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# Transdermal iontophoresis of sodium nonivamide acetate V. Combined effect of physical enhancement methods

Jia-You Fang [a,b], Tsong-Long Hwang [a], Yaw-Bin Huang [b], Yi-Hung Tsai [b,*]

[a] *Graduate Institute of Natural Products, Chang Gung University, Tao-Yuan, Taiwan*
[b] *School of Pharmacy, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan*

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**Abstract**

The effect of iontophoresis combined with treatment of other physical enhancement methods such as electroporation, low frequency ultrasound, and erbium:YAG (yttrium–aluminum–garnet) laser on the transdermal delivery of sodium nonivamide acetate (SNA) was examined in this present study. Iontophoresis increased the transdermal flux of SNA in vitro as compared to the passive diffusion without any enhancement. Furthermore, iontophoresis was always the most potent enhancement method for SNA permeation among the physical enhancement methods tested. Pulsing of high voltages (electroporation) followed by iontophoresis did not result in increased transport over iontophoresis alone. However, electroporation shortened the onset of transdermal iontophoretic delivery of SNA. Pretreatment of low frequency ultrasound (sonophoresis) alone on skin did not increase the skin permeation of SNA. The combination of iontophoresis and sonophoresis increased transdermal SNA transport more than each method by itself. The enhancement of drug transport across shunt routes and reduction of the threshold voltage in the presence of an electric field may contribute to this synergistic effect. Use of an erbium:YAG laser was a good method for enhancing transdermal absorption of SNA because it allows precise control of stratum corneum (SC) removal, and this ablation of SC could be reversible to the original normal status. The combination of laser treatment and iontophoresis also synergized the skin permeation of SNA, possibly due to a gradual drop in the electric resistance of the skin. The results in this present study point out that the choice of certain conditions with suitable physical enhancement methods can induce a synergistic effect on transdermal delivery of SNA during iontophoresis. 

*Keywords:* Sodium nonivamide acetate; Transdermal absorption; Iontophoresis; Electroporation; Sonophoresis; Erbium:YAG laser

## 1. Introduction

Sodium nonivamide acetate (sodium *N*-nonanoyl vanillylamide-4′-*O*-acetate; SNA; $C_{19}H_{28}NO_5Na$) is a newly designed derivative of capsaicin (Fang et al., 1995). The antinociceptive potency of this sodium salt was 1.75 and 27.50

* Corresponding author. Tel.: +886-7-27079823; fax: +886-7-27074804.
*E-mail address:* fajy@ms9.tisnet.net.tw (Y.-H. Tsai).

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times that of capsaicin and indomethacin (Chen et al., 1992). SNA could be extensively used in clinical therapy because of the lack of pungent skin sensation and burning pain which had been found in capsaicin to improve patients' compliance (Fang et al., 1997b). Previous investigations have suggested that the poor pharmacological effects of capsaicinoids following oral administration was due to the first-pass metabolism (Sietsema et al., 1988; Donnerer et al., 1990). Hence, transdermal drug delivery was suitable to be selected for SNA to accomplish better bioavailability.

Although transdermal drug delivery has the potential to be a noninvasive method of delivering drugs, its clinical use has found limited application due to the remarkable barrier properties of the skin's outermost layer, the stratum corneum (SC). Methods for transdermal enhancement such as permeation enhancers and iontophoresis (electric current density application) have been utilized to overcome the poor permeability of SNA (Fang et al., 1996a,b, 1998; Wu et al., 1995). The permeation enhancers and iontophoresis can be determined as the chemical and physical enhancement methods of transdermal delivery, respectively. These two methods have been shown to work synergistically when applied simultaneously for SNA permeation (Fang et al., 1997a). A variety of physical enhancements include electroporation, sonophoresis and laser irradiation have been successfully used. In this present study, a series of physical enhancement methods is selected to combine with transdermal iontophoretic delivery of SNA to elicit additive or synergistic effect.

Skin electroporation takes place at high voltages ( $\geq 100$ V) and is associated with micropore formation in the skin structure (Prausnitz, 1996). The use of electroporation in conjunction with iontophoresis can possibly enhance transport, allow rapid delivery of a bolus dose, and allow further control on modulation or programmability of delivery (Chang et al., 2000). Application of sonophoresis (ultrasound) reduces the required current in the presence of electric fields to achieve the desired drug flux (Le et al., 2000). Lasers have long been used for medical diagnosis and therapeutic purposes. The laser has been suggested for the controlled ablation or removal of SC in human skin (Marcells and Ellis, 2000). Because of the overall hydrophobic characteristic and net negative charge of SC, transdermal delivery of negatively charged drugs such as SNA is especially challenging. The skin permeation of SNA may be largely enhanced by laser irradiation alone or combination with iontophoresis. The aim of this study is to investigate the influence of other physical enhancement methods on the transdermal absorption of SNA across nude mouse skin during iontophoresis. The SNA permeation data of the combined methods was also elucidated to explain the possible mechanisms.

## 2. Materials and methods

### *2.1. Materials*

The synthetic procedure of SNA has been performed in our laboratory and reported earlier (Fang et al., 1995). All other chemicals and solvents were of analytical grade. All solutions were prepared in deionized bidistilled water purified in a Simplicity® water system (Millipore Co., USA).

### *2.2. Iontophoretic instruments and in vitro permeation procedures*

The in vitro permeation procedures of iontophoresis were determined using horizontal glass diffusion cells. The dorsal skin of excised female nude mouse (6 weeks old) was used as the model membrane. The receptor phase, containing 8 ml of 0.06 M; pH 7.4 citrate–phosphate buffer was used. The donor compartment of cell was filled with 8 ml of 0.06 M; pH 4.2 buffer containing 200 μg/ml SNA. The pH 4.2 selected was to neutralize skin's negative charges so as to avoid the interruption of skin charges during iontophoresis. The available diffusion surface area was 0.785 $cm^2$. The cells were agitated by magnetic stirrers at 600 rpm. A pair of Ag/AgCl wires, having an effective length of 15 mm, used as electrodes, were immersed in the cell with the cathode in the donor and the anode in the receptor. The electrodes were connected to a current power supplier (Yokogawa

Co., Model 7651, Japan). Current density of 0.5 mA/cm$^2$ was applied to stimulate the permeation of SNA. The 200 μl samples were withdrawn from the receptor at regular intervals and immediately replaced by an equal volume of fresh receptor solution. The samples were assayed by HPLC as described previously (Fang et al., 1995).

### 2.3. Electroporation protocols

Electroporation was performed using an exponential decay pulse generator (BTX Co., ECM 630 Electro Cell Manipulator®, USA). The platinum electrodes ($1 \times 2$ cm$^2$) were used, each located 3.0 cm from the skin. The cathode was positioned in the donor compartment, while the anode was in the receptor compartment, unless otherwise noted. Electroporation protocol was 1 pulse per 30 s, applied for 10 min. The pulse voltage was 300 V and pulse length was 200 ms. Voltages are expressed as applied values but not transdermal values. After 10 min of electroporation of skin, the iontophoresis was then applied in the in vitro experiments.

### 2.4. Sonophoresis protocols

The nude mouse skin was pretreated by ultrasound for 2 h prior to in vitro permeation experiments (Fang et al., 2001). Low frequency ultrasound was applied with a sonicator (Sonics and Materials Co., VCX 600, USA) with a transducer. The radiating diameter of transducer was 13 mm. The frequency was set at 20 kHz, and the estimated skin intensity was 0.2 W/cm$^2$. The ultrasound transducer was located approximately 0.5 cm from the surface of the skin. The coupling medium was pH 7.4 citrate–phosphate buffer.

### 2.5. Erbium:yttrium–aluminum–garnet (YAG) laser irradiation protocols

An erbium:YAG laser (Continuum Biomedical Co., USA) was used for pretreating skin in the present study. The laser has a wavelength of 2940 nm and a pulse duration of 250 μs. An articulated arm was used to deliver the laser beam onto the skin. Output energies of 0.35, 0.45, 0.55, and 0.60 J per pulse with a beam spot size of 7 mm in diameter would achieve fluences of 0.91, 1.17, 1.43, and 1.56 J/cm$^2$, respectively. The energy of laser pulse was monitored with an energy meter (Nova Display Co., Israel) before and after pretreatment. The laser hand-piece was located approximately 3.0 cm from the surface of the skin. Then the skin was irradiated by various doses of the laser pulse. After laser pretreatment, the skin surface was wiped with a cotton wool swab several times. Then the irradiated skin was mounted in the horizontal diffusion cells to perform in vitro permeation experiments.

### 2.6. Histological examination of skin

Histological changes in the nude mouse skin were examined after laser irradiation. Immediately after pretreatment of laser, a specimen of the exposed area was taken for histological examination. The adjacent untreated skin area was also assessed as the control. Each specimen was fixed in 10% pH 7.4 buffered formaldehyde solution for at least 48 h. The specimen was cut vertically against the skin surface. Each section was dehydrated using ethanol and then embedded in paraffin wax, stained with hematoxylin and eosin. In each skin sample, three different sites were examined and evaluated under an optiphoto light microscopy. The thickness of SC and epidermis of skin was determined.

### 2.7. Data analysis

In the in vitro permeation study, the total amount of drug permeating across the unit diffusion surface and into the receptor was calculated and plotted as a function of time. The flux was calculated by the slope of the linear portion of cumulative amount-time plots for pseudo zero-order model and expressed as the mass of drug passing across 1 cm$^2$ of skin over time. In the experiments of laser irradiation, the laser ablation pretreated the limited areas of SC by 49.02% of the total permeated skin surface area. Consequently the cumulative amount and flux data of the laser-irradiated area was extrapolated to an area of 100% ablation of permeated area.

The statistical analysis of the difference between different treatments was detected by using the unpaired Student's $t$-test. The 0.05 level of probability was taken as the level of significance. The ANOVA test was also utilized in this study.

## 3. Results and discussion

### 3.1. Iontophoresis combined with electroporation

The electric techniques of iontophoresis and electroporation can be used to enhance transdermal drug delivery. Iontophoresis applies a small low voltage (typically 10 V or less), continuous constant current (typically 0.5 mA/cm$^2$ or less) to push a charged drug into skin or other tissue. In contrast, electroporation applies a high voltage (typically $\geq 100$ V) pulse for a very short (μs to ms) duration to permeabilize the skin (Banga et al., 1999). Fig. 1 shows the cumulative amount of SNA (μg/cm$^2$) in the receptor compartment as a function of time with or without iontophoresis and electroporation. The slopes of the resulting plots were computed and the fluxes (μg/cm$^2$/h) were calculated from the slopes (Table 1). These profiles correspond well to the pseudo zero-order kinetics. As can be seen, there was no passive permeation (in the absence of electric enhancement) of SNA across nude mouse skin during a 6 h period. Application of iontophoresis, 0.5 mA/cm$^2$ for 6 h, greatly enhanced the transdermal SNA permeation from a flux of 0–23.02 μg/cm$^2$/h. The voltage (300 V), time constant (200 ms), and number of pulses (20 times) used in the electroporation experiments were selected because they had been previously used to provide dramatic enhancement of transdermal permeation of nalbuphine (Sung et al., 2001). According to the terminology cited by Prausnitz et al. (1996), forward polarity pulses correspond to the negative electrode in the donor and the positive electrode in the receptor. In contrast, alternating polarity pulses were applied such that the electrode polarity alternated with each pulse. As shown in Fig. 1, alternating polarity electroporation did not in-

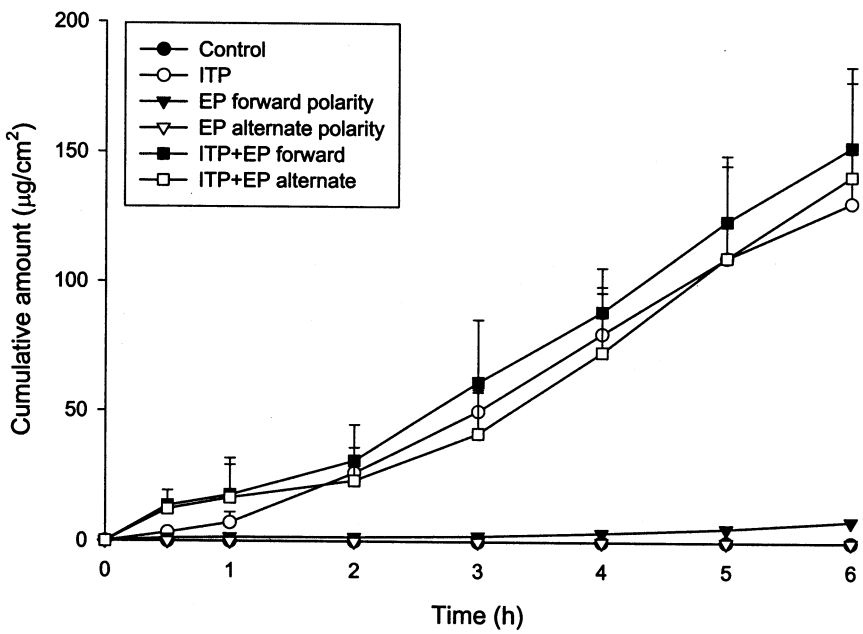


Fig. 1. Cumulative amount of SNA detected in the receptor compartment versus time by treating 0.5 mA/cm$^2$ iontophoresis combined with 300 V, 200 ms electroporation. All data represent the means of four experiments ± S.D. ITP, iontophoresis; EP, electroporation.

Table 1
The flux of SNA of various physical enhancement formulations

| Formulation | Flux ($\mu g/cm^2/h$) |
|---|---|
| Control | 0 |
| ITP[a] 0.5 $mA/cm^2$ | $23.02 \pm 2.37$ |
| EP[b] 300 V forward polarity | $1.14 \pm 0.23$ |
| EP 300 V alternate polarity | 0 |
| ITP+EP forward polarity | $25.26 \pm 3.56$ |
| ITP+EP alternate polarity | $22.68 \pm 7.91$ |
| Sonophoresis 0.2 $W/cm^2$ | 0 |
| ITP+Sonophoresis | $35.06 \pm 11.52$ |
| Laser 0.35 J | $1.66 \pm 0.67$ |
| Laser 0.45 J | $1.68 \pm 0.09$ |
| Laser 0.55 J | $0.94 \pm 0.43$ |
| ITP+laser 0.35 J | $32.55 \pm 4.58$ |
| ITP+laser 0.45 J | $34.90 \pm 3.27$ |
| ITP+laser 0.55 J | $17.76 \pm 1.75$ |

Each value represents the mean $\pm$ SD ($n = 4$).
[a] ITP, iontophoresis.
[b] EP, electroporation.

crease the passive permeation of SNA across skin. On the other hand, forward polarity resulted in the flux increased from 0 to 1.14 $\mu g/cm^2/h$.

The mechanism of transport is different for these two electric enhancement techniques (Banga et al., 1999). Iontophoresis is known to act primarily on the drug by an electrophoretic drift. The transfollicular and transappendageal (shunt) routes constitute the major penetration pathways for SNA passive diffusion (Fang et al., 1995). The permeation of ionic drugs by the shunt routes can be facilitated by the application of iontophoresis (Tyle, 1986). Accordingly the pathways for iontophoresis of SNA appear to be mainly follicles and other shunts. Electroporation involves the application of high voltage pulses which create transient aqueous pathways in cell membranes or lipid bilayers and permit transport of drugs across these pathways (Jadoul et al., 1998). As pores appear, molecules are rapidly moved across the pores by electrophoresis due to the local field. A previous study suggested that electroporation could enhance the skin permeation of nalbuphine both by micropores formation and electrophoretic movement under the same conditions as in the present study (Sung et al., 2001). The limited enhancement of SNA permeation by high voltage pulse treatment suggested that pore formation by skin electroporation could not be enough for SNA to transport across skin.

The influence of the polarity of the electrodes was also evaluated. Forward polarity pulsing can cause structural changes in the skin, possibly due to electroporation, as well as more drugs across the skin by electrophoresis through both previously existing and newly created pathways (Prausnitz et al., 1996). In contrast, alternating polarity pulsing can cause creation of structural changes in the skin, but has a zero total time-integral of voltage. Table 1 shows that forward polarity pulsing resulted in the SNA flux of 1.14 $\mu g/cm^2/h$; however, no cumulative amount of SNA was detected during the 6 h after alternating polarity pulsing. This result may indicate that the contribution of electroporated skin was negligible for SNA transport across skin, while electrophoretic movement was important for SNA after electroporation treatment.

Iontophoresis and electroporation showed different influences and mechanisms on the skin permeation of SNA. The combined use of these two electric enhancement methods may also result in some interesting results. No significant increase of SNA flux was provided by application of either forward polarity pulsing or alternating polarity pulsing prior to iontophoresis (ANOVA test, $P > 0.05$). Since the direct electrophoretic force by pulsing was the main mechanism acting on the SNA permeation across skin, this may suggest that the enhancement due to transdermal iontophoresis was so large that the enhancement due to 10 min electroporation treatment was small in comparison. Although there was no significant difference ($t$-test, $P > 0.05$) between the SNA flux of iontophoresis combined with electroporation and iontophoresis alone, the cumulative amount of SNA after 0.5 h application was significantly increased when treating electroporation prior to iontophoresis (ANOVA test, $P < 0.05$) (Fig. 1). This may indicate that electroporation accelerated the onset of transdermal iontophoresis of SNA. Typical iontophoretic exposure ($< 5$ V) might only be sufficient to electroporate a few bilayers of skin, perhaps affecting the lining of appendages (Chizmadzhev et al., 1995; Prausnitz, 1996). Ap-

plication of electroporation may amplify this effect, resulting in the increase of SNA cumulative amount of the initial stage of permeation. Another explanation is that the initial 0.5 h (30 min) application of SNA permeation includes 10 min electroporation and then following 20 min iontophoresis, the electrophoretic force by combination of both methods may be larger than that by iontophoretic treatment alone.

### 3.2. Iontophoresis combined with sonophoresis

Fig. 2 shows the influence of ultrasound on the in vitro permeation of SNA. The ultrasound was continuously pretreated to skin for 2 h with intensity of 0.2 W/cm$^2$, SNA was then applied to donor compartment for in vitro permeation. The result demonstrated that the low frequency ultrasound was not effective in enhancing the skin permeation of SNA. Our previous study showed that the transdermal flux and cumulative amount retained in skin of clobetasol 17-propionate could be enhanced significantly after ultrasound treatment under the similar conditions of this present experiment (Fang et al., 1999). This indicates that sonophoresis is a physical enhancement method for certain molecules under specific conditions (Meidan et al., 1995). It has been reported that the mechanism of sonophoretic enhancement is cavitation within the SC, a phenomenon that disorders the SC lipid bilayers (Mitragotri et al., 1996). Hydrophilic drugs such as SNA are not considered to be enhanced by sonophoresis since they may permeate the skin through aqueous pathways and, hence, would not be substantively affected by bilayer disordering (Johnson et al., 1996). It has also been shown in vitro that the skin absorption of molecules by ultrasound treatment can increase with increasing molecular weight (Meidan et al., 1995).

As shown in Fig. 2, a combination of iontophoresis and low frequency ultrasound offers significant enhancement of SNA flux over either of them alone ($t$-test, $P < 0.05$). It has been proposed that shunt routes are more susceptible to ultrasonic enhancement than are transcellular processes (Brucks et al., 1989; Meidan et al., 1995). Our previous study also suggested that ultrasound could enhance drug permeation through hair follicles to a greater extent than

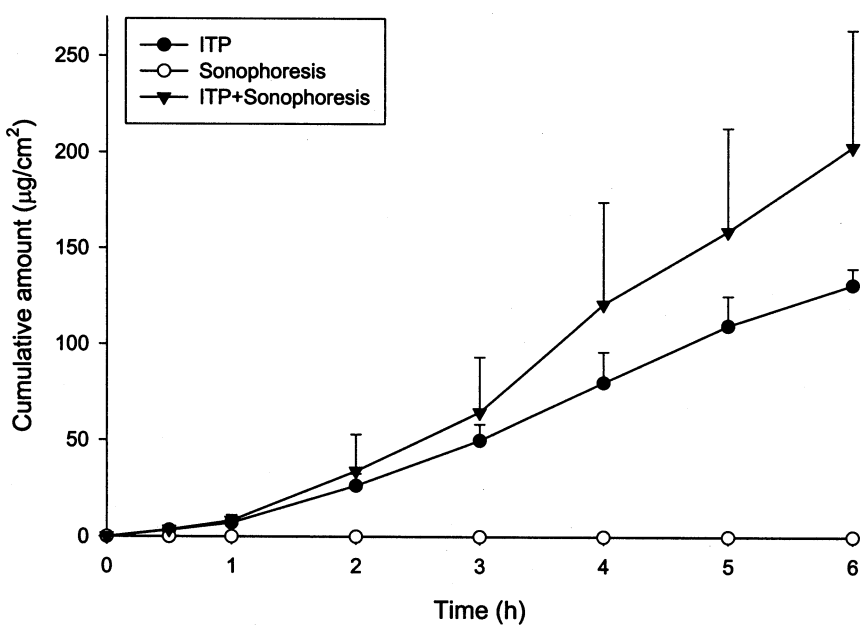


Fig. 2. Cumulative amount of SNA detected in the receptor compartment versus time by treating 0.5 mA/cm$^2$ iontophoresis combined with 0.2 W/cm$^2$ sonophoresis. All data represent the means of four experiments ± S.D. ITP, iontophoresis.

Table 2
SC and epidermal thickness (μm) of nude mouse skin treated by erbium:YAG laser

| Laser energy (J) | Fluence (J/cm$^2$) | SC thickness | Epidermal thickness |
|---|---|---|---|
| 0 (Control) | 0 | $11.11 \pm 0.51$ | $13.33 \pm 0.33$ |
| 0.35 | 0.91 | $6.22 \pm 0.19$ | $13.56 \pm 0.19$ |
| 0.45 | 1.17 | $3.55 \pm 0.19$ | $12.33 \pm 0.33$ |
| 0.55 | 1.43 | $1.78 \pm 0.19$ | $8.56 \pm 0.38$ |
| 0.60 | 1.56 | $0.67 \pm 0.33$ | $5.78 \pm 0.38$ |

Each value represents mean $\pm$ S.D. ($n = 3$).

through the bulk SC in the similar condition of this present experiment (Fang et al., 1999). Since SNA predominantly transport across skin by shunt routes during iontophoresis, pretreatment of ultrasound on the skin may expand this effect and result in the synergistic enhancement of these two physical methods. Application of ultrasound reduces the threshold voltage required for delivering drug transport in the presence of an electric field (Kost et al., 1996; Le et al., 2000). Accordingly, a lower voltage may be needed to deliver a given current during iontophoresis for SNA compared to that in controls.

### *3.3. Iontophoresis combined with erbium:YAG laser*

The skin barrier can be partially overcome by removal of the SC such as with tape stripping. However, the area and depth of SC treated by the tape stripping technique cannot be precisely controlled. The data and references about safety and recovery rate of skin of this method are rare. Lasers have long been used for medical diagnosis and therapeutic purposes. The laser has been suggested as a good method for the controlled ablation or removal of SC in human skin (Jacques et al., 1987). The erbium:YAG laser emits light with a 2940 nm wavelength which corresponds to the main peak of water absorption. This property enables the erbium:YAG laser to ablate the SC with minimal residual thermal damage, thereby potentially minimizing the risks of post-inflammatory hyperpigmentation (Manaloto and Alster, 1999). The erbium:YAG laser is currently used for the resurfacing of rhytides, scars, photodamage, and melasma (Polnikorn et al., 1998).

In order to assess the effect of erbium:YAG laser on the integrity of skin structure, nude mouse skin was irradiated by the laser with the energy from 0.35 to 0.60 J. The fluences used varied between 0.91 and 1.56 J/cm$^2$. After calculating the thickness of SC and epidermis by microscope as observed in Table 2, the etched thickness of the SC and epidermis after laser ablation appears to be proportional to the energy of treatment. Sections of epidermis appeared essentially the same and were within normal limits for 0.35 J laser-treated skin.

Fig. 3 shows the cumulative amount of SNA in the receptor as a function of time after laser treatment at various energies. The histological and structural alterations in skin could result in an increase in flux after exposure to the laser. The more significant changes in skin structure by higher laser fluences may lead to higher enhancement of drug permeation. However, the effect of enhancement was not proportional to the magnitude of laser intensity according to flux value (Table 1). SNA may firstly partition into the SC, after which it passes across the skin by various routes. The ablation of SC layer of the skin can reduce the inherent barrier properties of the SC and thus increase the skin permeation of SNA; however, the partition of drugs into the SC may be retarded because of the limited area of the SC after laser ablation. As a result, the increased permeation of the skin due to structural alteration may be partly offset by a reduction in the partition coefficient. Another explanation is that water itself acts as a transdermal enhancer. The permeation of drugs increased under occlusion because the diffusion resistance of the skin is reduced when free water present (Díez-Sales et al., 1996;

Ogiso et al., 1989). Erbium:YAG laser is reported to decrease retention of water in the skin because this laser emits light with a 2940 nm wavelength which is highly absorbed by water (Polnikorn et al., 1998). The water content should decrease as the dose of laser is increased. This characteristic of erbium:YAG laser makes the permeation of SNA rather difficult with less water in the skin under occlusion, resulting in the lower flux of SNA after irradiation of the highest laser energy (0.55 J) (Table 1).

Although the treatment of laser on skin could enhance the skin permeation of SNA by ablation of the SC layer, this effect was not large when compared with the treatment of iontophoresis on SNA permeation (Table 1). This result may confirm that SNA transport across the skin predominantly by shunt routes but not inter- or intra-cellular pathways of the SC directly. One of the characteristics of an ideal permeation enhancing method is that the skin should recover its normal barrier properties following removal of the enhancing method. Our previous study showed that the depths of the SC could completely recover within 5 days (Lee et al., 2001). The epidermal thickness remained constant after 0.35 and 0.45 J laser ablation (Table 2). On the other hand, the epidermal thickness recovered on the 4th day after laser treatment with higher doses (0.55 J) (Lee et al., 2001).

Fig. 4 shows the cumulative amount-time profiles of SNA after combination of laser pretreatment and iontophoresis. A synergistic effect was observed with 0.35 or 0.45 J laser exposure coupled with electric fields (Table 1). As the superficial layers of the SC were removed, there was a gradual drop in the electric resistance of the skin (Nelson et al., 1991). It is surprising that the application of iontophoresis on SNA transported across the skin after pretreating 0.55 J laser caused a reduction of SNA permeation (Fig. 4). During erbium:YAG laser irradiation on skin, the water in the substrate is raised to its boiling point, resulting in the loss of water inside the skin (Nelson et al., 1991). Since the iontophoretic flux of SNA could be increased by holding water in skin resulting in increased conductivity (Fang et al., 1997a). The extraction of water from skin may induce the opposite result of SNA permeation, causing the reduction of SNA flux after

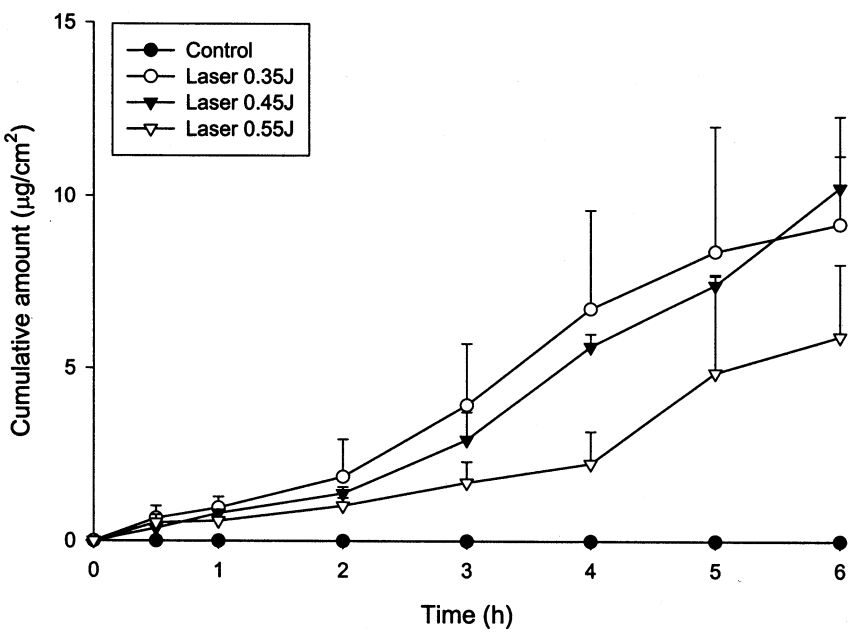


Fig. 3. Cumulative amount of SNA detected in the receptor compartment versus time by pretreating erbium:YAG laser on skin with various energies. All data represent the means of four experiments ± S.D. ITP, iontophoresis.

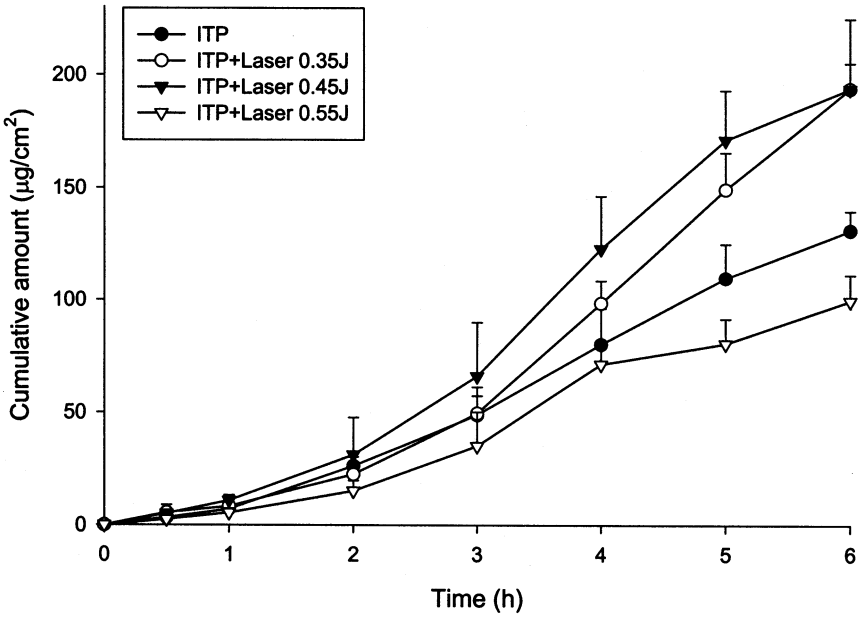


Fig. 4. Cumulative amount of SNA detected in the receptor compartment versus time by treating 0.5 mA/cm$^2$ iontophoresis combined with erbium:YAG laser with various energies. All data represent the means of four experiments ± S.D.

combining iontophoresis and laser with a higher dose.

## 4. Conclusion

This investigation illustrates the influence of iontophoresis combined with other physical enhancement methods on the transdermal absorption of SNA. The in vitro transport of SNA across skin without any enhancement was negligible. The permeation of SNA extensively increased following the application of current density. After comparing the physical enhancement methods tested in this present study, iontophoresis was still the most potent method to enhance SNA delivery across skin. Electroporation of skin did not increase transdermal iontophoretic delivery of SNA. However, the onset of transdermal iontophoresis of SNA could be accelerated by electroporation treatment. The contribution of electroporated skin was negligible for SNA transport across skin, while electrophoretic movement was important for SNA by high voltage pulsing. A synergistic effect of SNA permeation was observed after combining sonophoresis and iontophoresis. Since SNA predominantly is transported across skin by shunt routes during iontophoresis, pretreatment of low frequency ultrasound on skin may expand this effect. The erbium:YAG laser at lower intensities is safe and effective when used for the ablation of the SC to enhance the skin permeation of SNA. SC removal with this laser can be precisely controlled to a single pulse. Iontophoretic permeation of SNA across lower energies laser-treated skin could result in a synergistic effect on SNA flux. However, an opposite result was observed when combining iontophoresis and higher energy laser treatment. At this time, these methods of physical enhancement may require a relatively complex device for drug delivery (Le et al., 2000). Further studies should focus on performing in vivo tests to assess the applicability of transdermal drug delivery using these methods.

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