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Controlled (trans)dermal delivery of an antiviral agent (acyclovir). I: An in vivo animal model for efficacy evaluation in cutaneous HSV-1 infections

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Summary

An in vivo animal model using hairless mice was developed for the study of the dose/flux-efficacy relationship of antiviral agents in cutaneous herpes simplex virus-1 infections. After cutaneous virus inoculation of the mice, acyclovir (ACV), a widely used antiviral agent, was transdermally delivered in a quantitatively controlled fashion. Virus was inoculated into the skin of the mice at a site distant from the dermal patch area and the pattern of the induced lesion development was evaluated in a novel way which enabled the distinction between topical and systemic antiviral drug efficacy. Plots of the antiviral efficacy vs. the drug delivery rate gave typical sigmoidal curves of relatively high reproducibility, thus demonstrating a unique example of a dose/flux-response relationship in local antiviral treatment.

Introduction

The present work is part of a long-term effort to develop a quantitative approach for the study of topical and systemic treatments of cutaneous herpes simplex virus-1 (HSV-1) infections, the ultimate goal being to establish the quantitative pharmacokinetics-pharmacodynamics relationships for antiviral therapies of cutaneous herpes virus infections. In these studies the hairless mouse has been used as an animal model, since cutaneous HSV-1 infections in hairless mice induce skin lesions, which, when scored properly, can be used for the evaluation of antiviral drug efficacy. In an earlier report (Shannon et al., 1985) it was shown that different antiviral agents topically applied on the skin of hairless mice in the form of an ointment could prevent virus replication only when a permeation enhancer (Azone®) was incorporated together with the antiviral agent in the ointment formulation. However, in those studies, the amount of drug delivered into the skin could not be precisely controlled and, therefore, the interpretation of the results was only qualitative.

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In the experimental model described in the present paper, we have utilized the controlled release capabilities of dermal patches developed in our laboratories (Miyajima et al., 1987; Okano et al., 1987), to deliver the drug into skin in a quantitatively controlled fashion. Acyclovir (ACV) was selected as an antiviral agent as it has been shown to be effective in the treatment of many forms of HSV infections (Acyclovir Symposium, 1982), and has the additional advantage that it exhibits no known metabolism in the skin.

A new method for evaluating the antiviral efficacies of topically applied drugs is also reported here. In this new procedure, the antiviral drug treatment does not involve topical application at the site of the virus inoculation in the skin as before (Shannon et al., 1985), but instead at an area removed from the inoculation site. As will be shown, this technique together with appropriate data analysis provides the possibility of distinguishing between topical efficacy and systemic efficacy.

Materials and Methods

Drug and permeation enhancer

Acyclovir (ACV) was obtained from Zovirax® vials (Burroughs Wellcome Co., Research Triangle Park, NC), which contain the sodium salt of ACV. The acid form was prepared by dissolving an amount of the sodium salt powder corresponding to 500 mg of free ACV in 8 ml of deionized water and adding a slight excess of concentrated HCl to the solution. The precipitate was filtered, washed once with approx. 5 ml of deionized water and dried overnight at room temperature under vacuum.

The skin permeation enhancer Azone® (1-dodecylazacycloheptan-2-one) was a gift from Nelson Research Corp. (Irvine, CA) and was used as received.

Delivery rate controlling membranes

The delivery rate of the drug from the dermal patches was controlled by membranes consisting of crosslinked polymeric hydrogels. The preparation and characterization of these hydrogel membranes has been described previously (Miyajima et al., 1987). Briefly, the membranes were prepared by homopolymerization of 2-hydroxyethylmethacrylate (HEMA) or copolymerization of HEMA with styrene (Sty) or *N*-vinylpyrrolidone (VP) using ethylene glycol dimethacrylate (EGDMA) as crosslinker and 2,2′-azobisisobutyronitrile (AIBN) as initiator. The polymerizations were carried out in bulk in polyethylene molds. By varying the chemical composition, different hydration values of the membranes were obtained. It was shown that the permeability of the membranes to different solutes increased with their hydration over a wide range. The drug delivery rate of the dermal patches could be regulated using these hydrogel membranes.

All the starting materials for the preparation of the membranes were obtained from Polyscience, Warrington, PA. The monomers were purified by distillation under reduced pressure prior to use, while EGDMA and AIBN were used as received.

Fabrication of dermal patches

A schematic representation of a dermal patch is given in Fig. 1. The components of the patch consisted of the following materials: Backing film (I): Silastic® Sheeting 500-7; Spacer (II): Silastic® Sheeting 502-3; O-ring (III): Silastic® Sheeting 500-5 (Dow Corning Corp., Midland, MI); Stem (IV): Pharmaseal® Feeding Tube (American Hospital Supply Corp., Valencia, CA). The three Silastic Sheeting parts and the stem were glued together with Silastic® Silicone type A medical adhesive (Dow Corning) and cured overnight at room temperature. The rate controlling membrane (V) was attached in fully hydrated condition to

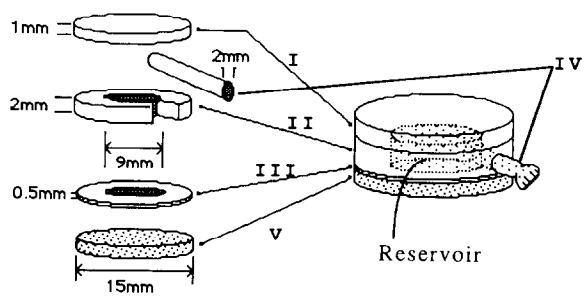

Fig. 1. Detailed sketch of the components of a dermal patch.

the O-ring using Silastic® 355 medical adhesive (Dow Corning) under light pressure applied for a few seconds. The reservoir, which was formed between the backing membrane, the hydrogel membrane, and the spacer was filled free of air bubbles with the drug formulation through the stem, which was then heat sealed. The formulation consisted of the suspension of ACV in 0.2% aqueous carbopol gel (pH 5.0). Carbopol® 934P (B.F. Goodrich Co., Cleveland, OH) was used as received. The patches contained sufficient amount of drug for a 7 day constant rate delivery and were stored in a high humidity chamber before they were used.

In vitro drug delivery rate of the dermal patches

Dermal patches were each placed in separate glass scintillation vials containing 5–10 ml of 0.1 M acetate buffer (pH 5). The vials were magnetically stirred and maintained at 37 °C. Samples of 1–2 ml were taken from the receiver solution at predetermined time intervals and were replaced immediately with equal volumes of fresh buffer. From the rate of increase of the ACV concentrations in the receiver samples, the delivery rate of the dermal patches and the permeability coefficients of the controlling membranes were deduced.

The concentration of ACV was determined using a Beckman HPLC system (Beckman Instruments Inc., Berkeley, CA) with a Cosmosil packed column (150 × 4.6 mm, C-18 reversed phase, Nacalai Tesque Inc., Kyoto, Japan). A methanol/acetate buffer pH 5, 7 : 93 mobile phase was used at a flow rate of 1 ml/min and the UV absorption was recorded at 254 nm.

Animals and virus

Hairless mice (SKH/HR-1 strain, Temple University, Philadelphia, PA), female, 6–7 weeks old with an average body weight of 23–24 g were used. The age of the mice was critical in these studies, since mice younger than 6–7 weeks suffered strong skin irritation after the Azone treatment and with mice older than 6–7 weeks the reproducibility of the virus infection was found to decline (unpublished data).

Herpes simplex virus 1 (HSV-1), strain E-377, with a final titer of 1.35×10^8 PFU/ml, was used for inoculation. The preparation and assay methods for the virus have been described previously (Kern et al., 1973).

Skin permeability of ACV

The permeability of Azone treated hairless mouse skin to ACV was measured in vitro using a Franz cell. A 2.25 cm^2 area on the back (left side) of hairless mice was treated with 25 mg of Azone for 24 h. The Azone was applied on a 1.5 × 1.5 cm piece of gauze (Topper® dressing sponge, Johnson and Johnson, New Brunswick, NJ), which was taped to the animals with a piece of Blenderm® surgical tape (3M, Medical Products Division, St. Paul, MN). This Azone treatment was further secured on the mice with a strip of OpSite® Incise drape (Smith and Nephew Medical Ltd., Hull, U.K.) by wrapping the strip a few times around the animals. Finally, the OpSite Incise drape was covered with Dermiform® hypo-allergenic knitted tape (Johnson and Johnson, New Brunswick, NJ), in order to prevent the Azone treatment from being torn away by the mice.

After 24 h the Azone treatment was removed and the animals were killed. The Azone treated skin area was excised and mounted in a Franz cell. A 0.5 ml aliquot of the same drug formulation used in the dermal patches was added to the donor compartment of the cell, which was then closed tightly with a plastic cap. The receiver compartment of the cell contained 4.5 ml of 0.1 M acetate buffer pH 5, which was stirred with a magnetic stirrer and maintained at 37 °C. At predetermined time intervals, 1 ml samples were withdrawn from the receiver compartment and replaced with the same volume of fresh buffer. The ACV concentrations of the samples were measured by HPLC as described above and the values obtained were used for the calculation of the ACV permeability coefficient of hairless mouse skin.

Calculations

The following calculations were involved in the measurement of the skin permeability in vitro and of the drug delivery rate of the dermal patches.

The cumulative drug permeation, Q_n, corresponding to the time of the n-th sample was calculated from the equation:

$$Q_n = V_R C_n + \sum_{i=0}^{n-1} V_s C_i \quad (1)$$

where C_n is the drug concentration of the receiver solution at the time of the n-th sampling point, C_i is the drug concentration of the i-th sample and V_R and V_s are the volumes of the receiver solution and the sample, respectively. C_0 is by definition equal to 0.

Eqn 1 takes into account the dilution of the receiver solution resulting from replacing the sampling volume with equal volume of fresh solvent at each sampling point. By plotting the cumulative drug amount Q_n vs time, the drug delivery rate (flux) was obtained as the slope ($\Delta Q/\Delta t$) of the steady-state part of the curve.

Permeability coefficients were calculated, assuming sink conditions, as follows:

$$P = \frac{\Delta Q/\Delta t}{A \cdot C_D} \quad (2)$$

where C_D is the donor concentration of ACV, which, in case of a suspension, is equal to its solubility (2.5 mg/ml at 37°C) and A is the effective diffusion area (Franz cell = 0.709 cm^2, dermal patches = 0.636 cm^2).

In vivo experimental model

The animal model used in the evaluation of the antiviral efficacy of ACV delivered by means of dermal patches was developed on the basis of a method described previously (Lieberman et al., 1973; Klein et al., 1977) for cutaneous HSV-1 infections.

For virus inoculation the mice were anesthetized with an i.p. injection of ketamine HCl, USP, 70 mg/kg body weight. A small area on the left lateral side of the body of the mice was scratched six times with a 27 gauge needle in a crossed-hatched pattern. 50 μl of the virus suspension were applied onto the scratched skin area and the virus suspension was rubbed for 10 s on the skin with a cotton tipped applicator. The infection induced by the virus inoculation generated skin lesions, which appeared at the site of inoculation as early as the third day after inoculation and progressed in the form of a 4–5 mm wide band first towards the back (dorsum) and then towards the abdomen of the mice. A lesion generally was fully developed 5–6 days after inoculation and formed a continuous band extending from the spinal area to the middle of the abdomen. The animals died from encephalitis by the seventh or eighth day after inoculation.

For antiviral treatment, a skin area adjacent to the inoculation site and towards the dorsum of the animals was first pretreated with Azone for 24 h (see Fig. 2). The conditions of the Azone pretreatment were described above. Virus inoculation was performed immediately after the Azone treatment and, following this, the ACV containing dermal patch was applied onto the Azone pretreated skin area. While the skin area on which the patch was applied was removed from the inoculation site, it was in the direct path of lesion development. The patches were firmly attached to the mice by a strip of OpSite Incise drape which was then covered with Dermiform hypo-allergenic knitted tape. The mice were labeled on the back and housed in cages in groups of five. The treatment groups consisted of 10 mice, and each group was treated with patches having a different drug release rate. A control group treated with placebo patches was included in every trial.

Five days after inoculation the dermal patches were removed and the antiviral drug efficacy was evaluated based on the extent of lesion development in the skin area adjacent to and underneath the dermal patches. The fifth day postinoculation

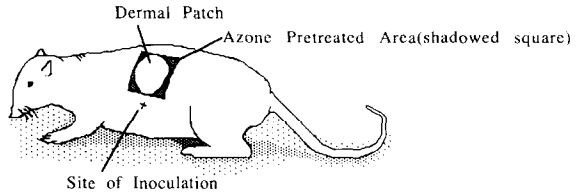

Fig. 2. Graphical representation showing the spatial relationship between the site of virus inoculation and the area of the dermal patch application on the mouse body.

represented an optimum time point for these observations, since it combined a high percentage of fully developed lesions in the control groups with still a very low animal mortality rate. As an additional efficacy criterion, the mortality of the animals was monitored over a time period of 10 days after patch removal.

Procedure for independently checking dermal patch performance

Following each in vivo efficacy experiment an attempt was made to check the drug delivery performance of the dermal patches. This was done by measuring the drug depletion of the patches. At the end of the experiments the patches were placed in scintillation vials containing 5 ml of diethyl ether, which had the functions of decontaminating and disassembling the patches. The ether was evaporated and ACV was extracted with 10 ml of pH 5 acetate buffer at room temperature for 24 h. ACV concentrations were measured by HPLC. In order to account for incomplete extraction, similar extraction experiments were conducted after in vitro release experiments with samples from every batch of dermal patches fabricated for use in the efficacy experiments.

Results and Discussion

Skin permeability of ACV

Fig. 3 shows typical results of the cumulative drug amounts permeating across the skin as a function of time. It is seen that, after a short lag time a steady-state flux was established. The permeability coefficient of ACV for Azone treated hairless mouse skin was calculated from the steady-state part of the curve using Eqn 2 to be 1.1×10^{-5} cm/s. This value is comparable to those reported previously for compounds chemically similar to ACV, e.g. vidarabine (Okano et al., 1987). The Azone treatment is found to cause a 100–500-fold enhancement of the skin permeability of these compounds over untreated full thickness skin. This enhancement is necessary in order to reach pharmacologically effective drug levels in the skin and in the systemic circulation.

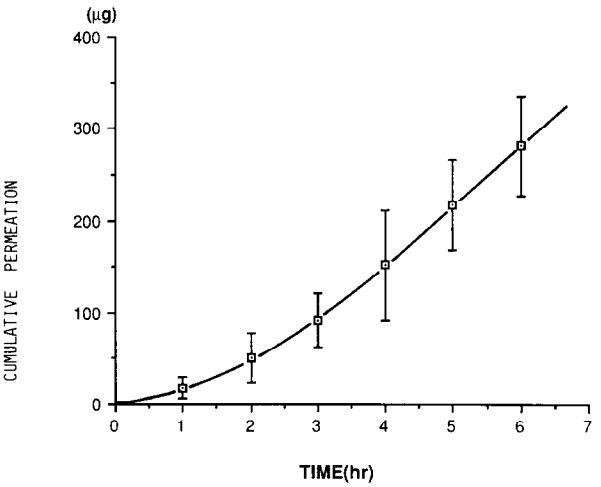

Fig. 3. Permeation of ACV across Azone pretreated hairless mouse skin as a function of time determined in a two-chamber cell. Error bars indicate standard deviation ($n = 3$).

Drug delivery rate of the dermal patches

Fig. 4 shows typical in vitro drug release profiles of dermal patches with four different compositions of controlling membranes. The results demonstrate that the patches provide constant zero-order release kinetics for several days with a wide range of release rates. The patches were stored in a high humidity chamber at 37 °C prior to their use in order to allow the controlling membranes to be saturated with ACV and therefore avoid lag time effects. The required equilibration time was determined experimentally. For the different membrane compositions shown in Fig. 4

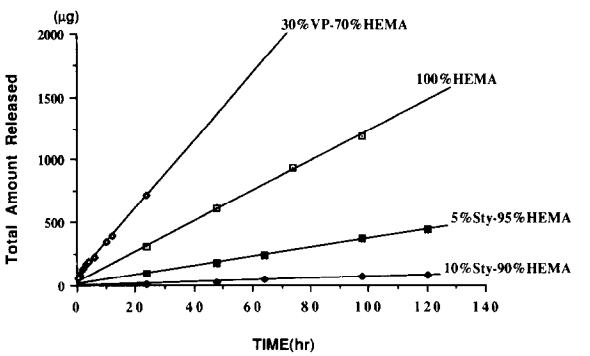

Fig. 4. Release curves of ACV from dermal patches with different compositions of controlling membranes. Membrane thickness approx. 1 mm.

the following equilibration times were applied: 30% VP–70% HEMA, 3 h; 100% HEMA, 5 h; 5% Sty–95% HEMA, 20 h; 10% Sty–90% HEMA, 2 days. The release curves in Fig. 4 show a small burst effect, which is probably due to the presaturation of the controlling membranes with the drug.

Table 1 lists the permeability coefficients (P_m) of ACV for release controlling membranes of four different chemical compositions at different membrane thicknesses. These P_m values were determined from in vitro patch release data. By varying the composition and the thickness of the membranes, their ACV permeability coefficients could be maintained within the range between 1×10^{-7} and 1×10^{-5} cm/s. When the patches are applied on the animals, the contribution of the permeability of the skin to the final delivery rate of the drug has to be taken into account. The total permeability coefficient P_T of ACV through the controlling membrane and the skin arranged in series is given by the following equation:

$$\frac{1}{P_T} = \frac{1}{P_S} + \frac{1}{P_M} \qquad (3)$$

where P_S and P_M are the permeability coefficients of ACV for the Azone treated skin and the controlling membrane, respectively.

Using a P_S value for Azone treated hairless mouse skin of 1.1×10^{-5} cm/s, the P_T values for the different membranes were calculated (Table 1). Note that the contribution of the skin is significant only for $P_M > 1 \times 10^{-6}$ cm/s. From these P_T values, the corresponding in vivo drug delivery rates of the dermal patches were estimated (last column, Table 1).

In vivo antiviral efficacy

Dermal patches, as drug delivery systems, can provide two different modes of skin drug delivery, topical (local) and systemic (see Fig. 5). In the case of topical delivery, drug is directly released into the skin area covered by the patch and this results in relatively high drug concentrations in this skin area. In the case of systemic delivery, drug which has already entered the systemic circulation can perfuse tissues including skin in areas removed from the patch area. For the same delivery rates, drug levels in the skin obtained sys-

TABLE 1

Drug delivery properties of dermal patches with different release controlling membranes

Composition of membrane	Thickness h (mm)	Permeability (cm/s)		Drug delivery rate (μg/cm^2 per day)
		P_M	P_T	
30% VP-HEMA	0.4	1.0×10^{-5}	5.2×10^{-6}	1123
	0.5	6.8×10^{-6}	4.2×10^{-6}	907
	0.5	6.4×10^{-6}	4.0×10^{-6}	864
100% HEMA	0.5	3.8×10^{-6}	2.8×10^{-6}	605
	1.0	2.2×10^{-6}	1.8×10^{-6}	389
	1.0	1.8×10^{-6}	1.5×10^{-6}	324
5% Sty-HEMA	0.5	8.5×10^{-7}	7.9×10^{-7}	171
	1.0	6.2×10^{-7}	5.9×10^{-7}	127
	2.0	3.2×10^{-7}	3.1×10^{-7}	67
10% Sty-HEMA	1.0	1.4×10^{-7}	1.4×10^{-7}	30

Membrane permeability coefficients (P_M), total permeability coefficients (P_T) of the membranes combined in series with Azone pretreated skin, and in vivo drug delivery rates of dermal patches calculated based on P_T and ACV saturated solution conditions. Results for membranes with different chemical compositions and different thicknesses. The variability in the drug delivery rates, originating from the variability in the P_M values, was in all cases $< 20\%$.

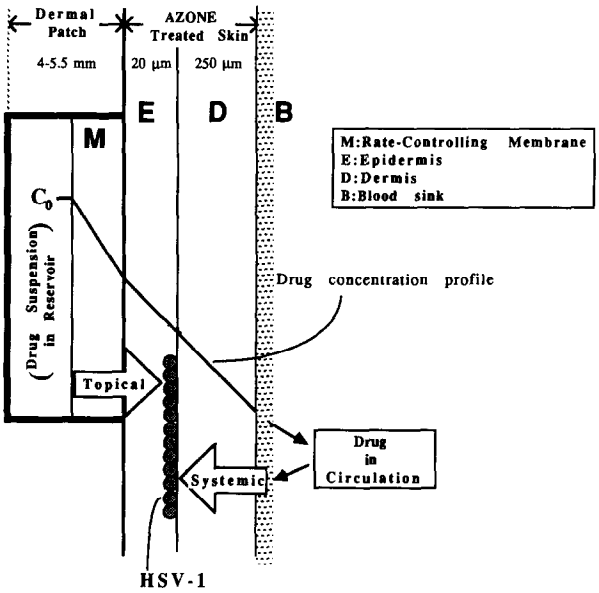

Fig. 5. Schematic representation of topical and systemic drug delivery into the skin using dermal patches. Shadowed circles denote the basal cell layer of the epidermis where symptoms of HSV-1 replication are first observed.

temically are expected to be significantly lower than those obtained by direct topical delivery.

For the purposes of evaluating both the topical and the systemic antiviral efficacies of the ACV patches, the patterns of lesion development were classified into the following categories (see also Fig. 6):

'NR' (not reach): The lesions started at the site of virus inoculation and developed only to a limited extent without reaching the dermal patch area.

'Th' (through): The lesions formed a continuous band which grew across (through) the skin area covered by the dermal patch.

'St' (stop): The lesions reached the edge of the dermal patch, but did not develop in the skin area covered by the patch.

'J' (jump): In a few cases lesions developed in a band form on both sides of the dermal patch but no lesions were visible in the area covered by the patch.

'M' (miss): The lesions grew irregularly, not forming a straight band and therefore missing the dermal patch area.

Inhibition of lesion development in a specific skin area implies suppression of virus replication in that skin area. Therefore, the above category designations of lesion development were interpreted in terms of antiviral drug effectiveness as follows:

St and J: Drug concentrations were high enough to inhibit virus replication only in the skin area covered by the dermal patch, i.e., in the area of topical drug delivery.

Th: Drug concentrations achieved by systemic or local drug delivery were not high enough to show an antiviral activity.

NR: Preventing lesion development at some distance from the dermal patch area suggests that drug in the systemic circulation has reached levels capable of inhibiting virus replication in the skin. Thus, NR was interpreted as a measure of systemic effectiveness.

M: The reason for this irregular pattern of lesion development is yet unknown. M was regarded as inconclusive with respect to the local drug effect; it indicates, however, the absence of a systemic drug effect.

Based on the above, the following two indices were used as measures of the topical and systemic antiviral effectiveness of the ACV patches:

$$\text{topical efficacy } (\%) = \frac{N_{St} + N_J}{N_{St} + N_J + N_{Th}} \times 100 \quad (4)$$

$$\text{systemic efficacy } (\%) = \frac{N_{NR}}{N_{St} + N_J + N_{Th} + N_M + N_{NR}} \times 100 \quad (5)$$

where N_{St}, N_J, N_{NR}, N_{Th} and N_M are the numbers of animals with the corresponding pattern of lesion development.

Table 2 shows the results of four antiviral efficacy trials with three to four treatment groups each. The number of animals corresponding to each of the different categories of lesion development were determined on the fifth day after virus inoculation, i.e., immediately after the removal of

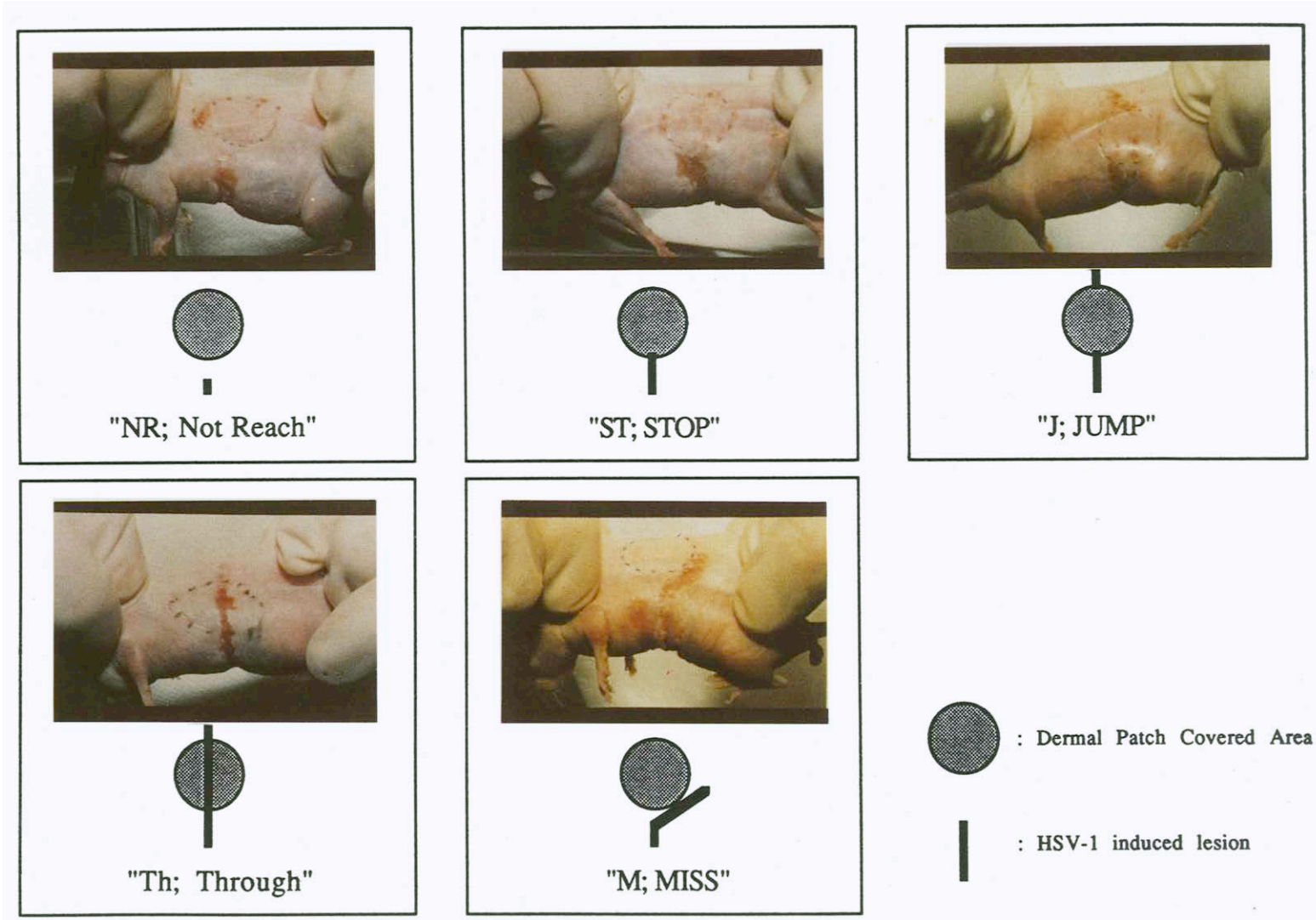

Fig. 6. Photographs and graphical representations of the five categories of the HSV-1 induced skin lesions.

TABLE 2

Results of antiviral efficacy experiments

Run	Group	Flux ($\mu g/cm^2$ per day)	n	Lesion categories					Topical efficacy (%)	Systemic efficacy (%)
				St	J	Th	NR	M		
1	1	907	10	6	0	0	4	0	100	40
	2	324	8	3	3	0	1	1	100	13
	3	placebo	7	0	0	7	0	0	0	0
2	1	864	10	3	1	0	5	1	100	50
	2	171	10	3	3	1	1	2	86	10
	3	67	10	3	1	5	0	1	44	0
	4	placebo	10	0	0	10	0	0	0	0
3	1	1123	10	3	1	0	5	1	100	50
	2	127	10	1	4	2	2	1	71	20
	3	30	9	0	0	8	0	1	0	0
	4	placebo	8	0	0	8	0	0	0	0
4	1	605	9	4	0	0	4	1	100	44
	2	389	9	3	3	0	2	1	100	22
	3	placebo	9	0	0	9	0	0	0	0

Topical and systemic antiviral efficacy of ACV dermal patches with different drug delivery rates (fluxes) (calculated values taken from Table 1). Efficacy was determined on the 5th day after cutaneous HSV-1 inoculation in hairless mice. n, number of animals per group; St, J, Th, NR and M defined in the text.

the dermal patches. Depending on the drug delivery rate, the topical drug efficacy ranged between 0 and 100% and the systemic efficacy between 0 and 50%. A zero placebo effect was obtained in all four trials, topically as well as systemically *.

In Fig. 7 the topical and the systemic drug efficacies are plotted vs the drug delivery rate of the dermal patches. In the case of topical efficacy, a sigmoidal dose (flux)-response (efficacy) curve was obtained with all of the experimental data points falling closely to this curve, this suggesting that this in vivo animal model and the method of lesion evaluation for topical efficacy may provide results of unusually high precision. The systemic efficacy results were shifted, as expected, towards higher delivery rates. This displacement of the systemic results toward higher fluxes is believed to be a direct measure of the lower steady-state skin concentrations of ACV achieved systemically (vs topically). A maximum systemic efficacy of about 50% was achieved with the highest drug delivery rate, i.e., approx. 1 mg/cm^2 per day. An increase of the delivery rate beyond this point was not possible with the present delivery system because, beyond this point, the Azone pretreated skin would become the permeation limiting barrier. The ex-

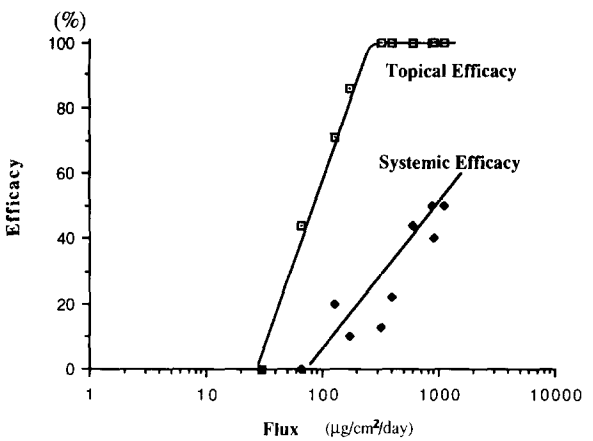

Fig. 7. Systemic and topical antiviral efficacy as a function of the drug delivery rate (flux) (data taken from Table 2).

* Results reported in the literature (Leonard et al., 1987) about the antiviral efficacy of the permeation enhancer Azone could not be confirmed in our studies.

perimental data in the case of the systemic efficacy showed a larger scatter than the results of the topical efficacy. This could be attributed, as discussed below, to variations in the skin permeability as a function of time after the removal of the Azone treatment, affecting predominantly the results at the higher drug delivery rates.

In addition to evaluating the topical and the systemic efficacy of the ACV patches in the skin infections, the mortality of the animals in the same experiments was recorded as a function of time. Death following cutaneous virus inoculation occurred due to encephalitis, caused by the spreading of the virus into the brain. In Fig. 8 the mean survival time is plotted vs the drug delivery rate. With placebo treatment a mean survival time of approx. 6.5 days was obtained. The survival time showed an increasing trend with increasing drug delivery rates and reached maxima of 9–10 days. Statistically significant increases of the mean survival time were obtained in several cases. This effect might be a measure of a delayed spreading of the virus into the brain, or could be suggesting that a certain level of systemic efficacy in virus encephalitis can be achieved with ACV dermal patches especially at high drug delivery rates. The latter would be consistent with the systemic efficacy of the ACV patches observed in the cutaneous infections, although comparison between the two cases at this point can be only qualitative due to the probable different distribution properties of ACV in the central nervous system and in the skin.

The results discussed so far demonstrate that the in vivo animal model developed here makes it possible to establish a quantitative relationship

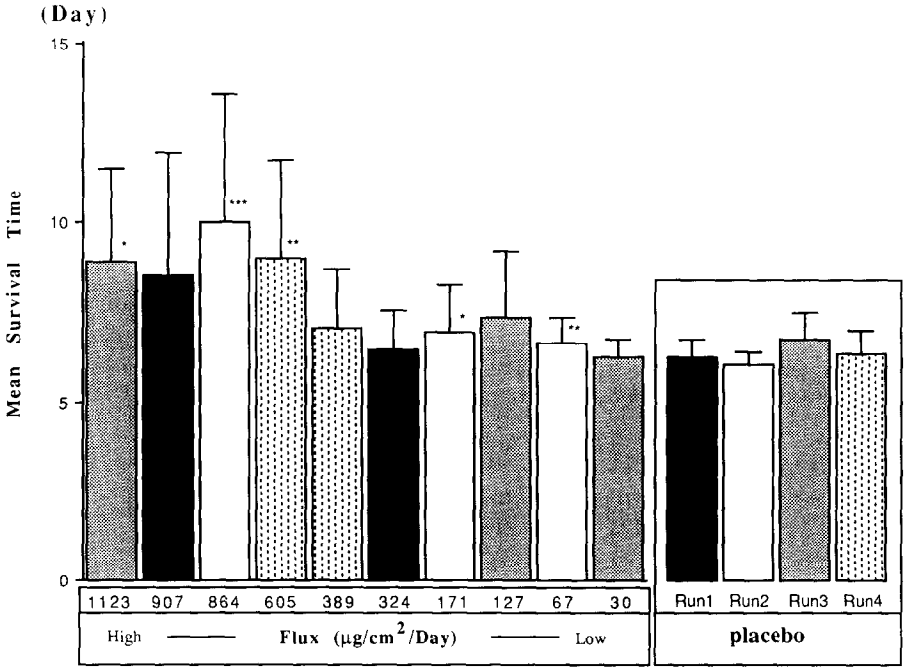

Fig. 8. Mean survival time as a function of drug delivery rate (flux) in four different experimental runs (same runs as in Table 2). Mean survival day calculated as:

$$\frac{\sum_{i=6}^{15} iN_i}{n}$$

where i denotes the i-th day, N_i is the number of animals having died on the i-th day, and n is the number of animals in the treatment group. Error bars represent standard deviation. Significance test performed by single-sided t-test; every flux group was compared with the placebo group of the same run. *** $p < 0.005$, ** $p < 0.025$, * $p < 0.05$.

between the drug delivery rate, controlled precisely by transdermal patches, and the topical and the systemic antiviral efficacies of the drug in cutaneous infections. The dose (flux) efficacy relationships obtained for topical and systemic delivery can be compared with each other by estimating the drug concentrations provided by the topical and the systemic delivery modes at the site of antiviral activity. According to histopathologic investigations (Huff et al., 1981; Spruance and McKeough, 1988) HSV-1 replication initially takes place in the cells of the basal layer of the epidermis and later extends to the rest of the epidermis, while no virus induced changes were seen in the dermis. Therefore, in the present model, the basal layer of the epidermis is considered to be the primary site of antiviral drug activity. Currently, pharmacokinetic studies are being conducted in order to determine the blood concentration and from there the concentration of ACV in the basal epidermal layer following systemic delivery and also a computational procedure developed earlier (Yu et al., 1979) is being employed for the calculation of the ACV concentration achieved topically in the basal layer in the area of the epidermis covered by the patch.

Studies for the assessment of the drug delivery performance of the dermal patches carried out after each efficacy experiment are summarized in Table 3. In the in vitro measurements, the cumulative drug amount (A_{rel}) released into the receiver medium in 5 days agrees very well with the drug amount depleted from the patches ($A_{ld} - A_{ext}$), except for the cases of low delivery rates. In these cases, which correspond to low drug contents of the patches, there appears to have been incomplete extraction of drug partitioned into the patch materials. The actual in vivo release could therefore be estimated from the in vivo depletion data using the correction factor F, which was obtained based on the in vitro data. The corrected in vivo release results (A_{rel}), presented in the last column of Table 3, may be compared to the theoretical values, Q_T, which were predicted based on the total permeability coefficient, P_T, of the Azone pretreated skin and the controlling membranes of the patches. It is seen, that, especially at the high drug delivery rates, A_{rel} in vivo is lower than the Q_T values. This difference may be attributed (at least in part) to the drop of the skin permeability coefficient occurring on the fifth day after the termination of the Azone treatment, as determined in independent experiments (Fig. 9). Efforts are currently underway to improve the Azone treatment procedure, so that constant skin permeability coefficients and therefore constant drug delivery rates are obtained over longer periods of time. This is necessary in order to obtain accurate

TABLE 3

Drug delivery rate performance of dermal patches

In vitro			P_T (cm/s)	Q_T (μg/5 days)	In vivo	
$A_{ld} - A_{ext}$ (μg/5 days)	A_{rel}	F			$A_{ld} - A_{ext}$ (μg/5 days)	A_{rel}
6494	6574	1.01	5.2×10^{-6}	3571	1258	1271
4920	4687	0.95	4.0×10^{-6}	2748	1870	1777
2413	2527	1.05	2.8×10^{-6}	1923	726	762
1305	1358	1.04	1.8×10^{-6}	1236	367	382
510	369	0.72	7.9×10^{-7}	542	246	177
482	441	0.91	5.9×10^{-7}	405	403	367
220	74	0.34	3.1×10^{-7}	213	225	77
190	86	0.45	1.4×10^{-7}	96	337	152

Drug delivery performance results of dermal patches in vitro and in vivo. A_{ld}, initial loaded drug amount; A_{ext}, extracted drug amount after 5 day release; ($A_{ld} - A_{ext}$), amount depleted; A_{rel}, drug amount released in five days; F, correction factor calculated from the in vitro data as follows: $F = A_{rel}/(A_{ld} - A_{ext})$; P_T, theoretical total permeability coefficient in vivo; Q_T, theoretical 5 day release amount in vivo.

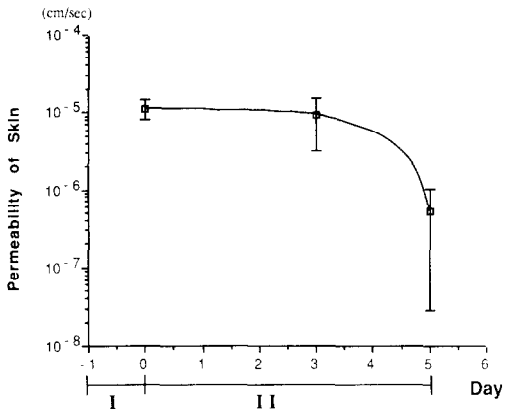

Fig. 9. ACV permeability coefficient of Azone pretreated hairless mouse skin as a function of time. (I) Treatment with 25 mg Azone. (II) Application of placebo dermal patch. Results obtained with a Franz cell. Error bars represent standard deviation; $n = 3$.

estimates of the drug concentration at the site of antiviral drug activity (i.e., the basal epidermal layer) over the entire treatment time.

Finally, it is pointed out that the present study has involved antiviral treatment which is started immediately after the virus inoculation. This protocol has demonstrated the applicability of the present animal model for quantitative antiviral efficacy studies, and will allow the investigation of basic questions such as the local concentration-antiviral activity relationship and the effect of prodrugs and other formulation factors on antiviral treatment. In future studies the effect of the length of time lapsing between virus inoculation and the beginning of treatment on the antiviral efficacy will be examined. A delayed start of treatment, possibly after the onset of the symptoms may simulate more accurately the situation encountered in real case therapy.

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