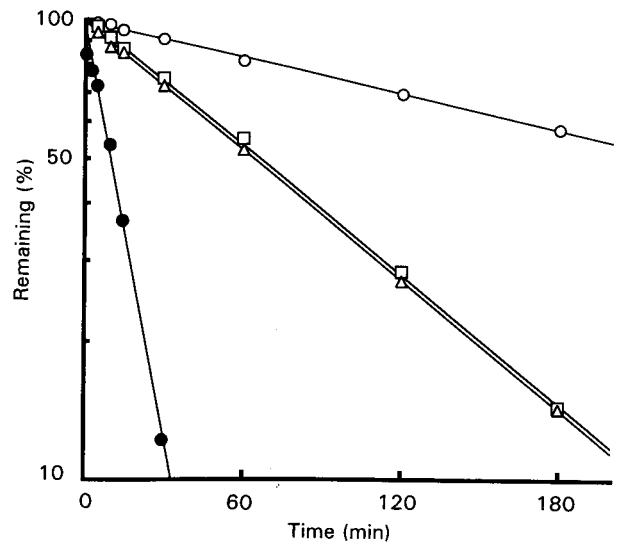

FIG. 4. Semilogarithmic plots of the proteolysis of tetragastrin (●) and its acetyl (○), butyroyl (△) and caproyl (□) derivatives in the rat-skin homogenate at 37°C.

Table 2. Half-lives of tetragastrin and its acyl derivatives in rat-skin homogenate.

	Half-life (min)
Tetragastrin	10
Acetyl tetragastrin	236
Butyroyl tetragastrin	64
Caproyl tetragastrin	64

Means of three experiments.

to the stratum corneum. These results suggest that for less lipophilic compounds such as tetragastrin, the stratum corneum plays an important role on limiting their transport across the skin, whereas viable epidermis and dermis offer a more significant resistance for the more lipophilic compounds such as caproyl-tetragastrin. From these findings, it may be considered that there is an optimal lipophilicity (length of acyl chain) of these acyl derivatives for improving their transdermal delivery.

Peptide and protein drugs are degraded by various peptidases before reaching the systemic circulation. In the skin, the proteolytic activity of stratum corneum was relatively low, but the viable dermis and epidermis have various kind of enzymes containing peptidases. Choi et al (1990) reported the metabolism of enkephalins both in rat skin homogenate and in-vitro transport studies. They proposed that at least two types of aminopeptidase activities were responsible for enkephalin metabolism. Samir & Jennifer (1994) reported that luteinizing hormone-releasing hormone was rapidly metabolized in mouse skin. Morimoto et al (1992) reported that proteolytic enzyme inhibitors enhanced the transdermal absorption of calcitonin with iontophoresis. Our findings suggest that various peptides were also metabolized in the skin as well as in the gastrointestinal tract. Indeed, our present stability experiments indicate that tetragastrin is rapidly degraded in the skin homogenate, which may account for its poor permeability across both intact and stripped skin.

The acyl derivatives were more stable than the native compound in rat skin homogenate. These results suggest that the stability of tetragastrin was improved by chemical modification with fatty acids, and this enhanced stability depends on the length of acyl chain. Such acylation effects for intestinal peptide stability have also been observed for other acyl derivatives of peptides such as thyrotropin-releasing hormone (Yamada et al 1992), insulin (Hashizume et al 1992; Asada et al 1994) and tetragastrin (Yodoya et al 1994). The mechanism whereby this acylation increased the stability of tetragastrin is not clear, but it may be possible that the alteration of binding affinity to the active center of peptidases may be related to the increased stability of tetragastrin.

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