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The Effects of Iontophoresis and Electroporation on Transdermal Delivery of Indomethacin Evaluated *In Vitro* and *In Vivo*

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ABSTRACT

The objective of this study was to evaluate the transdermal delivery of indomethacin *in vitro* and *in vivo* enhanced by iontophoresis and/or electroporation through several barriers. Excised Wistar rat skin, pig skin, human epidermal membrane and cellulose membrane were tested for the *in vitro* indomethacin permeation, whereas drug concentration of in the plasma was analyzed to monitor the percutaneous absorption in Wistar rat *in vivo*. Iontophoresis enhanced the *in vitro* transdermal delivery of indomethacin as compared to the passive diffusion. Pulsing of high voltages, as known as electroporation, followed by iontophoresis did result in a synergistic effect in contrast to iontophoresis or electroporation alone. The *in vitro* result indicated that higher drug permeation could be induced during and after the application of iontophoresis. In some case, the permeation could benefit from the electroporation in term of cumulative amount within 12 hr. Furthermore, the combination of electroporation and iontophoresis resulted in a higher permeation amount than iontophoresis or electroporation alone did. Both iontophoresis and the combination protocol could reduce interspecies difference *in vitro*. The *in vivo* results were similar to that of *in vitro* ones. Iontophoresis and electroporation/iontophoresis resulted in higher AUC (area under curve) of blood concentration profiles of indomethacin. In addition, the combination protocol significantly induced higher transepidermal water loss (TEWL). It is concluded that the *in vivo* permeation results did correspond to the enhancement efficiency of the *in vitro* experiments.

Key words: transdermal delivery, indomethacin, iontophoresis, electroporation, TEWL

INTRODUCTION

The delivery of drugs via skin routes has been studied extensively in the pharmaceutical field. Drug delivery across skin offers the advantages of accessibility, non-invasiveness, compliance, safety and effectiveness. Nevertheless, its clinical application has been limited due to the barrier properties of skin, especially the stratum corneum (SC). Several strategies have been developed to overcome the barriers of the SC and to enhance the transdermal drug delivery[(1)]. Iontophoresis and electroporation belong to the electrically assisted methods that have been demonstrated as effective means to enhance transdermal permeation of drugs[(2,3)]. In the iontophoresis, the electrical power of low voltage (typically 10 V or less) and constant current (typically 0.5 mA/cm^2 or less) was employed to push charged or uncharged molecules through skin or other tissues. Electroporation involves the application of high voltage (typically > 100 V) and short-duration (μs ~ ms) pulse to increase the permeability of skin[(4,5)]. Electroporation is usually coupled with iontophoresis so that transdermal drug delivery is further facilitated.

Indomethacin is a non-steroidal anti-inflammatory drug which can cause considerable gastrointestinal side effects when administered orally, whereas such side effects could be reduced when the compound is applied on skin. A previous study shows that the application of iontophoresis can elevate the plasma concentration of indomethacin through the transdermal routes[(6)]. Furthermore, the use of iontophoresis to facilitate the underlying deep tissue permeation of drugs after topical administration will be most beneficial in the treatment of osteoarthritis, soft tissue rheumatism, tendonitis, and other deep routed local inflammatory conditions[(7)]. Hence electrically assisted methods may be suitable for topical delivery of indomethacin to achieve both local and systemic effects.

The aim of this present study was to investigate the influence of iontophoresis or electroporation on the transdermal permeation of indomethacin of *in vitro* and *in vivo*. The effect of combined iontophoresis and electroporation combination protocol was also examined. Various membranes, including cellulose membrane,

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Wistar rat skin, pig skin and human epidermal membrane (HEM), were used as permeation barriers in the *in vitro* study. Wistar rat was used as the animal model in the *in vivo* study.

MATERIALS AND METHODS

I. *Materials and Skins*

Indomethacin was purchased from Sigma Chemical Co. (MO, USA). The cellulose membrane (Spectra-por® 2, molecule weight cut-off = 12,000 ~ 14,000) was supplied by Spectrum Co. (TX, USA). *In vitro* permeation experiments were performed using full skin removed from the back region of female Wistar rats (180 ~ 200 g) and that of pigs (one-week old). The hair of the skin was shaved using an electric clipper. Human skin from breast site was supplied by Chung-Ho Memorial Hospital of Kaohsiung Medical University, and the epidermal membrane was prepared by heat separation method[8]. All chemicals and solvents used in the study were of analytical grade.

II. *In Vitro Permeation Experiments*

The *in vitro* permeation studies were performed using horizontal glass diffusion cells. The skin or membrane was mounted between the two half horizontal cells. The receptor compartment contained 8 mL of 0.06 M citrate-phosphate buffer, pH 7.4. The donor compartment of the cell was filled with 8 mL of 0.06 M citrate-phosphate buffer, pH 6.5, containing 12.5 mM indomethacin. The available skin diffusion area was 0.785 cm^2. The cells were maintained at 37°C and agitated by magnetic stirrers at 600 rpm. Aliquotes (300 μL) were withdrawn from the receptor compartment at regular intervals and immediately replaced by an equal volume of fresh buffer solution. The indomethacin concentration was then analyzed by HPLC.

III. *Iontophoresis and Electroporation Protocols*

For the *in vitro* permeation experiments under iontophoresis, a pair of platinum wires (0.5 mm diameter) with an effective length of 15 mm was immersed in the buffer as electrodes, with cathode in donor site and anode in receptor site. The electrodes were each positioned 3 cm away from each side of the skin or membrane. The electrodes were connected to a direct current power supply (Yokogawa Electrical Co., Model 7651, Japan). The current density was set at 0.5 mA/cm^2. The iontophoresis was applied constantly and the application time was set 3 hr.

Electroporation was performed using an exponential decay pulse generator (BTX Co., ECM 630 Electro Cell Manipulator, USA). A pair of platinum electrodes (0.5 × 1.5 cm^2) were located each 3 cm away from the skin or membrane. The cathode was positioned in the donor site and the anode in the receptor site unless otherwise noted. The electroporation protocol was applied for 10 min with 1 pulse per 30 sec; the pulse voltage was 300 V and pulse duration was about 200 ms. The voltage was expressed as the applied value but not as the true transdermal value.

The combination protocol was composed of 10 min of electroporation followed by 3 hr of iontophoresis.

IV. *In Vivo Transdermal Study*

Female Wistar rat (180 ~ 200 g) was anesthetized by intraperitoneal injection of 25% urethane at a dose of 3 mL/kg and the back fur was shaved. Two glass cylinders with the available diffusion area of 1.539 cm^2 each were placed above the dorsal skin with glue (Instant Super glue®, Kokuyo Co., Japan). A 2 mL of citrate-phosphate buffer, pH 6.5, containing 12.5 mM indomethacin was added into the donor cylinder, while the receptor cylinder was filled with 2 mL of citrate-phosphate buffer, pH 7.4. The platinum plates were put in the center of the cylinders and vertical to the skin. The pair of platinum wires was bent to be horizontal to the skin and parallel to each other. After the electrical protocol started, about 0.4 mL of blood was withdrawn from the carotid every 1-hr intervals. The plasma was added with p-phenylphenol as an internal standard, extracted with dichloromethane, vacuum-dried and re-dissolved with mobile phase. All plasma samples were analyzed by HPLC.

V. *Sample Preparation and HPLC Analysis of Indomethacin*

The *in vitro* samples of indomethacin were acidified with 1N HCl and added with *p*-phenylphenol as internal standard before HPLC analysis. The plasma samples of *in vivo* study were added with 1N HCl and *p*-phenylphenol, and extracted with dichloromethane. The organic layer of each sample was, collected, vacuum vaporized and redissolved with the mobile phase. *In vitro* and *in vivo* samples were analyzed by a modified HPLC method[9]. The HPLC system consisting of a Hitachi L-7100 pump, a Hitachi L-7400 UV detector and a Hitachi L-7200 sample processor. A C18 column (LichroCart® 125-4, Merck) was used. The mobile phase consisted of 72% methanol and 28% de-ionized water with 0.05% acetic acid at a flow rate of 1 mL/min. The wavelength of the UV detector was set at 260 nm.

VI. *In Vivo TEWL Evaluation*

The method of setting up the glass cylinder and administrating the buffer containing indomethacin was the same as that of the *in vivo* transdermal study. After 3-hr administration, the formulation and buffer were removed, and the application site was gently cleaned with a cotton swab and allowed undisturbed for another 3 hr

due to the residual solution. TEWL was determined at the intervals of 6, 12, 24, 26 and 48 hr. TEWL of the donor and receptor site were measured quantitatively using a Tewa meter® 210 (Courage and Khazaka Co., Germany). Reference site of each rat was chosen close to both donor and receptor. The TEWL was calculated automatically and expressed in g/cm^2/hr. An adjacent untreated site was used as the basal standard for each determination.

VII. *Data Analysis*

Values of flux at the first 3 hr and the steady state flux calculated from the linear portion of the plot of cumulative amount versus time (6 ~ 12 hr, $R^2 > 0.9$) were analyzed. Penetration index (PI) was calculated from the ratio of cumulative amount of electrically assisted method to the cumulative amount of passive control. The Student's *t*-test and one-way ANOVA were utilized to test effects of various treatment effects. Subgroup comparisons were made using the Newman-Keuls multiple comparisons. The level of significance in all tests was set at 0.05.

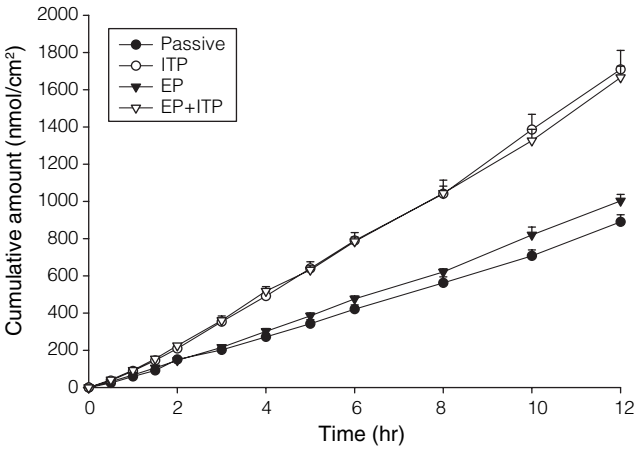

Figure 1. Cumulative amount of indomethacin in citrate-phosphate buffer, pH 6.5, detected in the receptor phase versus time by treating electrically assisted methods across cellulose membrane. All data represent the means of the experiments ± S.D. (n = 4). ITP, iontophoresis; EP, electroporation.

RESULTS AND DISCUSSION

I. *In vitro Permeation Experiments*

(I) *The Enhancement of Iontophoresis and Electroporation*

It has been reported in literatures that iontophoresis with platinum electrode can cause the pH change of buffer in the *in vitro* study. In our experiment, this phenomenon was examined by using diffusion cells with blank buffers and rat skins as barriers. The result demonstrated that pH values of both donor and receptor buffers remained stable even after 24 hr of iontophoresis of 0.5 mA/cm^2 and indicated that platinum electrode would not affect the result of the study.

The cumulative amounts of indomethacin penetration (nmol/cm^2) through the cellulose membrane with various methods including iontophoresis, electroporation and combination protocol were plotted against time as shown in Figure 1. The permeation parameters and the enhancement PI of each methods are listed in Table 1. The cumulative amount of indomethacin penetration through cellulose membrane at 12 hr increased in the order of combination protocol ≅ iontophoresis > electroporation (Newman-Keuls test).

The cumulative amount of indomethacin through cellulose membrane by iontophoresis at 3 hr or 12 hr is higher (ANOVA test, $p < 0.05$) than that by passive diffusion, indicating that the contribution of electrophoretic drift enhanced by iontophoresis was significant for indomethacin. However, both PI's of 3 hr and 12 hr by iontophoresis were relatively low in the membrane group. This may indicate the high porosity of the cellulose membrane, suggesting that water-filled pores or channels exist for drug molecules to diffuse freely at certain levels.

The diffusion of indomethacin across cellulose membrane by electroporation was similar to the control, as indicated by the 3 hr cumulative amount and 0 ~ 3 hr flux due to the existing pores of the cellulose membrane (Figure 1). This result confirmed that the permeation-enhancing effects of electroporation were predominantly attributed to the formation of micropore structures on the biological skin, and thereby making a permeabilized skin by the exposure of high-voltage pulses before applying iontophoresis. However, a 12 hr cumulative amount and steady-state flux achieved by electroporation were significantly higher than that of passive control, suggesting that long-term transport could benefit from electroporation. The enhancement mechanism by electroporation is the creation of aqueous pores in lipid bilayers of mammalian skin[10], which might significantly but weakly affect the cellulose membrane in the study. It is shown in Table 1 that the cumulative amount of cellulose membrane by a combination protocol approximated to that by iontophoresis alone (Newman-Keuls test, $p > 0.05$). The result indicated that the effect of electromotive force on the permeation of indomethacin after applying electroporation for the first 3 hr was negligible. Furthermore, the contribution of electroporation was also limited, which could be seen in results of the 12 hr cumulative amount and steady-state flux of indomethacin penetrating through cellulose membrane while applying electroporation with iontophoresis.

In order to simulate the *in vivo* transdermal delivery, rat skin was used as the permeation barrier for the *in vitro* study. The cumulative amount of indomethacin (nmol/cm2) in the receptor compartment was a function of time with or without iontophoresis and electropora-

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Table 1. The *in vitro* permeation profiles of transdermal indomethacin delivery by electrically-assisted methods across various skins and cellulose membrane

Skin types	Modes	3 hr C.A.a (nmol/cm^2)	3 hr PIb	12 hr C.A. (nmol/cm^2)	12 hr PI	0~3 hr flux (nmol/cm^2/hr)	Steady-state flux (nmol/cm^2/hr)
Membrane	Passive	202.11 ± 13.67$^{\alpha}$	1.00	890.0 ± 38.53$^{\alpha}$	1.00	70.68 ± 4.79$^{\alpha}$	77.62 ± 3.03$^{\alpha}$
	ITP[c]	353.25 ± 21.31$^{\beta}$	1.75	1709.03 ± 102.01$^{\beta}$	1.92	118.68 ± 6.49$^{\beta}$	155.20 ± 10.50$^{\beta}$
	EP[d]	215.83 ± 8.87$^{\alpha}$	1.07	1002.77 ± 34.60$^{\chi}$	1.13	72.98 ± 2.16$^{\alpha}$	88.94 ± 4.34$^{\chi}$
	EP+ITP	360.37 ± 23.86$^{\beta}$	1.78	1665.29 ± 61.38$^{\beta}$	1.87	121.81 ± 5.88$^{\beta}$	146.28 ± 7.17$^{\beta}$
Wistar rat	Passive	2.00 ± 2.18$^{\alpha}$	1.00	16.04 ± 11.41$^{\alpha}$	1.00	0.64 ± 0.56$^{\alpha}$	1.88 ± 1.26$^{\alpha}$
	ITP	26.39 ± 4.97$^{\beta}$	13.20	100.22 ± 7.46$^{\beta}$	6.25	12.17 ± 3.00$^{\beta}$	6.66 ± 0.20$^{\beta}$
	EP	4.45 ± 1.11$^{\alpha}$	2.23	57.92 ± 23.85$^{\chi}$	3.61	1.52 ± 0.32$^{\alpha}$	6.57 ± 2.59$^{\beta}$
	EP+ITP	159.91 ± 24.12$^{\chi}$	79.80	417.45 ± 5.50$^{\delta}$	26.03	54.82 ± 9.38$^{\chi}$	18.90 ± 1.90$^{\chi}$
Pig	Passive	1.41 ± 2.82$^{\alpha}$	1.00	6.28 ± 8.75$^{\alpha}$	1.00	0.87 ± 1.73$^{\alpha}$	0.68 ± 1.00$^{\alpha}$
	ITP	29.64 ± 10.27$^{\beta}$	21.02	81.23 ± 17.22$^{\beta}$	12.93	10.21 ± 4.19$^{\beta}$	3.68 ± 1.78$^{\beta}$
	EP	3.62 ± 2.60$^{\alpha}$	2.57	15.48 ± 9.28$^{\alpha}$	2.46	1.31 ± 0.84$^{\alpha}$	1.18 ± 0.75$^{\alpha}$
	EP+ITP	70.55 ± 23.29$^{\chi}$	50.04	161.62 ± 39.49$^{\chi}$	25.74	23.23 ± 7.98$^{\chi}$	4.26 ± 1.53$^{\beta}$
Human epidermal membrane (HEM)	Passive	1.63 ± 0.91$^{\alpha}$	1.00	13.64 ± 6.52$^{\alpha}$	1.00	0.82 ± 0.41$^{\alpha}$	1.47 ± 0.64$^{\alpha}$
	ITP	63.56 ± 25.72$^{\beta}$	39.00	107.08 ± 46.92$^{\beta}$	7.85	21.96 ± 9.21$^{\beta}$	3.59 ± 2.35$^{\alpha}$
	EP	12.10 ± 3.26$^{\alpha}$	7.42	52.33 ± 18.21$^{\alpha,\beta}$	3.84	3.84 ± 1.07$^{\alpha}$	4.77 ± 1.55$^{\alpha}$
	EP+ITP	126.21 ± 65.08$^{\chi}$	77.43	201.94 ± 86.36$^{\chi}$	13.35	43.27 ± 23.11$^{\chi}$	4.68 ± 2.35$^{\alpha}$

Each value represents the mean ± S.D. (n = 3~5).

$^{\alpha,\beta,\chi,\delta}$Modes with the same skin type and Latin letter are not significantly different and that with different letters are significantly different (ANOVA followed by Newman-Keuls test, $p < 0.05$).

[a]C.A.: cumulative amount.

[b]Penetration index (PI): cumulative amount of electrically-assisted method/cumulative amount of passive control.

[c]ITP: iontophoresis.

[d]EP: electroporation.

tion as shown in Figure 2 (A). There was limited passive permeation (in the absence of electric modulation) of indomethacin across rat skin during 12 hr. Application of electrically-assisted methods could greatly enhance the transdermal permeation of indomethacin. Iontophoresis, 0.5 mA/cm^2 for 3 hr, greatly enhanced the transdermal indomethacin permeation by 6.25 folds increase in terms of 12 hr cumulative amount as summarized in Table 1. Cumulative amount at 3hr and flux during the course of iontophoresis were also significantly increased (ANOVA test, $p < 0.05$). Moreover, steady state flux was significantly higher than control when the current was ceased (ANOVA test, $p < 0.05$). The current density was set at 0.5 mA/cm^2, which has been reported in the literature to be the maximum acceptable level concerning the minimal skin damage and irritation[11]. Indomethacin is an acidic drug with a pKa of 4.5 and should be almost completely ionized in the donor solution (pH 6.5). Therefore the observed enhancement of skin permeation of anionic indomethacin could be attributed to the electric potential gradient resulted from the application of iontophoresis. Although the current density was switched off after 3hr, indomethacin molecules continuously permeated across the skin as shown in Figure 2 (A). One explanation is the existence of a drug reservoir within the skin. In the current-off period, the permeating ions kept released from the skin until the drug reservoir drained off. Another explanation is the alteration of the nature of skin. The major route of iontophoretic transport is believed to be appendageal pores including the sweat ducts and hair follicles[7]. A non-appendageal pore pathway has also been proposed, which probably implies the current flow through "artificial shunts" as a result of disruption of the organized structure of SC[4]. The barrier function of the SC will be estimated by measuring TEWL as described below.

As shown in the time course of indomethacin permeation across rat skin by electroporation in Figure 2 (A), electroporation had no significant effect on transdermal delivery in the first 3 hr; however, the application of high voltage pulses significantly boosted the permeation amount of the drug at 12 hr as compared to the passive permeation. The results indicated that electroporation could benefit the long-term transdermal transport, since this phenomenon was also observed with cellulose membrane. The continuous accumulation of indomethacin post electroporation may indicate the formation of a drug reservoir or alteration of skin structure.

The increase of molecule transport by electroporation can be attributed to the creation of electropores as

well as by electrophoresis/iontophoresis due to the localization effect[12]. Therefore, in order to elucidate the influence of electroporation/iontophoresis, the polarity of electrodes was changed. Negative polarity pulsing for indomethacin may cause structural changes in the skin, possibly due to electroporation, so that more molecules move across the skin through both previously existing and newly created pathways[13]. In contrast, positive polarity pulsing for indomethacin can also create structural changes in the skin, but it has a zero time-integral of voltage. The positive polarity pulsing of indomethacin produced the total permeated amount of 71.93 ± 14.87 nmol/cm^2 across rat skin for 12 hr, which was approximate to the value of 57.92 ± 23.85 nmol/cm^2 of negative polarity as indicated in Table 1 (t-test, $p > 0.05$). This result indicated that the increases of indomethacin transport were hardly dependent on the pulse polarity, which suggested that the electrophoresis of electroporation did not significantly contribute to the transport since the phenomenon should depend on orientation of electrical field.

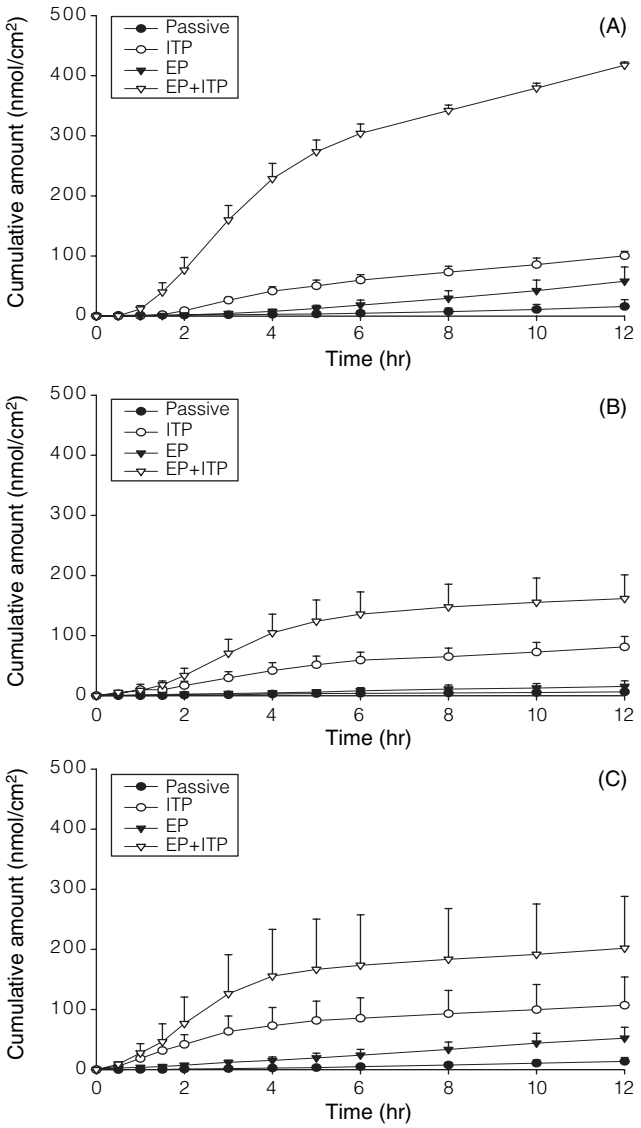

Figure 2. Cumulative amount of indomethacin from citrate-phosphate buffer, pH 6.5, detected in the receptor phase versus time by treating electrically-assisted methods across (A) Wistar rat skin, (B) pig skin and (C) human epidermal membrane. All data represent the means of the experiments ± S.D. (n = 3~5). ITP, iontophoresis; EP, electroporation.

The enhancement by the combination protocol was higher than that by individual protocols in terms of cumulative amount and flux (Newman-Keuls test, $p < 0.05$). Moreover, it accumulated 79.8 (3 hr) and 26.03 (12 hr) folds of amounts of passive control, and the cumulative amount was significantly higher than the sum produced by iontophoresis and by electroporation. The result suggested that the application of a high voltage pulse followed by low current density produced synergistic enhancement which was greater than the sum of individual protocol.

Rat skin is generally considered 2 ~ 5 times more permeable than human skin *in vitro* for some drugs, which hampers the quantitative prediction of cutaneous drug delivery in humans from rat studies[14]. Therefore, the excised pig skin was also used as a diffusion barrier in the permeation study because of the similarity of human and pig skins. As depicted in Figure 2 (B), the trends of drug permeation by various protocols were the same for rat skin and pig skin. The passive and iontophoretic permeation of indomethacin showed no significant difference (t-test, $p > 0.05$) between rat and pig skins (Table 1). On the other hand, the enhancement of skin permeation by electroporation in pig skin was not as effective as in rat skin, since electroporation mainly acts on the skin structure to induce indomethacin permeation, thus indicating more rigid characteristics of pig skin structure than rat skin.

Iontophoresis and iontophoresis/electroporation also significantly enhance the 0 ~ 3 hr flux, 3 hr and 12 hr cumulative amounts of the transdermal transport of indomethacin through HEM, as shown in Figure 2 (C), although the steady-state flux among four protocols exhibited no significant difference (ANOVA test, $p > 0.05$). Electroporation, however, could not produce significant enhancement in HEM, which was also found in pig skin.

(II) *Inter-species Comparison*

As shown in Table 1, it was noticed that 3-hr PI of iontophoresis/electroporation of HEM was about 77 folds, which was similar to that of Wistar rat skin and within the same magnitude order of that of pig skin. Iontophoresis enhanced the 3-hr cumulative amount through HEM up to 39 folds, which was about 3 times of that of Wistar rat skin and 2 times of that of pig skin. Electroporation was also found to enhance more in HEM than in Wistar rat skin and pig skin. Most of the 12 hr PI of the experiments were lower than that of 3-hr PI

since the electrical assistance was ceased after 3 hr. The results suggested that the combination protocol could narrow the interspecies difference.

It has been reported that iontophoretic flux measures were similar when delivering lithium ions through human, pig and rabbit skin[15]. Also, iontophoresis reduced interspecies difference in terms of cumulative amounts after 2 hr of iontophoresis with 22-hr post-iontophoretic passive diffusion and steady-state flux with delivery of timolol maleate[16]. In our study, it was found that the 3-hr cumulative amount of passive control, 3-hr cumulative amount of electroporation/iontophoresis, 12-hr cumulative amount of iontophoresis and 12-hr cumulative amount of electroporation/iontophoresis in Wistar skin, pig skin or HEM were not significantly different (ANOVA test, $p > 0.05$). This suggested that, not only iontophoresis, electroporation combined with iontophoresis could reduce the interspecies difference in terms of 3-hr cumulative amount and that with 9 hr of post current application, and also produced a synergistic effect. The results with short-term high voltage pulses alone varied among different species, however.

II. *In Vivo Permeation Experiments*

Wistar rat was chosen as an *in vivo* animal model. As shown in Figure 3, the AUC under 0 ~ 12 hr of indomethacin by passive, iontophoresis, electroporation and electroporation/iontophoresis were 0.42 ± 0.51, 12.26 ± 1.92, 2.65 ± 1.51 and 17.21 ± 2.07 μg/mL×hr, respectively. The result was similar to that of *in vitro* study. In addition, the AUC of first 3hr was linearly associated with the flux of the first 3hr *in vitro* ($R^2 = 0.88$), indicating that AUC proportionally increased as flux increased. The AUC of indomethacin under 0 ~ 12 hr by electroporation/iontophoresis was higher than that by individual protocol or passive control (Newman-Keuls test, $p < 0.05$). The synergistic effect of the combined physical enhancers could produce about 40-fold AUC of indomethacin by passive transport, suggesting that both *in vitro* and *in vivo* transdermal transport of indomethacin could benefit more from the combination protocol than individual protocols.

III. *In Vivo TEWL Evaluation*

TEWL assay was employed to assess SC damage, and subsequently the correlation between the damage to the skin barrier and an increase in TEWL[17]. It is also used to evaluate the post-iontophoresis water loss from human skin[18]. In order to elucidate the safety of electrically assisted methods on the *in vivo* permeation of indomethacin, TEWL values were determined as an index of skin disruption. Baseline values corresponding to the untreated sites need to be subtracted from the measurements in order to get the actual changes in TEWL (ΔTEWL).

At the donor site, iontophoresis and electroporation did not enhance ΔTEWL as compared to the control group (Figure 4) (Newman-keuls test, $p > 0.05$). This may suggest a negligible damage of SC barrier after iontophoresis and electroporation since skin hydration/alteration in SC barrier function are responsible for the increase of ΔTEWL. However, the combination protocol significantly induced the ΔTEWL values at the donor site at 12, 24 and 36 hr (Newman-keuls test, $p > 0.05$). At 48 hr, the ΔTEWL values of all protocols showed no marked difference. It was also noticed that the ΔTEWL values of all protocols at 6 hr were high. It might be due to the vaporization of the residual aqua solution. The results suggested that the synergistic effect from the combina-

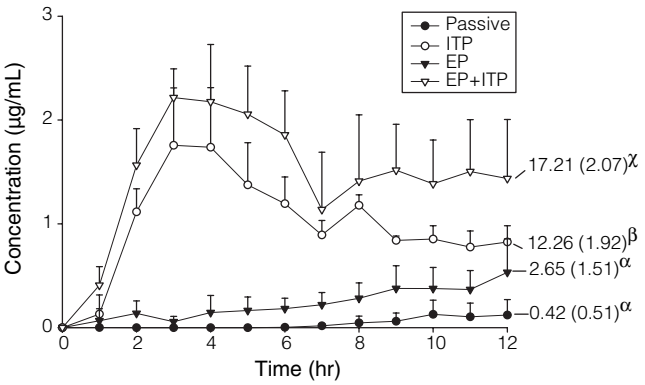

Figure 3. Indomethacin concentration in male Wistar rat plasma after treated with electrically assisted methods. All data represent the means of the experiments ± S.D. (n = 3~5, numbers represent the means of AUC ± S.D.). ITP, iontophoresis; EP, electroporation.
$^{\alpha,\beta,\chi}$Data with the same Latin letter are not significantly different and that with different letters are significantly different (ANOVA followed by Newman-Keuls test, $p < 0.05$).

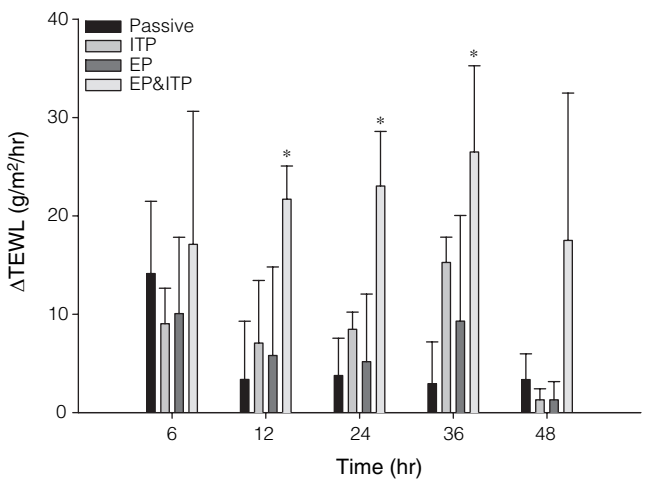

Figure 4. ΔTEWL measured at donor site on male Wistar rat. All data represent the means of the experiments ± S.D. (n = 3~4). ITP, iontophoresis; EP, electroporation.
*Significant difference from passive control (ANOVA followed by Newman-Keuls test, $p < 0.05$).

tion, rather than individual protocols, could enhance the reversible skin hydration and alteration in SC. On the contrary, all protocols applied did not significantly increase the ΔTEWL of receptor site (data not shown). Skin disruption thus occurred only when the combination protocol was applied at the donor site.

CONCLUSIONS

The *in vitro* and *in vivo* permeation characteristics of indomethacin by iontophoresis, electroporation, or a combination of the two, were examined in this study. Both iontophoresis and electroporation enhanced the *in vitro* and *in vivo* skin permeation of indomethacin. The *in vitro* and *in vivo* enhancement achieved by electroporation was less pronounced than that by iontophoresis. The combination protocol did further boost the permeated amount of indomethaicn. Iontophoresis and the combination protocol could reduce interspecies difference in terms of cumulative amounts *in vitro*. The *in vivo* permeation of indomethacin by electrically assisted methods was quite similar to the *in vitro* study. The results of the *in vivo* permeation study with n animal model corresponded to that of the *in vitro* experiment. While comparing the results of individual protocols and control, the combination of electroporation and iontophoresis significantly induced higher but reversible transepidermal water loss of the donor site.

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