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Enhancing effect of terpenes on the in vitro percutaneous absorption of diclofenac sodium.

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Abstract

The enhancing effect of naturally occurring terpenes on the in vitro percutaneous absorption of diclofenac sodium (DFS) from carbopol gels containing propylene glycol was investigated. Permeation experiments were performed on excised abdominal rat skin. Terpenes varied in their activities: the alcohol terpenes were effective accelerants for the drug whereas the ketones were much less efficient, providing only a 2-to-3-fold increase in DFS diffusion; limonene showed mild accelerant activity and 1,8-cineole was a poor accelerant. Acyclic alcohols were found to be the best enhancers for DFS, being geraniol, with an almost 20-fold increase, the most outstanding penetration enhancer. However, although the addition of terpenes increased DFS flux, diffusional lag times were longer than for the control gel.

Keywords: Diclofenac sodium; Skin penetration enhancer; Percutaneous absorption; Terpenes; Geraniol; Gel formulation

Diclofenac sodium (DFS) is a potent non-steroidal anti-inflammatory drug. It is extensively metabolized in the liver and because of its short biological half-live, the drug has to be given frequently. As a result, developing a therapeutic system to provide a transdermal delivery is beneficial. However, DFS is not easily absorbed on transdermal application (Nishihata et al., 1987).

Many strategies have been suggested in order to overcome the low permeability of drugs through the skin. A popular technique is the use of penetration enhancers which reduce reversibly the permeability barrier of the stratum corneum (Barry, 1983).

In this way, many compounds, such as isopropyl myristate (Naito and Tominaga, 1985), nicotinic acid esters (Yasukawa et al., 1985), hydrogenated soya phospholipid (Nishihata et al., 1987), ethanol (Nishihata et al., 1988; Obata et al., 1993), *n*-octanol and decanol (Takahashi et al., 1991a,b) and nonionic surfactants (Iwasa et

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al., 1991) have been reported to enhance the permeation of DFS through the skin.

Recently, a pronounced promoting effect of cyclic monoterpenes on the percutaneous absorption of DFS has been found (Obata et al., 1990, 1991). These chemicals are of low cutaneous irritancy and therefore good candidates for useful skin penetration enhancers. A variety of terpenes have been shown to increase the percutaneous absorption of both hydrophilic (Hori et al., 1991; Williams and Barry, 1991a; Cornwell and Barry, 1991a,b, 1994; Morimoto et al., 1993) and lipophilic drugs (Okabe et al., 1989, 1990; Hori et al., 1991; Takayama et al., 1991; Williams and Barry, 1991b; Priborsky et al., 1992).

In this study, the enhancing effect of naturally occurring terpenes on the in vitro percutaneous absorption of DFS from carbomer gels containing propylene glycol was investigated in rats. Terpenes were chosen from the broad chemical classes of hydrocarbons, alcohols, ketones and oxides; aromatic and aliphatic terpenes. A single sesquiterpene, nerolidol, was also used.

The structures of the terpenes used in this study are shown in Fig. 1. All were of extra pure reagent grade, and were obtained from Fluka

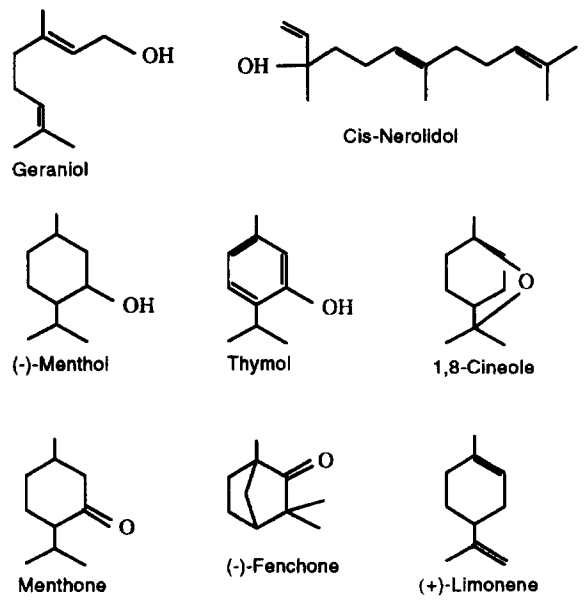

Fig. 1. The structural formulae of terpenes used in this study.

Table 1
Formulae of diclofenac sodium gels

Diclofenac sodium	1.0 g
Carbopol 940	1.0 g
Propylene glycol USP	40.0 g
Terpene	1.0 g
Triethanolamine 99%	1.2 g
Water	ad 100.0 g

(Buchs, Switzerland). DFS was generously supplied by FAES S.A. (Bilbao, Spain). Carboxypolymethylene (carbopol 940), triethanolamine and propylene glycol USP were purchased from Roig-Pharma S.A. (Barcelona, Spain). Other chemicals used were of analytical grade.

In vitro permeation study: Full-thickness abdominal skin was excised from male Wistar rats (200–250 g), the hair of which had been previously removed with an electric clipper. The excised skin was equilibrated in isotonic phosphate buffer (pH 7.2) for 1 h before being mounted on a Franz-type diffusion cell (FDC-400, Grown Glass Co., Somerville, NY) with an available diffusion area of 1.77 cm^2.The receptor phase consisted of a phosphate buffer solution (pH 7.2, 11 ml), stirred at 600 rpm and maintained at 37°C. 1 g of the test gel was placed on the donor side. The composition of the gels used in this study is shown in Table 1. At predetermined time intervals, 0.4 ml samples were taken from the receiver compartment and replaced by the same volume of fresh buffer.

DFS in samples, containing naproxen as an internal standard, was determined using HPLC apparatus (Model 1050, Hewlett Packard) equipped with a variable-wavelength UV monitor. The column was a Nova-Pak C18 (150 x 3.9 mm, 4 μm, Waters). Elution was carried out at room temperature with a mobile phase consisting of acetonitrile and water (50:50, v/v) adjusted to pH 2.2 with phosphoric acid; the flow rate was 1 ml/min. Detection was at 276 nm.

The permeation profiles of DFS through rat skin from carbopol gels containing the terpenes as penetration enhancers and without terpene (control gel) are given in Fig. 2. The flux, J, was determined from the slopes at steady-state and the

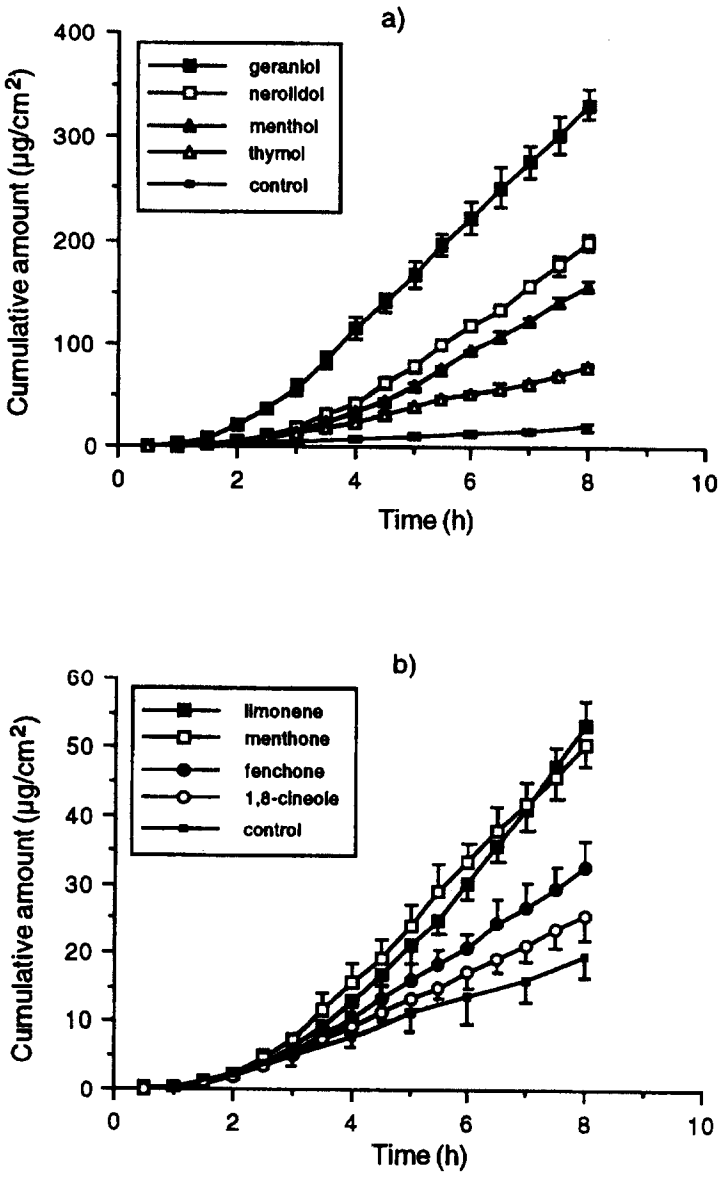

Fig. 2. Typical permeation profiles of diclofenac sodium through abdominal rat skin from carbopol gels containing terpenes as penetration enhancers. (a) Effect of alcohols; (b) Effect of ketone, oxide and hydrocarbon terpenes. Each point represents the mean ± S.E. of three to four experiments.

lag time from the x-intercept. The permeability coefficient, K_p, was estimated from the flux and the donor drug concentration. The effects of terpene enhancers on DFS permeation parameters are summarized in Table 2. Penetration-enhancing activities are expressed as enhancement ratios (ER) which is the ratio of the K_p value with enhancer to that obtained with control gel.

These results demonstrate that the alcohol terpenes were effective accelerants for the drug. The most outstanding penetration enhancer was geraniol, providing an almost 20-fold increase in DFS permeability coefficient, followed by nerolidol, with a 14-fold increase, and menthol,

Table 2
Effect of terpene enhancers on diclofenac sodium skin permeation parameters [a]

Enhancer	J (μg cm^{-2} h^{-1})	$K_p \times 10^4$ (cm h^{-1})	Lag time (h)	ER
control gel	2.86 ± 0.51	2.86 ± 0.51	1.26 ± 0.18	1 ± 0.35
geraniol	54.22 ± 2.13	54.22 ± 2.13	1.91 ± 0.12	18.97 ± 0.21
nerolidol	38.84 ± 1.32	38.84 ± 1.32	2.95 ± 0.11	13.60 ± 0.21
menthol	30.34 ± 1.07	30.34 ± 1.07	2.91 ± 0.05	10.63 ± 0.19
thymol	13.53 ± 0.28	13.53 ± 0.28	2.22 ± 0.24	4.74 ± 0.18
1,8-cineole	3.97 ± 0.48	3.97 ± 0.48	1.66 + 0.03	1.39 ± 0.30
menthone	8.76 ± 0.16	8.76 ± 0.16	2.22 ± 0.31	3.07 ± 0.20
fenchone	5.34 ± 0.57	5.34 ± 0.57	1.97 ± 0.08	1.87 ± 0.28
limonene	10.07 ± 0.37	10.07 ± 0.37	2.88 ± 0.13	3.53 ± 0.21

[a] Values are the mean ± S.E. of three to four determinations at 37°C.

with an 11-fold increase. However, thymol was not as effective as the aliphatic alcohols. The hydrocarbon terpene (limonene) showed mild accelerant activity, whereas the ketones (fenchone and menthone) were less effective, inducing a 2-to-3-fold increase in DFS diffusion. The oxide terpene (1,8-cineole) was a poor accelerant.

Of the alcohols, the best enhancers were the acyclic terpenes, geraniol and nerolidol. Both these compounds have structures suitable for disrupting the lipid packing of the stratum corneum because of the presence of definitive hydrocarbon tails besides a polar head group. These two terpenes were found to be the most effective promoters of 5-fluorouracil, a hydrophilic permeant, among a serie of terpene alcohols (Cornwell and Barry, 1991b).

On the other hand, menthol showed the largest effect of the cyclic terpenes. This concurs with Obata et al. (1990), who found menthol to be the most effective absorption promoter among cyclic monoterpenes on the percutaneous absorption of DFS. However, they also observed a pronounced promoting activity in the case of menthone.

In general, those terpenes with polar functional groups produce the best improvements in the absorption of hydrophilic drugs, whereas hydrocarbon terpenes are more active towards lipophilic drugs. Nevertheless, in this study ketones did not reveal a strong enhancing-activity, and neither did 1,8-cineole, while the hydrocarbon terpene was more effective. This may be related to the lower thermodynamic activity of the ketones in the gels. Both, menthone and fenchone were completely dissolved at the concentration of 1% in gels containing 40% propylene glycol; so did cineole and thymol. In contrast, the rest of the alcohols and the hydrocarbon terpene could not be dissolved. Therefore, the effective terpenes for the percutaneous absorption of different drugs are not the same, and drug absorption through the skin may be closely related to the physicochemical nature of drugs and terpenes and the vehicle in which they are formulated.

Table 2 shows that while the addition of terpenes increased DFS flux, diffusional lag-times were not reduced. It is likely that increased lag times were due to gradual increases in membrane permeability produced by the redistribution of the enhancers within the stratum corneum and consequently a conditioning of the membrane in the early stages of the diffusion process. Similar delayed onsets of action have been observed with other lipophilic enhancers such as long-chain fatty acids (Komata et al., 1992; Santoyo et al., 1995) and sesquiterpene compounds (Cornwell and Barry, 1994).

It has been shown that both mono- and sesquiterpene enhancers increase the percutaneous absorption of drugs primarily by increasing drug diffusivity within the stratum corneum (Williams and Barry, 1991a; Cornwell and Barry, 1991a). Differential scanning calorimetry (DSC) experiments indicate that this increase is accompanied by disruption of the intercellular lipid barrier, although no correlation has been observed between DSC results and the enhancing abilities of the terpenes (Williams and Barry, 1990; Cornwell and Barry, 1991b).

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