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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

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Abstract

Nonivamide (NVA) and non-pungent sodium nonivamide acetate (SNA) are synthetic derivatives of capsaicin. In this study, in vitro formulation evaluations in pigs and in vivo bioengineering methods in humans were performed to obtain the information on percutaneous absorption for capsaicin, NVA and SNA. After the consideration of penetration capacity, skin irritation and physicochemical properties of the formulations in vitro, 0.6% Carbopol 940® gel form for capsaicin and NVA as well as 3.8% isopropyl myristate-added hydrophilic o/w ointment for SNA were utilized to study the following in vivo test in humans. In the study of in vivo surface recovery techniques, SNA showed an equivalent therapeutic capability to that of NVA after calculation of the antinociceptive index. After the quantification of skin erythema by laser Doppler flowmetry (LDF), capsaicin caused more severe irritation than NVA in humans. In addition, SNA showed no skin irritation, toxicity and pungent sensation. The transepidermal water loss (TEWL) values were determined using an evaporimeter. A comparison of the gel form showed there was significantly higher TEWL AUC values in capsaicin and NVA than in the control group. The AUC value of the hydrophilic ointment control group was significantly higher than that of the gel control group. The reason was that isopropyl myristate and sodium laurylsulfate, two additives incorporated in the hydrophilic base, could cause slight skin irritation resulting in the increase of TEWL. The results of this study suggest that SNA possesses potent antinociceptive activities after transdermal application. Furthermore, SNA can be used extensively in clinical therapy because it avoids any pungent skin sensation and burning pain to improve patients' compliance.

Keywords: Capsaicin; Nonivamide; Sodium nonivamide acetate; Gel; Ointment; Percutaneous absorption; LDF; TEWL

1. Introduction

Capsaicin (8-methyl *N*-vanillyl-6-nonenamide; $C_{18}H_{27}NO_3$) is a pungent constituent from the

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Table 1
In vitro penetration data of capsaicin, NVA and SNA in various triethanolamine concentrations from gel bases through pig skin[a]

Trietnanolamine (%)	Capsaicin		NVA	
	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h$)	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h$)
2	143.3 ± 125.63	2.17 ± 0.43	149.98 ± 31.02	2.13 ± 0.43
4	170.35 ± 21.17	2.28 ± 0.47	181.48 ± 33.58	2.64 ± 0.71
8	132.54 ± 27.96	1.97 ± 0.43	164.52 ± 30.83	2.45 ± 0.52

[a] No SNA accumulative amount is detected during a 72 h period.
Each value represents the mean ± SD ($n = 3$).

Table 2
In vitro penetration data of capsaicin and NVA in various isopropanol concentrations from gel bases through pig skin[a]

Isopropanol (%)	Capsaicin		NVA	
	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h$)	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h$)
0	143.31 ± 25.63	2.17 ± 0.43	149.98 ± 31.02	2.13 ± 0.43
10	62.02 ± 19.25	0.93 ± 0.21	37.42 ± 7.66	0.55 ± 0.10
20	41.68 ± 19.67	0.57 ± 0.21	31.96 ± 9.04	0.43 ± 0.10
30	32.54 ± 27.96	0.56 ± 0.20	7.32 ± 1.68	0.10 ± 0.02

[a] Each value represents the mean ± SD ($n = 3$).

extraction of red pepper. Early studies of its pharmacological effects revealed a wide spectrum of activities including analgesic effects (Monsereenusorn et al., 1982; Tominack and Spyker, 1987; Crimi et al., 1992). Nonivamide (*N*-nonanoyl vanillylamide; NVA; $C_{17}H_{27}NO_3$), also called synthetic capsaicin, is a substitute of capsaicin which has similar chemical structure and pharmacological effects as those of capsaicin (Chen et al., 1992). However, the therapeutic value of these two analogues is limited by the concomitant irritation and toxicity accompanying its use (Hayes et al., 1984; Maggi et al., 1987). In order to improve these disadvantages of capsaicin and NVA and increase the compliance of the patients, NVA was reacted by replacing the phenolic hydroxyl group with hydrophilic substituents to form a newly synthetic analogue of capsaicin—sodium nonivamide acetate (sodium *N*-nonanoyl vanillylamide-4′-*O*-acetate; SNA; $C_{19}H_{28}NO_5Na$) (Fang et al., 1995a). Non-pungent SNA showed obvious antinociceptive and hypotensive activities as well as less acute and cardiac toxicity than those of capsaicin and NVA (Chen et al., 1992).

Capsaicin has a wide clinical use in topical diseases such as rheumatic arthritis. Since the obvious first-pass metabolism has been detected from capsaicinoids (Donnerer et al., 1990). Besides, the half-life values of capsaicin and its synthetic derivatives by intravenous administration were very short (Kawada et al., 1985; Fang et al., 1995b). So transdermal drug delivery system (TDDS) is suitable for the development of the capsaicin, NVA and SNA pharmaceutical dosage forms.

The aim of this study is to perform the in vitro evaluation of capsaicin, NVA and SNA so as to select the optimal formulations for in vivo bioengineering tests in humans. In our previous in vitro penetration study (Fang et al., 1995a), the Carbopol gel forms exhibited good fluxes and accumulative amounts for NVA and the gel base often provide a fast release of drug substance and high degree of clarity in the appearance. Further-

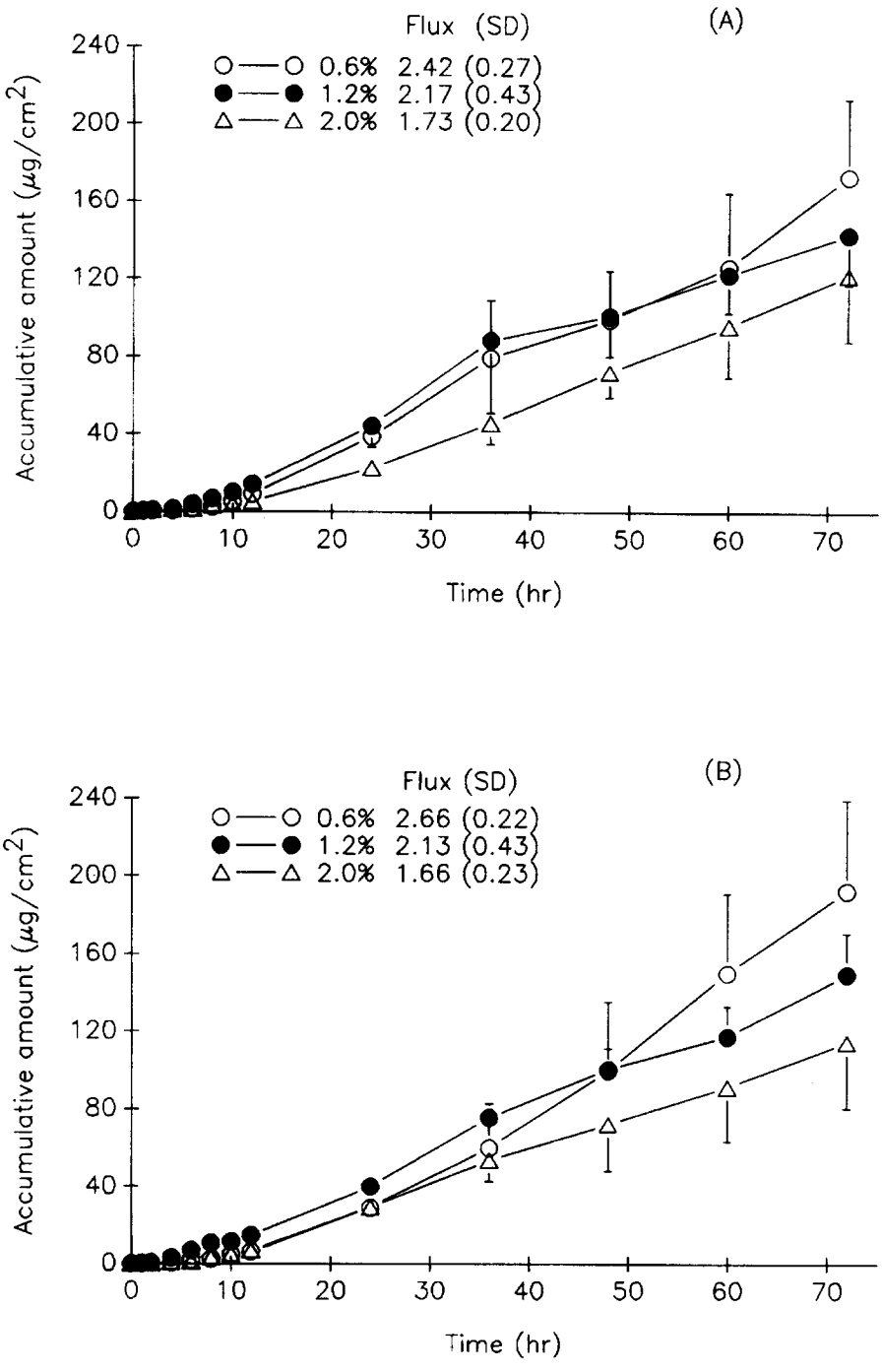

Fig. 1. In vitro percutaneous profiles of capsaicin (A) and NVA (B) at various Carbopol 940® concentrations from gel base in receptor phase. All data represent the means of three experiments ± SD.

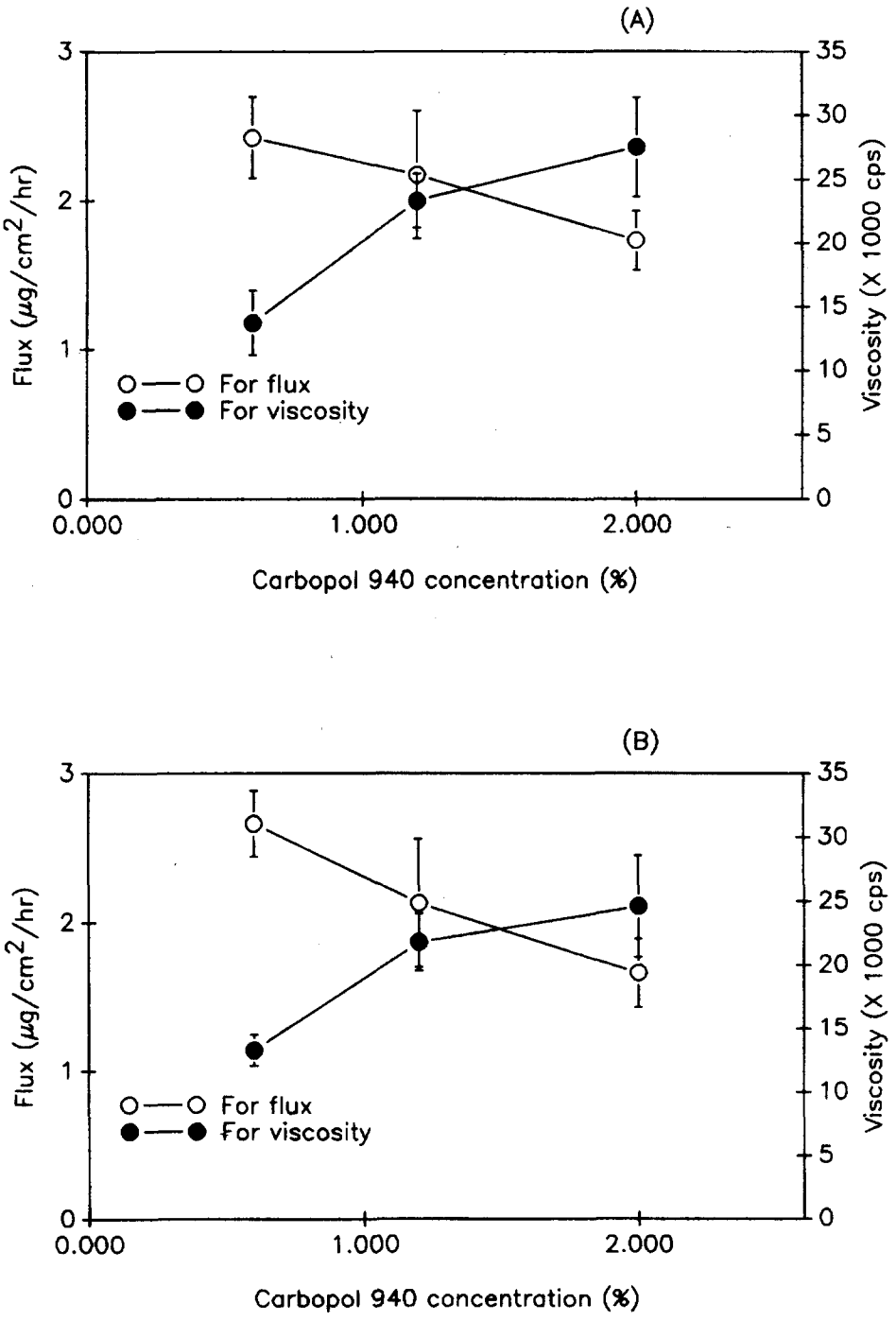

Fig. 2. Relationship between the fluxes of capsaicin (A), NVA (B) and apparent viscosity of each gel base. All data represent the means of three experiments ± SD.

Table 3
In vitro penetration data of capsaicin and NVA in various ethanol concentrations from gel bases through pig skin[a]

Ethanol (%)	Capsaicin		NVA	
	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h$)	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h$)
0	143.31 ± 25.63	2.17 ± 0.43	149.98 ± 31.02	2.13 ± 0.43
10	88.60 ± 19.47	1.22 ± 0.37	89.79 ± 13.25	1.28 ± 0.26
20	79.51 ± 25.38	1.07 ± 0.33	71.77 ± 22.15	1.04 ± 0.30
30	33.07 ± 7.76	0.49 ± 0.13	33.48 ± 10.80	0.42 ± 0.13

[a] Each value represents the mean ± SD ($n = 3$).

more, two o/w emulsion ointments—hydrophilic and myristic acid-added bases showed the highest percutaneous parameters for SNA among various NVA-SNA combined ointment bases. Accordingly, gel and o/w emulsion ointment bases were utilized for capsaicin, NVA and SNA to perform the in vitro formulation optimizations in this paper. The excised pig skin was utilized as the skin barrier for in vitro investigation since it could be successfully used as a substitutional model to study the percutaneous absorption of these three capsaicin analogues through human skin (Fang et al., 1995a). For the sake of evaluating the formulations selected from in vitro study, the non-invasive surface recovery technique and several bioengineering methods were used in the in vivo percutaneous experiment for humans (Agner and Serup, 1989; Chambin-Remoussenard et al., 1993).

Assessment of the skin irritant potential of chemicals has been performed using bioengineering methods in recent years (Berardesca and Maibach, 1988; Wahlberg, 1988; Agache and Dupond, 1994). The bioengineering methods carried out in this study include laser Doppler flowmetry (LDF) and transepidermal water loss (TEWL). New and sophisticated bioengineering techniques may offer valuable quantification for research purposes. The advantages of these methods are represented by the possibility of collecting data with objectivity and by monitoring readings on a linear scale with recorders. Furthermore, parametric statistics can be computed in order to analyze data and significance.

Promising attempts have been made to quantify skin irritant and erythema reactions by LDF and TEWL. Following topical application of capsaicin and NVA, the treated skin area was reddened and slightly oedematous in appearance (Culp et al., 1989). The levels of these skin erythema and inflammation can be determined by LDF since the cutaneous blood flow is enhanced after the treatment of capsaicin and NVA. Increases in skin temperature were also associated with capsaicin skin inflammation (Roberts et al., 1992; Fang et al., 1995c). Since the value of TEWL increased logarithmically with skin surface temperature, TEWL may be useful for evaluating the irritant degree of the capsaicin and its derivatives (Mathias et al., 1981).

In principle, LDF is an optical technique for estimation of microcirculation, especially cutaneous blood flow, based on the Doppler principle (Bircher et al., 1994). The theory of LDF has been described in detail elsewhere (Kohli et al., 1987; Schabauer and Rooke, 1994). TEWL is measured using the evaporimeter. A boundary layer about 10 mm in thickness develops around the skin, in which a water vapour gradient exists between the skin surface and the ambient air. The sensors of the evaporimeter determine the water vapour pressure gradient of this boundary layer in order to quantify the diffusion of water through the skin (Pinnagoda et al., 1990). The information obtained in this present study is helpful in establishing the in vitro and in vivo evaluation methods for the transdermal drug delivery formulations of capsaicin, NVA and SNA.

2. Materials and methods

2.1. Materials

The following reagents were used: Arlacel-*C*, capsaicin and NVA (TCI, Japan), *p*-phenylphenol, lauric acid and oleic acid (Sigma, USA), cetyl alcohol, triethanolamine, stearic acid and stearyl alcohol (Merck, Germany), sodium laurylsulfate and myristic acid (Wako, Japan), propylene glycol (Shimakyu, Japan), liquid paraffin (Riedel-de Haen, Germany), Carbopol 940® (B.F. Goodrich, USA). The synthetic procedure of SNA had been performed from our laboratory and reported earlier (Fang et al., 1995a). All other chemicals and solvents were of analytical grade.

2.2. Preparation of gel, hydrophilic and myristic acid-added bases

The original gel formulation was composed of propylene glycol (20.00%, w/w), triethanolamine (8.00%), Carbopol 940® (1.20%), and purified water was added to make a total amount of 100%. The original hydrophilic formulation was composed of white vaseline (20.00%), stearyl alcohol (18.00%), propylene glycol (10.00%), sodium laurylsulfate (0.45%), and purified water was added to make a total amount of 100%. The original myristic acid-added formulation was composed of cetyl alcohol (1.90%), stearyl alcohol (2.90%), Arlacel-*C* (0.95%), Brij-35 (0.95%), white vaseline (14.40%), liquid paraffin (4.80%), myristic acid (7.60%), and purified water was added to make a total amount of 100%. In the in vitro evaluations, capsaicin, NVA and SNA were incorporated in the bases to give a concentration of 0.060%, respectively. In the in vivo bioengineering methods, capsaicin, NVA and SNA were incorporated at a concentration of 0.075%, respectively.

2.3. In vitro formulation evaluations

2.3.1. In vitro percutaneous absorption experiments

Male pigs (1–2 week old; 5–6 kg) were obtained from Kaohsiung Medical College (Kaohsiung, Taiwan). The hair of the pig was removed with electric clippers and the dorsal skin was excised after careful shaving. The in vitro penetration flux for gel and ointment bases were determined by using a vertical Keshary-Chien glass diffusion cell (Keshary and Chien, 1984). The pig dorsal skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The top of the diffusion cells was covered with paraffin paper. The donor compartment of the cell was filled with 2 g gel or ointment containing drugs. 20 ml of 1:1 (v/v) ethanol-pH 7.4 McIlvaine buffer was used as the receptor medium. The available diffusion area of cell was 2.54 cm^2. The diffusion cell was carried out at 37°C and the receptor phase was stirred by a magnetic stirrer at 700 rpm. At appropriate intervals, 500 μl aliquots of the receptor fluid were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain a constant volume.

2.3.2. Determination of gel viscosity

Viscosity study was done in a cone and plate viscometer (Model DV-2, Brookfield, USA). 0.5 g of gel was placed in the sample cup of the viscometer and allowed to stand for 1 h to reach 37°C. To obtain stable display readings, viscosity measurements were made after 30 s.

2.4. In vivo percutaneous absorption evaluations

2.4.1. Subjects

Six healthy male volunteers participated in this study. Their average age was 24.7 years (range 24–25). Informed consent was obtained from all volunteers. None had any previous or existing history of skin diseases. The laboratory temperature was kept in the range of 22–23°C, and the relative humidity was 60–65%. Disturbances in the laboratory during measurements were kept at a minimum.

2.4.2. Surface recovery technique

An accurately weighted 0.2 g of the gel or ointment was spread uniformly over a sheet of cotton cloth (2 × 2 cm^2) for an 8 h administration

period by the occlusive dressing technique (ODT) (Hsu et al., 1991). Then these pieces of cloth were applied on both volar forearms of volunteers (4 pieces of each). The unabsorbed drug was randomly recovered at determined intervals after administration. After recovering, the residual base remaining on the skin was withdrawn by a sterile cotton wool swab immersed in methanol solution. The difference between applied and recovered amounts corresponded to the cumulated absorbed amount.

2.4.3. Laser Doppler flowmetry (LDF)

A laser Doppler flowmeter (PF3, Perimed, Sweden) was used for measuring the cutaneous blood flow. After withdrawal of the residual base from surface recovery technique at a determined interval for 2 min, the test was performed. The probe was gently held on the skin to avoid vascular compression. The depth of penetration of the light was approximately 1 mm into skin surface and the area illuminated was approximately 2 mm^2. The reading was calculated as average value after stabilization of the level. The output signal was expressed in relative and dimensionless blood flow values (arbitrary unit, a.u.). The perisoft® computer software program (Gastrosoft, Sweden) was used to aquire, graphically display and store the data obtained from LDF.

2.4.4. Transepidermal water loss (TEWL)

TEWL, determined by the diffusion capacity of the superficial epidermis, was measured by an evaporimeter (EP1, Servo Med, Sweden) after withdrawal of the residual base for 5 min. During all measurements, the probe was protected using a protection cover with the screen and the grid (no. 2107). TEWL was calculated automatically and expressed in g/m^2h. A reading was detected 30 s period after application of the probe onto the skin, when the level had stabilized (Agner and Serup, 1989).

2.5. Determination of capsaicin, NVA and SNA from gel and ointment

In the experiment of the surface recovery technique, one cotton cloth and one cotton wool swab contained base and 0.1 ml of 1.8 $\mu g/ml$ *p*-phenylphenol as an internal standard were mixed with 5 ml of methanol solution in the glass-stoppered centrifuge tube and then followed by mechanical shaking for 60 min. After centrifugation for 10 min at 3000 rpm, the supernatant organic layer was directly injected into the HPLC. The recoveries of capsaicin and NVA from the gel base and SNA from the ointment base were $86.10 \pm 0.32\%$, $99.10 \pm 1.17\%$ and $95.96 \pm 4.18\%$, respectively. The HPLC system for capsaicin, NVA and SNA was described previously (Fang et al., 1995a).

2.6. Statistical analysis

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

3. Results and discussion

3.1. In vitro formulation evaluations in pigs

In order to get the suitable semi-solid formulations of capsaicin, NVA and SNA for administration in humans, in vitro formulation evaluation was studied to screen these bases tested in this research. The various triethanolamine concentrations were incorporated into Carbopol 940® gel form for capsaicin, NVA and SNA. The result is shown in Table 1. No significant differences (ANOVA test, $p > 0.05$) were observed among the fluxes of three various triethanolamine concentrations for capsaicin and NVA. There was no SNA accumulative amount detected after in vitro percutaneous application for 72 h. This result was consistent with that of the previous investigation there was a higher percutaneous absorption amount for NVA in the NVA-SNA combined gel base but none for SNA through rat skin (Fang et al., 1995a). Since the *n*-octanol/buffer partition coefficients of SNA were lower than those of capsaicin and NVA (Tsai et al., 1994), SNA showed a hydrophilic inherence in its physico-

Table 4
In vitro penetration data of SNA in various skin penetration enhancers from various ointment bases through pig skin[a]

Enhancers	Myristic acid-added o/w base		Hydrophilic o/w base	
	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h) \times 10^{-2}$	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h) \times 10^{-2}$
Control	0.86 ± 0.32	0.88 ± 0.33	2.36 ± 0.96	1.96 ± 0.81
Isopropyl myrisrate	2.37 ± 0.56	2.70 ± 0.54	9.32 ± 3.16	11.36 ± 3.63
Lauric acid	6.35 ± 3.07	7.65 ± 2.30	7.11 ± 2.58	8.03 ± 2.41
Myristic acid	5.79 ± 1.56	6.41 ± 2.05	2.21 ± 0.50	1.96 ± 0.42
Stearic acid	0.83 ± 0.29	0.87 ± 0.36	2.06 ± 0.59	1.93 ± 0.57
Oleic acid	1.68 ± 0.46	2.00 ± 0.55	5.33 ± 2.05	6.00 ± 2.50

[a] The concentration of skin penetration enhancers utilized here is 7.6%.
Myristic acid is replaced by another enhancer in the myristic acid-added ointment.
Each value represents the mean ± SD ($n = 3$).

Table 5
In vitro penetration data of SNA in various isopropyl myristate concentrations from hydrophilic o/w bases through pig skin[a]

Isopropyl myristate (%)	72 h accumulative amount ± SD ($\mu g/cm^2$)	Flux ± SD ($\mu g/cm^2/h) \times 10^{-2}$
0	2.36 ± 0.96	1.96 ± 0.81
3.8	8.38 ± 1.67	10.08 ± 2.34
7.6	9.32 ± 3.16	11.36 ± 3.63
11.4	6.38 ± 2.16	6.43 ± 1.09

[a] Each value represents the mean ± SD ($n = 3$).

chemical character. The hydrogel base also exhibited the potent hydrophilic property. Consequently the SNA molecule and hydrogel base formed an intense affinity to each other, and then obstructed the release of the SNA molecule from the gel. The pH value of Carbopol 940® gel base after the addition of 2%, 4% and 8% triethanolamine was 6.37, 7.47 and 8.21, respectively. The pH value of skin surface is about 5.6, so the 2% triethanolamine was selected to perform the following study because of its pH value near that of skin.

The solubilizing agents—isopropanol and ethanol were incorporated into the gel base. As shown in Table 2 and Table 3, isopropanol and ethanol reduce the penetration fluxes and accumulative amounts of capsaicin and NVA. In addition, the percutaneous effect of capsaicin and NVA reduced with increasing concentrations of solubilizing agents. This phenomenon may be due to the fact that the addition of isopropanol and ethanol, a part of the hydrophobic organic solvent group, gave the reduction of hydrogel polarity. Since capsaicin and NVA showed high partition coefficient and were essentially insoluble or very slightly soluble in water (Tsai et al., 1994), these two analogues may participate into isopropanol or ethanol-added gel base so as to get a stronger affinity than that of hydrophilic non-solubilizing agents gel base. To compare Tables 2 and 3, the fluxes are lower after the addition of isopropanol than ethanol in the same proportions except the fluxes of 30% concentration for capsaicin gel. The explanation could be due to the fact that the physicochemical inherence of isopropanol is more hydrophobic than that of ethanol. Furthermore, alcohols and hydrocarbons induced changes in the structure of biological membranes and this change influenced the rate of drug transderrmal absorption (Inagi et al., 1981).

Fig. 1 depicts the effect of various Carbopol 940® amounts for capsaicin and NVA from gel bases. The fluxes of capsaicin and NVA increased following the decrease of Carbopol 940® content.

Table 6
In vivo skin surface recovery technique in humans from 0.075% drug concentration gel and ointment bases[a]

	Capsaicin	NVA	SNA
8 h absorbed amount ($\mu g/cm^2$)	22.81 ± 8.20	25.10 ± 9.08	14.54 ± 4.52
Flux ($\mu g/cm^2/h$)	2.71 ± 0.80	3.35 ± 1.07	1.68 ± 0.48
Antinociceptive potency ratio	1.14	1.00	2.00
Antinociceptive index	3.09	3.35	3.36

[a] The capsaicin and NVA formulation utilized here is Carbopol 940® gel base.
The SNA formulation utilized here is hydrophilic o/w ointment base.
Antinociceptive index is calculated by the antinociceptive potency ratio multplied by flux.
Each value represents the mean ± SD ($n = 6$).

This result found that the viscosity of the gel base played a very important role as shown in Fig. 2. The fluxes of capsaicin and NVA were observed to be inversely proportional to the viscosity of gel containing a determined amount of Carbopol 940®. Similar result had been reported for the percutaneous absorption of piroxicam from FAPG base (Hsu et al., 1994). The fluxes of 0.6% Carbopol 940® were significantly higher (t-test, $p < 0.05$) than those of 1.2% and 2.0% for both capsaicin and NVA. Hence the gel base containing the 0.6% Carbopol 940® amount was chosen to study the following in vivo test.

In our previous research (Fang et al., 1995a), the myristic acid-added ointment offered a high penetration effect for SNA through rat skin. Therefore, the determined concentration of 7.6% isopropyl myristate and fatty acids such as lauric acid, myristic acid, stearic acid and oleic acid were incorporated into two o/w ointment bases so as to investigate the penetration promotive effect of these agents. The above agents had been found to enhance transdermal delivery of some drugs and chemicals (Yamada and Uda, 1987; Green et al., 1988; Ruland and Kreuter, 1992). As shown in Table 4, the trends of steady-state flux of various enhancers between these two o/w ointments are quite different. It suggested the vehicles might influence the effect of penetration enhancers. The application of saturated, long-chain fatty acids (lauric, C_{12}; myristic, C_{14}; stearic, C_{18}) to the in vitro study showed a trend that a decrease in the number of carbon atoms (C_n) in the fatty acids resulted in an increase in enhancement of SNA permeation. Similar results had been published earlier and the mechanism was well described (Aungst et al., 1986; Komata et al., 1992). The flux of isopropyl myristate-added hydrophilic ointment was significantly higher (t-test, $p < 0.05$) than those of the other formulations as shown in Table 4. Moreover, isopropyl myristate revealed lower skin irritation and toxicity than fatty acids did (Aungst, 1989). Consequently isopropyl myristate was chosen to incorporate into the hydrophilic base in order to perform the next study.

In the following experiment, various concentrations of isopropyl myristate were evaluated using the hydrophilic ointment as the vehicle. As depicted in Table 5, the flux showed a maximum at 7.6%. However, there was no significant difference (t-test, $p > 0.05$) between the concentration of 3.8% and 7.6%. For this reason, 3.8% isopropyl myristate-added hydrophilic ointment for SNA at a concentration of 0.075% was utilized as the formulation to deliver for the in vivo test.

3.2. In vivo bioengineering methods in humans

In the study of surface recovery technique, cumulated absorbed amounts of capsaicin and its synthetic derivatives, which are the differences between applied and recovered amounts, are shown in Table 6. As compared with the previous research using the original hydrophilic o/w formulation for capsaicin, NVA and SNA so as to perform surface recovery techniques in the same conditions (Fang et al., 1995c), the same result was found that the percutaneous effect through human skin increased in the order of SNA < capsaicin < NVA. Besides, the fluxes of

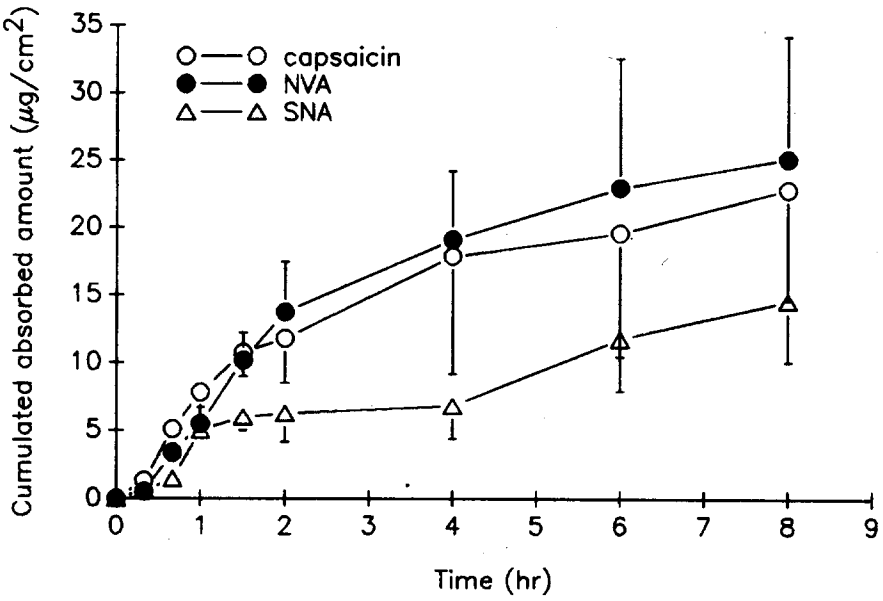

Fig. 3. Cumulated absorbed amounts versus time profiles of 0.075% capsaicin, NVA from gel base and SNA from hydrophilic base after percutaneous administration in humans. All data represent the means of six experiments ± SD.

capsaicin and NVA from gel and SNA from isopropyl myristate-added hydrophilic bases were all significantly higher (t-test, $p < 0.05$) than those of these three analogues from the original hydrophilic base. This suggested the fact that the percutaneous efficacy could be enhanced after the optimization and modification of in vitro formulation evaluations.

Although the flux of SNA was relatively lower than that of capsaicin and NVA, however, SNA revealed an approximately 2.00 fold greater potency of the antinociceptive activity than NVA (Chen et al., 1992). Consequently SNA may attain the same therapeutic effect as NVA in humans after the calculation of the antinociceptive index as shown in Table 6. The antinociceptive index was determined by the result of the steady-state flux multiplied by the antinociceptive potency ratio. The high cumulated absorbed amount of SNA was obtained in the first 1 h period as shown in Fig. 3. This result was consistent with that of the plasma profile of the SNA application through rabbit abdominal skin in the early 1 h which suggested the SNA molecules passed through skin via the intercellular and transappendageal routes (Illel et al., 1991; Fang et al., 1995b).

Fig. 4 and Table 7 depict the result of the skin erythema test quantified by LDF. The AUC value of capsaicin was significantly higher (t-test, $p < 0.05$) than that of NVA. This demonstrated the fact that capsaicin caused severer skin irritation than NVA. The fact that there was no significant difference between SNA and the control group in the hydrophilic base indicated no irritant and pungent properties were found in the formulation of SNA. The T_{max} value was attained quicker from gel than from the hydrophilic base for NVA (Fang et al., 1995c). Although the T_{max} of capsaicin gel was the same with that of the hydrophilic base, the capsaicin gel form may reach an earlier T_{max} value since there was no significant differences (ANOVA test, $p > 0.05$) among the a.u. levels of 0.33, 0.67 and 1 h. This demonstrated that gel base may provide a rapid onset and drug release for capsaicin and NVA as compared with those of the hydrophilic base.

The TEWL values for the formulations of capsaicin, NVA and SNA were calculated using an evaporimeter. As observed in Fig. 5, the TEWL

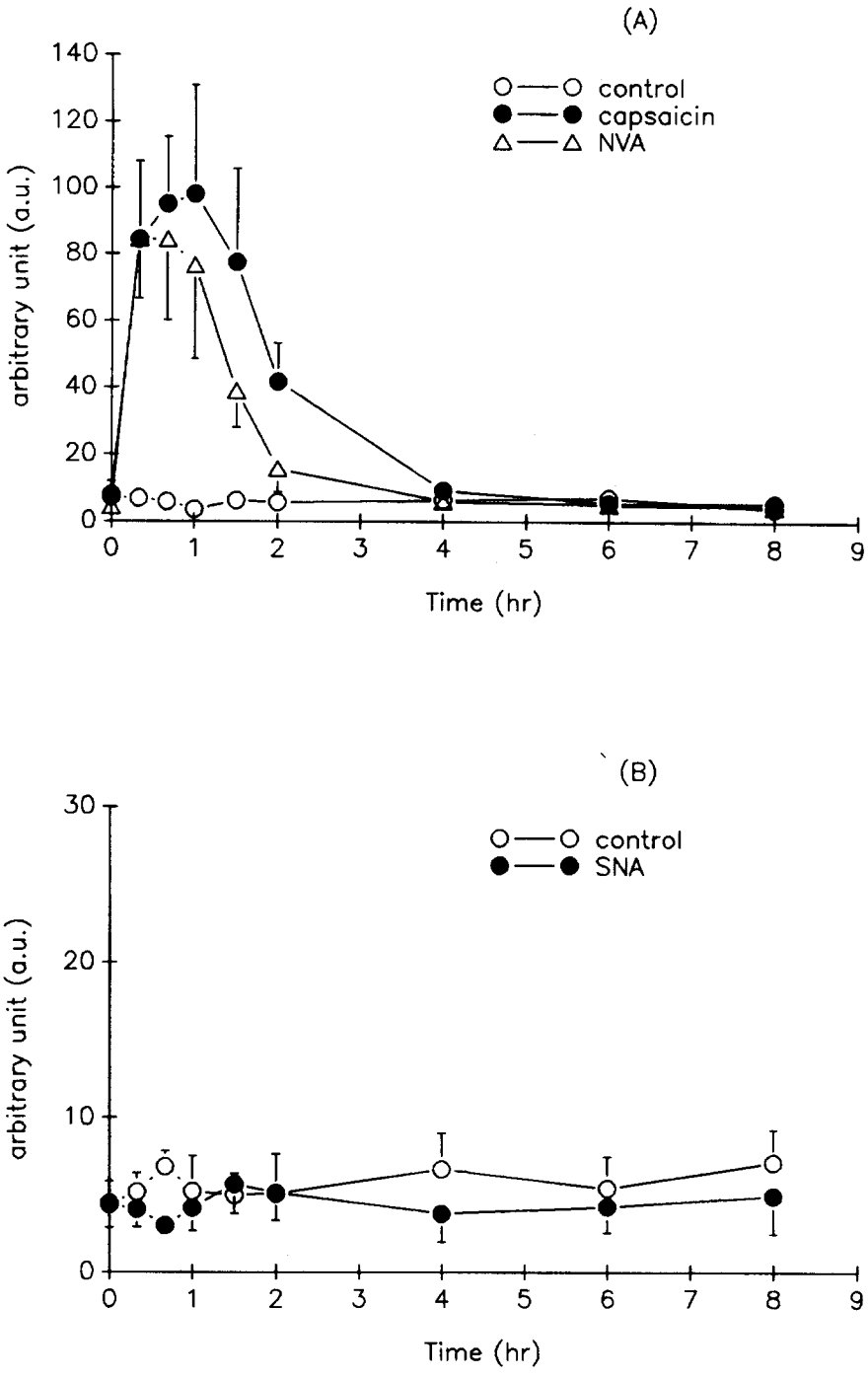

Fig. 4. Skin erythema test measured by LDF for 0.075% capsaicin, NVA from gel base (A) and SNA from hydrophilic base (B) after administration in humans. All data represent the means of six experiments ± SD.

Table 7
Skin erythema test of 0.075% capsaicin, NVA and SNA gel and ointment bases quantified by laser Doppler flowmetry[a]

	Control (gel)	Control (o/w)	Capsaicin	NVA	SNA
T_{max}(h)	0	8.0	1.0	0.33	1.5
a.u.$_{max}$	7.20 ± 0.56	7.10 ± 2.16	97.97 ± 32.90	84.43 ± 17.80	5.69 ± 1.90
AUC(a.u. × h)	48.10 ± 10.06	46.98 ± 14.28	226.92 ± 71.75	153.71 ± 40.15	35.07 ± 17.45

[a] The quantified unit represented here is the arbitrary unit (a.u.).
The capsaicin and NVA formulation utilized here is the Carbopol 940® gel base.
The SNA formulation utilized here is the hydrophilic o/w ointment base.
Each value represents the mean ± SD ($n = 6$).

Table 8
TEWL parameters of 0.075% capsaicin, NVA and SNA gel and ointment bases quantified by an evaporimeter[a]

	Control (gel)	Control (o/w)	Capsaicin	NVA	SNA
T_{max}(h)	0.67	4.0	0.33	0.67	6.0
g/m^{2}h$_{max}$	25.42 ± 5.77	33.02 ± 9.89	46.45 ± 16.57	36.87 ± 10.27	31.04 ± 3.97
AUC(g/m^2)	156.08 ± 32.46	204.14 ± 38.79	241.94 ± 72.69	227.67 ± 62.08	223.08 ± 43.63

[a] The quantified unit represented here is g/m^{2}h.
The capsaicin and NVA formulation utilized here is the Carbopol 940® gel base.
The SNA formulation utilized here is the hydrophilic o/w ointment base.
Each value represents the mean ± SD ($n = 6$).

values were raised after application of ODT for gel and hydrophilic formulations. For the transpiration of water from stratum corneum may be blocked by ODT. The TEWL values were determined 5 min after the cotton cloth removal, so the water content occluded by ODT evaporated and then resulted in the enhancement of TEWL values. Comparison of the gel form showed significantly higher (t-test, $p < 0.05$) TEWL AUC values in capsaicin and NVA than in the control group as shown in Table 8. Nevertheless, no significant difference (t-test, $p > 0.05$) was found between capsaicin and NVA. Unlike the a.u. values of LDF, the TEWL values kept higher in capsaicin and NVA than in the control group from the administration period of 4–8 h.

For the gel control group and the hydrophilic control group, a significant difference (t-test, $p < 0.05$) was demonstrated between these two drug-free bases. Furthermore, no significant difference (t-test, $p > 0.05$) was found between SNA and control hydrophilic bases, nor among SNA, capsaicin and NVA formulations (ANOVA test, $p > 0.05$). The reason of this phenomenon could be due to the fact that isopropyl myristate, the penetration enhancer added in the hydrophilic base, retarded the rate of loss of water from the skin resulted in the accumulative amount of water content in the epidermis and high TEWL values after the removal of ODT. This capacity of moisture retention of isopropyl myristate was proved in previous study (Dempski et al., 1965). In addition, sodium laurylsulfate, emulsifying agent of hydrophilic base, impaired the skin barrier resulted in the increase in TEWL (Agner and Serup, 1990; De Fine Olivarius et al., 1993).

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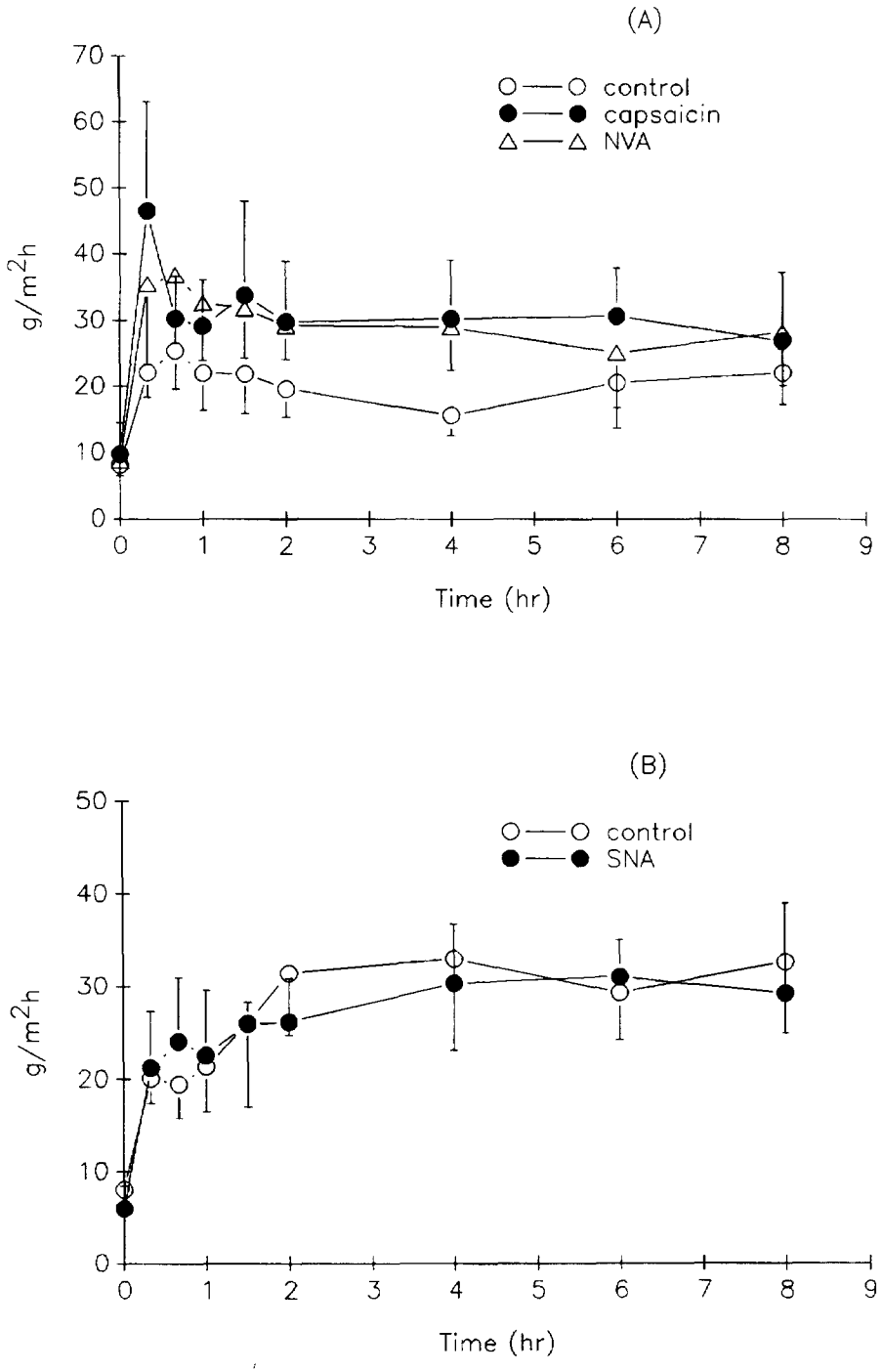

Fig. 5. TEWL values versus time profiles of 0.075% capsaicin, NVA from gel base (A) and SNA from hydrophilic base (B) after percutaneous administration in humans. All data represent the means of six experiments ± SD.

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