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

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ABSTRACT

The aim of this study was to evaluate the transdermal delivery of two meloxicam salts, meloxicam sodium and meloxicam potassium, enhanced by iontophoresis and/or electroporation *in vitro* with Wistar rat dorsal skin and human epidermal membrane (HEM) as barriers and *in vivo* with Wistar rat as the animal model. Iontophoresis and its combination with electroporation could significantly enhance the *in vitro* permeation of both salts, and two protocols had the same enhancement to each salt. Furthermore, the combination protocol had more enhancement to potassium salt than sodium salt. Interspecies difference was found in some electrical protocols. Electroporation might depress the transdermal delivery of meloxicam sodium while it might enhance that of meloxicam potassium. Iontophoresis, electroporation and their combination could increase the delivery of the two salts and result in significantly higher AUC of drug concentration to time *in vivo*. It was concluded that the *in vitro* and *in vivo* transdermal delivery of meloxicam potassium was more facilitated by the combination protocol than meloxicam sodium.

Key words: transdermal delivery, meloxicam salts, iontophoresis, electroporation

INTRODUCTION

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) of the acidic enolcarboxamide class. Meloxicam has faster anti-inflammatory and anti-arthritic activities as compared to piroxicam, indomethacin, diclofenac, tenoxicam, naproxen and aspirin after oral administration[1].

Another administration route, transdermal transport, has been shown to deliver the meloxicam successfully[2,3]. According to our previous study, iontophoresis could enhance the delivery of three salts of diclofenac[4,5], and this result led us to study the transdermal delivery of meloxicam salts by two physical enhancers, namely iontophoresis and electroporation.

Iontophoresis can enhance the transdermal delivery of compounds like drugs and peptides, and the synergistic effect of iontophoresis combined with electroporation has been reported in many studies. Several factors have been demonstrated to influence the transdermal permeation of drug molecules by iontophoresis or electroporation[6-8]; however, the influence of combination strategy of iontophoresis and electroporation on the transdermal delivery of various salts of one drug has rarely been discussed.

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This study was aimed to evaluate the enhancement of iontophoresis, electroporation and a combination protocol on the transdermal delivery of two meloxicam salts, meloxicam sodium and meloxicam potassium, as model drugs *in vitro* and *in vivo*. Human epidermal membrane and Wistar rat dorsal skin were used as barriers *in vitro* to examine the influence of the two physical enhancers and inter-species difference. The pharmacokinetics was also evaluated *in vivo* with Wistar rat as the animal model.

MATERIALS AND METHODS

I. *Materials and Barriers*

Two meloxicam salts, meloxicam sodium and meloxicam potassium, were custom-synthesized as follows: 10 g meloxicam was added into 1.2 L dichloromethane and the mixture was stirred for 5 min. Two and half liter of de-ionized water containing 20 g of NaOH/KOH was added into the solution and allowed to react for at least 6 hr. Afterwards, the water layer was heat-dried, and the residue was filtered and washed with sufficient dichloromethane and de-ionized water. The product was desiccated in the vacuum oven until the residual solvent vaporized. Two products of meloxicam salts were veri-

fied by GC/MS (JMS-700, JEOL, Japan) with FAB (fast atom bombardment), and it confirmed that the two salts were successfully synthesized.

In vitro permeation experiments were performed using the human epidermal membrane and the full and shaved rat skin removed from the back region of the male Wistar rat (6 ~ 8 weeks old). Human skin from the breast after breast reduction operation was supplied by Chung-Ho Memorial Hospital of Kaohsiung Medical University and its epidermal membrane was prepared by heat separation method[9]. All chemicals and solvents were of analytical grade.

II. *Determination of Partition Coefficient*

The partition coefficient was measured in *n*-octanol/buffer (v/v, 1:1). The n-octanol was saturated with 60 mM citrate-phosphate buffer (pH = 7.4) overnight before the partition measurement. Both saturated *n*-octanol and buffer containing meloxicam salt were added into the capped glass tube which was then horizontally shaken for 24 hr. After mixing well, the buffer layer was analyzed by HPLC. The partition coefficient was calculated as the ratio of the drug concentration in the *n*-octanol phase to that in the buffer.

III. *In Vitro Permeation Experiments*

Modified horizontal glass diffusion cells with an intercept area of 0.785 cm^2 were utilized in the *in vitro* permeation experiments. The skin was mounted between the two half horizontal cells. The donor compartment of the cell was filled with 8 mL of 60 mM citrate-phosphate buffer (pH 6.08) containing 12.5 mM meloxicam salt and the receptor phase contained 8 mL of 60 mM citrate-phosphate buffer (pH 7.4). Each cell was maintained at 37°C in a water jacket and agitated with a magnetic stirrer at 600 rpm. Sample (0.2 mL) was withdrawn from the receptor component at regular intervals and replaced by an equal volume of fresh buffer solution immediately. All samples were then analyzed by HPLC.

IV. *Iontophoresis and Electroporation Protocols*

An exponential decay pulse generator (ECM 630 Electro Cell Manipulator, BTX Co., USA) was used to perform the electroporation. A pair of platinum electrodes (0.5 × 1.5 cm^2) were used and each was located 3 cm from the skin. The cathode was positioned in the donor site and the anode in the receptor site. The electroporation protocol was 1 pulse per 30 sec, applied for 10 min; the pulse voltage was set at 300 V and pulse duration was about 200 ms. The voltage was expressed as an applied value but not as a transdermal value. A direct current power supply (Model 7651, Yokogawa Electrical Co., Japan) connected with a pair of platinum wires (0.5 mm diameter) was used in the *in vitro* permeation experiments. An effective length of 15 mm of platinum wire was immersed in the buffer as electrodes, with cathode in the donor site and anode in the receptor site. The electrodes were positioned 3 cm away from each side of the skin or membrane. The current density was set at 0.5 mA/cm^2. The iontophoresis was applied continuously and the application time was set at 3 hr. The combination protocol was consisted of 10 min of electroporation following by 3 hr of iontophoresis.

V. *In Vivo Transdermal Study*

Male Wistar rat (180 ~ 200 g) was anesthetized by intraperitoneal injection of 25% urethane at a dose of 3 mL/kg and the back fur of the rat was shaved. Two glass cylinders each with the available diffusion area of 1.539 cm^2 were placed above the dorsal skin with glue (Super Glue Gel®, 3M, USA). An aliquot of 2 mL of citrate-phosphate buffer (pH 6.08) containing 12.5 mM meloxicam salt was added into the donor (cathode-containing cylinder), while the receptor 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 0.3 ~ 0.4 mL of blood was withdrawn from the carotid at one hour interval. All samples were analyzed by HPLC. The animal experiment was reviewed and approved by the Institutional Animal Care Committee at Kaohsiung Medical University.

VI. *Sample Analysis*

The analytic method, HPLC, was modified from our previous study[3]. *In vitro* samples of meloxicam were acidified with aliquots of 1N HCl and prioxicam was added as internal standard for HPLC analysis. *In vivo* plasma samples were also supplemented by 1N HCl and prioxicam, and then extracted with dichloromethane. The organic layer of each sample was vacuum vaporized and re-dissolved with the mobile phase of HPLC. The HPLC system of Waters LC Module 1 with a C_{18} column (LichroCart® 125-4, Merck) as the stationary phase was employed. The mobile phase consisted of 40% methanol, 10% acetonitrile and 50% aqueous solution with 50 mM di-ammoniumhydrogenphosphate, pH = 7, at a flow rate of 1 mL/min. The wavelength of the UV detector was set at 363 nm. The *in vitro* calibration curves were linear (R^2 > 0.998 for sodium salt and R^2 > 0.999 for potassium salt) over the concentration range of 0.5 ~ 50 μg/mL (6 points, n = 6). The intraday coefficients of variation were less than 4.7% and 3.7% for sodium salt and potassium salt, respectively. The *in vivo* calibration curves were linear (R^2 > 0.999 for sodium and R^2 > 0.995 for potassium salt) over 0.5 ~ 50 μg/mL (6 points, n = 6). The intraday coefficients of variation were less than 4.9% for sodium and 12% for potassium salt.

VII. *Data Analysis*

The cumulative amount of drug permeating into the receptor through the unit diffusion area was calculated and plotted as a function of time. Values of the flux at the first 3 hr interval were analyzed. Penetration index (PI) was calculated from the ratio of cumulative amount of electrically-assisted method to that of passive control. The Student's *t*-test and one-way ANOVA were utilized to test the 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.

RESULTS

I. *In Vitro Permeation Experiments*

(I) *Wistar Rat Skin as the Barrier*

The passive transport of meloxicam sodium through Wistar rat skin was shown in Table 1. The 3 and 12 hr cumulative amount of meloxicam sodium via passive transport was higher than that of meloxicam potassium (*t*-test, $p < 0.05$). The flux of the first 3 hr of meloxicam sodium was almost 10 times higher than that of meloxicam potassium.

The results of meloxicam sodium permeation through Wistar rat skin and enhanced by iontophoresis / electroporation are presented in Figure 1A. During 12 hr of transdermal transport, electroporation did not enhance the permeation of meloxicam sodium in terms of both 3 hr and 12 hr cumulative amount (ANOVA test, $p > 0.05$). Moreover, in terms of PI, electroporation seemed to retard the permeation of meloxicam sodium through Wistar rat skin; on the other hand iontophoresis resulted in 11.26-fold of 3 hr cumulative amount and 19.56-fold of 12 hr cumulative amount, respectively, when compared with those of passive controls. Meloxicam sodium could benefit from the combination of electroporation and iontophoresis which resulted in 3 hr PI of 12.86 and 12 hr PI of 22.13 (ANOVA test, $p < 0.05$). However, the enhancement of electroporation/iontophoresis seemed the same as that of iontophoresis since there was no significant difference in terms of 3 hr and 12 hr cumulative amount, respectively.

Surprisingly, as shown as Table 1 and Figure 1B, the

Table 1. The *in vitro* transdermal permeation profiles of meloxicam sodium and meloxicam potassium delivered by electrically-assisted methods across Wistar rat dorsal skin and HEM

Skin types	Model drugs	Methods	3 hr CA[a] (nmol/cm^2)	3 hr PI	12 hr CA (nmol/cm^2)	12 hr PI	0~3 hr flux (nmol/cm^2/hr)
Wistar rat dorsal skin	Meloxicam sodium	Passive	$29.78 \pm 2.47^{\beta\theta*}$	–	$44.73 \pm 22.02^{\beta\theta*}$	–	$7.07 \pm 0.79^{\beta\theta*}$
		ITP	$335.44 \pm 130.76^{\alpha\gamma}$	11.26	$875.04 \pm 246.44^{\alpha\gamma}$	19.56	$112.75 \pm 44.64^{\alpha\gamma}$
		EP	$2.53 \pm 2.52^{\beta\theta*}$	0.08	$36.14 \pm 28.60^{\beta\theta*}$	0.81	$0.55 \pm 0.55^{\beta\theta*}$
		EP+ITP	$383.02 \pm 117.30^{\alpha\gamma}$	12.86	$989.69 \pm 303.58^{\alpha\gamma}$	22.13	$126.79 \pm 38.62^{\alpha\gamma}$
	Meloxicam potassium	Passive	$2.83 \pm 3.10^{\beta\theta*}$	–	$7.00 \pm 2.98^{\beta\theta*}$	–	$0.75 \pm 0.74^{\beta\theta*}$
		ITP	$320.20 \pm 57.14^{\alpha\gamma}$	113.01	$980.84 \pm 174.30^{\alpha\gamma}$	140.13	$106.95 \pm 17.94^{\alpha\gamma}$
		EP	$66.40 \pm 18.20^{\beta\theta*}$	23.43	$243.11 \pm 89.98^{\beta\theta*}$	34.45	$21.42 \pm 7.36^{\beta\theta*}$
		EP+ITP	$407.00 \pm 119.24^{\alpha\gamma}$	143.64	$1157.71 \pm 326.55^{\alpha\gamma}$	165.39	$133.90 \pm 37.70^{\alpha\gamma}$
HEM	Meloxicam sodium	Passive	$31.55 \pm 24.74^{\beta\theta}$	–	$33.16 \pm 25.01^{\beta\theta}$	–	$8.87 \pm 0.01^{\beta\theta*}$
		ITP	$189.33 \pm 67.60^{\alpha\gamma}$	8.82	$211.54 \pm 76.78^{\alpha\gamma}$	9.32	$64.23 \pm 24.13^{\alpha\gamma}$
		EP	$0.52 \pm 0.47^{\beta\theta*}$	0.02	$2.35 \pm 1.25^{\beta\theta}$	0.10	$0.15 \pm 0.19^{\beta\theta}$
		EP+ITP	$122.23 \pm 58.56^{\alpha\gamma*}$	5.70	$159.31 \pm 49.92^{\alpha\gamma*}$	7.02	$41.15 \pm 22.38^{\alpha\gamma}$
	Meloxicam potassium	Passive	$5.80 \pm 4.71^{\beta\theta}$	–	$7.58 \pm 6.90^{\beta\theta}$	–	$1.57 \pm 2.11^{\beta\theta*}$
		ITP	$196.09 \pm 38.68^{\alpha\gamma}$	33.79	$231.24 \pm 35.31^{\alpha\gamma}$	30.49	$67.84 \pm 13.65^{\alpha\gamma}$
		EP	$48.82 \pm 29.54^{\beta\theta*}$	8.41	$83.74 \pm 62.98^{\beta\theta}$	11.04	$15.67 \pm 11.52^{\beta\theta}$
		EP+ITP	$252.72 \pm 82.77^{\alpha\gamma*}$	43.55	$306.01 \pm 98.38^{\alpha\gamma*}$	40.34	$82.73 \pm 26.97^{\alpha\gamma}$

Each value represents the mean ± S.D. (n = 4).

$^{\alpha,\beta,\gamma,\theta}$ Data of one skin type and model drug were analyzed by ANOVA followed by Newman-Keuls test (significant level = 0.05).

$^{\alpha}$significantly different from passive; $^{\beta}$significantly different from ITP; $^{\gamma}$significantly different from EP;

$^{\theta}$significantly different from EP+ITP.

* Data of two model drugs with the same skin type and method were significantly different from each other (*t*-test, $p < 0.05$).

CA: cumulative amount; ITP: iontophoresis; EP: electroporation.

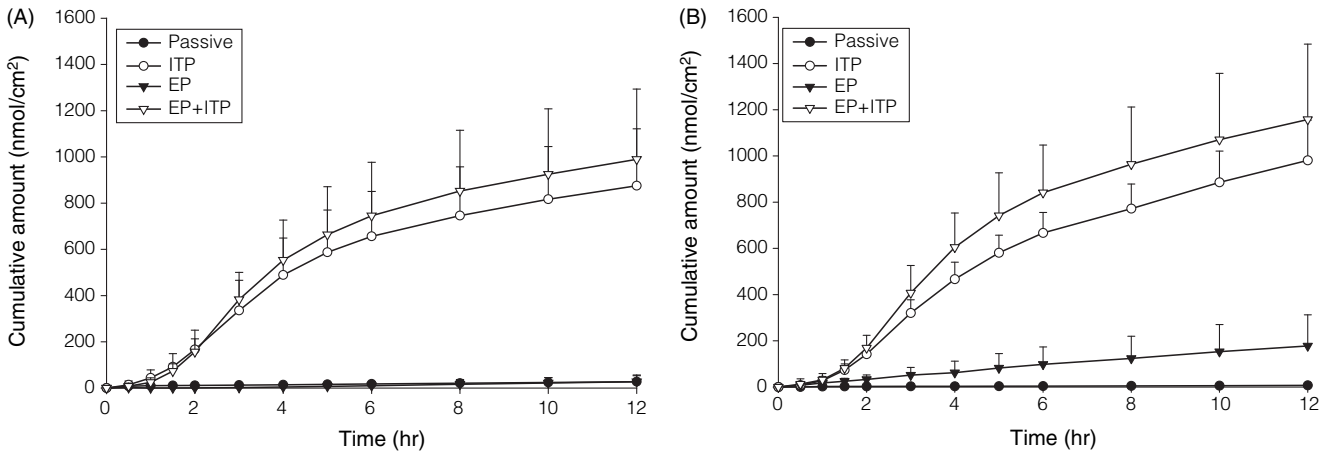

Figure 1. Cumulative amount versus time profile of (A) meloxicam sodium and (B) meloxicam potassium and enhanced by electrically assisted methods through Wistar rat skin. All data represent the means of the experiments ± S.D. (n = 4). ITP, iontophoresis; EP, electroporation.

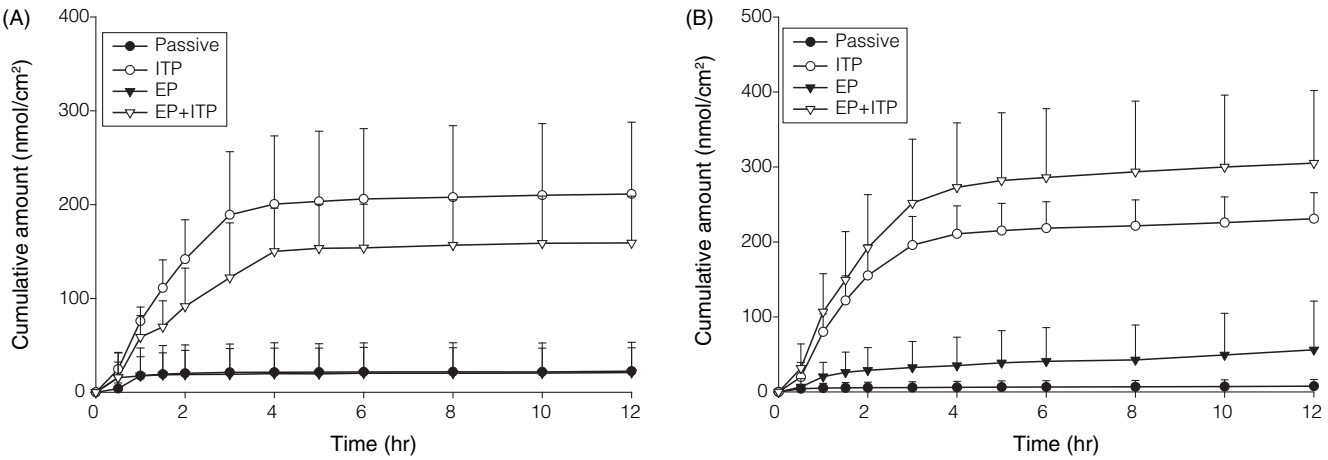

Figure 2. Cumulative amount versus time profile of (A) meloxicam sodium and (B) meloxicam potassium and enhanced by electrically assisted methods through HEM. All data represent the means of the experiments ± S.D. (n = 4). ITP, iontophoresis; EP, electroporation.

3 hr and 12 hr cumulative amounts of meloxicam potassium were both enhanced by electroporation by 23.43 and 34.45-fold when compared to those of passive control, respectively. Both the 3 hr and 12 hr PI of meloxicam potassium permeated through Wistar rat skin by electroporation were also higher than that of meloxicam sodium (*t*-test, $p < 0.05$), respectively.

Iontophoresis significantly enhanced the transport of meloxicam potassium through Wistar rat skin in terms of 3 hr cumulative amount (ANOVA test, $p < 0.05$). Moreover, at post-iontophoresis period (3 ~ 12 hr), the enhancing effect remained thus resulting in a significant enhancement of 12 hr cumulative amount. The 3 hr and 12 hr cumulative amounts of meloxicam potassium transported through Wistar rat skin enhanced by iontophoresis were similar to those of meloxicam sodium; however, both the 3 hr PI and 12 hr PI of meloxicam potassium were higher than those of meloxicam sodium. This phenomenon was due to a lower passive transdermal amount of meloxicam potassium than that of meloxicam sodium. (*t*-test for 3 hr and 12 hr cumulative amount, respectively, $p < 0.05$).

After 10 min of electroporation and 3 hr of iontophoresis, 3 hr cumulative amount of meloxicam sodium permeated through Wistar skin rat skin was similar to that enhanced by iontophoresis alone (ANOVA test, $p > 0.05$). This result was also found after 12 hr. The phenomenon was also found in the transdermal permeation of meloxicam potassium. It seemed that there was no synergistic effect of electroporation/iontophoresis in the enhancement of the two meloxicam salts permeated through Wistar rat skin.

(II) *HEM as the Barrier*

The result of passive permeation of meloxicam sodium through HEM was shown in Table 1 and Figure 2A. It was

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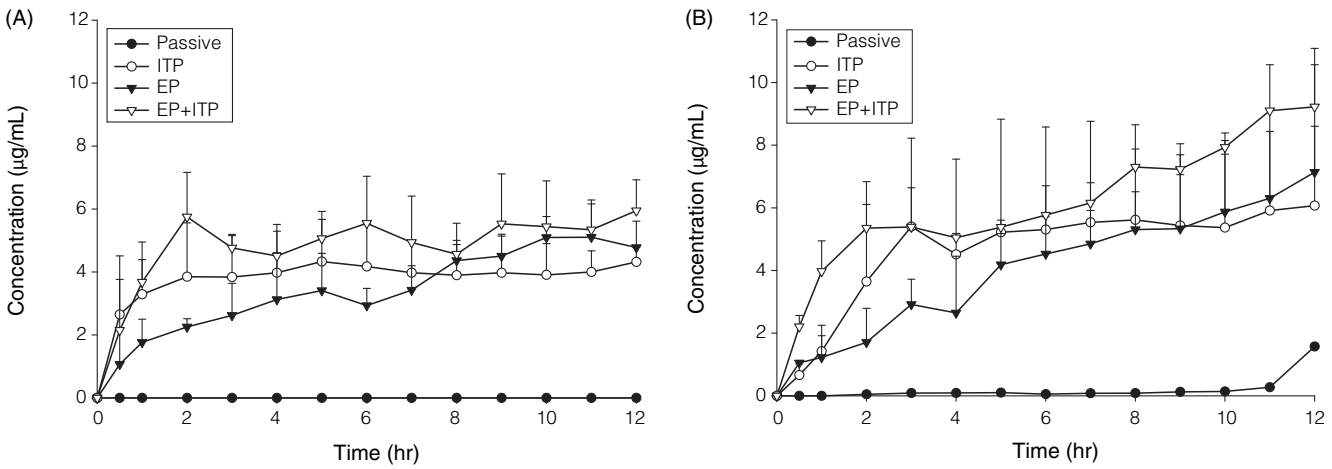

Figure 3. (A) meloxicam sodium and (B) meloxicam potassium concentration in plasma by treating electrically assisted methods with Wistar rat as an animal model. All data represent the means of the experiments ± S.D. (n = 4). ITP, iontophoresis; EP, electroporation.

observed that 3 hr cumulative amount of passive permeation was similar to that through Wistar rat skin (*t*-test, $p > 0.05$). After the application of iontophoresis, 3 hr and 12 hr PI were 8.82 and 9.32, respectively, and the enhancement was less than that of meloxicam sodium through Wistar rat skin. Electroporation, as discussed above, was found to reduce the transport of meloxicam sodium. Electroporation/iontophoresis could result in 3 hr PI of 5.70 and 12 hr PI of 7.02, and both were lower than those by iontophoresis. However, 3 hr cumulative amount of meloxicam sodium enhanced by electroporation/iontophoresis through HEM was similar to that enhanced by iontophoresis alone (ANOVA test, $p > 0.05$). The same was also found in 12 hr cumulative amount.

The cumulative amount of meloxicam potassium due to passive diffusion through HEM was lower than that of meloxicam sodium, but it showed no significant difference due to the variability of human skin. Iontophoresis greatly enhanced the cumulative amount of meloxicam potassium through HEM in terms of 3 hr PI of 33.79 and 12 hr PI of 30.49, even after iontophoresis ceased. When compared to the result of meloxicam sodium, electroporation produced higher 3 hr PI and 12 hr PI of meloxicam potassium. As the result of iontophoresis, electroporation/iontophoresis could enhance the transdermal permeation of meloxicam potassium in terms of 3 hr cumulative amount and 12 hr cumulative amount.

II. *In Vivo Permeation Experiments*

The plasma concentration-time curves of meloxicam salts after transdermal application of electrical enhancement are shown in Figure 3. Not only iontophoresis and combination protocol but also electroporation alone could significantly enhance the *in vivo* permeation of meloxicam sodium and meloxicam potassium when compared with the passive control in terms of 3 hr AUC, 12 hr AUC and C_{max} (ANOVA test, $p < 0.05$), as shown in Table 2. Combination protocol further enhanced the permeation of meloxicam salts and resulted in higher 3 hr AUC than electroporation did (ANOVA, $p < 0.05$). This phenomenon could also be found in 12 hr AUC of meloxicam potassium, but not in meloxicam sodium. Iontophoresis, however, resulted in the same enhancement with electroporation on potassium salt in terms of 3 hr and 12 hr AUC. It was also found that the combination protocol resulted in higher C_{max} of meloxicam potassium than that of meloxicam sodium (*t*-test, $p < 0.05$), while individual protocol had the same effect on both salts.

DISCUSSION

I. *In Vitro Permeation Experiments*

(I) *The Effect of Electroporation*

Iontophoresis is usually accompanied by electroporation, which can disorder the lipid bilayers of the skin and create new pathways into the skin and allow charged or neutral molecules transport through not only the subsistent appendageal pathway but also the new aqueous pathway under electric-field or by passive diffusion, thus resulting in enhanced transdermal transport[7]. Two skin barriers including the dorsal site of male Wistar rat skin and human epidermal membrane of breast site were used *in vitro* to elucidate the possible transdermal behavior of the sodium salt and potassium salt of meloxicam.

From the results mentioned above, it was found that the PIs of meloxicam potassium permeated through Wistar rat skin and HEM were generally higher than that of meloxicam sodium. The difference of transdermal behavior could be seen from the result of passive diffusion (*t*-test, $p < 0.05$). The electroporation, however,

Table 2. Parameters of the *in vivo* transdermal permeation of meloxicam sodium and meloxicam potassium enhanced by electrically-assisted methods with Wistar rat as an amimal model

Model drugs	Methods	0~3 hr AUC	0~12 hr AUC	C_{max} (μg/mL)	t_{max} (hr)
Meloxicam sodium	Passive	$0^{\beta\gamma\theta}$	$0^{\beta\gamma\theta}$	$0^{\beta\gamma\theta}$	NA
	ITP	$11.28 \pm 3.42^{\alpha\gamma}$	$46.67 \pm 14.44^{\alpha}$	$4.33 \pm 1.59^{\alpha}$	9.25 ± 3.4
	EP	$5.42 \pm 0.66^{\alpha\beta\theta}$	$41.26 \pm 7.21^{\alpha}$	$5.10 \pm 1.05^{\alpha}$	11 ± 1
	EP+ITP	$11.97 \pm 1.15^{\alpha\gamma}$	$57.51 \pm 10.42^{\alpha}$	$5.95 \pm 0.98^{\alpha}$*	9 ± 3
Meloxicam potassium	Passive	$0.09 \pm 0.01^{\beta\gamma\theta}$	$1.87 \pm 0.14^{\beta\gamma\theta}$	$1.57 \pm 0.10^{\beta\gamma\theta}$	12 ± 0
	ITP	$7.75 \pm 4.54^{\alpha}$	$56.42 \pm 26.96^{\alpha}$	$6.07 \pm 2.53^{\alpha}$	9 ± 5.2
	EP	$4.61 \pm 1.68^{\alpha\theta}$	$38.04 \pm 15.42^{\alpha\theta}$	$7.14 \pm 3.43^{\alpha}$	11.75 ± 0.5
	EP+ITP	$12.13 \pm 3.01^{\alpha\gamma,}$	$73.24 \pm 8.87^{\alpha\gamma}$	$9.23 \pm 1.86^{\alpha}$*	11 ± 1.41

Each value represents the mean ± S.D. (n = 4).
$^{\alpha\beta\gamma\theta}$ Data of one skin type and model drug were analyzed by ANOVA followed by Newman-Keuls test (significant level = 0.05).
$^{\alpha}$Significantly different from passive; $^{\beta}$significantly different from ITP; $^{\gamma}$significantly different from EP;
$^{\theta}$significantly different from EP+ITP.
*Data of two model drugs with the same skin type and method were significantly different from each other (*t*-test, $p < 0.05$).
AUC: area under curve; ITP: iontophoresis; EP: electroporation; NA: not available.

might retard the transdermal permeation of meloxicam sodium since the PI was below zero. Instead, the 3hr and 12hr cumulative amounts of meloxicam potassium through Wistar rat skin and 3hr cumulative amount of meloxicam potassium through HEM were significantly higher than those of meloxicam sodium, respectively (*t*-test, $p < 0.05$). This indicated that meloxicam potassium could benefit from the enhancement of electroporation.

Several physicochemical properties of the drugs, including charge, molecular weight, conformation and lipophilicity, could influence the drugs transport by electroporation[7]. Also, the mechanism, namely electrophoretic movement, diffusion and electroosmosis, of enhancement by electroporation should be considered. Meloxicam has a pKa of about 4.08 and almost ionized in citrate-phosphate buffer of pH 6.08. It has been suggested that the counter-ion in the formulation can influence the passive and iontophoretic permeation of the drug; however, the mechanism of how the counter-ion affects the permeation by electroporation remains unclear. Since the charge, M.W. and conformation of meloxicam sodium were similar to that of meloxicam potassium, the lipophilicity of the two salts was measured. The result showed that the partition coefficients (citrate-phosphate buffer, pH = 6.08/octanol) of meloxicam sodium and meloxicam potassium were 7.60 and 7.92, respectively, and the difference could merely explain the effect of electroporation on meloxicam sodium and meloxicam potassium. Further more, the protocol of electroporation applied in the study was 20 pulses within 10 min; hence the contribution of electrophoretic movement and electroosmosis could be negligible to molecules with low molecular weight like meloxicam sodium and meloxicam potassium. The enhancement of electroporation could be mainly due to the passive diffusion of meloxicam sodium and meloxicam potassium through the new pathway created by electroporation. According to the previous study, extraneous ions could influence the transdermal iontophoretic delivery of hydromorphone[10]. After the electrotransport of hydromorphone with a monovalent (Li^{+}) or a divalent cation (Ca^{+2}), the authors concluded that the difference of permeation behavior may result from the presence of more than one type of aqueous pathway or it may be due to differences in binding affinity of lithium and calcium to the charged sites within the pathway. However, we also observed this phenomenon in the presence of original and new pathways and two cations with equal valence. Since the transportation of the drug ion is in the form of ion-pair[10], our hypothesis was that the retention of hydrated sodium ion rather than potassium ion in the new pathways created by electroporation resulted in slower permeation of the meloxicam ion, and this result appeared to agree with the hypothesis of binding affinity.

(II) *Inter-species Difference*

In spite of the technologies of enhancement, the two salts showed different transdermal behavior in different barriers. It was reported that iontophoresis could reduce inter-species difference[11,12]. Our previous study showed that not only iontophoresis but also the combination of iontophoresis and electroporation could reduce interspecies difference in terms of flux and cumulative amount[13]. However, in some cases of both salts, iontophoresis and the combination protocol resulted in greater 3 hr and 12 hr cumulative amounts and 3 hr flux when permeating through Wistar rat skin than that through HEM (*t*-test, $p < 0.05$). Also, electroporation induced higher 12 hr cumulative amount of potassium salt perme-

ated through Wistar rat skin than that through HEM. This result suggested that the transdermal behavior of meloxicam sodium and meloxicam potassium through HEM was different from that through Wistar rat skin when enhanced by electrical protocols.

(III) *The Synergistic Effect of Electroporation/Iontophoresis*

A common strategy, the combination of iontophoresis and electroporation, was performed to produce synergistic effect on the enhancement of transdermal permeation. The synergistic effect was examined in this study. It was found that the enhancement of electroporation/iontophoresis of meloxicam sodium or meloxicam potassium respectively through Wistar rat skin was not significantly different from that of iontophoresis (ANOVA test, $p > 0.05$), and the same phenomenon was also found in two salts permeated through HEM. This indicated that electroporation/iontophoresis had no synergistic effect compared to iontophoresis alone on the enhancement of the transdermal delivery of the two meloxicam salts.

With the enhancement of the combination of electroporation/iontophoresis, meloxicam potassium seemed to be more facilitated than meloxicam sodium. The 3 hr and 12 hr cumulative amounts meloxicam potassium permeated through HEM and enhanced by combination protocol were significantly higher than that of meloxicam sodium (*t*-test, $p < 0.05$), but this result was not found in Wistar rat skin, which suggested that meloxicam potassium might have more advantage than meloxicam not only in oral administration but also in transdermal delivery enhanced by electrical protocols.

II. *In Vivo Permeation Experiments*

The synergistic effect of the combination of iontophoresis and electroporation was hardly found in the transdermal permeation of meloxicam salts. However, Our previous study demonstrated that the combination protocol could resulted in synergistic effect of enhancement of the *in vivo* transdermal permeation of indomethacin[13]. It was also found that electroporation alone could result in the same level of enhancement of iontophoresis and the combination protocol on meloxicam sodium in terms of 12 hr AUC (ANOVA test, $p > 0.05$). Although combination protocol had the same effect on both meloxicam salts in terms of 3 hr and 12 hr AUC (*t*-test, $p > 0.05$), it resulted in higher Cmax of potassium salt than that of sodium salt (*t*-test, $p < 0.05$). After the electrical protocols ceased, the concentration of potassium salt remained increasing, but not for sodium salt especially when enhanced by iontophoresis. The result indicated that meloxicam potassium might have more advantages than meloxicam sodium. The *in vivo* enhancement of electroporation was discrepant to that of *in vitro* study and further study is needed.

CONCLUSIONS

The present study established the iontophoretic and electroporatic properties of meloxicam salts through the transdermal experiments. Iontophoresis and the combination protocol enhanced the *in vitro* skin permeation of meloxicam sodium and meloxicam potassium and resulted in greater permeation than electroporation did, but there was also interspecies difference. Electroporation did not produce a synergistic enhancing effect when combined with iontophoresis *in vitro*. The *in vitro* transdermal permeation of meloxicam sodium might be depressed by electroporation while meloxicam potassium might profit from it, but this result was not found *in vivo*. Meloxicam potassium gained more from the combination of electroporation/iontophoresis than meloxicam sodium *in vitro*. Both meloxicam salts could benefit from iontophoresis, electroporation and the combination protocol, and meloxicam potassium might take more advantages from the combination protocol than meloxicam sodium *in vivo*.

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REFERENCES

1. Engelhardt, G., Homma, D., Schlegel, K., Utzmann, R. and Schnitzler, C. 1995. Anti-inflammatory, analgesic, antipyretic and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. Inflamm. Res. 44: 423-433.
2. Gupta, S. K., Bansal, P., Bhardwaj, R. K., Jaiswal, J. and Velpandian, T. 2002. Comparison of analgesic and anti-inflammatory activity of meloxicam gel with diclofenac and piroxicam gels in animal models: pharmacokinetic parameters after topical application. Skin Pharmacol. Appl. Skin Physiol. 15: 105-111.
3. Chang, J. S., Wu, P. C., Huang, Y. B. and Tsai, Y. H. 2006. *In vitro* evaluation of meloxicam permeation using response surface methodology. J. Food Drug Anal. 14: 236-241.
4. Fang, J. Y., Wang, R. J., Huang, Y. B., Wu, P. C. and Tsai, Y. H. 2000. Passive and iontophoretic delivery of three diclofenac salts across various skin types. Biol. Pharm. Bull. 11: 1357-1362.
5. Fang, J. Y., Wang, R. J., Huang, Y. B., Wu, P. C. and Tsai, Y. H. 2001. Influence of electrical and chemical factors on transdermal iontophoretic delivery of three diclofenac salts. Biol. Pharm. Bull. 24: 390-394.
6. Riviere, J. E. and Heit, M. C. 1997. Electrically-assisted transdermal drug delivery. Pharm. Res. 14: 687-697.
7. Denet, A. R., Vanbever, R. and Préat, V. 2004. Skin

electroporation for transdermal and topical delivery. Adv. Drug Deliv. Rev. 56: 659-674.

8. Banga, A. K., Bose, S. and Ghosh, T. K. 1999. Iontophoresis and electroporation: comparisons and contrasts. Int. J. Pharm. 179: 1-19.
9. Song, Y., Li, S. K., Peck, K. D., Zhu, H., Ghanem, A. and Higuchi, W. I. 2002. Human epidermal membrane constant conductance iontophoresis: alternating current to obtain reproducible enhanced permeation and reduced lag times of a nonionic polar permeant. Int. J. Pharm. 232: 45-57.
10. Phipps, J. B. and Gyory, J. R. 1992. Transdermal ion migration. Adv. Drug Deliv. Rev. 9: 137-176.
11. Phipps, J. B., Padmanabhan, R. V. and Lattin, G. A. 1989. Iontophoretic delivery of model inorganic and drug ions. J. Pharm. Sci. 78: 365-369.
12. Kanikkannan, N., Singh, J. and Ramarao, P. 2001. *In vitro* transdermal iontophoretic transport of timolol maleate: effect of age and species. J. Control. Release 71: 99-105.
13. Wang, R. J., Huang, Y. B., Wu, P. C. and Tsai, Y. H. 2007. The effects of iontophoresis and electroporation on transdermal delivery of indomethacin evaluated *in vitro* and *in vivo*. J. Food Drug Anal. 15: 126-132.