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Research Paper

Trypsin as a novel potential absorption enhancer for improving the transdermal delivery of macromolecules

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Abstract

Objectives The aim was to assess the effect of trypsin on the transdermal delivery of macromolecules by applying its specific biochemical properties to the stratum corneum of the skin.

Methods Fluorescein isothiocyanate (FITC)-labelled dextrans (FDs), with molecular weights of 4 to 250 kDa, and FITC-insulin were used as model macromolecules and a model polypeptide, and the in-vitro transdermal permeation experiments, with or without trypsin (0.1–2.5%), were carried out using rat skin and cultured human epidermis. The mechanism for the enhancement of trypsin was also studied using fluorescence and conventional light microscopy.

Key findings Trypsin significantly increased the transdermal permeability of all FDs through the rat skin (2.0- to 10.0-fold). It also markedly enhanced the permeation of FD4 through three-dimensional cultured human epidermis (3.1-fold), which was used to evaluate the transport pathways other than the transfollicular route. Furthermore, the permeation flux of FITC-insulin was increased by 10.0-fold with trypsin pretreatment (from 0.02 ± 0.00 to 0.20 ± 0.07 $\mu g/cm^2$ per h). Mechanistic studies indicated that trypsin affects both the intercellular pathway and the hair follicular route, and may alter stratum corneum protein structures, thereby affecting skin barrier properties.

Conclusions This study suggests that trypsin could be effective as a biochemical enhancer for the transdermal delivery of macromolecules including peptide and protein drugs.

Keywords absorption enhancer; insulin; macromolecule; transdermal delivery; trypsin

Introduction

An increasing number of macromolecules such as proteins and nucleic acids have been introduced as therapeutics in recent years. However, their potential usefulness is circumscribed by the parenteral route of delivery, which is the only one currently available for most of these agents. Alternative routes for the delivery of peptides and proteins including nasal, oral, transdermal, buccal and rectal pathways have been investigated[1] to avoid the invasive nature of parenteral administration and to improve the compliance of patients. However, delivery by alternative routes is difficult because of poor membrane permeability and proteolytic degradation at most biomembranes.[2]

As skin has relatively low proteolytic activity and has a large area compared with other tissues,[3] the transdermal route for the delivery of peptides and proteins seems to be attractive. However, transdermal delivery is limited due to the unique bioarchitecture of skin, which has primarily evolved as a protective barrier against the entry of microorganisms and water.[4] In order to overcome the barrier of the stratum corneum (SC), many approaches have been investigated, including chemical enhancer modification and physical disruption of barrier function. Many chemicals such as alcohols, fatty acids, fatty acid esters, polyols, amides and surfactants have been shown to enhance skin penetration. Their effects include an alteration of solvent potential of the SC biochemical environment and a disordering of the intercellular lipid matrix following insertion of the enhancer into the bilayer structure of the SC, which enhances penetration of drugs through the

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intercellular pathway.[5–8] However, these chemical enhancers are really only useful for small molecules,[9,10] and for large peptides it is still difficult to achieve meaningful permeation. On the other hand, proteolytic enzymes may offer favourable properties as skin penetration enhancers because their action is highly specific toward proteins. Sim *et al.* reported that SC-glucan conjugated papain increased the transdermal permeation of antipyrine and indometacin but did not show skin irritation.[11] However, no studies have applied such proteolytic enzymes for the transdermal permeation of macromolecules.

For more than 50 years, proteolytic enzymes such as trypsin have been extensively used in laboratory settings for in-vitro epidermal separation and keratinocyte isolation.[12–16] The unique ability of proteases to cause selective epidermal separation has been in part explained by the proteolytic degradation of desmosomal proteins in the SC, which leads to cell dissociation.[17,18] In clinic studies, several therapeutic applications have been attempted for wound debridement and epidermal ablation.[19–21] More recently, pancreatin, derived from pork or beef sources, has been developed as an enzyme supplement, which contains pancreatic trypsin, amylase and lipase. The trypsin found in pancreatin works to hydrolyse proteins into oligopeptides, and it could help people with pancreatic disorders in the digestion of food.

The aim of the present study was to develop a transdermal delivery system for hydrophilic macromolecules by applying trypsin as a biochemical enhancer. A series of fluorescein isothiocyanate (FITC)-labeled dextrans (FDs) were used as model compounds of hydrophilic macromolecules, and the enhancing effects of trypsin on the permeation of these compounds were studied *in vitro* using rat skin or a three-dimensional cultured human epidermis model. FITC-insulin, a polypeptide hormone, was used as a model large peptide in this study. The in-vitro permeation of FITC-insulin through rat skin with trypsin pretreatment was investigated to verify the enhancing effect of trypsin. In addition, microscopy and fluorescence microscopy were employed to visually characterise the morphology of the skin and the change in SC structure after treatment with trypsin.

Materials and Methods

Materials

Trypsin from porcine pancreas, FDs with average molecular weight of 4 kDa (FD4), 10 kDa (FD10), 20 kDa (FD20), 70 kDa (FD70), 250 kDa (FD250) and FITC-insulin (bovine) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals and reagents were of analytical grade. The three-dimensional cultured epidermis model, Labcyte EPI-model 12, was kindly provided by Japan Tissue Engineering Co. Ltd (Aichi, Japan).

Analytical method

The amount of FD and FITC-insulin in the samples was quantified using a fluorescence spectrometer (Spectrafluor Plus; Tecan Austria Gmbh, Grödig, Austria) at excitation and emission wavelengths of 485 and 535 nm, respectively. The method was validated by determination of linearity, precision, limits of detection, limits of quantification and recovery. Linearity was confirmed for concentrations ranging from 0.2 to 50 μg/ml of standard solutions and correlation coefficients for all FDs and FITC-insulin were greater than 0.998. The coefficient of variation was over the range of 0.45–1.82%. The limits of detection and limits of quantification were 0.0005 and 0.002 μg/ml, respectively. The mean recovery value for FD and FITC-insulin were over the range of 96.86–102.71%.

In-vitro permeation experiments with rat skin

All experiments were approved by the local review board of Kyoto University and were conducted in accordance with national and international guidelines for laboratory animal care. Male Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan), 220–240 g, were anaesthetised by intraperitoneal injection of sodium pentobarbital (4 mg/100 g body weight). The abdominal hair was removed using an animal hair clipper and full-thickness skin was excised. The excess fat adhering to the dermis side was removed using cotton.

Franz diffusion cells with an effective diffusion area of 0.785 cm^2 were used for the permeation studies. A piece of excised rat skin was mounted between receptor and donor chambers, with the SC facing the donor compartment. Trypsin was applied by pretreatment or co-administration methods. When the skin was pretreated with trypsin, 200 μl of trypsin solution (0.1% or 1.0%, w/v) was administered to the donor compartment and removed after 30 min, at which point the donor compartment was washed three times with 0.1 M, pH 7.4 phosphate-buffered saline (PBS). As a control, 200 μl of PBS was added to the donor compartment instead of trypsin. Subsequently, 200 μl of FD solution (2.5 mg/ml in PBS) was added to the donor compartment, and the receptor compartment was filled with 3.4 ml of PBS alone. Cells were maintained at 37°C and stirred with magnetic bars. When trypsin was co-administered with FDs, 200 μl of FD solution containing 0.1% (w/v) of trypsin was added to the donor compartment. Receptor solution (100 μl) was withdrawn periodically and replaced with an equal volume of fresh receptor solution. The amount of FD permeated through the rat skin at each time point was analysed using a fluorescence spectrometer as described above.

In the permeation experiment with FITC-insulin, 200 μl of 0.25% (w/v) trypsin was administered to the donor compartment. After 30 min pretreatment, it was removed and the donor compartment was washed three times with a mixture of ethanol and PBS (30 : 70, v/v) to protect against the decomposition of insulin by residual trypsin. The receptor solution contained urea (2 mg/ml) to prevent aggregation and adsorption of insulin to glass surfaces, as well as sodium azide (0.0025%, w/v) to prevent microbial growth. FITC-insulin (2 mg/ml) was used as the donor solution. Sampling and the analysis methods for the amount of FITC-insulin permeated at each time point were the same as the permeation experiment with FD.

To determine the integrity of FD and FITC-insulin during the permeation experiment, the donor and receptor solutions were analysed at the end of experiment using a computer-controlled HPLC system (Hitachi 7000; Hitachi, Tokyo,

Japan) with a TSKgel G2000 SW_{XL} column (Tosoh Bioscience, Tokyo, Japan) and a fluorescence detector at excitation and emission wavelengths of 485 and 535 nm, respectively. The mobile phase contained 7.1 g/l of Na_2SO_4, and the flow rate was set at 1 ml/min. The column temperature was kept at 40°C. The injection volume was 50 μl.

Permeation experiments in a three-dimensional cultured human epidermis model

Three-dimensional cultured human epidermis was used as a human skin model in this study. The permeation experiment was performed in cultures grown in a Transwell chamber (0.9 cm^2 growth area). Before the experiment, 0.5 and 1.5 ml of PBS were added to the donor and receptor chambers, respectively, and pre-incubated for 10 min. Then, 0.5 ml of 0.1% (w/v) trypsin solution was added to the donor chamber for pretreatment and removed after 30 min, at which point the donor chamber was washed three times with PBS. After pretreatment, 0.5 ml of FD4 solution (0.25 mg/ml in PBS) was added to the donor chamber, and the receptor chamber was filled with 1.5 ml of PBS. Then, 0.5 ml of PBS without trypsin was used as a control. The Transwell was maintained at 37°C in a shaking water bath. Samples (100 μl) were withdrawn from the receptor chamber at designated time intervals and immediately replaced with an equal volume of PBS solution. The amount of FD4 permeated through the three-dimensional cultured human epidermis was analysed as described in the rat skin permeation studies.

Histological and fluorescence microscopic examination

After setting the prepared rat skin in a Franz cell, 200 μl of PBS or 0.25% (w/v) trypsin solution was added to the donor compartment. After 30 min, the skin was washed with PBS and fixed in 10% (w/v) formalin. A section was cut from each sample and stained with haematoxylin and eosin for microscopic examination. The treated skin sections were observed under a light microscope to assess the changes in skin structure caused by trypsin treatment.

In the fluorescence microscopic examination, the skin was exposed to 200 μl of PBS or 0.25% (w/v) trypsin. After 30 min, the donor compartment was washed three times with a mixture of ethanol and PBS (30 : 70, v/v), then 200 μl of 2 mg/ml FITC-insulin was added. After 1 or 3 h the FITC-insulin solution left in the donor chamber was removed, and the skin was embedded in optimal cutting temperature compound and frozen in a freezer at –70°C. The skin samples were then sectioned using a cryostat microtome for fluorescence microscopy. Fluorescence photomicrographs of sections were obtained using a fluorescence microscope (BZ-8000; Keyence, Osaka, Japan) with excitation and emission filter wavelengths at 470–490 and 515–550 nm, respectively.

Data analyses

The cumulative amount of model compounds permeated per unit skin surface area was plotted against time, and the slope of the linear portion of the plot was estimated as the steady state flux (J_{ss}). The permeability coefficient, P, was calculated as $P = J_{ss}/C_0$, where C_0 is the donor concentration of model compounds.

The enhancement ratio (ER) of the permeation flux was expressed as: $ER = J_{\text{with trypsin}}/J_{\text{control}}$, where $J_{\text{with trypsin}}$ was the permeation flux of model compounds through the rat skin with trypsin treatment and J_{control} was the permeation flux of model compounds through the rat skin without trypsin treatment.

Statistical analysis

Results were expressed as the mean ± SE. Statistical analysis was performed to assess the significance of the differences among various treatments using one-way analysis variance, and the significance of the differences among the effect of molecular weight and treatment type on the permeation coefficient using two-way analysis variance. Tukey–Kramer post-test and Dunnett's test were used to compare the effects of different treatments and between the treatment and control data. $P < 0.05$ was considered statistically significant.

Results

To evaluate the enhancing effect of trypsin on the transdermal delivery of macromolecules, trypsin was applied by pretreatment or by co-administration with the model compound in the donor compartment. Trypsin (0.1%) increased the permeated amount of FD4 under both administration conditions (1.8- to 2.7-fold; $P < 0.05$), with the co-administration method showing a significantly greater enhancing effect compared with pretreatment ($P < 0.05$; Figure 1). Further, the enhancing effect of trypsin (0.1–2.5%) was increased with increasing pretreatment concentration (from 2.6- to 5.3-fold; $P < 0.01$), and the plot of permeation flux of FD4 versus trypsin concentration showed a rectangular hyperbola curve (Figure 2). Considering the stability of peptide and protein drugs in the presence of trypsin, the pretreatment method was used for all subsequent studies.

The effect of trypsin on the transdermal delivery of model macromolecules, FDs with molecular weights of 4 to 250 kDa, was investigated. Rat skin was pretreated with 0.1% and 1.0% trypsin *in vitro*. The permeation coefficients of these model compounds with or without trypsin are shown in Figure 3. The permeability of FDs without trypsin was low and was drastically reduced with increasing molecular weight (from 0.07×10^{-6} to 0.00×10^{-6} cm/s; $P < 0.0001$). Trypsin (0.1%, 1.0%) significantly increased the transdermal permeation of all FDs with molecular weights of 4 to 250 kDa (2.0- to 10.0-fold; $P < 0.05$). The enhancement ratio for pretreatment with a low concentration (0.1%) of trypsin was 2.0 for FD4 (from 0.59 ± 0.07 to 1.17 ± 0.19 $\mu g/cm^2$ per h), and with a high concentration (1.0%) of trypsin was 2.9 (from 0.59 ± 0.07 to 1.70 ± 0.38 $\mu g/cm^2$ per h). Furthermore, The permeation of FD250 was also increased from 0.00 ± 0.00 to 0.03 ± 0.01 $\mu g/cm^2$ per h with 0.1% of trypsin and from 0.00 ± 0.00 to 0.09 ± 0.02 $\mu g/cm^2$ per h with 1% trypsin.

In this study, a three-dimensional cultured human epidermis model was also used to evaluate the effect of trypsin on the FD4 transport pathway. The three-dimensional cultured

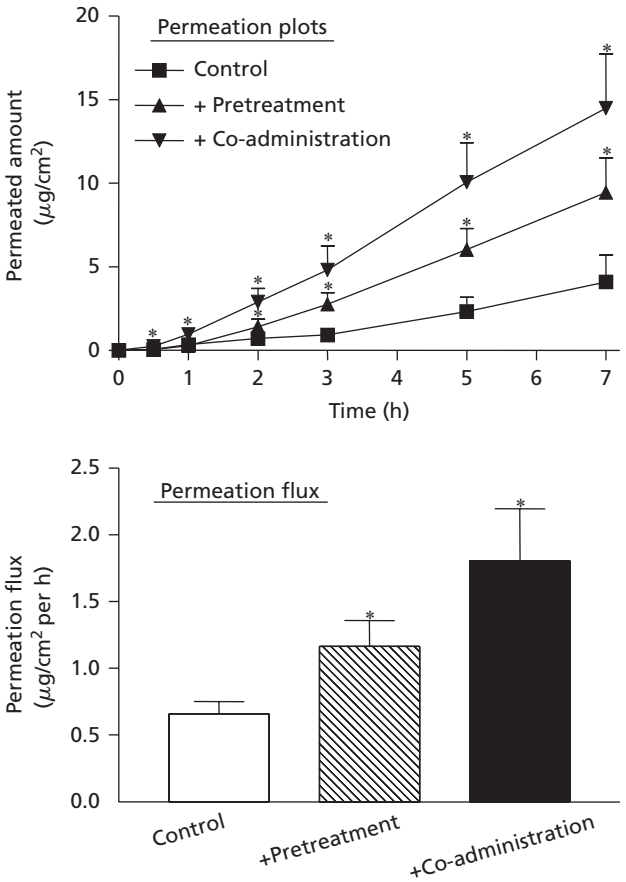

Figure 1 Enhancing effects of trypsin (0.1%) on the transdermal permeation of FD4 through rat skin using two treatment methods. Each value represents the mean ± SE of six or seven experiments. *$P < 0.05$, compared with the control.

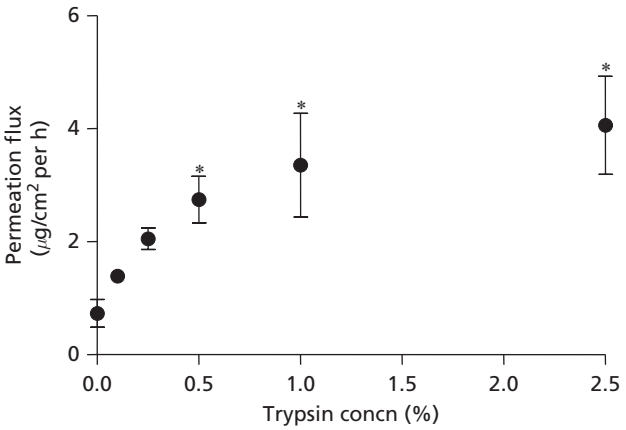

Figure 2 Effect of trypsin concentration (0.1–2.5%) on the transdermal permeation of FD4 through rat skin by pretreatment for 30 min. Each value represents the mean ± SE of four to six experiments. *$P < 0.05$, compared with the control.

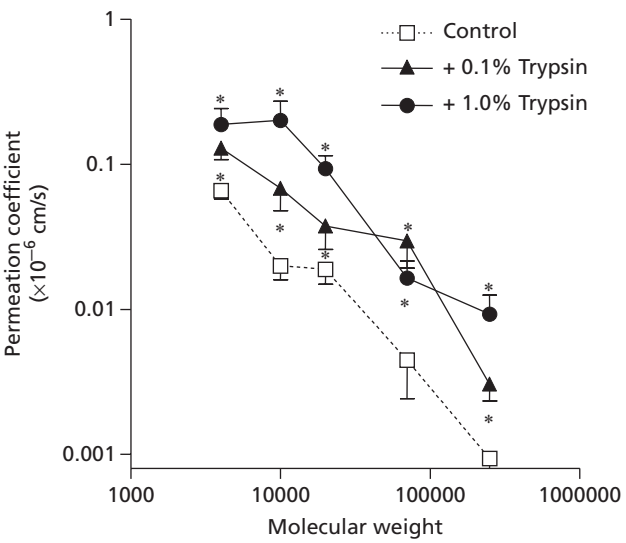

Figure 3 Relationship between the transdermal permeation coefficient of fluorescein isothiocyanate-labelled dextrans through rat skin and their molecular weights with or without trypsin pretreatment (0.1%, 1.0%). Each value represents the mean ± SE of five to seven experiments. *$P < 0.05$, compared with the control.

epidermis model derived from human normal keratinocytes has a multilayered SC and epidermis that blocked the permeation of the large FD4 molecule (Figure 4). Trypsin (0.1%) treatment generated a 3.1-fold increase in FD4 permeation flux (0.31 ± 0.02 μg/cm^2 per h) when compared with the control (0.10 ± 0.02 μg/cm^2 per h; $P < 0.05$).

FITC-insulin was used as a model polypeptide to assess the permeation enhancing ability of trypsin for peptide and protein drugs. The permeation of FITC-insulin through rat skin was low in the control, but a significant increase was obtained after pretreatment with 0.25% trypsin ($P < 0.05$; Figure 5). The permeability coefficient of FITC-insulin through the rat skin increased from 0.30×10^{-8} to 2.99×10^{-8} cm/s with trypsin pretreatment (0.25%) and the permeation flux increased by 10.0-fold (from 0.02 ± 0.00 to 0.20 ± 0.07 μg/cm^2 per h). These findings suggest the possibility that trypsin could be used to enhance the trandermal delivery of peptide and protein drugs.

Figure 6 shows fluorescence microscographs of rat skin after FITC-insulin administration and pretreatment with or without trypsin (0.25%) for 30 min. In the control, the fluorescence signal was mainly observed in the SC, and there was no marked fluorescence in the deeper layer of epidermis after 1 h (Figure 6a). After 3 h, diffusion of FITC-insulin into the epidermis, dermis, and hair follicles was observed (Figure 6c and 6e). Compared with the control, a broadly diffused fluorescence signal was observed in the epidermis and dermis of trypsin-treated skin even at 1 h (Figure 6b). After 3 h, a stronger and more continuous band of fluorescence was found in the epidermis and dermis (Figure 6d), as well as in hair follicles (Figure 6f).

Photomicrographs of rat skin sections treated with PBS (control) or 0.25% trypsin are shown in Figure 7. In the control, a clearly defined SC could be seen (Figure 7a), but after treatment with 0.25% trypsin, partial ablation and dilatation of the SC was observed (Figure 7b). Thickening and loosening of the SC were clearly induced by trypsin treatment.

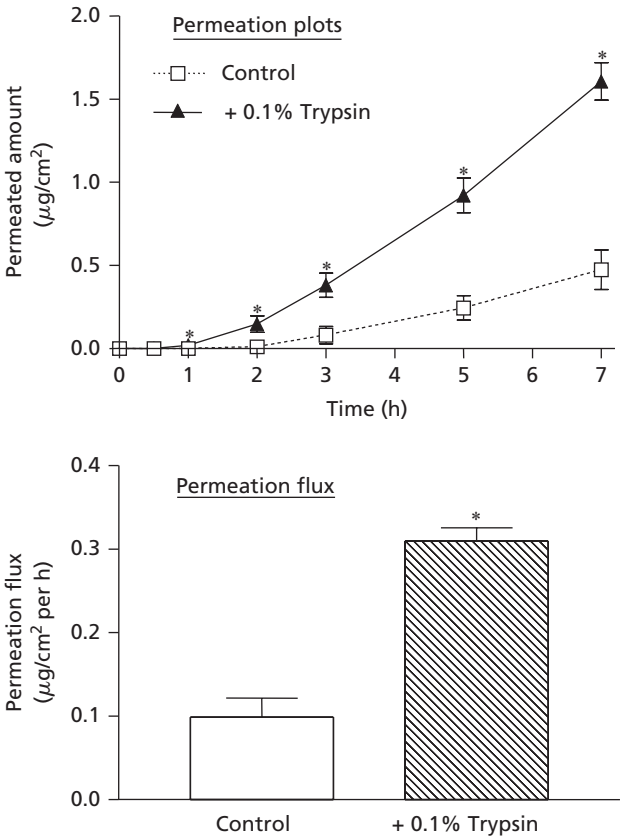

Figure 4 Transdermal permeation of FD4 through three-dimensional cultured epidermis with or without 0.1% trypsin pretreatment. Each value represents the mean ± SE of three experiments. $*P < 0.05$, compared with the control.

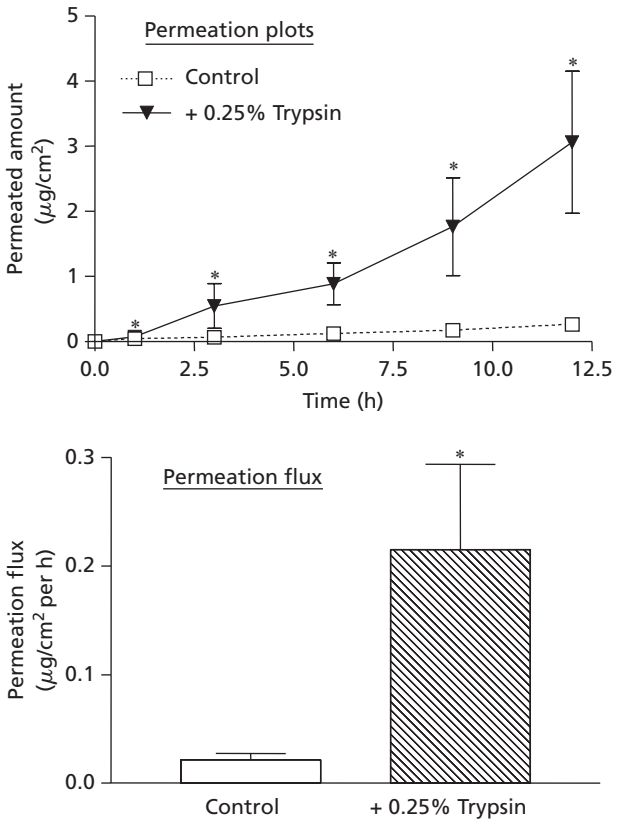

Figure 5 In-vitro transdermal permeation of fluorescein isothiocyanate-insulin through rat skin with or without 0.25% trypsin pretreatment for 30 min. Each value represents the mean ± SE of three experiments. $*P < 0.05$, compared with the control.

Discussion

It is well known that the endogenous proteases SC chymotrypic enzyme and tryptic enzyme are normally present in the granular layer of epidermis and hydrolyse intercorneocyte desmosomal adhesions.[22,23] They may therefore play an important role in epidermal desquamation.[24] It has been reported that the endogenous proteases trypsin and papain are capable of digesting intercellular desmosomal proteins in a similar fashion.[25] At a molecular level, at least 75 potential tryptic cleavage sites are predicted for the desmosomal proteins desmoglein 1 and 3.[26] Trypsin can cleave peptides on the C-terminal side of lysine and arginine amino acids residues, and it can also hydrolyse ester and amide linkages of synthetic derivatives of amino acids. Moreover, the in-vitro enzymatic digestion of desmosomes might cause epidermal acantholysis and dissolution.[17,18] In the present study, we attempted to reduce the barrier properties of the SC by application of the above hydrolytic mechanisms in order to promote the transdermal permeation of macromolecules.

We observed that the transdermal permeation of FDs was significantly enhanced by trypsin when using pretreatment or co-administration (Figures 1 and 3), and the dependence of the enhancing effect upon the trypsin concentration showed a rectangular hyperbola curve (Figure 2). We used trypsin concentrations of 0.1%, 0.25% and 1.0% in the in-vitro permeation studies of FDs and FITC-insulin, because these concentrations have been reported for treatment of human epidermal ablation *in vivo*[20] and are usually applied for epidermal separation and keratinocyte isolation.[12–16] The enhancing effect was greater for co-administration than for pretreatment (Figure 1). In our preliminary study, the permeation enhancing effect of trypsin was dependent on the pretreatment time (0.5–3 h). Trypsin might affect the barrier structure more strongly and extensively over longer periods of exposure to the skin, thereby permitting higher permeation flux of macromolecules in the case of co-administration. The permeability of FDs dramatically decreased with increased molecular weight (Figure 3). In the control without trypsin treatment, the permeability of these macromolecules was low (0.07×10^{-6} to 0.00×10^{-6} cm/s). Tokudome *et al.* reported that the permeation of FD4 through excised hairless rat skin was 6.42×10^{-7} cm/s,[27] which was higher than in our study (0.07×10^{-6} cm/s). However, they did not detect the permeation flux of FD10 and FD40. The difference in the permeation level compared with our result may be attributed to the different types of animals.

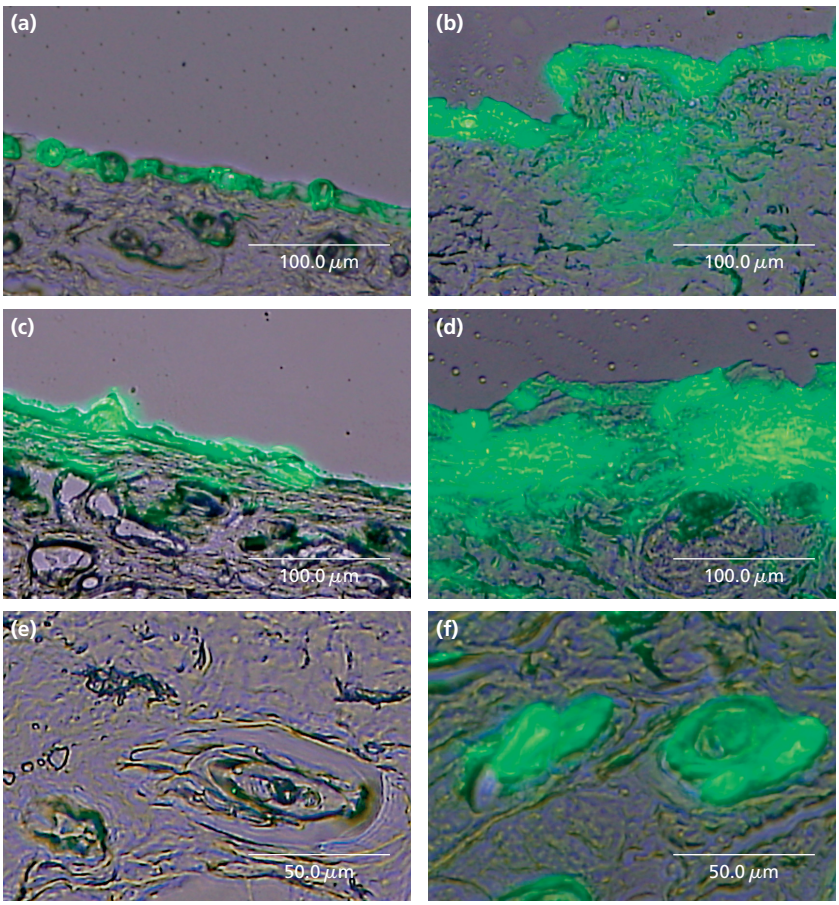

Figure 6 Fluorescence microscopic examination of rat skin in the presence of FITC-insulin with or without trypsin pretreatment. Rat skin was exposed to FITC-insulin for 1 h (a) or 3 h (c, e) after phosphate-buffered saline pretreatment for 30 min, and to FITC-insulin for 1 h (b) or 3 h (d, f) after 0.25% of trypsin pretreatment for 30 min. Original magnification: a–d ×100; e and f ×200.

Fang *et al.* found that the fluxes of FDs were different between furry mouse and nude mouse.[28] A morphological study revealed that the hair follicles of nude mouse skin greatly differ from those of the furry mouse.[29] In this study, we used Wistar furry rat skin, and the different amount of FD permeated may be related to follicular transport of these hydrophilic macromolecules. In this study, FD250 alone could almost not permeate the rat skin, but its permeation flux was dramatically increased after pretreatment with 1.0% trypsin. This finding suggests that the transdermal delivery of macromolecules with a molecular weight of up to 250 kDa can be achieved by trypsin treatment.

It has been hypothesised that hydrophilic drugs might diffuse across the SC via an intercellular pathway and that penetration through 'shunts', that is the transfollicular route, may be important for polar macromolecules. In the present study, we used a three-dimensional cultured human epidermis model derived from normal human keratinocytes to evaluate the effect of trypsin on the permeation of hydrophilic macromolecules and confirm their diffusion pathways. Because the cultured human epidermis model lacks hair follicles, which are an important transport pathway for hydrophilic molecules, it is a good model for evaluating transport pathways. The significant increase in the penetration of FD4 after trypsin pretreatment (Figure 4) indicates that trypsin may enhance passive diffusion through the intercellular pathway. On the other hand, we did not find histological layer separation of the SC and epidermis after the 7-h permeation experiment. Pretreatment with a low concentration of trypsin may not affect the viability of cultured epidermal cells.

Based on the marked enhancement of FD permeation, transdermal delivery of the polypeptide, FITC-insulin, was also performed. Similar to FD, a significant enhancement on the permeation flux was observed with FITC-insulin after trypsin pretreatment (Figure 5). In the control without trypsin treatment, the permeation flux of FITC-insulin (0.02 ± 0.00 $\mu g/cm^2$ per h) was lower than that of FD4 (0.59 ± 0.07 $\mu g/cm^2$ per h), although their molecular weight is similar. Langkjaer *et al.* found that insulin formed hexamers with a molecular weight of 36 kDa and showed a globular conformation with a radius of 2.5 nm,[30] which would have low skin permeability. It is known that dextrans are unbranched polyglucans[31] and FDs present a linear conformation. Therefore, our results may have been related to different sizes and conformations, and these factors should

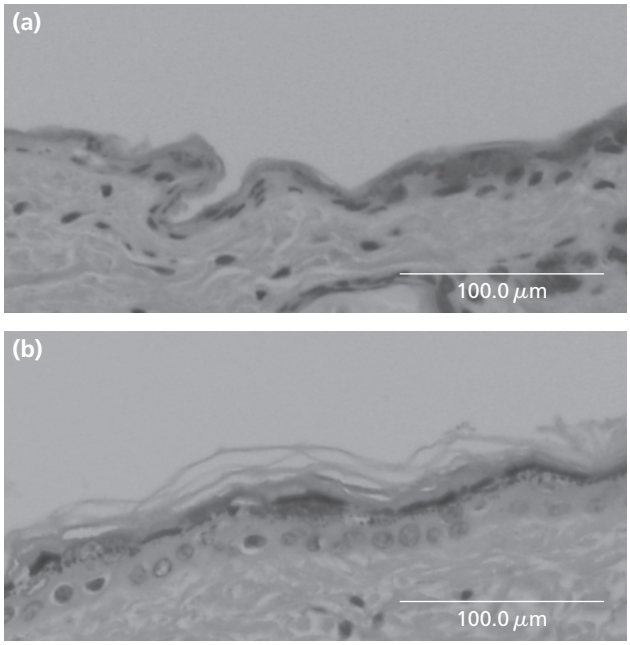

Figure 7 Histological examination of rat skin with or without trypsin treatment. (a) Rat skin treated with phosphate-buffered saline for 30 min. (b) Rat skin treated with 0.25% of trypsin for 30 min. Original magnification: ×200.

be taken into account when evaluating skin permeation.[32] On the integrity of FDs, it has been reported that FDs of various molecular weights were stable after the permeation through pig and nude mouse skin.[28] In this study, we monitored the degradation of FD and FITC-insulin at the end of experiment using a size exclusion gel chromatography. FD and FITC-insulin had the same elution profiles from both donor and receptor with or without trypsin pretreatment, and free FITC was not detected under our experimental conditions. Therefore, it could be noted that no FITC was degraded from intact FITC-labeled dextrans and insulin during the experiments. A previous investigation suggested that a depilatory cream could serve as permeation enhancer to increase the transdermal delivery of insulin.[33] Fang *et al.* enhanced the transdermal permeation of FITC-insulin using YAG laser treatment.[28] It was also reported that the recovery of damaged skin to a normal status required 3 to 7 days with these treatments. However, our in-vivo studies showed that insulin could reduce the plasma glucose level of diabetic rats with 0.5 to 2.5% trypsin pretreatment but no disorders were observed at the application sites.[34] This finding indicates that trypsin treatment, at the optimum concentration and pretreatment time, may be an efficient and safe way to enhance transdermal delivery of macromolecules such as insulin.

Fluorescence microscopy (Figure 6) revealed that FITC-insulin was markedly transported across the SC into the epidermis and dermis after trypsin pretreatment (Figure 6b and 6d). This result agreed with findings obtained with the three-dimensional cultured human epidermis model, showing that trypsin could enhance passive diffusion. On the other hand, the strong fluorescence signal in the hair follicles of trypsin-treated skin (Figure 6f) further suggests that appendageal routes are also important for FITC-insulin. Taken together, these findings indicate that trypsin affects both the intercellular pathway and the hair follicular route to increase the permeation of macromolecules.

On histological examination, partial thickening of the SC and viable epidermis were observed in rat skin treated with trypsin (Figure 7). It is well known that epidermal thickening can result from a disruption of the barrier function of the SC,[35] and that if only the upper SC is damaged, acceleration of cell proliferation and concomitant thickening of the epidermis can be induced. In our previous Fourier transform-infrared and attenuated total reflectance spectroscopy studies with trypsin-treated SC, broadness of amide I and amide II peaks toward higher wave numbers was obtained.[34] Trypsin may cleave the bonds of keratin-filled corneocytes in the superficial area, thus loosening and disordering the secondary structures of cellular proteins, especially at the surface of the corneocytes. These findings suggest that trypsin may alter SC protein structures, thereby inducing the reorganisation of peripheral SC lipids and resulting in the enhancing effect.

Conclusions

Pretreatment with, or co-administration of, trypsin markedly enhanced the transdermal permeation of FDs and FITC-insulin *in vitro*. It activates intercellular pathways and morphological alterations of SC structure by direct action on SC proteins. We can conclude that trypsin represents an advanced enhancement method with rapid action and low skin irritation that could be effective as a biochemical enhancer for the transdermal delivery of macromolecules including peptide and protein drugs.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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