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Cardamom oil as a skin permeation enhancer for indomethacin, piroxicam and diclofenac

Yaw-Bin Huang, Pao-Chu Wu, Hsiu-Man Ko, Yi-Hung Tsai*

School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China

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Abstract

The effect of the penetration enhancer, cardamom oil, on percutaneous absorption was investigated. Three kinds of drugs, indomethacin, piroxicam and diclofenac, which have different solubility in a mixed solvent system (phosphate buffer/alcohol, 50:50, v/v), were selected to evaluate the drug permeation with or without cardamom oil. For all drugs, the flux, lag time, accumulated amount (at 48 h) and penetration parameters were also determined at various pH values by using an in vitro permeation technique through rabbit abdominal skin. The solubility of each of the three drugs in the solvent system containing 1% cardamom oil was similar to that without enhancer. The penetration index of piroxicam was extremely increased by 1% cardamom oil, about 81.9-fold at the pH 5.8 solvent system, compared with that of indomethacin or diclofenac. The shorter lag time of indomethacin and diclofenac was observed in in vitro permeation experiments with 1% cardamom oil. These results suggest that the enhancing effect of cardamom oil is not due to changing the solubility of the drug in the solvent system and was dependent on the kind of drug, pH value of solvent system and concentration of cardamom oil.

Keywords: Cardamom oil; Indomethacin; Piroxicam; Diclofenac; Penetration enhancer; Penetration index

1. Introduction

Recently, in order to improve drug permeation through the skin, penetration enhancers have attracted a great deal of interest in increasing percutaneous absorption. In addition to many synthetic compounds (Morimoto et al., 1986; Sugibayashi et al., 1988; Catz and Friend, 1989; Wong et al., 1989), extracts of some crude drugs (Yamahara et al., 1989; Huang et al., 1993) were also found to be effective penetration enhancers. In previous studies in our laboratory the acetone extract of cardamom seed (*Amomum cardamomum*, Zingiberaceae) has been found to enhance absorption of indomethacin through rabbit skin (Huang et al., 1993). In order to confirm the composition of the acetone extract, a low-polar portion which had the best effect in enhancing the penetration of indomethacin was separated from the acetone extract with *n*-hexane. Based on the result generated from our preliminary screening study, it suggested that there was part of a volatile oil in the low-polar portion leading to an enhancing effect on

* Corresponding author.

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percutaneous absorption. Therefore, cardamom oil will be separated directly by a distillation method from seeds of *Amomum cardamomum* in the present work.

The purpose of this study was to investigate the effect of cardamom oil in enhancing percutaneous absorption. Indomethacin, piroxicam and diclofenac were selected as model drugs since they have been widely used, systematically and/or locally, as anti-inflammatory agents for percutaneous absorption (Hart and Huskisson, 1984). Using in vitro permeation techniques with rabbit skin as a model membrane, the properties of percutaneous absorption were evaluated. In addition, the effect of varying concentrations of cardamom oil was also investigated.

2. Materials and methods

2.1. Materials

The following reagents were used: indomethacin (Sumitomo Chemical, Osaka, Japan), piroxicam (Pfizer, USA), diclofenac (a gift of Yung Shin Pharmaceutical Ind. Co., Ltd. Taiwan, ROC), seeds of *Amomum cardamomum* (Hui-Chun-Tang Chinese Herb Store, Silo, Taiwan, ROC), *p*-phenylphenol (Sigma Chemical Company, USA), sodium dihydrogen phosphate (E. Merck, Germany), di-sodium hydrogen phosphate (E. Merck, Germany) and HPLC grade methanol (BDH Chemicals Ltd, Pool, UK). All other chemicals were of analytical reagent grade.

2.2. Drug formulations

The indomethacin, piroxicam and diclofenac suspensions were prepared by adding 2% drugs suspended in the formulations shown in Table 1.

2.3. Solubility measurement

The solubility of indomethacin, piroxicam and diclofenac in different pH value solvent systems was determined by addition of excess amount of the drug to the appropriate solvent (pH 5.8, 7.4) at 37 ± 0.5°C. These suspensions were shaken for 48 h at which time the samples were filtered through 0.45 μm PVDF membrane (Gelman Sciences) and diluted with methanol. The concentration of indomethacin, piroxicam and diclofenac in the supernatant were filtrate measured using HPLC.

2.4. Partition coefficient

The *n*-octanol/water partition coefficients of indomethacin, piroxicam and diclofenac were determined in different pH value buffer solutions at room temperature. The various aqueous phases were buffered to pH 5.8 and 7.4 with sodium phosphate. Equal portions (2 ml) of *n*-octanol saturated with buffer and different pH value buffer solutions were poured into the reservoir of the MIXXOR (Genex Co., USA). The MIXXOR, a separating cylinder, consists of a graduated glass mixing chamber, into which fits a glass piston containing an axial channel. The piston is slowly moved up and down forcing the liquid through the axial channel to cause intimate mixing of the two phases in a highly efficient transfer operation. After 20 strokes of the MIXXOR, the aqueous phase was collected and centrifuged (3000 rev./min, 10 min). The aqueous phase was analyzed by HPLC.

2.5. In vitro permeation studies

Skin permeation of indomethacin, piroxicam and diclofenac were measured a using diffusion cell which was similar to the Franz horizontal diffusion assembly (Franz, 1975). Rabbit abdominal skin was used as the barrier membrane. The receptor compartment had approximately 15 ml of the pH 7.4 phosphate buffer (0.02 M) solution containing 10% PEG 400 (v/v) as a solubilizing agent. The donor compartment also had approximately 15 ml of the drug suspension containing an enhancer or no enhancer. The temperature of the cell was maintained at 37 ± 0.5°C by 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

Table 1
Flux, lag time and accumulated amount of indomethacin, piroxicam and diclofenac through rabbit skin from different formulations in in vitro permeation studies

	Cardamom oil (%)	Flux (μg/cm^2.h)	Lag time (h)	Accumulated amount at 48 h(μg/cm^2)
Indomethacin				
pH 5.8[a]	—	2.76 ± 0.49	2.95 ± 0.13	132.29 ± 18.76
	0.1	4.29 ± 1.51	3.50 ± 0.49	204.32 ± 55.35
	0.5	20.82 ± 13.49	4.39 ± 0.02	1061.94 ± 525.24
	1.0	67.31 ± 5.73	1.17 ± 0.42	2967.21 ± 181.83
pH 7.4[a]	—	6.22 ± 2.99	4.16 ± 0.68	300.83 ± 114.72
	0.1	6.51 ± 4.55	3.66 ± 0.75	313.87 ± 167.64
	0.5	81.56 ± 23.04	5.07 ± 0.29	4169.39 ± 925.63
	1.0	254.58 ± 9.16	2.09 ± 0.51	11069.79 ± 228.55
Piroxicam				
pH 5.8[a]	—	0.53 ± 0.08	3.20 ± 0.40	25.30 ± 2.52
	0.1	0.46 ± 0.09	2.37 ± 1.10	20.43 ± 4.33
	0.5	7.15 ± 5.61	5.46 ± 0.55	403.30 ± 259.13
	1.0	43.42 ± 12.22	3.22 ± 1.60	1871.20 ± 334.91
pH 7.4[a]	—	0.99 ± 0.45	2.53 ± 0.01	51.17 ± 23.88
	0.1	0.87 ± 0.45	2.80 ± 0.64	42.12 ± 18.38
	0.5	9.49 ± 10.99	4.01 ± 1.82	526.25 ± 509.54
	1.0	54.14 ± 15.28	4.32 ± 1.02	2418.06 ± 280.43
Diclofenac				
pH 5.8[a]	—	8.73 ± 3.41	3.84 ± 0.34	430.40 ± 132.24
	0.1	11.47 ± 2.25	4.13 ± 0.27	517.83 ± 80.77
	0.5	62.87 ± 6.82	3.34 ± 0.85	2804.34 ± 236.86
	1.0	74.15 ± 7.85	1.17 ± 0.27	3323.48 ± 240.12
pH 7.4[a]	—	13.13 ± 8.52	3.83 ± 0.03	632.16 ± 322.68
	0.1	31.45 ± 6.31	3.48 ± 0.45	1460.82 ± 197.88
	0.5	198.40 ± 93.27	4.73 ± 0.34	9805.72 ± 3569.6
	1.0	336.49 ± 38.40	3.00 ± 0.26	15384.68 ± 1355.9

[a]Phosphate buffer/alcohol (50:50).

7.4) was added. Samples were assayed using high-performance liquid chromatography (HPLC) after subsequent dilution.

The amount of the drug diffused through the skin was plotted as a function of time and a linear regression analysis was used to determine the flux of the drug for each formulation. The permeability coefficients, K_p (cm/h), are given by

$$J = K_p \cdot A \cdot C_d \quad (1)$$

where J is the flux of the permeation traversing the skin of cross-sectional area A cm^2 and C_d is the drug concentration in the donor compartment.

The diffusion coefficient (D_m) of drug through the skin can be estimated from the lag time (t_L) (Flynn et al., 1974)

$$D_m = h^2/(6t_L) \quad (2)$$

where h is the skin thickness. Thus the skin/vehicle partition coefficient (K_m) can be calculated indirectly by the following equation:

$$K_m = (K_p \cdot h)/D_m \quad (3)$$

2.6. Chromatographic analysis

The HPLC analyses were performed on a Jasco system consisting of two Model 880 pumps, a Model 875 UV detector, a SIC chromatocorder 12 integrator and a 125 × 4 mm i.d. C-18 column (E. Merck).

The mobile phase for analyzing indomethacin and diclofenac consisted of a methanol/0.05% acetic acid solution (65:35, v/v) mixture. The op-

Table 2
Solubility of indomethacin and diclofenac in pH 5.8 and pH 7.4 buffer/alcohol system and *n*-octanol/buffer partition coefficient(PC)

Drug	pH	Solubility (mg/dl)[a]		Log(PC)[b]
		No enhancer	1% Cardamom oil	
Indomethacin	5.8	146.93	186.79	1.57
	7.4	788.12	810.45	0.81
Piroxicam	5.8	63.37	57.15	0.84
	7.4	422.40	431.74	−0.81
Diclofenac	5.8	150.38	157.02	*
	7.4	625.63	639.85	0.84

[a]Phosphate buffer/alcohol solvent system.[b]Phosphate buffer system.*Drug 100% in *n*-octanol phase.

erating temperature was ambient, and the flow rate was 1.0 ml/min with UV absorbency monitoring at 260 nm. The method for analyzing piroxicam was that described previously (Tsai et al., 1985).

3. Results and discussion

Cardamom oil is the volatile oil distilled from seeds of *Amomum cardamomum* (Zingiberaceae) and it is usually used as a flavoring agent (Gennaro, 1985). In previous studies, the acetone extracts of *Amomum cardamomum* and *Eletteria cardamomum* were found to enhance the penetration of prednisolone and indomethacin through mouse or rabbit skin in in vitro experiments (Yamahara et al., 1989; Huang et al., 1993). From the preliminary screening study, cardamom oil contained within the low-polar portion of the acetone extract was suspected of an effect in enhancing drug permeation. Therefore, we investigated it for its enhancing effect on percutaneous absorption of indomethacin, piroxicam and diclofenac using an in vitro permeation study through rabbit abdominal skin.

3.1. Solubility and partition coefficients of the three drugs

Table 2 shows the solubility of indomethacin, piroxicam and diclofenac in the solvent system (phosphate buffer/alcohol, 50:50, v/v) with or without 1% cardamom oil at two different pH values (5.8 and 7.4). The solubility of drugs was not much affected by the addition of 1% cardamom oil and dependent on the pH value of the solvent. A higher solubility was achieved at pH 7.4 and indomethacin showed the highest solubility among the three drugs. It was suggested that 1% cardamom oil would not affect the lipophilicity of the solvent system.

The partition coefficients (PC) of the drugs between *n*-octanol and buffer were presented as log PC. As shown in Table 2, the log PC values for the three drugs used covered a range from −0.27 to 1.57.

3.2. In vitro permeation study

The effect of cardamom oil on the permeation of indomethacin, piroxicam and diclofenac through rabbit abdominal skin was investigated. Most in vitro permeation experiments were done by using suspensions to ensure equal thermodynamic activity of drug in the donor compartment. In this study, in vitro permeation-time profiles of drug suspensions in the presence of 0.1%, 0.5% and 1.0% cardamom oil at various pH values are shown in Figs. 1–3. The flux data, lag time and accumulated amount (at 48 h) of the three drugs in the presence of cardamom oil are shown in Table 1. From the results, cardamom oil increased the permeation of these drugs remarkably in comparison with those without the enhancer. The enhancing effect was particularly noticeable in the case of piroxicam at pH 5.8. The shorter lag time of indomethacin and diclofenac was observed with 1% cardamom oil.

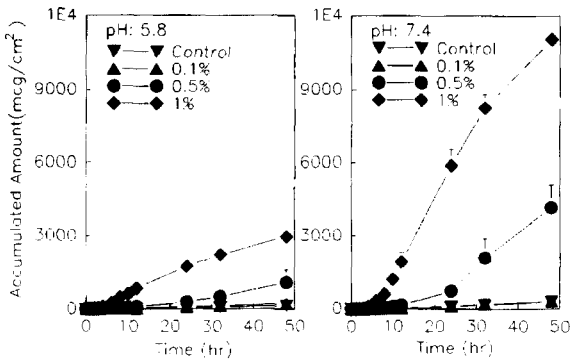

Fig. 1. Permeation-time profiles of indomethacin through rabbit abdominal skin with various concentration of cardamom oil.

However, the skin permeation profiles of indomethacin, piroxicam and diclofenac from aqueous formulations showed zero-order permeation at a constant penetration rate for rabbit skin as shown in Figs. 1–3. Table 3 shows the parameters calculated in the in vitro permeation experiments on indomethacin, piroxicam and diclofenac. In all cases the permeability coefficients (K_p) were increased by the addition of cardamom oil and the constant of piroxicam in the presence of 1% cardamom oil was about 90-fold higher than that in the absence of enhancer at pH 5.8. The diffusion coefficients (D_m) of the three drugs at the same pH value were slightly changed by the addition of 1% cardamom oil. The partition coefficients (K_m) of the three drugs were remarkably increased in the presence of 1% cardamom oil.

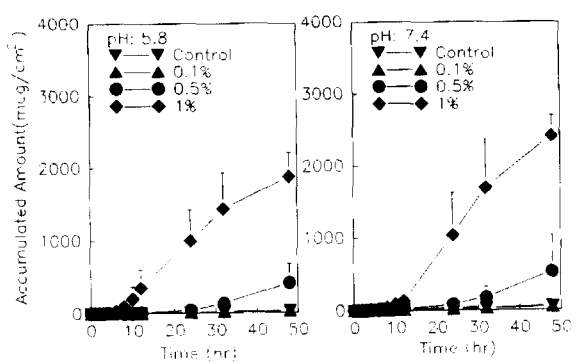

Fig. 2. Permeation-time profiles of piroxicam through rabbit abdominal skin withvarious concentration of cardamom oil.

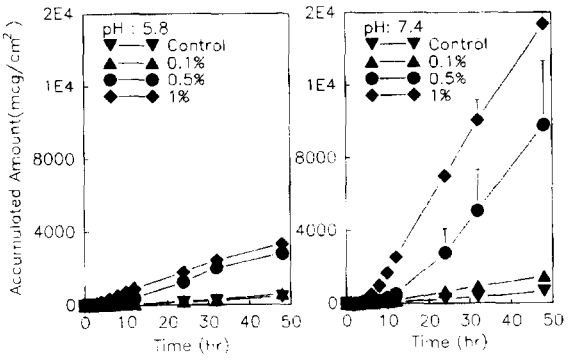

Fig. 3. Permeation-time profiles of diclofenac through rabbit abdominal skin with various concentration of cardamom oil.

Especially, the K_m value of piroxicam with enhancer was increased about 90-fold at various pH conditions.

The effect of pH value on the flux was probably sensitive. The low pH value of the aqueous donor phase (pH 5.8) led to a higher penetration index relative to high pH value (pH 7.4). The penetration index is a ratio whereby the flux of formulation containing enhancer is divided by the flux of control formulation containing no enhancer. The results are shown in Fig. 4. Although the drug solubility in the solvent system was considerably lower at pH 5.8 than at pH 7.4, the penetration index and permeability coefficient were higher. According to a previous study (Herzfeldt and Kümmel, 1983), the pK_a of piroxicam is 5.3 and close to the pH of experimental and skin conditions; hence, it was deduced from the results that the pH value close to pK_a of piroxicam would appear to provide the most favorable environment for permeation containing cardamom oil as a penetration enhancer. The penetration index of the three drugs decreased in the order of piroxicam > indomethacin > diclofenac either at pH 5.8 or pH 7.4.

Otherwise, the enhancing effect of cardamom oil was also dependent on concentration. The effect of cardamom oil at various concentrations (0.1%, 0.5% and 1.0%) is shown in Fig. 5. In general, the enhancement of cardamom oil at a 1% concentration was better than at 0.5%. An increase in concentration of cardamom oil was found to increase the penetration index followed

Table 3
The values of permeability coefficients (K_p), skin/vehicle partition coefficients (K_m) and diffusion coefficients (D_m) from different formulations in in vitro permeation studies

Drug	pH	K_p(cm/h:10^{-3})		D_m(cm^2/h:10^{-5})		K_m	
		Without[a]	With[b]	Without[a]	With[b]	Without[a]	With[b]
Indomethacin	5.8	1.88	36.04	10.84	27.33	0.76	5.78
	7.4	0.79	31.41	7.69	11.03	0.45	12.48
Proxicam	5.8	0.84	75.98	9.99	9.93	0.37	33.51
	7.4	0.23	12.54	12.64	7.40	0.08	7.42
Diclofenac	5.8	5.81	47.22	8.33	27.30	3.06	7.57
	7.4	2.10	52.59	8.35	10.66	1.10	21.61

[a]Containing no cardamom oil.[b]Containing 1% cardamom oil in formulations.

by a linear relationship and shortened the lag time of indomethacin and diclofenac (Table 1). It has been reported that the concentration of enhancer in a formulation markedly influences the promotion of transdermal drug delivery (Barry, 1983; Chow et al., 1984). Thus, the amount of enhancer present in the skin is an important factor in the enhancing effect.

In conclusion, cardamom oil was an effective enhancer of the penetration for indomethacin, piroxicam and diclofenac and it was suggested that the enhancing effect of cardamom oil was not due to changing the solubility of the drug in a solvent system. From these results, piroxicam had the highest penetration index (81.92 and 54.69) among the three drugs enhanced by 1% cardamom oil at various pH values (pH 5.8 and pH 7.4).

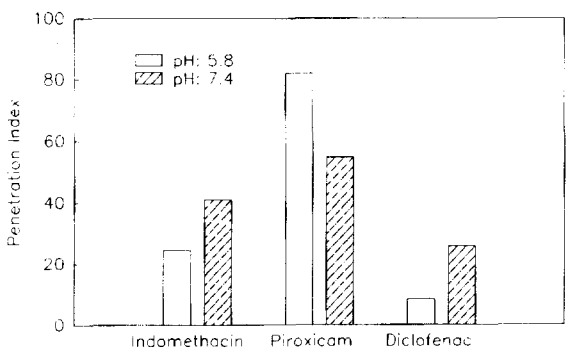

Fig. 4. Effect of pH value on penetration index of indomethacin, piroxicam and diclofenac formulations containing 1% cardamom oil.

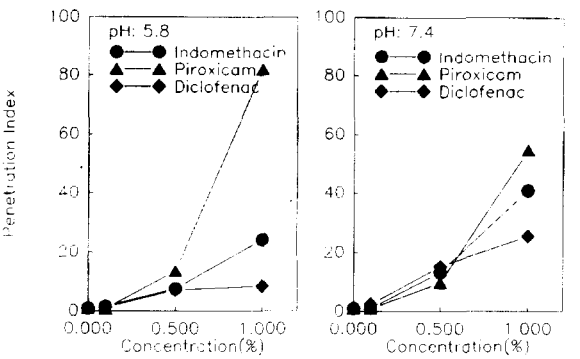

Fig. 5. Effect of increasing cardamom oil concentration on penetration index for indomethacin, piroxicam and diclofenac.

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