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Comparison of Depolarizing and Direct Current Systems on Iontophoretic Enhancement of Transport of Sodium Benzoate Through Human and Hairless Rat Skin

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Abstract—A direct current (DC) system and a pulsed depolarization (PD) system were evaluated for their iontophoretic permeation of sodium benzoate, as a model drug, through hairless rat and human skin. Approximately the same initial permeation of sodium benzoate through the hairless rat skin was obtained at 0·1 mA for the DC device and at 3·0 mA for the PD device. Study of the drug's permeation was performed using a two-chamber iontophoretic diffusion cell, over two cycles of three successive on–off experimental conditions [stage I (off) 0–4 h, II (on) 4–6 h, III (off) 6–10 h, saline washing 10–24 h, IV (off) 24–28 h, V (on) 28–30 h and VI (off) 30–34 h]. Skin permeation rate during stage IV of the iontophoresis as compared with the control group through hairless rat or human skin for the DC system was 2–4 times that in stage I, whereas in the same stage using the PD system it was almost the same as in stage I. Impedance of skin decreased during the application of either system (stage II); however, the value significantly recovered during stage III only in the case of the PD system use on human skin. Histological observation revealed no tissue alteration in the hairless rat skin after using either system. When the DC or PD system was applied to volunteers, the minimum current density producing pain was 0·016 or 2·7 mA cm^{-2}, respectively. These results suggested that the PD system was more appropriate for iontophoresis application than the DC system from the point of view of skin permeability of the drug and effect on the skin.

Iontophoresis can be defined as a process or method in which the permeation rate of ionic drugs into the body is enhanced by applying a voltage between viable tissues (Tyle 1986). It was reported that the absorption of not only low mol. wt drugs but also of high mol. wt drugs such as peptide hormones could be enhanced by this method (Banga & Chien 1988). The enhancing effect of iontophoretic drug transport may be analysed by the Nernst–Planck equation (Masada et al 1989). This equation indicates that the enhancement factor (iontophoresis/passive diffusion) is 37·38 times at 1 volt-linear electric field application across the membrane. The value is independent of the membrane resistance. However, the enhancement factor through the skin does not always obey the Nernst–Planck equation, because skin is a negatively charged membrane and convective flow or electro-osmosis through the skin may occur under iontophoresis application. In addition to such electrochemical and physicochemical problems, basic studies on pharmaceutical technology are also needed. Knowledge of power sources, electrodes, the composition of vehicles and phamaceutical additives for iontophoretic systems can prevent iontophoretic burns, electric shock and stimulus to the skin, and is thus essential for the practical use of iontophoretic systems and devices (Gangarosa et al 1978; Glikfeld et al 1988; Lelawongs et al 1990).

The present study compared the skin transport of sodium benzoate, as a model drug, by a pulsed depolarization (PD) iontophoretic system (recently developed by Advance Co. (Okabe et al 1986)) with that by a direct current (DC) system (a fundamental system) to accumulate data needed for development of the most efficacious and best-conceived iontophoretic systems and devices.

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Materials and Methods

Materials

Sodium benzoate (Japanese Pharmacopeia grade) was purchased from Yamada Pharmaceutical Co. Ltd (Ibaraki, Japan). Platinum wire (99·9% purity, 1 cm × 1 mm) was obtained from Tokuriki (Tokyo, Japan) for the electrodes. Other chemicals were of reagent grade and were used without further purification. All solutions were made with deionized water which had been passed through a water purifier (Eyela ER, Tokyo Rikakikai Co. Ltd, Tokyo, Japan). The resulting water had a pH of 6·74 and an electric conductivity of 0·39 μs cm^{-1}.

Skin membrane preparation

Excised full-thickness abdominal skin of male hairless rat (WBN/ILA-Ht, 6 weeks old, Life Science Research Center, Josai University, Saitama, Japan) was used for the study of iontophoretic permeation. Human skin was obtained from unrelated surgical operations (Department of Surgery, Kitasato University, School of Medicine and Sagamihara National Hospital, Kanagawa, Japan). Sources were the chest of 37- to 75-year old female patients. The human skin samples were stored at −20°C until use. Thickness of the skin was reduced to 750 μm by whittling the dermis side. This side was then washed with distilled water for 18 h before the permeation experiment. Skin was mounted on a two-chamber iontophoretic diffusion cell and only the dermis side

of the cell was filled with distilled water. Preliminary experiments confirmed that this treatment would not affect skin permeability of a drug.

Power source

A Phoresor (PM 600, IOMED, Inc., Salt Lake City, UT, USA) was used as the DC power source. This device generates a constant direct current. Advance 4030 (Advance Co. Ltd, Tokyo, Japan) was used as the power source for PD current, generating a constant pulsed direct current (frequency 40 kHz, on–off duty 30%) (Okabe et al 1986).

Determination of DC and PD values for similar skin permeation-enhancing effects

A piece of skin membrane (either hairless rat or human skin, each about 2 × 2 cm) was mounted between the cathode and anode sides of the two-chamber diffusion cell equipped with platinum electrodes and both sides of the cell were stirred with a star-head bar at 1200 rev min^{-1} by a constant-speed synchronous motor (MC-301, Scinics, Tokyo, Japan) (Morimoto et al 1991). The surface area of the membrane available for drug (ion) permeation was 0·95 cm^2. Experiments were performed at 37°C. Both platinum electrodes were immersed in the solution of both half-cells, and were then connected to either the DC or PD power source. The cathode side was filled with 4 mL sodium benzoate solution (10 w/v %) and the anode side of the cell with the same volume of saline (0·9% NaCl). At predetermined times, all of the solution in the anode side was removed and 4 mL saline was added to maintain a constant cell volume throughout the permeation experiment. Sodium benzoate was assayed by HPLC (Morimoto et al 1991). The value of the current applied was determined by the flux value of benzoate anion through the hairless rat skin in the first 2 h of the experiment.

On–off iontophoretic permeation

Permeation experiments were performed using an iontophoresis group and a control group. The on–off iontophoretic permeation study involved six stages as shown in Scheme 1. During stage I (0–4 h), a passive transport experiment was carried out by taking 250 μL samples from the anode side of the cell at predetermined time intervals, adding the same volume of saline and measuring skin impedance. During stage II (4–6 h), a constant current iontophoresis was applied across the skin membrane and the amount of benzoate anion permeated and skin impedance were recorded. During stage III (6–10 h), a second passive transport run was carried out by the same method as in stage I. At the end of stage III, all solution was withdrawn from both sides of the cell, saline (4 mL) was added to each side, and the cell assembly was incubated at 37°C for 14 h. The solution in the cathode side was then replaced with 4 mL sodium benzoate solution (10 w/v %) and that in the anode side of the cell with the same volume of saline. Stage IV (24–28 h), stage V (28–30 h) and stage VI (30–34 h) were repeats on the 2nd day of stages I, II and III, respectively. Each permeation experiment was carried out in triplicate.

Stage	Time (h)	Application
	0	Start (iontophoresis off)
I	↓	
	4	Iontophoresis on
II	↓	
	6	Iontophoresis off
III	↓	
	10	Wash out (all cells)
	24	Start (iontophoresis off)
IV	↓	
	28	Iontophoresis on
V	↓	
	30	Iontophoresis off
VI	↓	
	34	End

SCHEME 1.

Measurement of voltage drop across skin membrane

The cathode side of the cell was filled with 4 mL sodium benzoate solution (10 w/v %) and the anode side with the same volume of saline. A digital multimeter (TR 6843, Takedariken, Tokyo, Japan) serving as a voltmeter was connected in parallel with the platinum electrodes of the two-chamber iontophoretic diffusion cell and the voltage drop across skin was measured during a 2 h-constant current study. Each measurement was carried out in triplicate.

Measurement of impedance

Change of impedance of skin membrane was measured by an impedance meter (Advance Co. Ltd, Tokyo, Japan) (applied voltage, 7 V generating a 10 Hz sine wave). Each measurement was carried out in triplicate.

Histological observation of hairless rat skin

A hairless rat anaesthetized by pentobarbitone (50 mg kg^{-1}, i.p.) was fixed on its back to a supporting plate. An iontophoretic electrode unit with a disposable pad (EL 500, Iomed Inc, Salt Lake City, UT, USA) was affixed to the abdominal skin. The unit consisted of a treatment electrode (active electrode; application area, 6·15 cm^2) and a receptacle with a karaya-gum disposable pad (counter electrode; application area, 16·0 cm^2) as shown in Fig. 1. Saline (3 mL) was poured into the treatment electrode chamber. After a 2 h equilibration period, a 2 h iontophoresis was performed. Applied currents of the DC and PD devices were 0·1 and 3·0 mA constant current, respectively. Two hours after termination of iontophoresis, the abdominal skin was excised from the rat, fixed with 10% formalin, sliced on a microtome and stained using a conventional haematoxylin-eosin stain. Each skin sample was observed using an optical microscope (Model BHS-N, Olympus, Tokyo, Japan).

Experiments with human skin

A treatment electrode (active electrode) and a receptacle with karaya-gum disposable pad (counter electrode) were affixed to the forearm of 10 male volunteers (age 21–33). The distance between treatment electrode and receptacle was 6·5 cm. Three millilitres of saline was poured into the treatment electrode chamber. The electrodes were connected to the iontophoresis power source and the current was gradually increased. The applied current at which the volunteer experienced electrical sensation was recorded by an indicator which was part of the iontophoretic system.

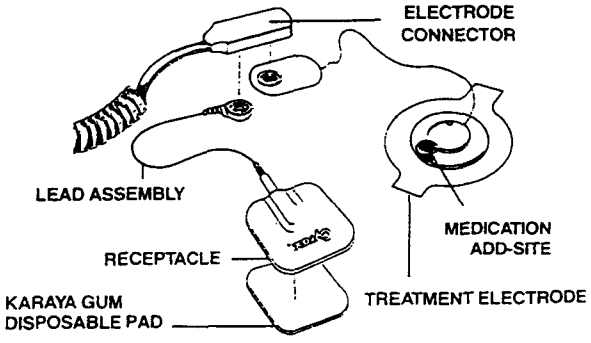

FIG. 1. Preparation of iontophoretic electrode (with permission of IOMED Inc.).

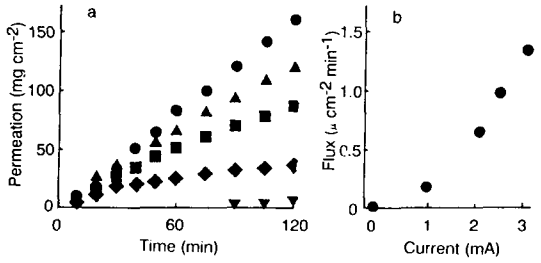

FIG. 3. Effect of PD on the permeation of sodium benzoate through excised hairless rat skin. a. Time course; ▼ control, ◆ 1·0, ■ 2·0, ▲ 2·5 and ● 3·0 mA. b. Flux–current relationship. Each point represents the mean of three experiments. The s.e. value is contained in the symbol.

Results and Discussion

Effect of applied current on permeation of benzoate anion

Fig. 2a shows the effect of applied current on the cumulative amount of benzoate anion which permeates through the hairless rat skin when the DC system is used, and Fig. 2b shows the relationship between the flux of benzoate anion and applied current. Fig. 3 shows the corresponding data for the PD system. In general, flux of an ion i, J_i (mol cm^{-2} s^{-1}), through membrane can be obtained using the following equation (Bockris & Reddy 1973):

$$J_i = C_i \times U_i \times E \quad (1)$$

where C_i and U_i are concentration (mol mL^{-1}) and ionic mobility (cm^2 s^{-1} V^{-1}) of ion i, respectively, and E is potential gradient (V cm^{-1}). It is clear from the equation that flux J_i is proportional to the potential gradient across the membrane, E, when C_i is nearly constant during the iontophoretic experiment. The relationship between the flux and applied current is similar to that between the flux and applied voltage, because applied current in the steady-state phase is proportional to applied voltage within the range in which Ohm's law applies. The relationships shown in Figs 2b, 3b did not strictly obey the Goldman–Hodgkin–Katz equation (Hodgkin & Katz 1949), which is a constant-field flux equation. However, the flux of benzoate anion increased with increasing applied current for both DC and PD systems. The convex curve for the DC system indicates that the benzoate anion transport decreased with increasing applied current, suggesting that skin may be damaged at a higher current (Burnette & Bagniefski 1988). The ionic mobility of benzoate anion in the skin may be greater when skin is damaged. Since electrical conductivity increases with greater ionic mobility, the potential gradient across the skin membrane should decrease with an increase in ionic mobility. This indicates that skin resistance is reduced when electrical conductivity of the skin is increased. The reason the efficacy of benzoate anion transport is reduced is the decrease in potential gradient as a driving force. The concave curve for the PD system indicates that the benzoate anion transport increased with increasing applied current, suggesting that skin was slightly damaged even at the higher current. Ionic mobility of benzoate anion in the skin was almost constant irrespective of the current applied; the potential gradient across the skin membrane increased with increase in the current. As a result, benzoate anion transport increased, because one of the driving forces, the potential gradient, was almost proportional to applied current at this value. The flux of benzoate anion (84 μg mL^{-1} h^{-1}) through hairless rat skin at 0·1 mA for the DC system was almost the same as that at 3·0 mA for the PD system. From the findings discussed above, we concluded that the effect of the DC system at 0·1 mA was comparable with that of the PD system at 3·0 mA for subsequent studies.

On–off iontophoretic permeation

Fig. 4 shows the effect of on–off switching of systems on the permeation of benzoate anion through the skin of hairless rats. Results for human skin (not shown) were similar. For the DC system, the permeation rate for the iontophoresis group at stage IV was about 1·6 times that for the control group, which suggested that both skin damage and skin hydration occurred. For the PD system, permeation rates for

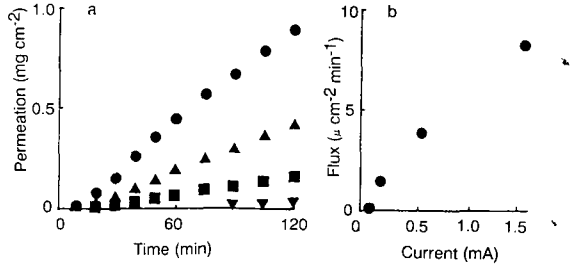

FIG. 2. Effect of DC on the permeation of sodium benzoate through excised hairless rat skin. a. Time course; ▼ control, ■ 0·1, ▲ 0·5 and ● 1·5 mA. b. Flux–current relationship. Each point represents the mean of three experiments. The s.e. value is contained in the symbol.

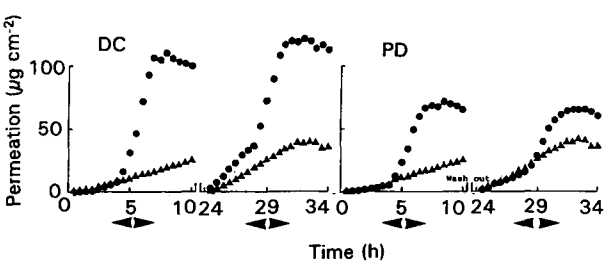

FIG. 4. The effect of on–off switching of iontophoresis on the permeation of sodium benzoate through excised hairless rat skin. ▲ Control, ● iontophoresis treatment. The arrows indicate the periods of iontophoresis.

both experimental and control groups at stage IV were almost the same as at stage I, which suggested little hydration or damage of the skin. The same tendency was reported by Sims et al (1991).

The findings that iontophoretic permeation rate at stage II (1st iontophoresis) was almost equal to that at stage V (2nd iontophoresis) made it clear that reproducible constant skin permeation can be obtained by both DC and PD systems in rat and human skins. Constant current iontophoresis has the advantage in chemotherapy of being capable of administering an exact dose at a designed dosage schedule. The PD system, however, was recognized as more suitable for iontophoresis application than the DC system from consideration of both drug permeability and skin damage.

Alteration in voltage across skin and skin impedance

The alteration in the voltage across hairless rat or human skin during DC and PD iontophoresis was measured in triplicate using an in-vitro diffusion cell technique (Fig. 5). For hairless rat skin during DC iontophoresis, voltage decreased to half the initial level in the first hour of the experiment, and thereafter was almost constant until the end of the experiment (2 h). In PD iontophoresis, except for an initial abrupt drop, the voltage also remained almost the same. For human skin, in DC iontophoresis, voltage decreased by 0·7 times in the first half of the experiment, and thereafter there was only a slight reduction. The DC voltage drop across both types of skin was larger than that of the PD voltage during the first hour. The decrease in voltage drop across the skin resulted from a reduction in skin resistance due to hydration and consequent impairment of the skin barrier. The hydration largely accounted for the lower initial drop in voltage. The results suggest that skin was slightly more damaged by the DC system than by the PD system. The decrease in skin impedance in the first hour of stage I suggests skin hydration. Impedance decrease during stage II was greater than at stage V. The former may be due to skin damage by the electrical current, and the latter to both skin damage and hydration.

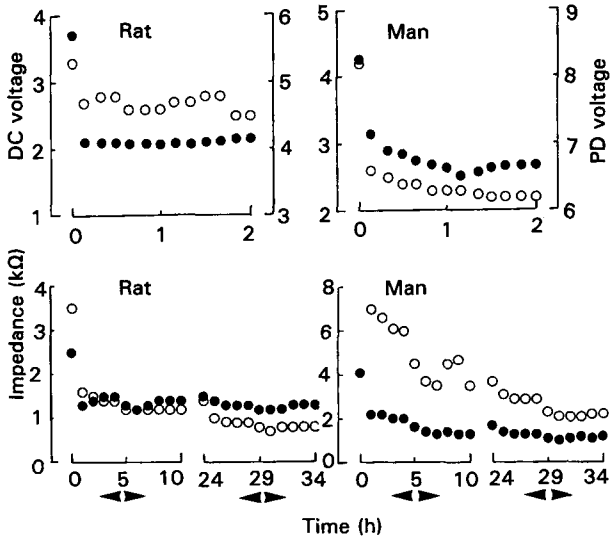

FIG. 5. Comparison of voltage drop and impedance across excised hairless rat and human skin during constant current DC and PD iontophoresis. ● DC iontophoresis, ○ PD iontophoresis. The arrows indicate the periods of iontophoresis.

Histological observation of hairless rat skin

No invasion of neutrophils was observed and no acute inflammation of the skin occurred as a result of DC or PD application. It was concluded that there was no severe damage to skin tissue at 0·1 mA for DC nor at 3·0 mA for PD iontophoresis.

Human skin

Nine of the ten volunteers felt weak electric pain when given a current of 0·1 mA/6·15 cm^2 by the DC system. The average current at which all volunteers felt such pain with the PD system, however, was much higher (16·8 mA/6·15 cm^2).

Iontophoresis is clearly a reproducible skin permeation-enhancing method. The pulsed depolarization system was found more suitable for iontophoretic application than the direct system.

Acknowledgement

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