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Research paper

Effect of skin pretreatment with fatty acids on percutaneous absorption and skin retention of piroxicam after its topical application

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Abstract

The enhancing effect of several fatty acids from different subclasses: saturated (lauric acid), mono-unsaturated (oleic acid) and poly-unsaturated (linoleic and linolenic acids) in the percutaneous absorption of piroxicam was investigated. These fatty acids were applied on the skin membrane in three different ways: included in the vehicle, as a pretreatment or both. An increase in piroxicam flux value was found for lauric and oleic acids in the following order: skin pretreatment with 5% fatty acids followed by application of gels containing 5% fatty acids > skin pretreatment with 5% fatty acids followed application of control gel > gel containing 5% fatty acids without skin pretreatment. For linoleic and linolenic acids, the piroxicam flux in the two pretreatment experiments was almost the same, although higher than when fatty acids were included in the formulation. Skin pretreatment with 5% linolenic acid in propylene glycol followed by application of control gel or a gel containing 5% linolenic acid, showed the highest enhancing capacity. After skin pretreatment with fatty acids, the lag time values decreased nearly three times compared to those obtained when the same fatty acids were included in the formulation. The amount of piroxicam retained in the skin after pretreatment with fatty acids was found to be very similar for all fatty acids and 3-fold higher than in the experiments without skin pretreatment.

Keywords: Piroxicam; Percutaneous absorption; Penetration enhancers; Fatty acids; Skin pretreatment

1. Introduction

Over the past few years, the field of transdermal drug delivery has experienced a rapid growth. One of the driving forces behind this growth is the increasing number of drugs that can be delivered to the systemic circulation, in clinically effective concentrations, via the skin portal. This is in spite of the inherent protective function of the stratum corneum, which is primarily one of excluding foreign substances from entering the body. Considerable research has been focused on discovering methods for increasing the permeability of human stratum corneum. One approach is to employ penetration enhancers, which can increase the permeability of the stratum corneum by increasing drug diffusivity within the membrane and/or by increasing drug partitioning from the applied formulation into the skin [1]. The effect of these compounds on the cutaneous absorption profile of drugs has been quantified by permeation parameters, such as the drug flux across the skin and the drug skin retention [2].

Fatty acids are currently receiving much attention as penetration enhancers [3,4]. This class of enhancers presents the advantage of being an endogenous component of human skin. Fatty acids can differ in several features: chain length, characteristics of the double bonds (position, number, configuration), branching schema and substituents, and these structural variations can influence their effects as skin penetration enhancers [5–8]. Fatty acids are capable of inserting between the hydrophobic tails of stratum corneum lipid bilayer, disturbing their packing, increasing their fluidity and subsequently, decreasing the diffusional resistance to permeants [9]. It has also been proposed that oleic acid exists as heterogeneously dispersed fluid domains within the intercellular multilayers and induces phase separation, thus reducing path length or resistance by the intercellular lipids [10,11].

The use of non-steroidal anti-inflammatory drugs is well recognized for regional inflammatory disorders such as muscle pain, low-back pain or osteoarthritis. 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

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use has been associated with a number of gastrointestinal disorders [12]. These side effects may be overcome by the topical administration of the drug [13], although piroxicam is not easily absorbed after transdermal application. Some studies have been carried out to predict the percutaneous absorption of piroxicam using different substances as effective permeation enhancers [14–20].

The present study evaluates the enhancing effect of several fatty acids, delivered in different ways, on the in vitro percutaneous absorption of piroxicam. Fatty acids in propylene glycol were applied as a pretreatment, added in the vehicle or both. The local tissue concentrations of piroxicam after pretreatment with fatty acids and application of Carbopol gels containing fatty acids were also measured.

2. Materials

Piroxicam was generously provided by Industrial Kern Española SA (Barcelona, Spain). Carbopol 940 (carboxypolymethylene), triethanolamine 85%, propylene glycol USP, lauric acid and oleic acid were supplied by Roig Pharma SA (Barcelona, Spain). Linoleic and linolenic acids were obtained from Sigma Aldrich Quimica SA (Steinheim, Germany). Other reagents used were of analytical grade.

3. Methods

3.1. Preparation of piroxicam gels

Gels were prepared by dispersing 1% w/w Carbopol 940 in a mixture of water and propylene glycol (40% w/w) with 1% w/w piroxicam, being kept under magnetic stirring for 12 h (control gel). 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.

3.2. In vitro permeation studies

All animal procedures were conducted in accordance with approved institutional protocols. The abdominal hair of Wistar male rats, weighing 200–250 g, was shaved using an electric razor. After sacrificing animals by CO_2 asphyxiation, the abdominal skin was surgically removed and adhering subcutaneous fat was carefully cleaned. Skins were allowed to hydrate for 1 h before being mounted on the Franz-type diffusion cells with an available diffusion area of 1.76 cm^2 (FDC-400, Crown Glass Co., Somerville, NY), with the stratum corneum facing the donor compartment. In this study, 11 ml of phosphate buffer solution (pH 7.4) was used as the receptor medium and 1 g of the gel was placed on the skin surface in the donor compartment. The receptor medium was maintained at 37 ± 1°C and magnetically stirred at 600 rev./min.

After application of the test formulation on the donor side, 400 μl aliquots were collected from the receptor side at designated time intervals, for a 9 h period, and replaced by the same volume of fresh buffer to maintain a constant volume. 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 ($r > 0.999$).

3.3. Pretreatment of skin for permeation studies

The solutions for the pretreatment were phosphate buffer, propylene glycol and different concentrations of fatty acids in propylene glycol. The following fatty acids were used in the skin permeation study: (i) saturated: lauric acid (12C); (ii) mono-unsaturated: oleic acid (C18:1); (iii) poly-unsaturated: linoleic acid (C18:2) and linolenic acid (C18:3).

The skin was pretreated by placing 1 ml of the pretreatment solution in the donor compartment of the diffusion cell, occluded with a sheet of film. The receptor compartment was filled with the buffer solution and thermostated at 37°C. The enhancer solution was removed after 12 h of incubation, the remaining enhancer on the surface of skin was eliminated and the percutaneous penetration experiment was carried out.

3.4. Determination of piroxicam retained in the skin

At the end of the permeation experiment, the skin was removed from the diffusion cell and washed using distilled water. The treated skin area was weighed, placed in 3 ml of phosphate buffer and homogenized using a tissue homogenizer (EuroTurrax, Ika Labortechink, Germany) for 2 min. 700 mg of potassium carbonate, 1 ml of tetrahydrofuran and 0.5 ml of ethanol were added to 1 ml of the resulting homogenized solution. The tubes were vortex-mixed for 30 s and then centrifuged for 15 min at $2550 \times g$. One thousand two hundred milliliters of the upper phase were placed in a second test tube and evaporated to dryness at 60°C under vacuum. The residue was then reconstituted in 1 ml of tetrahydrofuran, vortex-mixed for 30 s and filtered with a 0.5 μm filter (Millipore). The amount of piroxicam in the sample was assayed spectrophotometrically at 368 nm. For the calibration procedure, blank samples of skin homogenate were spiked with a known amount of piroxicam and extracted as previously described.

3.5. Data analysis

Cumulative amounts of drug (μg) penetrating the unit diffusion surface (cm^2) were plotted against time (h). The in vitro skin permeation rate or flux (J) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area as a function of time, in the steady state region. The lag time, $t_{L,}$ was determined from the x-intercept of the slope at the steady state. The permeability coefficient, k_p, was estimated from the flux and donor drug concentration. Penetration enhancing activities are expressed as

enhancement ratios (ER), i.e. the ratio of the flux value with enhancer to that obtained without enhancer.

Statistical comparisons were made using Student's *t*-test. The chosen level of significance was $P < 0.05$.

4. Results and discussion

The skin permeation enhancement of fatty acids is greatly dependent upon their structure, the characteristics of the permeant and the method used to deliver both the permeant and the fatty acid [21]. In this work, the enhancement effects of fatty acids on the penetration of piroxicam through rat skin were studied. Permeation enhancers were applied on the skin membrane in different ways, in order to obtain the higher and quicker piroxicam penetration.

4.1. Fatty acids incorporated in the gel formulation

The permeation data of piroxicam from different formulations containing fatty acids, over a 9 h time period, across abdominal rat skin are listed in Table 1. The flux data for oleic and linoleic acids were shown in a previous work [18] and they are included here for the sake of comparison. Piroxicam mean flux values at steady state from control gel (without enhancer) were found to be 4.59 ± 0.36 μg cm^{-2} h^{-1}. Oleic, linoleic and linolenic acids led to a large increase in piroxicam flux compared with control gel, while lauric acid slightly increased this flux. Since oleic acid has demonstrated to be the most efficient penetration enhancer, the effect of this acid concentration in the formulation was evaluated. The flux value reached a maximum at 5%.

These results indicate that when fatty acids are included in the formulation, all the C18 unsaturated fatty acids may be used to improve the percutaneous absorption of piroxicam. However, our data show that even if lauric acid (a saturated fatty acid, C12) has been previously described as an effective skin permeation enhancer for several substances [22,23], in the proposed experiments only a slight increase in piroxicam flux was observed. It was indicated that the possible mechanism of drug permeation enhancement by lauric acid might be due to a complex formation between drug and fatty acid, and a subsequent dissociation of this complex into each component in the interface between the stratum corneum and viable epidermis. The poor enhancing factor observed in our work for lauric acid may be explained because of the non-existence of an interaction between the fatty acid molecules and piroxicam. At pH 7.4, piroxicam and fatty acids are negatively charged and they probably do not form any complex.

Among unsaturated fatty acids, the highest enhancement factor was achieved by oleic acid which contains one double bond. The presence of additional double bonds as in linoleic and linolenic acids slightly decreased the enhancement ratio. It is clear from these results that the number of double bonds did not affect the fatty acid enhancing capacity when it was incorporated in the formulation. Besides, Lee et al. [24] have previously reported similar effects for tegafur when oleic and linoleic acids were applied from different vehicles.

The amount of piroxicam retained in the skin at the end of the experiments for oleic, linoleic and linolenic acids was nearly four times higher than for the control gel, indicating that tissue concentration could be related to the flux across skin from the vehicle applied.

The lag times for fatty acids were much longer than for the control gel, as they need some time to diffuse from the vehicle to the skin and also to penetrate through skin and interact with skin lipids. In order to avoid that, skin pretreatment studies with fatty acids in propylene glycol were carried out.

4.2. Skin pretreatment with fatty acids

The piroxicam permeation data from control gel after skin pretreatment with fatty acids are summarized in Table 2. To measure the enhancing effect due to the skin hydration, skin was pretreated for 12 h with a phosphate buffer solution (pH 7.4) before applying control gel. Also, as fatty acids were dissolved in propylene glycol, skin was pretreated with this substance in order to elucidate if propylene glycol itself acted as a percutaneous enhancer for piroxicam absorption. Piroxicam permeation through rat skin was not significantly enhanced ($P > 0.05$), neither by buffer phosphate nor by propylene glycol pretreatment. The percutaneous penetration flux for this drug was increased with

Table 1
The penetration data of piroxicam delivered from gels containing fatty acids through rat skin[a]

Enhancers	Steady state flux (μg cm^{-2} h^{-1})	Lag time (h)	Enhancement ratio	Skin retention at 9 h (μg mg^{-1})
Control gel	4.59 ± 0.36	0.50 ± 0.23	1.00	0.10 ± 0.02
5% lauric acid	6.79 ± 0.84	1.82 ± 0.39	1.48	0.16 ± 0.01
3% oleic acid	20.28 ± 2.57	2.68 ± 0.40	4.42	0.30 ± 0.03
5% oleic acid	33.06 ± 0.81	2.65 ± 0.36	7.20	0.33 ± 0.04
10% oleic acid	24.76 ± 1.05	1.60 ± 0.57	5.39	0.39 ± 0.03
5% linoleic acid	27.60 ± 1.26	2.18 ± 0.41	6.01	0.38 ± 0.12
5% linolenic acid	26.63 ± 1.02	2.13 ± 0.31	5.80	0.49 ± 0.03

[a] Values are the mean ± SD of three determinations.

Table 2
Effect of the different skin pretreatment on the penetration data of piroxicam delivered from various gels[a]

Pretreatment	Gel	Steady state flux ($\mu g\ cm^{-2}\ h^{-1}$)	Lag time (h)	Enhancement ratio	Skin retention at 9 h ($\mu g\ mg^{-1}$)
Buffer phosphate	Control	4.76 ± 0.67	0.96 ± 0.18	1.00	0.13 ± 0.04
Propylene glycol (PG)	Control	4.35 ± 0.19	1.21 ± 0.05	0.91	0.17 ± 0.05
5% lauric acid/PG	Control	42.20 ± 5.54	0.80 ± 0.10	8.86	0.87 ± 0.06
10% lauric acid/PG	Control	51.82 ± 2.52	0.53 ± 0.12	10.89	0.96 ± 0.04
3% oleic acid/PG	Control	51.91 ± 2.21	0.58 ± 0.07	10.91	0.83 ± 0.03
5% oleic acid/PG	Control	54.03 ± 3.85	0.58 ± 0.08	11.35	1.06 ± 0.08
10% oleic acid/PG	Control	56.26 ± 1.87	0.48 ± 0.22	11.82	1.02 ± 0.10
20% oleic acid/PG	Control	63.42 ± 3.71	0.79 ± 0.18	13.32	1.13 ± 0.15
5% linoleic acid/PG	Control	76.26 ± 2.42	0.67 ± 0.21	16.02	1.07 ± 0.23
5% linolenic acid/PG	Control	91.20 ± 3.56	0.71 ± 0.10	19.16	1.05 ± 0.20
5% lauric acid/PG	5% lauric acid	71.83 ± 3.56	0.51 ± 0.21	15.09	0.77 ± 0.04
5% oleic acid/PG	5% oleic acid	77.66 ± 4.88	0.51 ± 0.17	16.35	0.83 ± 0.07
5% linoleic acid/PG	5% linoleic acid	76.71 ± 5.54	0.65 ± 0.20	16.12	0.91 ± 0.03
5% linolenic acid/PG	5% linolenic acid	86.96 ± 1.26	0.56 ± 0.20	18.27	1.04 ± 0.08

[a] Values are the mean ± SD of three determinations.

skin pretreatment by 5% fatty acids in the following order: linolenic acid > linoleic acid > oleic acid > lauric acid. The pretreatment solution containing 5% linolenic acid in propylene glycol shown an enhancement ratio of 19.16 compared with the phosphate buffer solution.

The enhancing effect of fatty acid concentration in the pretreatment solution on the percutaneous absorption of piroxicam was evaluated. We have observed that by increasing the lauric acid concentration from 5 to 10%, the flux of piroxicam was slightly increased. For oleic acid (3%, 5%, 10%, 20%), the results obtained were similar to that for the lauric acid.

It is inferred from the experiments that skin pretreatment with propylene glycol did not increase piroxicam flux, although skin pretreatment with fatty acids in propylene glycol resulted in a great increase of piroxicam permeation. In this way, Takeuchi et al. [25] have already reported that when propylene glycol was applied to the skin surface, this substance showed almost no penetration into the skin barrier and was not distributed in the dermis. These authors also showed by Fourier transform infrared/attenuated total refection (FT-IR/ATR) that skin treatment with both lauric and oleic acids was effective in increasing propylene glycol dermal level by their structural alteration effect, but the action of lauric acid was significantly weaker. It has been proposed that oleic acid disrupts the packed structure of intercellular lipids because of the incorporation of its kinked structure (the kink is due to the cis double bond). This helps to explain why oleic acid is a much more effective penetration enhancer than saturated fatty acids [26].

According to our results, it has been reported that an increase in the degree of unsaturation in fatty acids causes greater perturbation of the stratum corneum and so enhances drug delivery [27]. Bhatia and Singh [7] and Tanojo et al. [3] also reported that skin pretreatment with poly-unsaturated fatty acids (linoleic and linolenic acids) shows a higher enhancement factor than oleic acid (mono-unsaturated). However, in contrast to our data, they proposed a similar enhancement capacity for linoleic and linolenic acids.

These data differ from those obtained when fatty acids were included in the vehicle, where all the unsaturated fatty acids presented almost the same enhancing factor. These results can be explained because, in the pretreatment experiments, unsaturated fatty acids were delivered directly on the skin surface, however, when these compounds are included in the gel they must diffuse out of the vehicle before reaching the skin surface. Since in the pretreatment experiments, linoleic and linolenic acids are able to disrupt the skin lipid structure more effectively than oleic acid, it can be thought that poly-unsaturated fatty acids may have difficulties in diffusing from the gel to the skin.

When the skin was pretreated with 5% fatty acids in propylene glycol followed by the application of control gel, the amount of piroxicam retained in rat skin was found to be very similar in all cases and much higher than after pretreatment with propylene glycol alone or phosphate buffer. These data confirmed that piroxicam retention was related to flux values across skin.

After skin pretreatment with fatty acids, the lag time values decreased nearly three times compared with the same values obtained when the same fatty acids are included in the formulation.

Our studies showed that pretreatment of skin with fatty acids increases the flux values and drug retention in the skin and decreases the lag time, compared with the values obtained when the same fatty acids are included in the formulation.

Therefore, we decided to carry out experiments consisting of skin pretreatment with 5% fatty acids for 12 h followed by the application of gels (9 h) containing the same fatty acid. Piroxicam permeation parameters for

these experiments are summarized in Table 2. The flux values observed in all cases were very similar. Comparing these results with those obtained when the control gel was applied after the pretreatment, an increase in piroxicam flux was observed for lauric and oleic acids, while for linoleic and linolenic acids there were no differences.

This increase in piroxicam flux observed for lauric and oleic acids was expected to be due to an addition effect between the skin pretreatment with the fatty acid and the action of fatty acid included in the formulation. However, gels containing 5% linoleic and linolenic acids did not increase piroxicam flux compared with control gel. These results are surprising because the same behaviour should be expected as for lauric and oleic acids. They might be explained because maximal piroxicam flux across rat skin was already achieved when employing linoleic and linolenic acids for skin pretreatment followed by application of control gel. Subsequently, the application of gels containing these fatty acids did not modify the flux value, as saturation was reached. Therefore the flux values obtained with lauric and oleic acids also reached the same saturation value.

The amount of piroxicam retained was almost the same for all fatty acids and very similar to that obtained with the control gel. A decrease in lag times was found for all fatty acids.

The differences in piroxicam permeation profiles for each fatty acid in the three different ways of application are depicted in Fig. 1. An increase in flux value was found for lauric and oleic acids in the following order: skin pretreatment with 5% fatty acids followed by application of gels containing 5% fatty acids > skin pretreatment with 5% fatty acids followed by application of control gel > gel containing 5% fatty acids without skin pretreatment. For linoleic and linolenic acids, the piroxicam flux in the two pretreatment experiments was almost the same, although it was observed to be higher than when the fatty acids are included in the formulation.

In the pretreatment experiments, the amount of piroxicam found in the skin was similar for all fatty acids employed in this work. Consequently, the differences in the flux values obtained with the different fatty acids can not be explained by the piroxicam retention in the skin. When increasing the flux values, the quantity of piroxicam retained in the skin was increased up to a saturation value. This result is in agreement with the previous ones of Singh and Roberts [28] who reported that the skin concentration after epider-

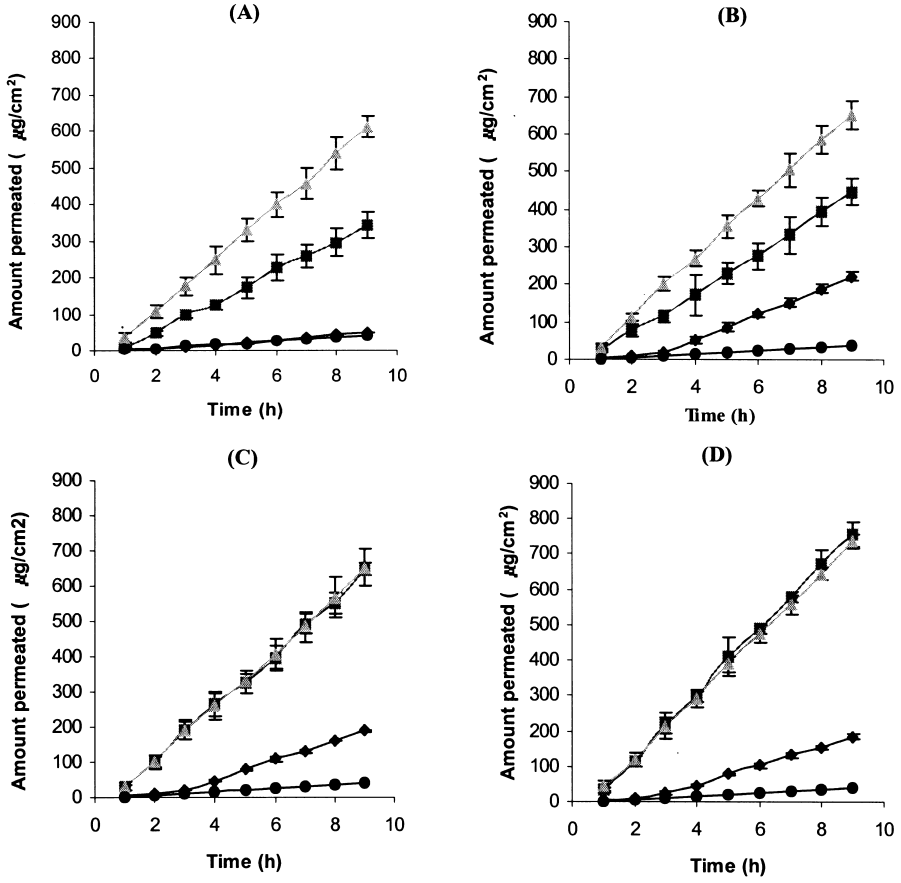

Fig. 1. Effect of fatty acids on piroxicam percutaneous absorption. (A) lauric acid; (B) oleic acid; (C) linoleic acid; (D) linolenic acid; ● control gel, ◆ gel containing 5% fatty acid without pretreatment; ■ pretreatment with 5% fatty acid/PG before application of gel control; ▲ pretreatment with 5% fatty acid/PG before application of gel containing 5% fatty acid. Each point represents the mean ± SD of three experiments.

mal application of several non-steroidal anti-inflammatory drugs could be related to their flux across epidermis.

5. Conclusions

The permeation of piroxicam through rat skin is remarkably enhanced by fatty acids. Among the tested fatty acids, the poly-unsaturated ones (linoleic and linolenic acids) were the most efficient penetration enhancers, although linolenic acid showed the greatest enhancement effect in piroxicam absorption.

Skin pretreatment with fatty acids followed by the subsequent application of control gel or gels containing fatty acids was found to increase piroxicam percutaneous absorption and piroxicam retention in the skin. These pretreatment experiments also decreased the lag time values for fatty acids, so a quicker piroxicam penetration can be obtained. Therefore, this protocol can open the way to obtain local delivery of piroxicam at the inflammation site and also a systemic absorption.

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