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Influence of low-frequency massage device on transdermal absorption of ionic materials

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Abstract

The influence of a low-frequency massage device on transdermal absorption of sodium benzoate, ketoprofen and diclofenac sodium was investigated in rats. Electrode pads spread with a hydroxypropyl cellulose gel containing the drug model were placed on excised skin in vitro. The transdermal permeation studies were carried out in the treatment group with the pulse applied through electrode pads spread with the gel, the pretreatment group with the gel applied after the application of the pulse and in the control group in which the gel was applied without the pulse. In vivo, transdermal absorption of ketoprofen was examined in the same groups used for the in vitro study. The pharmacokinetics of ketoprofen in plasma after intravenous injection was also studied. The treatment group showed higher cumulative permeated amounts of the drug models than the control in vitro. However, the enhancing effect was not observed in the pretreatment group. In vivo, the plasma ketoprofen level increased temporarily after the pulse was applied and then increased gradually as compared with the control. Since the distribution of ketoprofen from the central to the peripheral compartment was enhanced by the pulse in the injection study, enhancement of the biodistribution of ketoprofen by the low-frequency pulse was suggested.

Keywords: Low-frequency pulse; Low-frequency massage device; Skin permeation; Transdermal absorption; Enhancing effect

1. Introduction

Transdermal therapeutic forms have been used for the topical treatment of skin diseases. Recently, transdermal therapeutic systems, intended to deliver a drug across the skin for systemic activity, are receiving attention because they can: (1) avoid hepatic first-pass metabolism; (2) maintain blood drug levels for an extended period of time by controlling the drug-release rate; (3) interrupt drug-delivery easily (Yata, 1998). However, the stratum corneum is known to be a major barrier to drug permeation through the keratinized epithelia. Owing to the poor skin penetration of drugs, topical administration is often limited and not very effective (Lin et al., 1995). Since many drugs cannot permeate the skin in the amount necessary to cause

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therapeutic effects, prodrugs (Roberts and Sloan, 2000; Sung et al., 2000), chemical enhancers (Yamashita et al., 1993; Fang et al., 2003) and physical methods (Okabe et al., 1986; Tyle, 1986; Tashiro et al., 2000; Yoshida et al., 2000; Bose et al., 2001; Sugibayashi et al., 2001; Mori et al., 2003) have been used for enhancing transdermal drug transport. Iontophoresis, one such physical method, is a way to deliver ionic compounds to the body by an electrorepulsive force. The permeability of non-ionic compounds is also enhanced by iontophoresis via electroosmosis and convective water flow (Terzo et al., 1989; Sims et al., 1991; Hirvonen and Guy, 1998). Pulsed iontophoresis was reported to cause hardly any skin irritation and to enhance permeation (Okabe et al., 1986; Chien et al., 1990). Moreover, the permeation of peptides and compounds through the skin was reported to be enhanced by switching the polarity of the electrodes periodically (switching iontophoresis) (Tomohira et al., 1997; Ishikawa et al., 2002). However, for the development of transdermal therapeutic systems using iontophoresis, sufficient safety must be achieved using the current-supplying apparatus in addition to the drug reservoir.

A low-frequency massage device producing a low-frequency pulse (Sakai, 1996) corrects bioelectricity and induces continuous contraction and relaxation of muscles by providing an electric stimulus through the skin. The massage device has been approved as a medical device for the treatment of stiff shoulders and marketed widely. Since the safety of the massage device has already been proven, the possibility of its application to a transdermal drug-delivery system can be considered. However, few studies of the enhancing effect of the massage device on transdermal drug-delivery have been reported and many points still need to be investigated. Hence, in this study, the influence of the massage device on transdermal absorption of ionic materials was evaluated in vitro and in vivo and the usefulness of the massage device for transdermal drug-delivery was investigated.

2. Materials and methods

2.1. Materials

Sodium benzoate (MW = 144.11), ketoprofen (MW = 254.3) and diclofenac sodium (MW = 318.13) used as ionic drug models were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Acetonitrile (HPLC grade) was also purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Hydroxypropyl cellulose (type H, HPC-H) was obtained from Nippon Soda Co. Ltd. (Tokyo, Japan). All other chemicals were obtained commercially as the purest grade available.

2.2. Animals

Male Wistar rats weighing 200–260 g were purchased from Tokyo Laboratory Animals Science Co. Ltd. (Tokyo, Japan). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Hoshi University.

2.3. Low-frequency massage device

The Elepuls® HV-F125 (OMRON Corporation, Tokyo, Japan) used as a low-frequency massage device is shown in Fig. 1. The attached electrode pads contain-

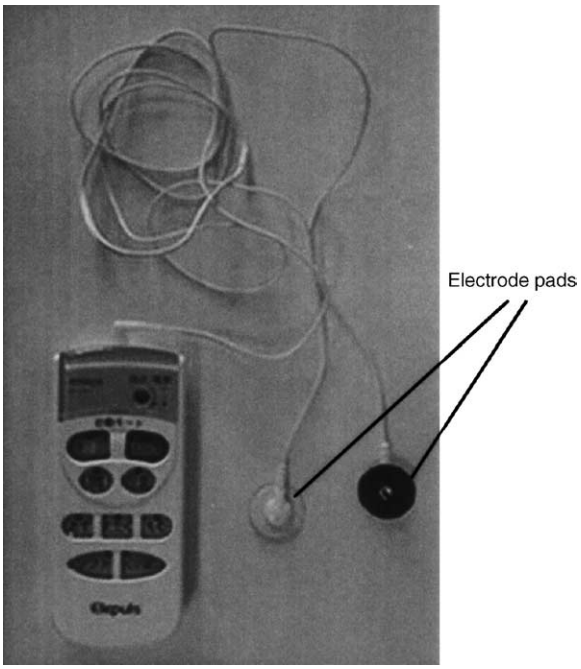

Fig. 1. Low-frequency massage device and electrode pads used for in vitro and in vivo studies.

Table 1
Characteristics of low-frequency pulse generated in three modes

	Mode		
	Push	Tap	Massage
Voltage (V)	1.5–2.0	0.5–2.0	1.0–2.0
Frequency (kHz)	1.0–4.0	<0.5	1.0–3.0
Switching interval (s)	3.0–6.0	<0.2	<3.0

ing carbon were circularly cut to a diameter of 2.2 cm. The adhesive, made of a cross-linked acrylic resin that was attached to the electrode pads at the time of purchase, was removed. The metal present at the center of the electrode was covered by vinyl tape with a diameter of 0.8 cm for insulation. The massage device has a function adjusting the stimulus intensity from 1 to 10. From the observation of the waveform by using an oscilloscope (CS-4025, KENWOOD Corporation, Tokyo, Japan), it was confirmed that voltage rose in proportion to the stimulus intensity. In this study, as standard use was assumed, the stimulus intensity was unified at 5 in vitro and in vivo. Moreover, the current application time was unified at 15 min according to the description indicating standard use. The massage device also has a function which can select either of three modes (push, tap or massage). The characteristics of the pulse in each mode measured by the oscilloscope are shown in Table 1. In all modes, switching polarity of the electrodes was confirmed. Since the studies of switching iontophoresis previously reported were carried out with switching at intervals of several minutes, the push mode, which had the longest switching intervals, was used in vitro and in vivo. The pulsed waveform generated in the push mode is shown in Fig. 2.

2.4. Preparation of gels containing drug models

Sodium benzoate, ketoprofen and diclofenac sodium were suspended in water at a concentration of 3% (w/w) under sonication for 30 min and stirred for 1 h. Then, HPC-H (10%, w/w) was added to the suspension and stirred until dissolved. A pH meter (pH BOY-P2, Shindengen Electric Manufacturing Co. Ltd., Tokyo, Japan) was used for measuring the pH of gels containing drug models.

2.5. In vitro transdermal permeation experiments

The acrylic diffusion cell used for permeation studies is shown schematically in Fig. 3 (Takahashi et al., 2001). After removal of the hair of rats using electric clippers, abdominal skin was excised and mounted on the diffusion cell with the epidermal side facing the electrode pads. The exposed skin area was 3.0 cm × 6.5 cm. The receiver side was filled with phosphate buffer (pH 7.4) and the receiver solution was magnetically stirred and maintained at 37 °C. The gels applied between electrode pads and the skin were 0.3 g each. The skin permeation studies were performed in three groups. In the treatment group, a pulsed electric current was applied through the electrode pads spread with the gel. In the pretreatment group, the gel was applied by using the electrode pads on the skin immediately after application of the pulse through the electrode pads attached with a cross-linked acrylic resin. In the control group, the electrode pads attached to the gel were applied without the pulse. A 200-μL aliquot of the receiver solution was sampled periodically over a period of 12 h and an equal volume of the buffer was added to compensate after each sampling. The

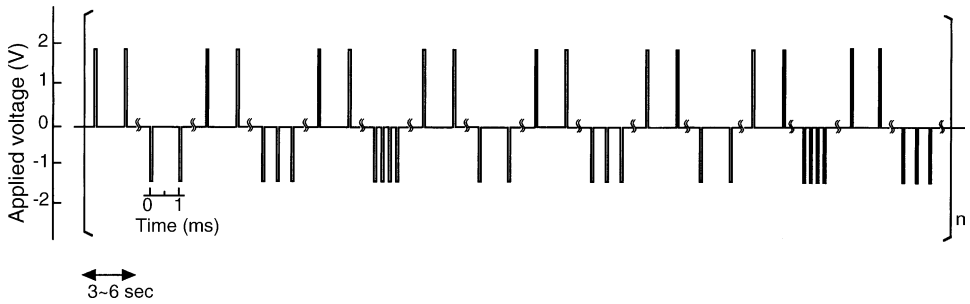

Fig. 2. Shape of the pulsed wave generated by the low-frequency massage device in the push mode.

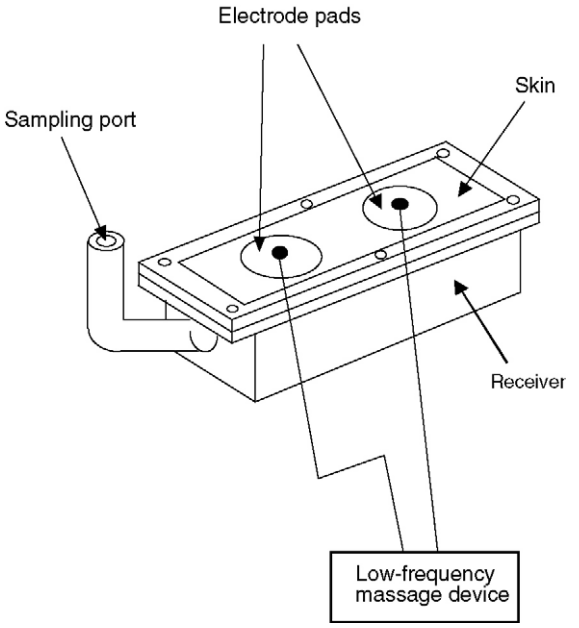

Fig. 3. Schematic representation of diffusion cell used for permeation studies.

concentration of the drug models in the receiver solutions was determined by HPLC after filtration by using Ekicrodisc® 13, 0.45 μm (Nihon Pall Ltd., Tokyo, Japan). The total amount of drug models that permeated through the unit skin area during 12 h was calculated. The flux was calculated from the slope of the linear portion of cumulative amount permeated–time plots for a zero-order model and expressed as the mass of drug models passing across 1 cm^2 of skin over time. The lag time was given by the *x*-intercept of the extrapolated linear portion.

2.6. HPLC assay

HPLC was carried out using an LC-6AD pump and a C-R7A plus chromatopac (Shimadzu, Kyoto, Japan) equipped with a Neopack C_{18} column (4.6 mm × 250 mm, Nishio Industry Co. Ltd., Tokyo, Japan) and a SPD-10AV UV detector (Shimadzu) set at 230 nm (benzoic acid), 254 nm (ketoprofen) and 283 nm (diclofenac). The mobile phases for benzoic acid, ketoprofen and diclofenac were a mixture of phosphate buffer (pH 7.4)–acetonitrile–phosphoric acid (60:40:0.2), a mixture of 0.02 M phosphoric acid–acetonitrile (65:35) and a mixture of 0.02 M phosphoric acid–acetonitrile (50:50), respectively. The flow rate was 1.0 mL/min. Chromatography was carried out at room temperature for benzoic acid and 30 °C for ketoprofen and diclofenac and the injection volume was 20 μL for benzoic acid and ketoprofen and 80 μL for diclofenac.

2.7. Determination of skin concentration of ketoprofen

In the in vitro experiments, the pieces of skin under the electrodes were excised and their weights were measured at 15 min after the application of the pulse through the electrodes spread with ketoprofen gel. The skin samples were cut and homogenized with phosphate buffered saline (PBS, pH 7.4) and were stored at 5 °C for 24 h to allow adequate extraction (Takehana et al., 2003). After centrifugation, the supernatant was injected into the HPLC system and the skin concentration of ketoprofen was determined and compared with that in the control group.

2.8. Determination of partition coefficient

The partition coefficients of sodium benzoate, ketoprofen and diclofenac sodium were determined using *n*-octanol and phosphate buffer (pH 7.4). The drug models were dissolved in phosphate buffer (4 μg/mL) and allowed to partition into equal volumes of *n*-octanol. The system was equilibrated in a shaker bath maintained at 37 °C for 24 h. After 24 h, the aqueous phase was separated and the concentration of the drug models in the aqueous phase was determined by HPLC. The partition coefficients (*P*) were calculated using the following equation:

$$P = \log\left(\frac{C_{\text{initial}} - C_{\text{aqueous}}}{C_{\text{aqueous}}}\right)$$

where C_{initial} is the initial concentration of the drug models in the aqueous phase and C_{aqueous} is the concentration of the drug models in the aqueous phase after equilibration.

2.9. In vivo transdermal absorption studies

Rats were intraperitoneally anesthetized with 25% (w/v) urethane in isotonic saline (4 mL/kg) and fixed on their backs. The hair of the abdominal skin was

removed carefully with electric clippers. The electrode pads spread with 0.3 g of ketoprofen gel were placed on the clipped region. The distance between the two electrode pads was 1 cm. The transdermal absorption of ketoprofen was examined in the same groups as in vitro. Blood samples of 300 μL were periodically collected from the jugular vein over a period of 4 h. After centrifugation, the plasma was separated and stored at −80 °C until analysis. The plasma concentration of ketoprofen was determined by HPLC. Methanol (200 μL) was added to the plasma (100 μL) and the mixture was centrifuged at 8000 rpm for 10 min after adequate agitation. Twenty microliters of the supernatant was injected into the HPLC system.

2.10. Studies of the effect of low-frequency pulse on the body distribution of ketoprofen

Rats were intraperitoneally anesthetized with pentobarbital sodium (40 mg/kg) and fixed on their backs. After the hair of the abdominal skin was removed, the left femoral aorta and the right jugular vein were catheterized with a polyethylene tube (PE-10). Ketoprofen dissolved in phosphate buffer (pH 7.4) at a concentration of 0.1 mg/mL was injected into the catheterized jugular vein (1 mL/kg). The pulsed electric current was applied through the electrode pads attached to a cross-linked acrylic resin immediately after the injection of the ketoprofen solution. As a control, the same amount of ketoprofen solution was injected without the pulse. A 200 μL aliquot of the blood sample was periodically collected from the femoral aorta over a period of 30 min after the administration of ketoprofen. To prevent coagulation, 100 μL of heparin sodium diluted three-fold with isotonic saline was infused through the tube before and after collecting the blood samples. The plasma concentration of ketoprofen was determined by HPLC. Methanol (100 μL) was added to the plasma (50 μL) and the mixture was centrifuged at 8000 rpm for 10 min after adequate agitation and the supernatant was injected into the HPLC system.

The time-course of the plasma concentration of ketoprofen was analyzed using the two-compartment model. The time-course of ketoprofen concentration is described by the following equation (Yamaoka et al., 1995):

$$C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$$

The parameters A, B, α and β in the equation were determined from experimental data by curve fitting using non-linear least squares. From the parameters, the area under the plasma concentration–time curve ($AUC_{0\rightarrow\infty}$), the distribution volume (V_d), the elimination rate constant (k_{10}) and the rate constants for exchange of drug between the central and peripheral compartments (k_{12}, k_{21}) were calculated.

3. Results and discussion

3.1. pH of the gels containing drug models

The pH of the sodium benzoate gel, the ketoprofen gel and the diclofenac sodium gel was 6.3, 6.2 and 6.8, respectively. Since the pK_a of ketoprofen listed in the drug information form prepared by Nichi-iko Pharmaceutical Co. Ltd. was 3.9 and the pK_a of benzoic acid and diclofenac sodium was 4.19, 4.0, respectively (O'Neil, 2001), the major fraction of the drug models in the gels existed as in the ionic state. In iontophoresis experiments, the ionization rate of drugs plays an important role in determining permeability through the skin. In this study, the three drug models were almost completely ionized, suggesting that the effect of differences in the ionization rate among the gels can be disregarded.

3.2. Influence of low-frequency massage device on skin permeation in vitro

The cumulative amount permeated–time profiles after application of the gels containing sodium benzoate, ketoprofen and diclofenac sodium are shown in Figs. 4–6, respectively. In the treatment group, the cumulative permeated amounts of the drug models were larger than those of the controls. Although it is known that the low-frequency massage device has the effect of increasing blood flow, the enhanced transdermal permeation in vitro was considered to be brought about by the electrorepulsive force of the pulsed electric current. After the current application for 15 min, the permeated amount of drug models increased gradually. Since it is considered that the skin concentration of the drug models was increased by the pulse, the skin ketoprofen concentrations were examined. The skin ketoprofen concentrations were 18.6 ± 0.6 μg/g in the

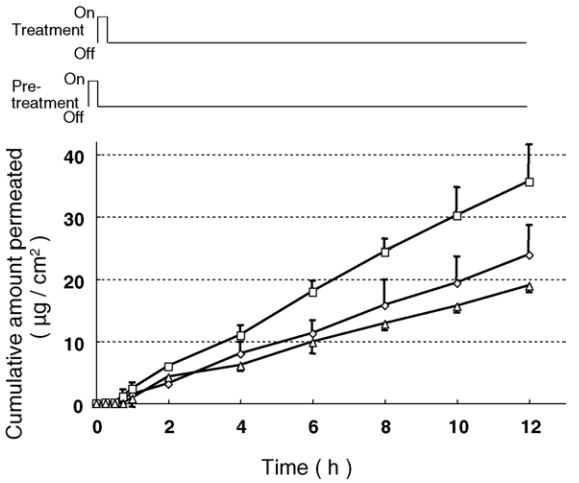

Fig. 4. Effect of low-frequency pulse on permeation of benzoate through rat skin. *Key:* control (◊); treatment (□); pretreatment (△). Each point represents the mean ± S.D. ($n = 3$).

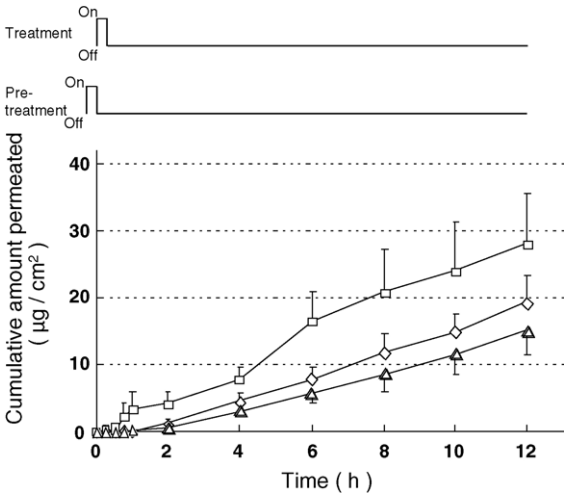

Fig. 6. Effect of low-frequency pulse on permeation of diclofenac through rat skin. *Key:* control (◊); treatment (□); pretreatment (△). Each point represents the mean ± S.D. ($n = 4$).

treatment group and 4.8 ± 1.0 μg/g in the control group (mean ± S.D., $n = 3$). Thus, it was confirmed that the application of the low-frequency pulse increased the skin concentration of ketoprofen.

The permeation parameters in each group are shown in Table 2. An increase of the cumulative amount permeated and a reduction of the lag time were observed in the treatment group. Two advantages of switching iontophoresis are considered to be the prevention of

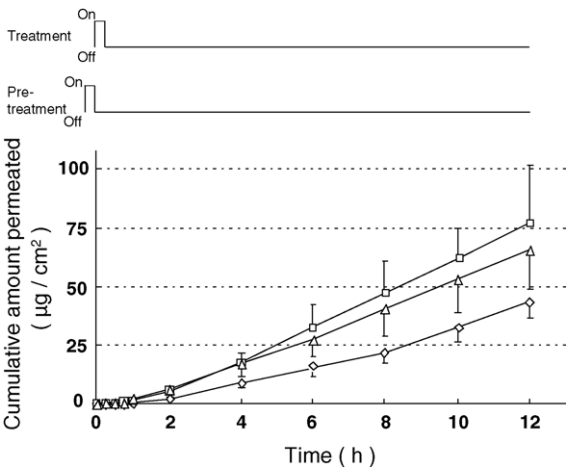

Fig. 5. Effect of low-frequency pulse on permeation of ketoprofen through rat skin. *Key:* control (◊); treatment (□); pretreatment (△). Each point represents the mean ± S.D. ($n = 3$).

polarization of the skin and skin hydration by polarity switching. Consequently, transdermal absorption of drugs is facilitated (Ishikawa et al., 2002; Sotoishi et al., 2003). The polarity of the pulsed electric current produced by the low-frequency massage device changes frequently. Though the polarization of the skin is prevented by frequent switching, a sufficient effect of the electrorepulsive force may not be achieved with the low-frequency pulse. In a previous report (Tomohira et al., 1997), iontophoresis of insulin at intervals of 20 min caused a blood glucose level reduction. However, no apparent effect of switching was observed with switching with intervals of 10 min. If the interval of switching is not sufficient for the transfer of the drug into the dermis, pull-back of the drug to the donor side by the switched current should be possible. It is necessary to consider a suitable drug for the low-frequency pulse switching with the intervals of 3–6 s. In this study, ketoprofen was the most suitable drug to be enhanced by the low-frequency pulse among the three materials. However, the low-frequency massage device was not much effective for diclofenac because the flux of diclofenac did not increase in the treatment group. It is known that the skin permeability of diclofenac is low. The C_{max} in human volunteers after transdermal administration of dicrofenac sodium gels (Voltaren® gel, 25 mg/body) was 1.3 ± 1.1 ng/mL according to the information provided by Novartis Pharma K.K. In

Table 2
Effect of low-frequency pulse on permeation parameters of three drug models through rat skin in vitro

Drug model	Condition	Cumulative amount permeated (μg/cm^2)	Flux (μg/(cm^2 h)	Lag time (h)
Sodium benzoate	Control	23.8 ± 4.7	2.0 ± 0.1	0.3 ± 1.0
	Treatment	35.6 ± 6.0	2.8 ± 1.0	0.2 ± 0.6
	Pretreatment	19.0 ± 1.2	1.5 ± 0.1	0.3 ± 0.1
Ketoprofen	Control	43.7 ± 7.2	11.0 ± 2.2	4.0 ± 1.0
	Treatment	77.5 ± 14.4*	14.9 ± 5.0	1.6 ± 1.2
	Pretreatment	61.0 ± 16.6	13.4 ± 1.4	2.5 ± 1.3
Diclofenac sodium	Control	19.3 ± 4.1	3.8 ± 0.7	2.0 ± 1.0
	Treatment	28.1 ± 7.3	3.6 ± 1.0	0.7 ± 0.5
	Pretreatment	15.2 ± 3.8	3.3 ± 0.8	2.5 ± 0.9

Each value represents the mean ± S.D. (n = 3–4). Statistical analysis was performed using the Student's t-test
* $p < 0.05$ vs. control.

contrast, the C_{max} in human volunteers after transdermal administration of ketoprofen tapes (Ketok® tape, 20 mg/body) was 135.85 ± 18.02 ng/mL according to the information provided by Nichi-iko Pharmaceutical Co. Ltd. It is considered that the penetration of diclofenac into the depths of skin is not effortless. The frequent switching of the polarity will be disadvantageous for the diclofenac penetration because the transfer of the diclofenac molecules into the dermis is interrupted. Besides, the voltage produced by the low-frequency massage device was low as compared with that in the iontophoresis reported previously (Okabe et al., 1986; Nakakura et al., 1996; Fang et al., 2001).

The octanol–water partition coefficient (P) is the most important parameter in determining mucosal and percutaneous absorption (Lien et al., 1971). The log P-values of sodium benzoate, ketoprofen and diclofenac sodium determined in this study were −0.81, 0.26 and 1.54, respectively. A parabolic dependence is often found between skin permeation and log P and ketoprofen absorbed favorably through human skin as it has the optimal P-value (Yano et al., 1986; Beetge et al., 2000). The ratios of the cumulative amount permeated in the treatment group to that in the control group were 1.49, 1.77 and 1.46 for sodium benzoate, ketoprofen and diclofenac, respectively. Hence, it is considered that the optimal P-value of ketoprofen is reflected in the higher enhancing effect by the low-frequency pulse.

In the pretreatment group, the cumulative amount of ketoprofen tended to increase and the cumulative amount of sodium benzoate and diclofenac sodium tended to decrease as compared with the control, though no significant differences were shown. It was reported that skin hydration was facilitated by switching iontophoresis and the hydration played an important role in the enhancement of skin permeation (Ishikawa et al., 2002). In this study, the electrodes with a cross-linked acrylic resin which contained a small amount of water were used for the pretreatment studies. For the skin hydration, the amount of water in the donor should be important. Then, it was considered that the skin permeation of ketoprofen was increased in consequent of the skin hydration by the pretreatment of the low-frequency pulse, though the skin hydration was too little to enhance the permeability of diclofenac. Since sodium benzoate is a hydrophilic material, it will be penetrated through a pore pathway. In this study, the skin permeability of benzoate tended to decrease as compared with control. It was considered that the condition of the pore, such as charge or gaps might be changed by the pretreatment of the low-frequency massage device. To clarify the skin condition after the pretreatment of the low-frequency pulse, further investigations will be needed.

3.3. *Effect of low-frequency pulse on the transdermal absorption and body distribution of ketoprofen*

The plasma ketoprofen level–time profiles on transdermal absorption in vivo are shown in Fig. 7. In the treatment group, the plasma ketoprofen level increased immediately and then, though the level decreased temporarily, the gradual increase was observed as compared with the control. The plasma ketoprofen level at 15 min after the application of the low-frequency pulse

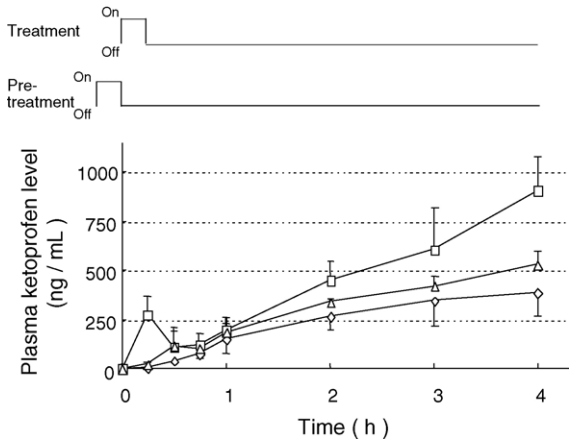

Fig. 7. Effect of low-frequency pulse on transdermal absorption of ketoprofen in vivo. *Key:* control (◊); treatment (□); pretreatment (△). Each point represents the mean ± S.D. ($n = 3$).

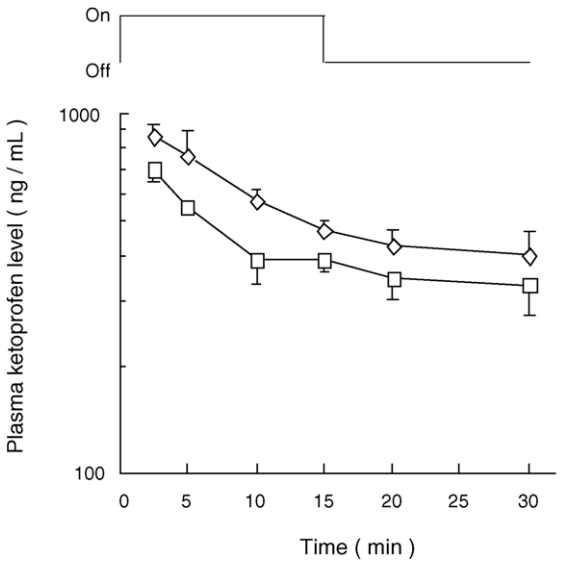

Fig. 8. Effect of low-frequency pulse on plasma concentration of ketoprofen after i.v. injection to rats. *Key:* control (◊); treatment (□). Each point represents the mean ± S.D. ($n = 3$).

was approximately 250 ng/mL. In contrast, ketoprofen was hardly detected at 15 min in the control group. As the reason for the temporal increase in the treatment group, it was considered that the pulsed electric current enhanced the initial absorption and the body distribution of ketoprofen by increasing blood flow.

To investigate the effects of the low-frequency pulse on the distribution of ketoprofen, ketoprofen solution was injected into rats. The plasma ketoprofen level–time profiles after intravenous (i.v.) injection are shown in Fig. 8. In the treatment group, the plasma ketoprofen level decreased significantly as compared with the control. Pharmacokinetic parameters of ketoprofen in the two-compartment model after i.v. injection are shown in Table 3. From the result, the increase of k_{12} and k_{21} with the application of the pulse was confirmed. Since the enhancement of the drug distribution between the central and the peripheral compartment was shown, it was suggested that the temporal decrease of plasma ketoprofen level in vivo was due to increase of distribution volume by improvement of the blood flow. However, the above mention cannot explain the initial increase of the plasma ketoprofen level. Then, it is considered that the blood flow in the skin under the electrode is increased and absorption of ketoprofen is enhanced at the beginning of the experiment and then the ketoprofen distribution to the peripheral compartment is enhanced by the application of the low-frequency pulse.

Table 3
Effect of low-frequency pulse on the pharmacokinetic parameters of ketoprofen in two-compartment model after i.v. injection to rats

Parameter	Control	Treatment
$AUC_{0\to\infty}$ ((h ng)/mL)	2141.5 ± 2381.4	2619.1 ± 2491.8
V_d (mL)	40.4 ± 7.4	48.1 ± 7.6
k_{10} (h^{-1})	1.0 ± 0.9	0.7 ± 0.7
k_{12} (h^{-1})	3.0 ± 1.2	4.7 ± 2.6
k_{21} (h^{-1})	5.3 ± 1.5	7.7 ± 3.2

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

In the pretreatment group, though the plasma ketoprofen levels increased slightly as compared with the control (Fig. 7), no significant improvement was observed. It is considered that the transdermal absorption of ketoprofen should be enhanced because the blood flow in the skin under the electrode is increased by the application of the low-frequency pulse. However, since the result was a disappointment, it is considered that the blood flow-increasing effect in the skin under the electrode is generated when the low-frequency pulse is applied and the effect is immediately lost after the pulse stops. It remains possible that the influence of the low-frequency pulse on the transdermal drug-delivery can be controlled by changing the application conditions, such as the stimulus intensity and the application area. Further investigations will be required

to clarify the influence of the application conditions on the skin permeability and the body distribution.

4. Conclusions

It was confirmed by in vitro and in vivo studies that the pulsed electric current produced by a low-frequency massage device enhanced the transdermal delivery of ketoprofen. However, no significant effect was achieved by pretreatment with the pulse. The plasma ketoprofen level was increased temporarily and then the gradual increase was observed by application of the pulse in vivo, because the blood flow in the skin under the electrode might be increased and then the ketoprofen distribution to the peripheral compartment was enhanced. Since the low-frequency massage device achieved rapid absorption and distribution of drugs in the body after transdermal application, it may be useful as an enhancing method for drugs required rapid and prolonged effect, such as an analgesic and a sedative. Further investigations of the influence of the application conditions, such as the stimulus intensity and the application area, will make it possible to optimize the extent of the enhancement of the drug permeability and the body distribution by using the low-frequency massage device.

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