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RESEARCH PAPER

Transdermal Iontophoresis and Skin Retention of Piroxicam from Gels Containing Piroxicam : Hydroxypropyl-β-cyclodextrin Complexes

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ABSTRACT

Iontophoretic transport of piroxicam (Px) across porcine ear skin in vitro was investigated. Cathodal iontophoresis of negatively charged Px was carried out from gel formulations containing Px as an inclusion complex with hydroxypropyl-β-cyclodextrin (HP-β-CD). From the gels, following a 7 h application period at 0.4 mA/cm^2, iontophoresis delivered 3.4 times more drug than passive diffusion. The formation of Px : HP-β-CD complexes did not increase the iontophoretic Px flux through the skin. However, Px complexation with HP-β-CD allowed us to increase the drug concentration in the gel; because of that, the amount of Px transported across the skin increased considerably. After iontophoretic experiments, the amount of Px retained in skin seemed to be related to the flux values obtained in each case. Skin pretreatment with 20% HP-β-CD, tested passively and iontophoretically for 3 h, followed by the application of gel containing Px : HP-β-CD complexes, showed no enhancing capacity in any case. The amount of Px retained in the skin after pretreatment experiments was found to be very similar to that obtained without skin pretreatment and was observed to be related to the Px flux through the skin.

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Key Words: *Carbopol gels; Iontophoresis; Percutaneous absorption; Piroxicam : hydroxypropyl-β-cyclodextrin complexes; Skin pretreatment; Skin retention*

INTRODUCTION

Recently, there has been considerable emphasis on drug delivery through the transdermal route. Since the skin is almost impermeable to most chemicals, researchers have been looking for ways to enhance skin permeability. In the literature, there are several enhancement techniques that have been developed to overcome the impervious nature of the stratum corneum (SC), such as chemical enhancers (1) and physical methods (2,3).

Cyclodextrins (CDs) and their derivatives play an important role in formulation development due to their effect on solubility, dissolution rate, chemical stability, and drug absorption. Because CDs form inclusion complexes with drugs, it has been suggested that they might act as transdermal enhancers by interacting with components of the skin. Some authors have proposed that CDs are able to modify the skin barrier by extraction of skin components such as cholesterol and triglycerides (4). Meanwhile, others have reported that CDs act as true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface, where they partition into the skin barrier (5). In this way, hydroxypropyl-β-cyclodextrin (HP-β-CD) has been extensively applied in pharmaceutical formulations to increase transdermal absorption of several drugs (6–8).

Iontophoresis may be defined as a process of transferring (usually) ionized drugs across a membrane, such as skin, into a tissue by the use of an electrical potential difference across two electrodes. Iontophoresis differs from passive transdermal transport in that an external electric potential gradient is imposed across the skin in addition to the existing concentration gradient (9).

Iontophoresis is a noninvasive technique and is being investigated for localized and systemic therapy. Furthermore, Ledger (10) reported that, in the treatment of pain and inflammatory conditions, it has been extensively utilized with steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs), which cause considerable gastrointestinal side effects after their oral administration. This is the case for piroxicam (Px), a NSAID widely used in the treatment of rheumatoid arthritis and other inflammatory disorders. Iontophoretic delivery of Px across the skin in vitro has been investigated by several authors (11,12). Depending on pH, the drug can exist in cationic, neutral (i.e., zwitterionic), or anionic forms. At pH 7.4, Px carries a net charge of −1. As the drug is negatively charged, its iontophoretic transport through the skin will be from the cathode chamber (cathodal iontophoresis). Other authors (13–16) have combined different enhancers (e.g., cyclodextrins, oleic acid, terpenes, azone), which act directly on the skin barrier, with iontophoresis to obtain greater enhancement in skin penetration than if the enhancer is used alone.

In this study, cathodal iontophoresis of negatively charged Px, forming inclusion complexes and physical mixtures with HP-β-CD, was carried out using pH 7.4 gel formulations. The aim of this study was to compare the passive and iontophoretic Px transport from Px : HP-β-CD complexes and to explore the influence of Px : HP-β-CD complexation on the transdermal iontophoretic transport of Px.

To evaluate the existence of interactions between this CD and the skin, this work also included skin pretreatment with 20% HP-β-CD. In addition, the amount of Px retained in the skin after all iontophoretic experiments was quantitatively evaluated.

EXPERIMENTAL

Materials

Px was generously provided by Industrial Kern Española S.A. (Barcelona, Spain). HP-β-CD was purchased from Sigma-Aldrich. Carbopol ETD 2001 (carboxypolymethylene), triethanolamine 85%, and propylene glycol (PG) USP were supplied by Roig Pharma S.A. (Barcelona, Spain). All other chemicals and reagents used were analytical grade.

Preparation of Piroxicam Gels

The compositions of Px gels used in this study are shown in Table 1. The solid inclusion complexes

Table 1

Composition (% w/w) of Piroxicam Gels

Gel	Control	A	B	C	D[a]
Piroxicam	1	1	1	2	2
HP-β-CD	0	4.4	4.4	8.8	8.8
Carbopol 2001	1	1	1	1	1
Propyleneglycol	40	40	10	40	40
Water ad	100	100	100	100	100

[a]Gel D contained Piroxicam and HP-β-CD as a physical mixture.

of Px with HP-β-CD, 1 : 1 molar ratio, were prepared by the coprecipitation method as described in a previous work (17). The Px : HP-β-CD 1 : 1 complexes and the physical mixtures were incorporated into carbopol gels. The gels were prepared by dispersing 1% w/w of Carbopol 2001 in a water : propylene glycol mixture. The pH of the carbopol dispersions was adjusted to 7.4 with triethanolamine.

The gel without HP-β-CD and with 1% Px was considered the control gel. However, due to the low solubility of Px, it was not possible to prepare solution gels containing 2% Px without HP-β-CD.

Transdermal Iontophoretic Permeation Studies

Diffusion Cell

The in vitro transdermal iontophoretic delivery of Px was conducted using a vertical glass diffusion cell. The vertical cells have a hollow chamber suitable for both solution and gel formulations. The capacity of the receptor was 7.5 ml, and the surface area of the skin exposed to the preparations was 0.7 cm^2. Similar cells have been employed when passive diffusion experiments have been developed. Concerning the electrodes, silver-silver chloride (Ag/AgCl) electrodes were used for the study as they have the property of avoiding electrolysis of water. A silver wire was used as the anode, and a silver-silver chloride wire was used as the cathode.

Current and voltage control with automatic crossover (model APH 1000 M, Kepco, Inc.) was used. This supply has a specified drift of less than 2 μA/8 h for its current-controlled output, an important consideration if the drug is sensitive to changes in current.

Animal Model

Porcine ears were obtained from the local slaughterhouse, and after cleaning under cold running water, the outer region of the ear was cut. The whole skin was dermatomed to 800 μm (Aesculap GA 630) and immediately frozen at −4°C. After a period of time, the skin samples were clamped between the two chambers of iontophoretic vertical cells, with the stratum corneum facing the donor compartment, and the dermis facing the receptor compartment.

Stabilization of Skin

During stabilization, the receptor cell was filled with 133 mM NaCl buffered to 7.4 with *N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES); 1 ml of the same HEPES buffered saline was placed in the donor cell; and the contents were stirred with a magnetic stirring bar at 300 rpm at ambient laboratory temperature. This was done to remove the ions inherently in the skin (K^+, Na^+, Cl^-), which interfere in the transport of drug and the estimation procedure.

In Vitro Permeation Experiments

After stabilization of the skin, 1.5 g of Px formulations were placed into the cathode chamber; 1 ml of fresh HEPES buffered saline was placed in the anode chamber; and constant current was applied at 0.4 mA/cm^2 in the case of iontophoretic experiments, but no current was applied in passive diffusions. All experiments were carried out for 7 h. The receptor fluid was continuously perfused using a peristaltic pump, and 1.3 ml were collected every hour.

The amount of Px in the samples was measured by ultraviolet (UV) spectrophotometer (Diode Array 8452 A, Hewlett-Packard) at 354 nm. The absorbance values were read against a linear standard plot to obtain the corresponding Px concentrations.

Skin Pretreatment Experiments

The pretreatment solution was 20% HP-β-CD dissolved in HEPES buffered saline. After mounting the skin in the diffusion cells and equilibrating for 1 h, 1 ml of the pretreatment solution was deposited onto the stratum corneum surface exposed in the cathode chamber. Anodal iontophoresis and passive diffusion of the pretreatment solution were carried

out. After 3 h, the pretreatment solution was removed from the cathode chamber and washed with distilled water. After this, the Px formulation and the anode buffer solution were placed in the iontophoretic cell, as described previously, and the iontophoretic run was initiated.

Determination of Piroxicam Retained in the Skin

At the end of the iontophoretic absorption experiment, the skin was removed from the vertical cell and washed with 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. To 1 ml of the resulting homogenized solution were added 700 mg of potassium carbonate, 1 ml of tetrahydrofuran, and 0.5 ml of ethanol. The tubes were vortex mixed for 1 min and then centrifuged for 10 min at 2500 g. Then, 1200 ml 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 Px 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 Px and extracted as previously described.

Statistical Analysis

Comparisons were made between groups by one analysis of variance (ANOVA) and post hoc Tamhane test. Statistical significance level was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Iontophoretic Transport of Piroxicam from Different Gels

In recent years, iontophoresis has been found to be an effective means for enhancing percutaneous penetration of several drugs. Fig. 1 shows the Px flux from a gel containing 2% Px complexed with HP-β-CD (gel C) after passive and iontophoretic transdermal delivery of the drug. Cathodal delivery of Px at pH 7.4 enhanced the transport of the drug from gels containing Px : HP-β-CD complexes 3.4 times relative to the passive control value. The other formulations prepared (gels A, B, and D) showed similar behavior (data not shown). Iontophoretic experiments also demonstrated that the steady state was reached relatively quickly, and the flux values remained practically constant during the time of the experiments.

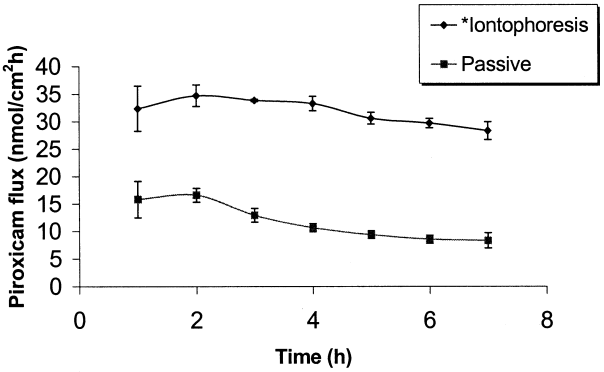

Figure 1. Piroxicam fluxes from gel C after passive and iontophoretic transdermal drug delivery. Each value represents the mean ± SD ($n = 4$). *$P < .05$, ANOVA and post hoc Tamhane test compared to the passive transport.

As to the causes of the enhancement in the absorption rate, when the current is activated, the negatively charged Px and other anions (predominantly Cl^-) in the cathodal chamber are driven by the electric field toward and into the skin. Gay et al. (11) reported similar results when Px was delivered from gel formulations, although in that case Px did not form complexes. However, this enhancement in Px flux due to iontophoretic transport was not too high compared with that for positively charged molecules. For anion iontophoresis, the electroosmotic flux was against the Px flux, suggesting that the absorption of this negatively charged species will be quite difficult. Likewise, Hirvonen and Guy (12) showed that the efficiency of iontophoresis depends on the structural and physicochemical properties of the drug.

The transdermal iontophoretic delivery of Px from gels containing Px : HP-β-CD complexes (gels A and B) and free Px (control gel) are compared in Fig. 2. These gels in all cases contained 1% Px and different percentages of PG. Due to the solubilizing effect of HP-β-CD, it was possible to reduce the quantity of the cosolvent (PG), resulting in gels with 10% PG (gel B), which showed higher

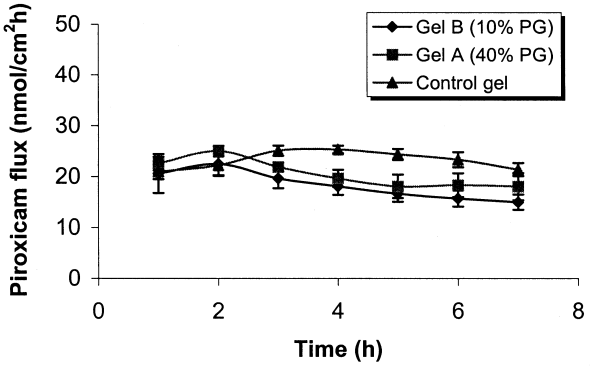

Figure 2. Iontophoretic flux of piroxicam delivered from gels containing 1% piroxicam and different percentages of propylene glycol (10–40%). Each data point shows the mean ± SD ($n = 4$). *$P < .05$, ANOVA and post hoc Tamhane test, compared to control gel.

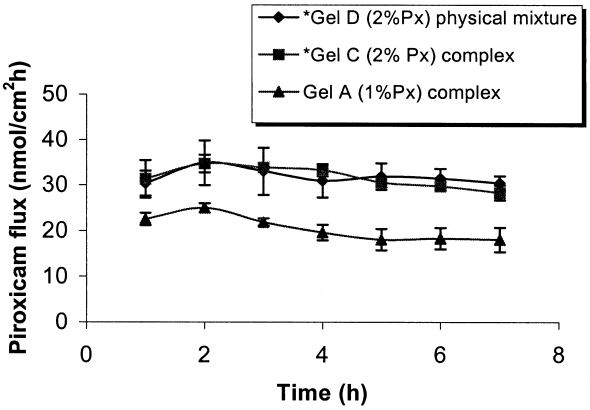

Figure 3. Iontophoretic flux of piroxicam delivered from gels containing 40% propylene glycol and different percentages of piroxicam. Each data point shows the mean ± SD ($n = 4$). *$P < .05$, ANOVA and post hoc Tamhane test, compared to gel A.

thermodynamic activity than gels containing 40% PG (gel A).

First, these results demonstrated that the flux of Px slightly decreased when the Px was complexed with HP-β-CD with either 10% or 40% PG. This phenomenon might be explained by the fact that, in the control gel, all the Px was free to diffuse through the skin, whereas in gels A and B, there was a quantity of drug complexed with the cyclodextrin that could not permeate through the skin. In line with these observations, Chang and Banga (13) reported that the iontophoretic delivery of hydrocortisone alone was found to be more efficient than in the presence of HP-β-CD, confirming that free hydrocortisone was delivered more readily by iontophoresis than its HP-β-CD complex. These results also suggest that iontophoresis did not enhance the penetration of the Px : HP-β-CD complex, which was possibly too bulky to permeate the skin.

The stoichiometry of the complex between Px and HP-β-CD has been determined to be 1 : 1, and the stability constants for Px complexes in the presence of 10% and 40% PG were shown to be 49.91 and 5.81 M^{-1}, respectively. From these data, it was possible to conclude that the iontophoretic flux of Px from gels containing 10% PG (gel B) was slightly lower than from gel A (40% PG) because in gel B (10% PG) there was more drug forming a complex and unable to be transported by the current through the skin.

The transdermal iontophoretic delivery of the drug from gels containing Px : HP-β-CD complexes with 1% Px (gel A), 2% Px (gel C), and Px : HP-β-CD physical mixtures with 2% Px (gel D) are compared in Fig. 3. The flux values obtained from gels containing 2% Px were larger than from those with 1% Px. The data revealed that, when doubling the drug concentration, the transport of Px was significantly increased, but not proportionally. These results can be explained because, in the formulation containing 2% Px : HP-β-CD, the percentage of the Px free (82.8%) was lower than in the case of the gel with 1% Px : HP-β-CD (90.58%) since the increase of Px displaced the equilibrium toward the complex formation.

Besides, according to the results shown in Fig. 3, there were no significant differences between the iontophoretic Px flux from Px : HP-β-CD complexes (gel C) and Px : HP-β-CD physical mixtures (gel D), in both cases with 2% w/w drug. Both complexes and physical mixtures increased the Px solubility in the same way; for this reason, it was possible to incorporate 2% w/w drug in the vehicle. Consequently, the iontophoretic flux of Px was not determined by inclusion of HP-β-CD in the vehicle, but rather by the Px concentration in the gel.

Piroxicam Retained in Skin

At the end of the iontophoretic experiments, the amount of Px retained in skin was quantitatively

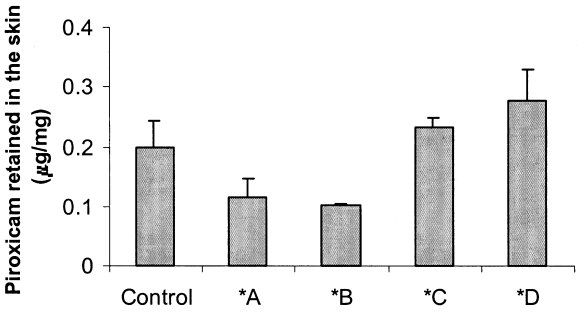

Figure 4. Amount of piroxicam retained in skin from various gel formulations following iontophoresis. Each value represents the mean ± SD ($n = 4$). *$P < .05$, ANOVA and post hoc Tamhane test, compared to control gel.

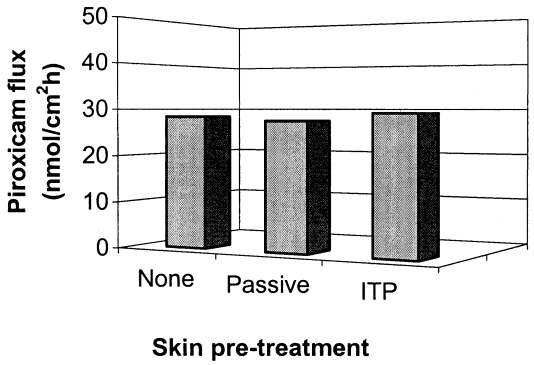

Figure 5. Transdermal iontophoretic fluxes of piroxicam at 7 h, after skin pretreatments (passive and iontophoretically) with 20% HP-β-CD. *$P < .05$, ANOVA and post hoc Tamhane test, compared to no skin pretreatment.

evaluated (Fig. 4). We could suggest that the quantity of Px retained in skin was in concordance with Px flux. These results agreed with those obtained by Santoyo and Ygartua (18) and by Singh and Roberts (19), who reported that the skin concentration after epidermal application of several nonsteroidal anti-inflammatory drugs could be related to their flux across the epidermis. In contrast, Tenjarla et al. (20) proposed that the reason for little skin permeation of the complex miconazole : HP-β-CD was that the amount of the drug retained in the skin increased via the formulation of inclusion complexes. Nevertheless, they suggested this skin retention could be related to the lipophilicity of the drug.

Skin Pretreatment with 20% Hydroxypropyl-β-cyclodextrin Solutions

Several authors have reported that cyclodextrins might affect the permeability of drugs through the skin via interaction with some components of the skin (21). Pretreatment studies have been developed to obtain a direct action of cyclodextrins on the skin surface because as when they are included in the vehicle, they need first to diffuse out of it. Furthermore, we also carried out iontophoretic pretreatments to force HP-β-CD penetration into the skin.

To study the effect of HP-β-CD as a penetration enhancer, pretreatment with 20% HP-β-CD in HEPES buffered saline was investigated. The pretreatment was tested passively and iontophoretically for 3 h; after this time, a gel containing Px : HP-β-CD (2% Px) (gel C) was placed in the cathode chamber, and the iontophoresis was carried out (Fig. 5). This figure shows that there were no statistical differences in Px iontophoretic fluxes in experiments without HP-β-CD pretreatment and those carried out with a conventional pretreatment.

From these results, we can postulate that HP-β-CD did not act as a penetration enhancer to disrupt any component of the skin. In the same way, Legendre et al. (22) reported that HP-β-CD did not affect either the differential scanning calorimetry (DSC) profile or the Fourier transform infrared spectrum of the stratum corneum of hairless rats. On the contrary, Vitória et al. (23) revealed that 20% HP-β-CD caused removal and possible disorganization of the lipid matrix that envelops the corneocytes of the stratum corneum of hairless mice. On the other hand, there were no statistical differences between Px flux after anodal iontophoretic pretreatment and conventional pretreatment.

From the above results, we could suggest that, as HP-β-CD has a high molecular weight and is a neutral molecule, its iontophoretic penetration was extremely difficult. This confirmed that, in our experimental conditions, HP-β-CD did not alter skin barrier properties.

In addition, the amount of Px retained in the skin after the pretreatment experiments was also evaluated. The quantity of Px found in the skin was similar in all cases, confirming that the amount of Px retained in the skin was in concordance with Px flux values.

CONCLUSIONS

In summary, transdermal iontophoresis of Px from carbopol gels increased the Px flux in comparison with passive diffusion. The results presented here indicate that the gels containing HP-β-CD were adequate vehicles for the topical iontophoretic delivery of Px. In addition, electrorepulsion could be the main force driving iontophoretic Px flux due to the negative charge of the drug.

In general, it was the free ion of the drug rather than complexes that was predominantly delivered iontophoretically through the skin. However, the presence of HP-β-CD allowed the incorporation of a higher Px "payload" into the gel, and this might result in increased drug delivery over a longer period of time.

After iontophoretic experiments, the amount of Px retained in the skin seemed to be in concordance with the flux values obtained in each case.

The conventional and iontophoretical skin pretreatment with 20% HP-β-CD did not increase the Px penetration through the skin. So, in our experimental conditions, HP-β-CD did not disrupt skin barrier properties.

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