ELSEVIER

International Journal of Pharmaceutics 131 (1996) 143–151

international journal of pharmaceutics

In vivo percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from ointment bases: skin erythema test and non-invasive surface recovery technique in humans

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Received 23 May 1995; accepted 21 August 1995

Abstract

Nonivamide (NVA) and sodium nonivamide acetate (SNA) are both synthetic analogues of capsaicin. In this present study, the in vivo skin erythema test and non-invasive surface recovery techniques were performed in humans for capsaicin, NVA and SNA after transdermal ointment application. In order to quantify the skin erythema and irritation caused by capsaicin and its analogues, laser Doppler flowmetry (LDF) was utilized for determining the cutaneous blood flow to assess the degree of irritant reaction. In the study of surface recovery technique, NVA hydrophilic ointment base showed higher flux and cumulative absorbed amount than the other formulations. In the study of skin erythema test, capsaicin caused severer skin irritation than NVA in humans. Moreover, there was no significant difference between the erythema levels of SNA and control group indicating that SNA produced no skin irritation or pungent sensation. In addition, increased skin temperatures were detected after transdermal application of capsaicin, NVA and SNA ointment bases. The trend of the skin temperature profiles was consistent with that of skin erythema test by laser Doppler flowmetry suggesting that increased skin temperatures may reflect the degree of vasodilation produced by irritation in the treated skin area. In the study of the skin erythema test using various concentration levels of capsaicin and NVA, the various doses from hydrophilic ointment did not markedly influence the pungent and irritant skin reactions after transdermal application.

Keywords: Capsaicin; Nonivamide; Sodium nonivamide acetate; Ointment; Percutaneous absorption; Surface recovery technique; Skin erythema test; Laser Doppler flowmetry

1. Introduction

Capsaicin (8-methyl *N*-vanillyl-6-nonenamide), a pungent principle of red pepper, reveals a variety of pharmacological actions on cardiovascular, respiratory and nerve systems (Monsereenusorn et al., 1982). *N*-Nonanoyl vanillylamide (nonivamide; NVA) is one of the synthetic analogues of capsaicin which has similar structure and pharmacological effects to those of capsaicin. Capsaicin

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SSDI 0374-2763(95)00178-G

and NVA act directly on peripheral sensory nerves, releasing and depleting potent vasodilator neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) from susceptible C-fibre primary afferents (Roberts et al., 1992). These small, naturally occurring peptides depolarize neurons causing marked vasodilation, stimulation of smooth muscle and secretions and activation of sensory nerve endings (Tominack and Spyker, 1987). Following topical application of capsaicin and NVA, heat sensitization, heat hyperalgesia and mechanical hyperalgesia have been well documented (Culp et al., 1989). Furthermore, the treated skin area was reddened and slightly oedematous in the appearance. A bright red flare and erythema were always observed after transdermal application of capsaicin and NVA. Increases in skin temperature were also associated with these skin inflammations (Roberts et al., 1992).

Sodium *N*-nonanoyl vanillylamide-4′-*O*-acetate (sodium nonivamide acetate; SNA) is a newly synthetic analogue of capsaicin. The antinociceptive potency of this sodium salt was 1.75- and 2.00-times than that of capsaicin and NVA (Chen et al., 1992). In addition, SNA did not even exhibit the pungent sensation and inflammation that have been found in capsaicin and NVA (Yang et al., 1992).

In order to quantify and compare the skin erythema and inflammation caused by capsaicin and its analogues from ointment bases, laser Doppler flowmetry (LDF) was utilized for determining the cutaneous blood flow to assess the degree of irritant reaction (Agner and Serup, 1989). Increased skin temperature following increased cutaneous blood flow in the erythema area of the treated site has also been detected by thermometric measurements. In principle, LDF is an optical technique for estimation of microcirculation, especially cutaneous blood flow, based on the Doppler principle. When the laser beam, a 632-nm He-Ne laser source, is directed toward the tissue. This laser light backscattered from moving red cells is shifted in frequency according to the Doppler principle. Then this shift is recorded and expressed in the quantitative units (Bircher et al., 1994). Its advantages are that it is non-invasive, simple to apply, provides a continuous record and objectivity compared with traditional methods such as visual scoring assessment (Schabauer and Rooke, 1994).

The present study also used a non-invasive surface recovery technique for estimating the residual drug concentration remaining in the ointment base after transdermal administration to human skin (Wester and Maibach, 1992; Chambin-Remoussenard et al., 1993). The information obtained in this present study is helpful in realizing the skin erythema and transdermal absorption data compared to capsaicin, NVA and SNA after percutaneous ointment application in humans.

2. Materials and methods

2.1. Materials

The following reagents were used: capsaicin and nonivamide (TCI, Japan), *p*-phenylphenol (Sigma, USA), stearic acid (Merck, Germany), sodium laurylsulfate (Wako, Japan), propylene glycol (Shimakyu, Japan). The method of synthesis for SNA has been performed in our laboratory and reported earlier (Fang et al., 1995). The capsaicin cream (Capderm®, 0.075%) was a gift from Mei-Shih Pharmaceutical Corp. (Taipei, Taiwan). The hydrophilic o/w ointment base (U.S.P.) was prepared in order to contain 0.075% of capsaicin, NVA and SNA, respectively. In the skin erythema test, various concentrations of capsaicin and NVA (0.025, 0.050 and 0.075%, respectively) were prepared in the hydrophilic ointment base. All solvents used were of HPLC grade.

2.2. Subjects

Six healthy male volunteers aged from 24 to 25 years participated in this study. Informed consent was obtained from all subjects. The laboratory temperature was kept in the range of 22–23°C, and the relative humidity was 50–60%. Disturbances in the laboratory during measurements were kept at a minimum.

2.3. Surface recovery technique

An accurately weighed amount (0.2 g) of the ointment base was spread uniformly over a sheet of cotton cloth (2 × 2 cm^2) for an 8-h administration using the occlusive dressing technique (ODT) (Naito and Tsai, 1981; Hsu et al., 1991). These pieces of cloth were then applied on both volar forearms of volunteers (four pieces on each forearm). The cloth was then covered with surgical adhesive tape (Micropore®, 3M, USA). The unabsorbed drug was randomly recovered at 0.33, 0.67, 1, 1.5, 2, 4, 6 and 8 h after transdermal administration. After recovering, the residual ointment base remaining on the skin surface was withdrawn by a sterile cottonwool swab immersed in methanol solution. The difference between applied and recovered amounts corresponded to the cumulated absorbed amount. The total amount of drug absorbing through the unit surface 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 from 0 to 8 h and expressed as the mass of drug passing across 1 cm^2 of skin over time.

2.4. Skin erythema test (LDF)

After withdrawal of the residual ointment base from the skin surface and after an interval of 5 min, a test was performed to determine skin erythema. The skin erythema test was carried out using a Periflux PF3 laser Doppler flowmeter (Perimed, Sweden). The laser Doppler probe was gently held on the treated skin to avoid vascular compression, and the readings were recorded as

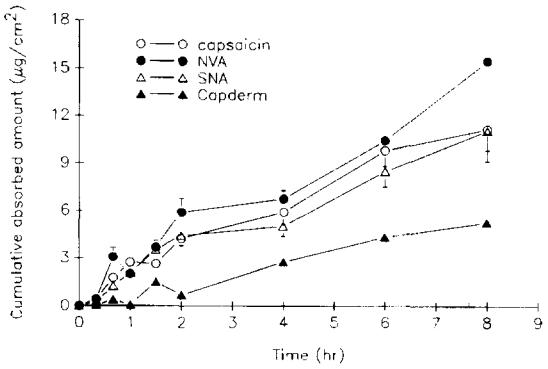


Fig. 1. Cumulative absorbed amouts versus time profiles of 0.075% capsaicin, NVA and SNA after percutaneous administration of ointment formulations in humans. All data represent the means of six experiments ± SD.

average values after stabilization of the level. The output signal of the shift directly observed in LDF was expressed in relative and dimensionless blood flow values (arbitrary units, a.u.). One a.u. on the display is equal to 10 mV. The Perisoft® software program (Gastrosoft, Sweden) was used to acquire, graphically display and store the data obtained from laser Doppler flowmeter.

2.5. Skin temperature

The increased skin temperature incorporated with the skin erythema in the treated skin site was also determined. A surface contact probe (Escort 100, Medical data electronic, USA) was used for estimating temperature. Raised skin temperature could be employed as an indirect measure of increased blood flow during inflammation and it has been assumed to reflect the level of vasodilation (Bjerring, 1991).

Table 1
In vivo skin surface recovery technique in humans from 0.075% drug concentration ointment bases[a]

	Capsaicin	NVA	SNA	Capderm®
8 h absorbed amount ($\mu g/cm^2$)	11.19 ± 1.35	15.49 ± 0.16	11.09 ± 1.93	5.27 ± 0.31
Flux ($\mu g/cm^2/h$)	1.40 ± 0.17	1.77 ± 0.21	1.32 ± 0.08	0.70 ± 0.04
AUC_{0-8} ($\mu g/h/cm^2$)	51.14 ± 6.21	61.08 ± 7.34	46.76 ± 4.79	21.21 ± 1.79

Each value represents the mean ± S.D. (n = 6).
[a]The ointment formulation utilized here is a hydrophilic base except for Capderm®.

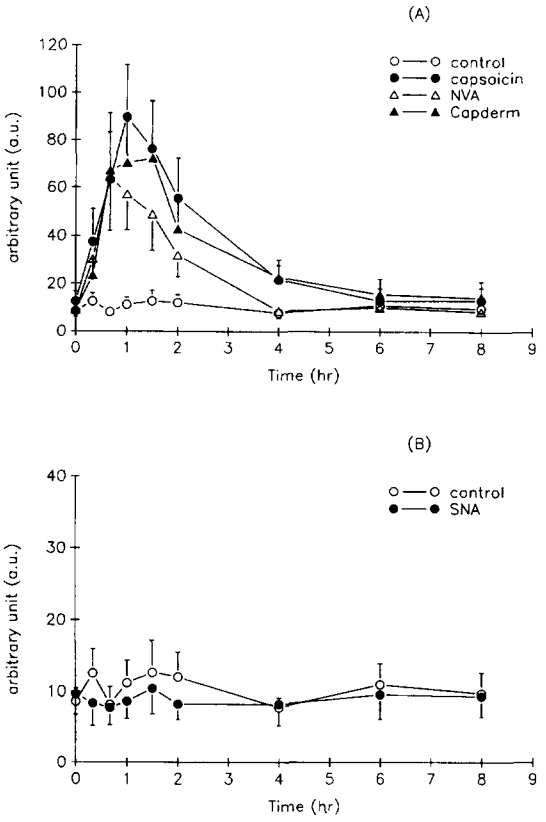


Fig. 2. Skin erythema test measured by laser Doppler flowmetry for 0.075% capsaicin, NVA (A) and SNA (B) after percutaneous administration of ointment formulations in humans. The quantified unit used here is the arbitrary unit (a.u.). All data represent the means of six experiments ± SD.

2.6. Determination of capsaicin, NVA and SNA from ointment bases

One cotton cloth and one cotton wool swab containing ointment base and 0.1 ml of 1.8 μg/ml *p*-phenylphenol were mixed with 5 ml of methanol in a glass-stoppered centrifuge tube for capsaicin, NVA and SNA 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 recovery rates of capsaicin, NVA and SNA from hydrophilic ointment, and capsaicin from commercial available cream, were 73.33 ± 4.89, 90.06 ± 5.42, 93.33 ± 5.01 and 98.97 ± 4.58%, respectively.

The HPLC system for capsaicin, NVA and SNA was described previously (Fang et al., 1995).

2.7. Statistical analysis

The statistical significance of the difference between various treatments was performed by using unpaired Student's *t*-test. The chosen level of significance was $p < 0.05$. The ANOVA statistical test was also utilized in this study.

3. Results and discussion

The results from using the surface recovery technique, cumulative absorbed amount of capsaicin and its synthetic analogues, which are the differences between applied and recovered amounts, are shown in Fig. 1. The applied amount was determined as the recovered amount in 0 h, which was calculated by the drug concentration from the ointment base applied on the skin and immediately peeled off. The cumulative amount-time profile of capsaicin from Capderm® cream was relatively low compared with that of capsaicin, NVA and SNA from hydrophilic ointment bases. The cumulative absorbed amounts in 8 h, the fluxes and the AUC_{0-8} of four ointment formulations are shown in Table 1. The fact that the flux of capsaicin from hydrophilic ointment was significantly higher (*t*-test, $p < 0.05$) than that of capsaicin from Capderm® cream suggested that the hydrophilic formulation offered a better ointment base for capsaicin to achieve effective percutaneous absorption through human skin in vivo. The flux of NVA from the hydrophilic base was significantly higher than that of capsaicin and SNA (*t*-test, $p < 0.05$), this result was consistent with the in vitro percutaneous absorption through cadaver human skin which revealed that NVA had the highest permeability coefficient (Fang et al., 1995).

The results of the skin erythema test using laser Doppler flowmetry, the erythema levels being quantified by arbitrary units (a.u.) after the transdermal application of capsaicin and its synthetic analogues for various periods, are presented in Fig. 2. The control group received the hydrophilic

Table 2
Skin erythema test of 0.075% capsaicin, NVA and SNA ointment bases quantified by laser Doppler flowmetry[a]

	Control	Capsaicin	NVA	SNA	Capderm®
T_{max} (h)	1.5	1.0	0.67	1.5	1.5
$a.u._{max}$	12.67 ± 4.49	89.93 ± 21.86	64.33 ± 22.13	10.44 ± 3.61	72.24 ± 24.14
$AUC_{(a.u. \times h)}$	81.47 ± 21.70	262.84 ± 79.05	166.75 ± 46.21	70.81 ± 23.04	241.06 ± 78.94

The ointment formulation utilized here is hydrophilic base except for Capderm®.
Each value represents the mean ± SD ($n = 6$).
[a]The quantified unit represented here is arbitrary unit (a.u.).

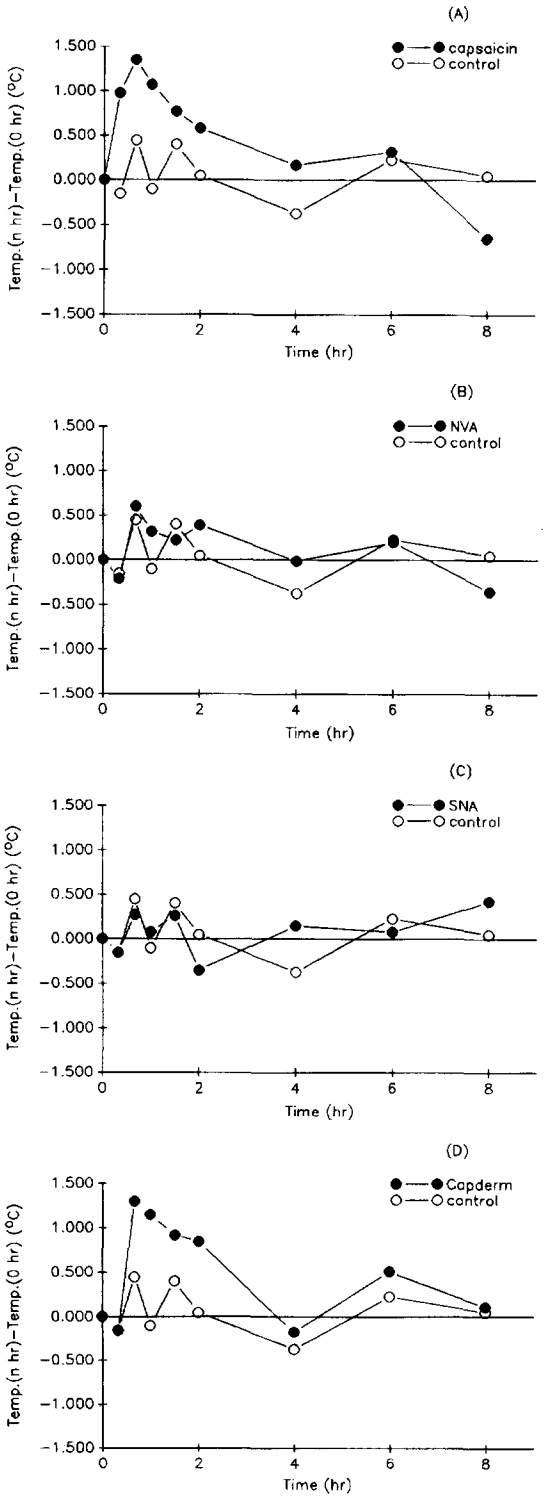
ointment base which contained no drug content. The skin erythema levels were all increased for capsaicin and NVA from hydrophilic bases as well as for Capderm® cream in the early periods after percutaneous application. After transdermal administration for 1 h, the a.u. level of NVA already declined but that of capsaicin and Capderm® still increased to higher levels. The skin erythema test for capsaicin from the hydrophilic base revealed a higher a.u. than the other formulations for the application periods from 1 to 4 h, demonstrating that capsaicin caused more severe skin irritation than NVA in humans (Table 2).

There was no significant difference observed between the application periods from 6 to 8 h for a.u. values of all formulations compared with that of the control group (ANOVA test, $p > 0.05$), suggesting that skin erythema and irritation decreased to normal dermal conditions after 6 h of transdermal administration. When the erythema levels between two capsaicin formulations were compared, no statistical difference was found between them, except for the values after the application period of 1 h. Furthermore, the area under the curve (AUC) values between these two capsaicin formulations showed no statistical difference (t-test, $p > 0.05$). Qualitatively, a slowly increasing sensation of burning pain was reported by the subjects about 15–20 min after transdermal application of capsaicin and NVA. The burning pungent sensation reported by subjects was particularly potent in hydrophilic ointment with capsaicin. This phenomenon may reflect the results from the skin erythema test after capsaicin hydrophilic formulation administration, which revealed higher a.u. values.

The skin erythema levels determined by laser Doppler flowmetry for SNA hydrophilic ointment base are also shown in Fig. 2. Apparently, no erythema or red flare were observed in the treated skin area. Qualitatively, the fact that there was no burning pain sensation reported by any of the subjects demonstrated that SNA did not produce the overt pungent sensation and skin irritation that has been found with capsaicin.

Increased skin temperature may be used as an indirect measure of skin erythema during inflammation, and it has been assumed to reflect the degree of vasodilation. The skin temperatures detected by a surface contact probe after transdermal application of various formulations are shown in Fig. 3. The evident increased skin temperatures were observed in 0.67 h after application of two capsaicin formulations. The skin temperature in the treated area was found to be 1.30–1.35°C higher than that of the pre-treated skin area at 0 h (Table 3). Previous research suggested that there was a 2–3°C increase in skin temperature after the administration of filter paper soaked in 1% capsaicin ethanol solution compared with the contralateral untreated side (Kilo et al., 1994). This phenomenon of a decrease in the increased skin temperature in the present study is due to the fact that the capsaicin concentration (0.075%) used in this study was lower, and the semisolid form of ointment bases may cause milder skin irritation than the solution type utilized in previous research.

The peak skin temperatures were found at 0.67 h after administration of both capsaicin formulations. Accordingly skin temperatures may reflect cutaneous vasodilation levels faster than laser Doppler flowmetry which showed peak a.u. values



at 1–1.5 h after percutaneous administration. The increased skin temperatures after NVA hydrophilic ointment base application were not evidently higher than those of control group, although a significant difference was found between skin temperatures of pre-treated and treated after 0.67 h application skin area (t-test, $p < 0.05$). Considering SNA hydrophilic ointment base in skin temperature measurement, there were no significant differences observed between pre-treated and treated skin areas during administration period and this result was consistent with that of the skin erythema test.

Considering the above results using the surface recovery technique, laser Doppler flowmetry and raised skin temperature, different experimental profiles were observed between capsaicin hydrophilic ointment and Capderm® cream formulations. This indicated that various formulations could influence the percutaneous absorption and pharmacological effects of capsaicin.

The results of the skin erythema test using various concentrations of capsaicin and NVA are depicted in Figs. 4 and 5. For capsaicin, the AUC values of 0.025 and 0.050% showed no statistical difference (t-test, $p > 0.05$), suggesting the same irritant reaction was observed with both doses of capsaicin, although the concentration was doubled. The AUC value of 0.075% capsaicin was significantly higher than those of 0.025 and 0.050% capsaicin (t-test, $p < 0.05$). There is a linear relationship between various capsaicin concentrations and $a.u._{max}$ values which displays a good correlation coefficient (r) of 0.9931 as shown in Fig. 5(B). As shown in Fig. 4, the various doses did not influence the a.u. levels in the earlier stage of percutaneous application until 1 h. The largest increase of a.u. levels was observed during the administration period from 1 to 4 h for the 0.075% dose of capsaicin compared with the other two low-dose formulations.

Fig. 3. Treated skin area temperature change versus time profiles of control group, capsaicin (A), NVA (B), SNA (C) from hydrophilic ointment bases and Capderm® cream (D) after percutaneous administration in humans. All data represent the means of six experiments ± S.D.

Table 3
Skin temperature data of capsaicin, NVA and SNA from 0.075% drug concentration ointment bases[a]

Time (h)	Control	Capsaicin	NVA	SNA	Capderm®
0	32.10 ± 0.19	32.10 ± 0.87	31.03 ± 0.60	31.80 ± 0.48	31.30 ± 0.75
0.33	31.95 ± 0.48	33.08 ± 0.48	30.82 ± 0.80	31.65 ± 0.67	31.13 ± 1.16
0.67	32.55 ± 1.15	33.45 ± 0.52	31.63 ± 0.56	32.07 ± 0.45	32.60 ± 1.26
1.00	32.00 ± 1.11	33.17 ± 0.55	31.35 ± 0.84	31.88 ± 0.84	32.45 ± 0.71
1.50	32.50 ± 0.35	32.87 ± 0.64	31.25 ± 1.12	32.06 ± 0.75	32.22 ± 0.78
2.00	32.15 ± 0.36	32.68 ± 0.44	31.42 ± 1.36	31.45 ± 0.39	32.15 ± 1.29
4.00	31.73 ± 0.69	32.27 ± 0.56	31.02 ± 0.69	31.95 ± 0.44	31.13 ± 1.27
6.00	32.33 ± 1.13	32.42 ± 0.62	31.23 ± 1.13	31.88 ± 0.29	31.81 ± 1.06
8.00	32.15 ± 1.11	31.25 ± 0.47	30.68 ± 1.11	32.22 ± 0.25	31.41 ± 1.13

The ointment formulation utilized here is a hydrophilic base except for Capderm®.
Each value represents the mean ± SD (n = 6).
[a]The unit represented here is °C.

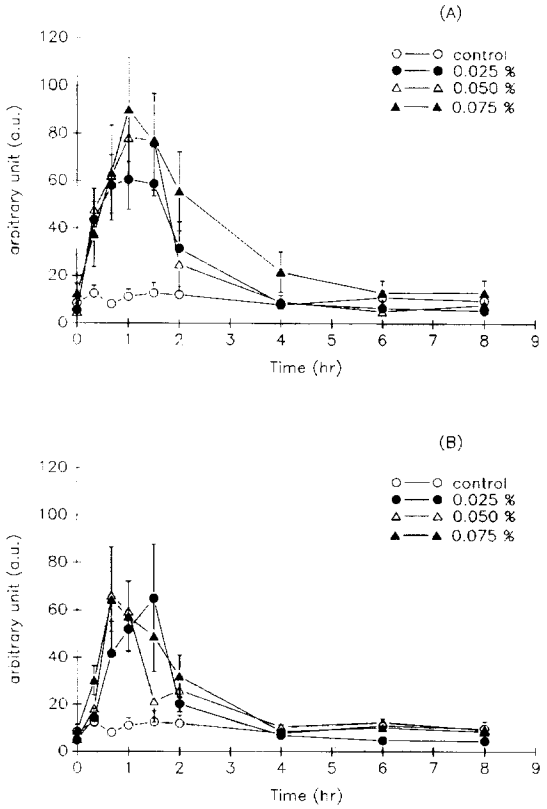


Fig. 4. Skin erythema test measured by laser Doppler flowmetry for capsaicin (A) and NVA (B) in various concentrations after percutaneous administration from hydrophilic ointments in humans. The quantified unit used here is the arbitrary unit (a.u.). All data represent the means of six experiments ± SD.

For NVA, Fig. 5(A) shows that the ointment base with 0.025% concentration produces delayed skin erythema compared with the other two high-dose formulations since the time to peak a.u. value decreases to 1.5 h. As shown in Fig. 5, there is a linear relationship between drug concentrations and AUC values, which displays a good correlation coefficient (r) of 0.9890, and this phenomenon is not observed in capsaicin. A comparison the AUC values of the same doses of capsaicin and NVA indicates that capsaicin caused higher skin erythema and pungent irritation than NVA. Qualitatively, the volunteers could not distinguish the degrees of pungent sensation of these various doses of NVA. The same sensations were reported for capsaicin. This demonstrated that these two analogues may produce a burning pain sensation although the dose increased from 0.025% to 0.075% (by 3 times). This phenomenon may be reflected in the skin temperature change after percutaneous application of various capsaicin and NVA concentrations (Fig. 6).

4. Conclusions

In conclusion, NVA revealed higher absorbed quantities and penetration flux after percutaneous application in humans. Moreover, it also produced lower a.u. values by laser Doppler

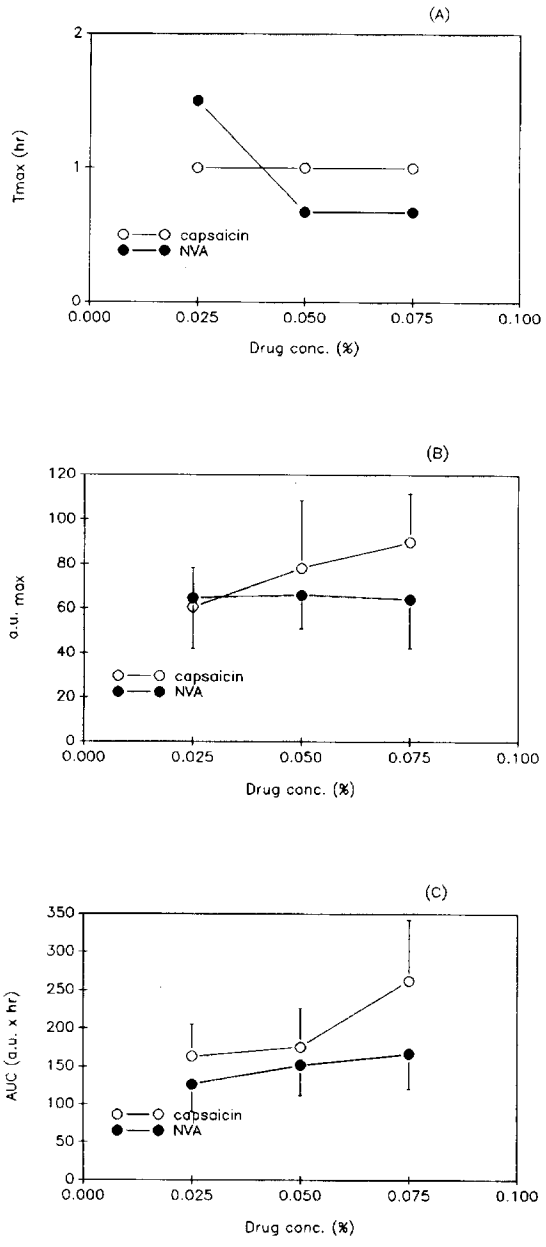


Fig. 5. T_{max} (A), $a.u._{max}$ (B) and area under the curve (AUC) values (C) of the skin erythema test of capsaicin and NVA in various concentrations after percutaneous administration of hydrophilic ointments in humans. The quantified unit used here is the arbitrary unit (a.u.) × h. All data represent the means of six experiments ± SD.

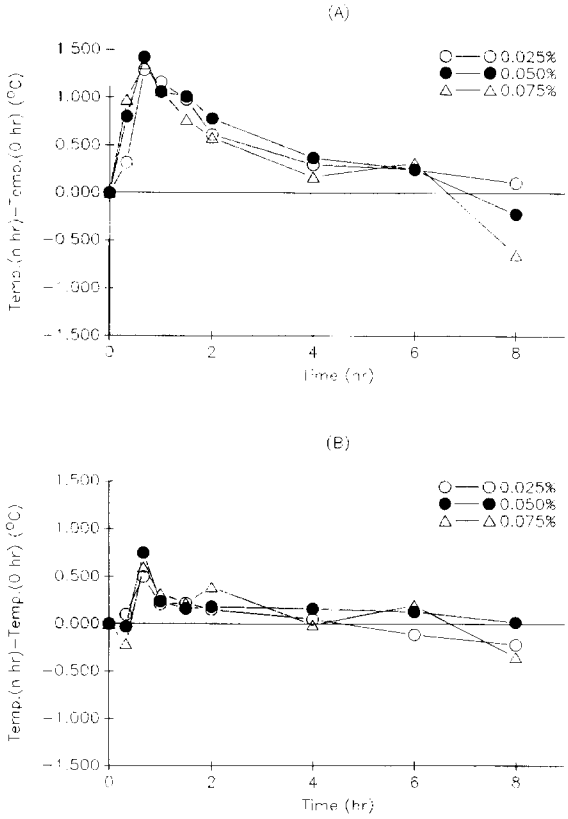


Fig. 6. Treated skin area temperature change versus time profiles of capsaicin (A) and NVA (B) in various concentrations after percutaneous administration of hydrophilic ointment bases in humans. All data represent the means of six experiments ± SD.

flowmetry in the skin erythema test than capsaicin from hydrophilic ointment bases at the same concentrations as NVA. Since the chemical structures and pharmacological activities of these two analogues are similar (Bucsics and Lembeck, 1981), synthetic NVA may be a better substitute for capsaicin because it reveals more effective transdermal absorption and lower skin inflammation in humans, as well as being cheaper to extracted capsaicin. Besides, the various concentrations of capsaicin and NVA from hydrophilic ointment did not markedly influence the pungent and irritant skin reactions after transdermal application. For SNA, moderate percutaneous absorption was observed with the surface recovery technique in the volar arms of humans. Furthermore, no burning pain sensation or skin irritation were detected with the skin erythema test. This result suggests

that SNA is a potent analogue of capsaicin because of its marked transdermal and pharmacological activities, as well as its lack of any pungent skin sensation which will improve patient compliance.

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