Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 253 (2003) 159–168
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Effect of Azone upon the in vivo antiviral efficacy of cidofovir or acyclovir topical formulations in treatment/prevention of cutaneous HSV-1 infections and its correlation with skin target site free drug concentration in hairless mice

Mohsen I. Afouna [a,b,*], Timothy K. Fincher [a], Abdel-Azim A. Zaghloul [b,c], Indra K. Reddy [a]

[a] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, 4301 W. Markham, St. No. 522-3, Little Rock, AR 72205, USA*
[b] *Department of Pharmaceutics, Al-Azhar University, Nasr City, Cairo, Egypt*
[c] *Department of Pharmaceutical Sciences, Texas Tech School of Pharmacy, 1300 Coulter, Amarillo, TX 79106, USA*

Received 20 September 2002; received in revised form 12 December 2002; accepted 12 December 2002

Abstract

The purpose of this study is to examine the influence of Azone upon the skin target site free drug concentration (C^*) and its correlation with the in vivo antiviral efficacies of cidofovir (HPMPC) and acyclovir (ACV) against HSV-1 infections. Formulations of HPMPC and ACV with or without Azone were used. The in vitro skin flux experiments were performed and the C^* values were calculated. For the in vivo efficacy studies, hairless mice cutaneously infected with HSV-1 were used and three different treatment protocols were carried out. The protocols were chosen based upon when therapy is initiated and terminated in such a way to assess the efficacy of the test drug to cure and/or prevent HSV-1 infections. A finite dose of the formulation was topically applied twice a day for the predetermined time course for each protocol and the lesions were scored on the fifth day. For ACV formulation with Azone, the C^* values and hence the in vivo efficacy were much higher than those for that without Azone. In protocol #1, however, early treatment did not increase the in vivo efficacy of ACV when compared with the standard treatment protocol #3. In protocol #2 where the treatment was terminated on the day of virus inoculation, the efficacies for both ACV formulations were completely absent. Although the estimated C^* values for HPMPC formulations with and without Azone were comparable, formulation with Azone was much more effective than that without Azone in all treatment protocols. HPMPC formulations with Azone at similar flux values were much more effective in "treating and preventing" HSV-1 infections than those without Azone. For ACV formulations, in contrast, addition of Azone has failed to show any effect on the preventive in vivo antiviral efficacy and the enhancement of ACV in vivo antiviral efficacy was merely the skin permeation enhancement effect of Azone.

Keywords: Azone; Penetration enhancers; C^* concept; In vitro flux; Cidofovir; HPMPC; Acyclovir; ACV; In vivo antiviral efficacy; HSV-1; Hairless mice

* Corresponding author. Tel.: +1-501-526-6937; fax: +1-501-526-6510.
E-mail address: afounamohsen@uams.edu (M.I. Afouna).

0378-5173/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-5173(02)00705-6

1. Introduction

Over the past several years, the relationship between the in vivo topical efficacy of some antiviral agents and skin target site free drug concentration (C^*) has been assessed (Afouna et al., 1998). Recently, it was found that the topical effectiveness of acyclovir (ACV, Fig. 1a) formulations was essentially the same when the therapy was initiated as early as 6 days prior to cutaneous herpes simplex virus type-1 (HSV-1) inoculation or later using hairless mice (Afouna et al., 1999). Similar results were obtained when the treatment with ACV in a transdermal delivery system was initiated on the day of HSV-1 virus inoculation (0-day), 1 or 2 days later using the same virus strain and the same animal model (Lee et al., 1992). The efficacy of ACV was lost when the treatment was terminated on the day of the virus inoculation, whereas the efficacy of cidofovir ((*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine (HPMPC, Fig. 1b) as a new class of antiviral agents, was found to be strongly dependent on how early the therapy was initiated. Unlike ACV, a relatively high in vivo efficacy was observed with HPMPC even when the treatment was terminated on the day of virus inoculation (Afouna et al., 1999). These results suggested that the in vivo topical efficacy for ACV is a single-valued function of C^* (Afouna et al., 1998, 1999; Lee et al., 1992; Mehta et al., 1997), whereas the topical efficacy for HPMPC is likely to be a function of C^* at a fixed treatment protocol (i.e., the in vivo antiviral efficacy of HPMPC is not a single function of C^*) (Afouna et al., 1999). The relatively straightforward behavior exhibited in case of ACV is thought to be the consequence of its relatively rapid local pharmacokinetics at the cellular level. HPMPC exhibits rather slow kinetics where the active metabolites remain intracellularly for longer period of time (Hitchcock, 1996; Cundy et al., 1995). In another but a related study with bromovinyldeoxyuridine (BVDU), we found that formulations with 5% Azone were much more effective in vivo than those without Azone at similar flux values (Afouna et al., 1998). Further, varying the Azone concentration did not affect the BVDU permeability parameters (Afouna, 2001). One of the goals of our research is to investigate the treatment and/or preventive therapy protocols of antiviral agents against HSV-1 infections with or without skin permeation enhancers. To this end, we have evaluated the applicability of the C^* concept using a variety of antiviral formulations and employing different treatment protocols, while using ACV as a control. The specific objectives of the current study are: (i) to examine the hypothesis that Azone "penetration enhancer" would remarkably increase the time-dependent in vivo efficacy of HPMPC against HSV-1 and hence shorten the course needed for maximum in vivo antiviral efficacy in treatment and/or prevention of such infections; (ii) to examine the effect of Azone upon the C^* estimates for HPMPC; and (iii) to assess the relationship between the in vitro C^* estimates and the in vivo antiviral efficacy for HPMPC using a set of formulations containing different drug concentrations with 5% Azone and compare that with our previous studies with HPMPC formulations without Azone (Afouna et al., 1998).

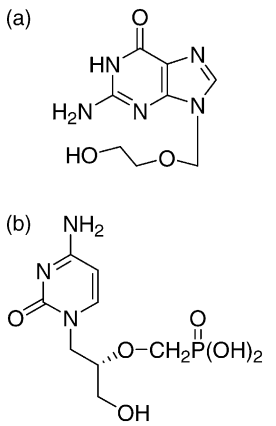

Fig. 1. (a) Chemical structure of acyclovir; 9-[(2-hydroxyethoxy)-methyl]guanine; and (b) chemical structure of cidofovir; ((*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine.

2. Materials and methods

2.1. Animal

Female hairless mice strain SKH/HR1 (Charles River, Bloomington, MA), 6–8 weeks old with average body weight of 22–27 g, were used throughout this study.

Table 1
Composition of formulation and mean C^* estimates from the in vivo–in vitro experiments for HPMPC and ACV

HPMPC formulations[a]						ACV formulations[a]					
Code	HPMPC (%)	Vehicle	Mean C^* (μg/ml)	±S.D.	n	Code	ACV (%)	Vehicle	Mean C^* (μg/ml)	±S.D.	n
H1	0.5	95% DMSO	1.2	0.04	6	A1	0.1	95% DMSO	0.12	0.01	9
H2[b]	0.1	95% DMSO + 5% Azone	0.26	0.06	3	A2	0.1	95% DMSO + 5% Azone	0.21	0.01	6
H3[b]	0.25	95% DMSO + 5% Azone	0.73	0.16	3						
H4[b]	0.5	95% DMSO + 5% Azone	1.02	0.41	6						
H5[b]	1	95% DMSO + 5% Azone	2.43	0.35	3						
H6[b]	5	95% DMSO + 5% Azone	4.31	0.70	3						

[a] All formulations contained 1% hydroxypropylcellulose as a thickening agent.
[b] A set of HPMPC formulations with Azone were used to asses correlation of C^* with the in vivo antiviral efficacy employing the standard treatment protocol (see text for details).

2.2. Virus

Samples from the same batch of herpes simplex virus type-1, strain E-377, with a final titer of 4×10^7 PFU/ml, were used for inoculation. Samples were stored at $-70\,^{\circ}C$ in aliquots until used. The preparation and assay methods of the virus have been previously reported (Kern et al., 1973).

2.3. Drug formulations

The compositions of the formulations used in this study are shown in Table 1. All HPMPC and ACV formulations contained 95% DMSO as a vehicle with or without 5% Azone. Also, 1% hydroxypropylcellulose (Sigma Chemical Co., St. Louis, MO) was used as a thickening agent in all formulations. ACV was obtained as a free acid from Thera Tech, Inc. (Salt Lake City, UT), and HPMPC was a generous gift from Gilead Sciences, Inc. (Foster City, CA). Azone was kindly supplied by Whiteby Inc., Richmond, VA and DMSO was purchased from Baker Chemical Co. (Phillipsburg, NJ).

2.4. In vitro flux measurement and C^* predictions

For the in vitro determination of HPMPC and ACV fluxes and C^* predictions, a combined in vivo–in vitro experimental procedure reported earlier (Lee et al., 1992; Patel et al., 1996) was adopted and performed at least in triplicate for each formulation. ACV and HPMPC concentrations were analyzed in accordance with the previously reported reversed-phase HPLC methods (Afouna et al., 1999; Lee et al., 1992; Afouna et al., 1998; Mehta et al., 1997).

The cumulative amount of the test drug transported into the receiver chamber was plotted as a function of time, and the instantaneous flux (J) was estimated from the slope of the line connecting the two consecutive points. The instantaneous C^* estimates were then calculated using the following equation:

$$C^* = \frac{J}{P_D} \quad (1)$$

where J is the skin flux and P_D is the in vivo dermis permeability coefficient of the drug. The details of the derivation of this equation have been previously reported and a P_D value of 1.4×10^{-3} cm/s was obtained for ACV (Lee et al., 1992; Patel et al., 1996; Imanidis et al., 1994). Since the molecular sizes of HPMPC and ACV are sufficiently close, it is reasonable to use the in vivo P_D value of ACV for HPMPC (for the present purpose) (Afouna et al., 1999).

2.5. In vivo antiviral efficacy studies

The in vivo antiviral efficacy was evaluated using a group of 8–10 hairless mice per experiment, and an average of two to six experiments were performed for each drug formulation. In each experiment, a group of animals treated with a placebo formulation similar to the test formulation without the drug was

included as a negative control. For the purpose of these experiments, the animals were dressed in a Velcro jacket to protect the topically applied dose from being licked off by the mice and/or scratched/rubbed off when the mouse comes to the cage walls. The details of dressing the animals in the Velcro jacket and virus inoculation have been described earlier (Lee et al., 1992; Mehta et al., 1997). Hairless mice that were cutaneously infected with HSV-1 were used and three general therapeutic protocols were carried out. In all the protocols, a finite dose of 20 μl of the test antiviral formulation was topically applied twice a day. In protocol #1, the treatment was started at days 4, 3, 2 and 1 before virus inoculation (−4, −3, −2 and −1 days, respectively) and the treatment was continued until the fourth day after virus inoculation (+4 day). In protocol #2, the treatment was initiated at days 4, 3, 2 and 1 before virus inoculation and was terminated on the day of virus inoculation. In protocol #3, which is a standard treatment protocol for cutaneous HSV-1 infection, the treatment was started a day after the virus inoculation and was continued until the fourth day after virus inoculation. The day of virus inoculation was always considered as day 0. For all protocols, the lesions were scored on day 5 as previously described (Gonsho et al., 1990) and the antiviral efficacies were calculated using the equations reported earlier (Lee et al., 1992).

During the treatment protocols, the dosing was performed over 4–9 days, and hence the animal was dressed with a Velcro jacket for that period of time. This raises the question of the possible effect of stress induced due to prolonged dressing of the mice in Velcro jackets on the efficacy data. To assess this, an experiment was performed in which the animals were dressed in Velcro jackets on day −4 and the treatment with HPMPC formulation H4 (0.5% HPMPC with 5% Azone) was initiated on day +1. The results of this experiment were compared with the results obtained with that of standard protocol (where the dressing in the jacket and the treatment were performed over 4 days). The results of these two experiments were comparable indicating that prolonged dressing in the Velcro jacket had no effect on the efficacy data (not shown). The same conclusion was drawn from separate experiments with ACV formulation A2 (0.1% ACV and 5% Azone).

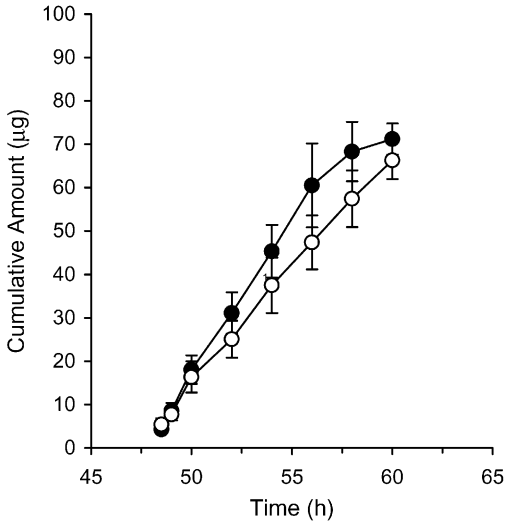

Fig. 2. Representative profiles of the mean cumulative amounts of HPMPC in the receiver chamber as a function of time for formulations H1 without Azone (●) and H4 with Azone (○).

3. Results and discussion

3.1. In vitro flux determination and C^* estimates

Fig. 2 shows representative profiles of the mean cumulative amounts of HPMPC delivered across hairless mice skin into the receiver chambers of Franz diffusion cells as a function of time for formulations H1 (without Azone) and H4 (with 5% Azone). Fig. 3 represents the temporal pattern of C^* estimates for the same formulations calculated according to Eq. (1) using the instantaneous fluxes of HPMPC estimated from the slopes of the lines connecting two consecutive points for both formulations. It must be noted that despite the presence of 5% Azone in formulation H4, its flux profile and temporal pattern of C^* estimates were comparable to that of formulation H1 without Azone.

In our previous study involving a series of in vivo–in vitro experiments conducted over a period of 5 days, it was shown that the in vitro HPMPC flux data, and hence the corresponding C^* values for HPMPC formulations without Azone, remained relatively unchanged over the entire treatment period (Afouna et al., 1999). Similar results (mean ± 25%) were also obtained for HPMPC formulations with Azone (data not shown).

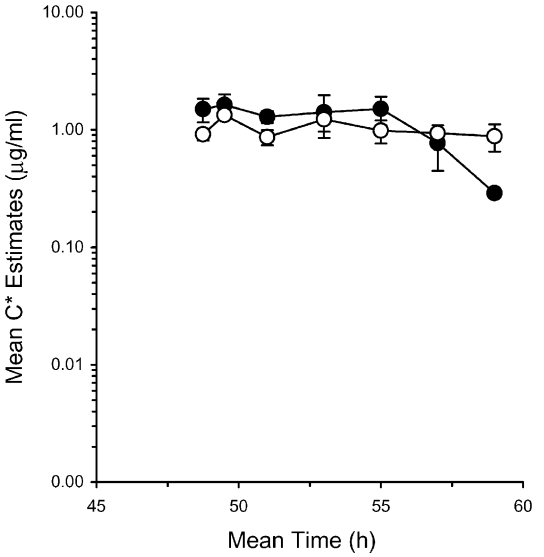

Fig. 3. The temporal pattern of the mean C^* values for formulations H1 (without Azone) (●) and H4 (with Azone) (○) calculated according to Eq. (1) using instantaneous HPMPC fluxes obtained from in vivo–in vitro experiments. (See text for details.)

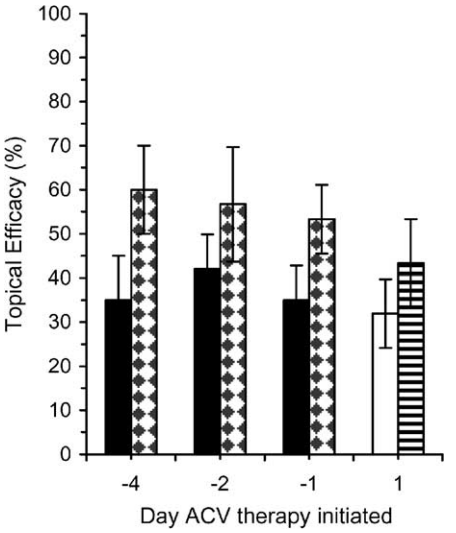

Fig. 4. The in vivo antiviral topical efficacy of 0.1% ACV formulations without Azone A1* (■) and with Azone A2 (◆) following treatment protocol #1 (when therapy was initiated at −4, −3, −2 and −1 day before virus inoculation). The in vivo antiviral topical efficacy of 0.1% ACV formulations without Azone A1* (□) and with Azone A2 (▤) following treatment protocol #3 (when therapy was initiated +1 day after virus inoculation (standard treatment protocol for cutaneous HSV-1 infections). *Data presented from our previous study for comparison.

Accordingly, it deemed reasonable to use the in vitro flux values measured after 2 days of pretreatment as representative for the in vivo flux values over the entire period of the in vivo efficacy experiment. Similarly, for a wide variety of ACV formulations with or without Azone, measuring the in vitro flux values after 2 days of in vivo pretreatment was believed to be an appropriate measure of the in vivo flux values over the entire treatment period (Afouna et al., 1998; Mehta et al., 1997). The mean values of the estimated C^* from the in vivo–in vitro experiments for HPMPC and ACV test formulations are shown in Table 1.

3.2. In vivo antiviral efficacy

3.2.1. ACV formulations

The observed in vivo topical efficacies for ACV formulations A1 and A2 in treatment protocols #1 and #3 are shown in Fig. 4. Each data point represents the mean observed in vivo topical antiviral efficacy for each formulation ($n = 2$–5). The in vivo topical antiviral efficacy for each formulation with or without Azone remained essentially unchanged in protocols #1 and #3, suggesting that starting treatment early with formulations A1 or A2 did not alter the efficacy of ACV against cutaneous HSV-1 infections. In treatment protocol #2 the efficacies for both ACV formulations were completely absent. These results are consistent with the earlier findings of Afouna et al. and Lee et al. where topical efficacies for ACV formulations without Azone were found to be independent of when the therapy was initiated (as early as 6 days before or as late as 2 days after virus inoculation) (Afouna et al., 1999; Lee et al., 1992). Therefore, the topical efficacy of ACV formulations was found to be a single-valued function of C^* and was explained on the basis of relatively rapid pharmacokinetics of ACV at the cellular level.

The increase in the in vivo antiviral efficacy of ACV formulation in presence of Azone can simply be attributed to its penetration enhancement effect. While it is apparent that Azone increases the in vivo antiviral efficacy of ACV formulation, it did not have any effect on the efficacy beyond enhancing the skin permeation rates. This result is consistent with our previous finding that Azone as a penetration enhancer did not

alter or shift the sigmoidal dose–response relationship curve of ACV established earlier (Lee et al., 1992; Afouna et al., 1998; Mehta et al., 1997; Imanidis et al., 1994).

Cell culture studies have shown that ACV (Fig. 1a) is converted to its mono-, di-, and triphosphorylated forms (ACVp, ACVpp, and ACVppp, respectively) in cells infected with HSV-1 (Fyfe et al., 1978). The initial phosphorylation of ACV to ACVp is catalyzed by the virally-induced thymidine kinase. Subsequent phosphorylations to ACVpp and ACVppp (which is the active metabolite that serves as a selective inhibitor of viral DNA polymerase), is carried out by endogenous cellular enzymes. In the uninfected cells, phosphorylations of ACV occur to a small extent (Furman et al., 1981). The intracellular concentration of the active species for ACV can be regarded as a function of: (i) C^*; (ii) the rate of cellular uptake of the drug; (iii) the rate of conversion of the drug to the active metabolite; and (iv) the rate of elimination of the active species. Since, the uninfected cells lack the virally-induced thymidine kinase enzyme that is responsible for conversion of ACV to ACVp (a precursor for the active species), the rate of formation of ACVppp in the uninfected cells is expected to be orders of magnitude smaller than that in the infected cells (Furman et al., 1981). Therefore, when the treatment with ACV formulations (with and without Azone) is started before virus inoculation, the rate of conversion of the drug to the active metabolite ACVppp (step iii) is very slow. Additionally, in cell culture studies, the levels of ACVppp declined rapidly after the removal of the drug from the medium. The initial half-life of the triphosphate was 1.2 h in the absence of ACV in the medium with the levels reaching a plateau after 6 h (Furman et al., 1981), suggesting that the rate of elimination of active species (step iv) is relatively rapid for ACV. The net effect of these findings is that the intracellular concentrations of ACVppp are negligible at the onset of infection. The results of protocol #3, in which no protective effect was seen with the pretreatment of ACV formulations with and without Azone, are consistent with these findings. In the case of infected cells, however, all the above steps are fairly rapid for ACV, resulting in a rapid attainment of steady-state. Consequently, the intracellular level of the active species as well as the in vivo efficacy appear to be a single-valued function of the C^* estimates.

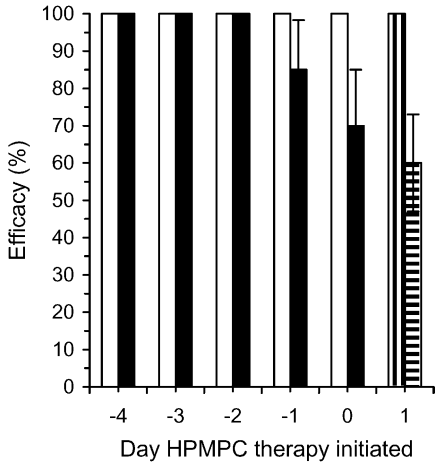

Fig. 5. Topical (□) and systemic (■) in vivo antiviral efficacies of HPMPC formulation containing Azone H4 following treatment protocol #1. Topical (▥) and systemic (▤) in vivo antiviral efficacies for the same formulation following treatment protocol #3.

3.2.2. HPMPC formulations

Although the permeation rates and the estimated C^* values for the two HPMPC formulations (H4 and H1) with and without Azone were approximately the same (1.2 and 1.02; Figs. 2 and 3), surprisingly, the in vivo topical and systemic antiviral efficacies for HPMPC formulation H4 in all protocols (Figs. 5 and 6) were much greater than that of a similar formulation containing no Azone reported earlier (Afouna et al., 1999). Fig. 5 shows the observed in vivo topical and systemic efficacies for HPMPC formulation H4 in treatment protocols #1 and #3. The observed in vivo efficacy for formulation H4 (0.5% HPMPC with 5% Azone) is relatively high when compared with that of our previous report using a similar HPMPC formulation but without Azone (Afouna et al., 1999). In addition, Azone has dramatically shortened the pretreatment period needed for the absolute (100%) topical and systemic efficacies from −6 days of pretreatment with HPMPC formulation without Azone reported earlier (Afouna et al., 1999) to −2 days of pretreatment with the similar formulation containing 5% Azone. Further, in this study with HPMPC formulation with Azone, the absolute (100%) topical efficacy and about 60% systemic efficacy were obtained in the standard treatment protocol, while only 17% topical efficacy and 0% systemic efficacy were obtained in our earlier report (Afouna

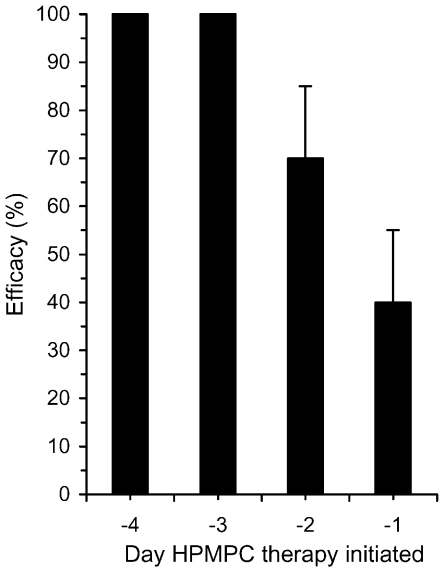

Fig. 6. The topical efficacy (■) of HPMPC in formulation H4 (with Azone) in treatment protocol #2.

et al., 1999) with similar HPMPC formulation without Azone employing the same treatment protocol.

Fig. 6 shows the in vivo antiviral efficacy for formulation H4 in protocol #2. It is obvious that the absolute (100%) topical efficacy was obtained when the treatment started 4 and 3 days prior to virus infection, while ~70 and 40% topical efficacy were obtained when the treatment started just 2 and 1 day before infection, respectively. These results are relatively higher than that obtained earlier (Afouna et al., 1999) with a similar formulation without Azone under similar experimental conditions, where only about 33 and 25% topical efficacies were observed when the treatment started 4 and 2 days before virus inoculation, respectively. In addition, no efficacy was observed when the pretreatment period was further reduced from 2 days.

HPMPC is a phosphonated nucleotide analogue (Fig. 1b), much like ACVp. However, in uninfected cells, unlike ACV, HPMPC is further phosphorylated by normal host enzymes to its mono- and di-phosphates (HPMPCp and HPMPCpp, respectively). HPMPCpp is the active metabolite of HPMPC which acts as selective inhibitor of viral DNA polymerase. Furthermore, the HPMPCp-choline adduct (with an exceptionally long half-life) is another intracellular moiety identified in cell culture studies and acts as a source (depot) for the active metabolite (Ho et al., 1992; Cihlar et al., 1992; Aduma et al., 1995; DeClercq and Holy, 1991). Therefore, there are notable differences between HPMPC and ACV with respect to mechanism of action, pharmacokinetics, and drug disposition parameters. Since the rate of formation of HPMPCpp does not depend upon the virus-encoded thymidine kinase enzyme as in case of ACV, no difference in the rate of conversion into active metabolite (step iii) is expected to exist between uninfected and infected cells. Moreover, in cell culture studies, the levels of HPMPCpp remained high for long time after the removal of the drug from the medium. The initial half-life of the triphosphate was 17 h while that for the HPMPCp-choline adduct was quite longer (>48 h) (DeClercq, 1993; Furman et al., 1979; St. Clair et al., 1980; Larsson et al., 1983). Therefore, the rate of elimination of active species (step iv) is relatively slow for HPMPC. The net outcome of such slow pharmacokinetic disposition of HPMPC is the attainment of sufficiently high intracellular concentration of HPMPCpp (active metabolite) before infection sets in. This can explain the results obtained with protocol #2, where a protective effect was obtained with the pretreatment of HPMPC formulations with and without Azone. In case of formulation H4 with Azone, however, the in vivo efficacies are much greater than that without Azone reported earlier under similar treatment protocols (Afouna et al., 1999). It is possible that Azone might affect one or more of the intrinsically slow steps mentioned earlier (i.e. the extracellular drug concentration (C^*), cellular uptake of the drug, conversion of drug to active metabolite, and/or drug elimination rate). Thus, the effect of Azone may be more than mere penetration enhancement because of possible interplay between Azone and one or more of the above steps, resulting in faster HPMPC steady-state and higher intracellular level of the active species as well as the in vivo efficacy.

3.2.3. *Correlation between HPMPC C^* estimates and the in vivo efficacy*

In a separate series of experiments, the relationship between the C^* estimates and the observed in vivo antiviral efficacy for HPMPC formulations containing different drug concentrations (0.1–5%) with 5% Azone (H2–H6) was assessed. In these experi-

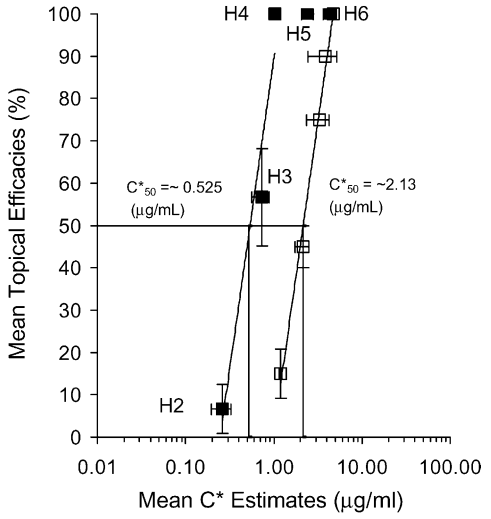

Fig. 7. Correlation between the mean C^* estimates and the in vivo topical antiviral efficacy for HPMPC topical formulations in the standard treatment protocol #3: formulations H2–H6 containing different drug concentrations (0.1–5%) with 5% Azone [closed symbols (■)], and set of formulations of similar compositions containing 0.5–10% HPMPC without Azone [open symbols* (□)] *data presented from our previous report for comparison.

ments, the standard treatment protocol for cutaneous HSV-1 infections was employed. The observed in vivo topical antiviral profiles of HPMPC formulations with and without Azone are depicted in Fig. 7. The dose–response relationship of HPMPC formulations with 5% Azone followed a straight line and shifted left in relation to the dose–response relationship obtained previously for HPMPC formulations containing different drug concentrations (0.5–10%) without Azone under similar experimental protocol (Afouna et al., 1999). In these studies, increasing the HPMPC concentration resulted in an increase in the in vitro flux and C^* values, which in turn increased topical in vivo efficacy. In order to obtain the comparable topical efficacy, therefore, a much higher flux is required for a HPMPC formulation without Azone compared to that for a formulation with Azone. Likewise, at similar flux values, the topical efficacies of HPMPC formulations with Azone are much greater than those of formulations without Azone. This finding further substantiates our previous finding with BVDU where it was pointed out that at similar flux value, BVDU formulations with Azone were much more effective than those without Azone (Afouna et al., 1998).

In the cell culture studies, the HPMPC concentration required for killing 50% (EC_{50}) of the HSV-1 virus, strain E-377 (the same virus type and strain used in this study), was 0.9 μg/ml (personal communication), which is relatively higher but fairly close to the C^*_{50} (extracellular drug concentration for 50% in vivo topical antiviral efficacy) predicted from the dose–response relationship curve for HPMPC formulations with Azone (~0.525 μg/ml) and much smaller than that predicted from similar curve for HPMPC formulations without Azone (~2.13 μg/ml) as shown in Fig. 7. It has been reported that for ACV, the estimated and predicted values of C^*_{50} from ACV dose–response relationship curve established earlier using formulations of different compositions, and the value of EC_{50} were comparable (Lee et al., 1992; Imanidis et al., 1994). In such cases, excipients in topical formulations may affect the topical bioefficacy of ACV, but they do not have any discernible effect upon the intrinsic in vivo potency of the drug.

The current results raise two basic questions for the second time (3). First, what role does Azone play in enhancing the efficacy of HPMPC formulations beyond enhancing the flux? Secondly, what factors prevented Azone from playing the same role with ACV formulations? It may be difficult to address both these questions with a single theory. It is unlikely that Azone has an intrinsic antiviral effect (Leonard et al., 1987) since placebo formulations with Azone showed no efficacy. It may be possible that an interplay between Azone and one or more of the clearly identified differences between HPMPC and ACV including: (a) the mechanisms of action; (b) membrane permeabilities; (c) phosphorylation kinetics; (d) rates of cellular uptake; and (e) drug disposition parameters can lead to such differential effects of Azone upon HPMPC and ACV. Such scenarios for example, may allow faster rate of conversion of HPMPC into the active metabolite and the HPMPC–choline adduct and/or faster HPMPC cellular uptake, which in turn may account for the higher in vivo efficacy seen in the presence of Azone. However, the role of the percutaneous enhancers is generally regarded as limited to enhancing the skin permeation rate of the drug. Therefore, topical formulations of similar flux values are expected to have comparable in vivo efficacies irrespective of their composition. The current example is in contrast with this theory since the excipients could affect the in vivo efficacy

by altering the intrinsic potency of the drug rather than simply increasing the skin permeation rates. Such findings should focus our attention in the future on the mechanistic understanding of the excipients' and skin penetration enhancers' roles upon the bioefficacy of a topical formulation.

4. Conclusion

In summary, HPMPC formulations with Azone at similar flux values were much more effective in "treating and preventing" of HSV-1 infections than those without Azone. The extracellular free HPMPC concentration (C^*), was a function (but not a single-valued function) of the observed in vivo efficacy. Moreover, the course of treatment with formulations exhibiting such higher efficacy is expected to be remarkably shorter than that with formulations without Azone. For ACV formulations, in contrast, addition of Azone has failed to show any effect on the preventive in vivo antiviral efficacy and the enhancement seen in the in vivo antiviral efficacy of ACV in treatment of HSV-1 infections, was merely the skin permeation enhancement effect of Azone.

Acknowledgements

Special thanks to Professors Dr. W.I. Higuchi and Dr. A.-H. Ghanem, Department of Pharmaceutics and Pharmaceutical Chemistry, The University of Utah, SLC for their generosity and partial support of this study. In addition, the authors greatly acknowledge the Department of Pharmaceutics and Pharmaceutical Technology, Al-Azhar University for its help and support.

References

Aduma, P., Connely, M.C., Srinivas, R.V., Fridland, A., 1995. Metabolism diversity and antiviral activities of acyclic nucleoside phosphonates. Mol. Pharmacol. 47, 816–822.

Afouna, M.I., 2001. Influence of various azone concentrations upon the permeability parameters for acyclovir (ACV) and (*E*)-5-(2-bromovinyl)-2-deoxyuridine (BVDU) topical formulations using hairless mice: a comparative study. Az. J. Pharm. Sci. 28, 26–37.

Afouna, M.I., Mehta, S.C., Ghanem, A.-H., Higuchi, W.I., Kern, E.R., DeClercq, E., El-Shattawy, H.H., 1998. Assessment of correlation between skin target site free drug concentration and the in vivo topical antiviral efficacy in hairless mice for (*E*)-5-(2-bromovinyl)-2-deoxyuridine and acyclovir formulations. J. Pharm. Sci. 87, 917–921.

Afouna, M.I., Mehta, S.C., Ghanem, A.-H., Higuchi, W.I., Kern, E.R., DeClercq, E., El-Shattawy, H.H., 1999. Influence of the treatment protocol upon the in vivo efficacy of cidofovir (HPMPC) and of acyclovir (ACV) formulations in topical treatment of cutaneous HSV-1 infection in hairless mice. J. Pharm. Sci. 88, 530–534.

Cihlar, T., Vortuba, I., Horska, K., Liboska, R., Rosenberg, I., Holy, A., 1992. Metabolism of ((*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) in human embryonic lung cells. Coll. Czech. Chem. Commun. 41, 661–671.

Cundy, K.C., Petty, B.G., Flaherty, J., Fisher, P.E., Polis, M.A., Wachsman, M., Leitman, P.S., Lalezari, J.P., Hitchcock, M.J.M., Jaffe, H.S., 1995. Clinical pharmacokinetics of cidofovir in human immunodificiency virus-infected patients. Antiviral Chem. Chemother. 39, 1247–1252.

DeClercq, E., 1993. Antivirals for treatment of herpesvirus infections. J. Antimicrob. Chemother. 32 (Suppl. A), 121–132.

DeClercq, E., Holy, A., 1991. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine in various models of herpes simplex virus infection in mice. Antimicrob. Agents Chemother. 35, 701–706.

Furman, P.A., St. Clair, M.H., Fyfe, J.A., Rideout, J.L., Keller, P.M., Elion, G.B., 1979. Inhibition of herpes simplex virus-induced DNA polymerase activity and viral DNA replication by 9-(2-hydroxyethoxymethyl)guanine and its triphosphate. J. Virol. 32, 72–77.

Furman, P.A., De Miranda, P., St. Clair, M.H., Elion, G.B., 1981. Metabolism of acyclovir in virus-infected and unifected cells. Antimicrob. Agents Chemother. 20, 518–524.

Fyfe, J.A., Keller, P.M., Furman, P.A., Miller, R.L., Elion, G.B., 1978. Thymidine kinase from herpes simplex virus phosphorylates in the new antiviral compound 9-(2-hydroxyethoxymethyl)guanine. J. Biol. Chem. 253, 8721–8727.

Gonsho, A., Imanidis, G., Vogt, P., Kern, E.R., Tsuge, H., Su, M.H., Choi, S.H., 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.

Hitchcock, M.J.M., 1996. Cidofovir, a new agent with potent antiherpesvirus activity. Antiviral Chem. Chemother. 7, 115–127.

Ho, H.-H.T., Woods, K.L., Bronson, J.J., De Boeck, H., Martin, J.C., Hitchcock, M.J.M., 1992. Interacellular metabolism of the antiherpes agent (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine. Mol. Pharmacol. 41, 197–202.

Imanidis, G., Song, W.Q., Lee, P.H., Suh, M.H., Kern, E.R., 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., Glasgow, L.A., 1973. Herpesvirus hominis infection in newborn mice. I. An experimental model and therapy with iododeoxyuridine. J. Infect. Dis. 128, 290–299.

Larsson, A., Brannstrom, G., Oberg, B., 1983. Kinetic analysis of cell culture of the reversal of antiherpes activity of nucleoside analogs by thymidine. Antimicrob. Agents Chemother. 24, 819–822.

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

Leonard, M.F., Kumar, A., Murray, D.L., Beaman, D.C., 1987. Inhibitory effect of azone® (1-dodecylazacycloheptan-2-one) on herpes simplex viruses. Chemotherapy (Basel) 33, 151–156.

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.

Patel, P.J., Ghanem, A.-H., Higuchi, W.I., Srinivasan, V., Kern, E.R., 1996. 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. Antiviral Res. 29, 279–286.

St. Clair, M.H., Furman, P.A., Lubbers, C.M., Elion, G.B., 1980. Inhibition of cellular and virally induced deoxyribonucleic acid polymerase by triphosphate of acyclovir. Antimicrob. Agents Chemother. 18, 741–745.