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International Journal of Pharmaceutics 117 (1995) 219–224

international journal of pharmaceutics

Penetration enhancer effects on the in vitro percutaneous absorption of piroxicam through rat skin

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Received 13 May 1994; accepted 26 September 1994

Abstract

The influence of various penetration enhancers such as urea, dimethyl sulfoxide (DMSO), isopropyl myristate, oleic acid, oleyl alcohol, oleic hexyl ester and linoleic acid on the percutaneous absorption of piroxicam from carbopol gels containing 40% propylene glycol was investigated. Skin permeation experiments were carried out using excised abdominal rat skin. Oleic acid was found to be the most efficient enhancer for piroxicam, followed by linoleic acid. With 5% oleic acid, piroxicam flux values increased 8–9-fold compared with control gel.

Keywords: Piroxicam; Penetration enhancer; Percutaneous absorption; Abdominal rat skin; Gel formulation; Oleic acid; Linoleic acid

1. Introduction

Piroxicam is one of the most potent non-steroidal, anti-inflammatory agents which also exhibits antipyretic activity. Piroxicam is well absorbed following oral administration, however, its use has been associated with a number of gastrointestinal disorders (Schiantarelli and Cadel, 1981). These side effects may be overcome by topical administration of the drug (Schiantarelli et al., 1982).

Some studies in vitro have been carried out in order to predict percutaneous absorption of piroxicam (Tsai et al., 1985; Wang et al., 1987; Sanghavi et al., 1989; Babar et al., 1990). Various skin permeation enhancers such as urea (Tsai et al., 1985), dimethyl sulfoxide (DMSO) (Babar et al., 1990) and fatty acids (Hsu et al., 1994) have been screened for their effects on piroxicam skin permeability.

The objective of the present paper was to examine the influence of some penetration enhancers on the in vitro permeation of the drug through abdominal rat skin.

Carbopol gels containing 40% propylene glycol as cosolvent were used in order to obtain a vehicle in which the drug was completely dissolved.

The release characteristics of these gels were evaluated in vitro using Franz diffusion cells.

2. Materials and methods

2.1. Materials

Piroxicam was gently provided by Industrial Kern Española S.A. (Barcelona, Spain). Carbopol

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SSDI 0378-5173(94)00344-0

Table 1
Composition (%w/w) of piroxicam gels

Constituents	Gel code									
	A	B	C	D	E	F	G	H	I	J
Piroxicam	1	1	1	1	1	1	1	1	1	1
Carbopol 940	1	1	1	1	1	1	1	1	1	1
Propylene glycol	40	40	40	40	40	40	40	40	40	40
Distilled water	58	53	54	55	53	48	53	53	53	53
Urea	–	5	–	–	–	–	–	–	–	–
DMSO	–	–	4	–	–	–	–	–	–	–
Oleic acid	–	–	–	3	5	10	–	–	–	–
Oleyl alcohol	–	–	–	–	–	–	5	–	–	–
Oleic hexyl ester	–	–	–	–	–	–	–	5	–	–
Isopropyl myristate	–	–	–	–	–	–	–	–	5	–
Linoleic acid	–	–	–	–	–	–	–	–	–	5
Butylhydroxytoluene	–	–	–	0.06	0.06	0.06	0.06	0.06	–	0.06

940 (carboxypolymethylene), triethanolamine 85%, propylene glycol USP, oleic acid, oleyl alcohol, isopropyl myristate and butylhydroxytoluene were supplied by Roig Pharma S.A. (Barcelona, Spain). Urea was purchased from Merck (Darmstadt, Germany). Linoleic acid and DMSO were obtained from Sigma Aldrich Química S.A. (Steinheim, Germany). Other reagents used were of analytical grade.

2.2. Preparation of piroxicam gels

The composition of the piroxicam gels used in this study is shown in Table 1.

Gels were prepared by dispersing 1% w/w Carbopol 940 in a mixture of water and propylene glycol with 1% w/w piroxicam, being kept under magnetic stirring for 12 h. The enhancers were added to the mixture before suspending the Carbopol. The dispersion was then neutralized (pH 7.4) and made viscous by the addition of triethanolamine.

2.3. Skin membrane preparation

The abdominal hair of Wistar male rats, weighing 200–250 g, was shaved using electric and hand razors. After anesthetizing the rats with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was in contact with a saline solution for 1 h before starting the diffusion experiment.

2.4. Permeation studies

A system employing three improved Franz diffusion cells with a diffusional area of 1.76 cm^2 (FDC-400, Grown Glass Co., Somerville, NY) was used for permeation studies. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis side facing the receptor.

1 g of the gel was placed on the skin surface in the donor compartment which was sealed from the atmosphere with plastic film (Parafilm). The receptor compartment of the cell was filled with 11 mL of phosphate buffer (pH 7.4). During the experiments, the solution in the receptor side was maintained at 37 ± 1°C and stirred at 600 rpm with teflon-coated magnetic stirring bars. After application of the test formulation on the donor side, 400-μl aliquots were collected from the receptor side at designated time intervals, and 400 μl of the phosphate buffer was added into the receptor side immediately after each sample collection.

2.5. Analytical method

The amount of piroxicam in the receiver phase was assayed spectrophotometrically (Diode Array Hewlett Packard 8452 A spectrophotometer) at 353 nm. The linearity interval established was 0.5–20 μg/ml ($r > 0.999$).

2.6. Data treatment

According to Fick's second law of diffusion, the total amount of drug (Q_t) appearing in the receptor solution in time t is expressed as:

$$Q_t = AKLC_o\left[\frac{Dt}{L^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \times \exp\left(\frac{Dn^2\pi^2 t}{L^2}\right)\right] \quad (1)$$

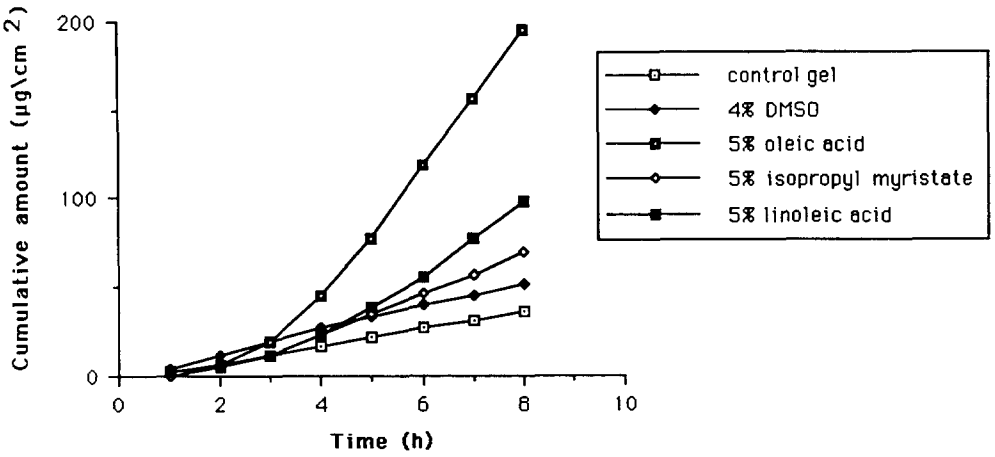

Fig. 1. Effect of different permeation enhancers on piroxicam percutaneous absorption. Each point represents the mean of three experiments.

where A is the effective diffusion area, C_0 represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of drug between membrane and vehicle. At steady state, Eq. 1 is expressed as follows:

$$\frac{Q_t}{A} = KLC_0\left(\frac{Dt}{L^2} - \frac{1}{6}\right) \tag{2}$$

The flux, J, was determined from the slope of the steady-state portion of the amount of the drug permeated divided by A vs time. The lag time values were determined from the x-intercept of the slope at steady state.

From Eq. 2 the flux is expressed as:

$$J = \frac{C_0 KD}{L} = C_0 K_p \tag{3}$$

where K_p is the permeability coefficient.

3. Results and discussion

The permeation profiles of piroxicam from the different formulations across abdominal rat skin are shown in the Fig. 1. The flux, J, permeability

Table 2
Piroxicam skin permeation parameters for various penetration enhancers [a]

Enhancers	Gel code	Steady-state flux (μg cm^{-2} h^{-1})	K_p ($\times 10^3$) (cm h^{-1})	Lag time (h)
Control gel	A	4.59 ± 0.36	0.45 ± 0.03	0.50 ± 0.23
4% DMSO	C	6.91 ± 0.18	0.69 ± 0.02	0.33 ± 0.13
3% oleic acid	D	20.28 ± 2.57	2.10 ± 0.26	2.68 ± 0.40
5% oleic acid	E	33.06 ± 0.81	3.33 ± 0.08	2.65 ± 0.36
10% oleic acid	F	24.76 ± 1.05	2.49 ± 0.10	1.60 ± 0.57
5% oleyl alcohol	G	9.66 ± 1.51	0.96 ± 0.15	1.05 ± 0.53
5% oleic hexyl ester	H	7.52 ± 1.74	0.76 ± 0.17	1.03 ± 0.52
5% isopropyl myristate	I	11.60 ± 1.25	1.16 ± 0.12	2.02 ± 0.35
5% linoleic acid	J	19.63 ± 2.44	1.96 ± 0.24	3.00 ± 0.48

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

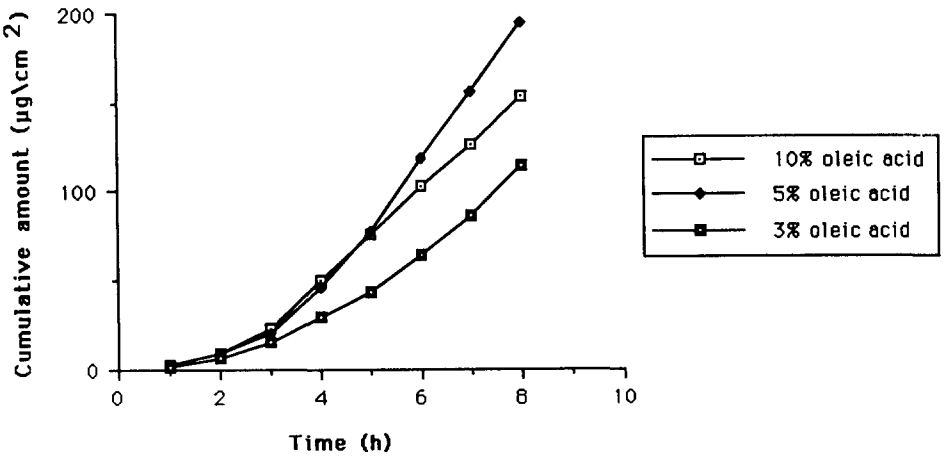

Fig. 2. Effect of oleic acid concentration on piroxicam percutaneous absorption. Each point represents the mean of three experiments.

coefficient, K_p and lag time for each of the different vehicles according to Eq. 2 and 3 are listed in Table 2. Piroxicam mean flux values at steady state from control gel were found to be 4.59 ± 0.36 $\mu g\ cm^{-2}\ h^{-1}$. Isopropyl myristate, oleic acid and linoleic acid led to a large increase in piroxicam flux compared with control gel, while DMSO, oleyl alcohol and oleic hexyl ester slightly increased this flux. However, urea decreased the flux to such an extent that it was not possible to measure it.

These results are inconsistent with the study of Babar et al. (1990) who reported a great increase in piroxicam penetration through hairless mouse skin using a hydroxypropylmethylcellulose gel containing 5% DMSO and also with the data of Tsai et al. (1985) who proposed urea as an optimal enhancer for piroxicam absorption from an ointment containing 12% propylene glycol.

Oleic acid was the most efficient penetration enhancer, increasing the flux of piroxicam 7–8-fold compared with control gel; linoleic acid also

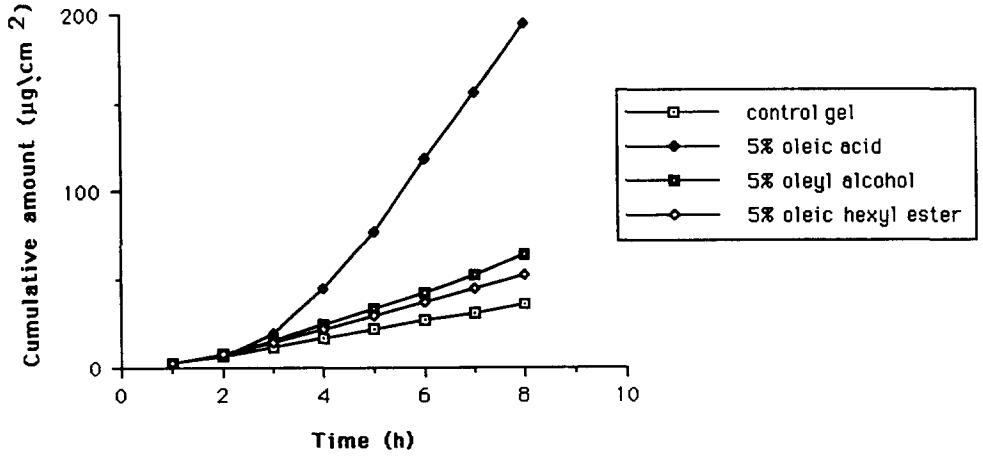

Fig. 3. Effect of oleic acid analogs on piroxicam percutaneous absorption. Each point represents the mean of three experiments.

showed a great enhancing effect. From the results, it was concluded that unsaturated fatty acids can be used to improve the percutaneous absorption of piroxicam from carbopol gels containing 40% propylene glycol.

Although the mechanism by which fatty acids enhance the permeation of drugs through the skin is not clearly understood, oleic acid can interact with stratum corneum lipids and disrupt their structures, increasing their fluidity and consequently, increasing the flux (Barry, 1991).

As shown in Table 2, the lag times for oleic and linoleic acids were longer than for the other enhancers. This effect indicates that fatty acids need time to penetrate through skin and interact with skin lipids. These results are confirmed by the study of Komata et al. (1992), who reported that fatty acids increase the lag time in the percutaneous absorption of thiamine disulfide.

The enhancing effect of oleic acid was measured as a function of the acid concentration (Fig. 2). The flux increased with increasing oleic acid concentration and reached a maximum at 5%, however, the value at 10% was less than at 5%. This decrease with the value at 10% is related to an increase in the lipophilicity of the gel. In contrast, Yamada et al. (1987) explained this decrease as being due to the reduction of the drug dissolved in the vehicle, however, this effect is not observed in piroxicam gels, where the total amount of the drug is always dissolved.

The enhancing effects of oleyl alcohol and oleic hexyl ester, shown in Fig. 3, were evaluated in order to determine the effects of the different polar groups on the permeation of piroxicam. As judged from the results, the percutaneous penetration rate (J) of piroxicam was significantly greater in the presence of oleic acid than in the presence of the alcohol and the ester, although the hexyl ester increased the number of carbon atoms compared with the alcohol and the acid. Fatty acids appear to be more effective in percutaneous absorption of some drugs than fatty alcohols and aliphatic esters (Yamada et al., 1987; Ogiso and Shintani, 1990; Komata et al., 1992). These compounds, fatty alcohols and aliphatic esters, increase lipid fluidization in the stratum corneum, but to a lesser extent than their acid analogs (Kitagawa et al., 1985; Sato et al., 1988; Friend et al., 1989).

The considerable enhancing effect of oleic acid on piroxicam absorption from gels containing 40% propylene glycol can also be explained by the combination of this acid with propylene glycol. The mixture of fatty acids with propylene glycol resulted in an optimum effect of fatty acids as permeation enhancers; this mixture may fluidize the stratum corneum lipids and reduce the resistance of the stratum corneum to permeation compounds (Mahjour et al., 1988).

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