ELSEVIER

International Journal of Pharmaceutics 143 (1996) 47–58

international journal of pharmaceutics

Transdermal iontophoresis of sodium nonivamide acetate I. Consideration of electrical and chemical factors

Jia-You Fang, Yaw-Bin Huang, Pao-Chu Wu, Yi-Hung Tsai*

School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, ROC

Received 17 April 1996; accepted 24 July 1996

Abstract

Transdermal iontophoresis is a process which enhances skin permeation of ionized species by an electrical field as driving force. The aim of this present study was to investigate the transdermal iontophoresis of a newly designed capsaicin derivative, sodium nonivamide acetate (SNA). Studies of electrical and physicochemical factors acting on the kinetics of in vitro iontophoresis were performed. Iontophoresis increased the transdermal penetration flux of SNA as compared to the passive diffusion in this study. Several application modes which possessed the same electrical energy had been researched. The iontophoretic flux of SNA increased following the decrease of donor buffer pH values. This trend could be due to the physiological property of skin and electro-osmotic flow presented. Comparing the various application modes, the discontinuous on/off cyclic current mode showed higher penetration capacity than did continuous mode which was due to the intensity of effective current which would not decay for on/off cyclic application of iontophoresis. The result of the present study is particularly helpful in the development of a SNA transdermal iontophoretic delivery system.

Keywords: Sodium nonivamide acetate; Transdermal absorption; Iontophoresis

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 which was synthesized by alkylation of the phenolic hydroxyl group of nonivamide with bromoacetic acid (Fang et al., 1995). The antinociceptive potency of this sodium salt was 1.75 and 27.50 times than that of capsaicin and indomethacin (Chen et al., 1992). The antinociceptive activities of capsaicinoids have been markedly influenced by various administration routes. Pre-

* Corresponding author.

PII S0378-5173(96)04681-9

vious investigations have suggested that the pharmacological effects of capsaicinoids following subcutaneous and intragastric administrations are significantly higher than that of oral administration in rats and mice (Sietsema et al., 1988; Donnerer et al., 1990). The poor antinociception after oral dosing of capsaicinoids was due to the first-pass metabolism. Accordingly transdermal drug delivery was suitable to be selected for SNA to accomplish better bioavailabilities. The previous studies suggested 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 (Yang et al., 1992; Fang et al., 1996). The percutaneous absorption of SNA and its analogues such as capsaicin and nonivamide from solution and ointment was well described previously (Tsai et al., 1994; Fang et al., 1995). Since the penetration capacity of SNA is lower than that of capsaicin because of its low *n*-octanol/buffer partition coefficient and high water solubility, methods for transdermal enhancement should be utilized to overcome this poor permeability.

The transfollicular and transappendageal routes constitute the major penetration pathways for SNA (Fang et al., 1995). Besides, the penetration of ionic drugs by the shunt route can be facilitated by the application of electricity (Tyle, 1986). Iontophoresis is defined as the migration of ions when an external electrical field is passed through a vehicle containing charged compounds (Masada et al., 1989; Singh et al., 1995). Some major limitations for passive transdermal delivery such as the requirements of low molecular weight, low dose and balanced oil–water partition coefficient can be easily overcome by iontophoretic delivery (Chien and Banga, 1989). In addition, the delivery rate can be controlled by the intensity of the electric current applied. This noninvasive drug delivery also minimizes trauma and risk of infection and is an alternative to the needle (Tyle, 1986). Moreover, transport of several chemicals with sodium salt through skin such as sodium iodide, sodium floride, sodium salicylate, sodium cromoglycate and diclofenac sodium (Tyle, 1986; Koizumi et al., 1990; Li et al., 1992; Nakhare et al., 1994) is increased by the application of an electrical current. So transdermal iontophoresis may be suitable for SNA to enhance its penetration capacity.

The aim of this present study was to investigate the influence of electrical factors and physicochemical factors on the transdermal iontophoresis of SNA through rat skin. Several application modes which possessed the same electrical energy (Coulombs) have also been researched (Lelawongs et al., 1990). 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). Accordingly the rat skin could be successfully used as a model to study in vitro percutaneous absorption of SNA through human skin. The information gained is particularly helpful in the development of SNA transdermal drug delivery system.

2. Materials and methods

2.1. Materials

Citric acid and di-sodium hydrogen phosphate dihydrate were purchased from E. Merck, Germany. The synthetic procedure of SNA has been performed from 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 Milli-Q® water system (Millipore, USA).

2.2. In vitro transdermal iontophoretic delivery

2.2.1. Instruments and penetration procedures

Male Wistar rats (200–250 g) were obtained from Kaohsiung Medical College (Kaohsiung, Taiwan). The hair of the rat was removed with electric clippers and the abdominal skin was excised after careful shaving. The in vitro penetration procedures of iontophoresis were determined by using a horizontal glass diffusion cell. The rat skin was mounted between the cell compartments with the stratum corneum facing towards the donor half cell. All SNA transports were carried

out in a McIlvaine buffer. The skin pieces were soaked in the receptor buffer solution for 45 min prior to placing in the cells (Miller and Smith, 1989). The receptor phase contained 8 ml of 0.06 M; pH 7.4 McIlvaine buffer (composition of citric acid and $Na_2HPO_4 \cdot 2H_2O$) was used. The available diffusion surface area was 0.785 cm^2. A pair of platinum wires having an effective working length of 15 mm (99.99% purity, 0.5 mm in diameter) were used as electrodes were immersed in the solutions 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 the rat skin membrane. The electrodes were connected to a constant current power supplier (Yokogawa, Model 7651, Japan).

The experiments were carried out at 37°C and the compartments of donor and receptor were agitated by magnetic stirrers at 600 rpm. 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 we were evaluating the penetration data. The samples were assayed by HPLC as described previously (Tsai et al., 1994).

2.2.2. Effect of donor pH value

The effect of pH value on the flux of SNA was determined using pH values of 4.2, 5.6, 7.0 and 8.0 while the ionic strength of donor solution was adjusted to 0.06 M. Current densities of 0, 0.2, 0.5 and 1.0 mA/cm^2 were applied in a continuous mode respectively.

2.2.3. Effect of donor SNA concentration

An increase in the concentration of SNA from 50 to 300 μg/ml in the donor compartment was conducted at pH values of 4.2 and 7.0 while adjusting the ionic strength of the donor solution to 0.06 M. A current density of 0.5 mA/cm^2 was applied to stimulate the penetration of SNA.

2.2.4. Effect of donor ionic strength

The ionic strength of donor solution was adjusted using McIlvaine buffer at pH values of 4.2 and 7.0 with the ionic strength ranging from 0.06 to 0.6 M. A current density of 0.5 mA/cm^2 was applied in a continuous mode.

2.2.5. Effect of the combination of varying current density and application duration

Current densities of 0.2, 0.4 and 0.8 mA/cm^2 were applied for 4, 2 and 1 h respectively to maintain the product of current density and application duration at a constant level. The drug concentration performed in this study was 200 μg/ml at a buffer pH value of 4.2.

2.2.6. Comparison of continuous application mode and discontinuous application mode

Three studies were conducted at a fixed current of 0.4 mA/cm^2 in buffer at pH 4.2. In the first study, continuous application of current was conducted for 4 h. In the second study, discontinuous application of current was conducted for a 10 min on/off cycle. In the third study, discontinuous application of current was conducted for 20 min on/off maintained for 4 h.

2.2.7. Effect of the combination of varying current density and SNA concentration

SNA concentrations of 50, 100 and 200 μg/ml at pH 4.2 were applied for 0.8, 0.4 and 0.2 mA/cm^2 respectively to maintain the product of drug concentration and current density at a constant level. The total application duration of continuous current was 4 h.

2.2.8. Effect of the combination of varying ionic strength, SNA concentration and current density

SNA concentrations of 200, 100 and 200 μg/ml at the buffer ionic strength of 0.06, 0.12 and 0.24 M were applied for 0.2, 0.8 and 0.8 mA/cm^2, respectively. The ratio of ionic strengths of the three groups was 1:2:4. Since higher buffer capacity may retard the iontophoretic penetration of drug, the electrical energy ratio of these groups could be considered as 4:2:1. Thereby the product of ionic strength, SNA concentration and current density was maintained at a constant electrical energy. The total application duration of continuous current was 4 h.

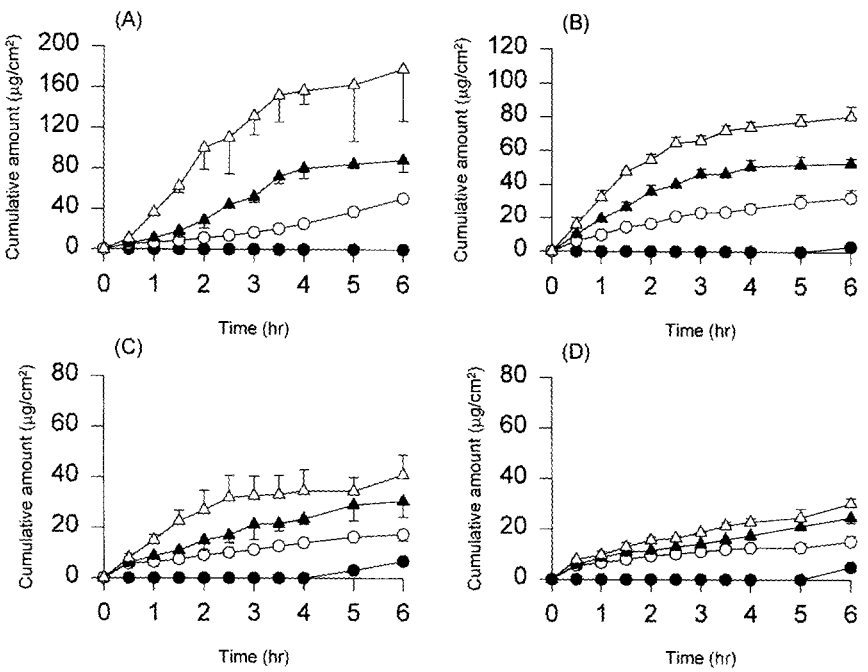

Fig. 1. Cumulative amount of SNA detected in the receptor compartment versus time following iontophoresis at various donor pH values: (●) 0 mA/cm², (○) 0.2 mA/cm², (▲) 0.5 mA/cm², (△) 1.0 mA/cm²; (A) pH 4.2, (B) pH 5.6, (C) pH 7.0, (D) pH 8.0. All data represent the means of three experiments ± SD.

2.3. Data analysis

The total amount of drug penetrating through the unit diffusion surface and into the receptor was calculated and plotted as a function of time. The Higuchi flux was calculated by the slope of the linear portion of cumulative amount–(time)$^{1/2}$ plots and expressed as the mass of drug passing across 1 cm^2 of skin over the square root of time (Higuchi, 1962). The permeability coefficient was calculated by dividing the flux by initial drug donor concentration. The area under the curve (AUC) of skin flux–time plots was calculated by the trapezoidal method.

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

3. Results and discussion

3.1. Effect of donor pH value

The SNA penetration with or without direct current density was performed at pH values of 4.2, 5.6, 7.0 and 8.0 as shown in Fig. 1. There were extremely low cumulative amounts observed in passive diffusion of SNA, no matter what the buffer pH values were. Because of the deficiencies of time plots in measuring the penetration flux of SNA without iontophoresis, the 6 h cumulative amounts were calculated as the capacity of passive penetration. This capacity of SNA increased in the order of pH 4.2 < pH 5.6 < pH 8.0 < pH 7.0 as shown in Table 1. During iontophoresis, the number of SNA molecules which passed through skin increased with the increase of strength of the current according to Faraday's law (Phipps et al., 1989). The cumulative amount–time profile fitted well with the Higuchi equation except for the data

Table 1
Effect of pH value of donor solution on the iontophoretic penetration of SNA

Current (mA/cm^2)	Cumulative amount ($\mu g/cm^2$) at 6 h				Flux ($\mu g/cm^2/h^{1/2}$)			
	pH 4.2	pH 5.6	pH 7.0	pH 8.0	pH 4.2	pH 5.6	pH 7.0	pH 8.0
0	0	3.22 ± 0.76	6.72 ± 1.16	4.98 ± 0.78	–*	–	–	
0.2	50.98 ± 1.93	32.46 ± 4.92	17.42 ± 2.49	15.28 ± 2.35	18.78 ± 0.75	13.98 ± 2.12	7.08 ± 1.00	5.90 ± 0.91
0.5	88.92 ± 11.76	53.03 ± 2.90	30.51 ± 6.25	24.74 ± 2.13	44.12 ± 5.92	24.87 ± 2.43	13.28 ± 2.45	9.59 ± 0.83
1.0	228.77 ± 51.11	80.73 ± 5.96	41.00 ± 7.90	30.31 ± 2.07	110.76 ± 24.44	36.60 ± 2.66	17.44 ± 3.37	11.89 ± 1.46

* Not determined in this experiment. Each value represents the mean $\pm$ S.D. ($n = 3$). The flux is calculated by the Higuchi model.

of pH 4.2 at a 0.2 mA/cm^2 current application which fitted with the zero-order equation. This phenomenon is consistent with the transdermal iontophoretic delivery of salbutamol by Bannon et al. (1988).

The degree of Higuchi flux during iontophoresis is found to be pH dependent at any current densities as shown in Table 1. The iontophoretic flux showed a trend of pH 8.0 < pH 7.0 < pH 5.6 < pH 4.2. Since the chemical structure of SNA is a carboxylate salt, the pK_a of the corresponding carboxylic acid should be in the range from 3 to 5; this molecule will be electrically neutral at pH values below the pK_a and ionized at higher pH values. According to previous research, iontophoresis was more effective at pH values where drug was mainly ionized (Siddiqui et al., 1985; Green et al., 1991). However, through the present experiment, exactly the opposite result is obtained. The pH of drug solution affects not only the ratio of ionized and non-ionized forms, but also the property of skin surface (Lelawongs et al., 1989). Firstly, the skin has a minimum charge density at a pH value of about 3–4 which is about the isoelectric point of keratin (Schade and Marchionini, 1927). As the pH approximates this value, the skin becomes positively charged and favors the transport of negatively charged species. Subsequently, transdermal iontophoresis favors anionic molecules at lower pH values, which results in the highest penetration amount of SNA at pH 4.2, approximately the isoelectric point of skin.

Secondly, the total flux of an ionized molecule at a given current can be calculated by the sum of passive diffusion, iontophoretic flux and electro-osmotic flux (Santi et al., 1993). The direction of electro-osmotic flow is usually toward the cathode (Gangarosa et al., 1980; Singh et al., 1995); thereafter, the equation of the total flux in this present study is the sum of passive diffusion and iontophoretic flux minus the electro-osmotic flux. The applied current-induced electro-osmotic flow of water across skin increased as the pH increased (Burnette and Marrero, 1986). This is a reasonable theory to explain the decrease in SNA flux as pH values increase.

Fig. 1 shows that there is a small increase in cumulative amount of SNA in all curves at the end-stage of application except for the curve at pH 4.2 and 0.2 mA/cm^2 current density. This is explained by the fact that SNA exhibits potent iontophoretic enhancement at pH 4.2, so the low current density of 0.2 mA/cm^2 may not product a mild curve as observed at high current density, yet during 6 h application. After the calculation of the correlation coefficient between current density and flux, a linear relationship of mainly nonionic form at pH values of both 4.2 and 5.6 can be observed ($r = 0.99$). However, the correlation coefficient is only 0.96 for mainly ionic form at pH 7.0 and 8.0.

The additional experiments, done at pH values of 4.2 and 7.0, have shown that no detectable amounts of SNA are transported across rat skin

Table 2
Effect of donor SNA concentration on the iontophoretic penetration at pH values of 4.2 and 7.0

Concentration (μm/ml)	pH 4.2		pH 7.0	
	Flux ($\mu g/cm^2/h^{1/2}$)	Permeability coefficient ($ml/cm^2/h^{1/2}$)	Flux ($\mu g/cm^2/h^{1/2}$)	Permeability coefficient ($ml/cm^2/h^{1/2}$)
50	19.45 ± 1.88	0.39 ± 0.04	3.19 ± 0.32	0.06 ± 0.01
100	29.07 ± 3.20	0.29 ± 0.03	6.04 ± 0.97	0.06 ± 0.01
200	44.12 ± 5.92	0.22 ± 0.03	13.28 ± 2.45	0.07 ± 0.01
300	53.66 ± 6.71	0.18 ± 0.02	18.34 ± 2.02	0.06 ± 0.01

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

as the anode is placed in the donor and the cathode is placed in the receptor.

3.2. Effect of donor SNA concentration

Drug concentration is an important parameter since it provides an easy way to control the rate of drug delivery in transdermal iontophoresis (Behl et al., 1989). One prerequisite is that the flux versus concentration relationship should be known in the beginning. The donor solutions of pH 4.2 and 7.0 were chosen to perform this study. The flux and permeability coefficient values are reported in Table 2. In the pH 4.2 solution, the concentration effect was not large although the flux was seen to increase with higher donor concentrations of SNA and showed a correlation coefficient of 0.99. On the other hand, the permeability coefficient decreased with increasing donor concentration. The skin is not an inert tissue and presents some resistivity to the movement of ions. Many small ions present in skin or buffer transport part of the current density. This could explain the controversial relationship between donor concentration and permeability coefficient (Kasting et al., 1988; Padmanabhan et al., 1990; Thysman et al., 1994). Moreover, the SNA molecules may act as the competitive ions for themselves in the high concentration during iontophoresis. This could cause the lower activity of the drug in more concentrated solution (Bellantone et al., 1986; Santi et al., 1993).

The result of pH 7.0 donor solution exhibits a different behavior compared with that of pH 4.2 as presented in Table 2. No statistical difference (ANOVA test, $P > 0.05$) is detected in the permeability coefficients among the various donor concentrations. This phenomenon indicates that an increase in SNA concentration in donor solution produces a proportional increase in SNA flux.

3.3. Effect of donor ionic strength

It is expected that the variation of ionic strength in the donor solution should be of importance for iontophoretic transport (Lelawongs et al., 1989, 1990). The result in Table 3 shows that the flux of SNA decreases as the ionic strength of the donor solution increases both at pH 4.2 and 7.0. A greater decline is observed at pH 4.2 than at pH 7.0. The low penetration at high ionic strength could be due to the competition of drug ions and buffer ions for the applied current. Most current would be carried by buffer ions with relatively high mobilities, so that only a small fraction of applied current was carried by SNA ions (Burnette and Bagniefski, 1988; Lelawongs et al., 1989).

In conclusion to the above, the extraneous ion concentration in the donor should be minimized so as to maximize the delivery efficiency (Phipps et al., 1989).

3.4. Effect of the combination of varying current density and application duration

Fig. 2 shows the flux (dQ/dt)–time profile–the combination of varying current and application duration. An earlier peak during the first hour is

Table 3
Effect of donor ionic strength on the iontophoretic penetration of SNA at pH values of 4.2 and 7.0

Ionic strength (M)	pH 4.2		pH 7.0	
	Cumulative amount at 6 h ($\mu g/cm^2$)	Flux ($\mu g/cm^2/h^{1/2}$)	Cumulative amount at 6 h ($\mu g/cm^2$)	Flux ($\mu g/cm^2/h^{1/2}$)
0.06	88.92 ± 11.76	44.12 ± 5.92	30.51 ± 6.25	13.28 ± 2.45
0.12	45.35 ± 6.95	17.78 ± 2.72	20.77 ± 3.15	8.77 ± 1.23
0.60	10.24 ± 0.40	3.65 ± 0.22	14.30 ± 0.81	5.20 ± 0.74

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

observed in all three iontophoretic modes. The shunt routes constitute the major penetration pathways for SNA (Fang et al., 1995). The higher cumulative amount of drug is obtained through shunts than that through stratum corneum directly in the early applied period (Illel et al., 1991; Williams and Barry, 1992). This could be the reason for the high penetration flux in the first hour. This enhanced effect in the earlier period is not commonly observed in the other researches using hairless mouse or human skin as the barrier membrane. This increased transport may be attributed to the higher number of hair follicles in furry rat skin than hairless mouse and human skin resulting in the amplification of this enhanced effect (Bronaugh et al., 1982). This also supports the mechanism of pore transport model. The other mechanism of enhancement under this condition could be attributed to the increase of water movement into the skin during electric field application. This increase in hydration may increase the permeation rate in the beginning (Wearley and Chien, 1990). At later time points the skin is likely to have completely hydrated. Hydration is therefore likely only to have played a small part in the results (Lashmar and Manger, 1994).

Judging from the second peak as shown in Fig. 2, the result indicates the second peak SNA flux increases with the increase in current density. Besides, the time of the second peak also shortened with the increase in current density. The skin flux of SNA levels off gradually at the end-stage of application. This result is always found in the cathodal iontophoresis of negatively charged permeants (Burnette and Ongpipattanakul, 1987; Green et al., 1991). Despite the effect of the cut of current application, the changes in the permselectivity of skin in the presence of an electric field may account for this result (Wearley and Chien, 1990; Green et al., 1991). Alternatively, direct current iontophoresis will always develop a skin polarisation potential, eventually decreasing the magnitude of effective current (Lashmar and Manger, 1994). After the calculation of the AUC_{0-6h}, the 0.8 mA/cm^2 current with 1 h applied duration revealed the highest value (ANOVA test, $P < 0.05$) among three different treatments inspite of the same amount of electrical energy (Coulombs) having been administered (Lelawongs et al., 1990). Nevertheless, in respect of calculating the value of AUC_{on-off} which showed a constant electrical energy in this period for all treatments, an adverse trend is seen as compared with that of AUC_{0-6h}.

After the cessation of current application, the flux still ascends as shown in Fig. 2B, C. In the beginning, when an electrical field with direct current is applied in a continuous manner to facilitate the permeation of charged molecules, an electrochemical polarization may occur in the skin. Afterwards, the 'flip-flop gating mechanism' reduces the impedance of skin barrier, resulting in the enhancement of skin permeability (Chien et al., 1989; Kasting and Bowman, 1990; Sims et al., 1991). When the electrical field is cut, the skin becomes depolarized but the resistance of skin may not recover immediately. Accordingly the SNA molecules continuously penetrate the skin even though the current has been stopped as shown in Fig. 2B,C. The other reason for this observation may be the existence of a drug reservoir inside the skin since the permeant ion is

desorbing from the skin until the drug reservoir is empty (Wearley et al., 1989).

The differences between the highest and the lowest fluxes during 6 h in from Fig. 2A to Fig. 2C are 6.48, 12.92 and 14.12 μg/(cm^2·h), respectively. This demonstrates that a steady-state like penetration rate of SNA could be achieved by applying a low current density for longer duration.

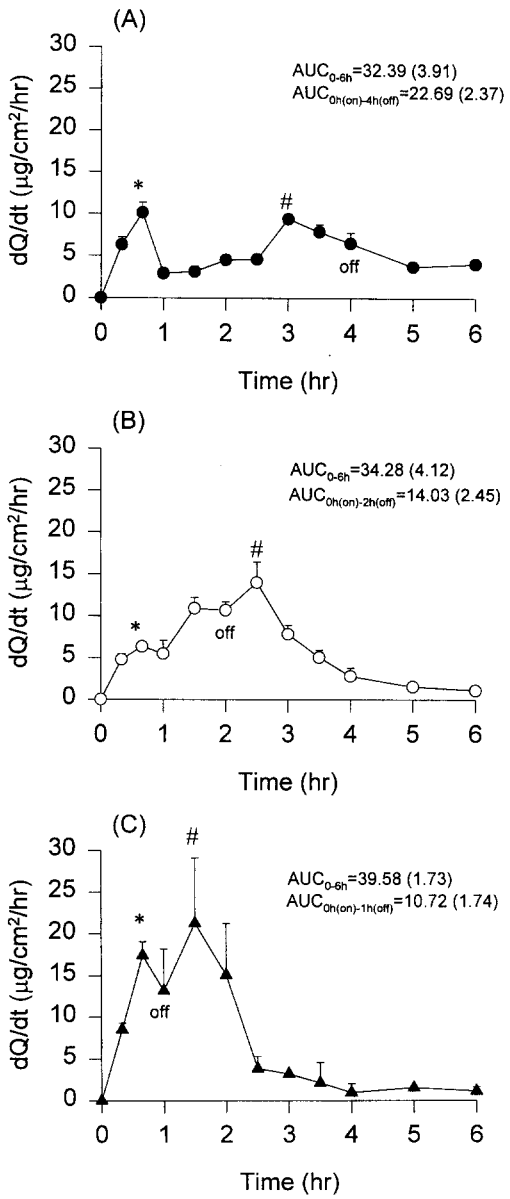

Fig. 2. Plot of flux versus time for SNA with varying current density and application duration: (A) 0.2 mA/cm^2 for 4 h iontophoretic application, (B) 0.4 mA/cm^2 for 2 h iontophoretic application, (C) 0.8 mA/cm^2 for 1 h iontophoretic application. *, first peak, #, second peak. All data represent the means of three experiments ± SD. The area under the curve (AUC) value represents the mean (S.D.).

3.5. Comparison of continuous application mode and discontinuous mode

The flux–time profile for the continuous and discontinuous modes during iontophoresis at pH 4.2 is shown in Fig. 3. The iontophoretic profile of 0.4 mA/cm^2 continuous mode for 2 h is redrawn from Fig. 2B for comparison. The high flux peak in the first hour is observed in discontinuous mode again. Although the applied electric energy was maintained at a constant value, the AUC_{0-6h} of the discontinuous mode exhibited a higher value than the continuous mode. The reason for this result is that the intensity of the effective current of the discontinuous mode across the skin would not decay exponentially as a function of treatment duration, which had been found in the continuous mode (Chien et al., 1989; Singh and Maibach, 1994).

The AUC_{0-6h} of 20 min on/off discontinuous mode is significantly higher (t-test, $P < 0.05$) than that of the 10 min on/off discontinuous mode. This is possibly due to the fact that the maximum SNA penetration capacity is reached during 20 min iontophoresis but never reached during 10 min current application.

3.6. Effect of the combination of varying current density and SNA concentration

Fig. 4 depicts the result of the product of varying current density and donor SNA concentration at a constant electrical energy of three different modes. The profile of the 0.2 mA/cm^2 × 200 μg/ml mode is redrawn from Fig. 2A for comparison. The AUC_{0-6h} of 0.4 mA/cm^2 × 100 μm/ml mode is significantly higher (t-test, $P <$ 0.05) than that of the 0.2 mA/cm^2 × 200 μg/ml and 0.8 mA/cm^2 × 50 μg/ml modes although this difference is not large. The low AUC_{0-6h} of the

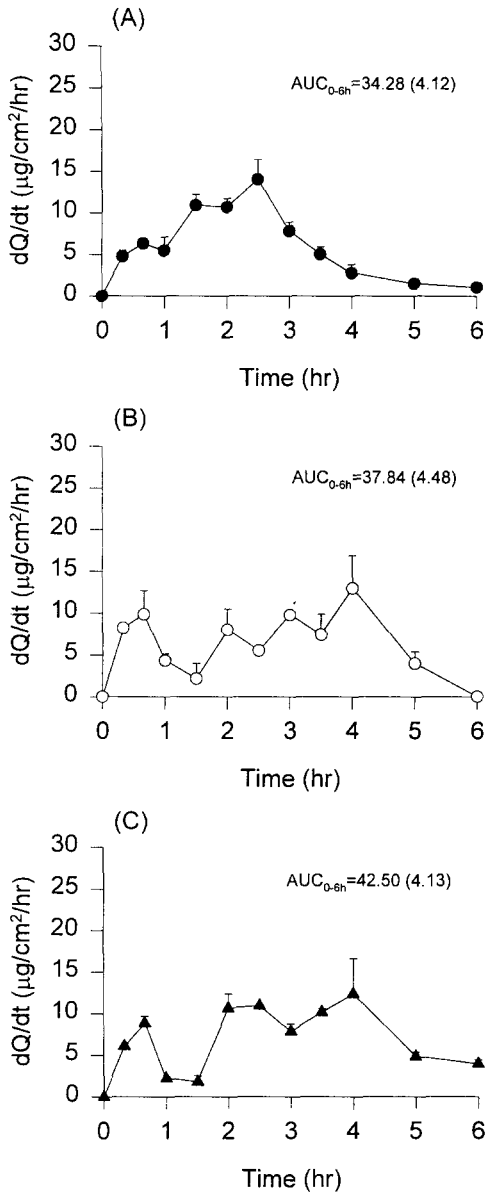

Fig. 3. Plot of flux versus time for SNA with continuous and discontinuous modes: (A) 0.4 mA/cm^2 for 2 h continuous application, (B) 0.4 mA/cm^2 for 10 min on/off cyclic discontinuous application, (C) 0.4 mA/cm^2 for 20 min on/off cyclic discontinuous application. The total current density application time was maintained for 2 h. All data represent the means of three experiments ± SD. The area under the curve (AUC) value represents mean (SD).

0.2 mA/cm^2 × 200 μg/ml mode could be due to the fact that the maximum steady-state flux is never attained at a current density of 0.2 mA/cm^2 during 4 h, as observed in Fig. 1A. In addition,

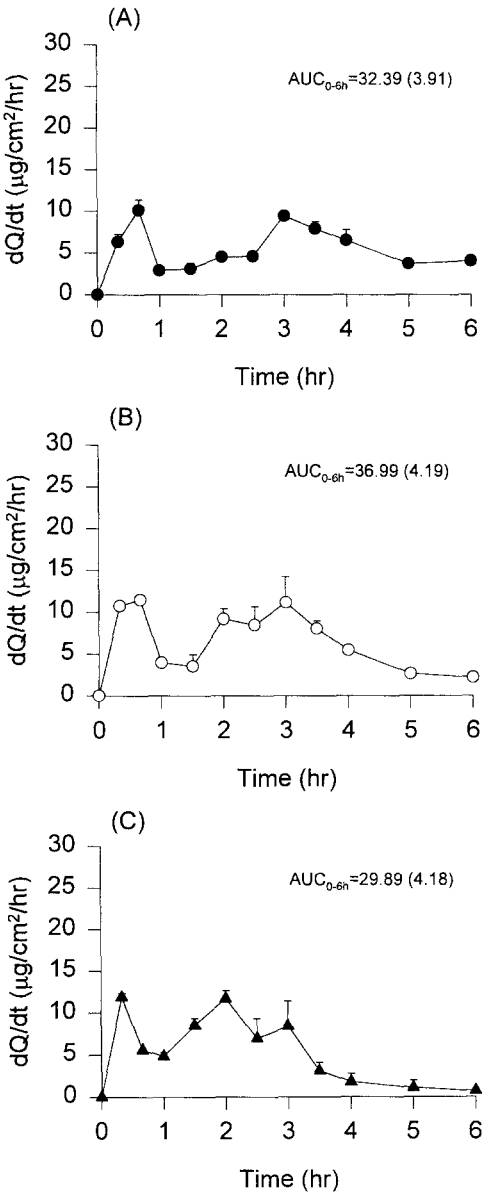

Fig. 4. Plot of flux versus time for SNA with varying current density and donor drug concentration at pH 4.2 solution: (A) 0.2 mA/cm^2 at 200 μg/ml dose, (B) 0.4 mA/cm^2 at 100 μg/ml dose, (C) 0.8 mA/cm^2 at 50 μg/ml dose. The total current density application time was maintained for 4 h. All data represent the means of three experiments ± SD. The area under the curve (AUC) value represents the mean (S.D.).

the permeability coefficient decreases following the increase of SNA concentration as seen in Table 2, which resulted in the low AUC_{0-6h} of this mode.

In the 0.8 $mA/cm^2 \times 50$ μg/ml mode, a lower AUC value at the last 2 h is calculated as compared with those of the other two modes which causes the poor penetration capacity during iontophoresis in this mode. This could be due to the effect of high current application and low administered SNA dose. A high portion of SNA molecules in the receptor compartment is detected as the high current of 0.8 mA/cm^2 is applied at the current cut-off time of 4 h. Therefore, a relatively smaller amount of SNA remains in the donor compartment in this mode as compared with the other two modes. After the cessation of iontophoresis at 4 h, the molecules penetrate the skin mostly by passive diffusion which shows a linear and proportional relationship between flux and donor concentration. Subsequently a lower amount of SNA passes through skin in this mode during the last 2 h. In conclusion, the mean values of current density and donor concentration may provide a good penetration capacity during iontophoresis as shown in Fig. 4B.

3.7. Effect of the combination of varying ionic strength, SNA concentration and current density

Although the delivery efficiency would be enhanced by minimizing donor ionic strength, as seen in Table 3, the platinum electrodes could cause a pH shift of the buffer solution with low ionic strength when a high current density is applied (Lelawongs et al., 1989; Phipps et al., 1989). Hence, it is important to optimize the concentration of buffer species, which should be sufficient to maintain a good buffer capacity.

Fig. 5 depicts the penetration effect of the combination of varying ionic strength, SNA concentration and current density of three electrical energy-constant modes at pH 4.2. Judging from the effect of ionic strength, there is a great reduction in flux when the buffer capacity is increased as shown in Table 3.

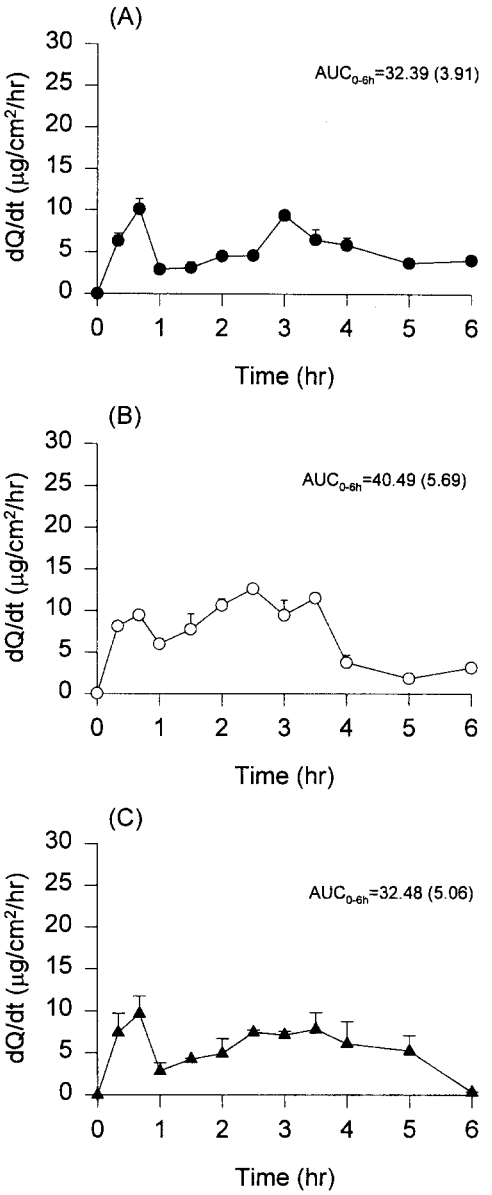

Fig. 5. Plot of flux versus time for SNA with the combination of ionic strength, donor drug concentration and current density: (A) 0.2 mA/cm^2 for 0.06 M buffer capacity at 50 μg/ml dose, (B) 0.8 mA/cm^2 for 0.12 M buffer capacity at 100 μg/ml dose, (C) 0.8 mA/cm^2 for 0.24 M buffer capacity at 200 μg/ml dose. All data represent the means of three experiments ± SD. The area under the curve (AUC) value represents the mean (S.D.).

However, the increase of flux from the administered dose of 100–200 μg/ml is not large

(Table 2). These two factors mean that the AUC_{0-6h} of 0.12 M × 100 μg/ml × 0.8 mA/cm^2 mode is significantly higher (t-test, $P < 0.05$) than that of the 0.24 M × 200 μg/ml × 0.8 mA/cm^2 mode. Consequently the effect of ionic strength on the penetration capacity of SNA is larger than that of donor SNA concentration itself during transdermal iontophoresis. In conclusion, the mode of 0.12 M × 100 μg/ml × 0.8 mA/cm^2 is a better choice to reduce the shift of donor pH value and increase the delivery efficiency of SNA simultaneously.

4. Conclusions

Transdermal iontophoretic delivery offers a strong penetration effect for SNA and, thereafter, short application time is expected to increase the convenience and compliance of patients clinically. This present study establishes the basic iontophoretic properties of SNA throughout the evaluation of electrical and chemical factors. Furthermore, in order to optimize and maximize the iontophoretic penetration capacity a series of application modes is studied to obtain a successful condition for SNA administered iontophoretically. The highest iontophoretic flux of SNA is observed at lower pH values which is due to the physiological property of skin and electro-osmotic flow presented. In varying the ionic strength of donor buffer, the low penetration capacity in high ionic strength could be due to the competition between drug and buffer ions during iontophoresis. Although the penetration capacity of SNA with lower current density for a longer duration is relatively low, a steady-state like penetration rate can be obtained which seems like a sustained release effect. The application of a discontinuous on/off current density mode can be a better choice than a continuous application mode since the intensity of effective current across the skin remains constant resulting in the higher AUC_{0-6h} value. In conclusion, the mean values of current density, drug concentration and ionic strength may provide a suitable penetration during iontophoresis as shown in the present experiment and the kinetic data observed.

Acknowledgements

The authors are grateful to the National Science Council, Republic of China, for the financial support of this study (NSC-85-2331-B037-035).

References

Bannon, Y.B., Corish, J., Corrigan O.I. and Masterson, J.G., Iontophoretically induced transdermal delivery of salbutamol. *Drug Dev. Ind. Pharm.*, 14 (1988) 2151–2166.

Behl, C.R., Kumar, S., Malick, A.W., Del Terzo, S., Higuchi, W.I. and Nash, R.A., Iontophoretic drug delivery: effects of physicochemical factors on the skin uptake of nonpeptide drugs. *J. Pharm. Sci.*, 78 (1989) 355–360.

Bellantone, N.H., Rim, S., Francoeur, M.L. and Rasadi, B., Enhanced percutaneous absorption via iontophoresis I. Evaluation of an in vitro system and transport of model compounds. *Int. J. Pharm.*, 30 (1986) 63–72.

Bronaugh, R.L., Stewart, R.F. and Congdon, E.R., Methods for in vitro percutaneous absorption studies I. Animal models for human skin. *Toxicol. Appl. Pharmacol.*, 62 (1982) 481–488.

Burnette, R.R. and Bagniefski, T.M., Influence of constant current iontophoresis on the impedence and passive Na-permeability of excised nude mouse skin. *J. Pharm. Sci.*, 77 (1988) 492–497.

Burnette, R.R. and Marrero, D., Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin. *J. Pharm. Sci.*, 75 (1986) 738–743.

Burnette, R.R. and Ongpipattanakul, B., Characterization of the permselective properties of excised human skin during iontophoresis. *J. Pharm. Sci.*, 76 (1987) 765–773.

Chen, I.J., Yang, J.M., Yeh, J.L., Wu, B.N., Lo, Y.C. and Chen, S.J., Hypotensive and antinociceptive effects of ether-linked and relatively non-pungent analogues of N-nonanoyl vanillylamide. *Eur. J. Med. Chem.*, 27 (1992) 187–192.

Chien, Y.W. and Banga, A.K., Iontophoretic (Transdermal) delivery of drugs: overview of historical development. *J. Pharm. Sci.*, 78 (1989) 353–354.

Chien, Y.W., Siddiqui, O., Shi, W.M., Lelawongs, P. and Liu, J.C., Direct current iontophoretic transdermal delivery of peptide and protein drugs. *J. Pharm. Sci.*, 78 (1989) 376–383.

Donnerer, J., Amann, R., Schuligoi, R. and Lembeck, F., Absorption and metabolism of capsaicinoids following intragastric administration in rats. *Arch. Pharmacol.*, 342 (1990) 357–361.

Fang, J.Y., Wu, P.C., Huang Y.B. and Tsai, Y.H., In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. *Int. J. Pharm.*, 126 (1995) 119–128.

Fang, J.Y., Wu, P.C., Huang Y.B. and Tsai, Y.H., Percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from gel and ointment bases: in vitro formulation evaluations in pigs and in vivo bioengineering methods in humans. *Int. J. Pharm.* 130 (1996) 121–135.

Gangarosa, L.P., Park, N.H., Wiggins, C.A. and Hill, J.M., Increased penetration of nonelectrolytes into mouse skin during iontophoretic water transport (iontohydrokinesis). *J. Pharmacol. Exp. Ther.*, 212 (1980) 377–381.

Green, P.G., Hinz, R.S., Cullander, C., Yamane, G. and Guy, R.H., Iontophoretic delivery of amino acids and amino acid derivatives across the skin in vitro. *Pharm. Res.*, 8 (1991) 1113–1120.

Higuchi, W.I., Diffusional models useful in biopharmaceutics. *J. Pharm. Sci.*, 51 (1962) 802–804.

Illel, B., Schaefer, H., Wepierre. J. and Doucet, O., Follicles play an important role in percutaneous absorption. *J. Pharm. Sci.*, 80 (1991) 424–427.

Kasting, G.B. and Bowman, L.A., DC electrical properties of frozen, excised human skin. *Pharm. Res.*, 7 (1990) 134–143.

Kasting, G.B., Merrit, E.W. and Keister, J.C., An in vitro method for studying the iontophoretic enhancement of drug transport through the skin. *J. Membr. Sci.*, 35 (1988) 137–159.

Koizumi, T., Kakemi, M., Katayama, K., Inada, H., Sudeji, K. and Kawasaki, M., Transfer of diclofenac sodium across excised guinea pig skin on high-frequency pulse iontophoresis. I. equivalent circuit model., *Chem. Pharm. Bull.*, 38 (1990) 1019–1021.

Lashmar, U.T. and Manger, J., Investigation into the potential for iontophoresis facilitated transdermal delivery of acyclovir. *Int. J. Pharm.*, 111 (1994) 73–82.

Lelawongs, P., Liu, J.C. and Chien, Y.W., Transdermal iontophoretic delivery (II): Evaluation of electrical and operational factors. *Int. J. Pharm.*, 61 (1990) 179–188.

Lelawongs, P., Liu, J.C., Siddiqui, O. and Chien, Y.W., Transdermal iontophoretic delivery of arginine-vasopressin (I): Physicochemical considerations. *Int. J. Pharm.*, 56 (1989) 13–22.

Li, L.C., Vu, N.T. and Allen, L.V., Iontophoretic permeation of sodium cromoglycate through synthetic membrane and excised hairless mouse skin. *J. Pharm. Pharmacol.*, 44 (1992) 444–446.

Masada, T., Higuchi, W.I., Srinivasan, V., Rohr, U., Fox, J., Behl, C. and Pons, S., Examination of iontophoretic transport of ionic drugs across skin: baseline studies with the four-electrode system. *Int. J. Pharm.*, 49 (1989) 57–62.

Miller, L.L. and Smith, G.A., Iontophoretic transport of acetate and carboxylate ions through hairless mouse skin. A cation exchange membrane model. *Int. J. Pharm.*, 49 (1989) 15–22.

Nakhare, S., Jain, N.K. and Verma, H.V., Iontophoretic cellophane membrane delivery of diclofenac sodium. *Pharmazie.*, 49 (1994) 672–675.

Padmanabhan, R.V., Phipps, J.B. and Lattin, G.A., In vitro and in vivo evaluation of transdermal iontophoretic delivery of hydromorphone. *J. Controlled Release*, 11 (1990) 123–135.

Phipps, J.B., Padmanabhan, R.V. and Lattin, G.A., Iontophoretic delivery of model inorganic and drug ions. *J. Pharm. Sci.*, 78 (1989) 365–369.

Santi, P., Catellani, P.L., Massimo, G., Zanardi, G. and Colombo, P., Iontophoretic transport of verapamil and melatonin I. cellophane membrane as a barrier. *Int. J. Pharm.*, 92 (1993) 23–28.

Schade, H. and Marchionini, A., Uber die azidose aut der normalen haut und ihre bedeutung zur abwehr der bakterien. *Munchen. Med. Wchnschr.*, 74 (1927) 1435–1436.

Siddiqui, O., Roberts, M.S. and Polack, A., The effect of iontophoresis and vehicle pH on the in-vitro permeation of lignocaine through human stratum corneum. *J. Pharm. Pharmacol.*, 37 (1985) 732–735.

Sietsema, K., Berman, F., Farmer, W. and Maddin, S., The antinociceptive effect and pharmacokinetics of olvanil following oral and subcutaneous dosing in the mouse. *Life Sci.*, 43 (1988) 1385–1391.

Sims, S.M., Higuchi, W.I. and Srinivasan, V., Interaction of electric field and electro-osmotic effects in determining iontophoretic enhancement of anions and cations. *Int. J. Pharm.*, 69 (1991) 109–121.

Singh, P., Anliker, M., Smith, G.A., Zavortink, D. and Maibach, H.I., Transdermal iontophoresis and solute penetration across excised human skin. *J. Pharm. Sci.*, 84 (1995) 1342–1346.

Singh, P. and Maibach, H.I., Transdermal iontophoresis. Pharmacokinetic considerations. *Clin. Pharmacokinet.*, 26 (1994) 327–334.

Thysman, S., Tasset, C. and Preat, V., Transdermal iontophoresis of fentanyl: delivery and mechanistic analysis. *Int. J. Pharm.*, 101 (1994) 105–113.

Tsai, Y.H., Huang, Y.B., Fang, J.Y. and Wu, P.C., Percutaneous absorption of capsaicin and its derivatives. *Drug Dev. Ind. Pharm.*, 20 (1994) 719–730.

Tyle, P., Iontophoretic devices for drug delivery. *Pharm. Res.*, 3 (1986) 318–326.

Wearley, L. and Chien, Y.W., Enhancement of the in vitro skin permeability of azidothymidine (AZT) via iontophoresis and chemical enhancer. *Pharm. Res.*, 7 (1990) 34–40.

Wearley, L., Liu, J.C. and Chien, Y.W., Iontophoresis facilitated transdermal delivery of verapamil I. In vitro evaluation and mechanistic studies. *J. Controlled Release*, 8 (1989) 237–250.

Williams, A.C. and Barry, B.W., Skin absorption enhancers. *Crit. Rev. Ther. Drug Carrier Syst.*, 9 (1992) 305–353.

Yang, J.M., Wu, B.N. and Chen, I.J., Depressor response of sodium nonivamide acetate: a newly synthesized nonpungent analogue of capsaicin. *Asia Pac. J. Pharmacol.*, 7 (1992) 95–102.