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Transdermal iontophoresis of sodium nonivamide acetate. III. Combined effect of pretreatment by penetration enhancers

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Abstract

The effect of iontophoresis combined with pretreatment of penetration enhancers such as benzalkonium chloride, cetylpyridinium chloride, sodium laurylsulfate and isopropyl myristate on the transdermal transport of sodium nonivamide acetate (SNA) and histologically structural properties of rat skin was undertaken. Cetylpyridinium chloride and isopropyl myristate showed the highest iontophoretic enhancement factor (E) and iontophoretic flux (J_I) on the transdermal penetration of SNA after pretreatment with skin, respectively. However, because of the severe change on the histological structure of rat skin irritated by isopropyl myristate, its clinical use is limited. The iontophoretic flux of SNA of sodium laurylsulfate pretreatment group was lower than that of control group. The reason for this phenomenon was that iontophoretic transport of SNA would be restricted by the hindrance of sodium laurylsulfate molecules inhibiting entry into pores of the skin. In the study of the different pretreatment duration of penetration enhancer for time ranging from 6 to 24 h, the total enhancement factor over passive diffusion was decreased following the increase of pretreatment duration both in cetylpyridinium chloride- and isopropyl myristate-pretreated skin. This illustrated that penetration enhancers could largely influence the passive transport of SNA but only showed a minor effect on the iontophoretic transport of SNA. In the result for anatomical skin structures treated by current density, there was almost no change observed in the structure of skin after iontophoretic treatment as compared with the control group. Accordingly, iontophoretic delivery is a transdermal enhancement method with high safety. A combination of physical iontophoresis with the chemical enhancer may be a potential route for transdermal delivery of drugs as this present study indicates. © 1997 Elsevier Science B.V.

Keywords: Sodium nonivamide acetate; Transdermal absorption; Iontophoresis; Penetration enhancer

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1. Introduction

Sodium nonivamide acetate (sodium *N*-nonanoyl vanillyl-4′-*O*-acetate; SNA; $C_{19}H_{28}NO_5Na$) is a recently designed derivative of capsaicin which was synthethized by alkylation of the phenolic sodium hydroxyl group of NVA with bromoacetic acid (Fang et al., 1995). When the antinociceptive potency, which is the mainly pharmacological activity of capsaicinoids, is measured, SNA showed a higher value than did capsaicin and indomethacin by 1.75 and 27.50 times, respectively (Chen et al., 1992). In our previous investigations, SNA showed an extensive use in clinical therapy as applied by transdermal route because of its avoiding any pungent skin sensation and burning pain which had been found in capsaicin to improve patients' compliance (Fang et al., 1996a,b,c). Nevertheless, the penetration capacity of SNA was lower than that of capsaicin because of its low *n*-octanol/buffer partition coefficient and high water solubility (Tsai et al., 1994). Accordingly, methods for transdermal enhancement such as penetration enhancer and iontophoresis (electrical current density application) had been utilized to overcome the poor permeability of SNA (Wu et al., 1995; Fang et al., 1996d,e).

The penetration enhancer and iontophoresis can be determined as the chemical and physical enhancement methods of transdermal absorption, respectively. In some cases, the chemical penetration enhancer can synergize with physical iontophoresis and provide an additional driving force to maintain and control the target flux (Srinivasan et al., 1989, 1990). Moreover, the combination of enhancers and iontophoresis not only slows the process of polarization of charged molecules but also reduces the possibility of skin damage or improves the tolerability of skin to the iontophoretic regimen (Rao and Misra, 1994). However, some researches found no additional enhancement and advantage were observed with the combination (Wearley and Chien, 1990; Gay et al., 1992).

In the presence of enhancers cathodal iontophoresis gave better results than did anodal iontophoresis, probably due to polarisation during anodal delivery (Lashmar and Manger, 1994). Since anionic SNA molecules penetrate skin barrier via cathodal iontophoresis, a positive effect of the combination of enhancer and iontophoresis may be expected for SNA molecules. In this present study, a series of enhancers such as cationic and anionic surfactants and isopropyl myristate was selected to combine with transdermal iontophoretic delivery for SNA to elicit additive or synergistic transport-promoting effects. The aim of this investigation also focuses on the skin structure changes caused by penetration enhancers and current density by means of a histopathological method. Determination of skin damage is important and necessary since the practical use of transdermal enhancements requires careful balancing of their benefits and risks such as penetration action and irritation (Quan et al., 1991).

2. Materials and methods

2.1. Materials

The following reagents were used: benzalkonium chloride (Tokyo Kasei, Japan), cetylpyridinium chloride (Sigma, US), cetrimide (hexadecyltrimethylammonium bromide) (Ferak, Germany), sodium laurylsulfate and isopropyl myristate (Wako, Japan), formaldehyde solution (Merck, Germany), hematoxylin and eosin (Schmidt, Germany). The synthetic procedure of SNA had been performed from our laboratory and reported earlier (Fang et al., 1995). All other chemicals and solvents were of analytical grade. All buffer solutions were prepared in deionized bidistilled water purified in a Milli-Q® water system (Millipore, US).

2.2. Pretreatment procedure of penetration enhancer

Male Wistar rats (200–250 g) were obtained from National Cheng Kung University (Tainan, Taiwan). The excised Wistar rat skin was used as the model membrane since the flux of SNA through rat skin was more similar to that through human skin than the other skin types (Fang et al.,

1995). After being killed in an ether chamber, the rat was removed its hair with electrical clippers and the abdominal skin was excised after careful shaving. The rat skin was mounted between the two half horizontal glass diffusion cells. The 8 ml of an enhancer solution was then deposited onto the stratum corneum surface. Skin samples were pretreated with the test enhancers for time ranging from 6–24 h. All enhancers were applied as aqueous solutions after dilution except isopropyl myristate as a neat liquid. After the duration of pretreatment, the enhancer solution was removed, then the skin was rinsed twice with 5 ml pH 7.4 McIlvaine buffer and the permeation experiment was conducted. Control experiments were performed under identical conditions without enhancer pretreatment. The variables examined were the effects of enhancer types, duration of pretreatment and concentration of enhancer.

2.3. Instruments and penetration procedures

All SNA transports were carried out in a McIlvaine buffer. The receptor phase contained 8 ml of 0.12 M; pH 7.4 buffer was used. Donor compartment of the cell was filled with 8 ml of 0.12 M; pH 4.2 buffer contained 50 mg/ml SNA as the active ingredient. The available diffusion surface area was 0.785 cm^2. A pair of platinum wires having an effective length of 15 mm (99.99% purity, 0.5 mm in diameter) used as electrodes was immersed in the solution with the cathode in the donor and the anode in the receptor. The anode and cathode were each positioned 3 cm from the side of rat skin membrane. The electrodes were connected to a current power supplier (Yokogawa, Model 7651, Japan).

There are two different application modes performed in this present investigation. The first application mode was that the current density of 0.5 mA/cm^2 and penetration experiment were conducted directly after the pretreatment procedure. The total duration of iontophoresis was maintained for 6 h.

The experimental procedure of the second iontophoretic application mode in this present work with SNA is identical to the three-stage experiment modified from Srinivasan et al. (1989, 1990). Current density of 0.3 mA/cm^2 was applied to stimulate the penetration of SNA in this experiments. In stage I, the initial passive flux (J_0) of SNA without applying current density was determined. In stage II, a 0.3 mA/cm^2 current density was carried out and the the iontophoretic flux (J_I) of SNA through the same skin was determined. In the last 4 h duration of stage III, the current density was turned off.

The 0.2-ml samples were withdrawn from the receptor at regular intervals and immediately replaced by an equal volume of fresh receptor solution to maintain a constant volume. This dilution of the receptor content was taken into account when evaluating the experimental data. The samples were assayed by high performance liquid chromatography (HPLC) method as described previously (Tsai et al., 1994).

2.4. Histological examination of rat skin

Histological changes in the skin were examined after the pretreatment of enhancers for 12 h. This examination was also evaluated by applying 0.3 mA/cm^2 current density for 5 h which was of the same duration with stage II of the second application mode. Pretreated skin with pH 4.2 McIlvaine buffer served as a control group. Each sample was fixed in 10% pH 7.4 buffered formaldehyde solution for at least 24 h. Then cut vertically against skin surface. Each section was dehydrated using a graded series of ethanol and embedded in paraffin wax, stained with hematoxylin and eosin. All sections were examined and evaluated under an optiphoto light microscopy (Olympus, Japan).

2.5. Data analysis

The total amount of drug penetrating through the unit diffusion surface and the cumulative amount of the receptor was calculated and plotted as a function of time. The flux (J) was calculated by the slope of the linear portion of cumulative amount-time plots for zero-order model and expressed as the mass of drug passing across 1 cm^2 of skin over time.

To discuss the effect of penetration enhancer on transdermal administration after iontophoretic

Table 1
Iontophoretic flux and enhancement factor of SNA through rat skin after treatment of a series of surfactants

Penetration enhancer	Concentration (%)	Iontophoretic flux (μg/cm^2/h)	Enhancement ratio (ER)
Control group	—	7.34 ± 0.80	—
Sodium laurylsulfate	0.10	6.31 ± 0.53	0.86
Benzalkonium Cl	0.10	8.66 ± 0.42	1.18
Cetrimide	0.10	8.92 ± 0.83	1.22
Cetrylpyridinium Cl	0.05	9.17 ± 0.55	1.25
Cetrylpyridinium Cl	0.10	10.66 ± 1.12	1.45
Cetrylpyridinium Cl	0.20	11.94 ± 1.77	1.63

Enhancement ratio (ER) was determined as iontophoretic flux with enhancer/iontophoretic flux enhancer (control group). Each value represents the mean $\pm$ S.D. ($n = 3$).

procedure in the first application mode, enhancement ratio (ER) is determined by the following equation:

$$ER = \textit{iontophoretic flux with enhancer/iontophoretic flux without enhancer} \quad (1)$$

To better interpret the results of enhancement after the combination of iontophoresis and penetration enhancer, the following equation can be defined (Srinivasan et al., 1989):

$$Enhancement\,factor(EF) = J_I/J_0 \quad (2)$$

where EF is the total enhancement factor of iontophoresis over passive diffusion or enhancer effect alone. In Eq. (2) it is assumed that J_0 is due solely to the chemical potential gradient across the skin. J_I is determined by the combined effect of enhancer and iontophoresis.

The statistical analysis of the differences between different treatment was detected by using unpaired Student's t-test. The 0.05 level of probability was taken as the level of significance.

3. Results and discussion

3.1. Effect of various enhancer types

To investigate the potential for iontophoresis facilitated transdermal transport of SNA in combination with the penetration enhancer, the method of pretreatment by enhancer was conducted since pretreating the skin before application of drugs would be a good method to promote the percutaneous absorption of drugs (Hosoya et al., 1987). Besides, pretreatment of enhancer could avoid the competitive ion effect caused by the direct incorporation of enhancer into the donor solution (Fang et al., 1996e). In this study, three types of enhancer were utilized: cationic surfactant-benzalkonium chloride, cetylpyridinium chloride and cetrimide; anionic surfactant-sodium laurylsulfate; lipophilic enhancer-isopropyl myristate. These agents had previously been found to enhance transdermal delivery of SNA (Wu et al., 1995; Fang et al., 1996a,c).

In the first iontophoretic application mode, a series of surfactants which showed the competitive ion effect when participating into donor compartment directly was utilized for SNA transdermal administration following the pretreatment with rat skin (Fang et al., 1996e). The flux and enhancement ratio (ER) after 6 h current density application were determined and summarized in Table 1. Similar to the previous data of passive diffusion (Wu et al., 1995), cationic surfactants revealed higher enhancing activity for SNA than did anionic surfactant in iontophoretic delivery. In addition, cetylpyridinium chloride showed the highest iontophoretic flux and enhancement ratio compared with control group. Although the iontophoretic fluxes after pretreating with cationic surfactants were all higher than that of control group (unpaired t-test, $P < 0.05$), however, this enhancer-iontophoresis combined effect was small and negligible. Various concentrations ranged from 0.05% to 0.20% of cetylpyridinium chloride were performed for 12 hpretreatment, the result is also shown in Table 1. The iontophoretic flux of

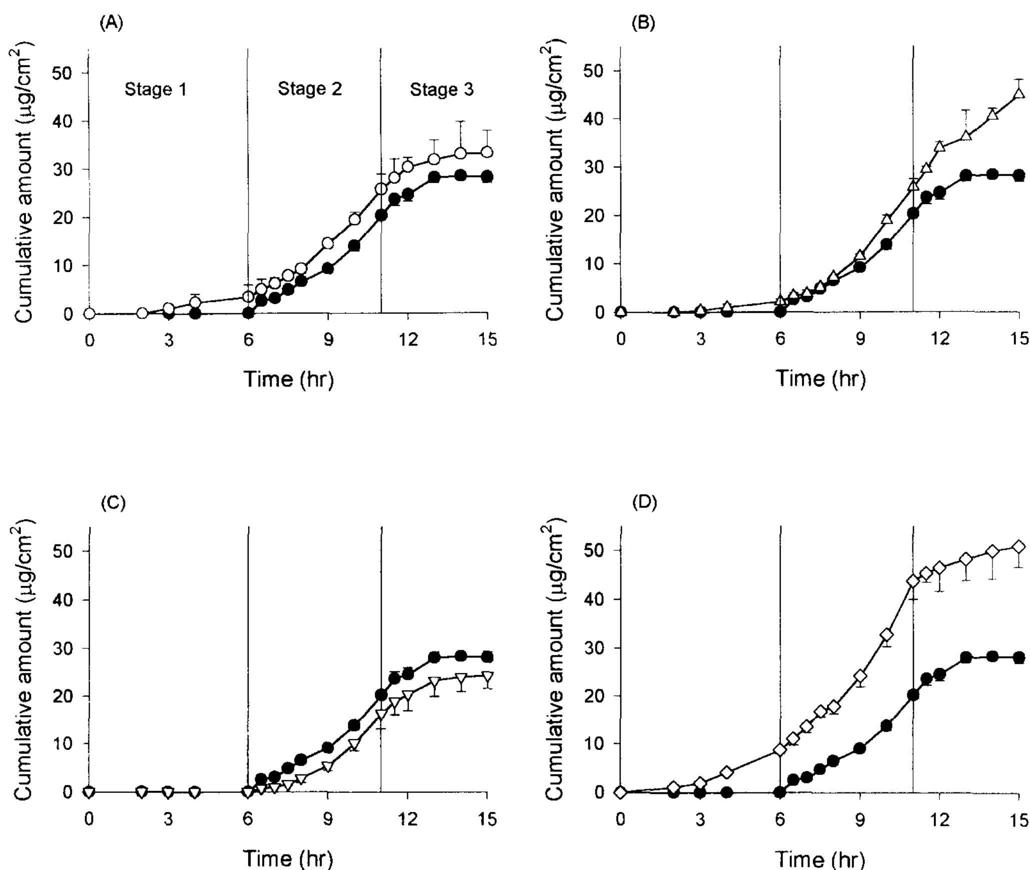

Fig. 1. Passive and iontophoretic penetration profile of SNA through rat skin after 12 h pretreatment of (a) 0.1% benzalkonium chloride (○), (b) 0.1% cetylpyridinium chloride (△), (c) 0.1% sodium laurylsulfate (▽), and (d) isopropyl myristate (◇). Control group (●). All data represent the means of three experiments ± S.D.

SNA increased with increasing concentration of cetylpyridinium chloride. But the enhancement ratio was only increased from 1.25 to 1.63 as the dose of cetylpyridinium chloride increased from 0.05% to 0.20% for 4 times. Since the current density conducted in this mode was 0.5 mA/cm_2, it might be that the enhancement due to transdermal iontophoresis was so large that the enhancement due to penetration enhancer was small in comparison. Moreover, the previous data indicated that the permeation of SNA incorporated with enhancers was significantly increased compared with non-enhancer experiment when the data were calculated beyond 6 h (Wu et al., 1995). Subsequently the SNA penetration capacities with and without enhancer were nonsignificant during the first 6 h permeation resulted in the limited enhancement after the combination of enhancer pretreatment and iontophoresis.

In order to minimize these adverse factors for penetration enhancer, lower current density of 0.3 mA/cm_2 was performed for the next iontophoretic application mode. Furthermore, current density (stage II) was conducted after 6 h duration of passive diffusion (stage I) since the capacity of penetration after pretreatment of enhancer may be significantly increased as the data were determined after 6 h permeation.

Fig. 1 shows a penetration profile for the three-stage experiment of the second application mode

Table 2
Iontophoretic penetration flux and enhancement factor of SNA through rat skin after pretreatment of enhancers

Penetration enhancer	J_o (μg/cm^2/h)	J_I (μg/cm^2/h)	Enhancement factor (EF)
Control group	0	4.15 ± 0.24	—
Benzalkonium Cl	0.84 ± 0.37	4.57 ± 0.57	5.44
Cetrylpyridinium Cl	0.55 ± 0.15	5.36 ± 0.38	9.75
Sodium laurylsulfate	0	3.72 ± 0.56	—
Isopropyl myristate	1.58 ± 0.17	6.36 ± 0.54	4.03

J_o, passive flux of SNA in stage I; J_I, iontophoretic flux of SNA determined from 6 h to 13 h.
Enhancement factor (EF) was determined as J_I/J_o. Each value represents the mean $\pm$ S.D. ($n = 3$).

which indicates the effect of iontophoresis in conjunction with the 12 h pretreatment of enhancers at the concentration of 0.1% except isopropyl myristate as a neat liquid. In consideration of the control group without pretreatment of enhancer, no cumulative amount was detected during stage I as observed in Fig. 1. The cumulative amount of SNA extensively increased following the application of current density during stage II. The SNA amount was still ascended after the cut of current for 2 h, then the flux of SNA leveled off at the last 2 h of stage III. When an electrical field with direct current was applied, the 'flip–flop' gating mechanism was created to reduce the impedance of skin barrier, which resulted in the enhancement of skin permeability (Chien et al., 1989; Kasting and Bowman, 1990; Sims et al., 1991). When the electrical field was cut, the skin became depolarized but the resistance of skin might not recover immediately. Accordingly the SNA molecules continuously penetrate the skin even though the current has been stopped as shown in Fig. 1. As a consequence, the J_I value should be calculated from 6 h to 13 h of experimental time since the influence of electrical field existed for 7 h duration although the application of current density only conducted for 5 h as shown in Table 2. At the last 2 h of stage III for control group, the cumulative amount of SNA remained constant which implied no disruptive effect on the skin after the treatment of iontophoresis. This result was similar to that of previous investigation using spectroscopic and calorimetric methods to evaluate the skin treated with electrical current (Clancy et al., 1994).

The application of cationic surfactants can promote the passive diffusion (J_0) of SNA as observed in Table 2. Subsequently the iontophoretic flux (J_I) also increased as compared with that of control group. Although the enhancement effect of benzalkonium chloride on passive diffusion was higher than that of cetylpyridinium chloride, the iontophoretic flux due to the combination of physical current density and chemical enhancer showed the obverse effect as compared with the flux of stage II. Statistically, the iontophoretic flux of benzalkonium chloride pretreatment group showed no significant difference (t-test, $P > 0.05$) as compared with that of control group. Accordingly the enhancement factor (EF) of cetylpyridinium chloride was higher than that of benzalkonium chloride. Treatment of the epidermis with cationic surfactants can markedly increase the transport of charged hydrophilic substances (Cooper, 1984). Application of even dilute solutions of cationic surfactants to the skin change the structure of the tight junctions in the skin (Rantuccio et al., 1979; Lashmar and Manger, 1994). Surfactants also effect the skin's permeability to water, possibly altering the nature of the barrier and promoting their own flux rates as they permeate the stratum corneum (Idson, 1985). Consequently benzalkonium chloride and cetylpyridinium chloride can lower the resistance of the stratum corneum, such that subsequently applied iontophoresis elicits a greater transport of SNA (Kushla and Zatz, 1991; Lashmar and Manger, 1994).

As shown in Table 2, there is still no cumulative amount of SNA in stage I after pretreatment of sodium laurylsulfate. This phenomenon demonstrates that 6 h duration of stage I is not enough for sodium laurylsulfate to enhance the penetra-

Table 3
Iontophoretic penetration flux and enhancement factor of SNA through rat skin after pretreatment of cetylpyridinium chloride and isopropyl myristate at various pretreatment duration

Penetration enhancer	Pretreatment enhancer	J_o (μg/cm^2/h)	J_I (μg/cm^2/h)	Enhancement factor (EF)
Cetylpyridinium Cl	6	0.30 ± 0.16	5.87 ± 0.20	19.57
	12	0.55 ± 0.15	5.36 ± 0.38	9.75
	24	1.45 ± 0.34	7.19 ± 0.88	4.96
Isopropyl myristate	6	1.05 ± 0.42	5.22 ± 1.20	4.97
	12	1.58 ± 0.17	6.36 ± 0.54	4.03
	24	1.59 ± 0.55	4.61 ± 0.52	2.90

J_o, passive flux of SNA in stage I; J_I, iontophoretic flux of SNA determined from 6 h to 13 h.
Enhancement factor (EF) was determined as J_I/J_o.
Each value represents the mean ± S.D. ($n = 3$).

tion capacity of SNA since previous data indicated that the passive transport of SNA incorporated with sodium laurylsulfate was significantly increased when the data were calculated beyond 6 h (Wu et al., 1995). Furthermore, the iontophoretic flux of sodium laurylsulfate pretreatment group was lower than that of control group although there was no significant difference between these two experiments (t-test, $P > 0.05$). The shunt routes constitute the major penetration pathways for transdermal iontophoresis (Grimnes, 1984; Tyle, 1986; Singh et al., 1995). Although the rat skin surface had been washed by buffer after pretreatment, however, the enhancer inside pores in the skin still remained. The iontophoretic transport of SNA would be restricted by the hindrance of sodium laurylsulfate molecules inhibiting entry into pore (Yoshida and Roberts, 1993). This effect might neutralize the penetration enhancing capacity of sodium laurylsulfate resulted in the lower iontophoretic flux as compared with control group.

The skin pretreated with isopropyl myristate shows the highest passive and iontophoretic fluxes of SNA among enhancers tested in this present study as depicted in Table 2. The effect of isopropyl myristate on the passive flux is thought to involve direct interaction of the ester with the skin (Inagi et al., 1981). So it is possible that isopropyl myristate enhances skin permeation of SNA by accelerating conformational changes of the stratum corneum (Miyazaki et al., 1995). The reason of the highest iontophoretic flux of isopropyl myristate pretreatment group could be due to the fact that isopropyl myristate retarded the rate of loss of water from the skin resulted in the cumulative amount of water content in the epidermis (Dempski et al., 1965). This capacity of moisture retention was proved by detecting transepidermal water loss (TEWL) value of isopropyl myristate treated skin (Fang et al., 1996a). The highest combined enhancement of iontophoresis and isopropyl myristate pretreatment may subsequently be attributed to the ability to hold water in skin resulting in increased conductivity (Rao and Misra, 1994). To optimize and maximize the combined capacity of iontophoresis and enhancer, cetylpyridinium chloride and isopropyl myristate which respectively showed the highest enhancement factor and iontophoretic flux on SNA molecules as shown in Table 2 were chosen as the model enhancers and performed in the next experiments.

3.2. Effect of various pretreatment duration

Table 3 shows the relationship between SNA flux and pretreatment durations of cetylpyridinium chloride and isopropyl myristate in the donor solution. The passive flux of SNA after pretreatment of cetylpyridinium chloride increased following the increase of pretreatment duration. After the calculation of correlation coefficient between pretreatment duration and J_0 value of SNA, a linear relationship was observed which had a high coefficient value of 0.9921. In

Table 4
Iontophoretic penetration flux and enhancement factor of SNA through rat skin after pretreatment of cetylpyridinium chloride at various concentrations

Concentration (%)	J_o (μg/cm^2/h)	J_I (μg/cm^2/h)	Enhancement factor (EF)
0.05	0.72 ± 0.21	3.14 ± 0.30	4.36
0.1	0.55 ± 0.15	5.36 ± 0.38	9.75
0.2	1.36 ± 0.5	4.59 ± 0.52	3.38

J_o, passive flux of SNA in stage I; J_I, iontophoretic flux of SNA determined from 6 h to 13 h.
Enhancement factor (EF) was determined as J_I/J_o.
Each value represents the mean $\pm$ S.D. ($n = 3$).

consideration of the passive flux of 12 h pretreatment, the enhancement of isopropyl myristate was similar to that of 24 h pretreatment (t-test, $P > 0.05$). This indicates that 12 h pretreatment of isopropyl myristate can act as a maximum duration to achieve the effective enhancement on SNA penetration since the longer durations may restrict their flux only to the level of 12 h pretreatment.

The highest J_I values of cetylpyridinium chloride and isopropyl myristate at various pretreatment durations were 24 h and 12 h respectively. After determining the total enhancement factor (EF) over passive duration, the result indicated EF value was decreased following the increase of pretreatment duration both in cetylpyridinium chloride and isopropyl myristate pretreated skin. This demonstrates that penetration enhancer could largely influence the passive transport of SNA molecules but only showed a minor effect on the iontophoretic transport of SNA. The skin appendages play the important roles on the transdermal transport of SNA and constitute the major pathway for iontophoretic delivery (Fang et al., 1995), but the surface area occupied by these pathways is relatively small. Although cetylpyridinium chloride and isopropyl myristate may arise the strong structural rearrangement within stratum corneum and epidermis, there is only a little effect on skin pores since this area is just 0.1% compared with total skin surface (Illel et al., 1991). The other reason of this phenomenon is that the enhancement due to transdermal iontophoresis is so large that the enhancement due to penetration enhancer is negligible in comparison.

3.3. Effect of various pretreatment concentration of cetylpyridinium chloride

The effect of cetylpyridinium chloride concentration in the pretreatment on the flux of SNA is seen from Table 4. The lowest enhancement factor of cetylpyridinium chloride is observed at the highest concentration of 0.2%. As discussed previously, the hindrance of ionic enhancer in the pore of rat skin inhibits the penetration of SNA during electrical field. This effect may be more significant at the concentrated cetylpyridinium chloride solution due to the larger amount of cetylpyridinium chloride molecules occupied into pores of skin. As shown in Table 4, the maximum iontophoretic flux and enhancement factor is detected at the concentration of 0.1%.

3.4. Histopathological study

The skin is a multilayered organ and has, anatomically, many histological layers. It is generally described in terms of three tissue layers: the stratified, avascular, cellular epidermis, the underlying dermis of connective tissue, and the subcutaneous fat layer (Quan et al., 1991). Moreover, the highly vascularized dermis and the epidermis support several skin appendages: ecrine apocrine, sebaceous glands, and hair follicles. In this present study, the influence of enhancers and iontophoresis on the anatomical structure will be discussed in detail with the aid of histopathological findings.

Fig. 2 shows the microscopic appearance of rat skin treated with isopropyl myristate, cetylpyridinium chloride and 0.3 mA/cm^2 current density. As compared to Fig. 2A of control group, iso-

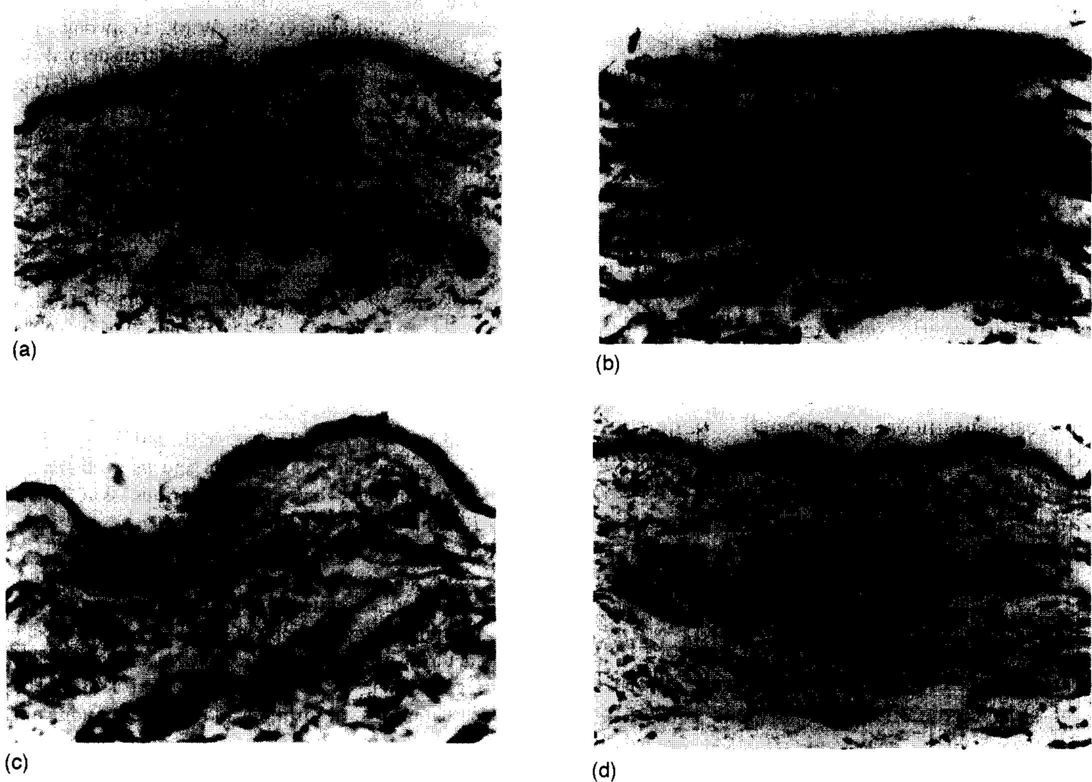

Fig. 2. Microscopic photos of rat skin after application of (a) nontreated group, (b) isopropyl myristate, (c) 0.1% cetylpyridinium chloride, and (d) 0.3 mA/cm^2 current density (original magnification ×200).

propyl myristate-treated skin showed collagen fiber swelling both in the stratum papillare and reticulare of the dermis as observed in Fig. 2B. Degeneration in skin appendages was also found in isopropyl myristate-treated skin. Furthermore, slight damage and exfoliation of the stratum corneum could be observed. Fig. 2C illustrates the microscopic appearance of rat skin after application of 0.1% cetylpyridinium chloride for 12 h. Slighter degeneration on skin appendages and stratum corneum exfoliation was also found for cetylpyridinium chloride-treated skin as compared with isopropyl myristate-treated skin. Since the shunt route constitutes the major pathway for the transdermal absorption of SNA, the degeneration of skin appendages caused by these two penetration enhancers may accelerate the transport of SNA resulting in the increase of passive diffusion and iontophoretic delivery.

Fig. 2D demonstrates microscopic result after treatment of iontophoresis for 5 h. There is almost no change observed in the anatomical structure of skin after current density treatment as compared with control group. This observation reflected the percutaneous absorption result of control group in Fig. 1. that no increase in cumulative amount of SNA at the last 2 h of experiment which confirmed no disruptive effect on the skin structure after iontophoretic treatment. This histological result of rat skin was similar to that of human skin in the previous study which illustrates electrical treatment exerts a less disruptive influence on the skin than treatment with penetration enhancers (Clancy et al., 1994).

4. Conclusion

This biopharmaceutical investigation clearly illustrates the influence of iontophoresis combined with pretreatment of penetration enhancer on the transdermal absorption of SNA and histopathology of rat skin. The in vitro passive transport of SNA without pretreatment of enhancer was negligible. The penetration of SNA extensively increased following the application of current density. Cetylpyridinium chloride showed the highest iontophoretic enhancement factor after pretreatment with skin indicating it can be used as an effective and potent enhancer utilized in the iontophoresis-enhancer combined penetration method. Pretreatment of isopropyl myristate also showed the highest iontophoretic flux on SNA penetration; however, because of the severer irritation on the histological structure of skin its clinical use is limited.

When histopathological examinations were used to evaluate the effect of electrical treatment on rat skin, no change was observed on three layers of skin tissue; it would appear that intact skin structure can be reserved after iontophoretic pretreatment. In this present study, the ability to enhance drug transport by both chemical and physical means was established and evaluated. Physical iontophoresis could accelerate the transdermal transport after being treated with chemical penetration enhancer. On the other hand, penetration enhancer could improve the tolerability of skin stimulated by iontophoresis (Rao and Misra, 1994).

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