Topical iontophoresis of valaciclovir hydrochloride improves cutaneous aciclovir delivery

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Research Paper

Topical Iontophoresis of Valaciclovir Hydrochloride Improves Cutaneous Aciclovir Delivery

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Purpose. To investigate the topical iontophoresis of valaciclovir (VCV) as a means to improve cutaneous aciclovir (ACV) delivery.
Methods. ACV and VCV electrotransport experiments were conducted using excised porcine skin *in vitro*.
Results. While the charged nature of the prodrug, VCV, enabled it to be more efficiently iontophoresed into the skin than the parent molecule, ACV, only the latter was detectable in the receptor chamber, suggesting that VCV was enzymatically cleaved into the active metabolite during skin transit. Iontophoresis of VCV was significantly more efficient than that of ACV; the cumulative permeation of ACV after 1, 2 and 3 h of VCV iontophoresis at 0.5 mA cm^{-2} and using an aqueous 2 mM (~0.06%) formulation was 20 ± 10, 104 ± 47 and 194 ± 82 μg cm^{-2}, respectively (cf. non-quantifiable levels, 0.1 and 1.0 ± 0.7 μg cm^{-2} after ACV iontophoresis).
Conclusions. These delivery rates provide ample room to reduce either current density or the duration of current application. Preliminary *in vitro* data serve to emphasize the potential of VCV iontophoresis to improve the topical therapy of cutaneous herpes simplex infections and merit further investigation to demonstrate clinical efficacy.

KEY WORDS: aciclovir; herpes simplex virus; iontophoresis; prodrug; transdermal delivery; valaciclovir hydrochloride.

INTRODUCTION

The successful topical treatment of cutaneous herpes simplex virus (HSV) infections using aciclovir (ACV; Fig. 1a) offers several advantages over systemic therapy: the drug can be directly targeted to its site of action, reducing circulating drug levels and hence, attendant adverse effects. However, topical ACV creams, though extensively evaluated, have demonstrated only modest efficacy that is also partly dependent on a number of pathophysiological parameters, e.g., the type and phase of infection (primary *vs*. recurrent; early *vs*. latent), the severity of infection and the patient's immune status (1,2). Moreover, most studies investigating ointment formulations have shown little or no clinical benefit in the treatment of cutaneous lesions e.g., herpes labialis (3–7). This vehicle effect has been attributed to the slightly improved permeation of ACV from creams relative to ointments as demonstrated across human (8) and guinea pig (9,10) skin *in vitro*. Although the inability of ACV to efficiently penetrate the stratum corneum (SC) barrier has been proposed as one of the principal reasons for inadequate topical ACV therapy (1,8), a dermatopharmacokinetic study has shown that while total epidermal concentrations of ACV subsequent to topical delivery are superior to those attained after oral administration, the latter appears to deliver more drug to the basal epidermis, the site of infection (11). Formulation strategies to enhance cutaneous ACV permeation have included the incorporation of enhancers such as dimethyl sulfoxide (8,9) and the use of polymeric vehicles (12).

Iontophoresis, the application of a small electrical current to facilitate the transport of charged molecules into and across the skin (13,14), has also been investigated as a means to increase cutaneous ACV bioavailability (15–19). This technique enables the non-invasive, controlled administration of therapeutic agents for either local or systemic action, and has recently culminated in the commercialization of a device incorporating lidocaine for local anaesthesia in infants prior to superficial dermatological procedures (LidoSite™, Vyteris, Inc., Fair Lawn, NJ) (13,20). The LidoSite™ device, in which the positively charged lidocaine participates in the electrical circuit by transporting charge from the anodal compartment into the skin, results in local anaesthesia within 10 min—within which period the drug reaches the nerves located in the dermis and epidermis.

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Although the delivery of therapeutic amounts of ACV into human skin *in vitro* after 30 min of current application followed by 5 h of passive delivery has been reported (albeit with a formulation pH of 3) (17), ACV is not an ideal candidate for topical iontophoresis as it is essentially uncharged at physiological pH (pK_{a1} 2.27; pK_{a2} 9.25) and has a low aqueous solubility (1.3 mg/ml at pH 7.4, 25°C (11)). In comparison, valaciclovir (VCV; Fig. 1a), the L-valyl ester prodrug of ACV (21,22) possesses three ionizable groups with pK_a values of 1.90, 7.47 and 9.43. Consequently, VCV is ~50% protonated at physiological pH, and more suited to iontophoresis relative to its active metabolite, ACV (Fig. 1c). VCV is rapidly and extensively converted to ACV by intestinal and/or first pass hepatic metabolism subsequent to oral administration (23,24). Although, to-date, VCV has not been used topically, the considerable esterase activity within the skin (25–27), together with the physical chemistry of VCV, suggest that this prodrug may prove to be an ideal candidate for cutaneous iontophoresis.

The iontophoresis of ester prodrugs, in particular lipophilic alkyl ester prodrugs, has been the subject of several studies (28–31). However, the use of an amino acid ester prodrug to augment the charged nature of a drug, and thus its iontophoretic permeation, has not been studied in detail. The concept has been exploited for delivering dehydroepiandrosterone (DHEA); iontophoresis of the ionized glycyl ester of DHEA led to a modest three-fold increase in permeation through rabbit skin, compared to the parent DHEA (32).

The aim of this study was to investigate the iontophoretic delivery of VCV, exploiting its positive charge to facilitate electrotransport across the skin and to rely on cutaneous esterase activity to release increased amounts of ACV at or near the site of infection—the basal epidermis. The prodrug approach would thus be expected to ameliorate treatment of cutaneous herpetic infections by targeting therapeutic levels of drug to this tissue layer without the undue systemic exposure associated with oral and parenteral delivery.

MATERIALS AND METHODS

Materials

ACV and MeCN (Acetonitrile Chromasolv® for HPLC, gradient grade) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Acetaminophen, sodium chloride (NaCl), di-sodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and trifluoroacetic acid (TFA) were purchased from Fluka (Saint Quentin Fallavier, France). VCV.HCl (99.5% purity) was purchased from Sequoia Research Products (Oxford, United Kingdom). All the solutions were prepared using de-ionized water (resistivity >18 MΩ cm).

Porcine ear skin, which is a well-accepted model for human skin (33,34), was used in these studies. Porcine ears were obtained from a local abattoir (Société d'Exploitation d'Abbatage, Annecy, France) a few hours after the sacrifice of the animals. The excised skin was then dermatomed (~750 μm) on the same day and stored at −20°C for a maximum period of up to 2 months.

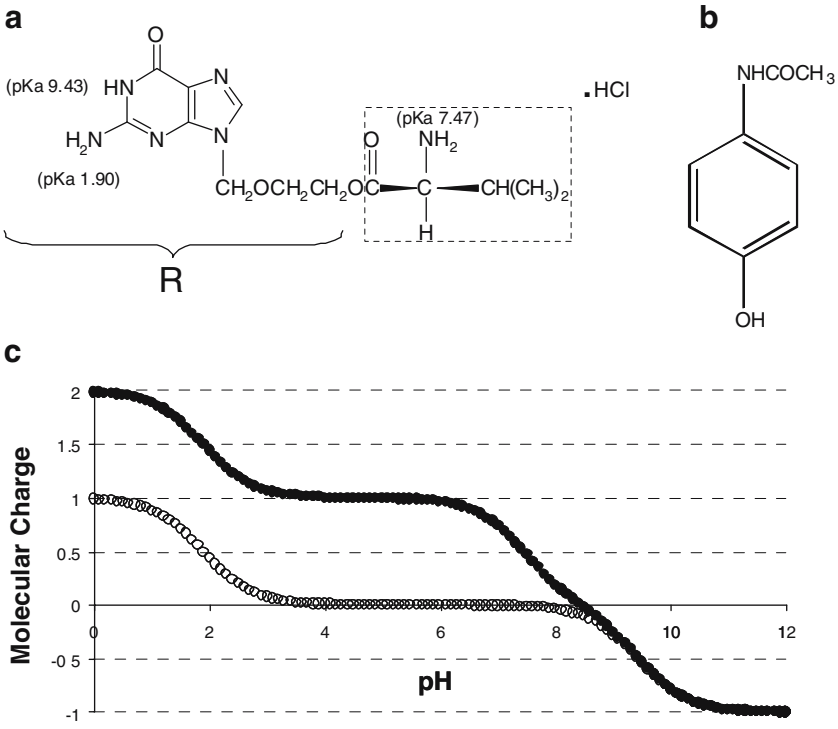

Fig. 1. Structure of (a) VCV (MW 324.4 Da) (R-H represents ACV; MW 225.2 Da) and (b) acetaminophen (MW 151.2 Da). (c) The molecular charge of ACV and VCV (*hollow and filled circles*, respectively) as a function of pH. Under typical iontophoretic conditions (i.e., pH 4–7), the prodrug, VCV, carries a net positive charge unlike its active metabolite, ACV.

Iontophoresis Experiments

Iontophoresis was performed using vertical three-compartment cells. The skin was placed between two half-cells: the upper half, in contact with the SC, comprised two electrode/donor compartments, while the lower receiver compartment was in contact with the dermis. A flow-through system circulated phosphate-buffered normal saline (PBS: 16.8 mM Na_2HPO_4, 1.4 mM KH_2PO_4 and 136.9 mM NaCl; pH 7.4) through the receiver chamber (volume ~4.7 ml) at a rate of 3 ml h^{-1}. Ag/AgCl electrodes were used throughout the study. The skin was allowed to equilibrate for 1 h prior to the iontophoresis experiment. In order to reduce competition from Na^+ ions present in the donor, and thus to increase the permeation of VCV, most experiments were performed using a salt bridge assembly. This strategy consists of physically separating the anodal chamber (Ag electrode immersed in PBS pH 7.4) from the donor compartment (drug solution in contact with the SC) and employing a salt bridge (prepared by filling a 12 cm tubing with a warm aqueous solution of 3% agarose and 0.1 M NaCl which is then allowed to cool) to electrically connect the two chambers. The donor contained either 2 or 10 mM VCV.HCl in water, or 2 mM ACV in 2 mM NaCl. For control experiments without the salt bridge, the anodal compartment contained 1 ml of 10 mM VCV.HCl in PBS pH 7.4. The cathodal and receiver compartment contained PBS pH 7.4 in all the experiments. A constant current of 0.34 mA was applied for 7 h (equivalent to a current density of 0.5 mA cm^{-2}). For passive controls, the donor contained 1 ml of 10 mM VCV.HCl in water or 2 mM ACV in 2 mM NaCl.

Acetaminophen (ACE, C_{ACE} = 15 mM) was included in the donor solution as a marker for electroosmotic flow (Fig. 1b). Being a small, polar and neutral molecule, it is transported mainly by convective solvent flow during iontophoresis. Therefore, its flux (J_{ACE}) can be used to determine the contribution of electroosmosis to the total iontophoretic flux of VCV,

$$J_{EO,VCV} = v_{A\rightarrow C} \cdot C_{VCV} \quad (1)$$

where $J_{EO,VCV}$ represents the contribution of electroosmosis (EO) to VCV delivery; $v_{A\rightarrow C}$ is the convective solvent flow from the anode to cathode (calculated from J_{ACE}/C_{ACE}) and C_{VCV} is the concentration of VCV in the donor compartment. Two assumptions are implicit in this analysis: (a) that drug and acetaminophen are transported in a similar fashion by convective solvent flow, and (b) that electroosmotic transport of the marker molecule is proportional to its concentration in the solvent.

The experiments were performed in sextuplicate, except for passive delivery (n = 3), using the skin of as many different animals as possible. Samples were collected hourly and analyzed by high pressure liquid chromatography (HPLC). The HPLC system consisted of a 600 E Controller pump, an Autosampler Injector 717-plus, an In Line Degasser, and a UV 2487 dual λ Detector (Waters, Saint-Quentin Yvelines, France). The separation was performed on a Nucleosil® 100-5 C18 Nautilus column (L:125 mm; ID: 4.6 mm; Macherey-Nagel, Hoerdt, France) with a 99:1 mixture of 0.1% trifluoroacetic acid (TFA) in water (pH 2.5): MeCN. The flow rate was 1 ml min^{-1}, the temperature was adjusted to 30°C, and 50 µl of sample were injected. ACV, ACE and VCV were separated after 5.6, 14.4 and 19.1 min, respectively, and detected at 252 nm (ACV and VCV) and 243 nm (ACE), enabling simultaneous analysis of the prodrug, drug and electroosmotic marker. The limits of detection/quantification for ACV, VCV and ACE were approximately 0.1/0.3, 0.25/0.8, and 0.2/0.6 µM, respectively.

VCV Stability

The aqueous stability of VCV in the donor formulations was investigated by periodic sampling of solutions (2 and 10 mM in water + 15 mM acetaminophen) over a period of 44 h. In addition, the impact of an electrical current (0.34 mA) on this stability was examined over the same period. The chemical hydrolysis of VCV was also investigated in the presence of PBS pH 7.4. The cutaneous conversion of VCV to ACV via hydrolytic cleavage of the ester bond was verified as follows. Cells were assembled as for an iontophoresis experiment. A 100 µM solution of VCV in PBS pH 7.4 was placed in all the cell compartments and left in contact with the skin (SC and dermis) for 4 h. The compartments were assayed for ACV and VCV immediately afterwards. Finally, the same experiment was performed with a 100 µM solution of ACV, to examine the stability of ACV when in contact with the skin.

RESULTS AND DISCUSSION

VCV Stability

Unbuffered aqueous solutions of VCV (2 mM: pH 5.65 and 10 mM: pH 5.24) were very stable in the presence of acetaminophen for the duration of the investigation (44 h). Less than 1% of the VCV was converted into ACV over this time-period in the absence of a current; the extent of degradation was slightly increased in the presence of a current but nevertheless represented only ~1% over the duration of an iontophoresis experiment (7 h). As mentioned above, VCV as supplied contained small amounts of ACV (0.5%). However, regeneration of ACV from the prodrug was significantly enhanced at physiological pH (in PBS pH 7.4) as previously reported (35) with ~12% of the prodrug being converted over 7 h.

After 4 h of contact with the dermis, VCV was completely hydrolyzed to ACV and only the latter was detected in the receiver (dermal) compartment, at physiological pH. In contrast, after an equivalent contact period with the SC, enzymatic conversion of VCV was not observed. When ACV was similarly tested, the drug placed in both donor and receptor chambers was stable for the duration of the experiment. Although the data indicate that esterase activity is present in the skin tissue despite prior storage of the skin samples at −20°C (as previously reported (36)), the extent of residual activity was not ascertained in the current investigation; it is of course possible that prodrug conversion *in vivo* proceeds with even greater efficiency.

Taken together, these preliminary observations supported the practical feasibility of VCV iontophoresis for enhanced delivery of ACV to the deeper skin layers. Since VCV was unaffected by the SC, it was reasonable to antic-

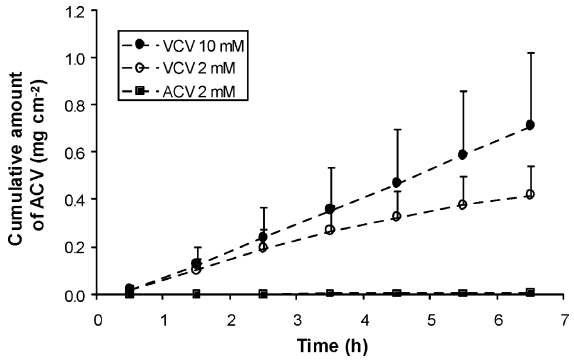

Fig. 2. Cumulative amount of ACV in the receptor compartment after iontophoresis of ACV (2 mM; in the presence of 2 mM NaCl; *filled squares*) and VCV (2 and 10 mM, in the absence of NaCl; *hollow and filled circles*, respectively) at 0.5 mA cm^{-2} across porcine skin *in vitro*. (Mean ± SD; $n = 6$).

ipate that this cationic prodrug could be iontophoretically delivered to the viable epidermis at enhanced rates and levels compared to the uncharged ACV, before being converted to the active moiety—either in the viable epidermis or in the dermis.

Passive Delivery

Neither ACV nor VCV could be measured in the receiver after passive delivery from formulations containing 10 mM VCV.HCl in water or 2 mM ACV in 2 mM NaCl. In view of the analytical limit of detection of ACV in the current study (~0.13 μM), the passive fluxes of ACV and VCV can be considered to be inferior to 1 nmol cm^{-2} h^{-1}. This is not surprising considering their polar nature (calculated log D_{pH7} values are −1.76 and −1.71, for ACV and VCV, respectively (37), and the aqueous formulation used, from which partitioning of these molecules into the lipidic stratum corneum is not favoured. These passive estimates are not dissimilar to the *in vitro* flux values of ACV observed across human (0.24 nmol cm^{-2} h^{-1}) and guinea pig (0.21 nmol cm^{-2} h^{-1}) skin from 5% w/w (~220 mM; 100-fold higher concentration compared to present work) ACV ointment (8). The topical application of a 220 mM ACV formulation to rabbits *in vivo* resulted in dermal concentrations (monitored by microdialysis) that were also below the limit of detection (18). Of course, this is not necessarily indicative of poor skin penetration. Indeed, topical formulations are designed to target the medicament to the site of action within the skin tissue and as such employ quantities which are far inferior to those administered systemically for equivalent effect. Drug reaching the highly vascularized dermal papillary layer subsequent to epidermal permeation is rapidly taken up into the general circulation but is difficult to detect by virtue of the rapid and extensive 'dilution' of these modest drug amounts. This has been demonstrated, to a certain extent, for commercially available topical ACV formulations (Zovirax®), which contain excipients (such as propylene glycol) to facilitate ACV penetration into the SC. In various *in vitro* and *in vivo* model systems, the topical administration of 5% w/w Zovirax® ointment and cream formulations resulted in *total* epidermal levels that far exceeded therapeutic levels but systemic levels that were analytically non-detectable. However, despite therapeutically acceptable epidermal levels of ACV, model predictions suggest that inadequate antiviral levels in the *basal epidermis*—the site of HSV type-1 infection, compromise topical therapy (11). In the experiments described here, ACV delivery was assessed by measurement of receptor levels subsequent to transport of the prodrug or drug across dermatomed porcine ear skin (~750 μm). Hence, although ACV levels at the site of action were not monitored, receptor concentrations adjacent to the dermis were considered to provide a "ballpark" estimate of those in the basal epidermis in view of previously reported skin distribution profiles for ACV (11,17,38).

Iontophoretic Permeation

Though neither ACV nor VCV could be delivered passively across the skin, application of an iontophoretic current (0.5 mA cm^2) enhanced the percutaneous transport of both these molecules, albeit to different extents. In certain isolated cases, minute amounts of VCV (<2% of ACV levels in receptor) could also be detected and measured in the receiver subsequent to VCV iontophoresis, implying incomplete VCV metabolism. In such cases, both ACV and VCV concentrations were summed to determine the total iontophoretic flux of VCV.

As shown in Fig. 2, receptor levels of ACV were significantly greater subsequent to the iontophoresis of VCV compared to that of ACV, when delivered from equimolar formulations (2 mM, 0.5 mA cm^{-2}). Although ACV was not detected in the receiver compartment after 1 h, and only modest amounts were present after 2 h of ACV iontophoresis (0.1 μg cm^{-2}), the cumulative permeation of ACV at the corresponding time-points was much greater upon VCV iontophoresis (20 ± 10 and 104 ± 47 μg cm^{-2}, respectively). After 3 h of current application nearly 200-fold greater levels of ACV (194 ± 82 *vs.* 1.0 ± 0.7 μg cm^{-2}) were monitored in the receptor using a 2 mM solution of the prodrug instead of the parent molecule. The experimental data, therefore, clearly demonstrate that VCV is better suited to iontophoretic delivery relative to its active metabolite, ACV. Moreover, the dissimilar transport kinetics, together with the preliminary hydrolysis assays, indicate that the prodrug is promptly converted to its active metabolite, *after* passage through the stratum corneum in the vicinity of the epidermal and/or dermal tissues. Since topical delivery of a therapeutic agent results in the establishment of a concentration gradient from the SC towards the subcutaneous tissues, we can expect (all else being equal) ACV concentrations in the receptor to reflect those in the basal epidermis.

Although, at first glance, cumulative ACV transport appeared to be enhanced upon increasing donor VCV concentration from 2 to 10 mM, strict comparison was confounded by donor depletion at the lower applied dose. Indeed, at the lower donor concentration, the amount that had reached the receptor after only 4 h was equivalent to 50% of the applied dose. Importantly, the average flux over the first 3 h of current application ($J_{t=0-3h}$) was not significantly different ($p < 0.05$) for the two formulations (2 mM: 87 ± 41 μg cm^{-2} h^{-1}; 10 mM: 108 ± 65 μg cm^{-2} h^{-1}). Given the virtual absence of a lag-phase and rapid attainment

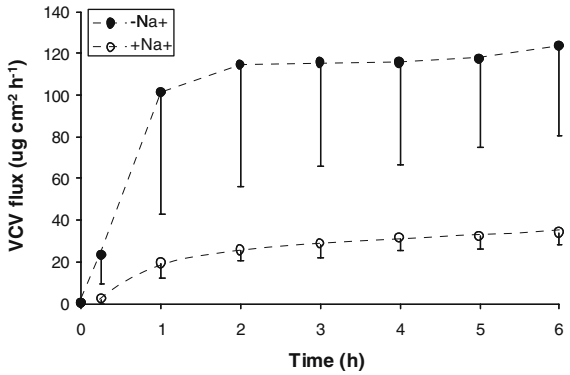

Fig. 3. The influence of competing ions in the 10 mM VCV formulation on VCV flux across porcine skin *in vitro* (estimated from ACV levels in the receptor compartment); the symbols ($+Na^+$, *hollow circles*) and ($-Na^+$, *filled circles*) denote the presence or absence of NaCl in the donor formulation, respectively. (Mean ± SD; $n = 6$).

of steady-state kinetics within the same time-frame for the 10 mM formulation ($J_{t=0-3h} = 108 \pm 65$ *vs.* $J_{t=0-7h} = 115 \pm 52$ μg cm^{-2} h^{-1}) the observed $J_{t=0-3}$ for the lower concentration was also assumed to approximate the steady-state value. That is to say, in the event of donor replenishment, the iontophoretic transport kinetics for VCV at 2 mM were not expected to differ from that observed for the 10 mM formulation. This non-dependence of flux on donor concentration has been previously reported for the hydrochloride salts of hydromorphone, methylphenidate, lidocaine, quinine and propranolol when delivered from formulations deficient in competitive charge carriers, e.g., those originating from buffer systems (39–41).

The efficiency of iontophoretic drug transport is influenced by formulation conditions including (a) donor electrolyte levels—mobile inorganic ions compete very effectively with higher molecular weight drug molecules to carry the current (42) and (b) pH and its effect on the degree of ionization of the drug molecule. The impact of these factors was evaluated in a series of control experiments conducted without a salt bridge assembly where the anodal/donor compartment contained 10 mM VCV.HCl in PBS pH 7.4 (170.5 mM Na^+; 1.4 mM K^+). The resulting fluxes are depicted in Fig. 3, which clearly shows the reduction in transport rates as competing ion levels and pH are increased (VCV steady-state flux decreased from ~120 to ~30 μg cm^{-2} h^{-1} in the presence of background electrolyte). In the PBS formulation, the mobile Na^+ ions were present in ~17-fold excess to the VCV; furthermore at pH 7.4, the degree of ionization of the latter is only 53% (cf. >99% at pH 5.2). The iontophoretic flux (J_{TOT}) of a charged species is considered to be the sum of two separate transport processes—electromigration (J_{EM}) and electroosmosis (J_{EO}), assuming negligible passive skin permeability (43):

$$J_{TOT,VCV} = J_{EM,VCV} + J_{EO,VCV} \quad (2)$$

Since the EO contribution, $J_{EO,VCV}$, was obtained as described in the Materials and Methods (using Eq. 1), the EM contribution, $J_{EM,VCV}$, could be calculated by subtracting $J_{EO,VCV}$ from the total flux, $J_{TOT,VCV}$ (using Eq. 2). Calculation of the respective EM and EO contributions to VCV transport under the different formulation conditions showed that VCV electromigration, $J_{EM,VCV}$ (after 7 h current application) decreased from 118.9 to 24.6 μg cm^{-2} h^{-1}, on passing from 10 mM VCV.HCl in unbuffered aqueous solution to 10 mM VCV.HCl in PBS at pH 7.4. This significant decrease could not be compensated by any increase in electroosmosis elicited by increasing formulation pH ($J_{EO,VCV}$ was 4.5 and 9.2 μg cm^{-2} h^{-1}, in unbuffered aqueous solution (pH 5.24) and in PBS at pH 7.4, respectively).

The iontophoretic delivery of ACV across human and nude mouse skin has been previously investigated at pH 3.0 (20% ionized) and at pH 7.4 (2% ionized) (16,17). The donor concentration-normalized ACV fluxes across porcine skin at pH 6.3, in the present study (2.4×10^{-3} cm h^{-1}) over 7 h of ACV iontophoresis compare favourably with those across human skin at physiological pH (2.7×10^{-3} cm h^{-1}) (17). In the studies with human skin, the distribution of ACV across various skin layers was also examined; although iontophoresis generally enhanced *total* skin concentrations of ACV, only formulations at pH 3.0 produced a significant impact on the *epidermal* levels of the antiviral agent. It should be noted that, in the current study, the donor concentration-normalized flux measured for the 2 mM VCV formulation over the initial 3 h of current application is approximately 50 times greater (0.13 cm h^{-1}) than the corresponding value for ACV.

Mechanism of Iontophoretic ACV and VCV Transport

The mechanism governing the iontophoretic transfer of each molecule across the skin was evaluated by virtue of the simultaneous measurement of acetaminophen (44), a neutral molecule, transported principally by electroosmosis (EO). While ACV was transported across the skin entirely by EO, delivery of the prodrug was due almost entirely to electromigration (EM). The two distinct transport mechanisms for each molecule reflect their ionization state in the respective donor formulations. The unbuffered ACV and VCV formulations had pH values of ~6.3 and 5.7, respectively; under these conditions, ACV is effectively uncharged (>99.9% neutral) while VCV is fully protonated in the donor phase. Consequently, the transport of uncharged ACV is mediated by convective solvent movement, whilst that of the protonated VCV is facilitated by electromigration from the anodal electrode. When donor electrolyte levels were increased, the EM contribution to the total VCV flux was reduced from 96 to 73%, due to competition from Na^+ ions in the formulation to transport charge across the membrane.

As Fig. 4 shows, in the absence of competing ions ($-Na^+$), acetaminophen transport (reflecting the EO contribution) was significantly influenced by donor levels of VCV ($p < 0.05$). Basal levels of acetaminophen transport were unaffected by the presence of either 2 mM VCV or ACV or 10 mM VCV in the presence of NaCl ($p < 0.05$). However, when NaCl was removed from the VCV formulation, and drug transport accounted for a greater proportion of current passage, acetaminophen transport decreased significantly ($p < 0.05$). This suggests that the increase in VCV transport, resulting in greater amounts of

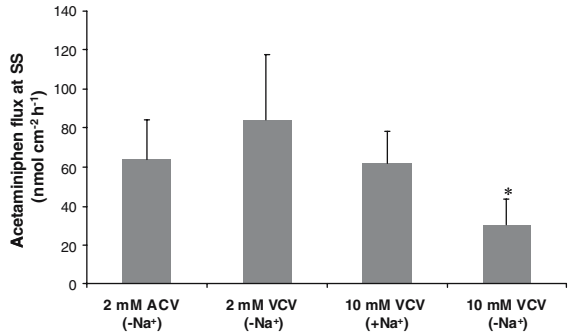

Fig. 4. Acetaminophen flux (J_{ACE}) reported on the effect of VCV iontophoresis on skin permselectivity. A statistically significant decrease in J_{ACE} and hence electroosmotic solvent flow, was only observed using the 10 mM VCV formulation in the absence of NaCl in the donor compartment (ANOVA, $p < 0.05$). The symbols ($+Na^+$) and ($-Na^+$) and denote the presence or absence of NaCl in the donor formulation, respectively. (Mean ± SD; $n = 6$).

VCV entering into the membrane, facilitated interactions with the skin that reduced convective solvent flow, as has been demonstrated previously for other cationic species (41,44). This phenomenon tends to have a negative impact on total iontophoretic transport (e.g., in the presence of background electrolyte, flux may not be linearly proportional to applied dose), and is considered to reduce the benefits of "controlled-delivery" generally offered by iontophoresis. However, for molecules such as VCV, which are overwhelmingly transported by EM (~96%), the impact of modifying EO on overall transport is virtually imperceptible.

The studies described here were conducted with porcine skin, therefore, we have to consider how these would correlate with their human equivalent. Although species dependent differences in hydrolysis rates have been observed for certain molecules, the hydrolysis of several structurally different ester derivatives suggests that human skin possesses considerable esterase activity (45–50): (a) Stinchcomb *et al.*, demonstrated that several 3-alkyl ester prodrugs of buprenorphine were competely hydrolysed during passage through human skin (45); (b) Bundgaard *et al.*, found that a series of acyloxymethyl ester prodrugs of nalidixic acid were completely hydrolysed during transit through human skin (46); (c) Nicolau and Yacobi showed that the prostaglandin analogue, viprostol, was 66% hydrolyzed to the free acid in human skin (47) (d) Seki *et al.*, found that the hexanoate ester of zidovidudine was completely hydrolyzed upon passage through human skin (however, the acetate derivative was more resistant to cleavage) (48); (e) studies have also shown that both animal and human skins are capable of hydrolyzing corticosteroid 21-monoesters (49); (f) similarly, glycerol trinitrate was found to be extensively hydrolyzed to the 1,2 and 1,3 dinitrate derivatives following application to human skin *in vitro* (50). Täuber and Rost have also demonstrated that in human skin, esterase activity in the epidermis (calculated per mg of tissue wet weight) was ~10-fold higher than that in the dermis (49), suggesting that there would be considerable VCV metabolism and its biotransformation into the active metabolite, ACV, during its transit through the epidermis and before reaching the basal layers—the site of infection.

Therapeutic Advantages of VCV Iontophoresis

Previous studies have shown that the iontophoretic administration of ACV across human skin results in epidermal concentrations (~80 μg cm^{-3}), which are well above those required for viral inhibition (17). Nevertheless, since iontophoretic efficiency is dependent on the electrical mobility of the drug, the electrotransport of charged species is clearly favoured; for ACV, this is only possible in relatively acidic formulations ($pK_a = 2.27$). In contrast, the dissociation profile of VCV is much more conducive to iontophoretic delivery, allowing anodal delivery from formulations at more physiologically acceptable pH (Fig. 1c). Indeed, our results show that VCV iontophoresis for 3 h is almost 200-times more efficient compared to iontophoretic ACV delivery under equivalent conditions. In view of the comparable ACV transport across porcine skin in the present investigation and that previously observed across human skin (17), which produced therapeutic levels *within*

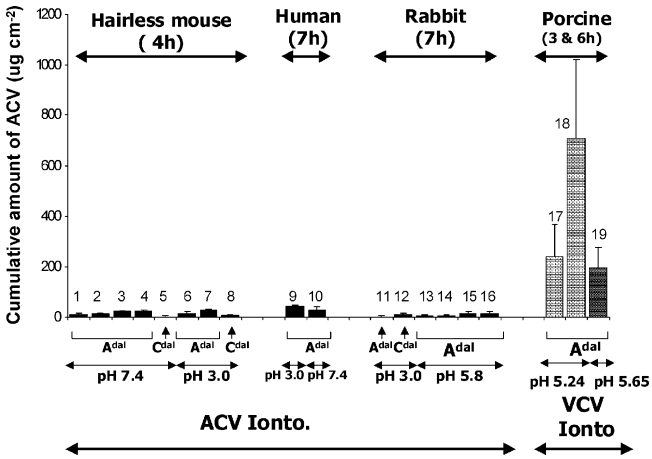

Fig. 5. Comparison of cumulative ACV permeation at steady state following ACV iontophoresis across hairless mouse (after 4 h, (16)), human (after 7 h (17)) and rabbit (after 7 h (19)) skin and VCV iontophoresis across porcine skin (after 3 and 6 h, this study). Columns 1–4 shows the cumulative ACV amounts after anodal iontophoresis at pH 7.4 (7.73 mM) at current densities of 0.18, 0.25, 0.36 and 0.5 mA cm^{-2}, respectively; column 5 shows the results after cathodal iontophoresis at pH 7.4 (7.73 mM) at 0.5 mA cm^{-2} across hairless mouse skin *in vitro*. Columns 6 and 7 show cumulative ACV amounts after anodal iontophoresis at pH 3.0 (6.88 mM) at current densities of 0.25 and 0.5 mA cm^{-2}, respectively; column 8 presents the results after cathodal iontophoresis at pH 3.0 (6.88 mM) at 0.5 mA cm^{-2}. Columns 9 and 10 represent the cumulative ACV permeation across human skin after anodal iontophoresis at pH 3.0 (6.88 mM) and pH 7.4 (7.73 mM), respectively, at a current density of 0.5 mA cm^{-2}. Columns 11 and 12 display anodal and cathodal ACV delivery, respectively, across rabbit skin (pH 3.0, 6.2 mM) at 0.125 mA cm^{-2}. Columns 13–16 show cumulative ACV permeation across rabbit skin from a formulation at pH 5.8 (4.2 mM) after anodal iontophoresis at 0.06, 0.125, 0.25 and 0.5 mA cm^{-2}, respectively. Columns 17 and 18 show the cumulative ACV permeation after 3 and 6 h, respectively, of VCV iontophoresis (pH 5.24, 10 mM, this study) at a current density of 0.5 mA cm^{-2}. Column 19 shows the cumulative ACV permeation after 3 h of VCV iontophoresis (pH 5.65, 2 mM, this study) at a current density of 0.5 mA cm^{-2}. The data confirm that cumulative ACV delivery is significantly more efficient following VCV iontophoresis.

the skin, the therapeutic superiority of VCV iontophoresis is evident. Figure 5 compares the cumulative amount of ACV permeated across hairless mouse (16), human (17) and rabbit (19) skin after ACV iontophoresis under a series of experimental conditions to that observed in the present study upon VCV iontophoresis across porcine skin. It demonstrates that ACV permeation was significantly greater following VCV iontophoresis using milder formulation conditions. A further advantage offered by the electrically mediated delivery of the prodrug is the rapidity with which steady-state kinetics are achieved, permitting short application times, which would obviously be a significant advantage in a therapeutic system (Fig. 3).

Given the iontophoretic transport efficiency of VCV (transport number ~0.03, calculated from $J_{EM,VCV}$ after iontophoresis for 7 h), it is clear that clinically efficacious dosing is likely to be achieved with significantly reduced doses and/or more innocuous currents. For example, in view of the results here, a 10-fold reduction in applied current density (to 0.05 $mA.cm^{-2}$, a value 10-fold lower than the so-called "limit of tolerability" (51)) is still likely to achieve skin concentrations well above the therapeutic levels previously reported.

CONCLUSIONS

This study demonstrates, unequivocally, the superiority of VCV iontophoresis over that of the parent molecule, ACV, to deliver the latter to the skin tissues. In doing so, it illustrates the pivotal role of drug physical chemistry in cutaneous iontophoretic transport and serves to emphasize the impact of drug design on therapeutic efficiency. Though drugs are rarely custom-designed for the chosen route of administration, it is clear that relatively minor chemical modifications can dramatically alter their transport behaviour across a given biological membrane.

With respect to topical applications, the presence of esterase and peptidase activity (principally) in the viable skin layers and, therefore, *after* passage across the rate-limiting stratum corneum, (27,36,45–50,52,53) opens the door for many iontophoretic applications using prodrugs comprising peptide or ester bonds which are susceptible to enzymatic hydrolysis.

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