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Effect of pretreatment by cardamom oil on in vivo percutaneous absorption of piroxicam gel: pharmacokinetic analysis in rabbits

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Abstract

The effect of pretreatment by 5% cardamom oil in an alcohol-H_2O vehicle on the percutaneous absorption of piroxicam gel was investigated. The area under the curve of plasma concentration from 0 to 24 h ($AUC_{0-24\ h}$) of piroxicam for 30 min pretreatment with cardamom oil was about 67.09-fold higher than that of non-pretreatment and the absolute bioavailability obtained was 83.23% and was similar to that for 60 min pretreatment. On increasing the pretreatment period from 0 to 30 min, the $AUC_{0-24\ h}$ value increased linearly. In addition, a pharmacokinetic model with two consecutive first-order processes was developed and successfully described the plasma level after topical administration of piroxicam by penetration enhancer for pretreatment. A good correlation was observed between the $AUC_{0-24\ h}$ and the absorption rate constant.

Keywords: Pretreatment; Cardamom oil; Piroxicam; Pharmacokinetic model

1. Introduction

Recently, considerable research is in progress on the penetration enhancer in order to improve the penetration of drugs (Hwang and Danti, 1983; Barry and Bennett, 1987; Yamada et al., 1987; Goodman and Barry, 1988). For liquid penetration enhancers incorporating a large quantity into the original formulation would influence the viscosity, solubility and other physicochemical properties of the semi-solid dosage forms. Hence, pretreating the skin with the penetration enhancers before application of drugs would be a good method to promote the percutaneous absorption of drugs (Hosoya et al., 1987; Hsu et al., 1991). In previous studies, we developed cardamom oil, a colorless volatile oil distilled from the seed of *Amomum cardamomum* (Zingiberaceae) and commonly used as a flavor (Gennaro, 1985), that was found to give remarkable enhancement of the penetration at increasing the flux of a number of drugs including indomethacin, piroxicam and diclofenac through rabbit skin in in-vitro experiments (Huang et al., 1995). The

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effect of pretreatment by cardamom oil on drug penetration through rabbit skin in an in vitro study was also investigated (Huang et al., 1996b).

The purpose of the present study was to evaluate the effect of pretreatment by cardamom oil on in vivo percutaneous absorption of piroxicam gel. In addition, a pharmacokinetic model with release and absorption processes was developed to elucidate the percutaneous absorption of piroxicam by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer for pretreatment.

2. Materials and methods

2.1. Materials

The following reagents were used: piroxicam gel (0.5%, Pfizer, Lot. No. 506-04712), piroxicam (Pfizer, USA), indomethacin (Sumitomo Chemical, Osaka, Japan), sodium dihydrogen phosphate (E. Merck, Germany), disodium hydrogen phosphate (E. Merck, Germany), citric acid (E. Merck, Germany), acetic acid (ALPS Chem Co., Ltd.), and HPLC grade acetonitrile (TEDIA Company, Inc., USA). Seeds of *Amomum cardamomum* were obtained from the Hui-Chun-Tang Chinese Herb Store (Silo, Taiwan). All other chemicals were of analytical reagent 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 topical application and kept in stainless cages during the period of drug administration.

2.3. Intravenous (i.v.) administration

The piroxicam solution for i.v. injection was prepared by dissolving 250 mg of piroxicam in 50 ml of a sodium bicarbonate-buffered solution (pH 9.2). Each rabbit was given an i.v. bolus of piroxicam into the marginal ear vein at a dose of 5 mg/kg. After injection, blood samples from all rabbits were obtained from an indwelling i.v. catheter (Insylate, Becton Dickinson, USA) which had been inserted into the central ear artery (Corbo et al., 1990; Fang et al., 1996). Arterial blood samples were withdrawn at 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min into heparinized centrifuge tubes and immediately centrifuged at 3000 rev./min for 10 min; 0.2 ml of 100 units/ml sodium heparin was injected into the indwelling catheter in the ear artery after sampling time to prevent blockage by blood clots. The plasma samples were then frozen at −20°C until assay. Each data point represents the average determination of five rabbits.

2.4. Stripping of the stratum corneum

The stratum corneum was stripped 30 times with a commercially available cellophane adhesive tape.

2.5. Wet dressing method for pretreatment and topical application of piroxicam gel

The hair was removed with electric hair clippers from the skin of the abdominal region prior to the application of the penetration enhancers. A piece of cotton cloth, 6 × 10 cm^2 (about 1 g), was wetted with about 10 ml of 5% cardamom oil in an alcohol-H_2O vehicle system (1:1). Then, the wetted cloth was applied to the shaved surface of the rabbit fixed on a plate for a specific time by the occlusive dressing technique (ODT) (Naito and Tsai, 1981; Hsu et al., 1991). The control experiment was pretreated with the alcohol-H_2O vehicle system (1:1) containing no enhancer. Subsequently, the wet dressing was peeled off and the applied area was gently swabbed clean with cotton to remove the residue solution without damaging the skin. An accurately weighed 6 g of piroxicam gel (0.5%) was spread uniformly over a sheet of cloth (6 × 10 cm^2) and applied to the treated skin immediately by the ODT method. As a control (no pretreatment), the same piroxicam gel was applied to the non-treated skin. Blood samples were taken at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. The method for collecting and storing plasma was the same as that of i.v. administra-

tion. Each data point represents the average determination of four rabbits.

2.6. Plasma analytical procedure of piroxicam

The method for the analysis of piroxicam, described previously by Tsai et al. (1985) was used with some modifications.

A 1-ml aliquot of plasma was pipetted into a glass-stoppered centrifuge tube, along with 1 ml of phosphate-citric acid buffer (pH 4) and 50 μl of internal standard solution (0.5 mg indomethacin/ml CH_3CN). The mixture was shaken for 10 s and extracted with 7 ml of ether-cyclohexane (8:2) by mechanical shaking for 20 min. After centrifugation for 10 min at 3000 rev./min, 5 ml of the ether-cyclohexane phase were transferred to another tube and evaporated to dryness on a water bath at 40°C. The residue was redissolved in 0.4 ml of mobile phase and then mixed for 15 s by a vortex mixer; 20 μl of this solution were injected into the HPLC. The HPLC analysis has been described previously (Huang et al., 1996b).

2.7. Data analysis

The plasma concentration data after i.v. administration were fitted to Eq. (1) by using the least-square fit program, PCNONLIN (SCI Software, USA),

$$C_p = AD \cdot e^{-\alpha t} + BD \cdot e^{-\beta t} \quad (1)$$

where C_p is the drug concentration at time t and A, B, α and β are the biexponential equation constants. Pharmacokinetic parameters for piroxicam in the rabbit were measured using the two-compartment model. The half-life of the terminal phase ($T_{1/2\,\beta}$), the area under the plasma concentration-time curve (AUC), the mean residence time (MRT), and clearance (Cl) were also calculated.

To explain the plasma concentration-time profiles after percutaneous absorption by pretreating, a pharmacokinetic model shown below (Scheme 1) was developed with two consecutive first-order rate constants including release and absorption processes. Differential equations required in the model are follows:

$$d(X)/dt = -K_r(X)$$

$$d(Y)/dt = K_r(X) - K_a(Y)$$

$$d(Z)/dt = K_a(Y) + K_{21}(W) - K_{10}(Z) - K_{12}(Z)$$

$$d(W)/dt = K_{12}(Z) - K_{21}(W)$$

where X, Y, Z and W are the amount of drug in the dosage form, skin, plasma compartment and peripheral compartment, K_r and K_a denote the first-order release rate constant and the first-order absorption rate constant, K_{10} is the elimination rate constant from the plasma compartment, K_{12} and K_{21} represent the transfer rate constants between plasma and peripheral compartments.

The following integrated equation (Eq. (2)) describing the plasma level-time curve (C1) for central compartment was obtained by solving the differential equations of the above model:

$$C_1 = \frac{D_0 K_a K_r}{V_d}(P \cdot e^{-\alpha t} + Q \cdot e^{-\beta t} + R \cdot e^{-K_a t} + S \cdot e^{-K_r t}) \quad (2)$$

$$P = \frac{(K_{21} - \alpha)}{(K_a - \alpha)(K_r - \alpha)(\beta - \alpha)}$$

$$Q = \frac{(K_{21} - \beta)}{(K_a - \beta)(K_r - \beta)(\alpha - \beta)}$$

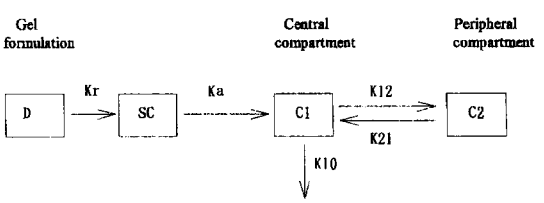
$$R = \frac{(K_{21} - K_a)}{(K_r - K_a)(\alpha - K_a)(\beta - K_a)}$$

$$S = \frac{(K_{21} - K_r)}{(K_a - K_r)(\alpha - K_r)(\beta - K_r)}$$

The $AUC_{0-24\,h}$ after percutaneous absorption was calculated by the trapezoidal method. The bioavailabilities ($F_{0-24\,h}$) through the percutaneous absorption of piroxicam were determined by the following equation:

$$F_{0-24\,h}(\%) = \frac{[AUC]_{0-24h}^{PA}/D_{PA}}{[AUC]_{0-\infty}^{IV}/D_{IV}} \times 100\% \quad (3)$$

where D_{PA} is the dose of piroxicam in 6 g of piroxicam gel (0.5%) and D_{IV} is the dose of piroxicam for i.v. administration.



Scheme 1.

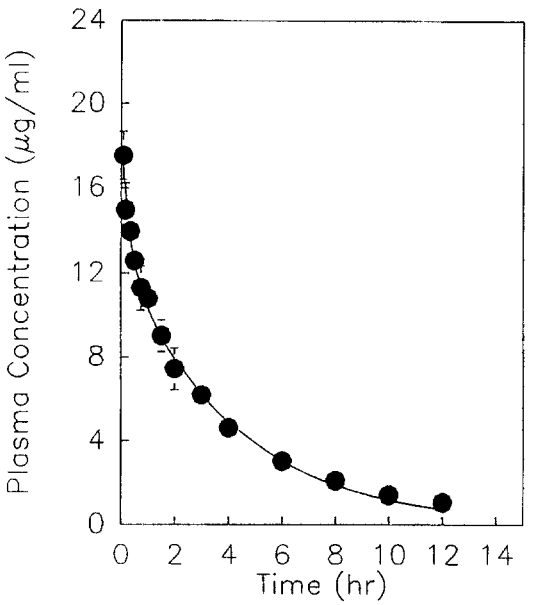


Fig. 1. Plasma concentration-time profile of piroxicam following intravenous administration of 5 mg/kg of piroxicam. Solid line shows simulated curve for piroxicam from Eq. (1). Each point represents the mean of five rabbits with the standard deviation.

Statistical analysis was performed by using the unpaired Student's t-test, and the significance level adopted was $P < 0.05$.

3. Results and discussion

3.1. Plasma concentration of piroxicam after i.v. administration

For the purpose of studying the biopharmaceutical aspects of percutaneous absorption of piroxicam, one prerequisite was that the pharmacokinetic parameters of the intravenous administration should be known to establish the basic kinetic knowledge.

The plasma concentration after i.v. administration of piroxicam (5 mg/kg) is shown in Fig. 1. The plasma curve of piroxicam appears to be adequately described by the two-compartment model. This model for piroxicam in rabbit was similar to that of previous studies (Tsai et al., 1985). A summary of the pharmacokinetic parameters of piroxicam after i.v. administration is listed in Table 1.

3.2. In vivo percutaneous absorption

From the results of the previous study (Huang et al., 1996), 5% cardamom oil in an alcohol-H_2O (1:1) vehicle system was selected as penetration enhancer. The enhancing effect of the vehicle system has been reported previously by other studies (Berner et al., 1989; Kurihara-Bergstrom et al., 1990; Obata et al., 1991; Cornwell and Barry, 1994). The enhancing activity of alcohol was significantly observed when the large amount of alcohol was formulated in the gel or solution. In another study (Hatanaka et al., 1992), neat alcohol markedly enhanced the skin permeability of drugs by pretreatment and the enhancing ratio of the pretreated skin for 24 h was higher than that for 2 h. In order to confirm the effect of pretreatment by an alcohol-H_2O vehicle system, a control experiment was performed. Table 2 shows the $AUC_{0-24\,h}$ and C_{max} values after pretreatment for 5, 15, 30 and 60 min with an alcohol-H_2O vehicle system containing no enhancer. Although slight increases in $AUC_{0-24\,h}$ and C_{max} values were observed, no significant difference was found among the results using analysis of variance (ANOVA, $P > 0.05$). It reveals that the percuta-

Table 1
Pharmacokinetic parameters of piroxicam following intravenous administration

Parameters	Estimate
A	7.2893 ± 1.4174
B	10.6757 ± 1.0138
α	1.7425 ± 1.0103
β	0.2080 ± 0.0252
AUC (μg/h per ml)	57.4813 ± 6.9898
$T_{1/2\,\beta}$ (h)	3.3915 ± 0.4883
K_{10} (h^{-1})	0.3185 ± 0.0561
K_{12} (h^{-1})	0.5197 ± 0.3768
K_{21} (h^{-1})	1.1122 ± 0.6116
V_d (l)	0.6648 ± 0.1148
Cl (l/h)	0.2059 ± 0.0205
MRT (h)	4.5095 ± 0.6253
Vss (l)	0.9178 ± 0.0588

Data are mean ± S.D. (n = 5).

Table 2
Comparision of $AUC_{0-24\ h}$ and C_{max} values by using an alcohol-H_2O vehicle system as the control experiment for pretreatment

Pretreatment period (min)	N	$AUC_{0-24\ h}$ (μg/h per ml)	C_{max} (μg/ml)
No pretreatment	6	1.8254 ± 0.2684	0.1257 ± 0.0173
5	4	3.1811 ± 0.5649	0.2267 ± 0.0528
15	4	3.9969 ± 0.6790	0.3059 ± 0.0513
30	4	4.1478 ± 0.7629	0.2615 ± 0.0503
60	7	4.9687 ± 0.9517	0.3558 ± 0.0687
P (α = 0.05)		NS	NS

Data are mean ± S.E.
NS, non-significant difference.

neous absorption of piroxicam through the pretreated skin with the alcohol-H_2O vehicle system was the same as that through the non-pretreated skin.

When the skin was pretreated with 5% cardamom oil in the alcohol-H_2O vehicle system for 5, 15, 30 and 60 min before topical administration of piroxicam gel, its plasma concentration-time profiles of piroxicam from 0 to 24 h for each condition and non-pretreatment were measured as shown in Fig. 2 (A–D). The $AUC_{0-24\ h}$, C_{max} and $F_{0-24\ h}$ values are shown in Table 3. The AUC_{0-24} h value of piroxicam for 30 min pretreatment by 5% cardamom oil in the alcohol-H_2O vehicle system was about 67.09-fold higher than that of non-pretreatment and the absolute bioavailability obtained was 83.23%.

A specific correlation between the $AUC_{0-24\ h}$ or C_{max} value of piroxicam and pretreatment period was found as shown in Fig. 3. On increasing the pretreatment period from 0 to 30 min, the $AUC_{0-24\ h}$ value increased linearly (r = 0.9936). A similar result was found between the C_{max} value and the pretreatment period (r = 0.9959). From the results, the $AUC_{0-24\ h}$ or C_{max} for 60 min pretreatment was not significantly different from that for 30 min pretreatment ($P > 0.05$).

For the skin irritation, the longer the duration of the pretreatment period will result in the greater extent of damage to the stratum corneum or epithelium. Hence, the $AUC_{0-24\ h}$ value of piroxicam for 5 min pretreatment was only about 13.63-fold higher than that of non-pretreatment and the absolute bioavailability obtained was 16.92%. However, the appearance of skin pretreated with 5% cardamom oil in an alcohol-H_2O vehicle system for 5 min revealed almost no significant conversion. Thus, it was suitable to pretreat with the penetration enhancer for 5 min.

To further examine the effect of the ODT method, 1 ml of 5% cardamom oil in the alcohol-H_2O vehicle system was applied to the skin surface for 5 min without ODT. The result is shown in Fig. 4 and Table 3. From the result, the $AUC_{0-24\ h}$ value with ODT was about 4.94-fold higher than that without ODT. This was confirmed by the volatile experiment which indicated that the weight of solution containing 5% cardamom oil in the alcohol-H_2O vehicle system was decreased about 75.90% during 5 min at 37°C. In addition, to evaluate the effect of the stratum corneum to percutaneous absorption of piroxicam, an investigation was performed in vivo where the stratum corneum had been stripped. The result is also shown in Fig. 4 and Table 3. Stripping of the stratum corneum resulted in a 14.16-fold increase in the $AUC_{0-24\ h}$ value of piroxicam and the plasma concentration-time profile was similar to that for 5 min pretreatment with the ODT method.

Many studies describe an interaction with some component of skin causing the stratum corneum to swell and/or leach out some of the structural components and thus increase drug penetration through the barrier membrane (Barry, 1983; Hadgraft, 1984). According to the literature (Gennaro, 1985), varieties of cardamom oil contain d-α-terpineol both free and as the acetate, 5–10% cineol and limonene. A number of investigations have focused on the terpene compounds as skin en-

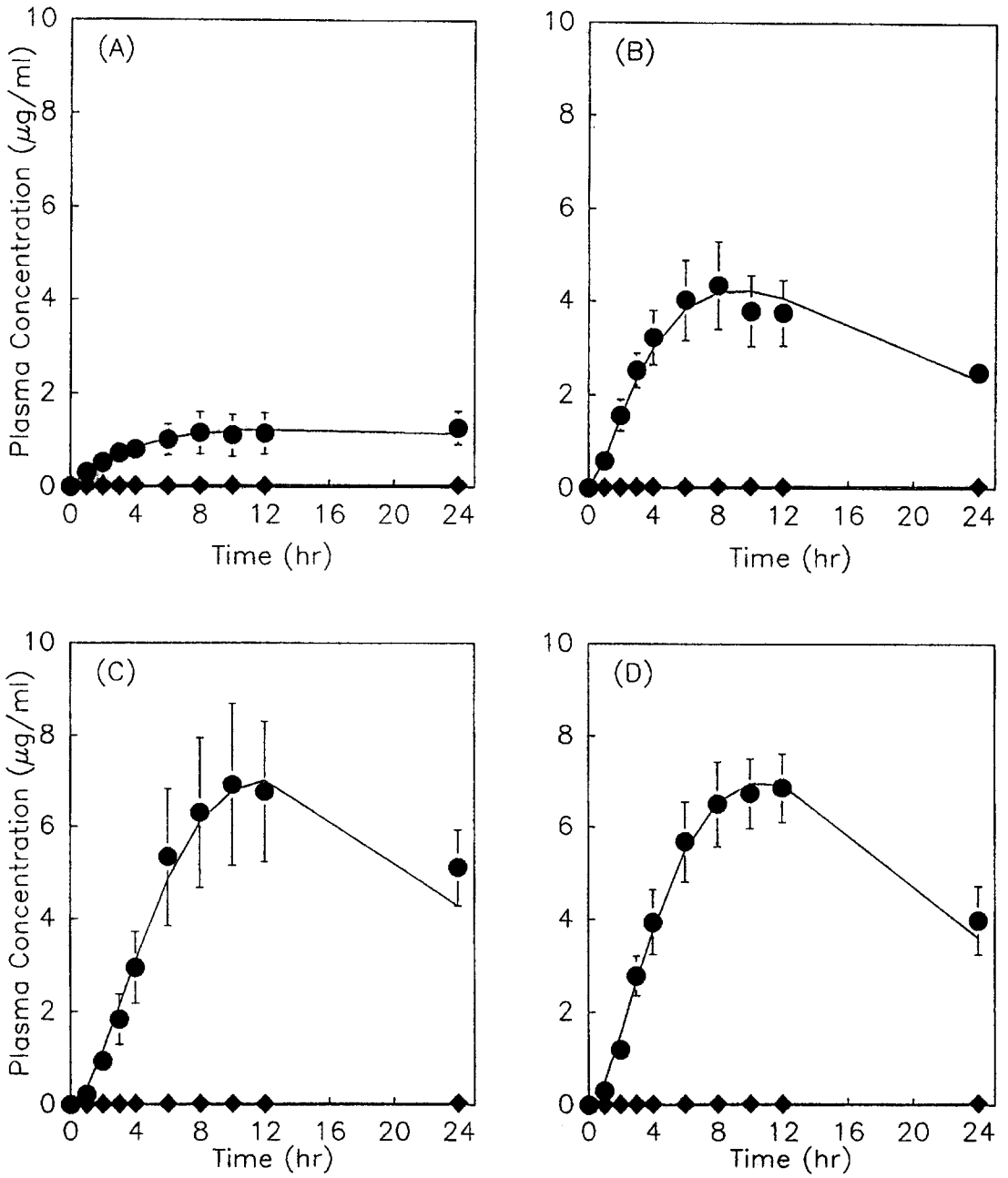


Fig. 2. Effect of pretreatment period (A, 5 min; B, 15 min; C, 30 min; D, 60 min) on the plasma concentration-time curve of piroxicam by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer for pretreatment. Key: ●—●, pretreatment; ◆—◆, no pretreatment; solid lines show simulated curves for piroxicam from Eq. (2). Each point represents the mean of four rabbits with the standard error.

Table 3
Comparision of $AUC_{0-24\ h}$, C_{max} and $F_{0-24\ h}$ values by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer for pretreatment

Pretreatment period (min)	$AUC_{0-24\ h}$ (μg/h per ml)	C_{max} (μg/ml)	$F_{0-24\ h}$ (%)
0: no pretreatment[a]	1.8254 ± 0.2684	0.1257 ± 0.0173	1.24
0: stripping	25.8480 ± 2.6498	1.3381 ± 0.1475	18.01
5: without ODT	5.0439 ± 0.6636	0.4083 ± 0.0472	3.76
5: with ODT	24.8922 ± 8.4024	1.3165 ± 0.4031	16.92
15: with ODT	74.9768 ± 11.9207	4.3514 ± 0.9357	52.32
30: with ODT	122.4594 ± 24.9938	7.4062 ± 1.5950	83.23
60: with ODT	119.8529 ± 9.5748	7.6777 ± 0.7978	81.11

Data are mean ± S.E. (n = 4).
[a] n = 6.

hancers for 5-fluorouracil (Williams and Barry, 1991a,b; Cornwell and Barry, 1994; Yamane et al., 1995), diclofenac and its sodium salt (Obata et al., 1990, 1991), ketoprofen (Okabe et al., 1990) and oestradiol (Williams and Barry, 1991b). 1,8-Cineol and *d*-limonene are among the substances that have been evaluated. For example, pretreatment of epidermal membranes with 1,8-cineol and *d*-limonene for 12 h, the enhancement ratio was 95.3 and 3.55, respectively (Yamane et al., 1995), showing similar results with the data in anther study (Williams and Barry, 1991a). Williams and Barry (1991a), and Yamane et al. (1995) presumed that the model of action of these compounds was probably described by the lipid-protein-partitioning theory; the terpenes interacted with the intercellular lipid lamellar structure to increase drug diffusivity. In previous reports by other workers, skin samples were pretreated with terpenes, usually applied as neat liquid for several hours. However, in the present studies, the percutaneous absorption of piroxicam through rabbit abdominal skin pretreated with 5% cardamom oil in the alcohol-H_2O vehicle system reached a maximum value of about 67-fold increase in $AUC_{0-24\ h}$, respectively, when the pretreatment period increased to 30 min. From these studies it is assumed that cardamom oil increased the percutaneous penetration of piroxicam by direct effects on the barrier nature of the skin.

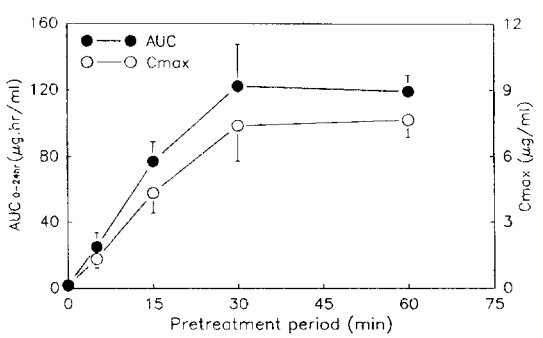


Fig. 3. Relationship between the pretreatment period and the $AUC_{0-24\ h}$ or C_{max} value of absorbed piroxicam by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer or pretreatment. Each point represents the mean of four rabbits with the standard error.

3.3. Pharmacokinetic analysis of plasma concentration data using the model

The pharmacokinetic model of percutaneous absorption of drugs has been widely discussed and

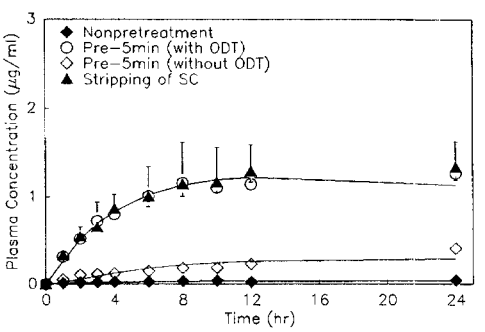


Fig. 4. Effect of ODT method on the plasma concentration-time curve of piroxicam by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer for pretreatment. Solid lines show simulated curves for piroxicam from Eq. (2). Each point represents the mean of four rabbits with the standard error.

Table 4
Pharmacokinetic parameters on percutaneous absorption from piroxicam gel by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer for pretreatment

Pretreatment period (min)	Release rate constant (K_r; h^{-1})	Absorption rate constant (K_a; h^{-1})
0	3.655	0.00028
5	2.395	0.013
15	0.741	0.061
30	0.229	0.093
60	0.329	0.094

many mathematical models have been developed (Naito and Tsai, 1981; Ogiso et al., 1989; Takada et al., 1990, Takayama and Nagai, 1991). In order to explain the plasma concentration-time data for piroxicam after topical administration, we previously analyzed the plasma profile of piroxicam administered percutaneously according to a pharmacokinetic model which includes two consecutive first-order processes (Tsai et al., 1985). In this study, the pharmacokinetic model gives the same model with the release rate constant and the absorption process which can account for the plasma concentration-time profiles after pretreatment by topical administration with 5% cardamom oil in an alcohol-H_2O vehicle system. All the fitting curves (Fig. 2, solid lines) were calculated from the experimental data by a non-linear least square fitting procedure according to the model using the parameters estimated following i.v. administration. The release rate constants (K_r) and absorption rate constants (K_a) of each condition are presented in Table 4.

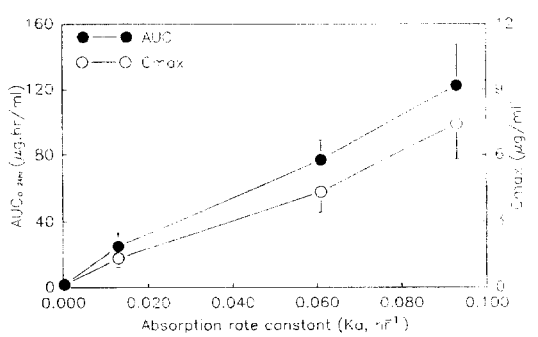


Fig. 5. Relationship between the absorption rate constant (K_a) and the $AUC_{0-24\ h}$ or C_{max} value of absorbed piroxicam by using 5% cardamom oil in an alcohol-H_2O vehicle system as the penetration enhancer for pretreatment (0–30 min). Each point presents the mean of four rabbits with the standard error.

From the results as shown in Fig. 2 (A–D), a satisfactory fit was obtained for experiments with 5, 15, 30 and 60 min pretreatment between the experimental data and calculated curves. Moreover, a good correlation ($r = 0.9972$) between the $AUC_{0-24\ h}$ and the absorption rate constant was observed after topical administration by 5% cardamom oil in the alcohol-H_2O vehicle system for pretreatment. The result is shown in Fig. 5. Thus, it would be reasonable to assume that the absorption process was considered to be the main rate-limiting factor in this model. In the present study, it was assumed that the stratum corneum and epithelium might gradually be influenced by pretreatment with cardamom oil and result in the absorption rate constant of piroxicam increasing linearly ($r = 0.9842$) with increase in the pretreatment period from 0 to 30 min. Furthermore, piroxicam could penetrate more extensively into the skin.

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