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Synergistic Effects of Iontophoresis and Jet Injector Pretreatment on the In-vitro Skin Permeation of Diclofenac and Angiotensin II

KENJI SUGIBAYASHI*†, MIDORI KAGINO*, SACHIHIKO NUMAJIRI*, NAOKO INOUE‡, DAISUKE KOBAYASHI*‡, MASAYUKI KIMURA‡, MASATOSHI YAMAGUCHI§ AND YASUNORI MORIMOTO*†

*Faculty of Pharmaceutical Sciences, †Life Science Research Center, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, ‡Department of Pharmacy Services, Saitama Medical Center, Saitama Medical School, 1981 Kamoda-Tsujido, Kawagoe, Saitama 350-8550 and §Department of Pharmacy, Niigata Prefectural Muikamachi Hospital, 636-2 Muikamachi, Muikamachi, Minamiuonuma, Niigata, 949-6623, Japan

Abstract

A non-needle syringe (jet injector) was utilized to increase skin permeation of drugs by iontophoresis. Briefly, physiological saline was initially flushed by the injector to make a pore in the stratum corneum of excised hairless rat skin, and the iontophoretic skin permeation of two model compounds, sodium diclofenac and angiotensin II, was followed using a 2-chamber diffusion cell. Constant voltage and constant current iontophoresis treatments were evaluated.

Pretreatment using the jet injector alone resulted in about 13- and 22-fold increases in the steady-state flux of diclofenac and angiotensin II, respectively, through the skin, compared with non-treated controls. Jet injector pretreatment with constant voltage iontophoresis further enhanced skin permeation of diclofenac and angiotensin II, and the enhancement was also greater than that by constant voltage iontophoresis alone. Thus, a synergistic effect was observed. The ratio of enhancement was greater compared with the control. Jet injector pretreatment with constant current iontophoresis, however, did not always yield higher skin permeation of the drugs than injector pretreatment alone, although the lag time was shortened. The difference in the enhancement between the constant voltage- and constant current iontophoresis can be explained by the electric current through the excised skin.

Constant current iontophoresis after a short period of constant voltage iontophoresis with multiple jet injector pretreatments may be the best way to increase drug permeability while preventing severe skin damage.

Several penetration enhancers and prodrugs have been utilized in an attempt to increase bioavailability of topically applied drugs with low skin permeability (Walters & Hadgraft 1993). These methods, however, have not been sufficiently successful in enhancing skin permeation, particularly of bioactive peptides and oligonucleotides, which form a promising new category of therapeutic drugs (Guy 1996). Physical means such as phonophoresis (Ueda et al 1995; Kost et al 1996) and electroporation (Prausnitz et al 1993; Mori et al 1999) have also been evaluated as methods to enhance skin penetration of drugs with low bioavailability via skin permeation. Iontophoresis using electrical power is one of the most promising physical skin penetration-enhancing methods (Tyle 1986; Green 1996; Numajiri et al 1998a). This method is particularly useful for promoting the skin permeation of high-molecular-weight electrolytes such as proteins, peptides and oligonucleotides. It can also be applied to non-electrolytes, because the solvent flux caused by voltage application onto the skin facilitates their skin permeation (Singh et al 1995). Since iontophoresis has the advantage of easy control of drug administration through adjustment of the applied voltage or current, it may become a useful method for home drug therapy.

Correspondence: K. Sugibayashi, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan.

The jet injector is a non-needle syringe that is used for self-injection of insulin and growth hormone (Lindmayer et al 1986). The injector flushes the drug solution into viable skin tissue through the skin barrier, the stratum corneum, using high pressure, so that the drug is rapidly absorbed into cutaneous blood vessels. Previously, we have applied such an injection system to the transdermal delivery of poorly absorbable drugs through the stratum corneum (Inoue et al 1996). Briefly, physiological saline without the drug was first administered by the jet injector to make a pore or permeation pathway and then the drug solution was applied over the pretreated region of skin. As a result, skin permeation of three poorly absorbable drugs, gentamicin, nicardipine and theophylline, showed marked enhancement.

In this study, a combination of iontophoresis and jet injector pretreatment was evaluated for the potential to increase skin permeation of drugs. Two model drugs with low and high molecular weight, diclofenac sodium (MW 318·13, pKa 4·0) and angiotensin II (MW 1046·2, pI 7·7) (Clemessy et al 1995), respectively, were used and their in-vitro permeabilities were evaluated through excised hairless rat skin using a 2-chamber diffusion cell. The enhancing effect was evaluated using the obtained skin permeation data with an electric current or voltage drop during iontophoresis.

Materials and Methods

Materials

Diclofenac sodium was obtained from Wako Pure Chemical Industries (Osaka, Japan). Cold and ^{3}H-labelled angiotensin II were purchased from Sigma Co. (St Louis, MO) and Daiichi Chemical Co. (Tokyo, Japan), respectively. Other chemicals used were of reagent grade.

Preparation of skin membrane

Male hairless rats (WBN/ILA-Ht; 200–240 g), 7–8 weeks old, were supplied by the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratories (Sugito, Saitama, Japan) and used in all animal experiments. The abdominal skin was excised from anaesthetized rats (50 mg kg^{-1} sodium pentobarbital i.p.). Physiological saline (60 μL) was then injected using a jet injector (Preci-Jet, Medi-Ject Corp., Minneapolis, USA) (Singh et al 1995) directly on the skin or using a spacer to adjust the distance to 10 mm between the injector tip and skin surface (Inoue et al 1996). The injection was carried out one or three times at different sites on the skin surface to make one or three pore(s) in the skin barrier, respectively. Skin specimens without injector pretreatment were also used for comparison.

In-vitro skin permeation experiment

The skin membrane was mounted between two half diffusion cells (each 4·0 mL in volume and 0·95 cm^2 in effective diffusion area) for iontophoresis, to conduct the permeation experiment (Morimoto et al 1991). Diclofenac sodium or angiotensin II in 1/30 M phosphate buffer solution (pH 7·4; 4 mL each) was added to the stratum corneum side of the cell and just the buffer added to the dermis side. Concentrations of diclofenac sodium and angiotensin II were adjusted to 5 mg mL^{-1} and 0·1%, respectively. ^{3}H-Labelled angiotensin II was used to assay skin permeation. Cathode and anode electrodes made of platinum were set in the drug-donor and receiver cells, respectively. An electrical power source (DMR-20-2, Metronix, Tokyo) was used, and the current or voltage drop was recorded using a digital multimeter (TR-6843, Takeda Riken Co., Tokyo) for iontophoresis treatment. Constant voltage and constant current iontophoresis were carried out. The constant voltages applied for the diclofenac and angiotensin II permeation experiments were 0·5 and 0·2 V, respectively, and the constant currents were set at 0·1 and 0·3 mA for diclofenac and 0·3 mA for angiotensin II. Relative electrodes were connected by a salt bridge. Both donor and receiver compartments were stirred with a star-head bar driven by a constant-speed synchronous motor (MC-301, Scinics, Tokyo) at about 1200 rev min^{-1}. The permeation study was conducted at 37°C. The receiver solution (0·1 mL) was withdrawn at predetermined times for analysis.

Assay

The receiver samples containing diclofenac were mixed with the same volume of methanol : 0·1% phosphoric acid (7 : 3) containing isopropyl *p*-hydroxybenzoate, as an internal standard and centrifuged. Samples of the supernatant (50 μL) were injected into an HPLC system composed of a pump (LC-6A, Shimadzu, Kyoto, Japan), a UV detector (SPD-6A, Shimadzu), an integrator (C-R3A, Shimadzu) and a reverse-phase column (Inertsil ODS, 4·6 mm × 250 mm, GL Sciences Inc.). The mobile phase was methanol : 0·1% phosphoric acid (7 : 3) and the flow rate was

1·0 mL min^{-1}. The detector was operated at a UV wavelength of 286 nm and the column was maintained at room temperature.

The receiver solution containing ^{3}H-labelled angiotensin II was mixed with 10 mL of general scintillation fluid and kept overnight. The obtained radioactivity was measured using a liquid scintillation counter (LSC-700, Aloka, Tokyo).

Results and Discussion

Effects of jet injector pretreatment on the skin permeation of diclofenac

The effect of pretreatment with the jet injector alone (single injection) on the in-vitro skin permeation of diclofenac was determined before evaluating the combined effect of jet injection and iontophoresis. Figure 1 shows the cumulative amounts of diclofenac sodium that permeated through the skin by setting the jet injector directly on the skin surface and 10 mm above the skin. The jet injector groups showed a shorter lag time followed by a higher pseudo-steady-state flux than the control group (without jet injector pretreatment). The results are summarized in Table 1. The steady-state flux was 4-fold greater with the injector set directly on the skin surface and 13-fold greater with the injector 10 mm above the skin as compared with the control. A shorter lag time was found when the jet injector was set 10 mm above the skin than when it was placed directly on the skin surface.

We reported previously that a changes in the distance between the injector tip and skin surface affects the size of pores produced by the jet injector in the stratum corneum (Inoue et al 1996). When the distance was adjusted to 10 mm, injected saline solution diffused into a larger area and a shallower

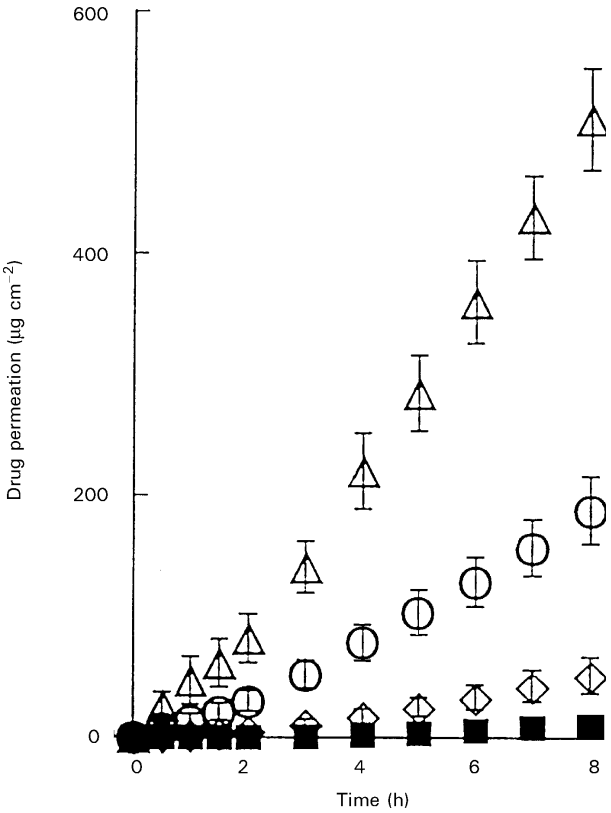

Figure 1. Effects of different jet injector pretreatment schedules on the permeation of diclofenac sodium through hairless rat skin. Control, ■; single jet injection directly onto the skin surface, ◇; single jet injection 10 mm above the skin surface, ○; triplicate jet injection 10 mm above the skin surface, △. Each data point represents the mean ± s.e.m. of 3–6 replicates.

Table 1. Effects of different types of jet injector and iontophoresis treatment on the permeation parameters of diclofenac sodium (steady-state flux (Jss) and lag time) through hairless rat skin and electrical parameters (potential difference (Vss), electric current (Iss) and membrane resistance (Rss)) at steady state.

	Jss ($\mu g\,cm^{-2}\,h^{-1}$)	Lag time (h)	Vss (V)	Iss (mA)	Rss (Ω)	Ref.
Control	1·96 ± 0·23	2·66 ± 0·32				Figure 1
1 × JI (0 mm)	7·81 ± 0·84	1·55 ± 0·18				
1 × JI (10 mm)	26·30 ± 3·65	0·98 ± 0·18				
3 × JI (10 mm)	71·83 ± 5·35	0·96 ± 0·17				
0·5 V IP	46·22 ± 10·04[a]	2·74 ± 0·47[a]	0·5	0·433 ± 0·038*	1·15	Figure 2
0·5 V IP + 1 × JI (10 mm)	98·76 ± 16·93[a]	2·16 ± 0·41[a]	0·5	0·688 ± 0·087*	0·72	
0·1 mA IP	14·76 ± 5·43	1·08 ± 0·07	0·092 ± 0·084	0·1	0·92	Figure 3
0·1 mA IP + 1 × JI (10 mm)	33·03 ± 4·61	0·84 ± 0·08	0·051 ± 0·031	0·1	0·51	
0·3 mA IP	57·26 ± 4·40	1·24 ± 0·17	0·360 ± 0·088	0·3	1·20	
0·3 mA IP + 1 × JI (10 mm)	45·96 ± 5·36	0·50 ± 0·05	0·246 ± 0·042	0·3	0·82	

Each value represents the mean or the mean ± s.e.m. of 3–6 replicates. n × JI (a mm): n times jet injection (JI) set at 1 mm over the skin surface; IP: iontophoresis. Rss was calculated according to Ohm's law. [a]Average value calculated from the last three sampling points (Figure 2).

region compared with close contact of the injector tip with the skin surface, resulting in a larger pore size in the stratum corneum. The pore areas in the skin surface were about 0·1 and 0·3 mm^2 for distances of 0 and 10 mm between the injection tip and skin, respectively. This is the reason for the differences in skin permeation rate between the two groups in Figure 1. Further experiments were then carried out with the injector 10 mm above the skin.

Next, jet injector pretreatment was performed in triplicate to obtain further enhancement of skin permeation of diclofenac, and the results were compared with those obtained following a single pretreatment. Figure 1 and Table 1 also show the skin permeation data of the drug for triplicate pretreatment: flux was about 3-fold higher compared with a single pretreatment and 37-fold higher compared with the control. These results suggested that the primary skin permeation pathway of diclofenac is through pores pierced by the injector and that jet injector pretreatment with physiological saline is a useful method to promote skin permeation of drugs.

Combined effects of jet injector pretreatment and constant voltage iontophoresis on the skin permeation of diclofenac

Constant voltage iontophoresis was first evaluated to investigate the combined effects of jet injector pretreatment and iontophoresis. A voltage of 0·5 V was selected based on the preliminary observation that much lower permeation was observed following 0·2 V iontophoresis compared with pretreatment with the jet injector alone. Figure 2 shows the cumulative amounts of diclofenac sodium that passed through skin following 0·5 V iontophoresis with and without jet injector pretreatment (single injection). The cumulative amount over 4 h for the combined treatment was about 3-fold higher than with iontophoresis alone. No steady-state flux was observed in either group. This may have been due to the gradual decrease in skin barrier resistance. The average flux and lag time were calculated only from the last three points in Figure 2 and are shown in Table 1. Since the combined treatment shown in Figure 2 exhibited a higher degree of permeation than injector pretreatment alone (Figure 1), the combination of treatments had a synergistic effect on the skin permeation of diclofenac.

This synergistic effect suggested that the pores produced by the jet injector become a new permeation pathway for diclofenac through the skin and have a role decreasing the electrical resistance of the main skin barrier, the stratum corneum. The average electric current through the skin

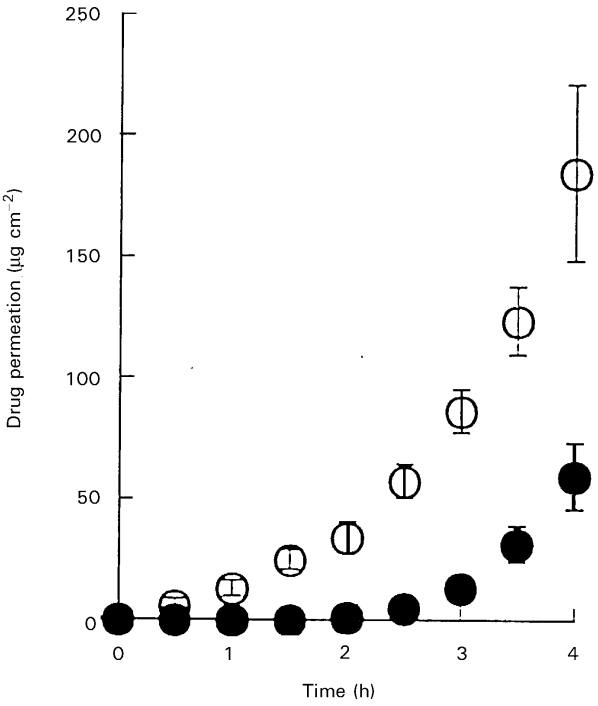

Figure 2. Combined effects of jet injector pretreatment and constant voltage iontophoresis on the permeation of diclofenac through hairless rat skin. Constant voltage (0·5 V) iontophoresis alone, ●; single jet injection 10 mm above the skin surface with constant voltage (0·5 V) iontophoresis, ○. Each data point represents the mean ± s.e.m. of 3–6 replicates.

(0·69 mA cm^{-2}) during constant voltage iontophoresis of the skin pretreated with the jet injector was greater than that without the jet injector (0·43 mA cm^{-2}), as shown in Table 1. The current is generally proportional to the sum of the product of valence and flux for each ion, including diclofenac ions, through the skin (Numajiri et al 1996). Thus, the electric current and diclofenac flux increased with the decrease in electrical resistance of the skin barrier (from 1·15 to 0·72 kΩ) during constant voltage iontophoresis in accordance with Ohm's law.

The electric current gradually increased with time during constant voltage iontophoresis. A good correlation was observed between the diclofenac flux and the electric current below the current density of 0·5 mA cm^{-2} with and without jet injector pretreatment (data not shown). The relationship became poor, however, at currents higher than 0·5 mA cm^{-2}. This phenomenon can be explained by the previous report (Ledger 1992) that a high electric current (> 0·5 mA cm^{-2}) decreases the integrity of the skin barrier and results in skin damage. Therefore, the permeation experiment run was stopped after 4 h for constant voltage iontophoresis.

Combined effects of jet injector pretreatment and constant current iontophoresis on the skin permeation of diclofenac

A gradual increase in drug flux was observed with the gradual increase in electric current during constant voltage iontophoresis, which made it difficult to control the input rate of the drug into skin and may have increased the possibility of skin damage. Therefore, we switched from constant voltage iontophoresis to constant current iontophoresis to obtain a more accurate dosage and reduce skin irritation, and evaluated the combined effects of jet injector pretreatment and iontophoresis.

Figures 3A and 3B show the cumulative amounts of diclofenac permeation through skin with a single jet injector pretreatment during 0·1 and 0·3 mA iontophoresis, respectively. Table 1 also shows the permeation and electrical data. All the permeation profiles for constant current iontophoresis showed a steady-state flux after a short lag time, unlike constant voltage iontophoresis. As shown in Figure 3A (0·1 mA iontophoresis), the iontophoretic flux with jet injection pretreatment was greater than that without pretreatment. This combined use, however, showed almost the same flux as jet injection treatment alone (Figure 1, Table 1). Thus, no synergistic effect was observed.

With 0·3 mA iontophoresis (Figure 3B, Table 1), however, the steady-state flux with the pretreatment was not higher than that without pretreatment, although the lag time was lessened by pretreatment. Also, no synergistic enhancing effect was observed for jet injector pretreatment in combination with the 0·3 mA iontophoresis.

Combined use of the jet injector and constant current iontophoresis (both for 0·1 and 0·3 mA) exhibited lower differences in electrical potential across skin than for constant current iontophoresis alone (Table 1). Jet injector pretreatment was useful to reduce the required electrical power, similar to iontophoretic delivery with a chemical enhancer (Numajiri et al 1998b). The electric current gradually increased with time during constant voltage iontophoresis, as noted above. Thus, the possibility of skin damage by constant current iontophoresis may be lower compared with constant voltage iontophoresis, especially over a long period of treatment.

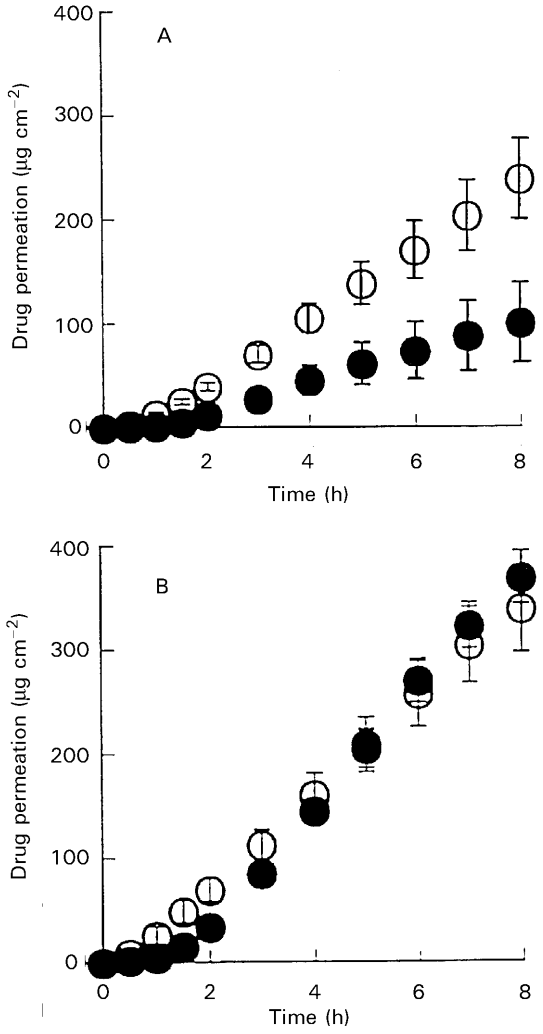

Figure 3. Combined effects of jet injector pretreatment and constant current iontophoresis on the permeation of diclofenac sodium through hairless rat skin. Constant current (0·1 (A) and 0·3 mA (B)) iontophoresis alone, ●; single jet injection 10 mm above the skin surface with constant current (0·1 (A) and 0·3 mA (B)) iontophoresis, ○. Each data point represents the mean ± s.e.m. of 3–6 replicates.

Combined effects of jet injector pretreatment and iontophoresis on the skin permeation of angiotensin II

The combined effects of jet injector and iontophoresis on the skin permeation of angiotensin II were examined. This compound has a molecular weight about 3-fold higher and is more hydrophilic than diclofenac, and therefore it is assumed to be less permeable through skin. Clemessy et al (1995) reported that no degradation products were found after applying 0·5 mA to a solution of angiotensin II on skin over 10 h, as determined by HPLC. In addition, no migration spot of radioactivity was observed on TLC after applying a current to ^{3}H-angiotensin II. Thus, its chemical and radiochemical stability was confirmed.

Figure 4 shows the cumulative amount of angiotensin II that permeated through skin for the control and the jet injector pretreatment groups (single injection). Steady-state permeation was found after a short lag time in both groups. Table 2

shows the steady-state flux and lag time. The flux was about 22-fold higher than that in the control group. Jet injector pretreatment probably enlarges the permeation pores for hydrophilic compounds and thus increases the drug delivery rate through the skin. Jet injector pretreatment is therefore useful for enhancing the skin permeation of molecules with relatively high molecular weights such as angiotensin II.

Next, the combined effects of jet injector and constant voltage iontophoresis were examined. Figure 5A shows the cumulative amounts of angiotensin II permeating through the skin during 0·2 V iontophoresis alone and 0·2 V iontophoresis with jet injection pretreatment. The combined treatment showed a 5·5-fold higher flux than iontophoresis alone, and was also about twice that observed with jet injector treatment alone (Table 2). Thus, a synergistic enhancing effect was also observed for angiotensin II delivery. Interestingly, the permeation profiles for both cases showed an almost straight line after an initial lag time, unlike diclofenac permeation. Skin resistance against diclofenac permeation during constant voltage iontophoresis decreased with time, whereas no decrease in resistance to the permeation of angiotensin II was observed. The gradual increase in the electric current or the gradual decrease in resistance during constant voltage iontophoresis may be related to gradual increases in skin permeation, especially of small drugs and endogenous ions. Further skin permeation studies using several penetrants are necessary to fully understand these phenomena. Combined use of a jet injector and constant current iontophoresis was also examined. Figure 5B shows the cumulative amounts of angiotensin II permeating through the skin for 0·3 mA iontophoresis with and without jet injector pretreatment. A linear relationship was observed after a short lag time, similar to the iontophoretic permeation of diclofenac. No significant difference was observed between the steady-state flux during constant current iontophoresis with and without jet injector pretreatment (Table 2). Jet injector pretreatment only decreased the lag time.

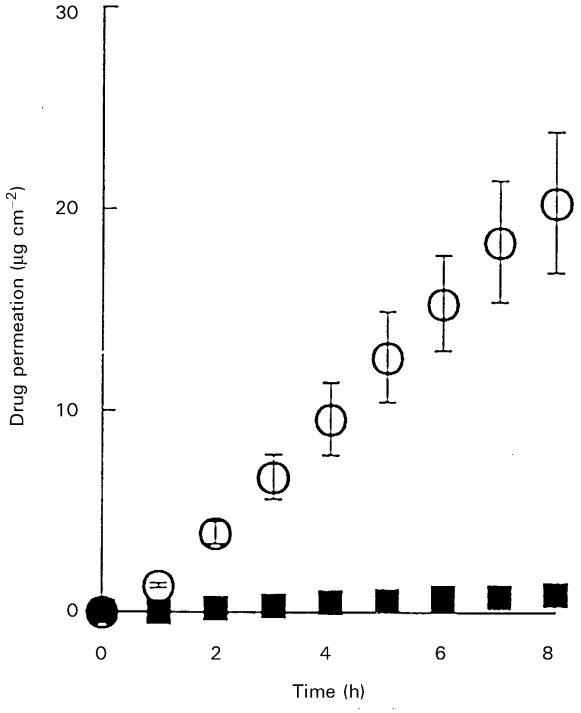

Figure 4. Effects of jet injector pretreatment on the permeation of angiotensin II through hairless rat skin. Control, ■; single jet injection 10 mm above the skin surface, ○. Each data point represents the mean ± s.e.m. of 3–6 replicates.

Conclusion

Both jet injection pretreatment of the skin and treatment by constant voltage iontophoresis increased the skin delivery rates of diclofenac and angiotensin II compared with controls (without jet injection; without iontophoresis). The combined use showed a higher flux than either jet injector pretreatment or constant voltage iontophoresis

Table 2. Effects of different types of jet injector and iontophoresis treatment on the permeation parameters of angiotensin II (steady-state flux (Jss) and lag time) through hairless rat skin and electrical parameters (potential difference (Vss), electric current (Iss) and membrane resistance (Rss)) at steady state.

	Jss (μg cm^{-2} h^{-1})	Lag time (h)	Vss (V)	Iss (mA)	Rss (Ω)	Ref.
Control	0·177 ± 0·013	0·37 ± 0·20				Figure 4
1 × JI (10 mm)	2·628 ± 0·445	0·23 ± 0·21				
0·2 V IP	0·878 ± 0·093	1·52 ± 0·15	0·2	0·068 ± 0·028	2·94	Figure 5
0·2 V IP + 1 × JI (10 mm)	4·868 ± 1·178	−0·09 ± 0·34	0·2	0·428 ± 0·103	0·47	
0·3 mA IP	4·007 ± 0·751	1·16 ± 0·14	0·647 ± 0·028	0·3	2·16	
0·3 mA IP + 1 × JI (10 mm)	4·979 ± 1·503	0·22 ± 0·17	0·185 ± 0·041	0·3	0·62	

Each value represents the mean or the mean ± s.e.m. of 3–6 replicates. n × JI (a mm): n times jet injection (JI) set at 1 mm over the skin surface; IP: iontophoresis. Rss was calculated according to Ohm's law.

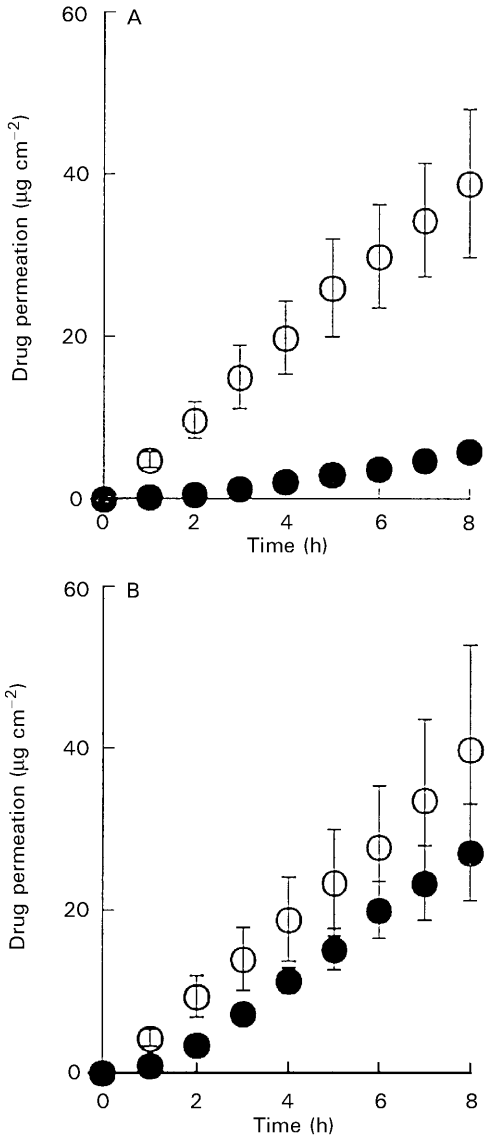

Figure 5. Combined effects of jet injector pretreatment and constant voltage and constant current iontophoresis on the permeation of angiotensin II through hairless rat skin. Constant voltage (0·2 V) (A) or constant current (0·3mA) (B) iontophoresis alone, ●; single jet injection 10 mm above the skin surface with constant voltage (0·2 V) (A) or constant current (0·3 mA) (B) iontophoresis, ○. Each data point represents the mean ± s.e.m. of 3–6 replicates.

alone. The synergistic effect observed here was probably due to lower skin resistance after jet injector pretreatment. The current increased, however, with time during constant voltage iontophoresis, which made it difficult to control drug delivery rate through the skin and may be related to skin damage. On the other hand, skin permeation by combined treatment with the injector and constant current iontophoresis was not always higher than with iontophoresis or jet injection alone. Constant current iontophoresis, after a short period of constant voltage iontophoresis with multiple jet injector pretreatments, may be the best way to increase drug permeability while preventing severe skin damage.

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