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Transdermal iontophoresis of sodium nonivamide acetate II: optimization and evaluation on solutions and gels

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Abstract

Sodium nonivamide acetate (SNA) is a newly designed derivative of capsaicin which reveals marked antinociceptive activity without producing an overt pungent sensation and skin irritation. The following iontophoretic drug delivery issues have been examined in this paper: (1) the competitive ion effect; (2) transdermal iontophoretic delivery from gel base; and (3) maximization of iontophoretic application mode from gel base. According to the theory of molal volume, divalent salt ions show higher buffering capacity on SNA iontophoretic transport than did monovalent salt ions. However, this effect also causes a great reduction of SNA transdermal flux. The experimental result of transdermal iontophoresis of gel indicated the flux of SNA decreased following the increase of viscosity. Using various polymers incorporated in gel formulations, indicated methyl cellulose and hydroxypropyl methyl cellulose showed higher capacity for SNA iontophoretic transport than the other materials. After a series of evaluation and optimization on the iontophoretic delivery of SNA, transdermal iontophoresis has provided a great capacity of enhancing SNA transport across the skin. The result of the present study is particularly helpful in the development of SNA transdermal delivery system and holds promise for the successful clinical development of an antinociceptive therapeutic regimen. Copyright © 1996 Elsevier Science B.V.

Keywords: Sodium nonivamide acetate; Transdermal absorption; Iontophoresis; Surfactant; Gel

1. Introduction

Capsaicin (8-methyl *N*-vanillyl-6-nonenamide) is a pungent principle from the extraction of red pepper. Several therapeutic advantages of capsaicin such as antinociceptive, hypotensive and hypolipidemia activities have been reported previously (Monsereenusorn et al., 1982; Wang et al., 1984; Clozel et al., 1985). However, the burning pain sensation and skin toxicity, result in the limit of its clinical use (Szolcsanyi and Jancso-Gabor, 1975; Hayes et al., 1984). Sodium nonivamide acetate (sodium *N*-nonanoyl vanillylamide-4′-*O*-acetate; SNA; $C_{19}H_{28}NO_5Na$)

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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 that of capsaicin and indomethacin (Chen et al., 1992). Previous studies suggested SNA could be extensively used in clinical therapy because it avoids any pungent skin sensation and burning pain which had been found in capsaicin to improve patients' compliance (Yang et al., 1992).

Transdermal drug delivery offers some advantages over other routes of drug administration such as its capability of avoiding the hepatic first-pass effect, patient convenience and improved patient compliance (Ledger and Nichols, 1989). In addition, it may be particularly useful for short-acting drugs since percutaneous absorption tends to be controlled and prolongs effects, and since the significant first-pass metabolism has been detected from many capsaicin derivatives (Sietsema et al., 1988; Donnerer et al., 1990). Besides, the half-life of SNA by intravenous administration from rabbits was only 16.80 ± 1.34 min (Fang et al., 1996a). Accordingly, transdermal drug delivery is suitable for SNA to achieve better bioavailability.

SNA becomes the ionized molecule because of the removal of sodium salt at appropriate pH values. So the transdermal iontophoresis may be suitable for SNA to enhance its penetration capacity. Some major limitations for passive transdermal delivery can be easily overcome by iontophoretic delivery (Chien and Banga, 1989). The delivery rate of this non-invasive transdermal system can also be controlled by the various electrical and physicochemical factors (Tyle, 1986). In our previous investigation, electrochemical factors and several current application modes which possess the same electrical energy acting on the kinetics of SNA iontophoresis were well established (Fang et al., 1996b). In order to extend the further investigation and mechanism of in vitro transdermal iontophoresis of SNA, the following drug delivery issues have been examined in this present paper: (1) the effect of competitive ion; (2) transdermal iontophoresis from gel formulation; and (3) optimization of various iontophoretic application modes from gel formulation.

The platinum (pt) wires are selected as the electrodes for the present experiments since they do not precipitate ions like Ag/AgCl electrodes do (Lelawongs et al., 1989). Besides, they can be utilized immediately without any oxidation–reduction pretreatment. Accordingly, the platinum electrodes are the convenient choice for iontophoretic delivery system. The platinum electrodes had been known to cause pH drift in solution as high current density was applied. So the extraneous ion should be added in the donor reservoir to minimize this effect (Phipps et al., 1989). Since the magnitude of SNA percutaneous capacity can be affected by the type and quantity of ions present in donor, the experimental data and mechanism of this effect is also performed and established in the present research.

When the transdermal iontophoretic delivery system is administered in vivo, the semisolid dosage formulation may be more applicable than solution. The gel base often provides a fast release of drug substance and a high degree of clarity in the appearance (Gennaro, 1990). Moreover, there is always a great volume of water employed in gel formulation which exhibits a high electrical conductivity. Hence the transdermal iontophoresis from gel base is developed to hold promise for further in vivo investigation.

2. Materials and methods

2.1. Materials

The following reagents were used: citric acid, di-sodium hydrogen phosphate dihydrate, triethanolamine, Tween 20® and sodium laurylsulfate (Merck); KCl, cetylpyridinium chloride (Sigma); LiCl and cetrimide (hexadecyltrimethylammonium bromide) (Ferak); NaCl and propylene glycol (Shimakyu); benzalkonium chloride, methyl cellulose (350–550 cps) and carboxymethyl cellulose sodium ($n = 500$) (Tokyo Kasei); hydroxypropyl methyl cellulose (Metolose®, 4000 cps) (Shin-Etsu); $MgCl_2$ (J.T. Baker); $MgSO_4$ (Nacalai); Carbopol 940® (B.F. Goodrich). The synthetic procedure of SNA had been performed from our laboratory (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).

2.2. Instruments and penetration procedures

The in vitro penetration procedures of iontophoresis were determined using a horizontal glass diffusion cell. 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 animal skin types (Fang et al., 1995). The hair of the rat was removed with electric clippers and the abdominal skin was excised after careful shaving. The skin pieces were soaked in the receptor buffer solution for 45 min prior to being placed in the cells (Miller and Smith, 1989). The rat skin was then mounted between the cell compartments with the stratum corneum facing towards the donor half cell. The donor compartment of the cell was filled with 8 ml solution or 8 g gel base containing SNA. The donor buffer solution was prepared so as to contain 100 mg/ml of SNA. The receptor phase contained 8 ml of 0.06 M; pH 7.4 McIlvaine buffer was used. The available diffusion surface area was 0.785 cm^2. All experiments were carried out at 37°C and the compartments of donor and receptor were agitated by magnetic stirrers at 600 rpm. 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 or gel 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 Co., Model 7651). Current density of 0.5 mA/cm^2 was applied to stimulate the penetration of SNA in experiments. 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).

2.3. Preparation of gel bases

The Carbopol 940® gel formulation was composed of Carbopol 940® (0.3–0.6%), triethanolamine (0.5–2.0%), propylene glycol (4.0–20.0%) and purified water was added to make a total amount of 100%. Another type of Carbopol 940® gel formulation was composed of Carbopol 940® (0.2–0.3%), triethanolamine (0.3–0.5%), Tween 20® (0.1%), and purified water was added to 100%. Cellulose gel formulation was composed of methyl cellulose, carboxymethyl cellulose sodium or hydroxypropyl methyl cellulose in various concentrations and then purified water was added to give 100%. SNA was participated in the base to give a concentration of 200 $\mu g/g$ (0.02%).

2.4. Viscosity of gel bases

Determination of gel viscosity was done in a cone and plate viscometer (Brookfield Co., Model DV-2). Gel (0.5 g) was placed in the sample cup of the viscometer and allowed to stand for 1 h to reach 37°C. Reading was detected 30 s after measurement was made, when the level had stabilized.

2.5. Optimization of iontophoretic application mode from gel

Four studies were conducted at a fixed current of 0.5 mA/cm^2. In the first study (A), continuous application of current was conducted for 2 h. In the second study (B), discontinuous application of current was conducted for 20:10 min on/off cycle. In the third study (C), discontinuous application of current was conducted for 20:20 min on/off cycle. The fourth study (D), discontinuous application of current was conducted for 20:30 min on/off cycle. The total current density application time was maintained for 2 h.

2.6. 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 was calculated by the slope of the linear portion of cumulative amount-time plots for zero-order model and cumulative amount-(time)$^{1/2}$ plots for Higuchi model and expressed as the mass of drug passing across 1 cm^2 of skin over time or square root of time (Higuchi, 1962). The area under the curve (AUC) of flux (dQ/dt)-time plots was calculated by the trapezoidal method. 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.

3. Results and discussion

3.1. Competitive ion effect

A series of 0.01 M salt ions such as LiCl, NaCl, KCl, $MgCl_2$ and $MgSO_4$ was added in donor compartment, respectively. The SNA concentration of 100 μg/ml at pH 4.2 with adjusting the ionic strength of donor buffer solution to 0.12 M was applied for 0.5 mA/cm^2 current density. As shown in Fig. 1, the curve of cumulative amount-time profile was suitable to fit by using zero-order equation (correlation coefficient, $r = 0.93–0.97$). This result is inconsistent with the previous data using ionic strength of 0.06 M in donor which was fitted by Higuchi equation (Fang et al., 1996b). The electrochemical decomposition of water with the production of H^+ ions at the anode and OH^- ions at the cathode is a common phenomenon when utilizing platinum wires as the electrodes (Wearley et al., 1989a). This reaction causes the pH of the donor compartment to increase during cathodal iontophoresis in this present study. So the ionic strength should be high enough to give sufficient buffering capacity and avoid pH drift (Burnette and Marrero, 1986). Accordingly the ionic strength of 0.06 M might shift the pH value to a higher degree than 0.12 M did during iontophoresis. The flux of SNA decreased with increasing donor pH values during iontophoresis. Thus the iontophoretic flux of SNA leveled off significantly when using 0.06 M ionic strength resulted in the limited increase on cumulative amount of SNA at the end-stage of the penetration experiment and the fit of Higuchi model.

The ionic mobilities are constant throughout the experiment, hence the permeant transport is a function of the total current and the fluxes of other ions present (Green et al., 1991). As the flux of the other ion species are also dependent upon the total current, the flux of SNA will be reduced as salt ion is added to the donor solution. The fraction of the current carried by a particular ion is given by its transference number (t_i) (Martin et al., 1993):

$$J_i = t_i \cdot I_T / z_i \cdot F \quad (1)$$

where J_i is the flux of ion species i; I_T, total current density; z_i, valence of ions i; F, Faraday's constant.

Judging from the effect of univalent salt ions of LiCl, NaCl and KCl as shown in Table 1, there are negligible pH differences after the addition of these three competitive ions as compared with the control group which had no competitive ions in the donor. Consequently univalent salt ion may

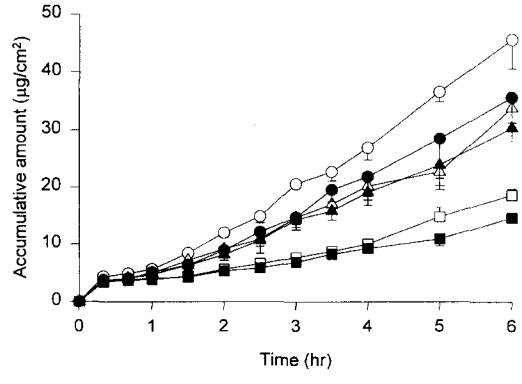

Fig. 1. Cumulative amount of SNA detected in the receptor compartment versus time following the iontophoresis at pH 4.2 for 0.5 mA/cm^2 incorporated with a series of salt ions: (○) control group, (●) KCl, (△) NaCl, (▲) LiCl, (□) $MgSO_4$, (■)$MgCl_2$. All data represent the means of three experiments ± S.D.

Table 1
Effect of 0.01 M salt ions added in donor buffer solution on the iontophoretic penetration of SNA at pH 4.2

Salt ion	Final pH of donor	Cumulative amount at 6 h (μg/cm^2)	Flux (μg/cm^2/h)
Control	6.109 ± 0.014	45.67 ± 5.01	7.34 ± 0.80
LiCl	6.093 ± 0.020	30.40 ± 2.32	4.80 ± 0.94
NaCl	5.994 ± 0.017	33.83 ± 2.53	5.07 ± 0.44
KCl	6.076 ± 0.014	35.67 ± 5.09	5.74 ± 0.82
$MgCl_2$	5.272 ± 0.015	14.62 ± 0.38	2.01 ± 0.31
$MgSO_4$	5.324 ± 0.040	18.60 ± 1.09	2.67 ± 0.58

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

not offer sufficient buffering capacity during iontophoresis. Furthermore, the inhibition of SNA flux increased in the order of KCl < NaCl < LiCl. When applying cathodal iontophoresis, the major ion competing with SNA anions from donor to receptor compartment is the negative ion (Cl^-). The fraction of total current carried by the cations or by anions is known as the transference number t_+ or t_-. The sum of the two transference numbers is obviously equal to unity (Martin et al., 1993):

$$t_+ + t_- = 1 \quad (2)$$

The transference numbers are related to the velocities of the ions and the faster-moving ion carrying the greater fraction of current. The velocities of ions also depend on hydration, ion size and ion charge. The transference number of sodium ion in NaCl solution is 0.385. Because it is greatly hydrated, the lithium ion in LiCl solution moves slower than the sodium ion and hence has a lower transference number of 0.317 (Martin et al., 1993). Because of the theory of Eq. (2), the transference number of chloride ion of LiCl is larger than that of NaCl resulted in a smaller fraction of current density carried by SNA anions in LiCl-added solution as depicted in Table 1. The same phenomenon is observed when comparing NaCl with KCl. It has been shown that Na^+ has a slower ionic velocity in an electrical field than dose K^+ since sodium ions attract more water of hydration resulting in a larger hydrated diameter of sodium ions (Gangarosa et al., 1980). Besides, the interionic interference between SNA negative ion and Na^+ increased after the addition of NaCl. The production of SNA molecules which is an uncharged substance impedes the penetration capacity of SNA during iontophoresis (Del Terzo et al., 1989).

Table 1 demonstrates that the iontophoretically induced SNA flux is markedly reduced when $MgCl_2$ is added into donor site. According to Faraday's law, the number of charges carried by chloride ion of $MgCl_2$ is twice that of univalent salt in the same 0.01 M condition. The fact that the efficiency of ion delivery of $MgCl_2$ is consequently larger than that of LiCl et al. results in the low fraction of total current density carried by SNA (Burnette and Ongpipattanakul, 1987; Phipps et al., 1989). $MgSO_4$ shows a weaker capacity of inhibiting SNA iontophoretic flux compared with $MgCl_2$, in other words, the transference number of $2Cl^{1-}$ is larger than that of SO_4^{2-}. This phenomenon can be explained by the theory of free volume model (Yoshida and Roberts, 1993). The ion sphere mobility has been assumed to be proportional to the fractional volume of the space that is accessible to the ion sphere (Smisek and Hoagland, 1990). So the iontophoretic permeability coefficient, PC_{iont}, of an ion solute has been shown to be directly related to the molal volume (MV) of the solute (Yoshida and Roberts, 1993):

$$\log PC_{iont} = \log B - MV/2.3\ V_{av} \quad (3)$$

where B is the empirical constants, V_{av} is the average free volume. The molal volume of an ion solute is related to its molecular weight. The free volume gives a measure of the size of a space into which a solute with an equal or smaller molal volume can enter readily (Yoshida and Roberts, 1993). According to Eq. (3), the transference

Table 2
Effect of 0.01% surfactants added in donor compartment on the iontophoretic penetration of SNA at pH 4.2

Enhancer	Final pH of donor	Cumulative amount at 6 h (μg/cm^2)	Flux (μg/cm^2/h)
Control group	6.109 ± 0.014	45.67 ± 5.01	7.34 ± 0.80
Sodium laurylsulfate	5.919 ± 0.010	22.92 ± 1.84	3.40 ± 0.27
Benzalkonium Cl	6.073 ± 0.022	14.76 ± 2.44	2.13 ± 0.15
Cetylpyridinium Cl	6.092 ± 0.020	9.20 ± 0.61	1.12 ± 0.07
Cetrimide	6.077 ± 0.012	8.29 ± 0.41	1.13 ± 0.06

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

number of the solute should be opposite to the size of molal volume. The molecular weight and molal volume of SO_4^{2-} is significantly larger than those of $2Cl^{1-}$, hence $2Cl^{1-}$ occupies higher transference number which is consistent with the results of Table 1. Considering $MgCl_2$ and $MgSO_4$, the high capacity of inhibiting SNA iontophoretic flux bring the result of high buffering capacity to slightly increase the pH of the donor compartment only from 4.2 to approximately 5.3 after 6 h iontophoretic application. In conclusion, although the divalent salt ions exhibit the strong buffering capacity, however, this effect may cause a great reduction of SNA iontophoretic flux.

The effect of surfactant adding in donor compartment was also studied. Anionic or cationic surfactant of 0.1% concentration participated with 100 μg/ml SNA as donor formulation was applied by 0.5 mA/cm^2 current density for 6 h duration. The result is shown in Table 2. In light of the final donor pH values, the data demonstrates that surfactants exhibit low buffering capacity since they are all univalent ions. The surfactants were used as penetration enhancers on the transdermal drug delivery (Williams and Barry, 1992), however, the data of iontophoretic flux indicate the competitive ion effect of surfactant is significantly larger than the penetration enhancing effect for SNA. According to free volume model (Yoshida and Roberts, 1993), the solute with low molecular weight shows high electrophoretic mobility which may play the role of a strong competitive ion. The fact that the molecular weight of chloride or bromide negative ion is significantly lower than that of laurylsulfate ion results in the stronger competitive ion effect of cationic surfactant than anionic surfactant.

Cetylpyridinium chloride has shown higher electrophoretic mobility and inhibitory activity for SNA transport than benzalkonium chloride as observed in Table 2. Since the molecular weights of these two surfactants are the same ($n = 340$), the Cl^- molal volumes of both surfactants should be judged by their steric structures so as to utilize the free volume model. The benzalkonium is a quaternary ammonium group which shows that a tetrahedron steric structure results in the increase of steric hindrance. On the other hand, the cetylpyridinium chloride is a plane-like structure. Accordingly, Cl^- can attach to the nitrogen cation of cetylpyridinium more easily and more strongly than that of benzalkonium. The radius or molal volume of Cl^- attached by cetylpyridinium is, for this reason, smaller than that of benzalkonium (Morrison and Boyd, 1983). Another possible reason is the density of the electron. The nitrogen atom holds four single chemical bonds in benzalkonium as well as two single bonds and one double bond in cetylpyridinium. For the cetylpyridinium ion, the fact that the loose π electrons cause lower electronic density than does the benzalkonium ion results in the possibility of seeking the Cl^- (Morrison and Boyd, 1983). Therefore, the same status as the above inference is obtained.

There is no significant difference (t-test, $p = 0.05$) between the iontophoretic flux of SNA incorporated with cetylpyridinium chloride and cetrimide which demonstrates that the chloride ion has the same molal volume as the bromide ion of cetrimide.

3.2. Transdermal iontophoresis of SNA from gel formulation

Various formulations of Carbopol 940® gel were performed for 0.5 mA/cm^2 iontophoretic delivery for 6 h. The cumulative amount-time curve suggested that transdermal iontophoretic flux of SNA from gel base was suitable to fit by using the Higuchi equation (correlation coefficient, $r = 0.94–0.99$) (Higuchi, 1962). In other words, at the later-stage of iontophoresis the flux appeared to level off. This phenomenon is consistent with previous research using hydrogel as the formulation for iontophoretic delivery such as salbutamol and piroxicam (Bannon et al., 1988; Gay et al., 1992). Despite the additives such as triethanolamine, propylene glycol or Tween 20®, there was no extraneous ions participated into Carbopol 940® gel base resulted in the lack of buffering capacity. Hence the final pH value of donor may be elevated to the higher levels to decrease the iontophoretic transport of SNA (Fang et al., 1996a; Fang et al., 1996b). Alternatively, continuous current iontophoresis will always develop a skin polarization potential, eventually decreasing the magnitude of effective current (Lashmar and Manger, 1994).

The passive transport experiments had shown that no detectable amounts of SNA were transported across rat skin from Carbopol 940® gel bases when the data was analyzed extensively for 72 h (Fang et al., 1995). As compared with the present data of Fig. 2 of iontophoretic delivery from gel bases, it is observed that iontophoresis has a great enhancement effect on the penetration of SNA across rat skin. As shown in Fig. 2 of various gel formulations, an increase in the viscosity results in a decrease in the formulation conductivity and the iontophoretic flux of SNA is decreased as a result of that (Chu et al., 1994). Consequently, the viscosity of semisolid formulation not only affected the passive transport of permeant (Hsu et al., 1994), but also the transdermal iontophoretic delivery of permeant.

The highest iontophoretic flux value of 15.78 ± 0.54 μg/cm^2/h$^{1/2}$ from Carbopol 940® gel which showed the pH value of 7.4 is significantly higher (t-test, $P < 0.05$) than that of 0.06 M; pH 7.0 buffer solution in donor site at the same iontophoretic condition (13.28 ± 2.45 μg/cm^2/h$^{1/2}$) (Fang et al., 1996b). The low penetration of SNA in the solution formulation could be due to the competition between SNA and buffer ions which were lacking in the gel formulation (Burnette and Bagniefski, 1988; Lelawongs et al., 1989).

The various Carbopol 940® formulations and cellulose gel formulations at proximate viscosities ($58.67 \pm 3.41 - 69.00 \pm 7.32$ cps $\times$ 10^3) are selected for transdermal iontophoresis as shown in Fig. 3. The cellulose gel formulations reveal greater iontophoretic capacity than Carbopol 940® gel bases except for carboxymethyl cellulose sodium gel. Carboxymethyl cellulose sodium is one of the ionic polymers. Partial current would be carried by the ionized polymer which resulted in the fact that a limited fraction of applied current was carried by SNA. The methyl cellulose and hydroxypropyl methyl cellulose gel bases reveal an almost 3-fold greater SNA iontophoretic flux than Carbopol gel bases. For maximizing the penetration capacity of SNA by iontophoretic application, afterwards, various concentrations of these two cellulose gels were chosen to perform transdermal experiments.

As shown in Fig. 4, the flux of SNA increased with the decrease of methyl cellulose which is due to the effect of viscosity. A similar result is observed in hydroxypropyl methyl cellulose al-

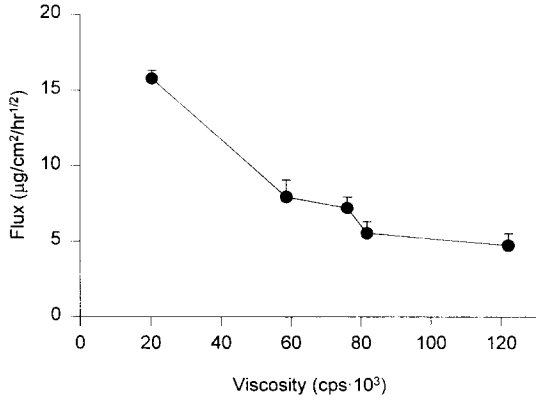

Fig. 2. Flux of SNA from Carbopol 940® gel formulations at various viscosities during transdermal iontophoresis. All data represent the means of three experiments ± S.D.

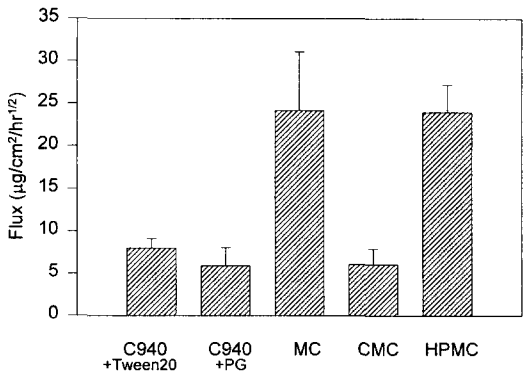

Fig. 3. Flux of SNA from various gel formulations at proximate viscosities during transdermal iontophoresis. C940 + Tween20: Carbopol 940® (0.3%), Tween 20 (0.1%), triethanolamine (0.3%); C940 + PG: Carbopol 940® (0.3%), propylene glycol (4.0%), triethanolamine (0.5%); MC, methyl cellulose (6.0%); CMC, carboxymethyl cellulose sodium (4.5%); HPMC, hydroxypropyl methyl cellulose (5.0%). All data represent the means of three experiments ± S.D.

though the flux of 6.5% concentration is higher than that of 6.0% which shows no significant difference (t-test, $P > 0.05$) between fluxes of these two groups. The methyl cellulose gels revealed higher iontophoretic capacity than did hydroxypropyl methyl cellulose gels compared at the same concentration. For maximizing the iontophoretic capacity of SNA by various application modes, the 5.0% methyl cellulose formulation was chosen as the model gel bases. Furthermore, the Carbopol formulation composed of 0.2% Carbopol 940®, 0.5% triethanolamine and 0.1% Tween 20 which shows the highest SNA flux in Fig. 2 was also performed in the next study.

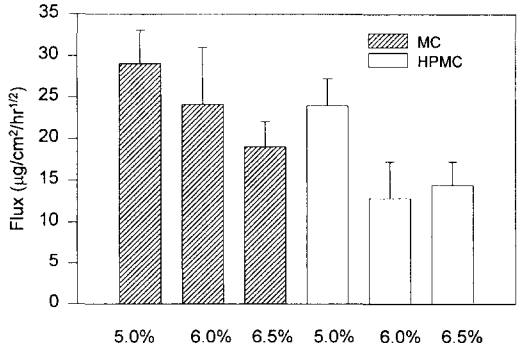

Fig. 4. Flux of SNA from cellulose gel formulations at various cellulose concentrations during transdermal iontophoresis. MC, methyl cellulose; HPMC, hydroxypropyl methyl cellulose. All data represent the means of three experiments ± S.D.

3.3. Optimization of iontophoretic application mode from gel

As mentioned previously, the iontophoretic penetration capacity by discontinuous current application was higher than that by continuous mode (Fang et al., 1996b). The reason for this result was that the intensity of the effective current of 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). Consequently, four modes were conducted at a fixed current of 0.5 mA/cm^2 in this present experiment for maximizing the penetration capacity of SNA during iontophoresis. Figs. 5 and 6, show the flux (dQ/dt)-time profile of the effect of four application modes of Carbopol gel and methyl cellulose gel respectively. The fact that the highest flux peak is observed at the first 20 min suggests there is a great burst effect for gel bases. SNA travel primarily through shunt pathways which carry a net negative wall charge (Burnette and Marrero, 1986) and that the shunts decrease in diameter as the tissue hydrates. The shunt will initially have a lower net negative charge density than at a later time, when it is more fully hydrated. As a consequence, SNA will initially show a higher flux through skin than later (Burnette and Ongpipattanakul, 1987; Lashmar and Manger, 1994). In addition, this increased transport may be attributed to the high number of hair follicles in furry rat skin than hairless mouse and human skin resulted in the amplification of this enhanced effect (Bronaugh et al., 1982).

With four application modes, the magnitude of $AUC_{0-6\ h}$ shows a trend of D < B for both gel formulations. In comparison to the discontinuous current application modes, the result suggests that 10 min-cessation of current density is enough for on/off cyclic iontophoretic application. Since the permeant continuously transport to the receptor compartment even though the current density has been stopped. At the current-off duration, the

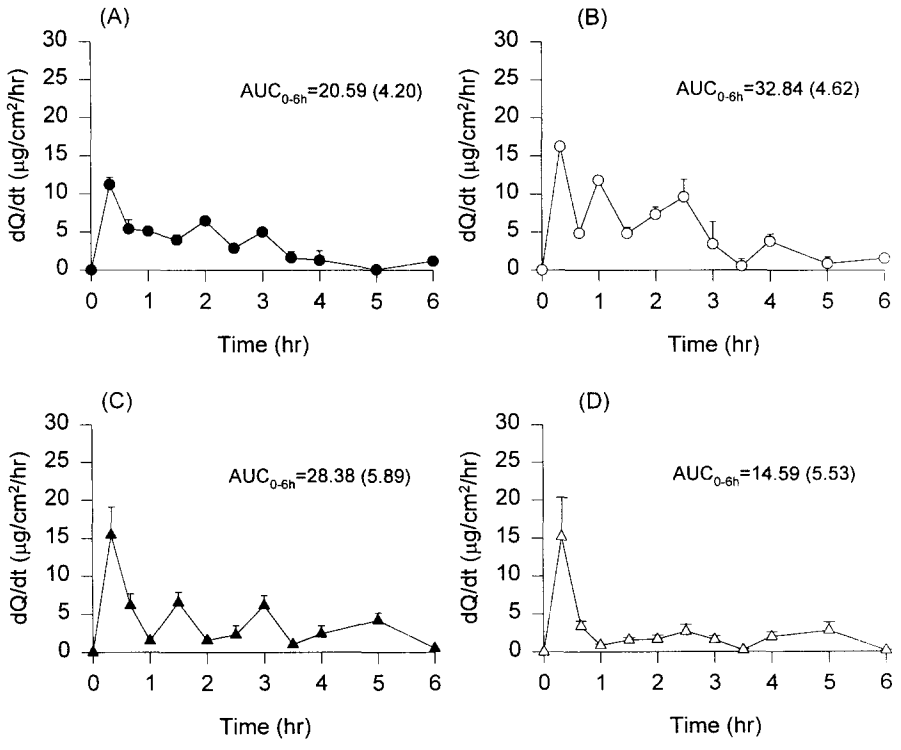

Fig. 5. Plot of flux versus time for SNA with various current application modes at 0.5 mA/cm^2 from Carbopol 940® gel formulation: (A) 2 h continuous application, (B) 20:10 min on/off cyclic discontinuous application, (C) 20:20 min on/off cyclic discontinuous application, (D) 20:30 min on/off cyclic discontinuous application. The total current density application time was maintained for 2 h. All data represent the means of three experiments ± S.D. The area under the curve ($AUC_{0-6\,h}$) value represents mean (S.D.).

permeant ion is desorbing from the membrane until the emptying of the drug reservoir inside the skin (Wearley et al., 1989a; Wearley et al., 1989b). So the desorption time of SNA from skin after current-off requires approximately 10 min, since the longer period of current-off time causes the reduction of $AUC_{0-6\,h}$. For three discontinuous current application modes, the $AUC_{0-6\,h}$ values from mode B to mode D show a ratio of 2.25:1.95:1 for Carbopol gel. This result is also found at methyl cellulose gel which shows a similar ratio of 2.38:1.83:1. Although the iontophoretic mode D is a discontinuous current application, nevertheless, its $AUC_{0-6\,h}$ is significantly lower (t-test, $p < 0.05$) than that of continuous current mode A. During the transdermal iontophoresis, the skin becomes depolarized but the resistance of skin may not recover immediately as the current is cut. Accordingly the SNA continuously penetrate the skin even though the current has been stopped. Then SNA passively transport across skin after the recovery of skin resistance. The cumulative amount of SNA from gel base across skin by passive diffusion is scarce and can be negligible. Therefore, the skin resistance may recover within 20 min since the 30 min current-off time of mode D caused the reduction of iontophoretic capacity which is due to there being a long duration of passive penetration for SNA in this mode.

4. Conclusions

A series of 0.01 M salt ions was added in the donor compartment respectively. According to the theory of molal volume, divalent salts show higher buffering capacity and inhibitory effect on

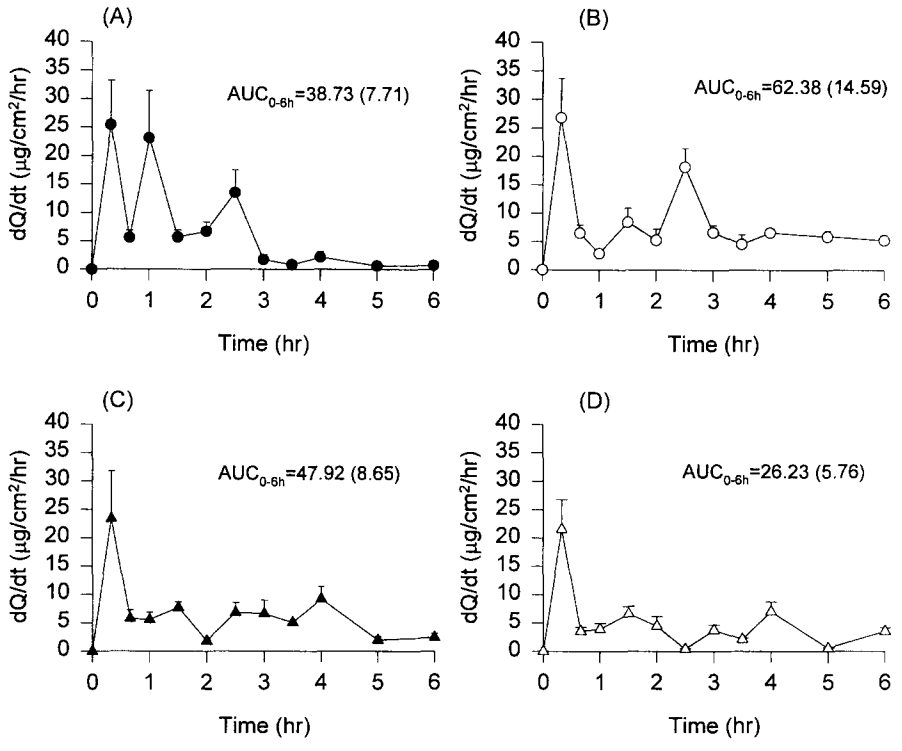

Fig. 6. Plot of flux versus time for SNA with various current application modes at 0.5 mA/cm^2 from methyl cellulose gel formulation: (A) 2 h continuous application, (B) 20:10 min on/off cyclic discontinuous application, (C) 20:20 min on/off cyclic discontinuous application, (D) 20:30 min on/off cyclic discontinuous application. The total current density application time was maintained for 2 h. All data represent the means of three experiments ± S.D. The area under the curve ($AUC_{0-6\,h}$) value represents mean (S.D.).

SNA penetration during iontophoresis than monovalent salts do. Various formulations of Carbopol 940® gel were performed for iontophoretic delivery. The iontophoretic transport of SNA is significantly larger than the passive transport from gel base. The iontophoretic transport of SNA from gel was even greater than that from solution formulation which is due to the lack of buffer ions in the gel formulation. After a series of optimization of iontophoretic delivery such as gel formulation and current application mode, transdermal iontophoresis has provided the great ability of enhancing SNA delivery across the skin. The discontinuous current application played a higher iontophoretic capacity than did continuous application. And the result suggested that 20:10 min on/off cycle of current density was enough for SNA so as to get the maximum $AUC_{0-6\,h}$ value. The result of this paper can hold promise for the successful clinical development of an antinociceptive therapeutic method.

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