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In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types

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Abstract

Nonivamide (NVA) and sodium nonivamide acetate (SNA) both are synthetic analogues of capsaicin. In the present study, in vitro penetration experiments through rat skin from ointment bases were performed in order to establish and develop the transdermal drug delivery system of these two capsaicin analogues. This study was also carried out to evaluate the relative skin permeability of capsaicin and its derivatives through different skin types. As regards the in vitro transdermal absorption of ointment bases, o/w emulsion-type bases (hydrophilic and University of California Hospital (UCH) ointment) revealed better percutaneous absorption effects than the others for both NVA and SNA. However, there was a higher accumulative amount for NVA in the gel base but none for SNA. There were no changes observed in the appearance and texture when the aqueous phase of the three ointment bases (hydrophilic, absorption and UCH ointment) was replaced by pH 4.2 buffer. In the steady-state flux of absorption ointment for NVA and UCH, absorption ointment for SNA was significantly higher ($P < 0.05$) in pH 4.2 buffer-replaced base than in the original. In the comparison of in vitro permeability through various animal skin types, full-thickness human skin showed the poorest permeability for NVA, SNA and capsaicin. The trends of steady-state flux through the various skin types for capsaicin, NVA and SNA were quite different. However, pig skin could be successfully used as a model to study in vitro percutaneous absorption of these three compounds through human skin.

Keywords: Percutaneous absorption; Ointment base; Skin; Capsaicin; Nonivamide; Sodium nonivamide acetate

1. Introduction

Capsaicin (8-methyl *N*-vanillyl-6-nonenamide, Fig. 1), a pungent principle of red pepper, has a variety of pharmacological actions on the cardiovascular, respiratory and nervous systems (Monsereenusorn et al., 1982; Clozel et al., 1985; Buck and Burk, 1986; Ordway and Pitetti, 1986). Several therapeutic advantages of capsaicin such as antinociceptive, hypotension and hypolipidemia activities have been reported previously (Makara

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et al., 1967; Hayes et al., 1981; Lahann and Farmer, 1982; Wang et al., 1984; Negulesco et al., 1987). However, because of the burning pain sensation and the concomitant hypothermia its clinical use is limited (Szolcsanyi and Jancso-Gabor, 1975).

N-nonanoyl vanillylamide (nonivamide; NVA, Fig. 1) and sodium *N*-nonanoyl vanillylamide-4′-*O*-acetate (sodium nonivamide acetate; SNA, Fig. 1) are both synthetic analogues of capsaicin. NVA, the pharmacological and pungent profiles of which were found similar to those of capsaicin, has been used as a substitude for capsaicin in neuro-physiological studies (Hayes et al., 1984). SNA was synthesized by alkylation of the phenolic hydroxyl group of NVA with bromoacetic acid. This sodium salt analogue revealed marked antinociceptive activities in mice without producing the overt pungent sensation and irritation that have been found with capsaicin (Yang et al., 1992).

The antinociceptive activities of capsaicinoids have been markedly influenced by various administration routes. Previous investigations have suggested that the pharmacological effects of capsaicinoids following subcutaneous and intragastric administration are significantly higher than those 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 NVA and SNA to accomplish better bioavailability.

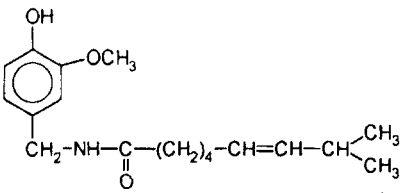
A transdermal drug delivery system (TDDS) offers several advantages over other routes of drug administration. Besides patient convenience, enhanced and controlled therapeutic responses have been reported (Ledger and Nichols, 1989). The purpose of this present investigation is to study the abilities of NVA and SNA in in vitro percutaneous absorption through rat skin from various ointment bases.

Moreover, this present study is an attempt to compare the relative skin permeability of capsaicin, NVA and SNA through various skin types from ointment bases. The information is particularly helpful in the development of capsaicin, NVA and SNA transdermal drug delivery systems.

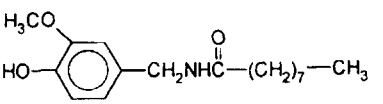
2. Materials and methods

2.1. Chemicals

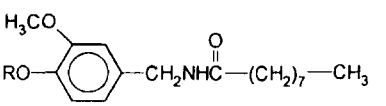
The following reagents were used: nonivamide and bromoacetic acid (TCI, Japan), *N*,*N*-dimethyl formamide (Fisher, USA), sodium hydroxide, citric acid, cetyl alcohol, 1,2,6-hexanetriol, disodium hydrogen phosphate, stearic acid, stearyl alcohol and PEG 4000 (Merck, Germany), lauric acid, oleic acid and *p*-phenylphenol (Sigma, USA), Liquid paraffin and PEG 400 (Riedel-de Haen, Ger-



Capsaicin



Nonivamide (NVA)



R : -CH2-C-O-Na
O

Sodium nonivamide acetate (SNA)

Fig. 1. Structures of capsaicin, nonivamide and sodium nonivamide acetate.

many), PEG 6000 (Hayashi, Japan), myristic acid, sodium laurylsulfate and triethanolamine (Wako, Japan), Carbopol 934, 940 and 941 (B.F. Goodrich, USA). All other chemicals were reagent grade obtained from commercial sources. The capsaicin cream (Capderm™) was a gift from Mei-Shih Parmaceutical Corp. (Taipei, Taiwan). All solvents used were of high-performance liquid chromatography (HPLC) grade. Water was purified in a Milli-Q water system (Millipore, USA).

2.2. Synthesis of sodium nonivamide acetate

This synthetic procedure was modified by the method described by Chen et al., 1992. NVA (2 g) was heated under reflux until dissolved with *N,N*-dimethyl formamide (DMF). Bromoacetic acid was added into NVA in the presence of sodium hydroxide which dissolved in water. The resulting solution was stirred at room temperature for 1 h. Excess DMF was removed under vacuum. The residue obtained was crystallized in an ice bath. SNA was obtained as colorless needles after purification by silica gel column chromatography. The NMR, mass spectral and IR data were consistent with their structures. Elemental analysis was consistent with the molecular formula.

2.3. Preparation of suspension-type ointments

NVA and SNA were incorporated into an ointment base representing each of the eight physical types. The bases selected were: simple ointment (U.S.P.), an oleaginous base; hydrophilic ointment (U.S.P.), an oil-in-water emulsion base; PEG ointment (U.S.P.), a water-soluble base; absorption ointment (U.S.P.), a water-in-oil emulsion base; FAPG ointment (U.S.P.), a cream-gel base; University of California Hospital (UCH) ointment (pharmaceutical science), an oil-in-water emulsion base; Carbopol gel ointment (U.S.N.F.), a gel base and a myristic acid-added ointment (Japan Patent, 58-39616, 8 March 1983). The ointment bases were prepared so as to contain 0.03% of NVA and 0.02% of SNA. In the comparison of the in vitro permeability through various animal skin types, the hydrophilic ointment was prepared so as to contain 0.06% NVA and 0.04% SNA. The hydrophilic ointment and commercially available cream (Capderm™) of 0.075% capsaicin were also used in this study.

2.4. Preparation of skin membranes

Samples of whole adult human skin (41–48 years old) were obtained from breast reduction operations and provided by the National Taiwan University (Taipei, Taiwan). Subcutaneous fat was carefully trimmed and the cadaver skin was rinsed with normal saline. The skin was then sealed in aluminium foil and a plastic bag and stored at −20°C.

Male Wistar rats (6–8 weeks old; 150–200 g), male BALB/c mice (4–6 weeks old; 25–35 g), male New Zealand rabbits (12–14 weeks old; 3.0–3.5 kg) and male Yorkshire pigs (1–2 weeks old; 5–6 kg) were obtained from Kaohsiung Medical College (Kaohsiung, Taiwan). Male severe combined immunodeficiency (SCID) mice (4–5 weeks old; 20–25 g) were obtained from the National Taiwan University (Taipei, Taiwan).

The artificial membranes (Visking™ 18/32 cellophane membranes) were used without any pretreatment. The BALB/c and SCID mice were killed by cervical dislocation; the other animals were sacrificed with ether. Full-thickness skin was excised from the abdominal region. The hair of the abdominal region was shaved with electric clippers. Rabbit pinna skin was separated from the middle of the inner side of the rabbit ear (Hirvonen et al., 1993). The pinna skin was peeled away from the underlying cartilage immediately after the rabbits were killed. After the removal of the subcutaneous fat and other extraneous tissues, the skin was immersed in pH 7.4 McIlvaine buffer for 45 min before experimentation.

2.5. In vitro percutaneous penetration experiments

The penetration experiments of different ethanol/pH 7.4 buffer proportions in the receptor phase were assessed in the modified horizontal diffusion cell (Liu et al., 1985). The rat skin was mounted between the cell compartments with the stratum corneum facing towards the donor half

cell; 15 ml of pH 7.4 McIlvaine buffer with 0%, 12.5%, 25% and 50% (v/v) absolute ethanol were added as the receptor phase. The effective diffusion surface area was 2.00 cm^2.

The in vitro penetration flux of capsaicin, NVA and SNA from ointment base was determined by using a vertical Keshary-Chien glass diffusion cell (Keshary and Chien, 1984). The 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 diffusion cells was covered with paraffin paper. The donor compartment of the cell was filled with 2 g of ointment containing drugs. The receptor phase contained 20 ml of 1:1 (v/v) ethanol–pH 7.4 buffer solution was used. The available diffusion area of the vertical diffusion cell was 2.54 cm^2.

The experiments were carried out at 37°C and the receptor compartment was agitated by a magnetic stirrer at 700 rev./min; 500-μl aliquots were taken from the receptor compartment at determined intervals and immediately replaced by an equal volume of fresh receptor solution to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the penetration data. The sample withdrawn from the receptor compartment was then analyzed by HPLC. Each data point represents the average of three determinations.

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 drug flux, J, was calculated by the slope of the linear portion of the penetration curves and expressed as the mass of drug passing across 1 cm^2 of skin over time. In vitro permeability coefficients (P) of capsaicin, NVA and SNA were then calculated using the relationship: $P = J/C_d D$, where C_d is the drug donor concentration (w/w) and D denotes the density of the ointment base. The ointment base density was estimated by using a cavate cylinder the same as the donor compartment of the vertical glass diffusion cell which was filled with the ointment base. The density was then computed using the equation: $D = W/V$, where W is the net weight of ointment in the cavate cylinder and V denotes the volume of the cavate cylinder.

Statistical analysis of the data was performed using Student's t-test.

2.6. Analytical methods

The drug content of the various samples was analyzed by a HPLC system consisting of a Waters Model M-45 HPLC pump, a Waters 715 sample processor and a Waters 470 fluorescence detector. A 12.5 cm long, 4.0 mm inner diameter stainless steel column with LichroCART 250-4 C-18 column (Merck) was used. An automated integrator system (Waters 740 data module) was used to determine the area under the curve. The drug sample was mixed with a suitable amount of p-phenylphenol as internal standard. The mobile phase for NVA and SNA consisting of 60% pH 4 McIlvaine buffer and 40% acetonitrile was used at a flow rate of 1.0 ml/min, and the mobile phase for capsaicin was 55% pH 4 McIlvaine buffer and 45% acetonitrile. The column effluent was passed through the fluorescence detector set at an excitation wavelength of 280 nm and an emission wavelength of 310 nm.

The retention time of SNA, p-phenylphenol and NVA were found to be 2.9 min, 7.6 min and 10.8 min, respectively. For the analytical condition of capsaicin, the retention time of p-phenylphenol and capsaicin were 5.4 min and 7.0 min, respectively. Linearity existed over a concentration of 0.3-6.0 μg/ml for NVA and SNA and 0.1-3.0 μg/ml for capsaicin. Samples were diluted prior to mixing with the internal standard if needed.

3. Results and discussion

It is known that full-thickness skin, when tested in vitro with hydrophobic compounds, may underestimate the permeability of the same skin in vivo (Bronaugh and Stewart, 1986; Catz and Friend, 1990). Compounds that are essentially insoluble in water may not partition freely from excised skin into an aqueous receptor fluid in vitro. When applied to skin in vivo, however,

Table 1
Solubility of capsaicin, NVA and SNA in various proportions of EtOH/pH 7.4 buffer solution

EtOH/pH 7.4 buffer (%)	Capsaicin (mg/l)	NVA (mg/l)	SNA (mg/l)
0%	32.15 ± 4.97	36.80 ± 1.92	270.11 ± 11.84
12.5%	433.93 ± 65.52	184.25 ± 15.47	2886.48 ± 128.28
25%	8699.41 ± 814.88	600.03 ± 37.84	3470.90 ± 507.74
50%	40657.39 ± 6955.22	40537.68 ± 4507.70	16578.60 ± 1026.60

these compounds may readily leave barrier layers because of their solubility in biological fluids (Bronaugh and Stewart, 1984). To obviate the problem of poor in vitro/in vivo relations when using full-thickness skin in vitro to evaluate hydrophobic compounds solubilizing agents are added to the receptor fluid. In this study, capsaicin and its synthetic derivatives which are essentially insoluble or very slightly soluble in water may partition only slightly from excised skin into pH 7.4 buffer. The solubility values of capsaicin and its synthetic derivatives in the solution of pH 7.4 buffer and ethanol are given in Table 1. The solubilities were much greater in the ethanol-pH 7.4 buffer solution than in pure pH 7.4 buffer. The solubility values increased with increasing ethanol proportions. Thus absolute ethanol as a solubilizing agent was chosen to add into the receptor compartment of the horizontal glass diffusion cell by using rat skin as the barrier.

Capsaicin (300 μg/ml), NVA (300 μg/ml) and SNA (200 μg/ml) were selected to be suspended individually in pH 7 buffer as a donor phase. The receptor fluid, in contact with the dermal side of the skin, was pH 7.4 McIlvaine buffer with 0%, 12.5%, 25%, 50% (v/v) absolute ethanol added to check the effect of the capsaicin, NVA and SNA permeability of different ethanol proportions in the receptor phase. As shown in Fig. 2, the penetration amount of capsaicin and NVA increased with increasing ethanol proportion. The same result is observed for SNA. There were almost no SNA accumulative amounts detected throughout 72 h when the ethanol proportions were $0 \sim 25\%$ in the receptor phase. However, a significant growth of SNA accumulative amounts were found in the 50% ethanol concentration. The result of this in vitro experiment was consistent with the solubility profiles of capsaicin and its synthetic derivatives. Therefore an ethanol-pH 7.4 buffer (1:1) was used as receptor solution to enhance the solubility of capsaicin and its derivatives and to maintain the pseudosink conditions throughout the in vitro experiments.

In our previous research (Tsai et al., 1994), the flux of the mixture of NVA and SNA was higher than that of NVA or SNA alone through excised rat skin in vitro. Therefore, a mixture of 0.03% NVA and 0.02% SNA was selected to add to the ointment base so as to promote the percutaneous penetration.

Table 2 depicts the influence of different types of ointment bases on the penetration of the NVA and SNA mixture through excised rat skin in vitro. For NVA, the three types of gel base, UCH ointment and hydrophilic ointment were found to yield higher steady-state flux and 72 h accunulative amount than the other ointment bases. There was no NVA percutaneous absorption in PEG ointment and FAPG ointment. The myristic acid-added ointment, hydrophilic ointment and UCH ointment were found to yield higher flux values of SNA than the other bases and no SNA accumulative amount penetrated through rat skin from simple ointment, PEG ointment, FAPG ointment and gel base. The poor penetration of SNA is largely due to the hydrophilic property of its structure which has a sodium salt in the ester group. SNA also becomes the ionized molecule because of the removal of the sodium salt in appropriate pH values. This result of much lower percutaneous profiles of SNA than of NVA can be attributed to the different transdermal routes between NVA and SNA. The stratum corneum is thought to be the primary barrier to the absorption of ionized and hydrophilic molecules. The

transappendageal routes constitute the major penetration pathways for these molecules. However, the surface area occupied by these pathways is relatively small, resulting in poor penetration of the ionized drugs (Tyle, 1986). Lipophilic molecules, such as NVA and capsaicin, directly penetrate the stratum corneum since the chief material of the stratum corneum is lipid. Comparing the steady-state flux of NVA and SNA, a higher penetration effect was observed for o/w emulsion type bases (UCH and hydrophilic ointments) both in NVA and SNA. The good permeation from UCH and hydrophilic bases may partly be due to the addition of sodium laurylsulfate, an anionic surfactant, which enhances the penetration of NVA and SNA. This phenomenon had been proven in our previous research (Tsai et al., 1994). However, there was quite a difference between NVA and SNA in the penetration of Carbopol gel bases. There was a higher percutaneous absorption accumulative amount for NVA in the gel base but none for SNA (Table 2). The highest flux of SNA was found in the myristic acid (3.8%)-added formulation. Therefore, myristic acid may act as a potent penetration enhancer on the permeation of SNA through skin.

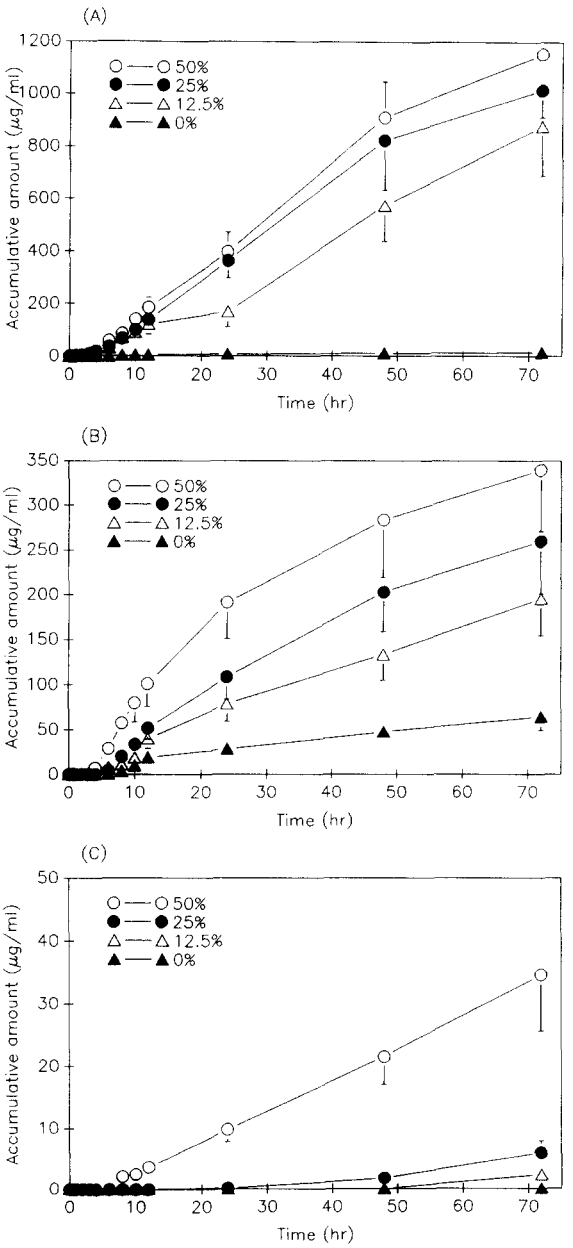


Fig. 2. In vitro penetration parameters of capsaicin (A) NVA (B) and SNA (C) at various ethanol proportions in the receptor phase. All data represent the means of three experiments ± S.D.

As mentioned previously (Tsai et al., 1994), the partition coefficients of NVA and SNA increased with decreasing pH values. Thus, a higher proportion of non-ionic form is present at lower pH. Thus, we replaced the purified water content of the aqueous phases of the hydrophilic, absorption and UCH ointment bases by the pH 4.2 McIlvaine buffer to perform the penetration experiment in vitro. There were no changes observed in the appearance and texture of these ointment bases after the aqueous phase replacement of purified water by pH 4.2 McIlvaine buffer. Fig. 3 shows the result of the effect of the aqueous phase replacement from the ointment base. The data presented here indicate that the flux of pH 4.2 buffer-replaced ointments for NVA and SNA was higher than that of the original ointment bases. In addition, the fluxes of these two different aqueous phase type ointments on percutaneous absorption were compared with statistical measurement. For NVA, only absorption ointment was significantly different between pH 4.2 buffer-replaced ointment and the original (t-test, $P < 0.05$). On the other hand, a significant deviation was observed between the two different aqueous phase type ointments from absorption and UCH bases for SNA.

Table 2
In vitro penetration of NVA and SNA from the various ointment bases through rat skin

Ointment bases	NVA		SNA	
	72 h accumulative amount ± S.D. ($\mu g/cm^2$)	Flux ± S.D. ($\mu g/cm^2$ per h)	72 h accumulative amount ± S.D. ($\mu g/cm^2$)	Flux ± S.D. ($\mu g/cm^2$ per h)
Simple	13.17 ± 4.32	0.14 ± 0.05	N.D.	N.D.
Hydrophilic	74.56 ± 21.42	1.01 ± 0.23	26.01 ± 7.71	0.43 ± 0.19
PEG	N.D.	N.D.	N.D.	N.D.
Absorption	7.71 ± 3.37	0.08 ± 0.03	7.45 ± 3.29	0.11 ± 0.03
FAPG	N.D	N.D.	N.D.	N.D.
UCH	74.39 ± 30.22	1.13 ± 0.34	8.46 ± 3.47	0.13 ± 0.03
Myristic acid–add	34.33 ± 11.96	0.49 ± 0.09	34.35 ± 16.60	0.52 ± 0.21
Carbopol-934 gel	75.35 ± 21.15	0.90 ± 0.14	N.D.	N.D.
Carbopol-940 gel	64.50 ± 17.66	0.79 ± 0.24	N.D.	N.D.
Carbopol-941 gel	59.86 ± 17.98	0.98 ± 0.30	N.D.	N.D.

[a]All data represent the means of three experiments ± S.D.N.D., no drug amount was detectable.

However, there were no significant deviations for NVA and SNA in the hydrophilic ointments between the purified water phase and the pH 7.4 buffer-replaced phase.

The steady-state fluxes of capsaicin, NVA and SNA through various types of skin from hydrophilic ointment are shown in Fig. 4. With capsaicin, the penetration flux through skins increased in the order of human < pig < rabbit SCID < rat < cellophane membrane < pinna < mouse. With NVA, the flux increased in the order of human < pinna < SCID < pig < rat < rabbit < mouse < cellophane membrane. With SNA, the flux through the eight skin types showed a trend of human < rat < pinna < pig < SCID < rabbit < mouse < cellophane membrane. The trends of steady-state flux through various skin types for capsaicin, NVA and SNA were quite different from each other. Statistical analysis of these data indicate no significant difference between human and pig skin for capsaicin, human and pinna skin for NVA, or human, rat, pinna and pig skin for SNA in this permeation study. All these three compounds showed that excised human skin was the least permeable of all the skin types. It is well known that rodent and pig skins are generally more permeable than is human skin (Catz and Friend, 1990). In addition, human breast skin showed a lower penetration rate than the other anatomic sites (Harada et al., 1993). Mouse skin showed great permeability since the thickness of whole skin and the size of the hair follicles are somewhat lower than that of the other animals (Bronaugh et al., 1982). The SCID mouse, a new laboratory animal model, is an autosomal recessive mouse mutant which lacks functional lymphocytes such as B and T cells (Bosma et al., 1988). The skin permeability of the SCID mouse was observed to be quite different from that of the BALB/c mouse and the reason for this needs further investigation. These findings will be a useful guide for selecting suitable model membranes for in vitro permeability experiments of capsaicin and its derivatives.

On the other hand, the steady-state flux of capsaicin from commercially available cream through skins increased in the order of human < pig < rabbit < rat < cellophane membrane < SCID < pinna < mouse (Fig. 5). Except for the cellophane membrane, the trends of capsaicin from hydrophilic ointment and commercially available cream through various types of skin were almost similar. This result shows that formulations of ointment base did not markedly influence the trend of capsaicin permeability through skins. There were higher fluxes for the commercial cream than for the hydrophilic ointment for capsaicin in all skin types except for the cellophane membrane. This result suggests there may be an

effective penetration enhancer in the commercial cream because the enhancer did not affect drug permeability for the artificial membranes such as cellophane but only for animal and human skins.

As shown in Fig. 4 and Fig. 5, the fluxes of capsaicin and SNA indicate no significant difference between pig and human skin. Besides, although there is a significant difference between pig and human skin for the NVA flux, the NVA flux through pig skin is similar to that through rabbit pinna skin which has no significant deviation compared with human skin. In conclusion, these results suggest that pig skin can be successfully used as a model to study the in vitro percutaneous absorption of capsaicin and its synthetic derivatives through human skin.

As shown in Table 3, the full-thickness human skin permeability coefficient of NVA from hydrophilic ointment is significantly higher than that of capsaicin. Since the pharmacological effects of

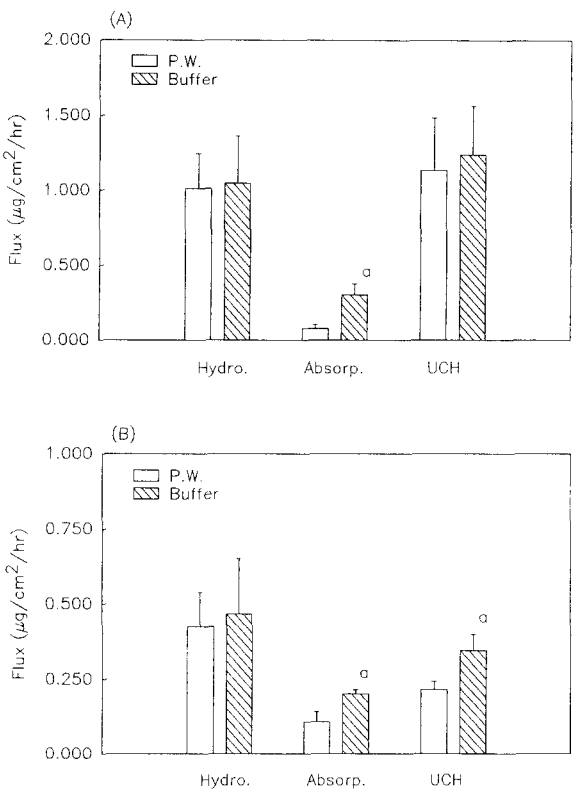


Fig. 3. Steady-state flux of NVA (A) and SNA (B) from pH 4.2 buffer replaced ointment as compared with original. P.W., purified water; buffer, pH 4.2 buffer; Hydro., hydrophilic ointment; Absorp., absorption ointment; UCH, UCH ointment. All data represent the means of three experiments ± S.D. [a]Significant difference vs. original ointment, $P < 0.05$.

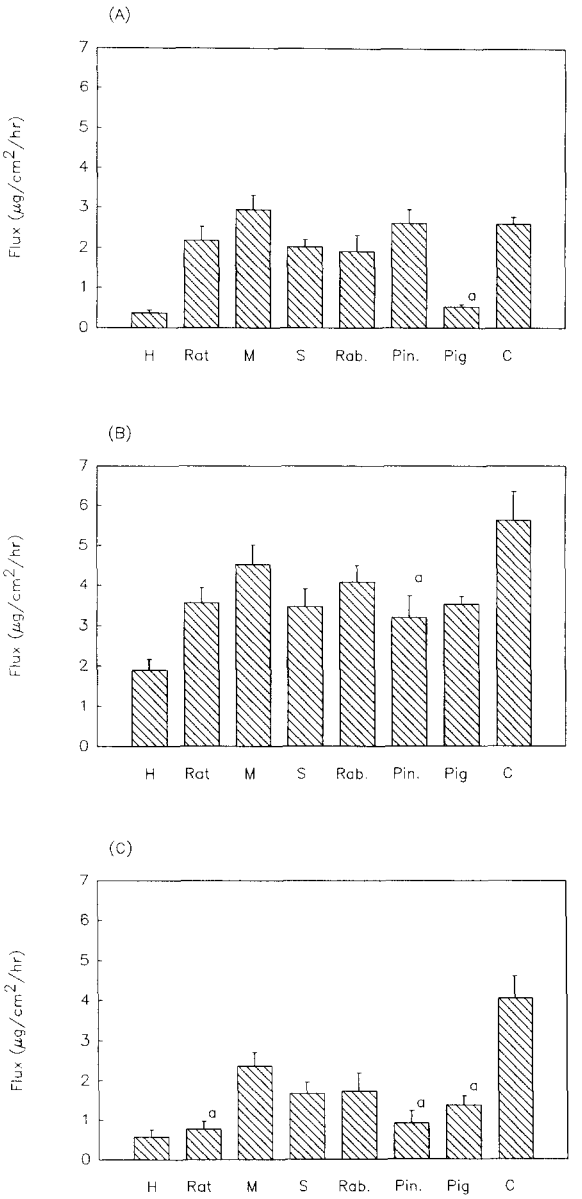


Fig. 4. Steady-state flux of capsaicin (A), NVA (B) and SNA (C) from hydrophilic ointment through various skin types. H, human; R, rat; M, mouse; S, SCID; Rab., rabbit; Pin., pinna; C, cellophane membrane. All data represent the means of three experiments ± S.D. [a]No significant difference vs. human skin.

Table 3
In vitro permeability coefficient (cm/h) of capsaicin, NVA and SNA through various skin types

	Capsaicin (hydrophilic)	Capsaicin (commercial)	NVA (hydrophilic)	SNA (hydrophilic)
Human	0.49 ± 0.09	0.88 ± 0.13	3.15 ± 0.45	1.43 ± 0.73
Rat	2.91 ± 0.47	3.17 ± 0.13	5.93 ± 0.63	1.93 ± 0.50
Mouse	3.92 ± 0.49	8.43 ± 0.51	7.53 ± 0.82	5.90 ± 0.85
SCID	2.69 ± 0.25	4.80 ± 0.44	5.77 ± 0.75	4.15 ± 0.73
Rabbit	2.55 ± 0.55	2.96 ± 1.01	6.80 ± 0.70	4.30 ± 1.18
Pinna	3.48 ± 0.47	5.05 ± 0.43	5.32 ± 0.90	2.25 ± 0.80
Pig	0.71 ± 0.08	1.27 ± 0.20	5.87 ± 0.33	3.40 ± 0.58
Cellophane	3.47 ± 0.23	3.25 ± 0.15	9.37 ± 1.22	10.13 ± 1.38

[a]The density of ointment base was 1 g/cm^3 when determined permeability coefficient. All data represent the means of three experiments ± S.D.

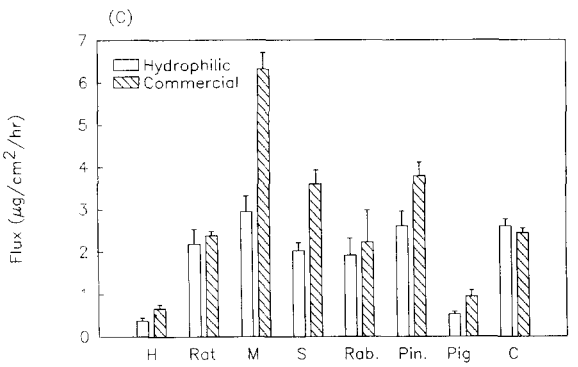


Fig. 5. Comparison of steady-state flux of capsaicin from hydrophilic ointment and commercial available cream through various skin types.

NVA are found to be similar to those of capsaicin (Bucsics and Lembeck, 1981; Northam and Jones, 1984), synthetic NVA can act as a commercially available substitute for capsaicin in the field of pharmaceutics because of it costs less and is more easily obtainable than extracted capsaicin. In the test of the antinociceptive effect of seven capsaicin analogues in mice, the ED_{50} values showed SNA to be the most potent in its activity and the potency was 1.75 times than that of capsaicin (Chen et al., 1992). Furthermore, the full-thickness human skin permeability coefficient of SNA from hydrophilic ointment is also 2.92 times higher than that of capsaicin. In conclusion, SNA will be a powerful antinociceptive agent because of its marked pharmacological activity and in vitro permeability through human skin without demonstrating overt irritant effects.

References

Bosma, G.C., Fried, M., Custer, R.P., Carroll, A., Gibson, D.M. and Bosma, M.J., Evidence of functional lymphocytes in some (leaky) scid mouse. *J. Exp. Med.*, 167 (1988) 1016–1033.

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

Bronaugh, R.L. and Stewart, R.F., Methods for in vitro percutaneous absorption studies III : Hydrophilic compounds. *J. Pharm. Sci.*, 73 (1984) 1255–1258.

Bronaugh, R.L. and Stewart, R.F., Methods for in vitro percutaneous absorption studies VI: Preparation of the barrier layer. *J. Pharm. Sci.*, 75 (1986) 487–491.

Buck, S.H. and Burk, T.F., The neuropharmacology of capsaicin : review of some recent observations., *Pharmacol. Rev.*, 38 (1986) 179–226.

Bucsics, A. and Lembeck, F., In vitro release of substance P from spinal cord slices by capsaicin congeners. *Eur. J. Pharmacol.*, 71 (1981) 71–77.

Catz, P. and Friend, D., Transdermal delivery of levonorgestrel VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin, and human skin. *Int. J. Pharm.*, 58 (1990) 93–102.

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.

Clozel, J.P., Roberts, A.M., Hoffman, J.I.E., Coleridge, H.M. and Coleridge, J.C.G., Vagal chemoreflex coronary vasodi-

lation evoked by stimulating pulmonary C-fibers in dogs. *Circ. Res.*, 57 (1985) 450–460.

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.

Harada, K., Murakami, T., Kawasaki, E.,Higashi, Y., Yamamoto, S., and Yata, N., In vitro permeability to salicylic acid of human, rodent, and shed snake skin. *J. Pharm. Pharmacol.*, 45 (1993) 414–418.

Hayes, A.G., Skingle, M. and Tyers, M.B., Effects of single dose of capsaicin on nociceptive thresholds in the rodents., *Neuropharmacology*, 20 (1981) 505–511.

Hayes, A.G., Orford, A., Reynolds, M., Skingler, A.H., Skingler, M., Smith, C. and Tyers, M.B., The effects of a series of capsaicin analogues on nociception and body temperature in the rat., *Life Sci.*, 34 (1984) 154–156.

Hirvonen, J., Sutinen, R., Paronen, P. and Urtti, A ., Transdermal penetration enhancers in rabbit pinna skin : duration of action, skin irritation, and in vivo/in vitro comparison. *Int. J. Pharm.*, 99 (1993) 253–261.

Keshary, P.R. and Chien, Y.W., Mechanism of transdermal controlled nitroglycerin administration : development of a finite-dosing skin permeation system. *Drug Dev. Ind. Pharm.*, 10 (1984) 883–913.

Lahann, T.R. and Farmer, R.W., Antinociceptive action of capsaicin in rodents. *Proc. West. Pharmacol. Soc.*, 26 (1982) 145–149.

Ledger, P.W. and Nichols, K.C., Transdermal drug delivery devices. *Clin. Dermatol.*, 7 (1989) 71–72.

Liu, J.L., Tan, E.L., Chiang, C.C., Tojo, K. and Chien Y.W., Mechanistical analysis of release kinetics for lipophilic drug from matrix-type drug delivery devices. *Drug Dev. Ind. Pharm.*, 11 (1985) 1373–1390.

Makara, G.B., Gyorgy, L. Molnar, J., Circulatory and respiratory to capsaicin, 5-hydroxytryptamine and histamine in rats pretreated with capsaicin. *Arch. Int. Pharmacodyn.*, 170 (1967) 39–45.

Monsereenusorn, Y., Kongsamut, S. and Pezalla, P., Capsaicin — a literature survey. *CRC Crit. Rev. Toxicol.*, 13 (1982) 321–339.

Negulesco, J.A., Noel, S.A., Newman, H.A.I., Nabor, E.C., Bhat, H.B. and Witiak, D.T., Effects of pure capsaicinoids (capsaicin and dihydrocapsaicin) on plasma lipid and lipoprotein concentrations of turkey poults. *Arthero.*, 64 (1987) 85–90.

Northam, W.J. and Jones, D.J., Comparison of capsaicin and substance P induced cyclic AMP accumulation in spinal cord tissue slices. *Life Sci.*, 35 (1984) 293–302.

Ordway, G.A. and Pitetti, K.H., Stimulation of pulmonary fibres decreases coronary arterial resistance in dogs. *J. Physiol.*, 371 (1986) 277–288.

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.

Szolcsanyi, J. and Jancso-Gabor, A., Sensory effects of capsaicin congeners part 1 : relationship between chemical structure and pain-producing potency of pungent agent. *Arzneim-Forsch (Drug Res.)*, 25 (1975) 1877–1881.

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.

Wang, J.P., Hsu, M.F. and Teng, C.M., Antiplatelet effect of capsaicin. *Trombos. Res.*, 36 (1984) 497–507.

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.