EFFECT OF MOLECULAR WEIGHT OF PENETRANTS ON IONTOPHORETIC TRANSDERMAL DELIVERY *In Vitro*

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Introduction

Transdermal delivery is preferable for some drugs to oral administration or intravenous injection. Macromolecules such as peptide drugs, however, little penetrate through the skin. Iontophoresis is an effective transdermal method for macromolecules. In this study the effect of duration of applying electric field and degree of current density has been investigated under *in vitro* conditions. The macromolecules whose molecular weights are over 1.0×10^4 Dalton have been thought not to quickly penetrate the viable skin by passive diffusion, even if the drugs could pass through stratum corneum by applying the advanced transdermal techniques (Tojo, 2003). The viable skin may become, therefore, a significant barrier layer to the macromolecules.

Methods

1.Passive diffusion

Intact skin or viable skin without stratum corneum stripped with adhesive tape completely was excised from the abdominal portion of a hairless mouse (Hr^-/Kud, 6-9 weeks old, Kyudo Co.). The skin was mounted on the vertical diffusion cell system which was filled with phosphate buffer solution. Two milliliters of blank gel was applied on one compartment, while the drug gel was applied on the other. The cell cap ring was put on the gel holder, and finally these parts were fixed with a clamp. At predetermined time intervals, receptor solution (0.5 mL or 2 mL) was withdrawn for assay and then equal volume of fresh solution without drug was returned into the receptor. Vitamin B_{12} (VB_{12} , Wako Pure Chemical Industries Ltd.) concentration of the sample was analyzed by HPLC (LC-10A System, Shimadzu Corp.), Fluorescein isothiocyanate dextrans (FITC-Dextrans, average M.W. 4.4×10^3 Dalton (FD-4), 11×10^3 Dalton (FD-10) and 19×10^3 Dalton (FD-20)) concentration of the sample was analyzed by fluorescence spectrophotometer (FP-6500, Jasco. Co., Ltd., or F-4010, HITACHI, Ltd.).

2. Iontophoresis and iontophoresis pretreatment

After the excised hairless mouse abdominal skin was mounted on the vertical diffusion cell system, the platinum wire net electrodes were placed. Iontophoresis was then performed at constant current density (0.15, 0.30, and 0.60 A/cm^2) from 1 h to 2 h under direct constant current source (direct current supplier, Model 6911, Metronix Co. Ltd.).

In iontophoresis pretreatment, 2 milliliters of blank gel was applied on both compartment of the gel holder, and the electric current of 0.15, 0.30, and 0.60 A/cm^2 was applied for 1 h. The cathode gel was removed immediately with the cotton bud carefully, and then 2 mL of drug gel was applied to start permeation experiment.

All permeation experiments described above were performed in triplicate (or more) on skin taken from different mice.

Results and Discussion

1. Passive diffusion through intact skin and viable skin

Fig. 1 shows the cumulative amount of the model drugs permeated through intact skin at 24 h (Q_{24}). Cumulative amounts of FD-10 and FD-20 permeated were about one-seventh of that of VB_{12}, suggesting these large molecules hardly penetrated through the intact skin. The diffusion coefficient of the compounds across viable skin ($D_{v.s.}$) is inversely proportional to molecular weight with the exponent of 0.38, $D_{v.s.} \propto (M.W.)^{-0.38}$, (Table 1), which are almost the same trend as in aqueous (i.e., for large molecule, $D \propto (M.W.)^{-1/3}$) (Tojo, 1987). Consequently, these experimental findings indicate that diffusion across the stratum corneum of hydrophilic large molecules used in this study is the rate limiting step, while diffusion into viable skin is little resistant to model drugs permeation.

Table 1 Molecular weight of penetrants and permeation parameters through viable skin

	VB_{12}	FD-4	FD-10	FD-20
M.W.	1.4×10^3	4.4×10^3	10×10^3	19×10^3
Q_{12} [μg/cm^2]$\times10^{-3}$	3.9±0.40	1.3±0.066	0.80±0.092	0.62±0.032
dQ/dt [μg/cm^2/h]$\times10^{-3}$	0.39±0.017	0.14±0.0051	0.074±0.0084	0.058±0.0029
$D_{v.s.}$ [cm^2/s]$\times10^7$	1.2±1.1	1.0±0.27	0.50±0.097	0.51±0.090

All the value represents mean ± S.D. (n=3).

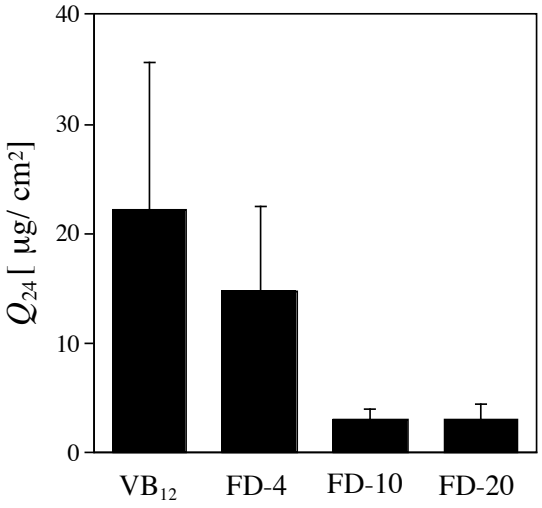

Fig.1 Cumulative amounts of model drugs permeated in 24 h through intact skin. Each data point represents mean + S.D. (*n*=3 or 5)

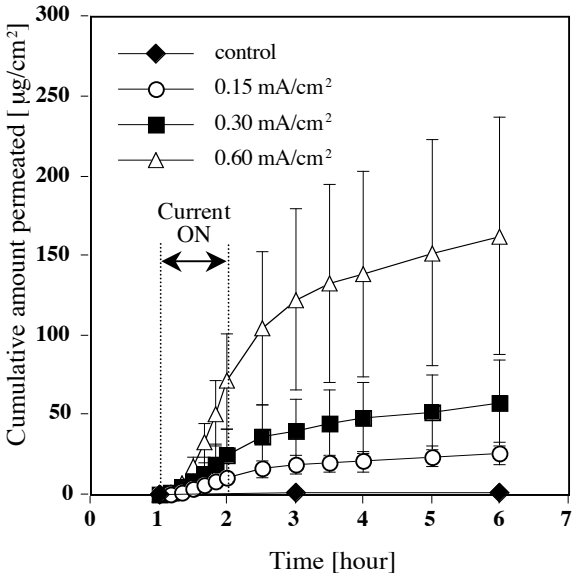

Fig. 2 Effect of current density on VB_{12} permeation. After 1 h of passive transport, the current was applied for 1 h. Each data point represents mean±S.D. (*n*=3 or 5)

2. Iontophoresis

2.1VB_{12} permeation

VB_{12} is a nonionic substance with the molecular weight of 1.4×10^3 Dalton. When the current density of 0.15, 0.30 and 0.60 mA/cm^2 were applied, the fluxes of VB_{12} were 14±5.2, 40±19 and 115±55 μg/cm^2/h, respectively (Fig. 2). Within the range of the current density applied, the flux was proportional to the current density. Permeation of non ionic substance such as VB_{12} could be increased mainly by convective solvent flow caused by electro-osmosis (Pikal, 2001). The flow could be proportional to current density applied, as shown above.

2.2 FITC-Dextrans (FD-4, FD-10, and FD-20) permeation

Since FITC-Dextrans are negatively charged under this experiment condition (Hämäläinen *et al.*, 1998); the cathode was placed on drug gel and the anode was placed on blank gel. The permeation profiles of FITC-Dextrans under iontophoresis differ clearly from the trend for charged small molecules; the cumulative amounts of FITC-Dextrans little increased during iontophoresis. The flux of FITC-Dextrans increased continuously after the electric current was removed.

Two hypotheses can be considered from the results of experiments. First, FITC-Dextrans might be pushed into stratum corneum temporarily by electro repulsion during

iontophoresis, and then released slowly from it (reservoir effect). The other hypothesis is that skin permeability of the substances might change during iontophoresis (decrease of skin barrier function). To confirm the reservoir effect, the drug gel was removed immediately after 1hour iontophoresis. The cumulative amount of FITC-Dextran slightly increased immediately after the iontophoresis, and then reached a plateau (Fig.3). Moreover, the result of iontophoresis pretreatment, cumulative amount of FITC-Dextran permeated was increased, and the trend of permeation profile was similar between iontophoresis and pretreatment experiments (Fig.4). The results indicate the decrease in the skin barrier function, which affects the permeation of hydrophilic large molecules and increases the penetrants diffusivity in stratum corneum.

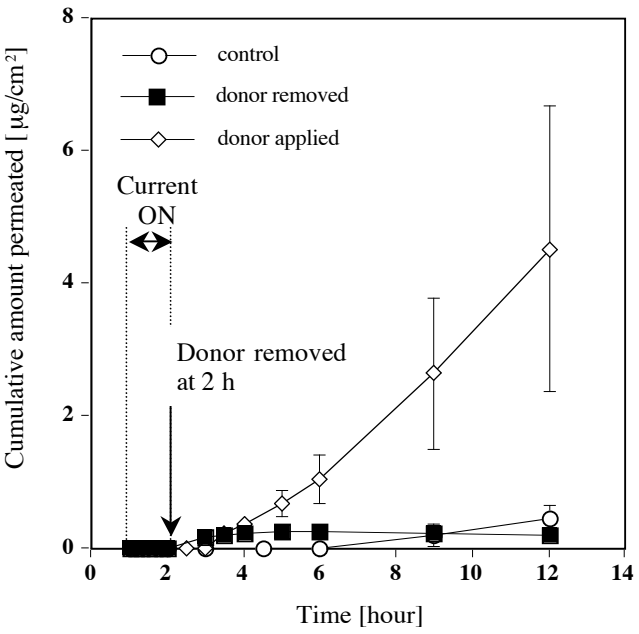

Fig. 3 Permeation profiles of FD-20 with and without donor gel after iontophoresis. After 1 h of passive transport, the current was applied for 1 h with 0.30mA/cm^2, and then after the donor gel was removed from skin surface at 2 h (■). With donor gel on skin during the permeation experiment (◇). Each data point represents mean ± S.D. (n=3)

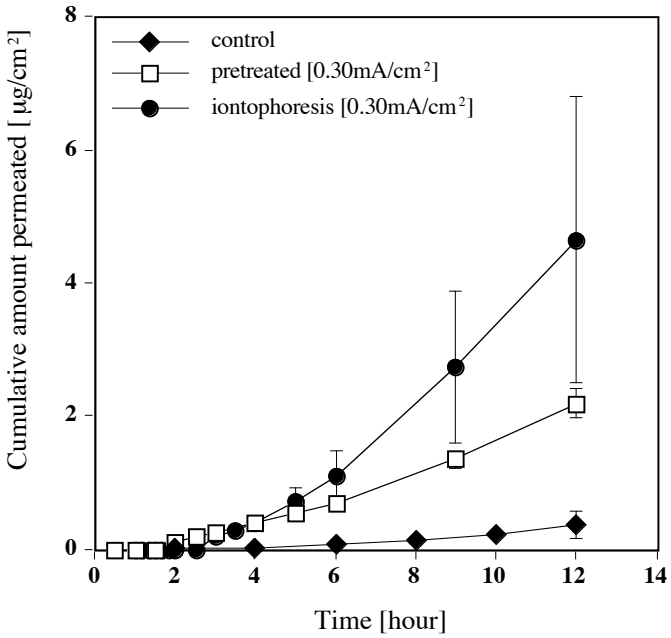

Fig. 4 Effect of iontophoresis on FD-20 permeation with 0.30 mA/cm^2 for 1h. (□) pretreated with current for 1 h before the permeation experiment. (●) current applied from 1 h to 2 h, and (◆) control. Each data point represents mean ± S.D. (n=3)

Concludion

From passive permeation experiments, the cumulative amounts of the model drugs permeated through viable skin are 100-fold larger than those through intact skin. The penetration across stratum corneum was found to be the rate limiting step for the drugs used although the barrier capacity of viable skin may increase gradually with the molecular weight of the penetrants.

Iontophoresis appreciably enhanced the cumulative amounts of the model drugs permeated. The trends of permeation profiles were, however, different between VB_{12} and FITC-Dextrans under electric field application. The flux of VB_{12} increased during iontophoresis application, and the flux was proportional to the current density applied. The flux of FITC-Dextrans little increased during iontophoresis, while the flux appreciably increased during the post-iontophoresis, probably due to skin hydration.

References

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