ELSEVIER

Antiviral Research 29 (1996) 279–286

Antiviral Research

Correlation of in vivo topical efficacies with in vitro predictions using acyclovir formulations in the treatment of cutaneous HSV-1 infections in hairless mice: an evaluation of the predictive value of the C^* concept

Phenil J. Patel[1a], Abdel-Halim Ghanem[a], William I. Higuchi[a,*], V. Srinivasan[b], Earl R. Kern[c]

[a]*Department of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, University of Utah, Salt Lake City, UT 84112, USA*

[b]*TheraTech, Inc., 417 Wakara Way, Suite 100, Salt Lake City, UT 84108, USA*

[c]*Department of Pediatrics and Microbiology, School of Medicine, University of Alabama, Birmingham, AL 35294, USA*

Received 16 May 1995; accepted 4 December 1995

Abstract

The purpose of this study was to carry out an extensive examination of the C^* concept for prediction of the topical antiviral efficacies of acyclovir (ACV) formulations in a hairless mouse model for the treatment of cutaneous herpes simplex virus type-1 (HSV-1) infections. This method is based on estimation of the free drug concentration at the target site (C^*), which is presumed to be the basal cell layer of the epidermis. Five different formulations (containing 5% ACV) were examined in a finite dose multiple dosing regimen (twice a day application) to simulate the clinical situation. For determination of C^*, in vitro ACV fluxes across the hairless mouse skin were measured in an in vivo–in vitro experimental design that approximated the in vivo antiviral treatment protocol. Then, the in vivo antiviral efficacies were measured using a 1-day delayed (after HSV-1 virus inoculation) 4-day treatment protocol. 10 $\mu L/cm^2$ dose of ACV formulation was applied every 12 h for 4 days after which the lesions were scored and efficacies were calculated. Our results indicate that, over a wide range of efficacies, the predictions based on C^* (estimated from the experimental fluxes) are in good agreement with the in vivo antiviral efficacies. These studies, therefore, support the validity of the C^* concept for various ACV formulations and suggest that the C^* approach has potential for future practical situations.

Keywords: Acyclovir; Topical antiviral efficacies; HSV-1; C^* concept; Animal model

1. Introduction

For past several years, our group has been working on the development of a non-clinical

[1] Current address: TheraTech, Inc., 417 Wakara Way, Suite 100, Salt Lake City, UT 84108.

* Corresponding author. Tel.: +1 801 581 7831; fax: +1 801 581 4499.

SSDI 0166-3542(95)00941-E

methodology to assess topical bioavailability and to predict efficacy of dermatological formulations, particularly those used for the treatment of local skin diseases. A novel approach, based on the concept of C^* (i.e., the free drug concentration at the skin target site), was recently proposed and then applied to predict the effectiveness of acyclovir (ACV) topical formulations in the treatment of cutaneous herpes simplex virus type-1 (HSV-1) infections in hairless mice (Su et al., 1991; Lee et al., 1993). Cutaneous HSV-1 infections in hairless mice induce narrow lesion bands which can be curtailed more or less by topical application of different antiviral agents, thereby providing a quantitative method for evaluation of topical and systemic antiviral efficacies (Gonsho et al., 1990). This method, which can distinguish between topical and systemic effects, was used to establish a relationship between ACV flux (using controlled delivery transdermal patches) and in vivo efficacies (both topical and systemic) (Gonsho et al., 1990; Lee et al., 1992; Imanidis et al., 1994). Subsequently, C^* for 50% antiviral efficacy, C^*_{50}, was determined from the plasma levels of ACV measured using the transdermal patches that provided fluxes resulting in 50% systemic efficacy (Imanidis et al., 1994). The C^*_{50} was found to be 0.25 μg/ml which is consistent with the literature values of ID_{50} for ACV obtained in vitro from the Vero cell cultures (Al-Hasani et al., 1986; Smee et al., 1985; McLaren et al., 1982). 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. The in vivo dermis permeability coefficient (P_D) for ACV was then estimated using C^*_{50} in the equation,

$$P_D = J_{50}/C^*_{50} \quad (1)$$

where J_{50} is the ACV flux resulting in 50% topical efficacy and is equal to 30 μg/cm^2/day (Lee et al., 1992; Imanidis et al., 1994). The above analysis gave a P_D value of 1.4×10^{-3} cm/s, which can then be used in Eq. 2 to calculate the predicted C^* achieved by a given topical formulation:

$$C^* = J/P_D \quad (2)$$

where J is the experimentally measured ACV flux produced by that formulation (Lee et al., 1993).

In an earlier report, the first attempts to utilize the C^* concept for predicting the effectiveness of topical ACV formulations were described (Lee et al., 1993). This initial study yielded encouraging results and it was decided to further investigate the applicability of this approach and an extensive examination of the predictive value of C^*, over a range of topical efficacies, was undertaken using several ACV formulations. The present report

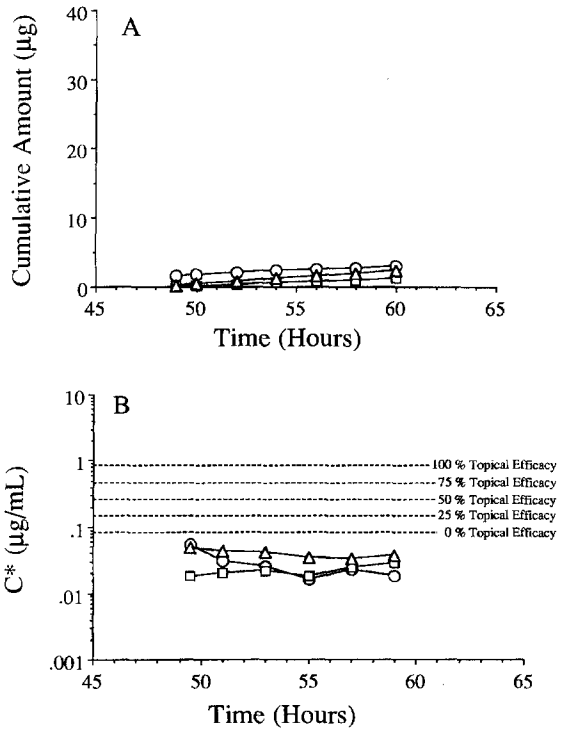

Fig. 1. (A) The cumulative amounts of ACV delivered topically across hairless mouse skin into Franz cell receiver chambers using a 10-μl finite dose of formulation I in an in vitro experiment carried out after 2 days of in vivo pretreatment. The data shown are from one representative set of three runs carried out at the same time. Four such sets of runs were conducted for this formulation. (B) The temporal pattern of C* assessed for formulation I according to Eq. 2 using the instantaneous ACV fluxes estimated from the slopes of the lines connecting two consecutive time points from the plots shown above in A. The broken horizontal lines (- - -) represent the C* values corresponding to the indicated topical antiviral efficacies calculated based on the results of Lee et al. (1992).

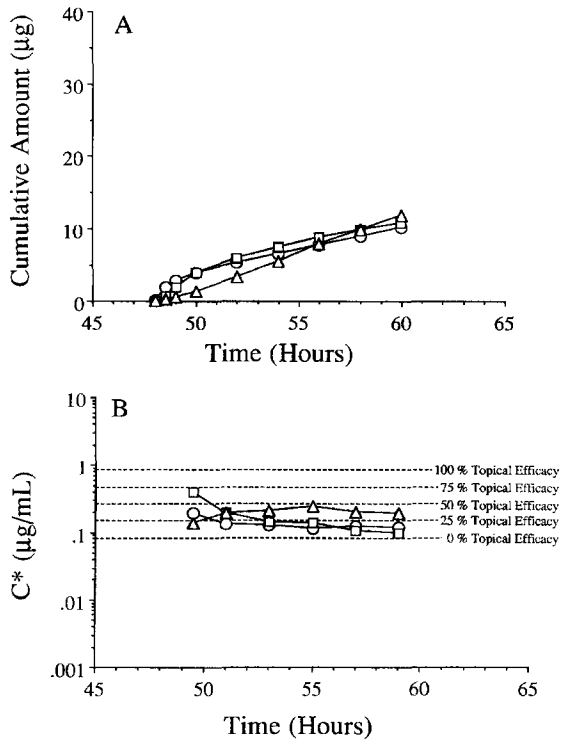

Fig. 2. Caption is the same as in Fig. 1, except the data shown are for a representative set of three runs (from five sets) with formulation II.

describes the application of the C^* concept using five different formulations (containing 5% ACV) in an attempt to see whether a correlation could be demonstrated between observed and predicted efficacies over a wide range of values.

2. Materials and methods

2.1. Drug formulations, virus and animals

The five topical formulations studied here (referred to as I, II, III, IV and V) contained 5% ACV and different proportions of the commonly used enhancers such as lauryl pyrrolidone, polyethylene glycol, sodium lauryl sulfate, methyl laurate and ethanol. HSV-1, strain E-377, with a final titer of 1.35×10^8 plaque forming units (PFU)/ml, stored at -70°C, was used for inoculation. The preparation and assay of virus have been reported previously (Kern et al., 1973). Female hairless mice (strain SKH/HR1), 5–6 weeks old with an average body weight of 20 ± 2 g, were purchased from Charles River, Bloomington, MA and were used for these studies.

2.2. In vitro ACV flux determinations and C^* predictions

In vitro ACV flux determinations with the hairless mouse skin and C^* predictions were carried out for each formulation using a combined in vivo–in vitro experimental design described by Lee et al. (1993). Each experiment was performed in triplicate. During the in vivo part of the experiment, the hairless mice were dressed in Velcro jackets and a finite dose (10 μl) of a formulation was applied every 12 h (after removal of residual formulation from the previous dose) onto a circular skin site with an area equivalent to the diffu-

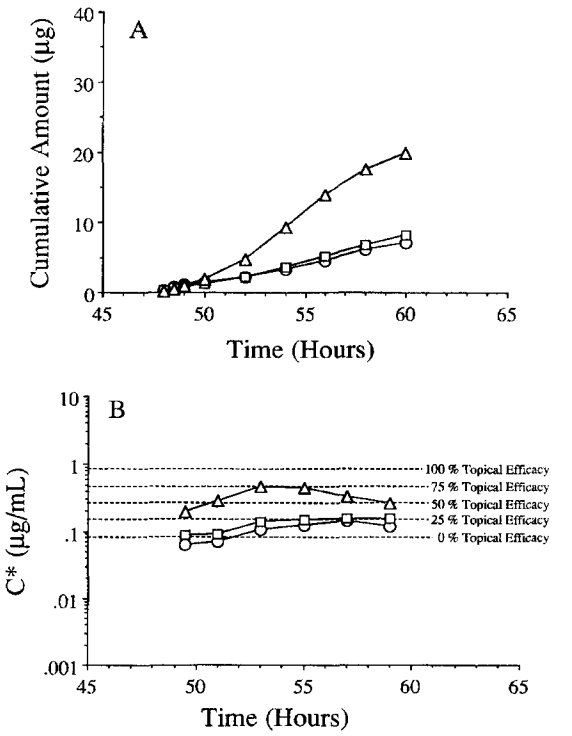

Fig. 3. Caption is the same as in Fig. 1, except the data shown are for a representative set of three runs (from three sets) with formulation III.

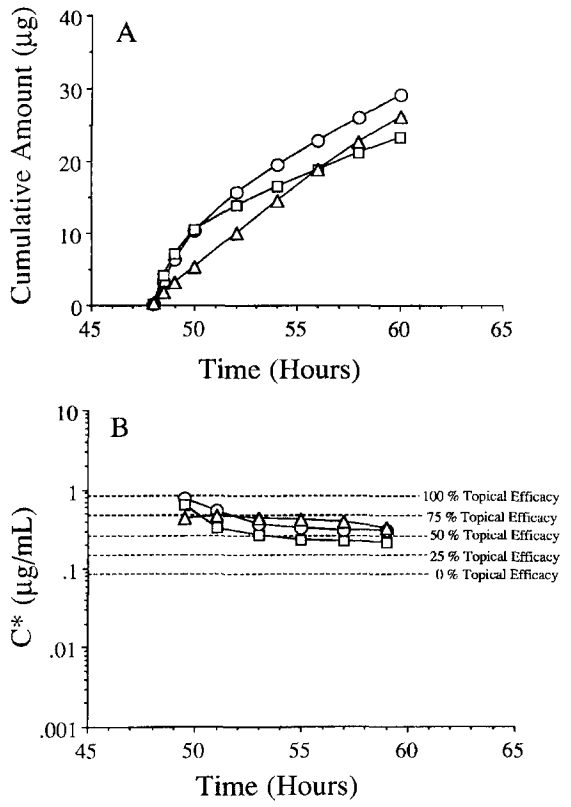

Fig. 4. Caption is the same as in Fig. 1, except the data shown are for a representative set of three runs (from four sets) with formulation IV.

sional area (1 cm^2) of the Franz cell. An in vitro flux experiment was carried out after 2 days of such in vivo pretreatment. For the in vitro part of the experiment, the Velcro jacket was removed from each mouse and the residual drug on the skin was wiped with a cotton tipped applicator. The mouse was then sacrificed by cervical dislocation and a new dose of 10 μl was applied to the formulation-pretreated skin site, which was then excised and mounted on a Franz cell. The receiver chamber was filled with saline containing 0.02% sodium azide. The sampling port of the receiver chamber was kept covered except during sampling, while the donor chamber was exposed to the atmosphere to mimic the conditions of the in vivo efficacy experiments (described below). The receiver chamber was stirred and maintained at 37°C during the experimental run. At each sampling time, a 1 ml aliquot was removed from the receiver chamber and was replaced with 1 ml of fresh saline. All samples were analyzed for ACV concentration using HPLC (150 mm × 4.6 mm C-18 reversed phase column; methanol/water '5 : 95' as mobile phase at flow rate of 0.8 ml/min using UV detection at 254 nm). The cumulative amount of ACV permeated into the receiver chamber was plotted as a function of time and instantaneous ACV fluxes were estimated from the slopes of lines connecting two consecutive time points. C^* was then calculated using Eq. 2, with $P_D = 1.4 \times 10^{-3}$ cm/s. Instantaneous C^* (calculated from instantaneous fluxes) were plotted against time, and the temporal pattern of C^* thus obtained was used along with the flux versus efficacy relationship established earlier (Lee et al., 1992; Imanidis et al., 1994) to predict topical efficacies for each formulation.

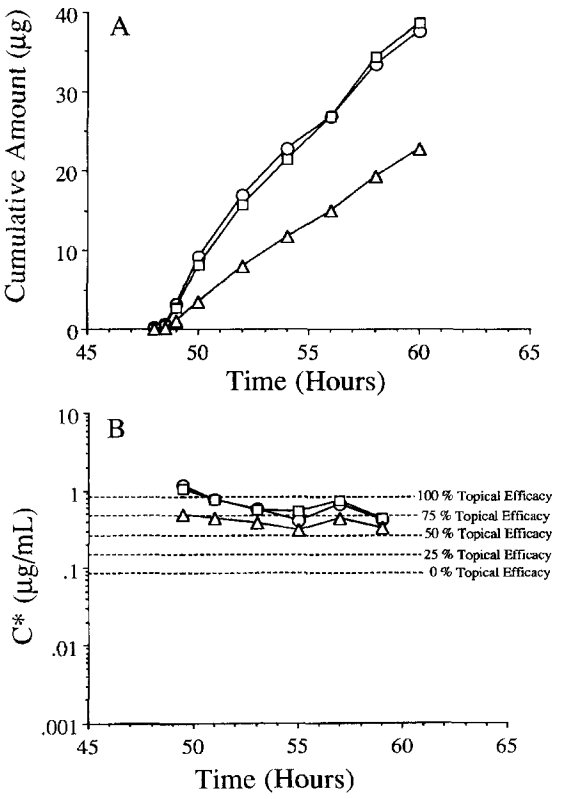

Fig. 5. Caption is the same as in Fig. 1, except the data shown are for a representative set of three runs (from three sets) with formulation V.

2.3. In vivo antiviral efficacy studies in hairless mice

The in vivo antiviral efficacy studies were carried out with minor modifications using the method described by Lee et al. (1993). For each study there were several treatment groups (nine or more mice per group) treated with the ACV formulations. For control, a placebo group treated with a formulation identical to the experimental formulation but containing no ACV, was always run along with the experimental group(s). On the first day, hairless mice were inoculated with HSV-1 using the procedures reported earlier (Gonsho et al., 1990; Lee et al., 1992). On the second day, a 4-day treatment was initiated. For the first few studies, a finite dose of 10 μl was applied every 12 h (after removal of residual formulation from the previous dose) onto a circular skin site (1 cm^2 in area) approximately 1 cm dorsal to the virus inoculation site in the predicted lesion development path. For later studies, a 20-μl dose was applied onto a rectangular skin site of 2 cm × 1 cm instead of the 1-cm^2 circular dosing site. This was done to minimize the possibility of the lesion development path missing the dosing site and thereby reduce the number of occurrences in the inconclusive lesion category of 'miss (M)' described below. Each mouse wore a Velcro protection jacket during the treatment period and was housed individually. Five days post-inoculation (i.e., after the fourth day of treatment), the lesion was scored for each mouse and the antiviral efficacy of the respective formulations calculated as described in the following section.

2.4. Measurement of antiviral efficacy

The antiviral efficacy was measured based on the five lesion categories described by Gonsho et al. (1990), namely, 'through' (Th), 'stop' (St), 'jump' (J), 'not reach' (NR) and 'miss' (M). Briefly, they represent the cases where the lesion passes through (Th), stops (St) at the edge of, jumps (J) over, does not reach (NR), or totally misses (M) the skin site treated with the formulation. Based on these lesion categories, the two antiviral efficacies were calculated using the following equations given by Lee et al. (1992):

topical efficacy (%)

$$= \frac{N_{St} + N_J + N_{NR}}{N_{St} + N_J + N_{NR} + N_{Th}} \times 100\% \quad (3)$$

and

systemic efficacy (%)

$$= \frac{N_{NR}}{N_{St} + N_J + N_{NR} + N_M + N_{Th}} \times 100\% \quad (4)$$

where N_{St}, N_J, N_{NR}, N_{Th} and N_M are the numbers of animals corresponding to each of the five lesion categories in the respective experimental groups.

3. Results and discussion

3.1. In vitro ACV flux determinations and C* predictions

Figs. 1A–5A are the results of the in vitro experiments showing the cumulative amounts of ACV transported across the hairless mouse skin into the Franz cell receiver chambers from 10 μl finite doses of formulations I–V. Since a twice-a-day dosing regimen (every 12 h) was followed for the in vivo efficacy studies, the in vitro runs were for 12 h. A 2-day pretreatment period was chosen for convenience because earlier results (Lee et al., 1993) suggested that the fluxes remained fairly constant after 1 day of pretreatment (this possibly due to the time needed for equilibration of enhancers in the skin).

Figs. 1B–5B show the temporal pattern of predicted C^* according to Eq. 2 (with $P_D = 1.4 \times 10^{-3}$ cm/s) using the instantaneous ACV fluxes estimated from the slopes of the respective lines connecting two consecutive points in the corresponding plots of Figs. 1A–5A. It was previously shown from in vivo–in vitro experiments conducted over a period of 5 days (Lee et al., 1993) that the C^* values were relatively constant over the entire treatment period of 4 days; therefore, it is believed reasonable to assume that the single in vitro flux determined (in the present study) after 2 days of pretreatment should be representative of the flux over the entire 4-day

Table 1
Antiviral efficacies in the topical treatment of HSV-1 cutaneously infected mice using different ACV formulations

Expt. group number [a,b]	Formulation	Number of mice in group	Number of mice in each lesion category (observed on day 5)					Topical efficacy(%)	Systemic efficacy(%)
			St	J	NR	M	Th		
1-A [c]	I	9	0	0	0	1	8	0	0
2-B	I	10	0	0	0	0	10	0	0
3-C	I	10	0	0	0	0	10	0	0
4-A [c]	II	10	3	1	1	1	4	56	10
5-B	II	10	3	1	1	0	5	50	10
6-C	II	10	3	1	1	0	5	50	10
7-D [c]	II	11	2	0	3	0	6	45	27
8-E [c]	II	10	2	1	0	0	7	30	0
9-F [c]	II	13	4	0	0	0	9	31	0
10-G [c]	II	10	2	0	0	1	7	22	0
11-B	III	10	2	2	0	0	6	40	0
12-C	III	10	2	1	0	0	7	30	0
13-G [c]	III	11	4	0	0	4	3	57	0
14-H	III	10	1	1	1	0	7	30	10
15-B	IV	10	1	6	0	0	3	70	0
16-C	IV	9	4	0	1	0	4	56	11
17-D [c]	IV	11	2	0	2	0	7	36	18
18-E [c]	IV	11	1	1	2	1	6	40	18
19-C	V	9	3	0	3	0	3	67	33
20-H	V	10	2	2	1	0	5	50	10
21-J	V	10	1	9	0	0	0	100	0
22-K	V	10	5	3	0	0	2	80	0
23-L	V	10	2	7	1	0	0	100	10

[a] For different formulations, data shown with the same capital letter next to the experimental group number are from the same set where all the groups were run at the same time with the same lot of mice.

[b] For each experimental set, a control group of nine or more mice treated with placebo was always run and all the mice in these groups were found to display a 'Th' lesion category.

[c] For these experimental groups, a 10-μl formulation was applied onto a circular dosing site of 1 cm^2. For all the other experimental groups, a 20-μl dose was applied onto a rectangular dosing site of 2 cm^2 and the systemic efficacies for these groups are reported without correction for this higher dose.

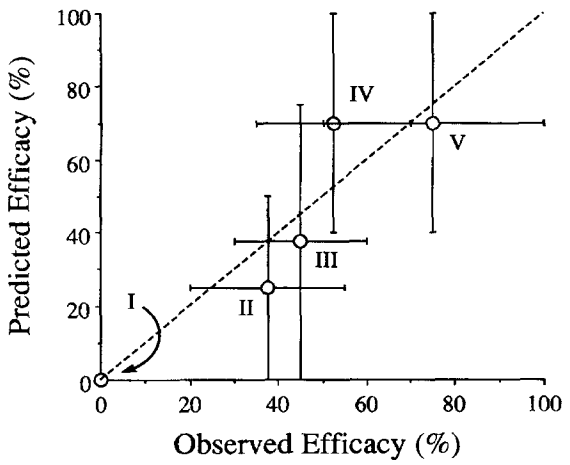

Fig. 6. Correlation of in vitro predicted efficacies with the observed in vivo efficacies. Here the observed in vivo efficacies for each formulation are taken from Table 1, and the predicted in vitro efficacies are based on three to five sets of three runs (such as shown in Figs. 1B–5B). Each point represents the midpoint of the range of all the experimental values (shown using error bars) obtained for each formulation. The broken line (- - -) represents the theoretical line for ideal correlation based on the C* concept. The correlation coefficient, $R^2 = 0.854$.

period of the in vivo experiments. The relationship between topical efficacy and C^* (i.e., - - - in Figs. 1B–5B) were previously deduced from the flux versus efficacy data (Gonsho et al., 1990; Imanidis et al., 1994) using Eq. 2 and a P_D value of 1.4×10^{-3} cm/s. It is clear from Figs. 1–5 that, except for formulation I, for which the C^* values remained below the 0% efficacy level, all the other formulations yielded fluxes resulting in the predicted C^* corresponding to efficacies ranging from 0–100%. It is also noted that none of the formulations resulted in C^* above the minimum level required for 100% topical efficacy.

Based on the predicted C^* values obtained from three or more separate experiments (each performed in triplicate), the range of predicted efficacies, for formulations I–V, respectively, are 0%, 0–50%, 0–75%, 40–100% and 40–100%. The high variability in the in vitro flux results is believed attributable, to a large extent, to animal-to-animal variability and, to a lesser extent, to the variability in the application of the topical formulation.

3.2. In vivo antiviral efficacy studies in hairless mice

The antiviral efficacies for each formulation were measured in three or more separate experiments and the results are summarized in Table 1. For each experiment, all the animals in the placebo group displayed a 'Th' lesion category (100% infection), confirming the viability of virus used and the reliability of the inoculation technique. For each experimental group, the antiviral efficacies were calculated using Eqs. 3 and 4 based on the number of animals corresponding to each of the five lesion categories as described earlier (Lee et al., 1992). It is important to note that for some of the experiments, the dose and the surface area of dose application site were both twice those of the others. The amount of ACV delivered, in these cases, will be doubled due to the larger diffusional surface area. Topical efficacy would not be expected to be affected by this, as it is expected to be dependent on the concentration of the ACV in the basal layer of the epidermis, which should be the same if both the amount of ACV delivered and the surface area are increased proportionally. However, by increasing the amount of ACV delivered into the systemic circulation, the concentration in blood is increased and the previously reported flux versus efficacy curve for systemic efficacy (Gonsho et al., 1990; Imanidis et al., 1994) would be expected to shift to the left. Consequently, the observed systemic efficacies in experimental groups treated with 20 μl of ACV applied on 2 cm^2 surface would be expected to be higher than those treated with 10 μl of ACV applied on 1 cm^2 surface. It is seen, however, that there is no consistent pattern (see last column of Table 1) of greater systemic efficacy when the 20-μl doses were used. This is probably due to the large inherent animal variabilities and to a factor of two in the dose being too small to show a significant difference in systemic efficacy.

3.3. Correlation of in vitro predicted efficacies with the observed in vivo efficacies

Fig. 6 shows the observed in vivo topical efficacies plotted against the predicted efficacies esti-

mated based on C^* assessed from the in vivo–in vitro data. The error bars (both vertical and horizontal) shown represent ranges of values obtained from three or more separate experiments. The high variability in the in vivo results was expected because of the statistics of small numbers, i.e., the data used to calculate efficacies involved small groups of mice (9 or 10) scored in various lesion categories, resulting in high statistical variation. As seen in Fig. 6, the theoretical line calculated based on the C^* concept passes through the range of values for each data point, suggesting that there is a good correlation between the in vitro ACV flux (and C^* predictions) and the in vivo antiviral efficacies.

4. Conclusions

The validity of C^* concept for prediction of topical efficacies has been supported with experiments involving various ACV topical formulations. The in vivo efficacies of ACV formulations were found to be in good agreement with the C^* predictions over a wide range of topical efficacies, and it is concluded that the C^* approach has potential for future practical situations. Future efforts should be directed to establish a further generalization of the C^* concept, using drugs other than ACV.

Acknowledgements

This work was supported by a Grant-in-Aid from TheraTech, Inc. and by NIH Grant AI 20161.

References

Al-Hasani, A.M., Barton, I.G., Al-Omer, L.S., Kinghorn, G.R. and Potter, C.W. (1986) Susceptibility of HSV strains from patients with genital herpes treated with various formulations of acyclovir. J. Antimicrob. Chemother. 18 (Suppl. B), 113–119.

Gonsho, A., Imanidis, G., Vogt, P., Kern, E.R., Tsuge, H., Su, M.-H., Choi, S.-H. and Higuchi, W.I. (1990) Controlled (trans)dermal delivery of an antiviral agent (acyclovir) I: an in vivo animal model for efficacy evaluation in cutaneous HSV-1 infections. Int. J. Pharm. 65, 183–194.

Imanidis, G., Song, W.-Q., Lee, P.H., Su, M.-H., Kern, E.R. and Higuchi, W.I. (1994) 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. Pharm. Res. 11, 1035–1041.

Kern, E.R., Overall, J.C. and Glasgow, L.A. (1973) Herpesvirus hominis infection of newborn mice. I. An experimental model and therapy with iodoxyuridine. J. Infect. Dis. 128, 290–299.

Lee, P.H., Su, M.-H., Kern, E.R. and Higuchi, W.I. (1992) Novel animal model for evaluating topical efficacy of antiviral agents: Flux versus efficacy correlations in the acyclovir treatment of cutaneous HSV-1 infections in hairless mice. Pharm. Res. 9, 979–989.

Lee, P.H., Su, M.-H., Ghanem, A.-H., Inamori, T., Kern, E.R. and Higuchi, W.I. (1993) An application of the C* concept in predicting the topical efficacy of finite dose acyclovir in the treatment of cutaneous HSV-1 infections in hairless mice. Int. J. Pharm. 93, 139–152.

McLaren, C., Sibrack, C.D. and Barry, D.W. (1982) Spectrum of sensitivity to acyclovir of herpes simplex virus clinical isolates. Acyclovir symposium. Am. J. Med. 73, 376–379.

Smee, D.F., Campbell, N.L. and Matthews, T.R. (1985) Comparative anti-herpes virus activities of 9-(1,3-dihydroxy-2-propoxymethyl) guanine, acyclovir and two 2′-fluoropyrimidine nucleosides. Antiviral Res. 5, 259–267.

Su, M.-H., Lee, P.H., Ghanem, A.-H. and Higuchi, W.I. (1991) A novel method to assess bioavailability and to predict efficacy for dermatological formulations. Chin. Pharm. J. 43, 265–274.