Estimation of Skin Target Site Acyclovir Concentrations Following Controlled (Trans)dermal Drug Delivery in Topical and Systemic Treatment of Cutaneous HSV-1 Infections in Hairless Mice

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The use of controlled transdermal delivery of acyclovir (ACV) in the treatment of cutaneous herpes simplex virus type 1 infections in hairless mice was investigated. Using an *in vivo* animal model (A. Gonsho, et al. *Int. J. Pharm.* 65:183–194 (1990)) made it possible to quantify both, the topical and the systemic antiviral efficacy of ACV transdermal patches as a function of the drug delivery rate of the patches. Drug delivery rates required to attain systemic efficacy were found to be higher than the rates required to attain the same magnitude of topical efficacy. The ACV concentrations in the basal cell layer of the epidermis for 50% topical efficacy and 50% systemic efficacy were estimated. The basal epidermis layer was considered to be the site of antiviral drug activity (skin target site). Systemic plasma levels were obtained from pharmacokinetic studies and were used to estimate the ACV concentration achieved systemically in the basal epidermis layer. A computational model for drug permeation across skin was employed to estimate the ACV concentration achieved topically in the basal epidermis layer. Equal topical and systemic efficacies were found to correspond to equal drug concentrations at the site of antiviral activity. The length of the effective diffusion pathway of drug molecules in the dermis prior to entering the blood circulation was assumed to be approximately equal to 1/20 of the anatomical dermis thickness because of dermis vascularization.

KEY WORDS: transdermal delivery; pharmacokinetics; skin target site; Herpes Simplex Virus-1; antiviral efficacy; animal model.

INTRODUCTION

The goal of this study has been to establish a quantitative relationship between locally achieved concentration and pharmacologic effect of antiviral agents in the treatment of cutaneous herpes simplex virus type 1 (HSV-1) infections. The hairless mouse has been used as an animal model because it provides a measure of the efficacy of antiviral chemotherapy in cutaneous HSV-1 infections based on the extent of band-shaped skin lesion development (1,2). The amount of antiviral agent delivered into the skin and into the systemic circulation of the mouse is quantitatively controlled by means of transdermal patches (3,4). Constant (zero order) drug delivery can be achieved over periods of several days with these transdermal patches and delivery rates can be controlled over at least a 100-fold range.

A quantitative relationship was established between the drug delivery rate and both the topical efficacy and the systemic efficacy of Acyclovir (ACV) in cutaneous HSV-1 infections in hairless mice (4). Patches loaded with ACV were fitted onto the mouse skin on an area distal to the site of virus inoculation but in the path of skin lesion development. This experimental approach followed by appropriate evaluation of lesion development made it possible to distinguish between topical and systemic antiviral efficacy of the ACV patches. Both types of efficacy were expressed as the percentage of mouse population responding to treatment. By plotting efficacy as a function of drug delivery rate of the patches, typical sigmoidal dose / response curves were obtained, the systemic efficacy curve being shifted towards higher delivery rates compared to the topical efficacy curve.

In the present study, estimation of drug concentration, C*, at the skin target site is introduced with the aim to establish an *in vivo* relationship between drug concentration and drug antiviral activity in cutaneous HSV-1 infections. The basal cell layer of the epidermis is used as the skin target site, since it appears to be the primary site of antiviral activity in cutaneous HSV-1 infections (5,6).

Experimental methods for determining drug levels in cutaneous tissue include punch biopsies of whole skin, tape stripping of stratum corneum and mechanical or heat separation of the epidermis and dermis layers. These techniques allow determination of the total drug content of each of the layers, stratum corneum, viable epidermis and dermis; they do not provide, however, the possibility to quantify drug concentration in the basal epidermis layer. In topical dermal delivery, drug concentration in the basal epidermis layer is expected to be lower than the average concentration in the viable epidermis because of a drug concentration gradient prevailing in the epidermis.

It is experimentally difficult to obtain sufficiently reliable drug concentrations in the basal epidermis cell layer while maintaining histological integrity of the tissue layers of hairless mouse skin. Therefore, computational methods are utilized in the present approach for estimating basal epidermis ACV concentrations following topical and systemic drug delivery. Pharmacokinetic studies with ACV-loaded transdermal patches were conducted in order to determine ACV plasma levels and to estimate the ACV concentration achieved systemically at the basal epidermis layer. Also, using a quantitative model for permeation of ACV across skin, the ACV concentration achieved topically at the basal epidermis layer was estimated. The role of the effective dermis thickness for drug transport in the latter case is discussed. It is proposed, that the skin target site drug concentration, C*, introduced here can be directly related to the pharmacologic effect of (trans)dermal formulations and, therefore, be utilized for the evaluation and prediction of the efficacy of these formulations.

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MATERIALS AND METHODS

Drug

Zovirax® sterile powder vials (Burroughs Wellcome Co., Research Triangle Park, NC) containing acyclovir (ACV) in its sodium salt form were used as the source of cold ACV. The free (uncharged) form of the drug was prepared by slowly adding a small excess of hydrochloric acid (analytical grade, 37%) to a concentrated aqueous solution of sodium-ACV. The precipitate was filtered, washed with a small amount of water and dried in vacuum over anhydrous calcium sulfate. No impurities were detectable by HPLC in the obtained product. The solubility of the free ACV in water at 37°C was 2.5 mg/ml.

^{3}H-labeled acyclovir with a specific activity of 67.6 mCi/mg was obtained in its free (uncharged) form in a 30/70 ethanol/water solution from New England Nuclear, Boston, MA. The radiochemical purity, tested by TLC, was found to be greater than 99%. The ethanol/water solvent was evaporated in a nitrogen jet stream before use.

To prepare radiolabeled powder of free ACV for use in the transdermal patch formulation, a trace amount of ^{3}H-ACV was added to a concentrated cold sodium-ACV solution in water and the solution was mixed thoroughly in a sonicator (Branson Cleaning Equipment Co., Shelton, CT). Radiolabeled crystals of free ACV were obtained by the addition of hydrochloric acid to this solution, as described above. The specific activity of the isotopic dilution was determined by weighing an exact amount of the dry ACV powder and measuring its radioactivity by liquid scintillation counting. A specific activity of the ACV powder of 17.3 μCi/mg was found.

Transdermal Patches

The transdermal patches used in the present study were identical to those described in detail previously (4). Drug release rate of the patches was controlled by a membrane consisting of crosslinked poly-(2-hydroxyethylmethacrylate) (p-HEMA) hydrogel prepared in this laboratory (3,4). The patches were made from an o-ring, a spacer, a backing membrane (all of Silastic Sheeting, Dow Corning Corp., Midland, MI) and the rate controlling membrane, these components being glued together with silastic medical adhesive to form the reservoir of the patch. The reservoir was filled with the drug formulation consisting of a suspension of free ACV in a 0.3%, pH 5 aqueous Carbopol® 934P gel (B. F. Goodrich Co., Cleveland, OH). Tritiated ACV powder was used in the formulation for pharmacokinetics studies and cold ACV powder was used in the formulation for antiviral efficacy studies. Each patch was loaded with a sufficient amount of solid drug to provide a 7 day constant release rate. The patches were equilibrated in a humidified chamber for 1 to 2 days before use in order to eliminate lag-time effects.

Drug Delivery Rate of Transdermal Patches

Transdermal patches containing a ^{3}H-ACV formulation were each submerged in 10 ml of 0.1 M, pH 5 acetate buffer in a glass scintillation vial. The vials were magnetically stirred and maintained at 37°C. Samples of 100 μl were taken from each vial at predetermined time intervals and replaced with the same volume of fresh buffer. The amount of ^{3}H-ACV in the samples was determined by liquid scintillation counting. From these data, the drug delivery rates of the patches and the permeability coefficients of their rate controlling membranes were calculated. The release experiments were carried out over a time period of 5 days.

Animals

Hairless mice, female, strain SKH/HR-1, 6 to 7 weeks old with body weights between 20 and 25 grams obtained from Temple University, Philadelphia, PA were used in this study.

In Vitro ACV Permeability of Azone Pretreated Hairless Mouse Skin

A 2.25 cm^2 skin area of the left lateral side of the mice was treated for 24 hours with 25 mg of the permeation enhancer Azone® (1-dodecylazacycloheptan-2-one, Nelson Research Corp., Irvine, CA). The Azone was applied on a 1.5 cm × 1.5 cm piece of gauze, which was attached to the animals with tape as described earlier (4). At the end of the 24 hour treatment the Azone gauze was removed and an additional 10 mg of Azone was applied on the exposed bare skin area which was immediately covered with a placebo transdermal patch. The ACV permeability coefficient of skin was determined at different time points after termination of the 24 hour Azone treatment period using different mice at each time point. No additional Azone and no placebo patch were applied when determining permeability coefficients immediately following the 24 hour Azone treatment.

The mice were sacrificed by being kept in an ether chamber for the appropriate period of time. The placebo patch was removed and the Azone pretreated skin area was excised and mounted immediately between the upper and lower chambers of a Franz cell. The lower (receiver) chamber contained 4.2 to 4.5 ml of 0.1 M, pH 5 acetate buffer, which was stirred magnetically at 150 rpm and maintained at 37°C. The upper (donor) chamber of the cell was loaded with approximately 200 μl of the non-radiolabeled free ACV formulation in Carbopol. The effective diffusion area of the cell was 0.68 cm^2. At predetermined time intervals, 100 μl samples were taken from the receiver chamber and replaced with the same volume of fresh buffer. The samples were assayed for ACV by HPLC and the data obtained were used to calculate the ACV permeability coefficient of Azone pretreated hairless mouse skin. The skin permeability experiments were carried out over an 8 hour period.

Intravenous Injection

For intravenous injection, the ethanol/water solvent of the purchased ^{3}H-ACV solution was evaporated and the radioactive material was taken up in normal saline. A 100 μl bolus, containing an exactly measured dose of 14 μCi of tritiated ACV (free form) and approximately 300 μg of Zovirax powder (sodium-ACV) in normal saline was injected into the tail vein of ether anesthetized hairless mice. Normal saline for injection was obtained from Abbott Laboratories, North Chicago, IL. Ten μl blood samples were taken with

micropipettes from the eye vein of the mice at predetermined time intervals following i.v. injection. The micropipettes had previously been soaked in a 1000 units/ml aqueous heparin solution and air dried. Radioactivity was determined in the blood samples and from the obtained data pharmacokinetic parameters were deduced.

In Vivo Transdermal Patch Application

A 2.25 cm^2 skin area of the left lateral side of hairless mice was treated for 24 hours with 25 mg of Azone as described above. After the Azone gauze was removed an additional 10 mg of Azone was applied directly onto the Azone pretreated skin and a drug loaded transdermal patch was immediately attached to this area. The patches were taped on the animals under occlusive conditions as described earlier (4).

For pharmacokinetics studies, mice wore the transdermal patches for 5 days while allowed to maintain a normal life pattern. During this time period 20 μl blood samples were taken at predetermined intervals from the eye vein of the mice and were used to determine the ^{3}H-ACV levels in blood.

For antiviral efficacy studies, mice were cutaneously inoculated with herpes simplex virus-1 and transdermal patches were applied either zero or one or two days post inoculation in an area distant from the inoculation site but in the path of the skin lesion development. The pattern of lesion development in the skin area near to or under the patch was used to assess antiviral drug efficacy based on a model described in detail in references (4) and (12).

Pharmacokinetics studies and antiviral efficacy studies were conducted separately using different groups of animals.

Assays

Quantitative analysis of non-radiolabeled ACV was performed by HPLC using a Beckman 330 system (Beckman Instruments Inc., Fullerton, CA). This system included a 110B solvent delivery module, a 210A injector and a 153 UV fixed wavelength detector. A cosmosil® packed C-18 reversed phase column, 150 × 4.6 mm, 5 μm particle size (Nakalai Tesque Inc., Kyoto, Japan) was used. The mobile phase was methanol/acetate buffer (pH 5) 7/93 and the flow rate was 1 ml/min. UV absorption was measured at 254 nm. The free form of ACV was used to prepare standard solutions in methanol and the ACV concentration in the samples was calculated based on a peak height calibration.

Samples of tritium labeled ACV were assayed by liquid scintillation counting. Aqueous samples were added to 10 ml of scintillation cocktail (Opti-Fluor, Packard Instrument Co., Downers Grove, IL) and radioactivities were measured in a Beckman LS 7500 counter (Beckman Institute, San Ramon, CA). Blood samples were treated as follows: 20 μl of a 20% w/v aqueous perchloric acid solution and 20 μl of deionized water were added to 10 μl of a blood sample and the mixture was agitated well in a shaker. The suspended solids were separated by centrifugation (12000g). The supernatant was transferred into a liquid scintillation vial and the pellet was washed twice with 40 μl of deionized water. The washings were added to the supernatant and the combined radioactivity was measured as above. In order to correct for the quenching of scintillation caused by the solubilized blood components, standard solutions of tritiated ACV were prepared in blood and treated as the samples. A 90% recovery of radioactivity from blood was obtained with this method.

RESULTS AND DISCUSSION

Flux/Efficacy Relationship

A positive correlation between drug flux (dose) and antiviral efficacy of the ACV patches in HSV-1 skin infections was found (Fig. 1), the systemic efficacy data being shifted towards higher fluxes compared to the topical efficacy data. A maximum systemic efficacy of 50% was obtained with the highest drug delivery rate. A further increase of the delivery rate was not possible with the present system, since beyond this point the skin would become the permeation limiting barrier. Similar relationships between drug flux and topical and systemic efficacies were reported earlier (4). In the present results, however, new data are included from studies in which patches were applied to the animals one or two days post virus inoculation. It is noted, that starting treatment zero, one or two days after virus inoculation provided essentially identical efficacy values. The issue of delayed start of treatment, however, is discussed extensively elsewhere (12).

The observed displacement of the systemic efficacy data towards higher fluxes is assumed to be due to lower drug concentrations achieved systemically at the skin target site compared to concentrations achieved topically at the target site, at comparable transdermal drug fluxes.

This hypothesis is tested in the present study by estimating the ACV concentrations, C*, obtained by topical and systemic drug delivery at the site of antiviral drug activity.

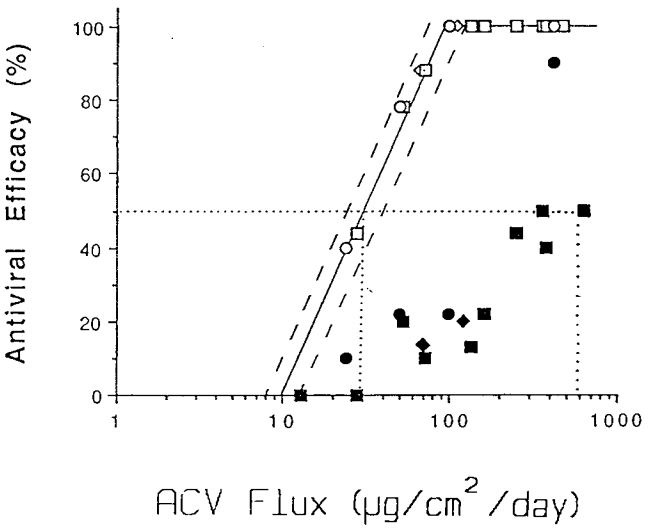

Fig. 1 Topical (open symbols) and systemic (closed symbols) antiviral efficacies of ACV as a function of experimental drug flux delivered with transdermal patches. Patches were applied zero (□,■), one (◇, ◆) and two (○,●) days post virus inoculation. Best line was empirically drawn through topical efficacy data. Dashed lines represent 95% confidence limits for the mean experimental flux. Dotted lines indicate drug fluxes corresponding to 50% topical and 50% systemic antiviral efficacy.

Histopathologic investigations of cutaneous HSV-1 infections in animal models (5) and in humans (6) indicate that infections initially localize in the basal cell layer of the epidermis and then progress towards the skin surface, while no signs of viral infection are apparent in the dermis. Therefore, the basal epidermis layer is considered here the primary site of antiviral drug activity.

In the following sections, the basal epidermis concentrations of ACV, C^*_{50}, for equal topical and systemic efficacies of 50% are estimated based on the corresponding transdermal drug fluxes. From Fig. 1 is obtained that the flux for 50% topical efficacy, $J_{50,top}$, is equal to 30 μg/cm²/day and the flux for 50% systemic efficacy, $J_{50,sys}$, is equal to 600 μg/cm²/day.

Estimation of Systemic C^*_{50}

For estimating the ACV concentration achieved systemically in the epidermis following drug delivery with transdermal patches, pharmacokinetics studies were conducted to determine the ACV concentration in the blood.

The pharmacokinetic parameters of radiolabeled ACV in the hairless mouse were deduced form i.v. bolus injection experiments. Fig. 2 shows the radioactivity concentration in blood as a function of time following i.v. injection of ^{3}H-ACV. This concentration versus time profile indicates that pharmacokinetics can be described by a two compartment model with central elimination and first order elimination kinetics (7). Hence, equation (1) was fitted to the i.v. experimental data of each mouse using non-linear regression.

$$C = A\exp(-\alpha t) + B\exp(-\beta t) \qquad \text{(Eq. 1)}$$

where C is the drug concentration in the central compartment, A and B are preexponential constants, α and β are disposition rate constants and t is time.

The calculated constants A, B, α and β are given in Table I. These constants were used to calculate the elimination rate constant, the apparent volume of distribution of the central compartment and the clearance from equation (2), equation (3) and equation (4), respectively.

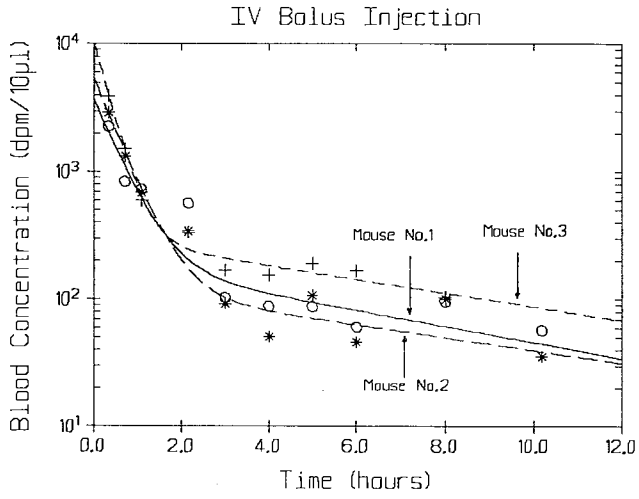

Fig. 2 Semilogarithmic plot of radioactivity concentration vs. time in the blood after i.v. bolus injection of tritiated ACV. Key: (○) Mouse No. 1; (*) Mouse No. 2; (+) Mouse No. 3. Lines represent best fit (two compartment model).

Table I. Pharmacokinetic Parameters[a] of ^{3}H-ACV

Mouse No.	1	2	3	mean	s.d.[b]
A (dpm/10μl)	3880	5350	10020	6420	3210
B (dpm/10μl)	250	158	302	237	73
α (hr^{-1})	1.6	1.9	3.1	2.2	0.8
β (hr^{-1})	0.15	0.12	0.12	0.13	0.02
k_{el} (hr^{-1})	1.0	1.3	1.8	1.4	0.4
V_c (ml)	75	56	30	54	23
Cl (ml/hr)	75	73	54	67	12

[a]Parameters obtained using Eq. (1), Eq. (2), Eq. (3) and Eq. (4); symbols defined in text; the i.v. bolus administered dose was in every mouse 14 μCi ≙ 3.1 · 10⁷ dpm. [b]Standard deviation.

$$k_{el} = \frac{\alpha\beta(A + B)}{A\beta + B\alpha} \qquad \text{(Eq. 2)}$$

$$V_c = \frac{D_{iv}}{A + B} \qquad \text{(Eq. 3)}$$

$$Cl = V_c k_{el} \qquad \text{(Eq. 4)}$$

where D_{iv} is the i.v. bolus administered dose, k_{el} is the elimination rate constant, V_c is the apparent volume of distribution of the central compartment and Cl is clearance.

The calculated pharmacokinetic parameters of tritiated ACV are given in Table I. A fairly good reproducibility between the three mice was obtained.

The parameters calculated above were used in equation (5) to predict the blood concentration profile of radioactivity achieved by a constant transdermal flux of ^{3}H-ACV of $2.3 \cdot 10^7$ dpm/cm²/day which corresponds to the drug flux of $J_{50,sys}$ = 600 μg/cm²/day (specific activity of drug in the patch formulation = 17.3 μCi/mg).

$$C = \frac{JS}{V_c k_{el}}\left[1 + \frac{\beta - k_{el}}{\alpha - \beta}\exp(-\alpha t) + \frac{k_{el} - \alpha}{\alpha - \beta}\exp(-\beta t)\right] \qquad \text{(Eq.5)}$$

where J is the transdermal flux, S is the diffusion surface area of the transdermal patch (= 0.636 cm²) and all other symbols are as defined above. In computing the predicted blood level profile, the average clearance value, Cl, of the three mice (Table I) was substituted for the product $V_c k_{el}$ in Eq. (5).

Experimental ^{3}H-ACV blood levels were determined in mice with transdermal patches providing a flux of $2.3 \cdot 10^7$ dpm/cm²/day (≙ 600 μg/cm²/day). The patches required to attain this flux value were determined as follows:

In vivo flux, J, obtained with transdermal patches can be expressed as

$$J = P_t C_s \qquad \text{(Eq. 6)}$$

with

$$\frac{1}{P_t} = \frac{1}{P_m} + \frac{1}{P_s} \qquad \text{(Eq. 7)}$$

where P_t is the total permeability coefficient, P_s is the ACV

permeability coefficient of skin, P_m is the ACV permeability coefficient of the rate controlling membrane of the patches and C_s is the concentration of dissolved radioactivity (= $9.5 \cdot 10^7$ dpm/ml) corresponding to the saturation concentration of ACV (= 2.5 mg/ml) prevailing in the transdermal patch formulation.

For a flux of $J_{50,sys}$ = 600 μg/cm²/day, a P_t of $2.8 \cdot 10^{-6}$ cm/sec is calculated using equation (6). Furthermore, the ACV permeability coefficient of Azone pretreated hairless mouse skin, P_s, was found in *in vitro* measurements immediately after Azone treatment to be equal to $1.4 \cdot 10^{-5}$ cm/sec. Thus, the required permeability coefficient of the controlling membrane, P_m, is calculated from equation (7) to be equal to $3.5 \cdot 10^{-6}$ cm/sec. To obtain this permeability coefficient, a p-HEMA release rate controlling membrane with a thickness of approximately 0.5 mm was used in the transdermal patches. P_m values of rate controlling membranes were determined *in vitro*.

In Fig. 3 the predicted radioactivity concentrations in blood are compared with the experimental data. A good agreement between calculated and experimental results is observed in the time interval between 0 and 72 hours. This demonstrates that, in this time interval, zero order delivery of ^{3}H-ACV is achieved *in vivo* through the hairless mouse skin with the transdermal patches. Blood concentration is precisely controlled by the drug delivery rate of the patches which is determined by their release controlling membrane taking into consideration the permeability of hairless mouse skin. Similar results were reported previously with vidarabine transdermal delivery systems (8).

Steady state radioactivity levels in the blood were reached within 24 hours from patch application and were maintained for up to 72 hours. A decline in the blood concentrations was observed after 72 hours of drug delivery. This decline was attributed to a decrease of the skin permeability which could be due to a partial depletion of Azone from the stratum corneum possibly associated with a progressing renewal of the stratum corneum; these effects starting to become apparent 72 hours after termination of Azone treatment. This interpretation was supported by the results of *in vitro* measurements of the ACV skin permeability as a function of time following Azone treatment. In Table II, a decrease of the skin permeability is evident on the 5th day post Azone treatment, in agreement with previous results (4). In the present study, a modified protocol of Azone treatment involving application of 10 mg of Azone on the animal skin in addition to the Azone soaked gauze (see *Materials and Methods*) did not prevent this decrease.

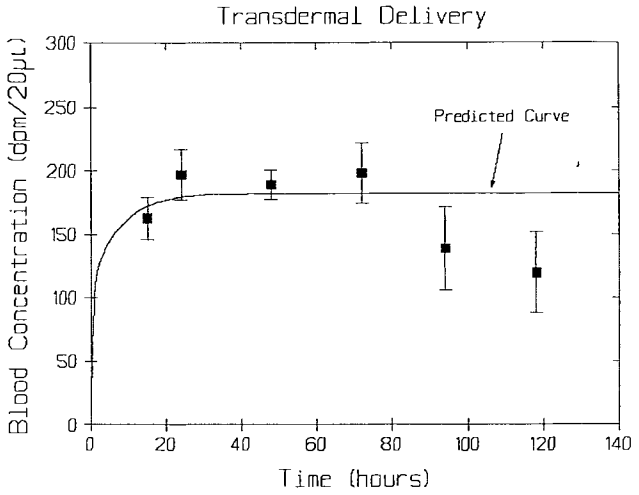

Fig. 3 Radioactivity concentration vs. time in the blood following application of transdermal patches containing radiolabeled ACV formulation. Solid line represents calculated prediction using Eq. (5). Points and vertical bars: mean ± standard deviation of experimental results, n = 3.

Estimation of the basal epidermis concentration of ACV, C*, responsible for systemic antiviral efficacy in cutaneous infections, is based on the steady state blood concentration prevailing over the treatment period. The ACV concentration in blood can be calculated from the radioactivity concentration using the specific activity of ACV in the patch formulation, since no major metabolite of ACV has been known to be formed in the body. Hence, from the measured steady state radioactivity concentration of 190 dpm/20 μl (Fig. 3), a steady state blood ACV concentration of 0.25 μg/ml is obtained (specific activity = 17.3 μCi/mg).

The ratio of ACV concentration between plasma and red blood cells equals one (13) and there is practically no binding of ACV to plasma proteins in mice at low drug concentrations (≤ 2.7 μg/ml) (9). Therefore, the steady state plasma concentration of unbound, i.e., pharmacologically active ACV is estimated to be 0.25 μg/ml. Because (a) there can be no significant outward flux of ACV through the skin and (b) there is negligible skin metabolism of ACV, it is reasonable to assume that ACV levels in various compartments of the skin will equilibrate with the steady-state plasma ACV. Thus, the steady state concentration of unbound ACV in plasma is expected to be equal to the steady state unbound ACV concentration in the aqueous compartments of the epidermis and of the basal epidermis layer. Therefore, the basal epidermis ACV concentration, C^*_{50}, responsible for a 50% systemic antiviral efficacy in cutaneous HSV-1 infections is estimated to be 0.25 μg/ml. This *in vivo* C^*_{50} value of 0.25 μg/ml agrees reasonably well with literature values of ID_{50} for ACV, obtained *in vitro* from Vero cell cultures (14,15,16). Here ID_{50} is the ACV concentration required *in vitro* to inhibit HSV-1 induced cytopathogenicity or viral plaques by 50% in these cell cultures.

Estimation of Topical C^*_{50}

For estimating the ACV concentration achieved topically in the basal epidermis layer by means of transdermal drug delivery, a quantitative model developed earlier (10) was employed. According to this model, skin is viewed as a

Table II. ACV Permeability Coefficients of Azone Pretreated Hairless Mouse Skin and ACV Total Permeability Coefficients of Skin and Release Rate Controlling Membrane

	day 0[c]	day 3[c]	day 4[c]	day 5[c]
P_s[a] (10^{-5} cm/sec)	1.4	4.2	1.4	0.19
P_t[b] (10^{-6} cm/sec)	2.8	3.2	2.8	1.2

[a]Permeability coefficient of Azone pretreated hairless mouse skin. [b]Total permeability coefficient calculated using Eq. (7) with P_m = $3.5 \cdot 10^{-6}$ cm/sec. [c]Days post termination of Azone treatment.

multilayer laminate and flux of drug across a diffusionally homogeneous skin layer can be expressed by the equation:

$$J = P(C_i - C_o) \qquad \text{(Eq. 8)}$$

where J is flux, P is the drug permeability coefficient of the skin layer and C_i and C_o are the drug concentrations at the inflow and outflow surfaces, respectively, of the skin layer. Equation (8) holds when no drug metabolism takes place in the skin, this condition being satisfied by Acyclovir.

When Eq. (8) is applied to the dermis layer of skin, drug concentration, C_o, at the outflow surface of this layer is assumed to be negligible, since this surface corresponds to the site at which drug enters the systemic circulation where sink conditions prevail. Furthermore, the inflow surface of the dermis layer corresponds to the epidermis/dermis interface which histologically is nearly coincidental with the basal cell layer of the epidermis. Therefore, C_i can be set equal to C^* and by rearranging Eq. (8) the ACV concentration, C^*_{50}, at the basal epidermis layer responsible for a 50% topical efficacy can be calculated from the corresponding flux $J_{50,top}$ = 30 μg/cm²/day as follows:

$$C^*_{50} = \frac{J_{50,top}}{P_D} \qquad \text{(Eq. 9)}$$

where P_D is the *in vivo* ACV permeability coefficient of the dermis.

In vitro experiments using heat separated full thickness dermis in a two chamber cell with aqueous donor and receiver solutions, gave an ACV permeability coefficient of the dermis of $P_D = 6.8 \cdot 10^{-5}$ cm/sec (A. Gonsho, unpublished data). However, the *in vivo* diffusion pathway of drug molecules in the dermis before they enter the systemic circulation may be shorter than the anatomical dermis thickness, due to the rather extensive vascularization of the dermis (11). Therefore, a shorter effective diffusion pathway in the dermis of, say, 1/20 of the anatomical dermis thickness may be considered. This would result in a 20-fold higher permeability coefficient value, i.e., $P_D = 1.4 \cdot 10^{-3}$ cm/sec.

By assigning the above two permeability coefficient values to P_D in Eq. (9), the ACV concentration, C^*_{50}, at the basal epidermis layer responsible for a 50% topical antiviral efficacy in cutaneous HSV-1 infections, is found to be 5.1 μg/ml in the case of full thickness dermis and 0.25 μg/ml in the case of 1/20 of the anatomical dermis thickness. These concentration values correspond to the unbound drug concentration in the aqueous compartment of the basal epidermis layer.

Comparison Between Systemically and Topically Obtained C^*_{50}. Implications for Future Work

The ACV concentrations, C^*_{50}, in the aqueous compartment of the basal epidermis layer providing equal topical and systemic antiviral efficacies of 50% were estimated above. The topical C^*_{50} value was found to be equal to the systemic C^*_{50} value, when it is assumed that the effective diffusion thickness of the dermis *in vivo* is approximately equal to 1/20 of the anatomical thickness of the dermis. This assumption implies that the depth at which absorption of ACV into the systemic circulation takes place lies approximately 1/20 of the anatomical dermis thickness below the epidermis/dermis interface. This is histologically reasonable considering the position of dermis blood microcirculation (11). This study provides therefore indirect, but strong experimental evidence that drug absorption into the systemic circulation following transdermal drug delivery takes place in the upper dermis layers, adjacent to the epidermis/dermis interface. Earlier studies support this finding (8).

In conclusion, *in vivo* estimated skin target site drug concentrations, C^*_{50}, correlate well with topical and systemic antiviral efficacies when reasonable assumptions are invoked, and they also agree with *in vitro* cell culture data. The employed computational approach allows the estimation of the pharmacologically significant drug concentrations in the aqueous compartment of the basal epidermis layer. C^*_{50} thus should be effective in the assessment and prediction of pharmacologic efficacies of topical formulations. Future studies will evaluate the predictive value of equation (9) with C^*_{50} = 0.25 μg/ml and $P_D = 1.4 \cdot 10^{-3}$ cm/sec for actual topical drug delivery systems (e.g., creams and ointments) applied in a finite dose regimen.

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