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# In vivo percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from ointment bases : Pharmacokinetic analysis in rabbits

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**Abstract**

Nonivamide (NVA) and nonpungent sodium nonivamide acetate (SNA) are both synthetic derivatives of capsaicin. In this study, in vivo systemic drug plasma data of capsaicin, NVA and SNA following intravenous and transdermal ointment base administration in rabbits were performed to establish the pharmacokinetic analysis of these analogues. In order to describe the capsaicin, NVA and SNA plasma profiles observed, one-compartment pharmacokinetic open model for capsaicin and NVA, and two-compartment model for SNA was used in the i.v. plasma data. After the percutaneous administration, the plasma profiles between capsaicin and NVA were quite different although these two analogues showed similar physicochemical properties and intravenous pharmacokinetic parameters. The high plasma concentrations of SNA were obtained in the early period after transdermal application. This phenomenon suggested that SNA passes through skin via the intercellular and transappendageal routes. In the study of the in vivo percutaneous effect of NVA — SNA combined ointments, the result indicated that NVA and SNA could often act as the potent penetration enhancer for each other to higher absorption amount and bioavailability than individual NVA or SNA ointment base. The information of the pharmacokinetic analysis of capsaicin and its analogues in rabbits are helpful for the development of capsaicin, NVA and SNA transdermal drug delivery system.

*Keywords:* Capsaicin; Nonivamide; Sodium nonivamide acetate; Rabbit; Pharmacokinetic model; Percutaneous absorption; Ointment

## 1. Introduction

Capsaicin (8-methyl N-vanillyl-6-nonenamide), which stimulates the release of vasoactive neuropeptides such as substance P (SP) and calcitonin generelated peptide (CGRP) from C-fibre nerve terminals (Manzini et al., 1989), has 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 the similar chemical structure to that of capsaicin. NVA has always been used as a substitute for capsaicin in neurophysiological studies because of the homogenerity of the phar-

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macological and pungent profiles between these two analogues (Hayes et al., 1984). The adverse effects of capsaicin and NVA, including severe burning pain, hyperalgesia and neurogenic vasodilation (flare), limit their clinical use.

Sodium N-nonanoyl vanillylamide-4′-O-acetate (sodium nonivami de acetate; SNA) is a newly designed derivatives of capsaicin which was synthesized by alkylation of the phenolic hydroxyl group of NVA with bromoacetic acid (Fang et al., 1995). This sodium salt analogue revealed marked antinociceptive activities in mice without producing the overt pungent sensation and irritation that have been found in capsaicin and NVA (Chen et al., 1992; Yang et al., 1992).

Transdermal drug delivery system (TDDS) offers some advantages over other routes of drug administration such as 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. The significant first pass metabolism has been detected from capsaicinoids in rats and mice (Sietsema et al., 1988; Donnerer et al., 1990). Besides, the half-life of capsaicin by intravenous administration from rats was very short (7.06 min) (Kawada et al., 1985). Accordingly, TDDS is suitable for capsaicin, NVA and SNA to achieve better bioavailabilities.

This present investigation utilizes the rabbit as an animal model for the in vivo evaluation of capsaicin and its synthetic derivatives since there were some pharmacokinetic analysis in rats but none in rabbits for capsaicin and NVA (Skofitch et al., 1984; Kawada et al., 1985). Moreover, though there were some investigations of the kinetic parameters of capsaicinoids but none for the newly designed analogue, SNA. Accordingly, this study evaluates the systemic plasma concentration of capsaicin, NVA and SNA following intravenous and transdermal ointment base administration. So the attempt to establish pharmacokinetic data for capsaicin and its synthetic derivatives in rabbits is accomplished in order to offer the information for the development of transdermal drug delivery system.

## 2. Materials and methods

### 2.1. Materials

The following reagents were used : Capsaicin and nonivamide (TCI, Japan), p-phenylphenol (Sigma, U.S.A.), stearic acid (Merck, Germany), sodium laurylsulfate (Wako, Japan), propylene glycol (Shimakyu, Japan), normal saline solution (Otsuka, Japan), sodium heparin (Greencross, Japan). The synthetic procedure of SNA had been performed from our laboratory (Fang et al., 1995). In this study, the hydrophilic o/w ointment base (U.S.P.) was prepared so as to contain 0.25% and 0.35% of capsaicin, NVA and SNA, individually. In the study of the in vivo percutaneous effect of NVA-SNA combined ointment, the hydrophilic ointment was prepared so as to contain 0.25%, 0.35% and 0.45% of NVA mixed with 0.45%, 0.35% and 0.25% of SNA, respectively. All solvents used were of HPLC grade.

### 2.2. Animals

Male New Zealand white rabbits (10–15 weeks old; 2.5–3.0 kg) were obtained from Kaohsiung Medical College (Kaohsiung, Taiwan). The rabbits were fasted for 24 h before intravenous and transdermal application and fixed in the stainless cages during the period of drug administration.

### 2.3. Intravenous (i.v.) administration

Stock solutions for intravenous injection contained 6 mg/ml capsaicin, 6 mg/ml NVA and 2 mg/ml SNA of each dissolved in 0.9% normal saline solution with 10% absolute ethanol and 10% Tween 80. Each rabbit was given an i.v. bolus of capsaicin, NVA or SNA into the marginal ear vein at a dose of 2 mg/kg. After injection, blood samples from all rabbits were obtained from an indwelling i.v. catheter (Insylate®, Becton Dickinson, U.S.A.) which had been inserted into the central ear artery (Corbo et al., 1990). Arterial blood samples were withdrawn at 0, 3, 6, 9, 12, 15, 25, 40, 60 and 120 min into heparinized centrifuge tubes and immediately centrifuged at 3000 rpm for 20 min; 0.2 ml of 100

U/ml sodium heparin was injected into the indwelling catheter in ear artery after sampling time to prevent the block of blood clogs. The plasma samples were frozen at −20°C until assay. Each data point represents the average determination of 6 rabbits.

### 2.4. In vivo percutaneous absorption experiment

The hair of rabbit was removed with a electric clipper from the skin of abdominal region prior to the application of hydrophilic ointment base. An accurately weighed 6 g of the ointment base was spread uniformly over a sheet of cotton cloth (6 × 10 $cm^2$). Then this piece of cloth was applied to the shaved skin surface of the rabbit for 14-h periods by the occlusive dressing technique (ODT) (Naito and Tsai, 1981; Hsu et al., 1991). Blood samples were taken at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 h. The method for collecting and storing plasma was the same as that of i.v. administration. Each data point represents the average determination of 5 rabbits.

### 2.5. Plasma analytical procedure of capsaicin, NVA and SNA

A 1.0 ml aliquot of plasma was pipetted into a glass-stoppered centrifuge tube. The centrifuge tube was coated with 10 μl of p-phenylphenol solution at a concentration of 4.5 μg/ml by centrifugal vaporizer previously. The solution was then extracted with 5 ml of chloroform for capsaicin and NVA and 2 ml methanol for SNA and then followed by mechanical shaking for 30 min. After centrifugation for 20 min at 3000 rpm, 4 ml of chloroform phase or 1.5 ml of methanol phase was transferred to another tube and evaporated to dryness by the centrifugal vaporizer under reduced pressure. The residue was redissolved in 0.5 ml of HPLC mobile phase; 20 μl of this solution was injected into the HPLC directly. The recoveries of capsaicin, NVA and SNA from rabbit plasma were 80.47 ± 4.82 %, 83.29 ± 4.11 % and 76.42 ± 3.14 %, respectively. In the study of the in vivo percutaneous effect of NVA — SNA combined ointment, 0.7 ml aliquots of plasma were pipetted for NVA and SNA extraction, respectively. The other analytical procedure was the same as the above method.

The HPLC instrument system was described previously (Tsai et al., 1994). The mobile phase consisted of a mixture of 55% pH 4 McIlvaine buffer and 45% acetonitrile for capsaicin and NVA, 60% pH 4 McIlvaine buffer and 40% acetonitrile for SNA, respectively.

### 2.6. Data analysis

Pharmacokinetic parameters for capsaicin and NVA in rabbits were measured using a one-compartment model fitting by utilizing the least-squares fit program (PCNONLIN, SCI Software, U.S.A.). The parameters of SNA in rabbits were calculated using a two-compartment model. The plasma concentration data of i.v. application were fitted to the following equations: $C = C_0 \cdot e^{-kt}$ $(one-compartment\ model) C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ $(two-compartment\ model)$

The total clearances (Cl) of capsaicin, NVA and SNA in plasma were measured as: $Cl = k \cdot Vd (one-compartment\ model)$ $Cl = \beta \cdot Vd (two-compartment\ model)$

The $AUC_{0-14\,h}$ after percutaneous administration was calculated by the trapezoidal method. The bioavailabilities (%) through transdermal absorption were determined by the following equation: $Bioavailability(\%) = (AUC_{0-14}/D_{ta}) \div (AUC_{0-\infty}/D_{iv})$

In order to obtain the best-fit curve for the calculated line compared with the percutaneous absorption experimental data, two models were used to suggest the most suitable theoretical values:

(I) Model for capsaicin and NVA, one-compartment model with first-order absorption. $C = KaFD(e^{-Kat} - e^{-Kelt})/Vd(Kel - Ka)$

(II) Model for SNA, two-compartment model with first-order absorption (Tsai et al., 1985). $C = KaFD(Pe^{-\alpha t} + Qe^{-\beta t} + Re^{-Kat})/Vd$, $P = (K_{21} - \alpha)/(Ka - \alpha)(\beta - \alpha)$, $Q = (K_{21} - \beta)/(Ka - \beta)(\alpha - \beta)$, $R = (K_{21} - Ka)/(\alpha - Ka)$, $(\beta - Ka)$D, dose administration; C, drug concentration in plasma; F, fraction of drug absorbed to topical dose; Ka, absorption rate constant; Kel, elimination rate constant; $K_{21}$, $K_{12}$, $\alpha$, $\beta$, distribution rate constant; Vd, volume of distribution.

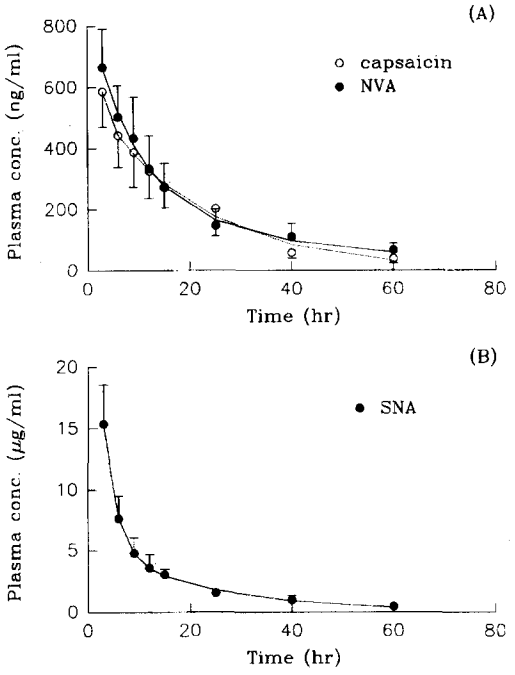


Fig. 1. Plasma concentration versus time profiles of capsaicin, NVA (A) and SNA (B) after i.v. bolus administration in rabbits. All data represent the means of six experiments ± S.D.

### 2.7. Statistical analysis

The statistical analysis of the difference between different treatments was performed by using the unpaired Student's *t*-test. The 0.05 level of probability was taken as the level of significant.

## 3. Results and discussion

For the purpose of studying the biopharmaceutical aspects of capsaicin and its synthetic analogues, one prerequisite was that the pharmacokinetic parameter of the intravenous administration should be known to establish the basic kinetic knowledge. After the single i.v. bogus application of 2 mg/kg of capsaicin, NVA and SNA, the plasma drug concentration-time curves for these analogues are shown in Fig. 1. The plasma levels of capsaicin and NVA appear to be consistent with and adequately described using a one-compartment open model. This model for capsaicin in rabbits was quite different from the result in rats using a biexponential equation to describe the time curve of capsaicin concentration in plasma (Kawada et al., 1985). However, after i.v. administration of 2 mg/kg capsaicin for 3 min, the plasma drug concentration was 585.68 ± 115 ng ml in rabbits, which approximate the data in rats (581 ± 230 ng/g) assumed the plasma density of about 1 g/ml (Saria et al., 1982).

A summary of the pharmacokinetic parameters of capsaicin, NVA and SNA after i.v. administration are shown in Table 1. The intravenous administered plasma profile of NVA was observed to be similar to that of capsaicin. Besides, the n-octanol/water partition coefficient and solubility profiles of capsaicin and NVA showed that capsaicin was more lipophilic than NVA (Tsai et al., 1994). This result may reflect the reason that the Vd of capsaicin was larger than that of NVA. The average terminal elimination half-life values for capsaicin, NVA and SNA were 12.44 ± 2.03,

Table 1
Pharmacokinetic parameters of capsaic in, NVA and SNA after intravenous administration[a]

| Parameter | Capsaicin | NVA | SNA |
|---|---|---|---|
| Compartment model | 1 | 1 | 2 |
| $\alpha$ ($min^{-1}$) | — | — | 0.38 ± 0.08 |
| $\beta$ (k) ($min^{-1}$) | 0.05 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 |
| $t_{1/2}$ (min) | 12.44 ± 2.03 | 10.54 ± 2.10 | 16.80 ± 1.34 |
| AUC ($\mu g \cdot min \cdot ml^{-1} \cdot kg^{-1}$) | 17.78 ± 4.45 | 18.79 ± 4.42 | 217.07 ± 57.74 |
| Clearance ($ml \cdot kg^{-1} \cdot min^{-1}$) | 150.80 ± 30.94 | 102.47 ± 23.67 | 9.55 ± 2.60 |
| Vd ($ml \cdot kg^{-1}$) | 3048.38 ± 521.01 | 2579.14 ± 418.74 | 231.57 ± 62.94 |

[a]Dose of 2.00 mg/kg. Each value represents the mean ± S.D. ($n = 6$).

10.54 ± 2.10 and 16.80 ± 1.34 min, respectively. This short half-life values suggested the rapid elimination of these capsaicin analogues in rabbits. Although SNA is one of the derivatives of capsaicin, the i.v. plasma profile and pharmacokinetic data of SNA were quite different from those of capsacin and this may be due to the fact that the sodium salt in its chemical structure caused various physicochemical constitution compared with capsaicin and NVA.

In order to study the effects of the various amounts of capsaicin, NVA and SNA on the in vivo percutaneous absorption, 0.25% and 0.35% of capsaicin and its analogues were individually prepared in the hydrophilic o/w ointment base. The plasma capsaicin, NVA and SNA concentrations via a single percutaneous administration of hydrophilic bases are presented in Fig. 2.

A summary of the $AUC_{0-14\,h}$ and bioavailabilities of capsaicin and NVA after transdermal administration is shown in Table 2. Results of the transdermal studies demonstrate that there was a proportional increase in the $AUC_{0-14\,h}$ of capsaicin in rabbits as the drug concentrations in ointment bases were increased from 0.25% to 0.35%, but this phenomenon was not observed for NVA since the bioavailabilities were significantly different (t-test, $P < 0.05$) between the ointment bases of 0.25% and 0.35% drug concentrations. The $AUC_{0-14\,h}$ of NVA was significantly higher (t-test, $P < 0.05$) than that of capsaicin by 1.86 times in 0.25% drug concentration ointment and 3.05 times in 0.35% drug concentration ointment. This result is consistent with that of in vitro percutaneous absorption studies which the permeability coefficient of NVA through rabbit abdominal skin from hydrophilic ointment formulation was higher than that of capsaicin by 2.30 times (Fang et al., 1995). The high bioavailabilities of NVA after transdermal administration indicated the hydrophilic o/w ointment could act as an effective reservoir for NVA to release the molecules from this base. The correlation coefficients of capsaicin and NVA, calculated by the regression of experiment concentrations and prediction concentrations in various administration periods, showed good agreements for the calculated curves compared with the experimental data except for the 0.25% NVA. So the best-fit curves obtained for the calculated curves compared with the experimental data indicate the one-compartment model with a first-order absorption is sufficient to describe the in vivo percutaneous absorption of capsaicin and NVA from ointment bases.

The plasma SNA concentration after in vivo transdermal application from hydrophilic ointment bases are also shown in Fig. 2C. The high plasma concentration of SNA was obtained in the early period after transdermal application. The theory of this phenomenon may be that the SNA molecules passed through skin via the intercellular and transappendageal routes since the flux through the hair follicles was higher than that through stratum corneum directly (Illel et al., 1991). This theory agrees to the skin penetration mechanism of SNA from the in vitro prediction (Fang et al., 1995). As shown in Fig. 2C, the plasma levels of SNA increased and declined slightly during the period of transdermal application and the levels generally remained near constant for a prolonged period such as betahistine (Ogiso et al., 1994). The $AUC_{0-14\,h}$ and bioavailabilities of SNA after percutaneous absorption application are shown in Table 2. As same as capsaicin, there was a proportional increase in the $AUC_{0-14\,h}$ of SNA when the drug concentrations were increased from 0.25% to 0.35%. Although the bioavailabilities of SNA were lower than those of capsaicin and NVA because of the much higher $AUC_{0-\infty}$ after the SNA i.v. administration, the SNA transdermal system offered high $AUC_{0-14\,h}$ and stable plasma drug concentration compared with those of capsaicin and NVA. The correlation coefficient of 0.25% and 0.35% SNA is 0.80 and 0.89 of each. So the two-compartment with a first-order absorption is sufficient to fit the in vivo transdermal data between experiment and prediction.

In the previous research (Tsai et al., 1994), the flux of NVA and SNA mixture was higher than that of individual NVA or SNA through excised rat skin in vitro. Therefore, 0.25% + 0.45%, 0.35% + 0.35% and 0.45% + 0.25% of NVA combined with SNA mixtures were selected to prepare into the hydrophilic ointment so as to test

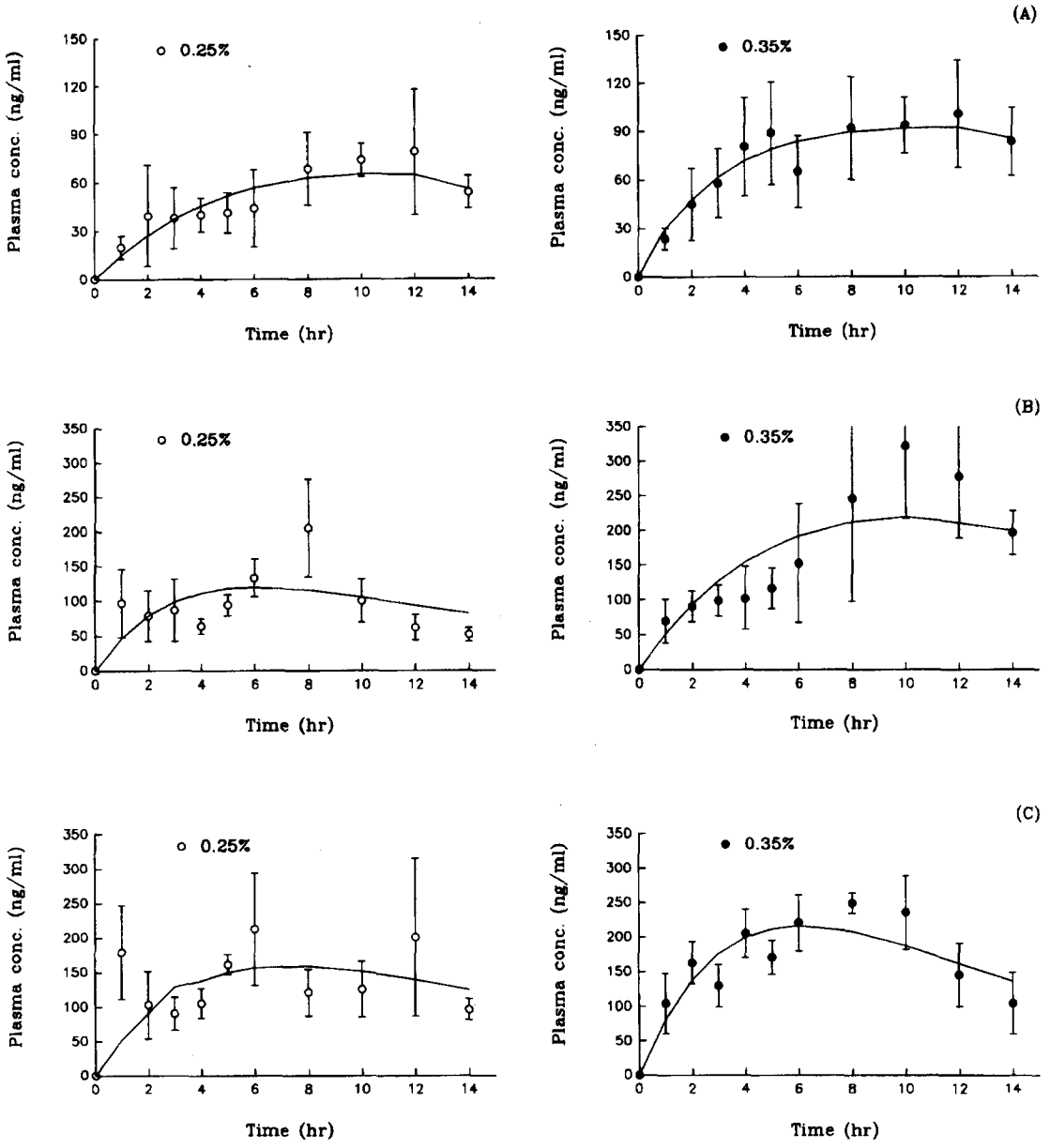


Fig. 2. Plasma concentration versus time profiles of capsaicin (A), NVA (B) and SNA (C) after percutaneous administration of hydrophilic ointment bases in rabbits. All data represent the means of five experiments ± S.D.

the percutaneous penetration effect compared with that of individual NVA or SNA ointment base.

The plasma profiles of NVA and SNA after transdermal penetration of NVA-SNA combined ointments are depicted in Fig. 3. When the bioavailabilities of NVA after percutaneous absorption of NVA-SNA combined ointment base were compared, 0.25% of combined base was significantly higher than 0.25% of individual base ($t$-test, $P < 0.05$) as shown in Fig. 4. As observed in Table 3, the $AUC_{0-14\,h}$ of 0.45% NVA was

Table 2
Pharmacokinetic parameters of capsaicin, NVA and SNA following in vivo percutaneous absorption application[a]

| Drug (%) concentration | Capsaicin | | NVA | | SNA | |
|---|---|---|---|---|---|---|
| | 0.25 | 0.35 | 0.25 | 0.35 | 0.25 | 0.35 |
| $AUC_{0-14\,h}$ ($\mu g \cdot h \cdot ml^{-1}$) | 0.76 ± 0.28 | 0.85 ± 0.23 | 1.41 ± 0.29 | 2.59 ± 0.67 | 2.04 ± 0.47 | 2.46 ± 0.59 |
| Bioavailability (%) | 34.22 ± 12.61 | 27.33 ± 7.40 | 47.24 ± 9.72 | 67.82 ± 17.54 | 7.51 ± 1.73 | 6.47 ± 1.55 |
| Correlation coefficient | 0.92 | 0.96 | 0.66 | 0.90 | 0.80 | 0.89 |

[a]Correlation coefficient is determined by the regression of $C_{exp}$ and $C_{pre}$ in various periods, where $C_{exp}$ mean concentration of experiment and $C_{pre}$ is the mean concentration of prediction. Each value represents the mean ± S.D. ($n$ = 5).

similar to that of 0.35% NVA ($t$-test, $P > 0.05$) after mixed with SNA. So the dose of 0.35% NVA may act as a maximum concentration to achieve the effective percutaneous absorption since the higher doses may restrict their absorption amounts only to the level of 0.35% NVA.

For SNA, the bioavailabilities after transdermal application of NVA-SNA combined base all

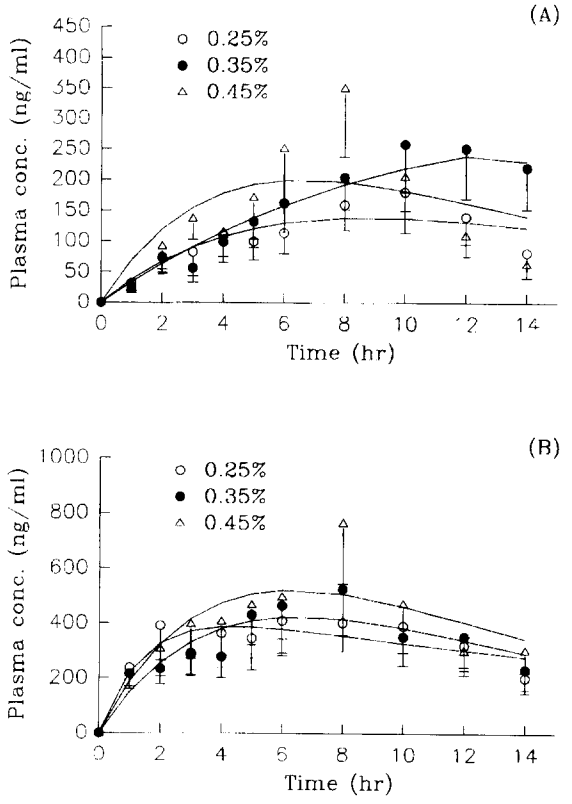


Fig. 3. Plasma concentration versus time profiles of NVA (A) and SNA (B) in various concentrations after percutaneous administration of NVA-SNA combined hydrophilic ointment bases in rabbits. All data represent the means of five experiments ± S.D.

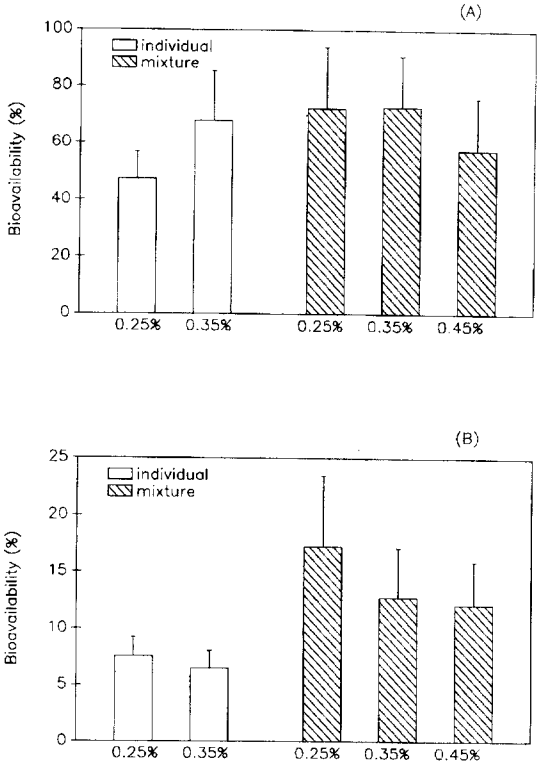


Fig. 4. Comparison of the bioavailabilities of NVA (A) and SNA (B) in various concentrations after percutaneous administration of individual and combined hydrophilic ointment bases in rabbits. All data represent the means of five experiments ± S.D.

Table 3
Pharmacokinetic parameters of NVA and SNA mixtures in various concentrations following in vivo percutaneous absorption application[a]

| Drug concentration (%) | NVA | | | SNA | | |
|---|---|---|---|---|---|---|
| | 0.25 | 0.35 | 0.45 | 0.25 | 0.35 | 0.45 |
| $AUC_{0-14\,h}$ ($\mu g \cdot h \cdot ml^{-1}$) | 1.61 ± 0.48 | 2.27 ± 0.57 | 2.31 ± 0.72 | 4.68 ± 1.69 | 4.85 ± 1.65 | 5.91 ± 1.86 |
| Bioavailability (%) | 72.31 ± 21.69 | 72.89 ± 18.22 | 57.64 ± 17.97 | 17.24 ± 6.23 | 12.77 ± 4.34 | 12.11 ± 3.81 |
| Correlation coefficient | 0.91 | 0.97 | 0.79 | 0.90 | 0.91 | 0.87 |

[a]Correlation coefficient is determined by the regression of $C_{exp}$ and $C_{pre}$ in various periods, where $C_{exp}$ is the mean concentration of experiment and $C_{pre}$ is the mean concentration of prediction. Each value represents the mean ± S.D. ($n$ = 5).

showed higher values than individual SNA ointment base in various concentrations. When the bioavailabilities of 0.25% SNA were compared, there was a largest increase of 2.30 times after mixed with NVA in the hydrophilic ointment. Accordingly, 0.45% + 0.25% of NVA-SNA combined ointment base was a better choice to enhance the SNA percutaneous amount in this study. This proportion was similar to that of previous in vitro investigation which suggested the ratio of 70/30 NVA: SNA mixture achieved the highest flux of SNA (Tsai et al., 1994). The similar $AUC_{0-14\,h}$ levels were observed between the individual NVA and SNA ointment base by percutaneous application. However, as observed in Table 3, the $AUC_{0-14\,h}$ values of SNA were significantly larger than those of NVA ($t$-test, $P < 0.05$) after transdermal application of NVA-SNA combined ointment. This result suggested that SNA could attain more effective enhancement in percutaneous amount than NVA after the combination of these two analogues in ointment base.

## 4. Conclusion

In conclusion, the plasma profile and bioavailability of NVA after percutaneous absorption were higher than those of capsaicin although there were similar physicochemical properties and intravenous pharmacokinetic parameters between these two analogues. Clinically, the pungent and irritant characters of capsaicin limit its percutaneous-administered dose in the low concentration (0.025% and 0.075% for the commercial available product) and hence restrict its use only in the topical treatment such as rheumatic arthritis. Because of the effective absorption amount of NVA and non-pungent property of SNA compared with those of capsaicin, these two synthetic derivatives may act as good substitutes for capsaicin and the systemic treatment could be accomplished. Furthermore, since the antinociceptive $ED_{50}$ value of SNA was 1.75 times than that of capsaicin (Chen et al., 1992). The transdermal delivery system of SNA deserves to be developed for its marked pharmacological activity and higher plasma levels without producing any appreciable skin irritation.

In the study of NVA-SNA combined ointment after transdermal administration, the result suggested that NVA and SNA could be utilized as the potent penetration enhancer for each other to attain higher absorption amount and bioavailability than individual NVA or SNA ointment base.

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