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Post-iontophoresis transport of ibuprofen lysine across rabbit ear skin

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Abstract

The aim of this work was to study in vitro the post-iontophoresis transport of ibuprofen lysine across rabbit ear skin, from ibuprofen lysine water solutions (20–200 mg/ml and pH 6.8–7.8). Current densities of 0.125, 0.25, and 0.5 mA/cm^2 were applied either continuously or for 15, 30, and 60 min followed by passive diffusion for up to 5 h. The results showed a significantly higher cathodal transport compared to passive flux. Anodal iontophoresis also increased ibuprofen permeation, even though the drug is negatively charged. The application of an electric current for a limited period of time, followed by passive diffusion from the reservoir in contact with the skin, produced much higher post-iontophoresis fluxes of ibuprofen than passive diffusion. Post-iontophoresis transport of ibuprofen from lysine salt solutions linearly depended on the total amount of current applied during iontophoresis, and in the absence of background ions was independent of donor drug concentration. The reason for this behavior was the creation of a drug reservoir in the skin owing to the short period of current application.

Keywords: Iontophoresis; Post-iontophoresis flux; Rabbit skin; Ibuprofen lysine

1. Introduction

Ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) characterized by low water solubility (Chiarini et al., 1984; Degim et al., 1998; Iervolino et al., 2000; Hadgraft and Valenta, 2000). The lysine salt of ibuprofen is more soluble and shows higher bioavailability after oral administration (Martin et al., 1990).

NSAIDs are often applied topically for the treatment of arthritis, arthrosis, and soft tissue inflammation, minimizing the systemic side effects of oral administration. Nevertheless, ibuprofen shows poor skin permeability (Beetge et al., 2000); several attempts have been made to increase its penetration through the skin with the use of enhancers (Bialik et al., 1993). Terpenes or phospholipids (Watkinson et al., 1993), polyoxyethylene alkyl ethers, eutectic mixtures (Stott et al., 1998), and supersaturated solutions (Iervolino et al., 2000) have also been proposed.

Ibuprofen transport through the skin can be physically enhanced by using iontophoresis, i.e. the application of an electric current through the porous membranes to promote the transport of ionic compounds. The mechanisms involved in transport during transdermal iontophoresis are electrorepulsion and electroosmosis (Guy et al., 2000). In addition, it has been shown that current application increases the

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skin's "passive" permeability, which persists in the post-iontophoresis phase (Kim et al., 1993; Volpato et al., 1995). The application of an electric current can cause the drug to accumulate in the skin, leading to the formation of a "reservoir" or "depot" (Volpato et al., 1998) from which the drug can be released during the post-iontophoresis phase. Therefore, by allowing the donor solution to remain in contact with the skin after a short application of current, the amount of drug permeating through the skin can be augmented without any further current application. This procedure could be exploited in the therapeutical application of ibuprofen iontophoresis across the skin.

The aim of this work was thus to study the post-iontophoresis transport of ibuprofen from lysine salt solutions, in order to elucidate the electrical and physico-chemical parameters affecting the amount of drug transported across the skin. In particular, the effect of polarity, density, and duration of current and drug concentration on ibuprofen post-iontophoresis transport was examined. As a reference, iontophoretic skin transport of ibuprofen was determined. Rabbit ear skin was used as a barrier, since it has been recently described as a reasonable model for transdermal iontophoresis (Nicoli et al., 2001).

2. Materials and methods

2.1. Materials

Ibuprofen lysine (MW 352.46) was a gift from Lisapharma S.p.A. (Erba, Italy). The ibuprofen (MW 206.27) content of lysine salt was 57.6%, and water solubility at room temperature was approximately 500 mg/ml, with a pH of 8.4.

Phosphate buffer solution at pH 7.4: 5.98 g of disodium hydrogen phosphate dodecahydrate (Carlo Erba Analyticals, Milan, Italy), 0.19 g of potassium dihydrogen phosphate (Carlo Erba Analyticals), and 8.8 g of NaCl were dissolved in water to 1000 ml (ionic strength 0.154 M). The solution was filtered and de-aerated before use.

2.2. Methods

Permeation experiments were conducted in Franz-type diffusion cells (Disa, Milan, Italy), with a surface area available for permeation of 0.6 cm^2. The donor compartment contained 1 ml of the appropriate ibuprofen lysine water solution (concentrations 20–200 mg/ml).

The receptor compartment was filled up with 4.5 ml of phosphate buffer at pH 7.4 and was kept under continuous magnetic stirring to avoid any boundary layer effect.

Skin from the inner part of mixed breed rabbit ear, obtained from a local slaughterhouse, was mounted between the two compartments with the corneal site facing the donor compartment. All experiments were conducted at room temperature and lasted 5 h.

Passive diffusion and both anodal and cathodal iontophoresis experiments were performed. In the cases of the iontophoretic experiments, the current was applied by means of a constant current generator (Iono1, Cosmic, Pesaro, Italy), using silver/silver chloride electrodes, made from silver wires (diameter 1 mm, purity 99.9%) and silver chloride (Sigma Chemical, St. Louis, MO) (Green et al., 1991).

The electric current (typically 0.5 mA/cm^2) was applied following two different experimental settings. In the first, the current was continuously applied for 5 h, whereas in the second setting, after current application of 15, 30, or 60 min, the donor solution was left on the skin and the ibuprofen passive permeation was monitored up to 5 h.

At predetermined time intervals, 300 μl of receptor solution were sampled for analysis and replaced with the same volume of fresh buffer. The quantitative determination of ibuprofen permeating across the skin was performed by HPLC analytical assay modified from (Degim et al., 1998), using a Shimadsu instrument (isocratic pump LC-10AS, UV-Vis detector SPD-10A, integrator C-R6A Chromatopac, Shimadsu, Kyoto, Japan) and a Waters C18 Novapack® 150 mm × 3.9 mm column (Millipore Corporation, Milford, MA). The mobile phase consisted of acetonitrile (50% in volume) and 100 mM dipotassium phosphate brought to pH 5 with phosphoric acid, pumped at 1 ml/min. The UV detector was set at 225 nm. In these conditions, the retention time of ibuprofen was about 5.5 min.

The method was validated according to USP25. The response was linear in the concentration interval from 0.03 to 1.0 μg/ml. The relative standard deviation of six injections of the same solution was 1.56%. The

tailing factor was 1.17 ± 0.07 and the theoretical plates 327 ± 5. The limit of quantification was 0.03 μg/ml.

Each experiment was replicated at least four times. The statistical analysis was performed using ANOVA and two-sided *t*-test.

3. Results and discussion

3.1. Ibuprofen iontophoretic transport

Using a saline solution of ibuprofen lysine (concentration 133 mg/ml) as donor, permeation experiments across rabbit ear skin were performed under the application of 0.5 mA/cm^2 current for 5 h. This donor solution had a pH of 7.8. Since the pH value was compatible with the skin, buffering agents were not used. Fig. 1 shows ibuprofen permeation profiles during anodal and cathodal iontophoresis, in comparison with the passive diffusion. The curves obtained show that the iontophoresis significantly enhanced the ibuprofen skin flux. The cathodal iontophoretic transport, i.e. drug donor at negative electrode, was higher than the anodal. In fact, in 5 h, more than 1 mg/cm^2 of ibuprofen was transported from the cathode, compared to approximately 0.3 mg/cm^2 from the anode and 0.01 mg/cm^2 passively. The enhancement obtained with cathodal iontophoresis was justified by the negative charge carried by the ibuprofen molecule, which allowed for the electric transport from the negative electrode.

However, although the drug is negatively charged, even anodal iontophoresis was sufficient to increase the amount of ibuprofen transported to about 30 times that of passive diffusion. This effect was attributed to the presence of an electroosmotic contribution to drug transport (Guy et al., 2000), which at pH 7.8 was directed from anode to cathode. From anodal transport data of ibuprofen, the electroosmotic volume flow involved was calculated, taking into account the ibuprofen donor concentration (Marro et al., 2001). The value obtained was 1.03 ± 0.38 μl/cm^2/h, which was in agreement with the value calculated from mannitol transport studies (Nicoli et al., 2001) in the same experimental conditions (1.72 ± 0.17 μl/cm^2/h). This result supported the claim that the anodal transport of ibuprofen was driven by the electroosmotic flow in the same direction as drug passive migration.

3.2. Post-iontophoresis transport of ibuprofen

In these post-iontophoresis experiments, after a short period of current application ibuprofen transport was measured up to the end of the experiment,

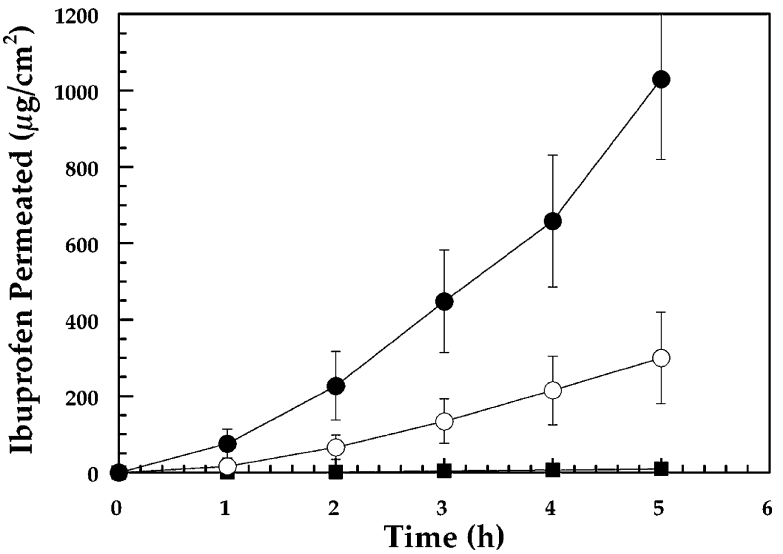

Fig. 1. Permeation profiles of ibuprofen from 133 mg/ml ibuprofen lysine solution in water. (■) Passive diffusion, (○) anodal iontophoresis, and (●) cathodal iontophoresis applied for 5 h. Mean values ± S.E.M., $n \geq 4$.

i.e. 5 h. The donor solutions were prepared by dissolving ibuprofen lysine in water without buffers or electrolytes. The pH of these solutions ranged from 6.8 to 7.8, depending on the drug concentration. The increase in pH could negatively affect the passive transport of ibuprofen (Hadgraft and Valenta, 2000). However, from pH 6.8 to 7.8, the percentages of drug ionized were 99.56 and 99.96%, respectively, making any effect negligible.

In transdermal iontophoresis, the use of donor solutions without competing ions has been shown to be more effective in delivering drugs across the skin (Marro et al., 1999). In our case, the avoidance of extraneous ions was possible, since the cathode was the most appropriate polarity, and chloride ions were not required in the donor compartment for the electrochemical functioning of Ag/AgCl electrodes. Even in the case of anodal iontophoresis, no sodium chloride was added to the donor solution. With this polarity, the amount of chloride ion required for the reversibility of the Ag/AgCl electrodes in the experimental conditions used (0.3 mA for 30 min) was very small, namely 5.6 μmol. Therefore, the chloride ions in the skin, attracted by the anode into the donor compartment, could guarantee the reversibility of the electrodes.

Initially, the transport of ibuprofen was studied after 30 min of iontophoresis (0.5 mA/cm^2), using a donor solution of ibuprofen lysine (133 mg/ml; pH 7.8). The curves of amount permeating versus time obtained with the donor at the anode or at the cathode are reported in Fig. 2. After the current was switched off (30 min), ibuprofen transport across the skin from the cathode or anode continued. The profiles were lower than the corresponding ones obtained during continuous iontophoresis experiments, and the amount of ibuprofen transported from the cathode was at least three times higher than from the anode. After cathodal and anodal iontophoresis, the ibuprofen flux (slope of the curves) did not vary significantly during the 5 h of the experiments. In the case of cathodal post-iontophoresis, the formation of a drug reservoir in the skin could be envisaged. In fact, the application of electric current has been shown to accumulate the applied drug in the skin (Volpato et al., 1998; Marconi et al., 1999), forming a "reservoir" or "depot" from which the drug can be released to the receptor compartment. As a consequence, the ibuprofen flux in cathodal post-iontophoresis was contributed by release from skin "reservoir" concomitantly with passive transport. In the case of ibuprofen anodal post-iontophoresis, the flux was contributed solely by

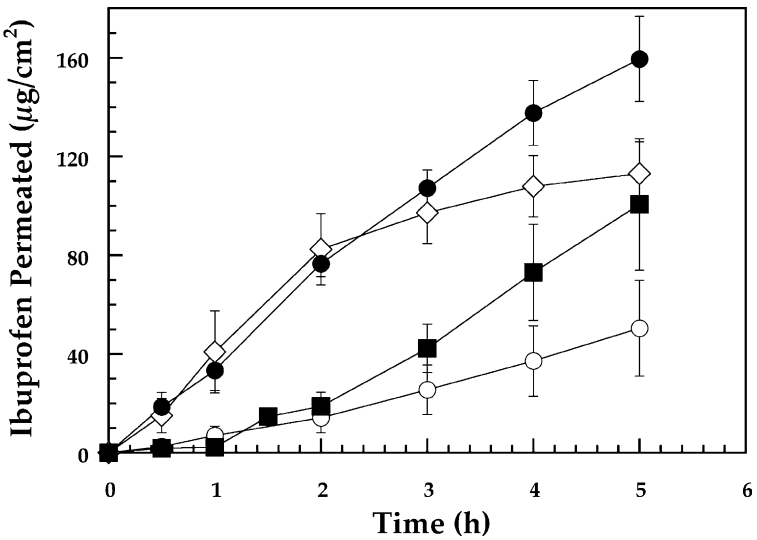

Fig. 2. Ibuprofen permeation from 133 mg/ml ibuprofen lysine solution in water. (●) Cathodal iontophoresis with reservoir kept on the skin for 5 h, (◇) cathodal iontophoresis with reservoir kept on the skin for 30 min, (○) anodal iontophoresis with reservoir kept on the skin for 5 h, and (■) pretreatment with cathodal iontophoresis. The current (0.5 mA/cm^2) was stopped at 30 min. Mean values ± S.E.M., $n \geq 4$.

Table 1
Cumulative amount of ibuprofen permeating from 133 mg/ml ibuprofen lysine solution left in contact with the skin for up to 5 h (mean values ± S.E.M., $n \geq 4$)

Current density (mA/cm^2)	Polarity	Application time (min)	Cumulative amount permeating after 5 h ($\mu g/cm^2$)
0	–	–	12.8 ± 3.5
0.125	Cathodal	30	85.3 ± 18.7
0.25	Cathodal	30	73.3 ± 23.6
0.5	Cathodal	30	135.1 ± 23.9
0.5	Cathodal	15	71.6 ± 11.8
0.5	Cathodal	60	249.9 ± 25.4
0.5	Anodal	30	50.4 ± 19.4

passive transport, since the conditions for creating a reservoir in the skin were not favored by the polarity.

The effect of current density and duration on the amount of ibuprofen permeating after cathodal iontophoresis was then examined. Current densities of 0.125, 0.25, and 0.5 mA/cm^2 were applied for 30 min to a donor solution containing 133 mg/ml of ibuprofen lysine. The effect of current application time (current density 0.5 mA/cm^2 applied for 15, 30, or 60 min) was also evaluated, using the same donor solution. The amounts permeating after 5 h are reported in Table 1. The values show that, as current density and duration increased, the transport of ibuprofen increased in proportion.

The overall effect of the electric current was quantified by plotting the cumulative amount permeating after 5 h versus the quantity of electric charge applied (Santi et al., 1997). The results in Fig. 3 show a linear dependence of the amount of ibuprofen transported in 5 h from the amount of current applied. The linear relationship indicated that, in the 5 h, the contribution to post-iontophoresis ibuprofen transport of passive diffusion was less relevant than the electrotransport taking place at the beginning of the experiment.

We also tested the effect of donor concentration on the post-iontophoresis flux of ibuprofen, in the range between 20 and 200 mg/ml of ibuprofen lysine. Cathodal iontophoresis with current density of 0.5 mA/cm^2 was applied for 30 min, using donor solutions with increasing concentration. It can be seen in Fig. 4 that the permeation profiles did not correlate with concentration and were not significantly different. A drug flux across the skin during iontophoresis, independent of donor concentration, in the absence of background electrolyte, has already been reported for lidocaine (Marro et al., 1999). The explanation was that all the electric current was transported by drug ions. In our case, the current was applied only for a short time,

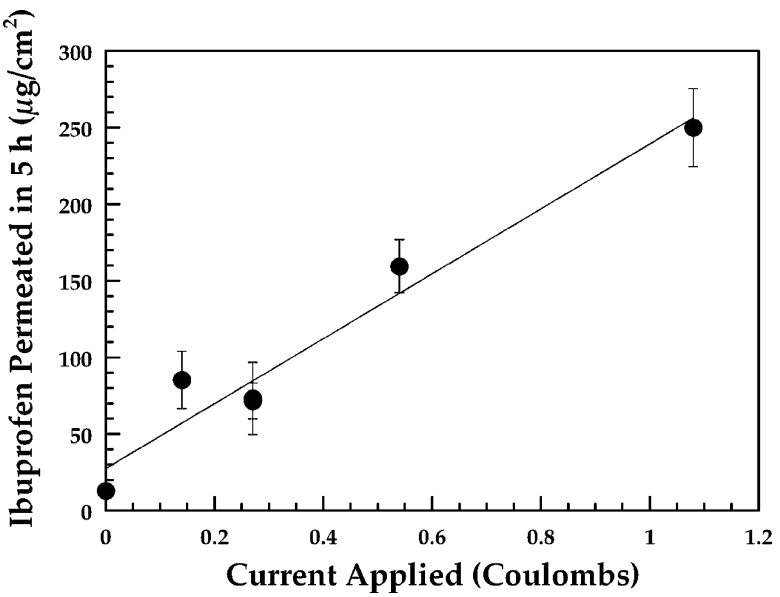

Fig. 3. Cumulative amount of ibuprofen permeating in 5 h, as a function of the total amount of current applied, from 133 mg/ml ibuprofen lysine solution. The regression equation is: $y = 27.575 + 211.71x$ ($R = 0.97592$). Mean values ± S.E.M., $n \geq 4$.

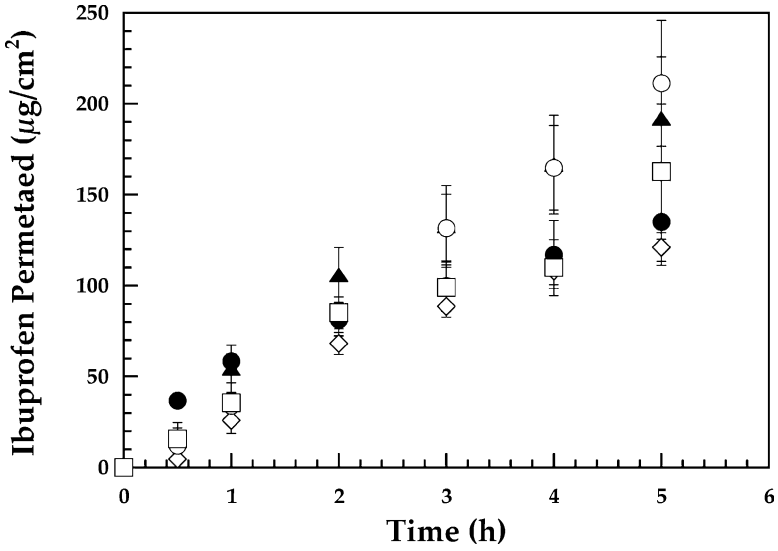

Fig. 4. Ibuprofen permeation profiles from ibuprofen lysine solutions at different concentrations: (◇) 20, (▲) 40, (○) 100, (●) 133, and (□) 200 mg/ml in water. Cathodal iontophoresis 0.5 mA/cm^2 applied for 30 min. Mean values ± S.E.M., $n \geq 4$.

but the post-iontophoresis transport did not significantly depend on drug concentration either. In the post-iontophoresis phase the drug diffusive transport through the "permeabilized" skin was expected to be dependent on drug concentration. Again, the result indicated that the passive transport was masked by the current effect. This behavior supported the formation of a drug reservoir in the skin owing to the short application of the electric current. Since no electrolytes were present, the same amount of current gave rise to similar skin reservoirs, despite the differing ibuprofen concentrations in the donor compartment. In the time the experiment was being carried out, similar amounts of ibuprofen accumulated in the skin after current application led to similar post-iontophoresis fluxes.

The effect of donor concentration and current, and the shape of the post-iontophoresis curves (see Fig. 2), strongly suggest the formation of a reservoir in the skin owing to current application. Further experiments were carried out to differentiate the contribution of the reservoir effect from skin "permeabilization." Firstly, an experiment was performed (cathodal iontophoresis, 0.5 mA/cm^2 applied for 30 min, 133 mg/ml ibuprofen lysine solution), in which the donor solution was removed after current application. In a second experiment, the skin was pretreated with current (cathodal iontophoresis, 0.5 mA/cm^2 applied for 30 min), and the ibuprofen solution (133 mg/ml ibuprofen lysine) was then placed on the treated skin. Fig. 2 shows both of these permeation profiles. When the donor solution was removed after the current application, the permeation profile during the first 2 h remained superimposed on the curve in which the donor solution was kept on the skin. Thereafter, the profile clearly tended to flatten, indicating the exhaustion of the reservoir accumulated in the skin. The difference between the two profiles shows the advantage of leaving the donor solution in contact with the skin after iontophoresis. In the pretreatment curve, a time lag of approximately 1.5 h was observed, followed by a steady increase in the flux. It is interesting to observe that, after approximately 3 h, the current pretreatment curve shows a slope comparable to the cathodal post-iontophoresis curve with the donor on the skin, confirming the exhaustion of the reservoir.

4. Conclusions

From the results obtained, it can be concluded that iontophoretic transport of ibuprofen from lysine salt solutions across rabbit ear skin is enhanced by

cathodal iontophoresis, but also to a certain extent by anodal iontophoresis, owing to the presence of an electroosmotic contribution.

The post-iontophoresis transport of ibuprofen was highly dependent on current polarity, density, and application time. Cathodal iontophoresis was more effective than anodal iontophoresis in increasing post-iontophoretic flux. The amount of ibuprofen transported after cathodal iontophoresis was dependent on the amount of current applied, but independent of drug donor concentration. Skin pretreatment and skin desorption experiments demonstrated the formation of an ibuprofen reservoir in the skin, able to sustain the ibuprofen post-iontophoresis transport for more than 2 h.

The substantial improvement in ibuprofen transported through the skin, obtained by applying iontophoretic current for a short time and leaving the drug donor in contact with the skin during post-iontophoresis, could be of vale for ibuprofen topical therapy.

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