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Enhancement of transdermal absorption by switching iontophoresis

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Abstract

The enhancing effect of switching iontophoresis on transdermal absorption of phthalic acid (PA), benzoic acid (BA), salicylic acid (SA), *p*-phenylenediamine (PD), aniline (AN) and verapamil (VR) and its mechanism were examined. An electric current with pulsed waveform (4 kHz, 50% duty) was passed through the skin for 2 h at 10 V. Iontophoretic application was carried out with switching at intervals of 5, 10 and 20 min, or without switching. Each drug solution was injected into the donor side of the cell, and phosphate buffer (pH 7.4) was injected into the receiver side. Transport of PA, BA and VR was affected by switching the polarity of electrodes but no effect was observed on that of SA, PD and AN. Cumulative amount permeated and apparent permeability coefficients were apparently high at switching intervals with a short period. The partition coefficient suggested that there was no interrelation between the affinity for skin and the permeability of each drug. The resistance values of PA and glucose were low at intervals of 5 min suggesting the participation of enhanced hydration of the skin. These results suggested that enhancement of skin hydration plays an important role in the enhancing effect of switching iontophoresis on skin permeation.

Keywords: Switching iontophoresis; Transdermal absorption; Mechanism; Skin hydration; Skin resistance

1. Introduction

Iontophoresis can be classified into three forms, direct current, pulsed current and pulse-depolarization iontophoresis. The pulsed iontophoresis has been reported to show hardly skin irritation and to enhance permeation (Okabe et al., 1986; Chien et al., 1990). In recent years, attempts to combine use of chemical enhancers and iontophoresis (Wearley and Chien, 1990; Tomohira et al., 1997) and non-invasive sampling of biological fluid by iontophoresis (Glikfeld et al., 1989) have been reported. Moreover, the permeation of insulin and calcitonin through the skin was reported to be enhanced by switching polarity of the electrodes periodically (Tomohira et al., 1997). In the present study, the enhancing effect of switching iontophoresis on transdermal absorption of model drugs, i.e. phthalic acid (PA), benzoic acid (BA), *p*-phenylenediamine (PD), aniline (AN) and ver-

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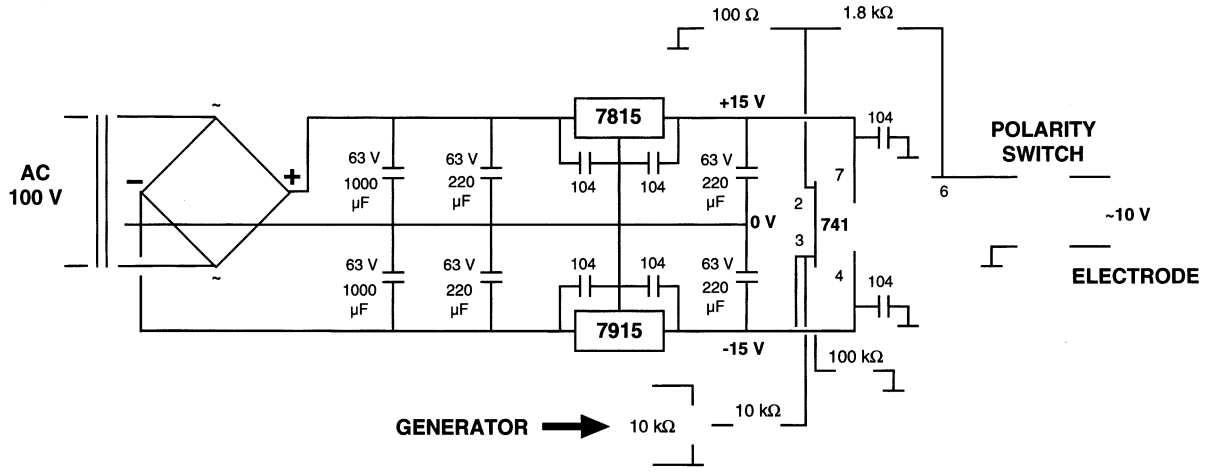

Fig. 1. Block diagram of the pulsed voltage generator in which the average voltage limit can be set.

apamil (VR), was investigated. Furthermore, the mechanism of the enhancement was also investigated in vitro using PA and glucose as model drugs.

2. Materials and methods

2.1. Materials

Phthalic acid (PA: MW = 166.13), benzoic acid (BA: MW = 122.12), *p*-phenylenediamine (PD: MW = 108.14), aniline (AN: MW = 93.13), glucose anhydrous (Glu: MW = 180.16) and a Glucose B-test kit were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Salicylic acid (SA: MW = 138.12) and verapamil hydrochloride (VR: MW = 491.1) were from Koso Chemical Co., Ltd. (Tokyo, Japan) and Sigma Chemical Co., respectively (St. Louis, USA). VR, PD and AN were used as cationic model drugs, and PA, BA and SA were used as anionic model drugs. All other chemicals were obtained commercially as reagent-grade products.

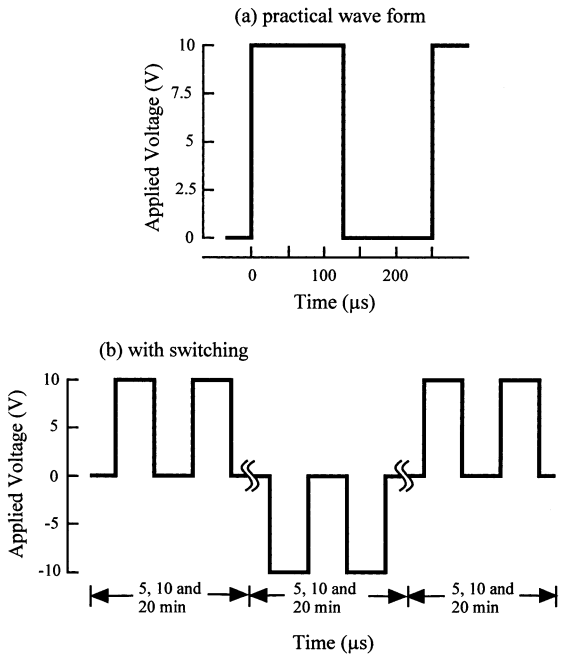

Fig. 2. Shape of the pulsed wave used in iontophoresis.

2.2. Animals

Male Wistar rats weighing approximately 180–200 g were purchased from Saitama Experimental Animal Supply Co. (Saitama, Japan). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Hoshi University.

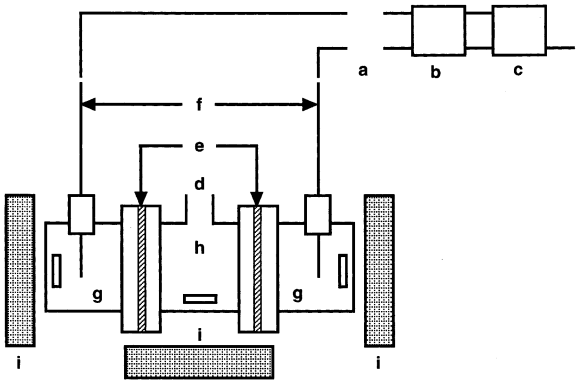

Fig. 3. Schematic representation of the diffusion cell used for the permeation studies. (a) switch, (b) amplifier, (c) oscillator, (d) sampling port, (e) skin (3.53 cm^2), (f) electrodes, (g) donor (2.65 ml), (h) receiver (6.00 ml), (i) magnetic stirrer.

2.3. Apparatus and pulse waveform

The circuit used for iontophoresis is illustrated in Fig. 1. In this study, a pulsed wave (4 kHz, 50% duty) was generated in this circuit. The pulsed waveform is shown in Fig. 2(a). As shown in Fig. 2(b), the polarity of the pulsed waveform was reversed periodically by switching.

2.4. Enhancing effect of switching iontophoresis on skin permeation of ionic drugs

Anionic drugs (PA, BA and SA) and cationic drugs (VR, PD and AN) were dissolved in phosphate buffer (0.07 M, pH 8.0) and in acetate buffer (0.1 M, pH 5.0), respectively. The final concentrations of both types of solutions were fixed at 15 mM. It has been reported that ionization rate of drugs plays an important role in permeability through skin (Kamath and Gangarosa, 1995). Consequently, anionic and cationic drug solutions were adjusted to the above respective pH values.

After removal of abdominal hair of rats using electric clippers, abdominal skin freshly excised from rats was mounted on the modified skin permeation cells as shown in Fig. 3. The epidermal side faced to the donor chamber. Each drug solution was injected into the donor side of the cell, and phosphate buffer (pH 7.4) was injected into the receiver side. Two bars of platinum were used as electrodes, and an electric current with a pulsed waveform was passed through the skin for 2 h at 10 V. Although it was presumed that pH of donor solution was changed in case that platinum was used as an electrode (Clemessy et al., 1991), pH of donor compartment was little changed before and after iontophoretic application (data not shown). Iontophoresis was carried out with switching at intervals of 10 and 20 min or without switching. Application of similar cells without current was also performed as a control. The receiver solution was sampled periodically for 6 h, and an equal volume of the same buffer was compensated after each sampling. Incubation temperature was maintained at 37 °C throughout the experiments.

The amounts of PA, BA and VR in the receiver solution were quantified by high performance liquid chromatography (HPLC). HPLC was carried out using a Shimadzu LC-5A apparatus equipped with a Nucleosil $5C_{18}$ column (4.6 × 150 mm) and an SSC UV detector 3000B (Senshu Scientific Co., Tokyo) set at 254 nm (PA and BA) and 278 nm (VR). The mobile phase was a mixture of water–methanol (70:30, 500 ml) containing phosphoric acid (1 ml), a mixture of water–methanol (60:40, 500 ml) containing phosphoric acid (1 ml) and a mixture of phosphate buffer (pH 7.4)–methanol (80:20) for PA, BA and VR, respectively. The flow rate was 1.0 ml/min. The amounts of SA, PD and AN in receiver solution were measured spectrophotometrically at 295, 239 and 230 nm, respectively.

The partition coefficient of octanol/buffer (pH 5.0 or 8.0) was measured spectrophotometrically after incubation at 37 °C for 72 h.

2.5. Mechanism of enhancement by switching iontophoresis

About 15 mM PA solution in phosphate buffer (pH 8.0) and 300 mM glucose solution in acetate buffer (pH 5.0) were prepared. PA was injected into the donor side of the cell, and phosphate buffer (pH 7.4) was injected into the receiver side. Incubation temperature was maintained at 37 °C throughout the experiments. Iontophoresis was carried out with switching at intervals of 5, 10

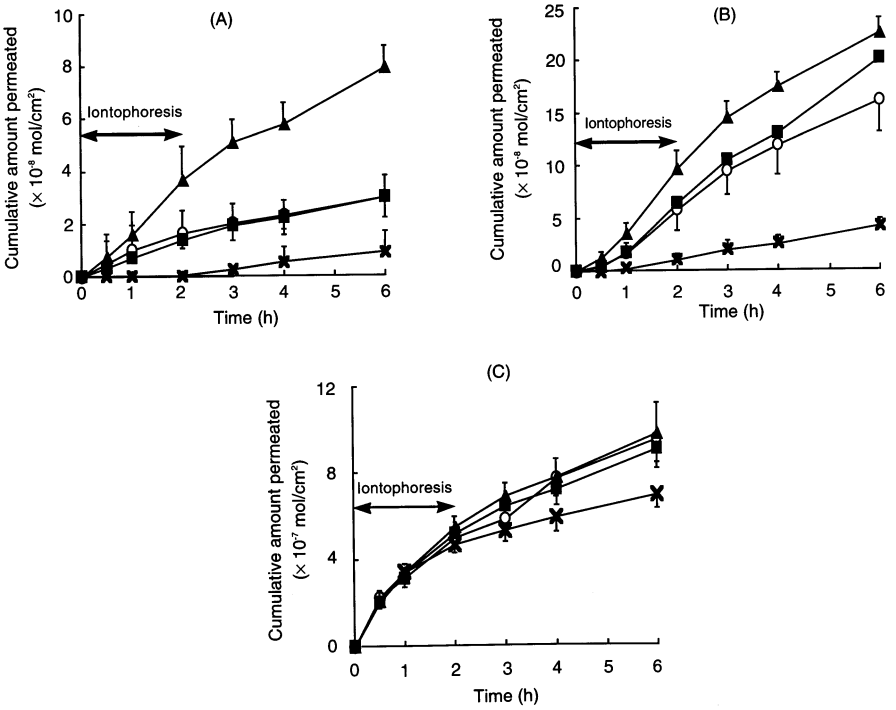

Fig. 4. Effects of switching the polarity of electrodes on skin permeation of (A) PA, (B) BA and (C) SA. Crosses (×), open circles (○), closed triangles (▲) and squares (■) represent control, non-switching, switching at intervals of 10 and 20 min, respectively. Each point represents the mean ± S.D. ($n = 3–5$).

and 20 min or without switching. The receiver solution was sampled periodically, and an equal volume of the same buffer was added after each sample was withdrawn. To evaluate the effect of the switch in polarity of electrodes on the skin, passive diffusion after iontophoresis with or without PA was examined. Further, to examine the effects of switching on the skin, the skin resistance was measured at 1 and 2 h after application of iontophoresis using a YX-360TR tester (Sanwa Electric Instrument Co., Tokyo, Japan).

Similarly, the skin permeation of glucose was examined to confirm the participation of skin hydration. Glucose solution and phosphate buffer (pH 7.4) were injected into the donor side and the receiver side of the cell, respectively. The subsequent procedures were similar to those described for PA, except for the quantification of glucose by the glucose oxidase method using a Glucose B-test kit.

3. Results and discussion

3.1. Effects of switching iontophoresis on skin permeation of ionic drugs

In this study, the voltage was set at 10 V based on the previous observation that no skin burning was observed at this voltage (Sudeji et al., 1989). Fig. 4 shows the effects of switching iontophoresis on skin permeation of anionic drugs. In the case of PA, no lag time was observed by application of iontophoresis (Fig. 4(A)). The cumulative amount of PA permeated at 6 h was increased by approximately 2.7-fold by switching at an interval of 10 min compared with that at an interval of 20 min. The permeation of BA was higher than that of PA and the lag time observed in controls was about 1 h (Fig. 4(B)). For PA and BA, the permeation was enhanced by application of iontophoresis; the cumulative amount of both drugs

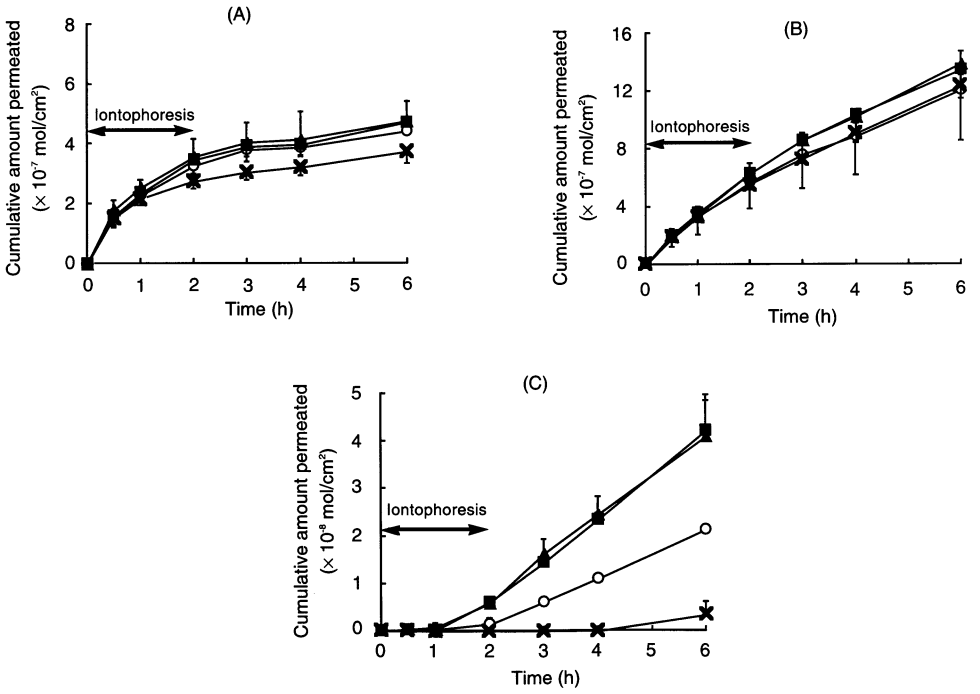

Fig. 5. Effects of switching the polarity of electrodes on skin permeation of (A) PD, (B) AN and (C) VR. Crosses (×), open circles (○), closed triangles (▲) and squares (■) represent control, non-switching, switching at intervals of 10 and 20 min, respectively. Each point represents the mean ± S.D. ($n = 3$).

permeated tended to increase with switching at an interval of 10 min as compared with switching at an interval of 20 min, without switching and controls. The permeation of SA was markedly higher than those of PA and BA (Fig. 4(C)). The permeation of SA was only slightly enhanced by application of iontophoresis as compared with controls.

Fig. 5 shows the effects of switching iontophoresis on skin permeation of cationic drugs. The permeation of PD was enhanced only slightly by application of iontophoresis as compared with controls (Fig. 5(A)). The permeation of AN without iontophoresis (control) was markedly high, and was higher than that of PD indicating that the skin has selective permeability to monovalent ions (Phipps et al., 1989). This observation suggested that divalent ions migrate more slowly than monovalent ions, and as a consequence the former might interact more strongly with charged sites in the skin as compared with the latter. It was

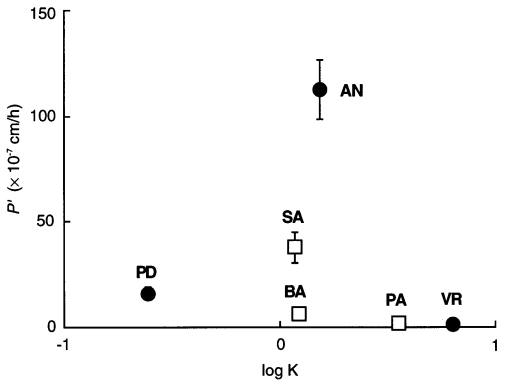

Fig. 6. Apparent permeability coefficients of six permeants plotted as a function of the octanol/water partition coefficient (log K). Closed circles (●) and open squares (□) represent cationic and anionic drugs, respectively. Each point represents the mean ± S.D. ($n = 3$–5).

difficult for VR to permeate through the skin as indicated in Fig. 5(C) because its molecular weight is relatively high. The permeation of VR was

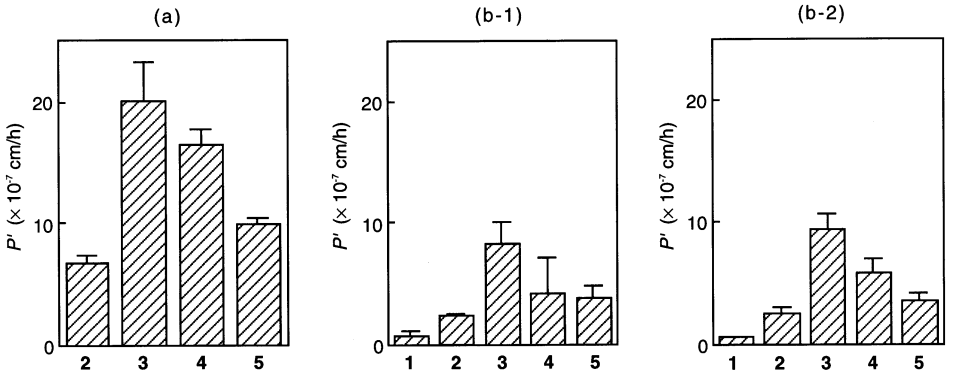

Fig. 7. Apparent permeability coefficients of permeants in Wistar rat skin. (a) During iontophoresis, (b-1) After iontophoresis with PA, (b-2) After iontophoresis without PA. 1–5 Represent control, non-switching, switching at intervals of 5, 10 and 20 min, respectively. Each point represents the mean ± S.D. ($n = 3$).

improved by application of iontophoresis and the profile with switching iontophoresis at an interval of 10 min was almost equivalent to that with switching at an interval of 20 min. On the whole, the switching polarity of electrodes contributed to the improvement of permeability although the effect was dependent on the drug; i.e. the permeation of PA, BA and VR was affected by switching the polarity of the electrodes, but that of SA, PD and AN was not.

The relationship between skin permeability and partition coefficient of drugs was investigated. Apparent permeability coefficients (P') of drugs without iontophoresis (control) plotted as a function of the partition coefficient (log K) are shown in Fig. 6. P' was calculated from the slope of linear line plotted in Figs. 4 and 5 using the equation based on Fick's first law: $dQ/dt = P' \times S \times (C_d - C_r)$, where Q, t, S, C_d and C_r are the amount of drug permeated, time, the area in membrane applied, the drug concentration in donor chamber and that in receiver one, respectively. There was no interrelation between the permeability and the affinity to skin. However, from this figure, skin permeation of the drug with low P' and with low log K tended to be increased by iontophoresis, especially with switching. Moreover, factors other than hydration were considered to affect skin permeability in vivo study although this report focused on skin hydration. Further studies are needed to clarify in vivo enhancing effect of switching iontophoresis on transdermal absorption.

3.2. Mechanism of enhancement by switching iontophoresis

Fig. 7 summarizes apparent permeability coefficients in the above experiments. As can be seen in this figure, these values with switching at an interval of 5 min were the highest. The switching was considered to affect the skin rather than the drug because passive diffusion from the donor solution after application of iontophoresis with or without drug showed little difference.

A system in which one influence (X) generates one flux (J), assuming that the flux is proportion to the influence, can be described by the following formula:

$$J = L \times X \tag{1}$$

where L is the proportional constant. This relationship can hold true over a wide range of values of X and J. Here, the proportional constant is in inverse relation to resistance and can be expressed as follows:

$$L = \frac{1}{R} \tag{2}$$

where R is the resistance and L is the conductance. Thus, the flux would be increased with reduction of resistance if there was a definite constant

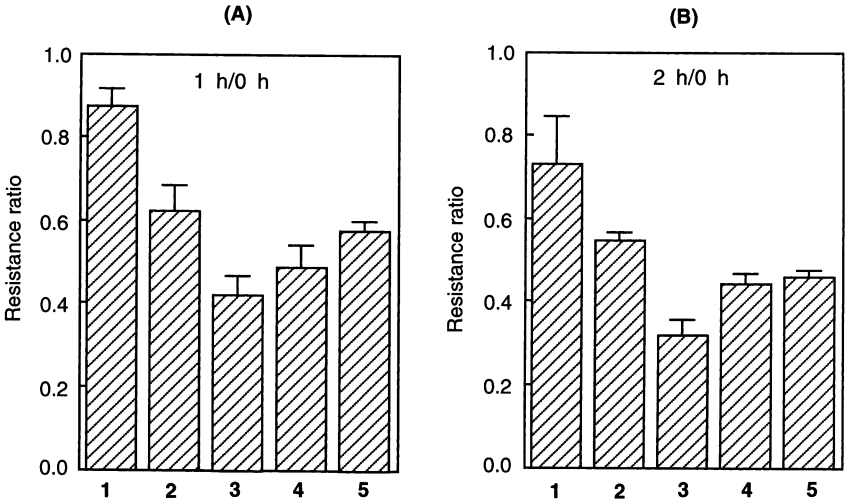

Fig. 8. Effects of switching polarity of electrodes on resistance of Wistar rat skin. 1–5 represent control, non-switching, switching at intervals of 5, 10 and 20 min, respectively. Each point represents the mean ± S.D. ($n = 3$).

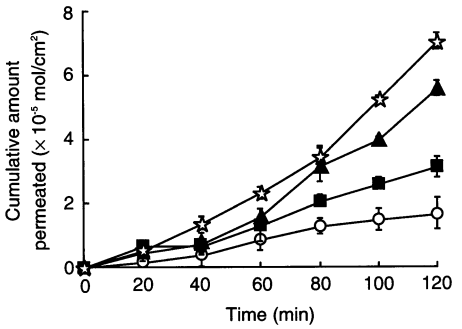

Fig. 9. Effect of switching polarity of electrodes on skin permeation of glucose during iontophoresis. Open circles (○), open stars (☆), closed triangles (▲) and squares (■) represent non-switching, switching at intervals of 5, 10 min and 20 min, respectively. Each point represents the mean ± S.D. ($n = 3$).

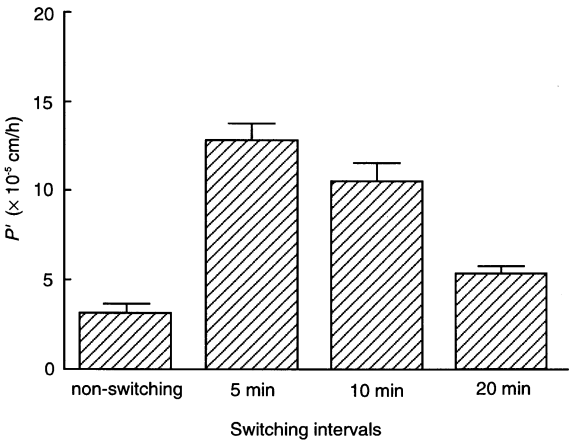

Fig. 10. Apparent permeability coefficient of glucose in Wistar rat skin. Each point represents the mean ± S.D. ($n = 3$).

influence. Pikal and Shah reported that the skin resistance was altered by hydration when iontophoresis was applied (Pikal and Shah, 1991). Further, Burnette and Ongpipattanakul reported that (i) skin resistance was decreased by hydration, (ii) conductance was increased and (iii) the flux of ions was increased linearly by hydration (Burnette and Ongpipattanakul, 1988). In view of the above points, the enhancement of skin hydration was discussed in terms of skin resistance. Fig. 8 shows the ratio of skin resistance at 1 or 2 h after application of iontophoresis to that prior to application of iontophoresis. As indicated, skin resistance was markedly reduced by application of iontophoresis. The skin resistance at 2 h when the interval of the polarity switch was 5 min became approximately 40 and 60% as compared with controls and non-switching iontophoresis, respectively. This result suggested that switching with a short interval enhanced hydration to a greater extent than switching with a long interval. It was

conceivable that the enhancement of skin hydration by switching iontophoresis was concerned with repeat between relaxation and tension of skin by current.

Figs. 9 and 10 reveal the effect of switching on skin permeation of glucose during iontophoresis. Similar to PA, apparent permeability coefficient of glucose showed the maximum value when switching was carried out at an interval of 5 min. Permeation of neutral materials such as glucose was accelerated by the increase in solvent flux caused by electric permeation (Srinivasan et al., 1989). Further, the increase in solvent flux has a skin hydration enhancing effect (Tyle, 1986). For all that pH in the donor solution may influence the solvent flux, pH in the donor solution used in these studies was different (pH 8 or 5). The effect of pH in the donor solution on skin permeability will be reported in the near future. It was possible that the enhancement of skin hydration was most responsible for the improvement of permeability. Therefore, the results in the present study verified that hydration played an important role in the enhancement of skin permeation by polarity switching.

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