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Effect of pretreatment by cardamom oil on in vitro percutaneous penetration of piroxicam gel

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Abstract

The effect of pretreatment by the penetration enhancer, cardamom oil, on the percutaneous penetration of piroxicam from gel through rabbit abdominal skin was investigated using an in vitro technique. The flux and the cumulative amount (at the 48th h) after 1 h pretreatment with 10% cardamom oil in three vehicle systems (alcohol, alcohol/pH 5.8 buffer and alcohol/pH 7.4 buffer) were higher than that of nonpretreatment, and were similar to that of 3 or 6 h pretreatment. A specific correlation between the flux of piroxicam and the pretreatment period was found. Compared to the lag time of skin penetration of piroxicam for nonpretreatment, the lag time for pretreatment was remarkably diminished. The penetration index (PI) of piroxicam after 1 h pretreatment with 10% cardamom oil in alcohol/pH 7.4 buffer (50/40) was about 340.9-fold higher than that of nonpretreatment. In contrast to previous results, 1 h pretreatment with 10% cardamom oil in alcohol had no significant enhancing effect on the percutaneous penetration of piroxicam from gel dosage form. From these results, the alcohol proportion of the vehicle system was the more effective factor for penetration index.

Keywords: Cardamom oil; Pretreatment; Piroxicam gel; Penetration enhancer; Penetration index

1. Introduction

Recently, considerable research is in progress on the penetration enhancer in order to improve the penetration of drugs. Many established penetration enhancers are synthetic chemicals that are taken as additives incorporated into a formulation (Hwang and Danti, 1983; Yamada et al., 1987; Barry and Bennett, 1987; Goodman and Barry, 1988). During our previous studies, we developed cardamom oil as a potential penetration enhancer. Cardamom oil, a colorless volatile oil distilled from the seed of Amomum cardamomum (Zingiberaceae), is commonly used as a flavor and miscible with alcohol (Gennaro, 1985). This volatile oil was found to remarkably enhance the penetration when increasing the flux of a number of drugs including indomethacin, piroxicam and diclofenac through rabbit skin.

For liquid penetration enhancers such as dimethyl sulfoxide, oleic acid and cardamom oil, incorporating a large quantity into the original formulation would influence the viscosity, solubility and other physicochemical properties of the

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semisolid 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).

The purpose of this study was to investigate the effect of pretreatment by cardamom oil on drug penetration through rabbit skin. A commercial piroxicam gel was selected as the model dosage form to evaluate the relationship between the concentration of cardamom oil, proportions of vehicle and pretreatment period on the skin penetration behaviour.

2. Materials and methods

2.1. Materials

The following reagents were used: piroxicam gel (0.5%, Pfizer, Lot. No. 406-04711B), indomethacin (Sumitomo Chemical, Osaka, Japan), sodium dihydrogen phosphate (E. Merck, Germany), disodium hydrogen phosphate (E. Merck, Germany), acetic acid (ALPS Chem Co., Ltd.), polyethylene glycol 400 (RDH, Germany) and HPLC grade acetonitrile (TEDIA Company, Inc. USA). Seed of Amomum cardamomum was obtained from Hui-Chun-Tang Chinese Herb Store (Silo, Taiwan). All other chemicals were of analytical reagent grade.

2.2. Pretreatment with cardamom oil and in vitro penetration study

The diffusion cell used was similar to the Franz diffusion assembly (Franz, 1975). Rabbit abdominal skin was used as the barrier membrane. The effect of in vitro pretreatment with cardamom oil on the penetration of piroxicam from gel was investigated. One ml of cardamom oil in different vehicle (alcohol, alcohol/pH 5.8 buffer, alcohol/pH 7.4 buffer and alcohol/H_2O) was applied to the skin surface for a specific time by the occlusive dressing technique (ODT) (Naito and Tsai, 1981). Subsequently, the applied area was gently swabbed clean with cotton to remove the residue solution without damaging the skin. Then 2 g of piroxicam gel (0.5%) was added to the open cap and tamped down on the treated skin membrane. The area of the skin available for permeation was 2.54 cm^2. The donor compartment was covered with parafilm by the ODT method. The receptor compartment contained approximately 20 ml of pH 7.4 phosphate buffer (0.02 M) solution containing 10% PEG 400 (v/v) as a solubilizing agent. The temperature of the cell was maintained at 37 $\pm$ 0.5°C by a thermostatically controlled water which was circulated through a jacket surrounding the cell body. Samples (0.5 ml) were removed from the receptor compartment at regular intervals and an equal volume of fresh phosphate buffer solution (pH 7.4) was added. Samples were assayed using a high-performance liquid chromatography (HPLC) after subsequent dilution.

The cumulative amount of the drug penetration through the skin was plotted as a function of time and a linear regression analysis was used to determine the flux of the drug The effectiveness of penetration enhancers can be determined by comparing the flux of pretreated skin to that for untreated skin. This was defined as the penetration index (PI): PI = (Flux of drug through pretreated skin)/(Flux of drug through nonpretreated skin).

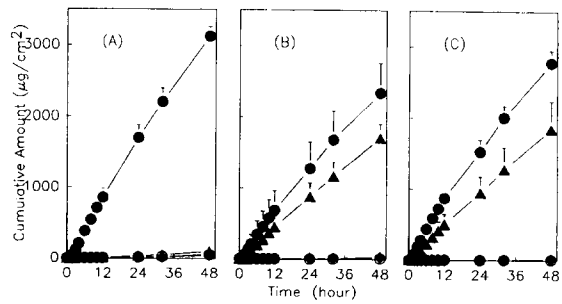

Fig. 1. Effect of different concentration of cardamom oil as the penetration enhancer on the permeation-time profile of piroxicam after 12 h pretreating in different vehicle systems (A) alcohol, (B) alcohol/pH 5.8 buffer, (C) alcohol/pH 7.4 buffer. ○ - ○, control; ◆—◆, 1%; ▲—▲, 5%; ●—●, 10%. Each point represents the mean (n = 3) with the standard deviation.

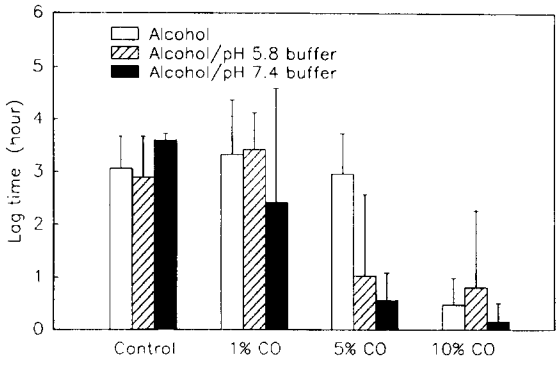

Fig. 2. Effect of different concentration of cardamom oil as the penetration enhancer on the lag time (h) after 12 h pretreating in different vehicle systems.

2.3. Chromatographic analysis

The HPLC analysis, which has been described previously (Tsai et al., 1985) with some modification, were performed on a Jasco system consisting of a Model 880 pump, a Model 875 UY detector, a SIC chromatocorder 12 integrator and a 3.9 × 150 mm i.d. NOVA-Pak C_{18} column (Waters, USA).

The mobile phase consisted of an acetonitrile-water-acetic acid (58:40:1) mixture. The operating temperature was ambient, and the flow rate was 1.0 ml/min with absorbance monitoring at 365 nm.

3. Results and discussion

In previous studies, the acetone extracts of Elettaria cardamomum and Ammonum cardamomum (Zingiberaceae) were found to enhance the percutaneous penetration of prednisolone through mouse skin in vitro (Yamahara et al., 1989) and of indomethacin through rabbit skin in vitro and in vivo (Huang et al., 1993). For preliminary studies, cardamom oil was found out to be an effective enhancer of the penetration of a number of drugs.

In this study, the percutaneous penetration of piroxicam from gel by pretreating the skin with cardamom oil was investigated.

3.1. Effect of different concentration of cardamom oil

Fig. 1 shows the permeation-time profiles of piroxicam after 12 h pretreating with different concentration of cardamom oil in three different vehicle systems: alcohol, alcohol/pH 5.8 buffer and alcohol/pH 7.4 buffer. In the alcohol vehicle, there was no significant enhancement with 1% or 5% cardamom oil and similar to the nonpretreatment. The flux of piroxicam was increased when the concentration of cardamom oil up to 10%. In contrast, a marked enhancement was found with 5% cardamom oil in either alcohol/pH 5.8 buffer or alcohol/pH 7.4 buffer. The lag time was also estimated by extrapolation of the cumulative amount per cm^2 versus time plot at steady state and it was remarkably shortened after pretreatment with 10% cardamom oil in the three vehicle system. The result is shown in Fig. 2 and Table 1.

The penetration index at each concentration of cardamom oil is also shown in Table 1. It can be seen that 10% cardamom oil in alcohol produced only a 62.8-fold increase and 10% cardamom oil in alcohol/pH 7.4 buffer was the most effective pretreatment, yielding a 265.2-fold increase. A similar result was found in the alcohol/pH 5.8 buffer system.

3.2. Effect of pretreatment period

Using 10% cardamom oil as a penetration enhancer in different vehicle systems such as alcohol, alcohol/pH 5.8 buffer(50/40) and alcohol/pH 7.4 buffer(50/40), we pretreated the skin for 0.25, 0.5, 1, 3, 6 and 12 h before topical application of the piroxicam gel. A specific correlation between the flux of piroxicam and the pretreatment period was found, as shown in Fig. 3. The fluxes for 1 h pretreatment by 10 % cardamom oil in alcohol/pH 5.8 buffer and alcohol/pH 7.4 buffer were about 248.2-fold and 311.4-fold higher than that of nonpretreatment and were not significantly different from that of the 3 or 6 h pretreatment (ANOVA test, $P > 0.05$). The flux and cumulative amount (at the 48th h) of piroxicam increased linearly as the pretreatment period increased from 0 to 1 h.

Table 1
The penetration index (PI) and lag time (T_L) of piroxicam from gel after 12 h pretreating 1%, 5% and 10% cardamom oil in different vehicle systems

Cardamom oil concentration	Alcohol		Alcohol/pH 5.8 buffer		Alcohol/pH 7.4 buffer	
	PI	T_L	PI	T_L	PI	T_L
1%	0.84	3.33	2.12	3.42	1.24	2.42
5%	1.84	2.96	136.71	1.03	168.47	0.56
10%	62.83	0.48	187.97	0.81	265.19	0.15

T_L; hour.

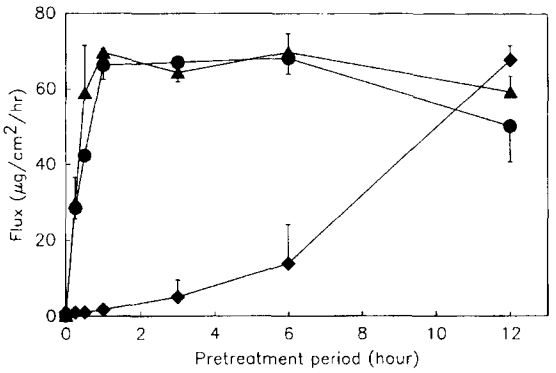

Fig. 3. Effect of pretreatment period on the flux by using 10% cardamom oil as the penetration enhancer in different vehicles. ●—●, alcohol/pH 5.8 buffer (50/40); ▲—▲, alcohol/pH 7.4 buffer (50/40); ◆—◆, alcohol. Each point represents the mean (n = 3) with the standard deviation.

On the other hand, 10 % cardamom oil in alcohol had no significant enhancement for 1 h pretreatment. The flux of piroxicam for 12 h pretreatment was about 62.8-fold higher than that of nonpretreatment. On increasing the pretreatment period from 0 to 12 h, the flux and cumulative amount of piroxicam were increased slowly.

3.3. Effect of vehicle system

To further examine the effect of vehicle system on the flux and lag time, changing proportion of alcohol and buffer containing 10% cardamom oil was investigated and was compared with alcohol/H_2O system.

The penetration indexes and lag times for each condition are shown in Table 2. When the proportion of alcohol decreased in vehicle system from 70% to 30%, the penetration index increased about 300-fold and the lag time for the penetration of piroxicam was close to zero. Comparison of the results in Table 2 indicated that the alcohol proportion of vehicle system was the more effective factor for the penetration index than that of a different pH buffer in the vehicle system.

Table 2
The penetration index (PI) and lag time (T_L) of piroxicam from gel after 1 h pretreating 10% cardamom oil in different ratios of vehicle

Ratio of vehicle system	Alcohol/pH 5.8 buffer/CO		Alcohol/pH 7.4 buffer/CO		Alcohol/H_2O/CO	
	PI	T_L	PI	T_L	PI	T_L
70/20/10	3.23	3.96	3.81	3.90	4.13	3.88
50/40/10	319.15	0.39	340.89	0.00	266.41	0.06
30/60/10	256.12	0.00	294.51	0.00	337.93	0.00

CO, Cardamom oil; T_L, hour.

According to many studies, penetration enhancers are thought to interact 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). Also, 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. Although the part of components such as terpenes or terpenoids had been assessed as skin penetration enhancers (Williams and Barry, 1989, 1991; Takayama and Nagai, 1991), the compositions of cardamom oil are not fully understood. From these studies, it is assumed that cardamom oil increased percutaneous penetration of piroxicam by direct effects on the barrier nature of skin. However, additional studies are needed to elucidate the mechanism and mode of the action of cardamom oil.

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