Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 274 (2004) 201–211
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmacokinetics of acyclovir in rabbit skin after IV-bolus, ointment, and iontophoretic administrations

Grazia Stagni*, Md Ehsan Ali, Daniella Weng

Division of Pharmaceutics and Industrial Pharmacy, Arnold and Marie Schwartz College of Pharmacy, Long Island University, 75 DeKalb Avenue, Brooklyn, NY 11201, USA

Received 17 September 2003; received in revised form 16 December 2003; accepted 13 January 2004

Abstract

The aim of this study was to characterize and compare the pharmacokinetics of acyclovir (ACV) in skin and plasma after iontophoresis, IV-bolus, and ointment administrations in rabbit. On five occasions, each separated by at least 1-week washout, rabbits received a 10 mg/kg dose of ACV as IV-bolus, ACV iontophoresis for 1 h at different current densities (100, 200, 300 $\mu A/cm^2$) or a commercially available ointment for two hours. Blood samples were collected serially up to 6 h. Skin ACV concentrations were monitored via microdialysis using linear microdialysis probes (1 cm window). Cathodic iontophoresis was performed using commercially available patches (10 cm^2 contact area). Following IV-bolus, C_{max} in skin occurred with a delay of 38 ± 4 min compared with plasma. No quantifiable concentration of ACV was detected in the skin on passive drug delivery. Following iontophoresis, skin exposure to ACV was 40, 22, and 11% of that following IV-bolus. Conversely, systemic exposure to ACV was negligible and plasma concentrations were below the limit of quantification at any time-point. In skin dialysate, C_{max}, AUC, and half-life increased with current density. During ointment application, ACV in dermis was detectable only for the first 30 min thereafter ACV skin concentrations were below the LOQ (30 ng/ml).

Keywords: Acyclovir; Iontophoresis; Microdialysis; Rabbit; Pharmacokinetics; Skin

1. Introduction

Microdialysis is a technique used to monitor in vivo the concentration time course of drugs and endogenous substances in tissue's extracellular fluid (Stahl et al., 2002). Among several applications, microdialysis has gained popularity in the past few years to study drug pharmacokinetics in dermis (Ault et al., 1992; Benfeldt, 1999). Dermis is the target of several diseases and the site from which a drug is absorbed into blood from transdermal delivery systems. Some of the advantages of microdialysis over existing methods to study pharmacokinetics in skin are the capability to perform sampling at the same site for several hours, the physiological conditions of the sampling, and the low invasiveness of the method (Groth, 1998). The most relevant limitation is the fact that the concentration detected in the dialysate is proportional and not equal to the actual extracellular concentration. Therefore, if the study requires the knowledge of an accurate estimate of the extracellular fluid concentration, a standardization method is required (Krogstad et al.,

* Corresponding author. Tel.: +1-718-488-1231; fax: +1-718-780-4586.
E-mail address: gstagni@liu.edu (G. Stagni).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.01.024

1996). A better understanding of the pharmacokinetic of drug in skin would promote the development of improved therapy schedules, drug formulations, or molecular structure for drugs meant to act on skin diseases. In addition, the estimation of pharmacokinetics both in dermis and in plasma would help to sort out the role of skin in transdermal delivery.

Acyclovir (ACV) is an antiviral agent used in the treatment of Herpes simplex infection that occurs at the lowest epidermis. ACV is commercially available for intravenous, oral, and topical administration. Oral bioavailability is low ($F = 20\%$) and its dipeptide prodrug valacyclovir is now preferred for oral therapy. Systemically administered ACV may cause mild to severe side effects (e.g., nephrotoxicity). The availability of topical formulation is therefore desirable. However, the high polarity of the compound makes topical delivery difficult and the efficacy of local administration has been questioned (Parry et al., 1992). Iontophoretic delivery of ACV was shown effective in the treatment of localized herpetic lesions in 1984 (Henley-Cohn and Hausfeld, 1984). Iontophoresis is a penetration enhancer technique that uses an electrical gradient as a driving force for the delivery of electrically charged drugs into the skin (Banga, 1998).

In the present work, microdialysis was used to study the dermatopharmacokinetics (Shah, 2001) of acyclovir in rabbit skin following three different types of delivery: IV-bolus, iontophoresis, and topical ointment. Microdialysis is particularly appropriate to study electrically assisted delivery systems like iontophoresis because both microdialysis and iontophoresis perform better with water-soluble molecules. Microdialysis recovery is not affected by the presence of iontophoretic current (Stagni et al., 1999) and can be safely used to study the pharmacokinetics in dermis of drugs delivered by iontophoresis (Stagni et al., 2000). The selection of the drug ACV for this study was determined by the fact that the therapeutical site of action of ACV is at the interface between epidermis and dermis therefore the measure of dermis pharmacokinetics is therapeutically relevant. Iontophoresis was performed under the simplest condition that is using a commercially available patch and a standard ACV formulation for I.V.-injection. The results of this study will provide a more thorough understanding of the iontophoretic process in vivo.

2. Materials and methods

2.1. Chemicals and reagents

All chemical were analytical grade or higher in quality. Acyclovir 99% for standard solutions, monobasic and dibasic sodium phosphates were purchased from Sigma Chemical, CO (St. Louis, MO). Acyclovir sodium for injection (1000 mg/20 ml) was from American Pharmaceutical Partners Inc. (Los Angeles, CA). Zovirax® ointment (50 mg/g) was from a local Pharmacy. Acepromazine® was from Boehringer Ingelheim Vetmedica, Inc. (St. Joseph, MO). HPLC grade water was from Mallinckrodt, Inc. (Phillipsburg, NJ). Acetonitrile and Perchloric acid were purchased from EM Science (Gibbstown, NJ); Lactated Ringer's solution USP was from B. Barun, (Irvine, CA).

2.2. HPLC assay

Acyclovir concentrations were determined using a modified reverse phase HPLC method (Hedaya and Sawchuk, 1990). The chromatographic apparatus consisted of Waters™ 717 Autosampler, Hitachi L-4250 UV-Vis Detector, Hitachi L-6200 Intelligent Pump, and a PE Nelson 900 series interface. The chromatography was performed on a C_{18} (Discovery, Supelco, 10 cm × 4.6 mm, 5 μm, Bellefonte, PA) column equipped with a 1 cm guard column (Upchurch Scientific C-750, 5 μm). Data acquisition and chromatographic analysis were carried out using a Turbochrom Nevigator Version 4.1(1L22). Detection wavelength was 254 nm. The isocratic flow rate was 1.2 ml/min. The mobile phase consisted of 97%, 0.01 M phosphate buffer (pH 6.78) and 3% acetonitrile for dialysate samples. The mobile phase for plasma samples consisted of 99.5%, 0.01 M phosphate buffer (pH 6.78) and 0.05% acetonitrile. The mobile phase was modified for plasma samples to allow the separation of the ACV peak from the peak of an endogenous substance that was not present in skin dialysates. Microdialysis samples were injected directly onto the column. Injection volumes were 10 μl for samples collected after iontophoresis or IV-bolus and 70 μl for dialysate samples collected after ointment applications. In plasma samples (500 μl) proteins were precipitated by adding 50 μl of 35% (v/v) freshly prepared

perchloric acid and thoroughly mixing by vortexing for 1 min (Boulieu et al., 1991). Samples were then centrifuged at 11,000 rpm for 6 min at room temperature and the supernatants were transferred to 0.2 ml vials for injection. A 100 μl aliquot was injected. Extraction efficiency tested versus distilled water was 100%. Typically, the retention time was 3.5 min for microdialysis samples and 6.8 min for plasma samples. The CV (%) for inter-day dialysate assays at 30 ng/ml (lowest limit of quantification, LLOQ) and 20,000 ng/ml were 6.4 and 0.2, respectively. The CV (%) for inter-day plasma assays at 100 ng/ml (LLOQ) and 20,000 ng/ml were 13 and 0.3, respectively.

2.3. Microdialysis system

The microdialysis system consisted of a CMA/102 microdialysis pump (CMA/Microdialysis AB, Stockholm, Sweden) equipped with 2.5 ml EXMIRE micro syringe type I (ITO Corporation, Fuji, Japan) and a CMA/142 fraction collector (CMA/Microdialysis AB, Stockholm, Sweden). Disposable microdialysis probes (Fig. 1) were made in our laboratory according with the procedure described by Stagni et al. (1999). They consisted of two 7–8 cm segments of polyimide tubing, 200 μm diameter (MicroLumen, Tampa, FL), connected by a tubular microdialysis membrane with a 20 kDa molecular cutoff (Spectrum, Medical Industries, Inc., Los Angeles, CA). The microdialysis membrane was fixed to the polyimide tubes by cyanoacrylate glue leaving a membrane window 1 cm long. An internal stainless steel wire (0.0015 in. diameter, Molecu-Wire Corp., Wall Township, NJ) reinforced the probes.

2.4. In vitro microdialysis

The concentration detected in the dialysates is always lower than the actual extracellular fluid concentration, because the continuous flowing of fluid throughout the probe does not allow the reaching of a thermodynamic equilibrium with the peri-probe environment. Therefore, it is necessary to estimate the recovery of the probe to calculate the actual extracellular fluid concentration of the analyte (Kehr, 1993). There are two ways to perform microdialysis. One, called simply “microdialysis” is when the probe is perfused with a solution without the analyte and is used to recover the analyte from a bulk solution (in vitro) or a tissue (in vivo). In this case, the recovery of the probe is the ratio between the concentration of the analyte in the dialysate and that in the bulk solution. The other way, called “retrodialysis”, is when the analyte is added to the perfusing solution and the probe is placed in a solution (in vitro) or a tissue (in vivo) that does not contain the drug. In this case, the analyte is lost from the perfusing solution and the concentration in the dialysate is lower than that in the initial solution. The ratio between the difference of the concentrations and the initial concentration is also called recovery. Theoretically, the recovery estimated by either method should be equal (Krogstad

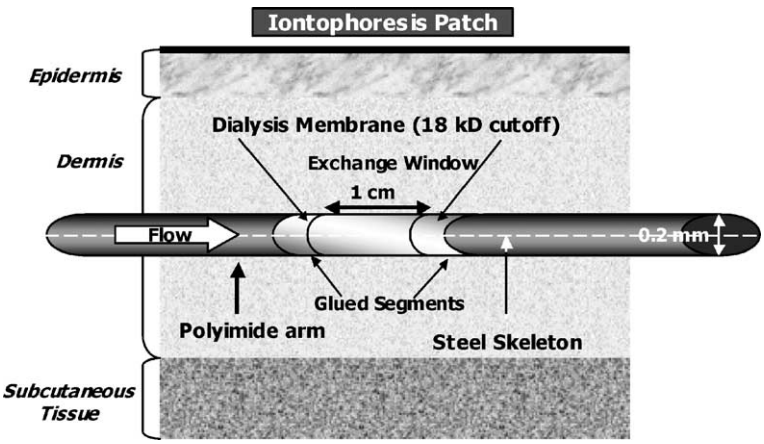

Fig. 1. Schematic representation of the microdialysis probe and of its location in the skin.

et al., 1996). Usually, when the microdialysis method is applied in vivo the concentration in the tissue is unknown, and the retrodialysis method is used to estimate the recovery, under the assumption that the two are equal. This in vitro study allows validating the assumption that the retrodialysis method can be used in vivo to estimate microdialysis relative recovery of ACV.

One microdialysis probe was inserted in the inner chamber (2 ml total volume) of a custom-made glass cell, donated by Novartis (Somerville, NJ) in such a way that the membrane window was placed exactly in the center of the chamber. A circulator (Vanderkamp, Edison, NJ) maintained the temperature of the circulating water in the cell jacket at 37 °C to mimic the skin temperature. The solution in the cell was stirred continuously with a star headed magnetic stirrer. The microdialysis flow rate was 3 μl/min and sampling was carried out at 8 min interval for 64 min. When studying recovery from bulk solution, the cell was filled with 2 ml of the following concentrations of acyclovir in Lactated Ringer's solution: 0.100, 0.400, 0.800, 1000, 5000, 10,000, 20,000 μg/ml; and the probe was perfused with blank Lactated Ringer's solution. Relative recovery (RR) was calculated as the slope of the linear regression of drug concentration in the dialysate ($C_{dialysate}$) and drug concentration in the cell (C_{bulk}):

$$C_{dialysate} = RR \cdot C_{bulk} \quad (1)$$

For the retrodialysis studies, the cell was filled with a blank Lactated Ringer's solution and the probe was perfused with 0.100, 0.400, 0.800, 1000, 5000, 10,000, 20,000 μg/ml concentrations of acyclovir in Lactated Ringer's solutions. Recovery was calculated as:

$$C_{perfusate} - C_{dialysate} = RR \cdot C_{perfusate} \quad (2)$$

where $C_{perfusate}$ is the concentration of the perfusing solution.

2.5. Iontophoresis

The iontophoresis delivery system consisted of pH stabilized, disposable electrodes (TransQ^{E}; IOMED, Inc., Salt Lake city, Utah) with a contact surface area of 10 cm^2 and a constant current source (Phoresor II, Model no PM 700, IOMED, Inc., Salt Lake City, Utah). Using a syringe, the GelSponge® pad was saturated with 1.5 ml of acyclovir sodium injection containing 75 mg of acyclovir. The dispersive pad was then applied on the skin. The drug delivery electrode was applied about 4 in. away from the dispersive pad on top of one of the microdialysis probes. Both the pads were applied in such a way that the entire surface area was in contact with the skin. Also, particular care was paid to assure that the inlet and the outlet of the probe where always outside the patch. Excess pressure was avoided on the drug delivery pad to prevent leaking of the medication and no tapes were used to stick the pads to the skin. The cathode from the iontophoresis current device (Phoresor II) was connected to the acyclovir-delivery electrode since acyclovir is negatively charged at the pH of the solution and the anode was attached to the dispersive pad.

2.6. In vivo studies

The institutional Animal Care and Use Committee (IACUC) at Long Island University, Brooklyn, New York, approved all the animal procedures. The experiments were performed in four female, pathogen free New Zealand albino rabbits weighing 3.5–5.0 kg. The rabbits were housed under standard laboratory conditions (22 ± 1 °C; relative humidity 40–60%) and were fed with normal rabbit chow and provided with regular drinking water from the tap. At the time when the experiments were performed, the animal's age ranged from 3 to 6 months. Each rabbit received the following four treatments according to a randomized, crossover design (separated by at least one week washout period): one IV-bolus administration (10 mg/kg) and three transdermal Iontophoresis administered for a total of 60 min on different occasions (Current density: 100, 200, or 300 μA/cm^2, respectively). Passive delivery of acyclovir from the iontophoretic patch (0 μA/cm^2 current density) was tested in one experiment. Three rabbits also received Zovirax® ointment (50 mg/g). 3M® wall-mounting tapes were used to delimit the ointment application area to 10 cm^2. The resulting window was filled with one gram of the ointment and left in place for 2 h. Microdialysis samples were collected every 30 min over a 6-h period. No blood samples were collected.

The day before the experiment, the dorsum of the rabbits was shaved carefully with an electrical animal hair clipper. On the day of the experiment, rabbits were tranquilized with 1 mg/kg I.M. acetopromazine maleate injection (ACE) and allowed a 20-min period for the tranquilization to take place. Two microdialysis probes were implanted according to the technique described by Stagni et al. (2000) as superficially as possible, using a 25 G × 1.5 in. needle as a guide. Actual depth of the probe could not be measured because the appropriate instrumentation was not available. However, the needle was carefully inserted under the skin in such a way that consistently resulted in the needle being clearly visible through the superficial skin layer. In these conditions, the depth of the probe is not greater than approximately 2 mm that corresponds to the deep dermis. After about 45 min to allow the skin to recover from the insertion trauma, the probes were connected to the pump via Teflon tubing and perfused at a constant flow rate of 3 μl/min. Microdialysis samples were collected every 8 min for 6 h. Microdialysis samples were analyzed the same day without any further extraction. Blood was collected from the auricular artery into K_2-EDTA tubes and centrifuged at 3000 rpm, 5 °C for 10 min, immediately after collection. The plasma was separated, frozen, and stored at −25 °C until assayed. A 1 ml blood sample was collected immediately before dosing and then at 15, 30, 45, 60, 65, 70, 80, 100, and 120 min during and after iontophoresis. Sampling schedule for the IV-bolus was 5, 10, 15, 20, 30, 60, 120, 180, 240, 300, and 360 min after drug administration. Retrodialysis was performed at the end of the experiment at the iontophoresis site or during the experiment at the second microdialysis probe, once it was verified that iontophoretic delivery did not produce detectable concentrations in the skin at sites other than under the iontophoresis patch. The probes were perfused with 5 μg/ml acyclovir solution. The retrodialysis samples were collected over 8 min interval for a period of 100 min, and analyzed the same day without any further extraction. When available, the recovery estimated from the same probe was used to calculate the actual extracellular acyclovir concentration. At the end of the IV-bolus experiment, retrodialysis could not be performed due to the time length of the experiment. Hence, the average of all the recoveries (12.5%) from the other experiments was used to estimate the actual extracellular skin concentrations after the IV-bolus experiments.

2.7. Data analysis

Statistics analysis was performed with SPSS 9.0 for Windows. Pharmacokinetics analysis was performed with WinNonlin Professional 4.0.1 (Pharsight Corporation, Mountain View, CA).

Plasma data obtained from IV-bolus were fitted with a bi-exponential model (Gibaldi and Perrier, 1982) ($C = Ae^{-\alpha t} + Be^{-\beta t}$) using the Gauss–Newton minimization algorithm. The following parameters were estimated A, B, α (the distribution rate constant), β (the elimination rate constant), area under the plasma concentration curve (AUC), volume of distribution at steady state (V_{ss}), and total body clearance (CL). Dialysate skin data were corrected by the recovery value calculated by retrodialysis to estimate the actual peri-probe concentration of acyclovir in the skin. Pharmacokinetic analysis on skin data collected from IV-bolus and iontophoresis delivery was performed using a non-compartmental approach (Gillespie, 1991). The following parameters were estimated: elimination rate constant (lambda-z), elimination half-life ($t_{1/2-\text{lambda-}z}$), area under the skin concentration curve (AUC), maximum skin concentration (C_{max}), time to maximum skin concentration (T_{max}), and the time delay in the appearance of ACV in skin (t-lag). Area under the concentration curve for the ointment application was estimated by multiplying the average concentration detected over each sampling interval by the length of such interval (30 min), and adding them together.

3. Results

3.1. In vitro/in vivo microdialysis recovery studies

The in vitro recovery of acyclovir estimated from the bulk solution (Eq. (1), mean ± standard deviation) was 31 ± 7% ($r^2 > 0.99$) and in vitro recovery estimated from retrodialysis (Eq. (2)) was 30 ± 9% ($r^2 > 0.99$). Both "dialysate" versus "bulk" concentrations and "loss" versus "perfusing" concentrations exhibited a linear relationship at the concentration tested demonstrating no dependence of recovery from concentration

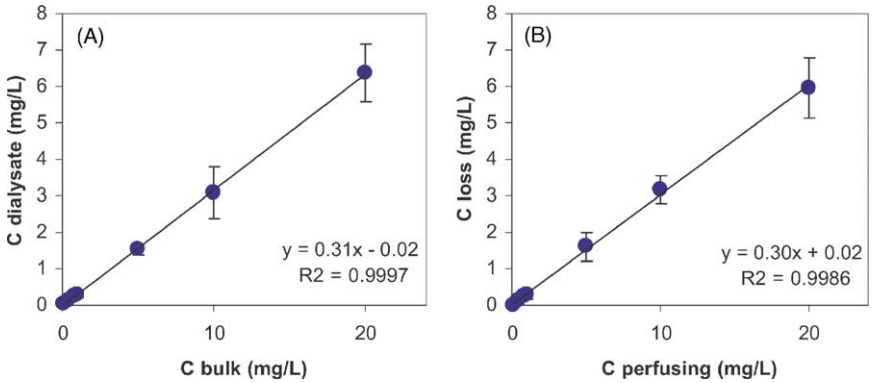

Fig. 2. (A) Plot of ACV concentrations (mean ± S.D.; $n = 12$) recovered in the dialysate versus the respective bulk concentrations. The slope of the line represents the recovery (Eq. (1)). (B) Plot of the difference (C_{loss}; mean ± S.D.; $n = 12$) between the ACV concentration entering the probe (C_{in}) and the ACV concentration leaving the probe (C_{out}) when the probe was immersed in plain Ringer's solution. The slope of the line represents the recovery (Eq. (2)). The linearity of both plots shows that recovery is not concentration dependent in the range of concentrations tested.

(Fig. 2). In vivo recovery estimated using the retrodialysis method (Eq. (2)) was 12.5 ± 3.5% with a range of 9.4–18.5%.

3.2. In vivo experiments

3.2.1. General

Rabbits did not show any signs of intolerance for the microdialysis probes during the 7–8 h long experiments. Microdialysis probes were easily inserted in the skin without local anesthesia. Visual inspection of rabbit skin responses at the end of the experiments and after 24 and 48 h showed that iontophoresis did not induce any severe irritation of the skin such as rash, erythema, or edema.

3.2.2. IV-bolus

Fig. 3 shows the average acyclovir concentration–time profiles in plasma and skin, after IV-bolus administration. All the plasma concentration–time profiles showed a remarkable distribution phase and were fitted with a bi-exponential equation. Relevant pharmacokinetic parameters estimated in plasma for the IV-bolus are reported in Table 1. Skin concentration data did not follow clearly any compartmental model. Data from rabbits #1 and 2 exhibited a bi-exponential decay after reaching the peak concentration, while data from rabbits #3 and 4 showed a mono-exponential decay. Therefore, skin concentration data were analyzed by a non-compartmental approach. Table 2 reports the relevant parameters estimated for skin data. Two data sets were available for each IV-bolus administration because a microdialysis probe was inserted at two different sites on the rabbit dorsum. Only in one case, one of the probes broke during the experiment and collection of data was not completed. There was no statistically significant ($P > 0.05$) difference between the data collected at the two sites as demonstrated by a *t*-test performed between the two sets of data. The peak concentration in the skin occurred with an average delay of 38 ± 4 min. The

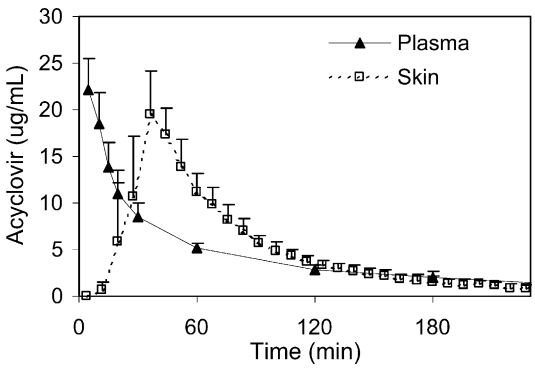

Fig. 3. Acyclovir concentration (mean±S.D.) versus time following IV-bolus in rabbit plasma ($n = 4$) and skin ($n = 7$). The skin concentrations reported are estimated from dialysate concentrations corrected by the recovery factor (Eq. (1)).

Table 1
Acyclovir pharmacokinetics parameters estimated in plasma following the administration of IV-bolus

Parameter ($n = 4$)	Mean ± S.D.
A (mg/l)	23 ± 5
Alpha (min^{-1})	0.074 ± 0.023
B (mg/l)	6.9 ± 0.64
Beta (min^{-1})	0.007 ±0.002
$t_{1/2}$ alpha (min)	10 ± 3
$t_{1/2}$ beta (min)	104 ±29
C_0 (mg/l)	30 ± 4
Clearance (ml/min)	38 ± 7
V_{ss} (ml/kg)	4.343 ± 425
AUC (mg/l min)	1.351 ± 313

terminal elimination half-life was greater in plasma than in skin (104 ± 29 min versus 48 ± 11 min).

3.2.3. Iontophoresis

Plasma concentrations collected during and after the iontophoresis treatments were below the LLOQ at all time points. Therefore, no pharmacokinetics analysis was done on plasma data. Skin dialysates showed that without the administration of electrical current, passive delivery of ACV did not produce any detectable concentration in skin when an iontophoresis patch filled with the acyclovir solution was applied for 60 min. In addition, no acyclovir was detected in skin dialysates at the control site during iontophoresis delivery, showing that the amount of drug absorbed into the systemic circulation and redistributed to the skin was negligible. Acyclovir was detectable in the dialysate under the iontophoresis patch during the delivery of electric current. Fig. 4 shows the average skin concentrations corrected for microdialysis recovery following the iontophoretic treatments. Acyclovir concentrations in skin increased immediately with the current delivery. However, dialysate concentrations did not decreased sharply after the current was stopped and the skin-concentration profiles showed an "absorption phase" lagging behind the discontinuation of current (Fig. 4). In some cases, the peak concentration was reached after the stop of current administration. Because these pharmacokinetic profiles did not follow any conventional model, a non-compartmental pharmacokinetic analysis was performed on skin dialysate data from the iontophoresis experiments. Table 3 lists the relevant pharmacokinetic parameters estimated for this administration.

Table 2
Acyclovir pharmacokinetics parameters estimated in skin (data corrected for microdialysis recovery) following administration of IV-bolus

Parameter	Mean ± S.D. ($n = 7$)
Lambda-z (min^{-1})	0.015 ± 0.004
$t_{1/2}$-Lambda-z (min)	48 ± 11
AUC (mg/l min)	1194 ± 245
C_{max} (mg/l)	20 ± 3
t_{max} (min)	38 ± 4
t_{lag} (min)	7 ± 4

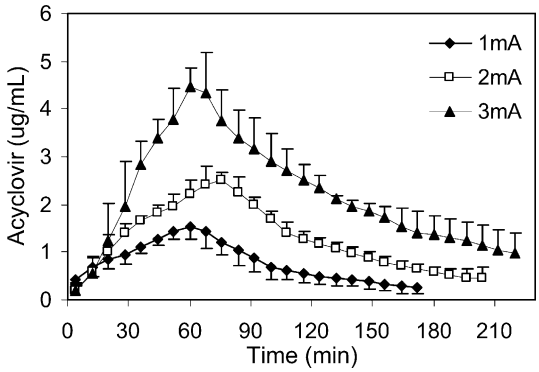

Fig. 4. Skin concentrations (mean ± standard deviation; $n = 4$) of acyclovir in rabbits after iontophoretic administration for one hour of 1, 2, and 3 mA, respectively. The skin concentrations reported are estimated from dialysate concentrations corrected by the recovery factor (Eq. (1)).

3.2.4. Ointment

Skin concentrations after ointment treatment were very low. Two experiments were performed using a microdialysis-collecting interval of 8 min to be consistent with the iontophoretic studies. However, no acyclovir was detectable in the dialysates. In order to achieve detectable concentrations, the microdialysis sampling time was then increased to 30 min. Acyclovir was detectable only in the first three samples of the six samples collected. Only the concentration of the first sample was above the limit of detection of our assay. In Fig. 5 data below the detection limit are also included. During the first 30 min the average skin concentration was 0.54 mg/l, during the second collection interval average skin concentration was 0.12 mg/l, and skin concentration fell to about zero during the third

Table 3
Acyclovir pharmacokinetic parameters in skin following iontophoresis (data presented are corrected by the recovery factor)

Parameter	1 mA ($n = 4$)	2 mA ($n = 4$)	3 mA ($n = 4$)
Lambda-z (min^{-1})	0.017 ± 0.003	0.012 ± 0.003	0.010 ± 0.005
Half-life lambda-z (min)	41 ± 6	62 ± 19	94 ± 53
AUC (μg/ml min)	133 ± 30	263 ± 18	484 ± 31
C_{max} (μg/ml)	1.60 ± 0.29	2.68 ± 0.17	4.86 ± 0.32
T_{max} (min)	60 ± 6	76 ± 6	64 ± 5

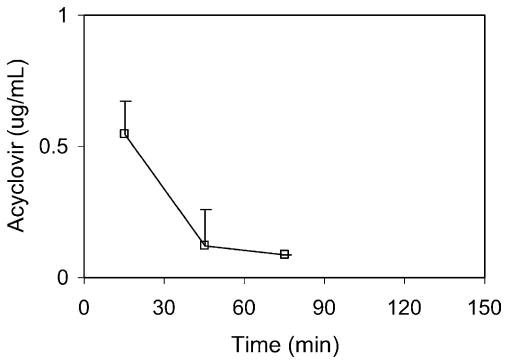

Fig. 5. Skin concentrations (corrected by the recovery factor) detected during the ointment application.

collection interval when it was detectable only in one of the three collected samples. It should be noted that skin ointment was left in place on the rabbit skin for two hours. The ointment application exposure (AUC) was 23 mg/l h.

4. Discussion

The potential of the microdialysis technique to study pharmacokinetics in skin of drug was used in this study to evaluate the different skin-concentration time profiles produced by three different types of administrations of the drug acyclovir in a rabbit model.

The skin concentrations produced by the ointment application, whose efficacy has been questioned in several papers (Parry et al., 1992; Qureshi et al., 1998), were very low and decreased while the ointment was still in place. However, ACV was detectable in the skin dialysate without the help of a vasoconstrictor. Morgan et al. (2003) recently showed, in human volunteers, that ACV concentrations were below the detection limits of 0.050 ng/ml in skin dialysates unless noradrenaline was delivered with the microdialysis perfusate to induce vasoconstriction in the peri-probe region. The differences between ACV dermis concentrations observed in this study and those in the Morgan's study, may be due to the dissimilar experimental conditions of the microdialysis sampling, to the diversity between human and rabbit skin, or to the differences in the ointment formulations. Mehta et al. (1997) reported that excipients in a topical formulation of ACV altered significantly the rate and extent of available ACV at the target site.

The concentrations of ACV in dermis following IV-bolus administration showed that ACV reaches the skin with an average delay of 10 min and reaches a peak about 38 min after plasma. Exposure to ACV concentrations in plasma and skin was similar ($AUC_{skin}/AUC_p \sim 90\%$) consistently with the fact that ACV binding to plasma proteins is low. It should be remembered that microdialysis measures only unbound ACV concentration while in plasma the total ACV concentration was measured. Actual protein binding of ACV to plasma protein was not estimated in this study. However, protein binding of ACV in rabbit is similar to that found in humans (de Miranda et al., 1982). The elimination half-life in dermis (48 min) was shorter than that estimated in plasma (108 min). In a similar study, where pharmacokinetics of the drug penciclovir was studied in dermis, via microdialysis, and in plasma of human volunteers, Borg et al. (1999) find that penciclovir has a longer half-life in skin than in plasma and suggested the presence of an accumulation process in the skin. With this respect, ACV in rabbit differs from penciclovir in human.

The possibility to improve ACV permeability through the skin via iontophoresis has been the target of several investigations in vitro (Lashmar and

Manger, 1994; Volpato et al., 1995). ACV contains two ionisable groups ($pK_a = 2.52$ and 9.35) and at physiological pHs most of the drug is neutral with a 2–5% of negatively charged ions and a negligible amount of positively charged ions. Iontophoresis enhances transport via electrorepulsion and electroosmosis (Banga, 1998). With electrorepulsion being usually several orders of magnitude more effective than electroosmosis. For ACV iontophoresis, the application of a negative current to the drug reservoir would facilitate electrorepulsion (penetration of the ACV^- ions) and application of positive current would favor electroosmosis (Volpato et al., 1995), that is the transport of ACV into the skin with the flux of liquid moving from anode to cathode. In vitro studies showed that both anodic and cathodic current may improve ACV penetration over passive delivery and the experimental conditions may favor one or the other mechanism. In the present study, we selected a cathodic current because the ACV sodium for injection used to wet the commercial electrode has a pH of 10.85–11.50. In these experimental conditions most of the ACV has a negative charge and is probably more efficiently delivered by electrorepulsion than by electroosmosis.

The results of this study demonstrate that ACV can be safely delivered to rabbit skin by iontophoresis using commercially available patches under cathodal transport. No irritation occurred at the application sites even at the highest current density (300 $\mu A/cm^2$) for the 60-min application. However, systemic exposure after iontophoretic delivery was negligible even at the higher current density showing that the iontophoretic conditions applied here are not sufficient to produce a systemic effect.

Conversely, iontophoresis of ACV remarkably increased the delivery rate of ACV to the skin over passive delivery from the patch and from a commercial topical formulation. Skin exposure to ACV after iontophoresis was 6, 11, and 20 times (at 1, 2, and 3 mA, respectively) greater than exposure to the commercial ointment. Following iontophoresis, skin exposure to ACV was 40, 22, and 11% of that following IV-bolus at 3, 2, and 1 mA, respectively. Parry et al. (1992) reported that a concentration of 0.35–0.79 μg/ml ACV is useful for a 50% inhibition of the cytopathic effect in Herpes simplex type 1 (HSV-1) infections. Mehta et al., (1997) found that a 1 μg/ml ACV in mouse skin correlates with 100% therapeutical efficacy against HSV-1. Therefore, a possibly clinically relevant concentration was reached within 30 min from the application of electrical current (Fig. 4). AUC and peak concentrations (C_{max}) increased with the current density applied. However, the plots of C_{max} and AUC versus current applied (Fig. 6) suggest that the increase may not be linear and tends to be larger at the higher current density. Interestingly, the elimination half-life increased with current density. This may explain the fact that AUC is increasing more than expected at the higher current densities. Another possible explanation for the nonlinear increase in AUC is that the electrode lost its buffer capability at the higher current densities and generated hydroxide ions. The resulting increase in pH would have increased the concentration of the

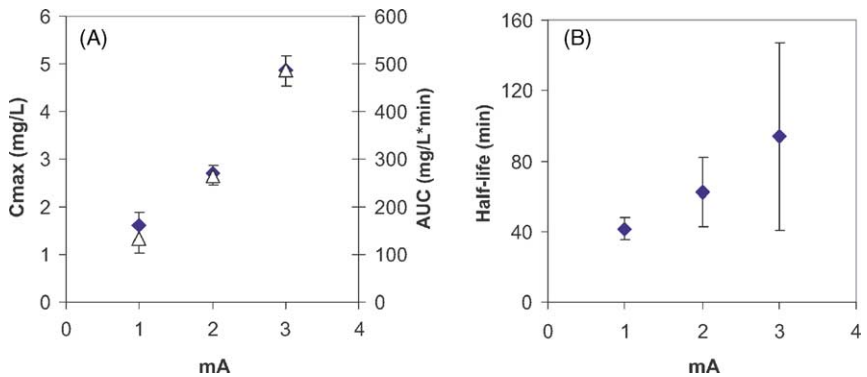

Fig. 6. (A) Plot of C_{max} (◆) and AUC (△) in skin (mean ± S.D.) versus current density. (B) Plot of terminal half-life in skin (HL-lambda-z; mean ± S.D.) vs. current density.

negatively charged ACV and enhanced its delivery by electrorepulsion.

The elimination half-life for the 1 mA iontophoresis is similar to that observed in skin after the IV-bolus (41 ± 6 min versus 48 ± 11 min) confirming an effect of current on half-life. In these studies, we removed the iontophoretic patch from the treatment site and cleaned the skin just after discontinuation of current delivery. In this way, it was possible to distinguish between a "facilitated transport" due to a prolonged activation of the skin pores that improve passive delivery from the patch after the application of electrical current (Delgado-Charro and Guy, 1994) and a "depot" effect, that is the storage of ACV in the skin. In some preliminary experiments, the patches were left in place after the discontinuation of current delivery and a sizeable prolongation of ACV delivery was observed as the dialysate concentration kept rising for another 20 min after discontinuation of current. In the experiments presented here, a prolonged delivery was still present even though of lesser extent. Therefore, the results of these experiments suggest the possibility that ACV deposits in the skin and that the extent of this deposit is a function of the current density applied. However, more experiments are necessary to confirm this hypothesis.

5. Conclusions

This study further confirms the potential of ACV iontophoresis for the treatment of skin diseases. A therapeutically relevant dermis concentration was reached within 30 min of current application with commercially available iontophoretic patches and a standard ACV I.V.-injection formulation. In particular, the use of microdialysis to study ACV pharmacokinetics in skin showed to be a powerful tool to evaluate and compare the concentration–time profiles of different delivery systems.

Acknowledgements

This study was partially supported by AACP-2000 New Investigator award to Dr. Grazia Stagni. The Authors are grateful to Pharsight Corporation (Mountain View, CA) for the award of Pharsight Academic License to A&M Schwartz College of Pharmacy, to Dr. Lindsay Lloyd of IOMED, Inc., Salt Lake City, Utah for the donation of Phoresor II units, and to Ms. Karen Jackson for the valuable help in animal handling.

References

Ault, J.M., Lunte, C.E., Meltzer, N.M., Riley, C.M., 1992. Microdialysis sampling for the investigation of dermal drug transport. Pharm. Res. 9, 1256–1261.

Banga, A.K., 1998. Electrically Assisted Transdermal and Topical Drug Delivery. Taylor & Francis Group, London, Bristol, PA.

Benfeldt, E., 1999. In vivo microdialysis for the investigation of drug levels in the dermis and the effect of barrier perturbation on cutaneous drug penetration. Studies in hairless rats and human subjects. Acta Derm. Venereol. Suppl. (Stockh.) 206, 1–59.

Borg, N., Gotharson, E., Benfeldt, E., Groth, L., Stahle, L., 1999. Distribution to the skin of penciclovir after oral famciclovir administration in healthy volunteers: comparison of the suction blister technique and cutaneous microdialysis. Acta Derm. Venereol. 79, 274–277.

Boulieu, R., Bleyzac, N., Ferry, S., 1991. High-performance liquid chromatographic determination of ganciclovir in plasma. J. Chromatogr. 567, 481–484.

de Miranda, P., Krasny, H.C., Page, D.A., Elion, G.B., 1982. Species differences in the disposition of acyclovir. Am. J. Med. 73, 31–35.

Delgado-Charro, M.B., Guy, R.H., 1994. Characterization of convective solvent flow during iontophoresis. Pharm. Res. 11, 929–935.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics. Marcel Dekker, New York.

Gillespie, W.R., 1991. Noncompartmental versus compartmental modelling in clinical pharmacokinetics. Clin. Pharmacokinet. 20, 253–262.

Groth, L., 1998. Cutaneous microdialysis. A new technique for the assessment of skin penetration. Curr. Prob. Dermatol. 26, 90–98.

Hedaya, M.A., Sawchuk, R.J., 1990. A sensitive and specific liquid-chromatographic assay for determination of ganciclovir in plasma and urine and its application to pharmacokinetic studies in the rabbit. Pharm. Res. 7, 1113–1118.

Henley-Cohn, J., Hausfeld, J.N., 1984. Iontophoretic treatment of oral herpes. Laryngoscope 94, 118–121.

Kehr, J., 1993. A survey on quantitative microdialysis: theoretical models and practical implications. J. Neurosci. Methods 48, 251–261.

Krogstad, A.L., Jansson, P.A., Gisslen, P., Lonnroth, P., 1996. Microdialysis methodology for the measurement of dermal interstitial fluid in humans. Br. J. Dermatol. 134, 1005–1012.

Lashmar, U.T., Manger, J., 1994. Investigation into the potential for iontophoresis facilitated transdermal delivery of acyclovir. Int. J. Pharm. 111, 73–82.

Mehta, S.C., Afouna, M.I., Ghanem, A.H., Higuchi, W.I., Kern, E.R., 1997. Relationship of skin target site free drug concentration (C^*) to the in vivo efficacy: an extensive evaluation of the predictive value of the C^* concept using acyclovir as a model drug. J. Pharm. Sci. 86, 797–801.

Morgan, C.J., Renwick, A.G., Friedmann, P.S., 2003. The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. Br. J. Dermatol. 148, 434–443.

Parry, G.E., Dunn, P., Shah, V.P., Pershing, L.K., 1992. Acyclovir bioavailability in human skin. J. Invest. Dermatol. 98, 856–863.

Qureshi, S.A., Jiang, M., Midha, K.K., Skelly, J.P., 1998. In vitro evaluation of percutaneous absorption of an acyclovir product using intact and tape-stripped human skin. J. Pharm. Pharm. Sci. 1, 102–107.

Shah, V.P., 2001. Progress in methodologies for evaluating bioequivalence of topical formulations. Am. J. Clin. Dermatol. 2, 275–280.

Stagni, G., O'Donnell, D., Liu, Y.J., Kellogg Jr., D.L., Shepherd, A.M., 1999. Iontophoretic current and intradermal microdialysis recovery in humans. J. Pharmacol. Toxicol. Methods 41, 49–54.

Stagni, G., O'Donnell, D., Liu, Y.J., Kellogg, D.L., Morgan, T., Shepherd, A.M., 2000. Intradermal microdialysis: kinetics of iontophoretically delivered propranolol in forearm dermis. J. Control Rel. 63, 331–339.

Stahl, M., Bouw, R., Jackson, A., Pay, V., 2002. Human microdialysis. Curr. Pharm. Biotechnol. 3, 165–178.

Volpato, N.M., Santi, P., Colombo, P., 1995. Iontophoresis enhances the transport of acyclovir through nude mouse skin by electrorepulsion and electroosmosis. Pharm. Res. 12, 1623–1627.