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The effects of iontophoresis and electroporation on transdermal delivery of buprenorphine from solutions and hydrogels

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Abstract

The in-vitro permeation of buprenorphine across skin was investigated to assess the effects of iontophoresis and electroporation on drug permeation from solutions as well as from hydrogels. Iontophoresis (0.3 mA cm^{-2}) increased the buprenorphine permeation from solution by a factor of 14.27 as compared with passive diffusion; the application of electroporation increased the buprenorphine permeation from solutions by a factor of 8.45. The permeation experiments using cellulose membrane and stratum corneum (SC)-stripped skin as permeation barriers suggested that the enhancement with iontophoresis was primarily due to strong electrophoretic drift of buprenorphine molecules, whereas the enhancement seen with electroporation was mainly attributed to the creation of transient aqueous pores in the SC layer. Application of high-voltage pulses followed by iontophoresis resulted in a shorter permeation onset time from both solutions and hydrogels as compared with iontophoresis or electroporation alone. The charge repulsion between buprenorphine and chitosan vehicles as well as the competition effects of counter-ions for carboxymethylcellulose (CMC)-based polymers may account for the different permeation rates under electrical field. This study demonstrates the feasibility of using hydrogels for delivery of buprenorphine under the application of iontophoresis or electroporation, separately or together.

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Introduction

Buprenorphine is a morphine-like drug with partial agonist activity at the μ-opiate receptor and antagonist activity at the κ-opiate receptor. It is approximately 25–30 times more potent than morphine and has been widely used in the treatment of acute and chronic pain (Ho et al 1994). Its half-life after parenteral administration is estimated to be 3–5 h and the recommended dosing frequency is 3 or 4 times daily (Wilding et al 1996). Orally administered buprenorphine is reported to undergo extensive first-pass metabolism, with only 10–15% bioavailability (Roy et al 1994). Thus, to circumvent those unfavoured biopharmaceutical characteristics and to improve therapeutic effectiveness in pain management, the development of controlled-release or alternative delivery systems for buprenorphine is desirable. The sublingual tablet and the transdermal system of buprenorphine have been developed and used frequently in various countries.

Several approaches have been utilized to drive drug molecules through the skin barrier, including passive diffusion, iontophoresis, sonophoresis and electroporation. Of these, electrically modulated delivery by iontophoresis or electroporation provides the advantage of programming the dose in proportion to the desired therapeutic effect. Iontophoresis utilizes a low voltage (typically 10 V or less) and constant current (typically 0.5 mA cm^{-2} or less) to push a charged molecule into skin or other tissues. Electroporation involves the application of high voltage (typically >100 V) and short-duration (μs–ms) pulses to increase the permeability of skin (Banga et al 1999). Previous results have demonstrated that, with the application of electrically modulated transdermal systems, the steady-state opioid concentration may be rapidly achieved and

the delivery rates can be adjusted by varying several electrical factors (Grond et al 2000). Accordingly, the transdermal delivery system modulated by iontophoresis or electroporation (or both) may also be utilized to deliver buprenorphine to maintain its blood concentration and therapeutic effectiveness.

Though the delivery of buprenorphine via electrically modulated methods is very promising, the choice of appropriate vehicles may affect the performance (such as permeation kinetics) as well as the drug stability. Most previous permeation studies utilized aqueous solutions as vehicle. However, when administered clinically, the patch or hydrogel dosage form may be more applicable than solution. For successful transdermal delivery of buprenorphine utilizing electrically modulated methods, the compositions of vehicles and their effects on permeation kinetics have to be characterized in a systematic way (Fang et al 1999).

In this study, three major goals are to be achieved: firstly, to evaluate the skin-permeation kinetics of buprenorphine under the application of iontophoresis or electroporation (or both); secondly, to explore the transdermal permeation mechanisms of buprenorphine under the application of iontophoresis, as well as electroporation; and finally, to evaluate the use of hydrogels as vehicles for electrically modulated systems, the effects of various hydrogel polymers on transdermal permeation kinetics of buprenorphine under iontophoresis or electroporation (or both) were also examined.

Materials and Methods

Materials

Buprenorphine hydrochloride was purchased from Macfarlan Smith Co. (UK). Carboxymethyl cellulose sodium (CMC-Na), carboxymethyl cellulose ammonium (CMC-NH_4) and chitosan (Chitosan 10) were purchased from Wako Chemical Co. (Japan). All other chemicals and solvents were of analytical grade (E. Merck, Germany) and used as received.

In-vitro permeation experiments

The in-vitro permeation studies were performed using horizontal glass diffusion cells. The dorsal skin of excised female nude mouse (Balb-c/nu strain, 6 weeks old) was used as the model skin membrane. The designed protocol for using mouse in this study was according to the international recognized ethical guidelines and was approved by the Institutional Animal Study Committee of Chang Gung University. The skin stripped of stratum corneum (SC-stripped skin) was obtained by applying the adhesive tape (Four Pillars Co., Taiwan) to nude mouse skin with uniform pressure and then removed; this procedure was repeated 20 times. The receptor phase contained 8 mL of 0.06 M citrate-phosphate buffer (pH 6). For buprenorphine permeation from drug solutions, the donor compartment was filled with 8 mL of 0.06 M citrate–phosphate buffer (pH 5) containing 0.5% (w/v) of buprenorphine. For buprenorphine permeation studies from hydrogel vehicles, 8 g of hydrogel containing 0.5% (w/w) of buprenorphine was used as the donor vehicle. The available skin diffusion area was 0.785 cm^2. The cells were agitated by magnetic stirrers at 600 rev min^{-1}. The samples (300 μL) were withdrawn from the receptor compartment at regular intervals and immediately replaced by an equal volume of fresh buffer solution. The sample concentration was then analysed by HPLC.

Iontophoresis and electroporation protocols

For the in-vitro permeation experiments under iontophoresis, a pair of Ag/AgCl wires (0.5 mm diameter) with an effective length of 15 mm were immersed in the buffer as electrodes, with the anode in the donor site and the cathode in the receptor site. The electrodes were each positioned 3 cm from the side of the skin. The electrodes were connected to a current power supplier (Yokogawa Co., Model 7651, Japan). The current density was set at 0.3 or 0.5 mA cm^{-2}. The iontophoresis was applied continuously and the application time was set at 8 h.

Electroporation was performed using an exponential decay pulse generator (BTX Co., ECM 630 Electro Cell Manipulator, USA). A pair of platinum electrodes (1×2 cm^2) were used and each located 3 cm from the skin membrane. The anode was positioned in the donor compartment and the cathode was in the receptor compartment. Unless otherwise noted, the electroporation protocol was 1 pulse per 30 s, applied for 10 min; the pulse voltage was 500 V and pulse duration was 200 ms. The selection of this relatively high voltage and long duration of pulses was referred to in several previous publications (Prausnitz 1996; Vanbever et al 1999; Bose et al 2001) and was intended to allow observation of the more significant permeation-enhancing effect via electroporation. The voltages were expressed as applied values but not transdermal values. In the study combining electroporation and iontophoresis, the iontophoresis was started after applying 10 min of skin electroporation and applied continuously until the end of the 8 h experiment.

HPLC analysis

Buprenorphine concentration was analysed by an HPLC system consisting of a Hitachi L-7110 pump, a Hitachi L-7400 UV detector and a Hitachi L-7200 sample processor. A C18 column (Kanto Mightysil, 250×4.6 mm) with a guard column was used. The mobile phase consisted of 55% acetonitrile and 45% 0.06 M citrate–phosphate buffer (pH 5). The flow rate was set at 1.0 mL min^{-1} and the UV wavelength was set at 210 nm.

Preparation of hydrogels

For the preparation of CMC-based hydrogels, the 5% (w/w) of aqueous polymer was added into half of the pH 5 citrate–phosphate buffer and the mixture was continuously

stirred for 1 h. The weight ratio of 5% aqueous polymer to citrate–phosphate buffer was 52.75/47.25. After 24 h, the other half of the pH 5 citrate–phosphate buffer and buprenorphine were added into the mixture with continuous stirring for 1 h. The final buprenorphine concentration in hydrogel was 0.5% (w/w). To prepare chitosan hydrogels, suitable amounts of chitosan and lactic acid were added in double-distilled water to obtain a mixture containing 5% (w/w) of chitosan and 5% (w/w) lactic acid. The mixture was stirred for 1 h. Buprenorphine was then added into the mixture to give a drug-containing hydrogel with a final buprenorphine concentration of 0.5% (w/w).

Viscosity measurement

Viscosity measurements were carried out on hydrogels before and after performing the in-vitro permeation experiments. The viscosity was determined in a cone and plate viscometer (Brookfield Co., Model DV-2). Hydrogel (0.5 g) was placed in the sample cup of the viscometer and allowed to stand for 30 min to reach equilibrium temperature (37°C). For each measurement, the readings were recorded for 20 s; the stablized values were then averaged and converted to the hydrogel viscosity.

Data analysis

In the permeation study, the total amount of buprenorphine permeated across the unit diffusion surface and into the receptor was calculated and plotted as a function of time. The permeation data were analysed using the following equation:

$$J_{ss} = dQ/(dt \times A) \quad (1)$$

Where J_{ss} is the flux at apparent steady state, Q is the cumulative mass of drug transferred to the receptor compartment in time t and A is the membrane surface area. The enhancement ratio (ER) was defined as the flux under iontophoresis or electroporation (or both) divided by the flux under passive permeation. The lag time was determined by extrapolating the cumulative mass per unit area versus time profile at apparent steady state to the x-axis.

Statistical analysis

The Students' *t*-test and one-way analysis of variance were utilized as appropriate to test the various treatment effects; the Student's *t*-test was to compare the mean of two treatment effects whereas the one-way analysis of variance was used to compare the mean of two or more treatment effects. The level of significance in all tests was set at 0.05. A subsequent multiple comparison (LSD test) was used to quantify the individual differences between means following analysis by analysis of variance.

Results and Discussion

The effects of iontophoresis or electroporation on permeation of buprenorphine from solutions

Figure 1 shows the cumulative amount of buprenorphine ($\mu g/cm^2$) in the receptor compartment as a function of time after applying iontophoresis. Steady-state flux and lag time calculated from linear portions (apparent steady state) of the curves are summarized in Table 1. The passive permeation rate of buprenorphine was extremely low, although the flux was increased significantly (*t*-test, $P < 0.05$) by application of iontophoresis. Buprenorphine is a weak basic drug with a pK_a of 8.24 (Roy et al 1994); the drug was almost completely ionized in the donor solution (pH 5). Therefore, the observed enhancement of skin permeation of cationic buprenorphine can be attributed to the electrical potential gradient resulting from application of iontophoresis (Sung et al 2000). These results are similar to those of previous studies on transdermal iontophoretic delivery of fentanyl (Vanbever et al 1996a). Table 1 shows the increase in current density had no effect on drug flux (*t*-test, $P > 0.05$), which may be explained by the charge saturation of the aqueous conducting pathways in the skin (Singh et al 1999). Despite the current density having no influence on permeation flux, a shortened lag time was observed (*t*-test, $P < 0.05$) after applying higher current density (0.5 mA cm^{-2}) (Table 1). Moreover, the coefficient of variation of flux values from iontophoretic permeation through intact skin were lower than the values from passive diffusion through intact skin (Table 1), suggesting a higher and more stable transdermal buprenorphine delivery rate can be obtained by applying iontophoresis.

Transdermal buprenorphine delivery was also investigated by applying electroporation. The application of 300-V or 500-V pulses (200 ms) for 10 min increased buprenorphine flux to 5.5 and 8.45 times that of passive diffusion, respectively (Table 1 and Figure 1). The application of higher voltage pulses (500 V) resulted in higher flux (*t*-test, $P < 0.05$). After 8 h, the amount of buprenorphine delivered by applying electroporation was significantly lower than the amount delivered by applying iontophoresis (*t*-test, $P < 0.05$) (Figure 1). The increase in molecular transport by electroporation can be attributed to the creation of electropores as well as electrophoresis/iontophoresis (electrophoretic drift) by the local field (Prausnitz 1996; Riviere & Heit 1997). Despite the pulsing time only being 10 min, the cumulative amount of buprenorphine in the receptor compartment increased constantly until the end of the experiment (Figure 1). Some previous studies have also shown that the affinity of lipophilic moieties to SC may lead to the formation of a large drug reservoir after electroporation (Vanbever et al 1996b; Jadoul et al 1998; Regnier et al 1999; Bose et al 2001). Accordingly, the constantly increased buprenorphine permeation after the 10 min of application suggests the creation of a drug reservoir within the skin and the buprenorphine may permeate from skin reservoir to receptor site after 10 min of application.

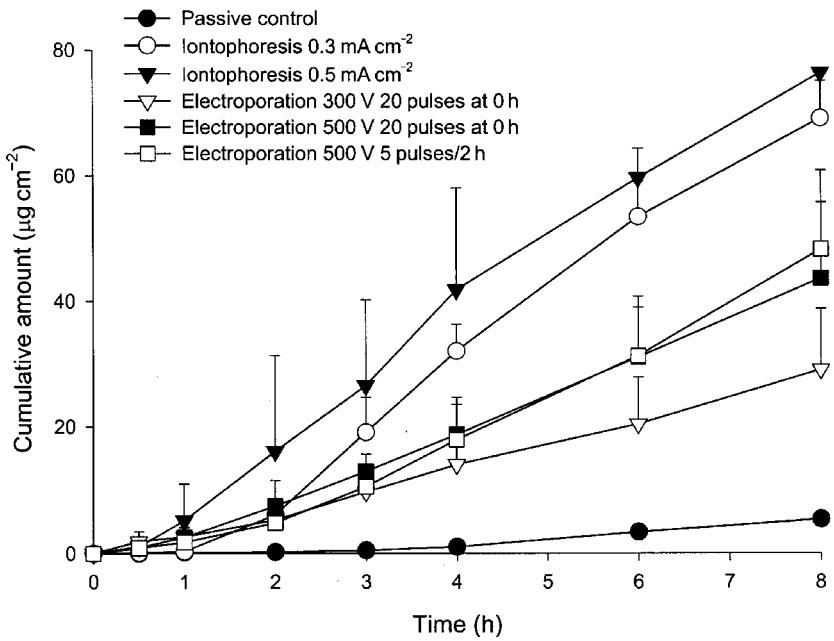

Figure 1 Cumulative amount versus time profiles for buprenorphine permeated across nude mouse skin from pH 5 buffer by applying iontophoresis or electroporation. All data represent the means of four experiments±s.d.

Table 1 Flux, lag time and enhancement ratio (ER) for permeation of buprenorphine from pH 5 buffer across various types of barrier by iontophoresis or electroporation, or a combination of the two.

Mode	Intact skin			Cellulose membrane			SC-stripped skin		
	Flux (μg cm^{-2} h^{-1})	ER	Lag time (h)	Flux (μg cm^{-2} h^{-1})	ER	Lag time (h)	Flux (μg cm^{-2} h^{-1})	ER	Lag time (h)
Passive control	0.66±0.11	–	1.08±0.17	236.12±37.48	–	−0.47±0.06	21.06±3.96	–	0.71±0.15
ITP 0.3 mA cm^{-2}	9.42±0.93	14.27	0.67±0.10	317.56±54.42	1.34	−0.44±0.09	35.00±10.66	1.66	−0.85±0.31
ITP 0.5 mA cm^{-2}	10.14±0.05	15.36	0.27±0.02	–	–	–	–	–	–
EP 300 V, 20 pulses at 0 h	3.63±1.25	5.50	0.20±0.07	–	–	–	–	–	–
EP 500 V, 20 pulses at 0 h	5.58±1.55	8.45	0.44±0.19	199.58±33.98	0.85	−1.31±0.21	54.74±11.36	2.60	−0.29±0.07
EP 500 V, 5 pulses/2 h	6.08±1.62	9.21	0.69±0.16	–	–	–	–	–	–
ITP 0.3 mA cm^{-2} +EP 500 V, 20 pulses at 0 h	8.35±2.06	12.65	−0.09±0.03	327.40±23.89	1.39	−0.50±0.12	60.62±3.49	2.88	−1.74±0.23
ITP 0.3 mA cm^{-2} +EP 500 V, 5 pulses/2 h	8.11±0.53	12.29	0.61±0.08	–	–	–	–	–	–

ER, enhancement ratio (ratio of the flux by iontophoresis or electroporation (or both) to the flux by passive diffusion); ITP, iontophoresis; EP, electroporation. Each value represents the mean±s.d. (n = 4).

Figure 1 also compares the permeation rates of various pulsing protocols. The pulse voltage and pulse duration was set at 500 V and 200 ms, respectively. The application of 10 min of pulsing at the beginning of the experiment resulted in essentially the same permeation rate and lag time as the application of 2.5 min of pulsing every 2 h (t-test, $P > 0.05$) (Table 1).

The effects of combining iontophoresis and electroporation on the permeation of buprenorphine from solutions

Previous studies, as well as the above results, demonstrate that iontophoresis and electroporation can enhance transdermal permeation via different mechanisms. Therefore, application of both iontophoresis and electroporation may have a synergistic effect on the permeation rate. Figure 2 shows the permeation resulting from application of twenty 500-V pulses initially followed by the application of iontophoresis (0.3 mA cm^{-2}) until the end of the in-vitro experiment. The combination of two driving forces did not enhance the flux as compared with iontophoresis alone (t-test, $P > 0.05$) (Table 1). Nevertheless, the permeation lag time was significantly reduced (Table 1), suggesting a more rapid onset was achieved by combining iontophoresis and electroporation. The rapid onset may be attributed to the creation of a permeabilized skin by the exposure of high-voltage pulses before applying iontophoresis (Jadoul &

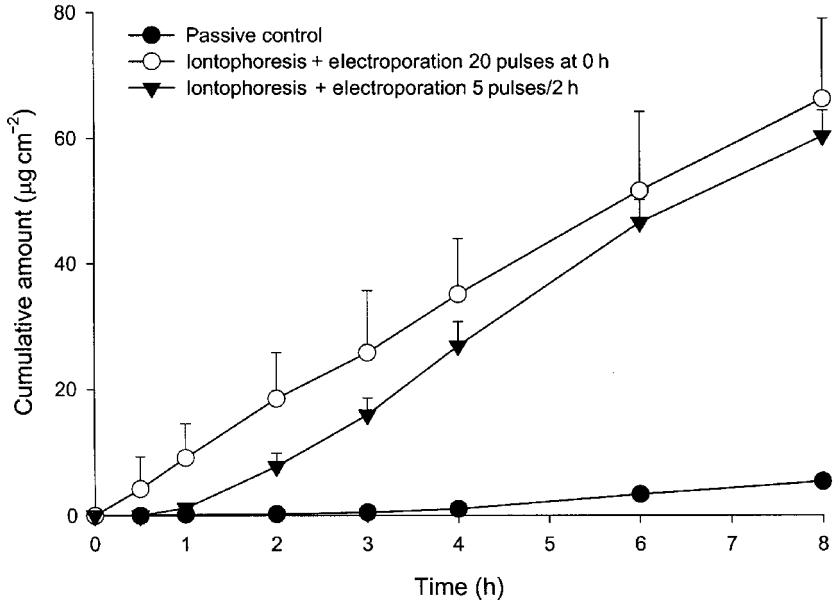

Figure 2 Cumulative amount versus time profiles for buprenorphine permeated across nude mouse skin from pH 5 buffer by applying iontophoresis (0.3 mA cm^{-2}) combined with electroporation. All data represent the means of four experiments±s.d.

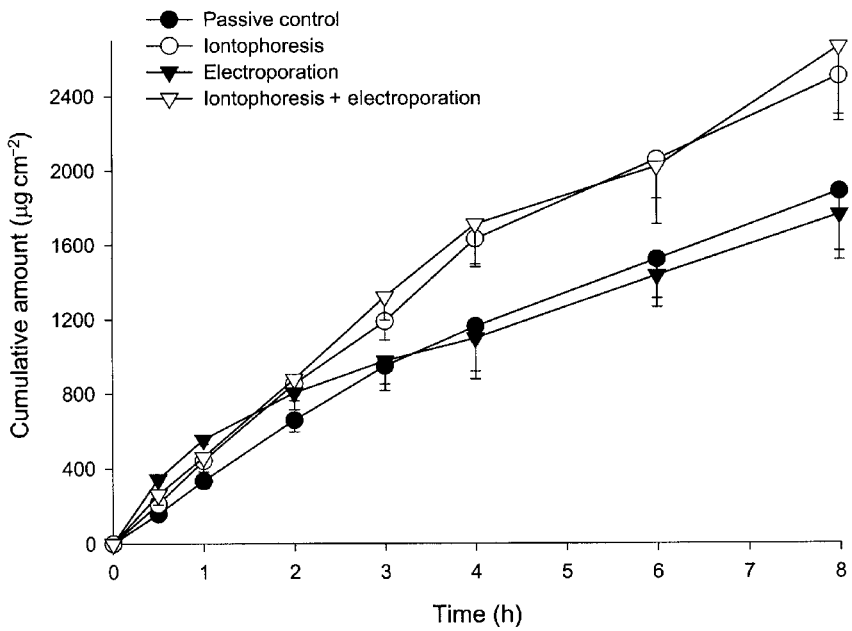

Figure 3 Cumulative amount versus time profiles for buprenorphine permeated across cellulose membrane from pH 5 buffer by applying iontophoresis (0.3 mA cm^{-2}) or electroporation (20 pulses of 500 V at 0 h), or a combination of the two. All data represent the means of four experiments±s.d.

Préat 1997). The application of 0.3 mA cm^{-2} current density of iontophoresis may extend the permeabilized state (Mitragotri 2000), resulting in the rapid onset and similar flux to the iontophoretic permeation. Figure 2 also demonstrates that the lag time was not significantly shortened by application of 2.5 min electroporation every 2 h combined with iontophoresis. The results indicate that the application of 2.5 min of high-voltage pulses at the beginning was not sufficient to produce a highly permeabilized skin for buprenorphine permeation.

Various types of skin were used as permeation barriers to obtain mechanistic information on the transdermal delivery of buprenorphine. Figure 3 shows the cumulative amount versus time profile for buprenorphine permeated

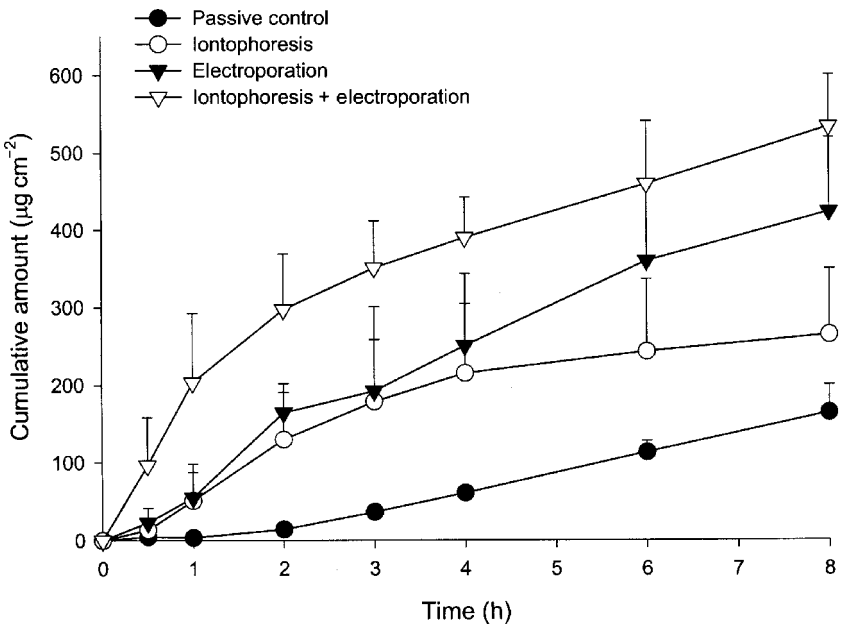

Figure 4 Cumulative amount versus time profiles for buprenorphine permeated across SC-stripped skin from pH 5 buffer by applying iontophoresis (0.3 mA cm^{-2}) or electroporation (20 pulses of 500 V at 0 h), or a combination of the two. All data represent the means of four experiments±s.d.

through cellulose membrane (weight cut-off value 12000–14000; Spectra/Por, Spectrum Co., USA) via various driving forces. The permeation of buprenorphine through cellulose membrane via diffusion was significantly faster than through intact skin, demonstrating that the skin indeed provides barrier characteristics in the permeation studies (Table 1). The permeation rate of buprenorphine through cellulose membrane achieved by iontophoresis was higher (t-test, $P < 0.05$) than that by passive diffusion, indicating that the contribution of electrophoretic drift enhanced by iontophoresis was significant for buprenorphine. Nevertheless, the permeation rate of buprenorphine across cellulose membranes achieved by applying electroporation was similar to that by passive diffusion (Figure 3). The results suggest that the permeation-enhancing effects of electroporation were mainly attributed to the formation of micropores structure in the skin; the direct electromotive force on the drug was not as important. Figure 3 also shows that the cumulative amount versus time profile of combined iontophoresis/electroporation approximated to that of iontophoresis. The results again confirm that the effect of electromotive force on permeation of buprenorphine after applying electroporation was negligible.

Figure 4 shows the cumulative amount versus time profile for buprenorphine across SC-stripped skin. The buprenorphine flux by passive diffusion increased 31.91 times after removing the SC layer (Table 1), suggesting that the SC layer was the principal diffusion barrier for the permeation process. Both iontophoresis and electroporation enhanced the buprenorphine permeation and their corresponding lag times were also shorter (t-test, $P < 0.05$) than with passive diffusion. The permeation-enhancing effect of iontophoresis and electroporation was less significant in SC-stripped skin than in intact skin. These data again indicate that the rate-limiting characteristics of the SC layer in passive diffusion of buprenorphine could be partially overcome by application of electric field.

Contrary to results obtained from the permeation studies through intact skin, the ER obtained by applying iontophoresis across SC-stripped skin was lower than that by electroporation. This observation indicates that the enhancement of buprenorphine permeation through SC-stripped skin was more pronounced by applying electroporation. The buprenorphine flux across SC-stripped skin by combining iontophoresis and electroporation was similar to the flux obtained by applying electroporation alone, although the lag time obtained from the combined method was significantly shorter (t-test, $P < 0.05$) (Table 1). The observations were similar to those obtained with buprenorphine permeation across intact skin.

The effects of iontophoresis or electroporation (or both) on permeation of buprenorphine from hydrogels

The polymers CMCNa and $CMCNH_4$ and chitosan were utilized in this study as hydrogel vehicles for buprenorphine transdermal delivery. The permeation data of buprenorphine from various hydrogels are shown in Table 2. There was no statistically significant difference among the flux and lag times of buprenorphine permeation from the three hydrogel formulations via passive diffusion (analysis of variance test, $P > 0.05$). Moreover, the permeation flux, as well as lag time, for buprenorphine permeation from pH 5 buffer solution and hydrogels (Tables 1 and 2) were also

Table 2 Flux, lag time and enhancement ratio (ER) for permeation of buprenorphine from hydrogels across skin barrier by iontophoresis and/or electroporation.

Hydrogel	Mode	Flux (μg cm^{-2} h^{-1})	ER	Lag time (h)
CMCNa	Passive control	0.71±0.36	–	1.06±0.57
	ITP 0.3 mA cm^{-2}	1.75±0.07	2.46	0.80±0.06
$CMCNH_4$	Passive control	0.87±0.43	–	0.94±0.43
	ITP 0.3 mA cm^{-2}	3.74±1.46	4.30	0.62±0.27
Chitosan	Passive control	0.90±0.30	–	1.08±0.36
	ITP 0.3 mA cm^{-2}	11.17±2.51	12.41	0.37±0.11
	EP 500 V, 20 pulses at 0 h	1.47±0.17	1.63	0.58±0.09
	ITP 0.3 mA cm^{-2} +EP 500 V, 20 pulses at 0 h	10.89±2.76	12.10	−0.43±0.17

ER, enhancement ratio (ratio of the flux by iontophoresis or electroporation to the flux by passive diffusion); ITP iontophoresis; EP, electroporation. Each value represents the mean±s.d. (n = 4).

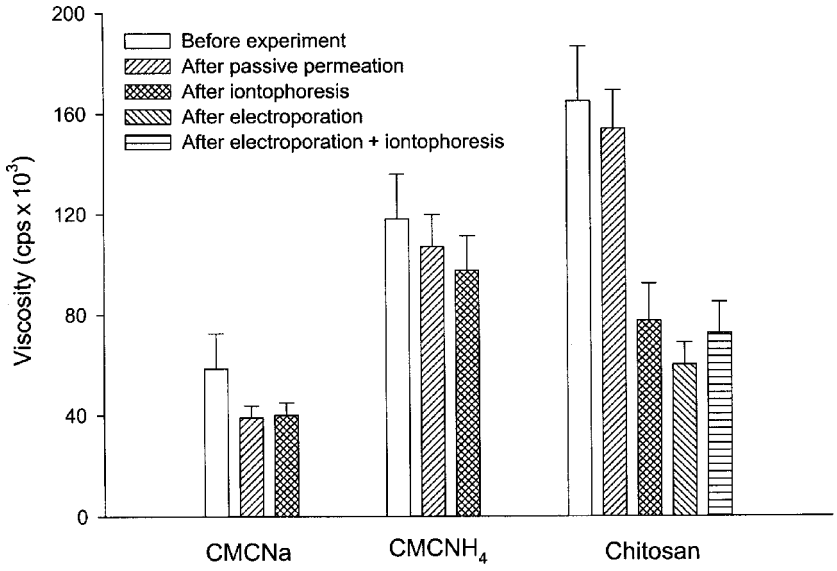

Figure 5 Viscosity of hydrogels before and after the in-vitro permeation experiments. All data represent the means of four experiments±s.d.

similar, suggesting that the diffusion of buprenorphine was not hindered by the hydrated polymer structure.

Figure 5 demonstrates that the hydrogel viscosity before in-vitro experiments was in the order of chitosan > $CMCNH_4$ > CMCNa. The viscosity of drug-loaded hydrogels was slightly reduced after performing 8 h of passive diffusion experiments, although no statistically significant difference was observed before and after permeation experiments (t-test, $P > 0.05$). Since hydrogel viscosity showed no impact on the permeation rates, it can be inferred that the whole permeation process of buprenorphine via passive diffusion was consistent with the skin-controlled mechanism but not vehicle-controlled mechanism (Ho et al 1994).

Table 2 summarizes the results of in-vitro buprenorphine permeation by iontophoresis from hydrogels. These results demonstrate that hydrogel composition had a significant effect on the permeation rate of buprenorphine via iontophoresis. The flux values of buprenorphine from various vehicles were significantly different (one-way analysis of variance) and increased in the order of chitosan > $CMCNH_4$ > CMCNa. The flux of buprenorphine from chitosan hydrogel was also higher than that from pH 5 buffer solution (t-test, $P < 0.05$) (Tables 1 and 2). Except for the polymer backbone, the major difference between chitosan hydrogel and the other two hydrogel vehicles was that its aqueous phase contained 5% lactic acid. Therefore, the medium was acidic in nature and the chitosan backbone possessed positive charges. Since the ionic mobility is opposite to its size (molal volume or molecular weight) under application of iontophoresis (Yoshida & Roberts 1993), the high molecular weight of the chitosan polymer could not act as a strong competitive ion for buprenorphine permeation. On the contrary, the repulsion force between

the positively charged buprenorphine and chitosan polymer may increase the drug permeation, as observed in Table 1 and Table 2.

The application of iontophoresis may also reduce the viscosity of chitosan hydrogels; but a similar phenomenon was not observed in CMC-based hydrogels (Figure 5). Since there was repulsion force between the anode in the donor and the positively charged chitosan, the application of electric field may collapse the chitosan network and subsequently enhance drug permeation (Hsu & Block 1996; Ramanathan & Block 2001). Accordingly, the charge repulsion between buprenorphine and chitosan, as well as viscosity reduction of chitosan vehicles, contributes to the higher buprenorphine iontophoretic permeation rates.

The iontophoretic permeation of buprenorphine from CMC-based hydrogels containing different counter-ions was also compared. Under the application of current, the counter-ions of the CMC-based polymer may compete with buprenorphine for permeation. Since sodium ions (Na^+) are less bulky than ammonium ions (NH_4^+) (Maitani et al 1994) and cationic buprenorphine, sodium ions may possess higher mobility. As a result, the enhancement of buprenorphine permeation by iontophoresis was significantly lower from CMCNa vehicle than from $CMCNH_4$ vehicle (Table 2).

Since the enhancement mechanism of electroporation on permeation was not mainly based on charge repulsion, a less pronounced enhancement effect would be expected by applying electroporation. Indeed, despite the viscosity of the chitosan hydrogel being significantly reduced (t-test, $P < 0.05$) after electroporation (Figure 5), a slight increase (1.63 fold) in buprenorphine flux was observed (Table 2). Similar to the results obtained previously using solution as vehicles, the combined iontophoresis and electroporation did not further increase buprenorphine flux from hydrogels by iontophoresis, although the lag time for drug permeation was significantly reduced (t-test, $P < 0.05$) after combining the two electrically modulated methods (Tables 1 and 2). The results again demonstrate that the application of electroporation has limited influence on the permeation of buprenorphine from hydrogels.

Conclusions

The permeation characteristics of buprenorphine by iontophoresis, electroporation, or a combination of the two, from solution as well as various hydrogels were examined in this study. Both iontophoresis and electroporation enhanced the skin permeation of buprenorphine from solution. The enhancement achieved with electroporation was less pronounced than that with iontophoresis. The combination of electroporation and iontophoresis did not further increase the buprenorphine flux from solution, although the lag time was significantly shortened. The in-vitro experiments using cellulose membrane and SC-stripped skin as permeation barriers suggested that enhancement by iontophoresis was primarily due to the strong electrophoretic drift of buprenorphine, whereas electroporation mainly affected SC layers to produce transient aqueous pores for buprenorphine permeation. The similar permeation rates observed among the passive diffusion of buprenorphine from various hydrogel matrices indicated that the hydrogel composition did not influence the passive permeation of buprenorphine. However, the iontophoretic permeation of buprenorphine from various hydrogels increased in the order of chitosan > $CMCNH_4$ > CMCNa. The repulsion between positively charged buprenorphine and chitosan vehicles as well as counter-ions of CMC-based polymers may account for the different permeation rates from various vehicles in electric field. This study showed the feasibility of applying hydrogel for delivery of buprenorphine under iontophoresis or electroporation, or a combination of the two.

References

Banga, A. K., Bose, S., Ghosh, T. K. (1999) Iontophoresis and electroporation: comparisons and contrasts. *Int. J. Pharmaceutics* **179**: 1–19

Bose, S., Ravis, W. R., Lin, Y. J., Zhang, L., Hofmann, G. A., Banga, A. K. (2001) Electrically-assisted transdermal delivery of buprenorphine. *J. Control. Release* **73**: 197–203

Fang, J. Y., Hsu, L. R., Huang, Y. B., Tsai, Y. H. (1999) Evaluation of transdermal iontophoresis of enoxacin from polymer formulations: in vitro skin permeation and in vivo microdialysis using Wistar rat as an animal model. *Int. J. Pharmaceutics* **180**: 137–149

Grond, S., Radbruch, L., Lehmann, K. A. (2000) Clinical pharmacokinetics of transdermal opioids. *Clin. Pharmacokinet.* **38**: 59–89

Ho, H. O., Huang, F. C., Sokoloski, T. D., Sheu, M. T. (1994) The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system. *J. Pharm. Pharmacol.* **46**: 636–642

Hsu, C. S., Block, L. H. (1996) Anionic gels as vehicles modulated drug delivery. I. Solvent and drug transport phenomena. *Pharm. Res.* **13**: 1865–1870

Jadoul, A., Préat, V. (1997) Electrically enhanced transdermal delivery of domperidone. *Int. J. Pharmaceutics* **154**: 229–234

Jadoul, A., Lecouturier, N., Mesens, J., Caers, W., Préat, V. (1998) Transdermal alniditan delivery by skin electroporation. *J. Control. Release* **54**: 265–272

Maitani, Y., Kugo, M., Nagai, T. (1994) Permeation of diclofenac salts through silicone membrane: a mechanistic study of percutaneous absorption of ionizable drugs. *Chem. Pharm. Bull.* **42**: 1297–1301

Mitragotri, S. (2000) Synergistic effect of enhancers for transdermal drug delivery. *Pharm. Res.* **17**: 1354–1359

Prausnitz, M. R. (1996) Do high-voltage pulses cause changes in skin structure? *J. Control. Release* **40**: 321–326

Ramanathan, S., Block, L. H. (2001) The use of chitosan gels as matrices for electrically-modulated drug delivery. *J. Control. Release* **70**: 109–123

Regnier, V., De Moore, N., Jadoul, A., Préat, V. (1999) Mechanisms of a phosphorothioate oligonucleotide delivery by skin electroporation. *Int. J. Pharmaceutics* **184**: 147–156

Riviere, J. E., Heit, M. C. (1997) Electrically-assisted transdermal drug delivery. *Pharm. Res.* **14**: 687–697

Roy, S. D., Roos, E., Sharma, K. (1994) Transdermal delivery of buprenorphine through cadaver skin. *J. Pharm. Sci.* **83**: 126–130

Singh, P., Boniello, S., Liu, P., Dinh, S. (1999) Transdermal iontophoretic delivery of methylphenidate HCl in vitro. *Int. J. Pharmaceutics* **178**: 121–128

Sung, K. C., Fang, J. Y., Hu, O. Y. P. (2000) Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis. *J. Control. Release* **67**: 1–8

Vanbever, R., Le Boulenge, E., Préat, V. (1996a) Transdermal delivery of fentanyl by electroporation I. Influence of electrical factors. *Pharm. Res.* **13**: 559–565

Vanbever, R., De Morre, N., Préat, V. (1996b) Transdermal delivery of fentanyl by electroporation II. Mechanisms involved in drug transport. *Pharm. Res.* **13**: 1360–1366

Vanbever, R., Pliquett, U. F., Préat, V., Weaver, J. C. (1999) Comparison of the effects of short, high-voltage and long, medium-voltage pulses on skin electrical and transport properties. *J. Control. Release* **69**: 35–47

Wilding, I. R., Davis, S. S., Rimoy, G. H., Rubin, P., Kurihara-Bergstrom, T., Tipnis, V., Berner, B., Nightingale, J. (1996) Pharmacokinetic evaluation of transdermal buprenorphine in man. *Int. J. Pharmaceutics* **132**: 81–87

Yoshida, N. H., Roberts, M. S. (1993) Solute molecular size and transdermal iontophoresis across excised human skin. *J. Control. Release* **25**: 177–195