Iontophoresis Enhances the Transport of Acyclovir Through Nude Mouse Skin by Electrorepulsion and Electroosmosis

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Purpose. Iontophoresis was employed for enhancing the transdermal delivery of acyclovir through nude mouse skin *in vitro*, with the aim of understanding the mechanisms responsible for drug transport, in order to properly set the conditions of therapeutical application.
Methods. Experiments were done in horizontal diffusion cells, using as donor a saturated solution of acyclovir at two different pH values (3.0 and 7.4). Different electrical conditions (current density and polarity) were employed.
Results. At pH 3.0, acyclovir anodal transport was due to electrorepulsion, since acyclovir was 20% in the protonated form. In acyclovir anodal iontophoresis at pH 7.4 the main mechanism involved was electroosmosis, since the drug was substantially unionized and the negative charge of the skin at this pH caused the electroosmotic flow to be from anode to cathode. In the case of cathodal iontophoresis at pH 3.0, acyclovir transport was enhanced approx. seven times, due to the presence of an electroosmotic contribution caused by the reversal of the charge of the skin. At pH 7.4 during cathodal iontophoresis acyclovir transport was not enhanced because the electroosmotic flow was in the opposite direction, compared to drug electric transport, i.e. anode to cathode. The increased skin permeability caused by current application was demonstrated to be less important than electrorepulsion and electroosmosis.
Conclusions. Anodal iontophoresis shows potential applicability for enhancing acyclovir transport to the skin, considering that both electric transport and electroosmosis can be used by appropriately setting the pH of the donor.

KEY WORDS: iontophoresis; acyclovir; electroosmosis; electrorepulsion; transdermal delivery; nude mouse skin.

INTRODUCTION

During transdermal iontophoresis the main transport mechanism involved is the electric transport through the annexial appendages, due to the repulsion between ions and the electrode having the same charge (1). Furthermore, when a continuous electric current is applied across a porous membrane containing fixed charges, such as skin, a flow of liquid occurs through the pores in the direction of counter ion migration. Human and mouse skin is negatively charged at physiological pH values, therefore the volume flow occurs from the anode to the cathode. This movement of liquid, known as electroosmotic flow, contributes to the iontophoretic transport, though to a lesser degree than the electric contribution (2).

Acyclovir (ACV), a synthetic analogue of 2'-deoxyguanosine, is active in the treatment of cutaneous herpes virus infections. ACV is an ampholyte drug containing two ionizable groups (Figure 1). The imidazole moiety possesses weak basic properties whereas the 1-NH moiety is weakly acidic. The pKa values are 2.4 for protonation at N7 and 9.2 for deprotonation at N1, respectively (3).

It has been suggested that the low efficacy of dermatological formulations of ACV may be attributed to inadequate drug penetration through the stratum corneum and to the heterogeneous distribution of drug in skin layers. The site of herpes virus lesions is the basal epidermis, but following topical administration ACV concentration in the basal epidermis is lower than after oral administration (4-5).

The delivery of greater amounts of acyclovir to the target site should provide improved topical efficacy. Recently the possibility of using absorption enhancers and iontophoresis for effective acyclovir local delivery has been investigated (6).

The present study set out to explore the iontophoretic delivery of acyclovir to the skin with the aim of understanding the mechanisms responsible for drug transport, in order to properly set the conditions of therapeutical application. The pKa values imply that ACV may be a substance suitable to be transported by anodal and cathodal iontophoresis and/or by electroosmosis. Therefore by changing the pH value of donor solution, or current density and/or electrode polarity, the electrical and formulation variables important in ACV iontophoretic transport could be assessed and the mechanisms of transport deduced.

MATERIALS AND METHODS

Materials

Acyclovir (mol wt. 225.2) was a gift from Drug Research, Milan, Italy (batch B34/93). Acetonitrile (Carlo Erba Reagenti, Milan, I) and acetic acid (J.T. Baker, Deventer, Netherlands) were HPLC grade.

Saline pH 7.4 phosphate-buffered was prepared with 5.98 g of disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$, Carlo Erba Analyticals, Milan, I), 0.19 g of potassium dihydrogen phosphate (KH_2PO_4, Carlo Erba Analyticals, Milan, I) and 10.4 g of NaCl (Carlo Erba Codex, Milan, I) in sufficient water to 1000 ml (ionic strength 0.229 M). Saline pH 3.0 citro-phosphate-buffered was prepared mixing 794 ml of 0.01 M citric acid (Carlo Erba Analyticals, Milan, I) with 206 ml of 0.02 M disodium hydrogen phosphate and adding 10.4 g of NaCl (ionic strength 0.238 M). BP 1993 saline pH 7.4 phosphate-buffered was prepared with 5.98 g of disodium hydrogen phosphate dodecahydrate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of NaCl in sufficient water to 1000 ml (ionic strength 0.188 M) (the amount of chloride ions guaranteed the reversibility of Ag/AgCl electrodes).

Both abdominal and dorsal, freshly excised, full-

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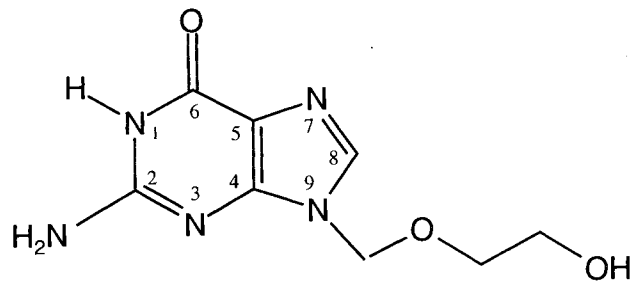

Fig. 1. Chemical structure of acyclovir.

thickness skin of male nude mice of 4-6 weeks old (crl: nu/nu CD-1® BR, Charles River Italia, Calco, Italy) were used. Two skin samples for each mouse were taken and conditioned in BP 1993 saline pH 7.4 phosphate-buffered for 1 hour before mounting in the diffusion cells.

Iontophoretic Experiments

Horizontal diffusion cells (Iomed, Salt Lake City, Utah, USA) were used. The skin was mounted between the donor and the receptor chambers with the stratum corneum facing the donor. The donor compartment volume was 1.5 ml and the receptor compartment 10 ml. The electrodes used were Ag electrode in the anode and Ag/AgCl electrode in the cathode (Iomed, Salt Lake City, Utah, USA). These electrodes in the presence of Cl^- are reversible to the current (1,7), i.e. they do not produce pH shift in the donor and receptor solutions. Two cells were connected in series to a current supply module (Neuromed CBR2, IREM, Parma, I) capable of furnishing constant direct current. Current densities of 0.18, 0.25, 0.36 and 0.50 $mA \cdot cm^{-2}$ were applied for 4 h. The potential difference across each cell during iontophoretic experiment was continuously monitored, using an oscilloscope (Hitachi Denshi, Tokyo, Japan) connected to the electrodes.

In order to have the maximal driving force for permeation, the donor solutions were either saline pH 7.4 phosphate-buffered saturated at 25°C with acyclovir (7.73 mM) or saline pH 3.0 citro-phosphate-buffered saturated at 25°C with acyclovir (6.88 mM).

Twenty-five ml of BP 1993 saline pH 7.4 phosphate-buffered were recirculated in the receptor chamber through an external water bath, using a peristaltic pump, in order to maintain the temperature of the diffusion cells thermostated at 36 ± 1°C. A magnetic stirrer guaranteed the homogeneity of the receptor chamber solution, avoiding boundary layer effects. At pre-determined time intervals, 500 µl of the receptor solution were sampled and replaced with the same volume of fresh buffer. Four replicates were performed for each experiment. The ACV passive diffusion experiments, made in the same conditions without current application, lasted for 9 h.

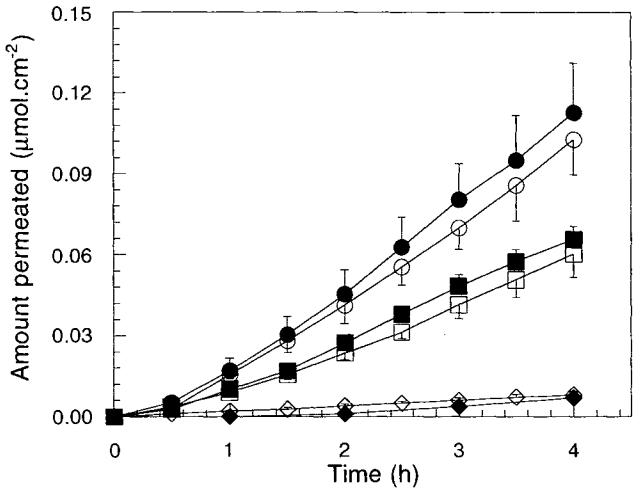

Fig. 2. Amount of acyclovir permeated through nude mouse skin as a function of time from pH 7.4 donor solution at different current densities and polarity. Passive diffusion (♦); anodal iontophoresis: 0.18 (□), 0.25 (■), 0.36 (○) and 0.50 (●) mA cm^{-2}; cathodal iontophoresis: 0.5 mA cm^{-2} (◇) (mean ± s.e.m.).

Analysis of Acyclovir Permeated

The amount of acyclovir in the receptor solution samples was assayed by high performance liquid chromatography (Isocratic LC pump 250, Perkin Elmer, Norwalk, Connecticut, USA), using a reverse-phase column (Pecosphere 5C-C18, Perkin Elmer) and eluant composed of 98 parts acetic acid 0.1% (v/v) and 2 parts of acetonitrile at flow rate of 1.0 ml/min. The detection was performed at 254 nm (UV/VIS Spectrophotometric detector LC290, Perkin Elmer).

To compare the relative iontophoretic enhancement of acyclovir, the transport was expressed in terms of "normalized flux", defined as the amount permeated per unit area per unit time (slope of permeation curve between the third and the fourth hour) divided by the initial concentration of drug in the donor compartment (8). Normalized flux was used instead of permeability coefficient because true steady state conditions could not reasonably be assumed, since the skin is not a homogeneous membrane and its permeability characteristics change over the course of the experiment. The results plotted in the figures are the mean ± s.e.m. of four experiments.

RESULTS AND DISCUSSION

Two pH values of 3.0 and 7.4, considered as limiting values for practical ACV transdermal delivery, were selected for anodal and cathodal iontophoretic experiments.

pH 7.4 Anodal Iontophoresis

The amounts of acyclovir permeated per unit area through nude mouse skin, obtained at different current density and polarity from pH 7.4 donor solution, are shown as a function of time in Figure 2. The amount of acyclovir reaching the receptor compartment in the absence of current (passive diffusion) was quite low. When the positive electrode was in the donor (anodal iontophoresis), the application of current at densities from 0.18 $mA \cdot cm^{-2}$ to 0.50 $mA \cdot cm^{-2}$ increased the amount of ACV transported after an initial lag time phase. Table I shows the normalized fluxes obtained as described above, indicating that the application of electric current enhanced acyclovir penetration through the skin, but no significant differences were observed between the permeation curves obtained at current densities of 0.18 and 0.25 $mA \cdot cm^{-2}$ and between 0.36 and 0.50 $mA \cdot cm^{-2}$, whereas the

Table I. Normalized Flux Values of Acyclovir Iontophoretic Transport Through Nude Mouse Skin from pH 7.4 and pH 3.0 Donor Solutions (mean ± s.e.m.)

Current density ($mA \cdot cm^{-2}$)	pH 7.4 Normalized flux ($cm \cdot h^{-1}$)	pH 3.0 Normalized flux ($cm \cdot h^{-1}$)
0	$3.29 \cdot 10^{-4} \pm 9.32 \cdot 10^{-5}$	$1.98 \cdot 10^{-4} \pm 5.29 \cdot 10^{-5}$
anodal		
0.18	$2.44 \cdot 10^{-3} \pm 4.71 \cdot 10^{-4}$	—
0.25	$2.56 \cdot 10^{-3} \pm 3.18 \cdot 10^{-4}$	$3.64 \cdot 10^{-3} \pm 4.93 \cdot 10^{-4}$
0.36	$4.23 \cdot 10^{-3} \pm 6.49 \cdot 10^{-4}$	—
0.50	$4.30 \cdot 10^{-3} \pm 7.37 \cdot 10^{-4}$	$6.81 \cdot 10^{-3} \pm 5.49 \cdot 10^{-4}$
0.25[a]	$8.62 \cdot 10^{-4} \pm 1.20 \cdot 10^{-5}$	$6.79 \cdot 10^{-4} \pm 2.97 \cdot 10^{-4}$
0.50[a]	$9.42 \cdot 10^{-4} \pm 7.46 \cdot 10^{-4}$	$8.38 \cdot 10^{-4} \pm 2.52 \cdot 10^{-4}$
cathodal		
0.50	$2.63 \cdot 10^{-4} \pm 4.98 \cdot 10^{-5}$	$1.42 \cdot 10^{-3} \pm 1.25 \cdot 10^{-4}$

[a] The skin was pretreated for 4 hours with the current before the ACV transport experiment.

flux significantly increased between 0.25 and 0.36 $mA \cdot cm^{-2}$. The normalized flux of ACV through nude mouse skin increased by one order of magnitude during four hours of anodal iontophoresis at current density of 0.50 $mA \cdot cm^{-2}$, compared to passive diffusion.

These results gave the opportunity to examine the transport mechanisms. Given that acyclovir was 98.4% in the unionized form at pH 7.4 and the actively transported protonated form was at a negligible concentration (<0.01%), it seemed reasonable to attribute the amount of acyclovir permeated in these conditions to the electroosmotic solvent flow (2), without undervaluing a possible increase of skin permeability due to the current. In fact, during the monitorage of the voltage across each cell, it was constantly observed that the voltage applied decreased during the first 30 minutes of iontophoresis, reaching a lower stable value for the remainder of the experiment. This behaviour was interpreted as a decrease in skin resistance due to the current application (as electrolyte solution and electrode resistance did not change appreciably under the conditions of the iontophoretic experiment) (9) and possibly to an alteration of the barrier properties of the skin.

It has been previously reported that the passive flux after iontophoresis is higher than that measured before iontophoresis, allowing an evaluation of the changes in skin barrier function due to current application (9-10). Therefore the skin was pretreated for 4 h with a current density of 0.25 and 0.50 $mA \cdot cm^{-2}$, respectively, using a donor compartment filled with saline pH 7.4 phosphate-buffered and connected to the positive electrode. After this skin pre-treatment with current, the saline in the donor was substituted with ACV pH 7.4 saturated solution and acyclovir passive permeation was measured. With this experiment the contribution of the increased skin permeability on ACV iontophoretic transport was quantified (11). The results obtained, for the two current densities employed, expressed as normalized fluxes, were not significantly different between them, being approx. 2.5 times higher than passive diffusion (see Table I). On the other hand, the normalized fluxes previously obtained under iontophoresis, at the same current densities of the skin pretreatments, were 7 and 13 times higher than passive diffusion (see Table I). Therefore it could be deduced that, from a donor solution at pH 7.4 connected to the anode, the iontophoretic flux of acyclovir resulted mainly contributed by electroosmotic transport.

pH 7.4 Cathodal Iontophoresis

When the negative electrode was connected to donor solution (cathodal iontophoresis), the form of acyclovir transported by electrorepulsion was the deprotonated one and the pH 7.4 donor solution contained 1.55% of ACV^-. Furthermore, remembering that the electroosmotic flux occurred in the direction of counter-ions movement and that at pH 7.4 the skin was negatively charged, during pH 7.4 cathodal iontophoresis the electroosmotic flow in the skin was expected from the receptor to the donor, i.e. opposite to the direction of ACV^- transport. The results obtained showed that the normalized flux measured during pH 7.4 cathodal iontophoresis (see Table I and Figure 2) was slightly lower, but not significantly different, from passive diffusion, indicating an electroosmotic flow opposing the electric transport of ACV negative ions from the donor.

These findings at pH 7.4 are quite different from the data published by Lashmar and Manger (6). In fact they obtained an enhancement in ACV transport higher with cathodal than with anodal iontophoresis, using no buffered pH 7 ± 1 donor solution (ACV concentration: 6.66 mM) and current density of 0.67 $mA \cdot cm^{-2}$. A possible explanation of this difference is the absence in their experiments of chloride ions in the donor chamber, which in anodal iontophoresis are necessary for the reversibility of Ag/AgCl electrodes (7). Additionally, these authors used frozen nude mouse skin, whereas freshly excised skin was used for the purpose of this study.

pH 3.0 Anodal Iontophoresis

Having assessed the importance of electroosmosis at pH 7.4, where acyclovir is substantially unionized, the pH of acyclovir donor solution was modified in order to increase $ACVH^+$ concentration. Moreover applying a donor solution having a pH lower than 4, the possibility existed of reversing

to positive the skin charge (2, 9) thus inducing the electroosmotic flow from cathode to anode. Figure 3 shows the permeation profiles of acyclovir obtained performing iontophoretic experiments from a pH 3.0 donor solution connected to the positive electrode, at different current densities (0, 0.25 and 0.50 mA·cm^{-2}). At pH 3.0, acyclovir was 20% in protonated form, so a substantial electrical transport was expected. The normalized flux values reported in Table I indicate that the application of anodal iontophoresis enhanced acyclovir penetration through the skin to an extent greater than at pH 7.4, in particular considering that the passive permeation of ACV was lower at this pH. A lower passive diffusion at pH 3.0, where the unionized form was 80%, i.e. less than at pH 7.4 where it was 98%, was in agreement with pH-partition theory and reflected also the findings reported by Préat and Thysman (12).

Again, to quantify the contribution of the increased skin permeability due to the current, the normalized fluxes of ACV after anodal pre-treatment of the skin were also measured at pH 3.0 at both current densities studied. The values obtained were not significantly different from the ones obtained after pH 7.4 anodal pre-treatment (see Table I). Additionally, when the passive permeation of acyclovir was measured for 4 hours subsequent to 4 hours of current application (0.50 mA·cm^{-2}) with the drug (see Figure 4), the amount permeated decreased when the current was turned off and the calculated normalized flux ($1.01 \cdot 10^{-3} \pm 5.50 \cdot 10^{-5}$ cm.h^{-1}) was not significantly different from the value obtained after anodal pre-treatment of the skin.

The results of acyclovir iontophoretic transport at pH 3.0 must be attributed to the electrical transport (electrorepulsion), since a contributing electroosmotic flow could not be expected. In fact it has been previously observed that electroosmotic flow from anode to cathode in hairless mouse skin was significantly low at pH close to 3.0 (9). Already at pH 4.0, transference number data suggested that the skin pores were slightly positively charged (13). Therefore in these experimental conditions the main contribution to the

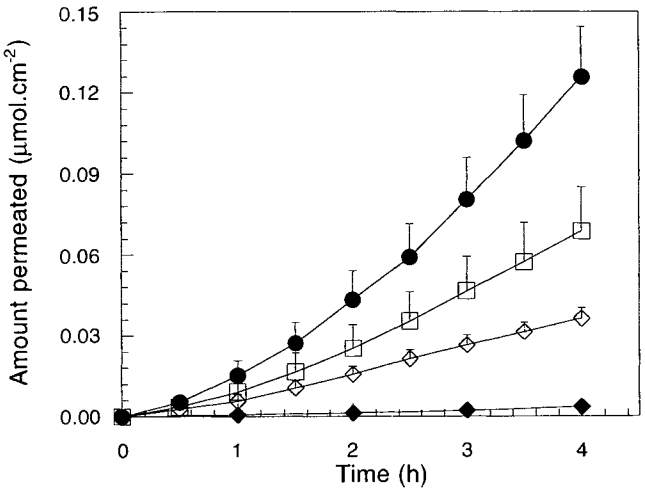

Fig. 3. Amount of acyclovir permeated through nude mouse skin as a function of time from pH 3.0 donor solution at different current densities and polarity. Passive diffusion (♦); anodal iontophoresis: 0.25 (□) and 0.50 (●) mA cm^{-2}; cathodal iontophoresis: 0.5 mA cm^{-2}. (◇) (mean ± s.e.m.).

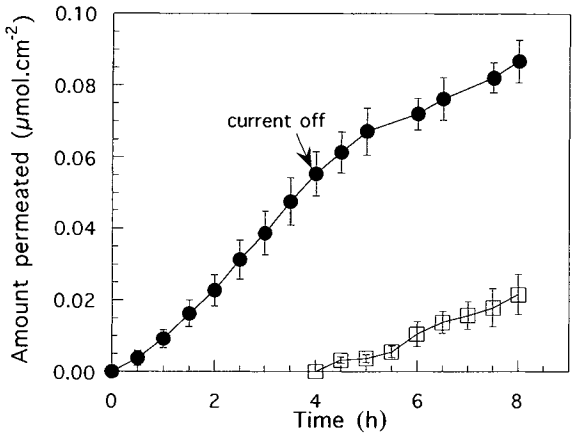

Fig. 4. Amount of acyclovir permeated through nude mouse skin as a function of time from pH 3.0 donor solution: (●) anodal iontophoresis 0.50 mA cm^{-2} for 4 h and subsequent passive diffusion for 4 h and (□) passive diffusion after anodal pre-treatment of the skin with 0.50 mA cm^{-2} for 4 h (mean ± s.e.m.).

acyclovir permeation remained the electric transport of $ACVH^+$.

Delgado and Guy (14) studied the effect of current application on mannitol iontophoresis and found that the current-dependence of electroosmotic mannitol flux was not particularly strong. At pH 3.0, where $ACVH^+$ was present at a considerable concentration, the normalized fluxes obtained were linearly related to current density. At pH 7.4, where the ACV transport is mainly electroosmotic, the normalized fluxes still increase with current density, but not to the same extent as pH 3.0.

pH 3.0 Cathodal Iontophoresis

By performing a cathodal iontophoresis at pH 3.0, the 20% of protonated ACV could not contribute to the amount permeated and the deprotonated form was at an irrelevant concentration (<0.01%). This experiment was also particularly interesting because at pH 3.0 the reversal of the fixed charges in the skin could cause a "reverse" electroosmosis favourable to the drug transport, i.e. volume flow from cathode to anode (15). The normalized flux measured (Figure 3 and Table I) showed an enhancement of the passive permeation of approx. 7 times. Bearing in mind that current pretreatment gave an enhancement of about 2.5 times and that no possibility existed for the electric transport in this situation, a "reverse" electroosmotic flow was developed. Thus at pH 3.0 during cathodal iontophoresis, the transport of ACV was mainly supported by electroosmosis from cathode to anode. Finally, it might be also observed that the enhancement due to "reverse" electroosmosis at pH 3.0 was lower than the one due to "normal" electroosmosis at pH 7.4 for the same current density (0.50 mA.cm^{-2}).

CONCLUSIONS

From the results obtained it was found that iontophoresis promoted the transport of ACV through the nude mouse skin, enhancing up to 30 times the passive transport, depending on the experimental conditions adopted.

Transdermal permeation of ACV is driven by electric

transport at pH 3.0 during anodal iontophoresis. At pH 3.0 during cathodal iontophoresis, "reverse" electroosmosis is activated, whereas "normal" electroosmosis is the main transport mechanism at pH 7.4 anodal iontophoresis. However, electroosmosis contribution is significantly lower than the transport due to electrorepulsion.

At pH 7.4 during cathodal iontophoresis, the electric transport of the low amount of ACV^- was suppressed by the opposite electroosmotic flow; such flow in the charge-reversed skin at pH 3.0 was not observed during anodal iontophoresis, probably due to the higher amount of protonated ACV.

ACV transport is also enhanced by the permeability increase of the skin due to current application, but the enhancement measured was significantly lower than that due to electric transport and/or electroosmosis.

Anodal iontophoresis shows potential applicability for enhancing ACV transport to the skin, considering that both electric transport and electroosmosis can be used by appropriately setting the pH of the donor.

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