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Effect of lactic acid and iontophoresis on drug permeation across rabbit ear skin

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Abstract

The aim of this paper was to explore the efficacy of lactic acid as permeation enhancer for drug molecules across the skin. Three model permeants were chosen: acetaminophen (non-ionized), buspirone hydrochloride (cationic drug) and ibuprofen lysine (anionic drug). We also explored the association of lactic acid and iontophoresis as a means of enhancing drug delivery. Permeation experiments were performed in vitro, using rabbit ear skin as barrier. The results obtained indicate that lactic acid has some effects on model drug permeation across the skin. The effect was more evident with the anionic drug ibuprofen. Cathodal intophoresis increased ibuprofen transport, but when lactic acid was associated with cathodal iontophoresis, a concentration-dependent reduction of ibuprofen iontophoretic flux was observed, probably for the competition by the co-ion. The application of electric current (anodal iontophoresis) to a solution of acetaminophen produced an increase in its transport, due to the presence of an electroosmotic contribution; however, the effect of the association of anodal iontophoresis and lactic acid produced no further enhancement.

Keywords: Transdermal; Permeation enhancer; Iontophoresis; Permeability; Skin

1. Introduction

Drug penetration enhancement across the skin is a key issue for the development of a transdermal drug delivery system, because the excellent barrier properties of the skin can reduce the applicability of this administration route. Several approaches have been attempted, such as the use of chemical enhancers and of physical techniques. The use of chemical penetration enhancers has been recently reviewed by Williams and Barry (2004). The authors underline the difficulty to select rationally a penetration enhancer for a specific permeant.

Among physical techniques, iontophoresis, i.e. the application of an external electric field to enhance drug transport across the skin, although efficient primarily

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for ionized molecules, can also be useful for non-ionized molecules (Naik et al., 2000). In fact, one of the transport mechanisms, electroosmosis, is a convective solvent flow, which transports all solutes present, regardless of their charge (Pikal, 1992; Delgado-Charro and Guy, 1994).

A combined approach can also be used. Ethanol (Srinivasan et al., 1990), fatty acids (Oh et al., 1998; Valjakka-Kostla et al., 2000; Nicoli et al., 2001; Wang et al., 2003; Smyth et al., 2002), benzalkonium chloride (Costa et al., 1997; Fang et al., 1998), therpenes (Bhatia and Singh, 1998b) and Azone® (Meidan et al., 2003) have been successfully associated with iontophoresis. The use of iontophoresis together with a chemical enhancer can also produce a synergic effect. The main drawback is that skin irritation can be increased as well. Additionally, due to the different individual response to the enhancer, the overall variability of the effect can be augmented.

Lactic acid belongs to the class of α-hydroxy acids, widely used in cosmetic products as exfoliants, moisturizers and emollients. The specific action of α-hydroxy acids on the skin is not completely known. It has been suggested that α-hydroxy acids can reduce stratum corneum corneocyte cohesion by interference with ionic bonding (Kraeling and Bronaugh, 1999). Given this mechanism of action, lactic acid could be effective in increasing the permeation of drugs across the skin. Kraeling and Bronaugh (1997) showed that the permeability of the skin to tritiated water was increased by a factor of 2 after treatment with glycolic acid. Nakamura et al. (1996) used a combination of lactic acid, ethanol and isopropyl myristate to enhance the permeation across the skin of ketotifen fumarate, although the effect of each enhancer was not determined individually.

We explore in this paper the efficacy of lactic acid as permeation enhancer for drug molecules across the skin. Three model permeants were chosen: acetaminophen (non-ionized), buspirone hydrochloride (cationic drug) and ibuprofen lysine (anionic drug). We also explored the association of lactic acid and iontophoresis as a means of enhancing drug delivery. Rabbit ear skin was used as barrier, since it has been shown to be a reasonable model for human skin in vitro (Hirvonen et al., 1991; Nicoli et al., 2003; Blanco et al., 2003; Artusi et al., 2004).

2. Materials and methods

2.1. Materials

Ibuprofen lysine was a gift from Lisapharma S.p.A. (Erba, I). For HPLC solvent preparation, distilled water and HPLC grade acetonitrile were employed. L-Lactic acid 85 % w/v was used. Acetaminophen and buspirone were obtained from ACEF (Fiorenzuola D'Arda, I) and Sigma (Sigma Chemical, St. Louis, MO, USA), respectively.

2.2. Drug analysis

2.2.1. Acetaminophen

Acetaminophen analysis was performed by HPLC (Perkin-Elmer, Norwalk, USA), using a Waters C18 Novapack® 150 mm × 3.9 mm column (Millipore Corporation, Milford, MA) and a mobile phase composed of 10 mM sodium acetate (pH 4):acetonitrile 60:40 (v:v), at 1 ml/min. UV detection at 254 nm was employed.

2.2.2. Buspirone

Buspirone analysis was performed by HPLC (Perkin-Elmer, Norwalk, USA), using a Waters C18 Novapack® 150 mm × 3.9 mm column (Millipore Corporation, Milford, MA) and a mobile phase composed of phosphate buffer (pH 7.5):acetonitrile 60:40 (v:v), at 1 ml/min. The column was thermostated at 25 °C. The UV detector was set at 235 nm.

2.2.3. Ibuprofen

The quantitative determination of ibuprofen permeating across the skin was performed by HPLC analytical assay according to Santi et al. (2003), using a Shimadsu instrument (isocratic pump LC-10AS, UV–vis detector SPD-10A, integrator C-R6A Chromatopac, Shimadsu, Kyoto, J) and a Waters C18 Novapack® 150 mm × 3.9 mm column (Millipore Corporation, Milford, MA, USA). 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.

2.3. Skin sample preparation

Rabbit skin was excised post-sacrifice from the inner part of rabbit ears (6 months old) obtained from a local

slaughter's house. When not used immediately, the skin was kept refrigerated (2–5 °C) and used within 3 days. It has been shown that storage in the refrigerator keeps the metabolic activity of the skin. This leads also to lactic acid production, although the amount produced (approximately 0.1 mmol/24 h, Wester et al., 1998) was very small compared to the concentration used in the donor compartment.

2.4. Permeation experiments

Permeation experiments were conducted at 37 °C in glass Franz type diffusion cells (Disa, Milan, I, permeation area 0.6 cm^2). The donor compartment was filled with 1 ml of 25 mM HEPES buffer at pH 6.0 containing:

(a) buspirone hydrochloride (26 mM);
(b) acetaminophen (33 mM);
(c) ibuprofen lysine (1.4 mM).

In a second set of experiments, the donor solutions contained the model permeants (at the concentrations previously reported) and lactic acid 1.7 M. In the case of ibuprofen lysine, the effect of 14.4 and 1.4 mM of lactic acid was also explored. All donor solutions were brought to pH 6.0 by the addition of sodium hydroxide 4N. The receptor compartment was filled with 4.3 ml of saline solution, degassed under vacuum before use and magnetically stirred to avoid boundary layer effects. At predetermined time intervals, 300 μl of receptor solution were sampled for analysis and replaced with the same volume of fresh saline.

In the iontophoretic experiments, the current was applied by means of a constant current generator (Iono1, Cosmic, Pesaro, I), using silver/silver chloride electrodes made from silver wires (diameter 1 mm, purity 99.9 %) and silver chloride (Sigma Chemical Co., St. Louis, MO, USA), in accordance with Green et al. (1993). Direct current (0.5 mA/cm^2) was applied for 7 h; anodal iontophoresis was used for acetaminophen while in the case of ibuprofen cathodal iontophoresis was used. In the case of acetaminophen iontophoretic experiments, 0.9% of sodium chloride was added to the donor compartment, to guarantee the reversibility of the electrodes.

The permeation profiles were fitted to Eq. (1) (Moser et al., 2001):

$$Q = KHC_{\text{veh}} \times \left[\frac{D}{H^2}t - \frac{1}{6}\frac{2}{\pi^2}\sum_{n=1}^{7}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{H^2}\right)\right] \quad (1)$$

where Q is the cumulative amount of drug permeated per unit area at time t, C_{veh} is the concentration of the drug in the donor, K is the stratum corneum/vehicle partition coefficient, D the diffusion coefficient and H the diffusion path-length. The fitting was performed using KaleidaGraph 3.6.2 (Synergy Software) running on a MacIntosh Power Book G4.

The permeability coefficient P was calculated as:

$$P = KH\frac{D}{H^2} \quad (2)$$

steady-state flux (J_{ss}) as

$$J_{\text{ss}} = PC_{\text{veh}} \quad (3)$$

and time-lag as

$$\text{lag time} = \frac{H^2}{6D} \quad (4)$$

2.5. Statistical analysis

All experiments were replicated at least four times; the results were expressed as the mean ± standard deviation. The statistical differences were determined by Kruskal–Wallis test.

3. Results and discussion

Fig. 1 reports the structural formula and the relevant properties of the three model drugs. They differ not only for the molecular weight but also for the ionization characteristics, since acetaminophen is non-ionizable, buspirone is positively charged and ibuprofen lysine is negatively charged at the pH of the experiments (pH 6.0). We chose pH 6.0 because it is compatible with the skin and guarantees the presence, in the donor solution, of high percentages of buspirone and ibuprofen in the ionized form, which are required in the iontophoretic experiments.

The effect of lactic acid on the passive permeation of the model drugs was studied, using a concentration of α-hydroxy acid of 1.7 M. In all cases the molar

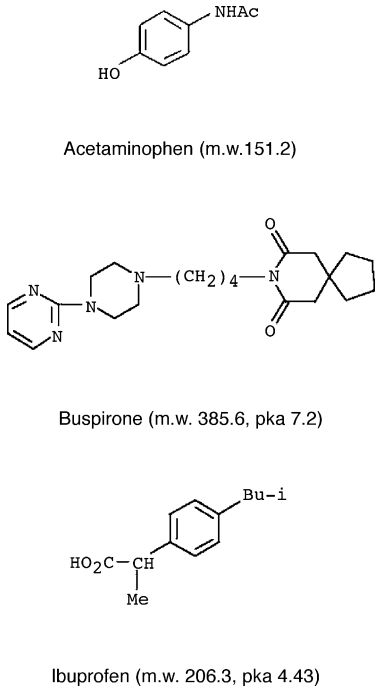

Fig. 1. Structural formula of the model permeants.

concentration of lactic acid exceeded largely that of the permeant in the donor solution. Fig. 2 reports the permeation profiles obtained. Acetaminophen and buspirone permeation profiles were only slightly affected by the presence of lactic acid, while the permeation of ibuprofen was significantly increased. From the permeation profiles it appears that steady-state diffusion has not been achieved over the course of the experiment. The calculation of permeability coefficient and flux values from the last three points has been shown to produce great errors. For this reason, the permeation profiles were fitted to the appropriate solution of Fick's law for un-steady-state permeation (Eq. (1)) to calculate the relevant permeation parameters, such as time-lag (Eq. (4)), permeability coefficient (Eq. (2)) and steady-state flux (Eq. (3)). The values are reported in Table 1. Acetaminophen permeation parameters were practically un-changed by the presence of lactic acid, except for the lag time that was significantly reduced ($p < 0.05$). In the case of buspirone, the permeability coefficient and flux were slightly reduced by the presence of the organic acid, although the difference was not significant. Comparing these results with literature data, it appears that

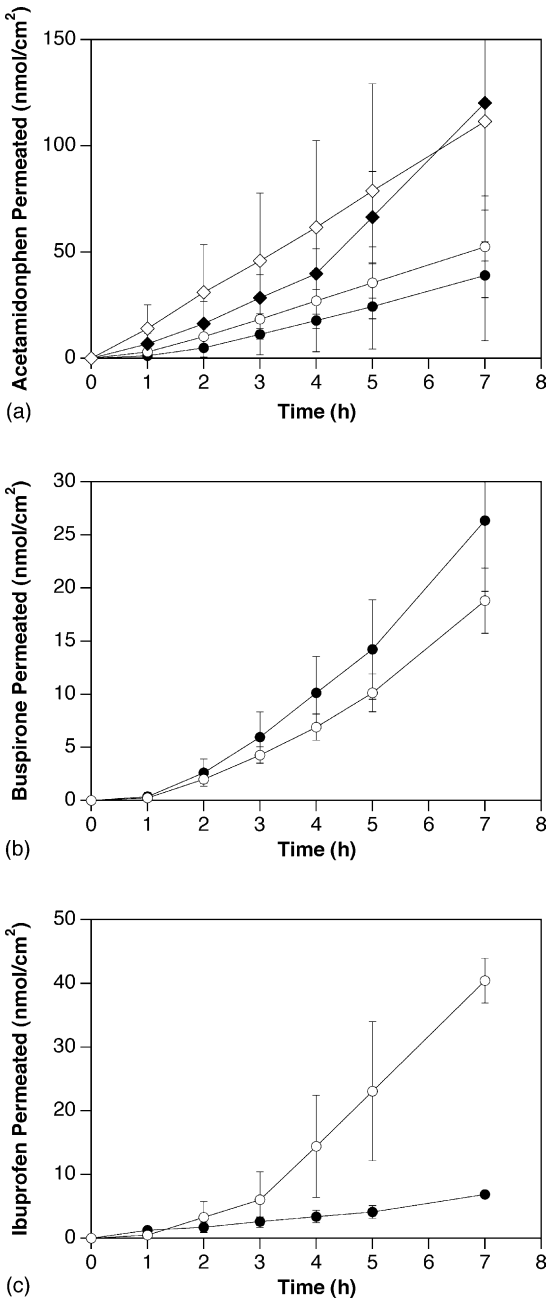

Fig. 2. Permeation profiles of acetaminophen (Panel a), buspirone (Panel b) and ibuprofen (Panel c) across rabbit ear skin in the presence (empty symbols) and absence (full symbols) of 1.7 M lactic acid at pH 6.0. In Panel a circles refer to passive diffusion, diamonds to anodal iontophoresis (0.5 mA/cm^2). Mean values ± S.D.

Table 1
Permeation parameters of the model drugs across rabbit ear skin (mean values ± S.D.)

	Lactic acid concentration (M)	Lag time (h)	P (cm/h) × 10^4	J_{ss} (nmol cm^{-2} h^{-1})
Acetaminophen	0	1.8 ± 0.8*	2.15 ± 1.62	7.10 ± 5.36
	1.7	0.9 ± 0.2*	2.56 ± 1.13	8.48 ± 3.72
Buspirone	0	2.5 ± 1.6	2.36 ± 1.00	6.13 ± 2.60
	1.7	2.4 ± 0.2	1.53 ± 0.26	3.98 ± 0.68
Ibuprofen	0	7.6 ± 11.5	5.16 ± 4.54*	0.73 ± 0.65*
	1.7	4.1 ± 0.9	89.4 ± 12.29*	12.74 ± 1.75*

* Significantly different ($p < 0.05$).

buspirone steady-state flux obtained in the present work is in agreement with the value obtained by Meidan et al. (2003) across human epidermis, confirming that rabbit ear skin is a predictive animal model for human skin.

From this set of results it is clear that ibuprofen lysine was the only molecule really affected by the presence of lactic acid, since the permeability coefficient was increased approximately 20 times ($p < 0.05$). Since acetaminophen and buspirone permeation was not augmented by the presence of lactic acid, the effect of lactic acid on ibuprofen is probably specific on this drug. Since the pH of the solution was 6.0 both in the presence and absence of lactic acid, an effect of the organic acid on ibuprofen ionization should not be the cause. To try to explain this result, we also examined the effect of the presence of lactic acid on the individual permeation parameters calculated using Eq. (1), i.e. partitioning (KH) and diffusive parameters (D/H^2). The partitioning parameter resulted two orders of magnitude higher in the presence of lactic acid compared to the control (0.23 ± 0.08 versus 0.003 ± 0.002 cm; $p < 0.05$); on the contrary, the diffusive parameter did not change significantly (0.04 ± 0.01 versus 0.13 ± 0.11 h^{-1}; $p = 0.5$). This result suggests that lactic acid increases the partitioning of ibuprofen between the skin and the formulation, assuming that the diffusion path-length remains unchanged. The reason why lactic acid can increase the partitioning of ibuprofen into the stratum corneum, but does not change the permeation parameters of the other permeants tested, is still unclear. Possible reasons are the following: (i) lactic acid interacts with lysine, thus changing the partitioning of ibuprofen lysine. It has been shown that ibuprofen lysine can aggregate forming either micelles or a hexagonal liquid crystalline phase (Stoye et al., 1998). However, the aggregation of ibuprofen lysine in water has been demonstrated for concentrations (2.67–50% w/v) well above the concentration used in our experiments; (ii) lactic acid penetrates the skin, thus reducing the lipophilicity of the stratum corneum and this facilitates the partitioning of the ionized form of ibuprofen in the stratum corneum; (iii) the polarity of the donor vehicle is decreased by the presence of lactic acid. This last hypothesis is supported by some considerations on the solubility parameter of the solvents. Lactic acid has a solubility parameter of 30.2 MPa$^{0.5}$ (Hancock et al., 1997) while water solubility parameter is 47.9 MPa$^{0.5}$ (Hancock et al., 1997). Given that the solubility parameter is an indicator of the lipophilic parameter of a solvent or mixture of solvents, then the mixture lactic acid and water is less hydrophilic than water and this can facilitate the partitioning of the ionized (hydrophilic) form of ibuprofen in the stratum corneum. This interpretation can also justify the (modest) reduction of penetration of buspirone across the skin in the presence of lactic acid. In fact, buspirone is more lipophilic than ibuprofen (the percentage ionized being similar for ibuprofen (97%) and buspirone (94%) in the conditions of the experiment) then a decrease in the solubility parameter leads to a decreased partitioning of buspirone between the vehicle and skin. The partitioning parameter of buspirone, calculated using Eq. (1), was effectively decreased in the presence of lactic acid by a factor of 2 (although the difference was not statistically significant).

Then, the combined effect of lactic acid and iontophoresis was tested. First of all, the effect of iontophoresis alone was studied. When the electric current (cathodal iontophoresis) was applied to the solution of ibuprofen lysine (Fig. 3), an evident improvement in ibuprofen permeation was observed, the mechanism of action being primarily electrorepulsion (Santi et al.,

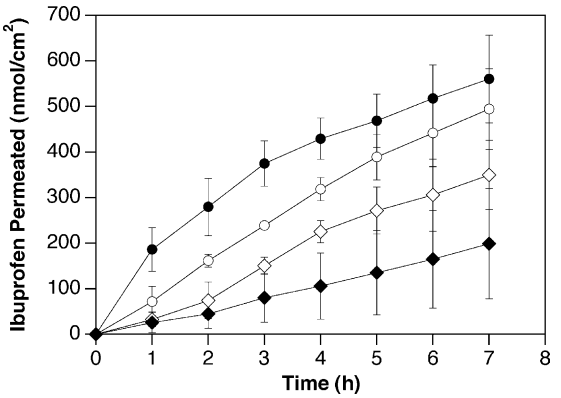

Fig. 3. Permeation profiles of ibuprofen in the presence of lactic acid: 0 mM (●), 1.4 mM (◯), 14.4 mM (◊) and 1.7 M (♦). All experiments are under cathodal iontophoresis (0.5 mA/cm^2). Mean values ± S.D.

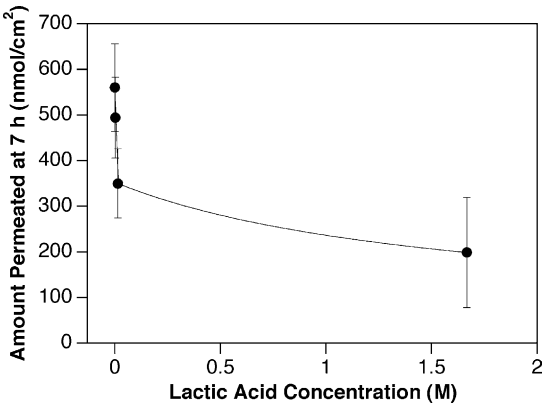

Fig. 4. Relationship between the amount of ibuprofen permeated at 7 h and lactic acid concentration in the donor solution during cathodal iontophoresis. Mean values ± S.D.

2003). In fact ibuprofen, whose pK_a value is 4.43, is almost completely (97%) in its anionic form at pH 6. The shape of the permeation curve was not linear with time, but the curve tended progressively to flatten over time, probably because there was an important depletion of ibuprofen from the donor solution. When 1.7 M lactic acid was present in the donor compartment, the permeation profile of ibuprofen was dramatically decreased compared to iontophoresis alone. A possible explanation for this phenomenon is the competition between lactic acid and ibuprofen for current transport. In fact it is well known that the presence of co-ions with higher or equal mobility can decrease the iontophoretic transport of ionized permeants (Bellantone et al., 1986). Lactic acid has a low molecular weight (m.w. = 90.08), is negatively charged at the pH value of the experiment (pK_a 3.86) and is present in a molar concentration approximately 1000 times higher than ibuprofen. To confirm the hypothesis of ionic competition, additional experiments were performed with varying concentrations of lactic acid in the donor compartment, keeping the pH at 6.0. The concentrations chosen were 14.4 and 1.4 mM, corresponding to lactic acid:ibuprofen molar ratio of 10:1 and 1:1, respectively. The results obtained by applying cathodal iontophoresis to a solution of ibuprofen with varying concentrations of lactic acid are summarized in Fig. 3. It is quite evident that lactic acid produces a concentration proportional reduction of ibuprofen transport, due to lactic acid competition for current transport. The cumulative amount of ibuprofen permeated at 7 h was then plotted versus lactic acid concentration in the donor solution (Fig. 4). The amount of ibuprofen permeated decreased exponentially with lactic acid concentration.

The combined effect of iontophoresis and lactic acid was tested also on acetaminophen permeation. When anodal iontophoresis was applied to the solution containing acetaminophen alone (see Fig. 2a), the amount permeated increased compared to the no current control, although the effect was evident only after 4 h. The mechanism by which a non-ionized molecule such as acetaminophen can take advantage of iontophoretic application is electroosmosis (Pikal, 1992) and for this reason acetaminophen has been used as marker for electroosmosis by Bath et al. (2000) in hairless mouse skin. The water volume flow calculated from the acetaminophen profiles reported in Fig. 2a was 0.54 ± 0.1 μl cm^{-2} h^{-1}, in agreement with data previously obtained in similar experimental conditions across rabbit ear skin (Artusi et al., 2004). This result confirms that the iontophoretic transport of acetaminophen is due to electroosmosis. When iontophoresis was associated with lactic acid the result was not significantly different from iontophoresis alone, suggesting that the α-hydroxyacid did not influence the electroosmotic transport of acetaminophen.

Since the flux of buspirone was not enhanced by the presence of lactic acid, no further attempts in associating it with iontophoresis were tested. It has already been shown (Meidan et al., 2003) that electric current is able to increase buspirone flux across the skin.

4. Conclusions

From the results obtained it can be concluded that lactic acid has some effects on model drug passive permeation across the skin. The effect was evident only with the anionic drug ibuprofen, although the reason remains unknown and is worth future investigations. A possible hypothesis is a change in the solubility parameter of the vehicle, due to the presence of lactic acid, which leads to an increased partitioning of ibuprofen lysine in the stratum corneum. Cathodal intophoresis increased ibuprofen transport, but when lactic acid was associated with cathodal iontophoresis, a concentration-dependent reduction of ibuprofen iontophoretic transport was observed, probably for the competition by the co-ion on current transport. The application of electric current (anodal iontophoresis) to a solution of acetaminophen produced an increase in its transport, due to the presence of an electroosmotic contribution. The association of anodal iontophoresis and lactic acid produced no further enhancement, indicating that lactic acid do not modify the electroosmotic contribution to the overall iontophoretic transport.

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