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# Enhanced transdermal delivery of phenylalanyl-glycine by chemical modification with various fatty acids

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**Abstract**

We synthesized three novel lipophilic derivatives of phenylalanyl-glycine (Phe-Gly), C4-Phe-Gly, C6-Phe-Gly and C8-Phe-Gly by chemical modification with butyric acid (C4), caproic acid (C6) and octanoic acid (C8). The effect of the acylation on the stability, permeability and accumulation of Phe-Gly in the skin was investigated by in vitro studies. The stability of Phe-Gly in skin homogenates was low, but was significantly improved by the acylation. In the transport studies, a Franz-type diffusion cell was used for the permeability experiments with Phe-Gly and its acyl derivatives. The permeability of acyl-Phe-Gly derivatives across the intact skin was higher than that of native Phe-Gly. Of all the acyl-Phe-Gly derivatives, C6-Phe-Gly was the most permeable compounds across the intact skin. On the other hand, the permeability of acyl-Phe-Gly derivatives across stripped skin was less than that of native Phe-Gly in the initial time period of transport studies, but their permeability was higher than that of native Phe-Gly at the end of the transport studies. When the skin was pretreated with ethanol, which could inactivate the peptidases responsible for the degradation of Phe-Gly, the permeability of native Phe-Gly was higher than that of acyl derivatives. These findings indicated the involvement of peptidases on the permeability of Phe-Gly across the skin. The relationship between the lipophilic indexes of Phe-Gly derivatives and the permeability coefficients indicated that there is an optimal carbon number of fatty acid for improving the transdermal permeability of Phe-Gly by the acylation. A good correlation was found between the accumulation of these acyl-Phe-Gly derivatives in the intact skin and their lipophilicity. These results suggest that the stability and permeability of Phe-Gly were improved by chemical modification with fatty acids and this enhanced permeability of Phe-Gly by the acylation may be attributed to the protection of Phe-Gly from the enzymatic degradation in the skin and the increase in the partition of Phe-Gly to the stratum corneum.


*Keywords:* Percutaneous absorption; Skin permeation; Peptide drug delivery; Chemical modification; Skin metabolism; Transdermal drug delivery

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## 1. Introduction

The oral administration of peptide and protein drugs is often not practical or reliable, since many peptides are either extensively degraded by proteases in the gut lumen or generally have low permeability characteristics because of their large molecular size and their hydrophilicity (Lee and Yamamoto, 1990). Therefore, alternative routes such as rectal (Yamamoto et al., 1992), vaginal (Okada et al., 1983), nasal (Hirai et al., 1981) pulmonary (Yamamoto et al., 1994), and transdermal routes (Choi et al., 1990), are being investigated for peptide delivery.

Transdermal delivery of peptides is an attractive route, since this route has many advantages such as 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 like peptides could easily permeate across the skin, especially the stratum corneum. In addition, previous studies suggested that peptide drugs might also undergo extensive enzymatic degradation in the viable skin (Choi et al., 1990; Banerjee and Ritschel, 1989; Shah and 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 and/or metabolic inhibitors (Ruland et al., 1994; Choi et al., 1990). These invasive approaches might prove to be useful, but there are still some problems in their safety and skin irritation.

Another promising approach is to use prodrugs and analogues to improve the transdermal absorption of peptide and protein drugs. We previously showed that intestinal absorption of insulin (Asada et al., 1994, 1995), calcitonin (Fujita et al., 1996), DADLE, an enkephalin analogue (Uchiyama et al., 2000), tetragastrin (Tenma et al., 1993; Yodoya et al., 1994; Fujita et al., 1998), thyrotropin releasing hormone (TRH) (Tanaka et al., 1996) and Phenylalanyl-glycine (Phe-Gly) (Fujita et al., 1996) was improved by chemical modification with various fatty acids. Furthermore, it was also indicated that the transdermal delivery of tetragastrin was improved by lipophilic modification with fatty acids (Setoh et al., 1995). An acylation of tetragastrin increased its transdermal delivery by overcoming the poor permeability and the enzymatic instability of native tetragastrin. However, the effect of chemical modification on the transdermal absorption and stability of other peptide drugs was not investigated. Moreover, the effect of chemical modification with fatty acids on the accumulation of peptide in the skin was not fully understood.

In this study, therefore, the contribution of acylation to the transdermal delivery of peptides was examined. Phe-Gly was chosen as a model drug, and the in vitro stability, permeability and accumulation of Phe-Gly and its acyl derivatives were investigated in rat skin.

## 2. Materials and methods

### 2.1. Materials

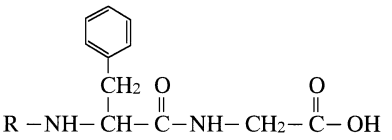
Phe-Gly, Boc-Phe-OH, and H-Gly-OEt·HCl were purchased from Peptide Institute Inc. (Osaka, Japan). *n*-Butyric anhydride, *n*-caproic anhydride, *n*-octanoic anhydride, trifluoroacetic acid (TFA), ethanol, and acetonitrile were obtained from Nacalai Tesque Inc (Kyoto, Japan). Dimethylacetoamide (DMAc) was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). All other chemicals were of the finest reagent grade available.

### 2.2. Synthesis of the acyl derivatives

Standard methodology was used for the preparation of Phe-Gly (structure shown in Table 1). After purification and characterization, the acyl derivatives were prepared.

Boc-Phe-Gly-OEt: H-Gly-OEt·HCI (1.0 g, 7.16 mmol) was dissolved in 8 ml of DMF and triethylamine (1.0 ml, 7.16 mmol), and then Boc-Phe-OH (2.09 g, 7.88 mmol), 1-hydroxybenzotriazole (1.26 g, 7.88 mmol), and dicyclohexylcarbodiimide (1.63 g, 7.88 mmol) were added and stirred

Table 1
Physicochemical properties of Phe-Gly and its acyl derivatives



| Peptide | Side-chain length ($R$) | M.W. | Solubility(mg/ml)[a] | Lipophilic index[b] |
|---|---|---|---|---|
| Phe-Gly | | 222.2 | 750 | 0.58 |
| C4-Phe-Gly | C4 | 292.3 | 8.80 | 1.94 |
| C6-Phe-Gly | C6 | 320.4 | 1.27 | 2.79 |
| C8-Phe-Gly | C8 | 348.4 | 0.36 | 3.71 |

[a] Drug solubility in PBS was determined at 37 °C.
[b] The lipophilic indices were determined by the method of Yamana et al. (1977).

overnight at room temperature. The extract was washed with 10% acetic acid, 5% $NaHCO_3$ and a saturated NaCl solution, dried over $Na_2SO_4$, and the product was concentrated in an evaporator. The resulting solution was applied to TLC and developed with chloroform and methanol (20:1) in order to confirm the reaction. The resulting concentrate was recrystallized with ether and hexane (1:3).

R-CO-Phe-Gly-OEt: Boc-Phe-Gly-OEt (500 mg, 1.43 mmol) was treated with anisole (30 ml, 2.86 mmol) in 8 ml of TFA in an ice-bath for 1 h, then the TFA was removed by evaporation at room temperature. The product was dissolved in DMF (6 ml) and its pH was adjusted to 8 with $Et_3N$. *n*-Butyric anhydride or *n*-caproic anhydride or *n*-octanoic anhydrate was added to the solution and stirred overnight at 0 °C. The resulting solution was concentrated by evaporation and solubilized in AcOEt. The extract was then washed with 5% $NaHCO_3$, 10% acetic acid, and a saturated NaCl solution, dried over $Na_2SO_4$, and concentrated. The resulting solution was applied to TLC and developed with chloroform and methanol (20:1) in order to confirm the reaction. The resulting concentrate was recrystallized with ether and hexane (1:3).

R-CO-Phe-Gly-OH: R-CO-Phe-Gly-OEt (230 mg, 0.72 mmol) was dissolved in methanol (5 ml), then 1 M NaOH (0.864 ml, 0.864 mmol) was added to the reaction mixture and stirred at 4 °C for 30 min. These solutions were extracted with AcOEt and were washed with 10% acetic acid and a saturated NaCl solution, dried over $Na_2SO_4$ and recrystallized with ether and hexane (1:3). The product was purified by FPLC.

### 2.3. Determination of the lipophilic indexes of Phe-Gly and its acyl derivatives

The lipophilic index was determined by HPLC (Yamana et al., 1977). A mixture (pH 7.4) of methanol (40–70%) and phosphate buffered saline (PBS) (30–60%) was used as a mobile phase. The elution time of the solvent ($t_0$) and the retention times of the acyl-Phe-Gly derivatives ($t_R$) were determined for each of the mobile phase. The log $K'$ value, defined by Eq. (1) was plotted against the methanol concentration in the mobile phase and the log $K'$ value extrapolated to 0% methanol was used as the index of the lipophilicity of the acyl-Phe-Gly derivatives (log $K_0'$).

$$\text{Lipophilic index } (\log K') = \log(t_R - t_0)/t_0 \qquad (1)$$

### 2.4. In vitro permeation studies

Full-thickness abdominal skin excised from a male Wistar rat (Japan SLC, Shizuoka, Japan) ( ~ 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-stripping 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 sulfate (50 mg/ml, Nacalai Tesque, Inc.) and penicillin G potassium salt (30 mg/ml, 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 of the test solution containing Phe-Gly or its acyl derivatives was administered to the donor compartment (the epidermis side) of a Franz-type diffusion cell, 150 μl of reservoir solution was periodically collected. The drug concentrations were determined by reversed phase HPLC.

### 2.5. Skin homogenate experiments

#### 2.5.1. Preparation of the 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 POLYTRON homogenizer (Kinematica, GmbH, Switzerland). The homogenate was centrifuged for 5 min at $3000 \times g$ at 4 °C, and the supernatant was recovered and centrifuged for 30 min at $20\,000 \times g$ at 4 °C. Then, the supernatant was collected and adjusted with PBS to a protein concentration of 5 mg/ml. The protein concentration in the tissue supernatant was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

#### 2.5.2. Stability of Phe-Gly and its acyl derivatives in rat skin homogenates

The stability of Phe-Gly and its acyl derivatives was determined by incubating tissue supernatants (final protein conc., 2 mg/ml) with a test solution (final conc., 0.4 mM). The test solution and skin homogenates had been preincubated at 37 °C for 10 min. Fifty microliters of the incubation mixture were taken at various time intervals, and 100 μl of methanol was added to terminate the reaction. The resulting mixture was centrifuged at $10\,000 \times g$ for 5 min to remove the precipitated proteins. Supernatants were assayed for the amount of Phe-Gly and its acyl derivatives remaining by HPLC.

### 2.6. Analyses

The concentrations of Phe-Gly and its acyl derivatives were assayed by reversed phase HPLC in Cosmosil $5C_{18}$ packed columns ($250 \times 4.6$ mm$^2$). The HPLC consisted of a Shimadzu LC-10A pump, a Shimadzu SPD-10A UV detector operated, at 210 nm, and a Shimadzu SIL-10B auto injector. The mobile phases for Phe-Gly, C4-Phe-Gly, C6-Phe-Gly and C8-Phe-Gly were mixtures of methanol and water (95:5, 8:2, 7:3 and 6:4, respectively) with a flow rate of at 1.0 ml/min.

### 2.7. Statistical analyses

Results are expressed as the mean $\pm$ S.E. and statistical significance was assessed using the Student's $t$-test with $P < 0.05$ as the minimum level of significance.

## 3. Results

### 3.1. Stability of Phe-Gly and its acyl derivatives in a rat skin homogenate

Fig. 1 shows the degradation profiles of Phe-Gly and its acyl derivatives in a rat skin homogenate. Phe-Gly was rapidly degraded in the skin homogenate. Conversely, acyl-Phe-Gly derivatives were more stable than native Phe-Gly. However, we found no significant difference in the stability of acyl-Phe-Gly derivatives.

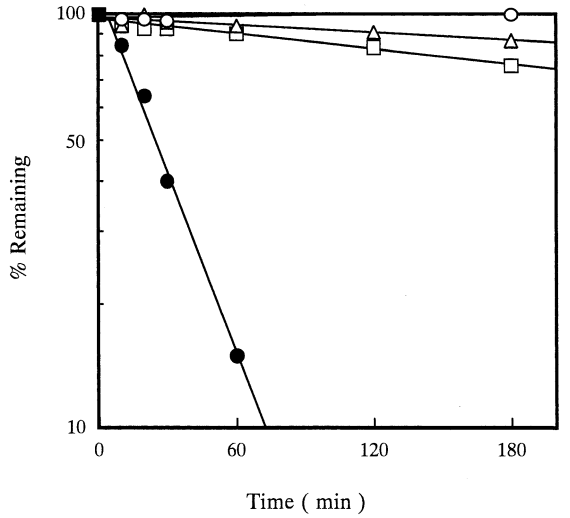



Fig. 1. Degradation profiles of Phe-Gly and its acyl derivatives in rat skin homogenates. Results are expressed as the mean of three experiments. Key: Phe-Gly (●), C4-Phe-Gly (○), C6-Phe-Gly (△), C8-Phe-Gly (□).

### 3.2. The in vitro permeability of Phe-Gly and its acyl derivatives in the skin

Fig. 2 shows the time course of permeation of Phe-Gly and its acyl derivatives across intact and stripped skin. A flux of native Phe-Gly across intact skin was not detected during the period of observation. In contrast, the three derivatives, C4-Phe-Gly, C6-Phe-Gly and C8-Phe-Gly permeated through the intact skin, and C6-Phe-Gly was the most permeable. The cumulative amounts of C4-Phe-Gly, C6-Phe-Gly and C8-Phe-Gly transported after 24 h were about 2.5, 4 and 2% of the initial dose, respectively. The permeability of these drugs through the stripped rat skin was also compared and shown in Fig. 2. Overall, the permeability of Phe-Gly and its acyl derivatives across the stripped skin was much higher than that in the intact skin. The permeability of acyl derivatives was less than that of native Phe-Gly in the initial time period of transport studies, but was higher than that of native Phe-Gly at the end of the transport studies. On the other hand, phenylalanine, which is a metabolite of Phe-Gly, was detected during the transport studies in the stripped skin. The cumulative amounts (percentage of the initial dose) of acyl-Phe-Gly derivatives, C4-Phe-Gly, C6-Phe-Gly, and C8-Phe-Gly transported across the stripped skin after 6 h were about 50%, respectively. The cumulative amount (percentage of the initial dose) of native Phe-Gly transported across the stripped skin after 6 h was about 30%. However, when the skin was pretreated with

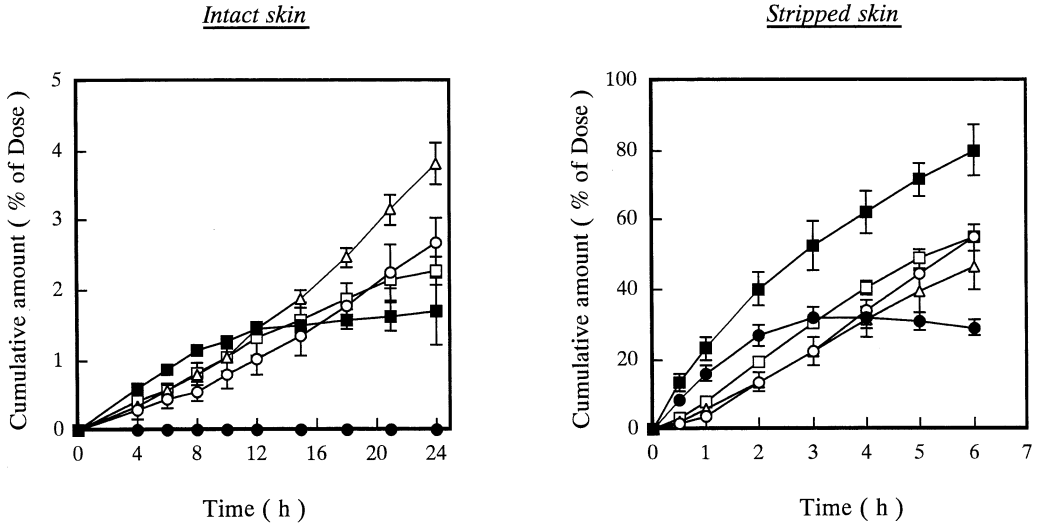



Fig. 2. Permeation profiles of Phe-Gly and its acyl derivatives across the intact and stripped skin. Results are expressed as the mean ± S.E. of 3–5 experiments. Key: Phe-Gly (●), Phe-Gly (ethanol) (■), C4-Phe-Gly (○), C6-Phe-Gly (△), C8-Phe-Gly (□).

ethanol, which could inactivate the peptidases responsible for the degradation of Phe-Gly in the skin, the permeability of native Phe-Gly was higher than that of acyl derivatives.

The skin fluxes and permeability coefficients of Phe-Gly and its acyl derivatives across intact and stripped skin are summarized in Table 2. The permeation rate and permeability coefficient ($K_p$) of Phe-Gly increased by the chemical modification with various fatty acids and in particular, C6-Phe-Gly was the most permeable compound of all these acyl derivatives. The permeability of Phe-Gly and its acyl derivatives across the intact skin was in the following order: C6-Phe-Gly > C4-Phe-Gly ≧ C8-Phe-Gly ≧ Phe-Gly.

On the other hand, the permeability of acyl-Phe-Gly across the stripped skin was higher than that of native Phe-Gly at 6 h without ethanol pretreatment. However, the permeability of acyl-Phe-Gly was lower than native Phe-Gly when the skin was pretreated with ethanol.

### 3.3. Relationship between the lipophilicity of Phe-Gly and its acyl derivatives and their permeability coefficients across rat skin

Fig. 3 shows the relationship between the lipophilicity of Phe-Gly and its acyl derivatives, defined as the lipophilic index, and their permeability coefficients for rat skin. The lipophilic indexes for Phe-Gly and its acyl derivatives are summarized in Table 1. A bell-shaped profile was observed between the lipophilic indexes and the permeability coefficients of Phe-Gly and its acyl derivatives in the intact skin. There exists an optimal lipophilicity for improving the transdermal permeability of Phe-Gly by chemical modification with various fatty acids. However, in the stripped skin, the permeability coefficients of acyl-Phe-Gly derivatives decreased as their lipophilicity increased.

### 3.4. The amount of Phe-Gly and its acyl derivatives in the skin after the permeation experiments

Fig. 4 indicates the amounts of Phe-Gly and its acyl derivatives in the skin after the permeation experiments. As shown in this figure, the amount of Phe-Gly in the skin increased as the carbon number of fatty acid attached to the Phe-Gly molecule increased in the intact skin. On the other hand, there was no significant difference on the drug amounts in the skin among these three acyl derivatives.

## 4. Discussion

In this study, we synthesized three novel lipophilic derivatives of Phe-Gly to improve the transdermal absorption of this model dipeptide. We indicated that the lipophilicity of acyl-Phe-Gly derivatives increased as the carbon number of fatty acid that was chemically attached to the native Phe-Gly increased. This suggested that it is possible to adjust the lipophilicity of Phe-Gly derivatives by modifying them with various fatty acids. We calculated and obtained the lipophilic index by

Table 2
Permeability of Phe-Gly and its acyl derivatives across intact and stripped skin

| | Intact skin | | | Stripped skin | | |
|---|---|---|---|---|---|---|
| | Permeation rate ($\times 10^{-1}$%/h)[a] | $K_p$ value ($\times 10^{-4}$ cm/h)[b] | Lag time (h) | Permeation rate (%/h) | $K_p$ value ($\times 10^{-2}$ cm/h) | Lag time (h) |
| Phe-Gly (ethanol) | 1.19±0.08 | 2.03±0.46 | – | 15.8±2.3 | 5.53±0.66 | – |
| C4-Phe-Gly | 1.43±0.17 | 2.43±0.41 | 5.9±1.2 | 10.6±0.3 | 2.81±0.01 | 0.82±0.08 |
| C6-Phe-Gly | 2.09±0.28 | 3.55±0.33 | 5.6±0.9 | 8.6±1.6 | 2.19±0.37 | 0.40±0.16 |
| C8-Phe-Gly | 1.24±0.17 | 2.14±0.17 | 1.5±0.1 | 10.8±0.5 | 2.97±0.12 | 0.23±0.03 |

[a] Permeation rate $= dC/dt$.
[b] $K_p = (X_R/\int_0^t C_D dt)1/A$.

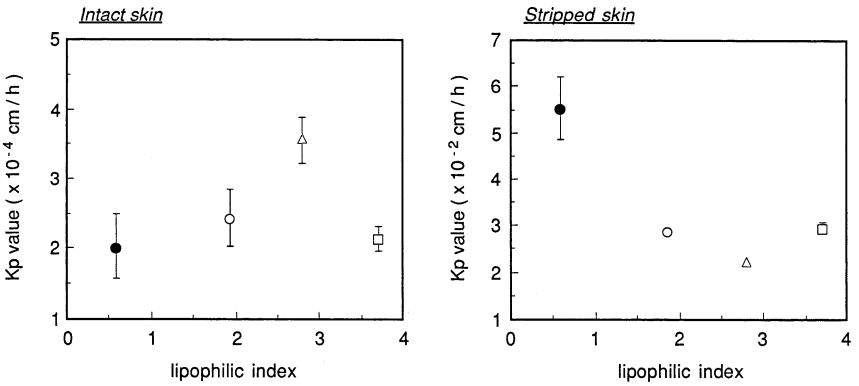



Fig. 3. Relationship between the lipophilic indices and the permeability coefficient ($K_p$) of Phe-Gly and its acyl derivatives across the rat skin. Key: Phe-Gly (●), C4-Phe-Gly (○), C6-Phe-Gly (△), C8-Phe-Gly (□).

the method of Yamana et al. (1977) because: (1) it is easy and repeatable, (2) it is easy to adjust the pH, (3) it can be used for unstable drugs in solution, (4) it can measure drugs dissolved with the assistance of a dissolving agent. In addition, the lipophilic index is closely correlated with the octanol–water partition coefficient. Therefore, we adopted the lipophilic index as a suitable index of lipophilicity in this study.

In general, peptide and protein drugs are easily degraded by various peptidases and proteases before reaching the systemic circulation (Lee and Yamamoto, 1990). In the skin, the proteolytic activity of stratum corneum was relatively low, but the viable dermis and epidermis have various kinds 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 and 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. In the present study, we demonstrated that Phe-Gly was easily degraded in rat skin homogenates and acyl-Phe-Gly derivatives were more stable than native Phe-Gly. These findings suggested that the stability of Phe-Gly was improved by chemically modifying it with fatty acids. This finding was

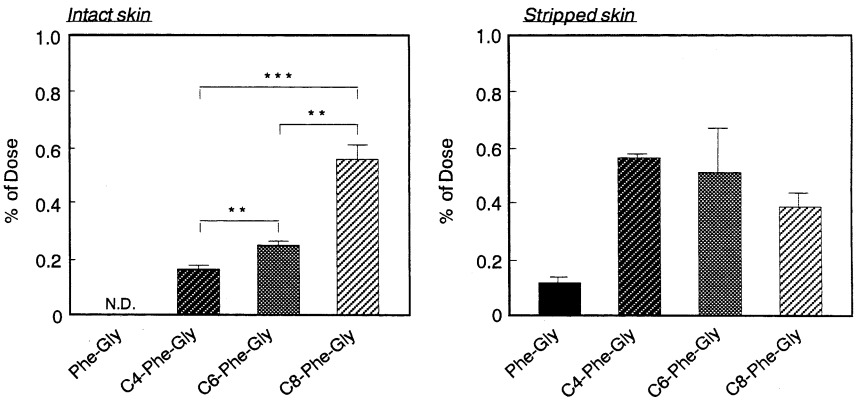



Fig. 4. Amount of Phe-Gly and its acyl derivatives in the skin after the permeation experiments. Results are expressed as the mean ± S.E. of four experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with each control.

well correlated with our previous findings of the stability of various acyl-peptide derivatives in the skin and the gastrointestinal tract including TRH (Tanaka et al., 1996), tetragastrin (Yodoya et al., 1994; Setoh et al., 1995), and insulin (Asada et al., 1994) modified with various fatty acids. The mechanism whereby the stability of Phe-Gly increased by acylation is not clearly understood. However, since the N-terminal portion of Phe-Gly is modified with the fatty acid and various proteolytic enzymes are known to be distributed in the skin (Choi et al., 1990), we consider that the N-terminal modification of Phe-Gly may protect against enzymatic degradation including various aminopeptidases localized in the skin.

In the skin permeability experiments, we demonstrated that the permeability of acyl-Phe-Gly derivatives across the intact skin was much higher than that of native Phe-Gly. In particular, we found the greatest permeability of C6-Phe-Gly across the intact skin, of all the acyl-Phe-Gly derivatives. This result was in good agreement with our previous finding that the transdermal absorption of tetragastrin was enhanced by its chemical modification with fatty acids (Setoh et al., 1995). Similar results were also observed in the intestinal absorption of various acyl derivatives of peptides and proteins including insulin (Asada et al., 1995), calcitonin (Fujita et al., 1996), DADLE, an enkephalin derivative (Uchiyama et al., 2000), tetragastrin (Tenma et al., 1993; Yodoya et al., 1994) and TRH (Tanaka et al., 1996). These findings suggest that the acylation may increase the permeability of Phe-Gly across the intact skin due to the increased lipophilicity and stability of this dipeptide by the acylation. The lack of permeability of native Phe-Gly across the intact skin was maybe mainly attributed to its extensive metabolism in the skin and low lipophilicity. Indeed, our present finding indicated that native Phe-Gly was easily metabolized in the skin homogenate as discussed above and its lipophilicity was relatively low as compared with that of the acyl-Phe-Gly derivatives. This former proposed mechanism is also supported by the fact that the transport of Phe-Gly was observed across intact skin which had been treated with ethanol to inactivate the enzymes in the skin.

The reason why the permeability of C6-Phe-Gly was the highest of all the acyl derivatives used in the study is not clearly understood. However, in our previous study, we demonstrated that the permeability of the acyl-tetragastrin derivatives was not correlated with their lipophilicity. That is, the permeability of acetyl-TG was higher than that of butyroyl- and caproyl-derivatives across the intact and stripped skin. Therefore, the low permeability of higher lipophilic derivatives of Phe-Gly (C8-Phe-Gly) may be due to its strong partition and binding to the stratum corneum. This speculation was supported by the fact that the amount of C8-Phe-Gly in the skin was much higher than the native Phe-Gly and the other acyl derivatives. From these findings, it may be considered that there is an optimal lipophilicity (length of acyl chain) of the acyl derivatives for improving their transdermal delivery.

We demonstrated that the permeability of Phe-Gly and its acyl derivatives across the stripped skin was much higher than that across the intact skin in which the stratum corneum exists. The cumulative amounts of acyl-Phe-Gly derivatives across the intact skin were less than 4% at 24 h, whereas those of native Phe-Gly and its acyl derivatives across the stripped skin were about 30–50%. These findings suggest that like other drugs, stratum corneum acts as a major barrier for the transport of Phe-Gly and its acyl derivatives.

Unlike the intact skin, the permeability of acyl derivatives was less than that of native Phe-Gly in the initial period of transport studies, although it was higher than that of native Phe-Gly at the end of transport studies. This is mainly due to the metabolic degradation of native Phe-Gly in the skin, since the permeability of Phe-Gly was enhanced across the stripped skin pretreated with ethanol, which could inactivate the peptidases responsible for the degradation of Phe-Gly in the skin. Alternatively, it may be plausible that the stratum corneum mostly acts as a major transport barrier for the hydrophilic drugs rather than lipophilic compounds. Therefore, the increase in the transdermal absorption of Phe-Gly across the stripped skin without stratum corneum is more remarkably observed than that of acyl-Phe-Gly derivatives.

We showed that the amount of drug remaining in the intact skin increased as the number of carbons in the fatty acids that were chemically attached to the native Phe-Gly increased. In particular, the amount of C8-Phe-Gly was the highest of all the acyl derivatives. Therefore, an optimum lipophilicity (the carbon number of the fatty acids) is thought to exist for the drug accumulation in the skin. Walter and Kurz (1988) investigated the binding of 10 kinds of drugs to human skin (epidermis and dermis) and suggested that binding to the epidermis was higher than that to the dermis for 9 kinds of drugs. They also found a good correlation between the partition coefficients of these drugs and their skin affinities and concluded that these drugs might combine with components such as proteins, but not lipids, because binding was not affected even if lipids were removed. These findings were in good agreement with the present finding of the highest accumulation of C8-Phe-Gly. Therefore, we speculate that the permeability of C8-Phe-Gly through intact skin might be inhibited due to its high binding characteristics to the skin, since C8-Phe-Gly has the highest lipophilicity of all the acyl derivatives. Indeed, our present finding demonstrated that the permeability of C8-Phe-Gly was the lowest, which can support our speculation as indicated above.

In conclusion, we indicated that the stability and permeability of Phe-Gly across the skin was improved by the chemical modification with various fatty acids. This chemical modification approach may be useful for the transdermal delivery of peptide drugs as well as the oral delivery of these drugs.

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