

## Failure of Topical Acyclovir in Ointment to Penetrate Human Skin

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**Topical acyclovir (ACV) in polyethylene glycol (PEG) ointment has been disappointing in the treatment of recurrent herpes simplex virus infections in immunocompetent patients. To investigate the possible role of poor drug delivery from this formulation, we studied the penetration of ACV through excised human skin from three vehicles; PEG ointment, modified aqueous cream, and dimethyl sulfoxide. A second antiviral agent, idoxuridine, was studied in the same formulations, and drug delivery through excised guinea pig skin was also assessed for comparison. The delivery of ACV from PEG ointment was very slow for both human and guinea pig skin (drug flux, 0.055 and 0.047  $\mu\text{g}/\text{cm}^2$  per h, respectively). Formulation of ACV in modified aqueous cream and in dimethyl sulfoxide resulted in an 8- and 60-fold increase, respectively, in the flux of ACV through human skin. Idoxuridine behaved similarly to ACV in the three vehicles. The poor clinical results seen with topical use of ACV ointment may be due in part to retarded drug delivery from this formulation.**

Topically administered acyclovir (ACV) in polyethylene glycol (PEG; Zovirax Ointment; Burroughs Wellcome Co., Research Triangle Park, N.C.) has proved disappointing in the therapy of recurrent herpes simplex virus (HSV) infections in immunocompetent patients, including trials in which therapy was patient initiated (3, 10, 12, 15, 17). Investigators have speculated from this experience that the failure of topical ACV therapy is due in part to the inability of ACV to penetrate the stratum corneum barrier layer of the skin (10, 11, 15, 20).

In this study we examined this question by measuring the penetration of ACV and idoxuridine (IDU) through excised human and guinea pig skin from three different vehicles: PEG, a modified aqueous cream (MAC), and dimethyl sulfoxide (DMSO). The results show that PEG is an inferior vehicle for the percutaneous delivery of nucleoside antiviral agents and that drug delivery is markedly enhanced by formulation in other vehicles.

### MATERIALS AND METHODS

**Drugs and formulations.** ACV powder, PEG ointment base, and MAC base were supplied by Burroughs Wellcome Co. (Research Triangle Park, N.C.). IDU powder was obtained from Sigma Chemical Co. (St. Louis, Mo.). Research Industries (Salt Lake City, Utah) supplied DMSO. Tritium-labeled nucleosides, [<sup>3</sup>H]ACV, and [<sup>3</sup>H]IDU were obtained from Moravek Biochemicals (Brea, Calif.). Batches of radiolabeled ACV and IDU were prepared by recrystallization. [<sup>3</sup>H]ACV (200  $\mu\text{l}$ ) was dissolved with an excess of unlabeled ACV (3 g) in an ethanol-water (70:30) solution at 60°C and recrystallized overnight at room temperature. The recrystallized [<sup>3</sup>H]ACV had a specific activity of 80 cpm/ $\mu\text{g}$ . Similarly, 100  $\mu\text{l}$  of [<sup>3</sup>H]IDU was added to 900 mg of IDU in 5 ml of DMSO and stirred for 1 h at 60°C. IDU was recrystallized by the addition of 10 ml of cold water and holding overnight at room temperature. The recrystallized IDU had a specific activity of 60 cpm/ $\mu\text{g}$ . The purity of the

recrystallized nucleosides was confirmed by thin-layer chromatography (5).

Formulations containing either 5% ACV or 5% IDU in either PEG or MAC were made by melting the ointment or cream base at 40°C; adding 5% (wt/wt) recrystallized, radio-labeled drug; and completely mixing at room temperature until the formulation resumed the semisolid characteristics of the base. Consistency of drug mixing was confirmed by serial sampling of the formulations and measurement of radioactivity in a liquid scintillation counter. DMSO was diluted to 95% (wt/wt) by the addition of water, and 5% recrystallized radiolabeled drug was then added to the 95% DMSO vehicle.

**Skin penetration experiments.** Drug penetration was measured through excised human and guinea pig skin by using methods described previously (6, 16). Human skin was obtained from surgical specimens excised during facelift or abdominoplasty procedures and was used within 24 to 48 h of surgery. Subcutaneous fat was dissected from the dermal side as thoroughly as possible. Care was taken not to damage the epidermal side. Hair follicles on the specimens were sparse or absent. Closely clipped (Professional Animal Grooming Clipper; Oster, Milwaukee, Wis.) guinea pig skin was removed from Hartley outbred albino guinea pigs (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) which were sacrificed with ether.

Skin specimens were mounted with the epidermal side up in single-chamber, jacketed diffusion cells. The temperature of the receiver chamber was controlled by circulating 37°C water through the external jacket, and the epidermal surface was left exposed to ambient conditions. Single doses of drugs were applied to the epidermal surface as either 100  $\mu\text{l}$  of solution or 200 mg of ointment or cream; these quantities were sufficient to completely cover the exposed epidermal surface.

Drug concentration in the receiver chamber was determined by withdrawing samples over time and measuring labeled drug by scintillation counting. Drug flux ( $J$ ; in micrograms per square centimeter per hour) was calculated from the slope of a plot of drug concentration versus time, the area of the treated skin, and the volume of the receiver chamber (16).

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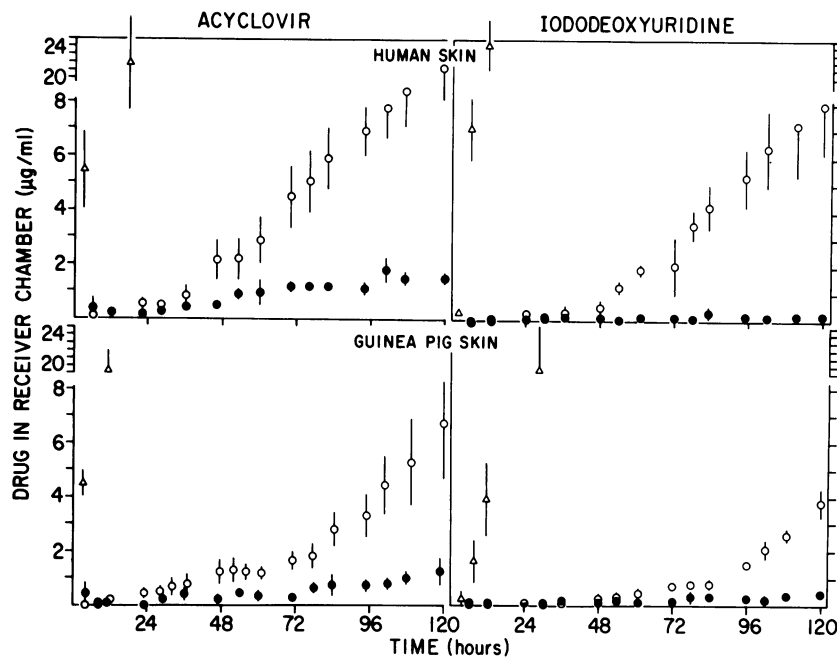


FIG. 1. Concentration of ACV and IDU in the receiver chamber of a drug diffusion apparatus as a function of time. The upper panels show the results for human skin, and the lower panels are the results for guinea pig skin. The points shown represent the mean values  $\pm$  standard error of the mean for three to five experiments each. Symbols indicate different drug vehicles:  $\Delta$ , 95% DMSO;  $\circ$ , MAC;  $\bullet$ , PEG.

**Statistics.** Differences in drug delivery were compared by Student's *t* test. All probability determinations were two-tailed, and *P* values  $\leq 0.05$  were considered significant.

**RESULTS**

The results of the skin penetration studies with ACV and IDU are shown in Fig. 1, and the calculated flux values are given in Table 1. Each drug formulation was studied three to five times with both human and guinea pig skin. The delivery of ACV through human skin from the PEG ointment was very slow, and drug levels in the receiver chamber did not reach 2  $\mu\text{g/ml}$ , even after 120 h. Drug delivery was notably improved by formulation in MAC, with which the concentration of ACV in the receiver chamber exceeded 2  $\mu\text{g/ml}$  in less than 48 h and reached 10  $\mu\text{g/ml}$  after 120 h. The rate of ACV penetration with MAC was eight times higher than that obtained with PEG (*P* = 0.02). From 95% DMSO, a vehicle with known skin penetration-enhancing properties (16), the flux for ACV was 60-fold higher than from PEG (*P* = 0.01).

The second antiviral nucleoside analog, IDU, showed rates of penetration through human skin similar to those of

ACV for the different formulations (Table 1). IDU flux from PEG was minimal, but it was enhanced 36 times with MAC (*P* = 0.04), and 1,000-fold with 95% DMSO (*P* = 0.001). The penetration of ACV and IDU through guinea pig skin from each formulation was of similar magnitude to the values obtained with human skin.

**DISCUSSION**

Results of these studies establish that ACV formulated in PEG penetrates human skin very slowly. This attribute of ACV ointment likely causes a clinically significant delay in the achievement of HSV-inhibitory concentrations of ACV in the epidermis. We have also shown that poor skin penetration by ACV in PEG is not an inherent feature of the drug but is attributable to PEG, because formulation of the antiviral agent in an aqueous cream base was associated with an eightfold increase in drug flux. The negative features of the PEG preparation were further substantiated by studies with a second nucleoside antiviral agent, IDU, which also penetrated skin poorly in PEG, and by duplication of the findings in a second series of experiments with guinea pig skin.

Work in our laboratory with topical therapy of experimental cutaneous HSV type 1 infection in guinea pigs has shown that ACV ointment has only a limited therapeutic benefit, while therapy with 5% ACV in 95% DMSO (16) or 5% ACV in MAC (S. L. Spruance, D. J. Freeman, and N. V. Sheth, submitted for publication) is more effective. Because of the observation in this report that ACV penetrates human and guinea pig skin to a similar degree, it is reasonable to consider that the efficacy of topical therapy of human cutaneous HSV type 1 disease could also be increased by the use of new formulations that exhibit improved drug delivery. Clinical evidence is growing that ACV in MAC is superior to ACV ointment for the treatment of herpes labialis (21).

Effective drug delivery through intact skin is necessary in recurrent HSV disease because major, virus-induced, epi-

TABLE 1. Penetration of ACV and IDU from different vehicles through human and guinea pig skin in vitro

Drug	Vehicle	Skin penetration ( $\mu\text{g/cm}^2$ per h) <sup>a</sup> :	
		Human skin	Guinea pig skin
5% ACV	PEG	0.055 $\pm$ 0.044	0.047 $\pm$ 0.011
	MAC	0.42 $\pm$ 0.05	0.36 $\pm$ 0.12
	95% DMSO	3.31 $\pm$ 0.79	4.10 $\pm$ 0.26
5% IDU	PEG	0.01 $\pm$ 0.002	0.023 $\pm$ 0.006
	MAC	0.36 $\pm$ 0.01	0.17 $\pm$ 0.05
	95% DMSO	10.39 $\pm$ 1.08	3.69 $\pm$ 0.33

<sup>a</sup> Values are the mean  $\pm$  standard error of the mean (*n* = 3 to 5).

dermal pathology occurs in the erythema and papule lesion stages before the overlying stratum corneum is disrupted. Later in the course of the disease, when the stratum corneum has been eroded, the rapid ingress of host resistance factors makes chemotherapy redundant and of questionable value (18). The early stages of viral infection (prodrome, erythema, papule) do not increase the permeability of the stratum corneum to antiviral agents. We have measured the flux of 5% ACV in PEG and 5% ACV in MAC through both infected and uninfected guinea pig skin and found no differences (7). In contrast to recurrent disease in immunocompetent subjects, primary cutaneous HSV disease and cutaneous HSV infection in immunocompromised patients have a more prolonged course characterized by predominantly ulcerated lesions. Because of inadequate host resistance, these diseases can more readily benefit from antiviral therapy, and the absence of stratum corneum over the infection simplifies effective topical drug administration (3, 20).

Evidence that ACV delivery from PEG ointment is inadequate can also be derived indirectly from the positive results found with orally administered ACV in the treatment of recurrent HSV genital disease (13). Results of this study demonstrate that if adequate drug levels in the skin are achieved through systemic drug administration, the clinical course of the disease can be altered.

The use of PEG as a topical drug delivery vehicle has been associated with poor drug delivery in other settings, including various corticosteroid preparations (1, 14, 19) and formulations of the antiviral agent trifluorothymidine (14a). The effect of PEG on skin penetration has been postulated to be due to a drug-vehicle interaction that results in a lower thermodynamic activity of the drug (4, 8, 9). Other researchers have suggested that the retardant effect of PEG is due to its inability to hydrate the stratum corneum or to a relative osmotic effect which tends to dehydrate the stratum corneum (2).

In this report we have identified a problem that occurs in the majority of trials with topical ACV therapy in immunocompetent hosts, which may help to explain some of the negative results: formulation of ACV in an ointment vehicle which retards topical drug delivery. Our data also identify other formulations that enable good drug penetration through skin and which would provide a better test of the topical route of ACV administration. Our results support the use of pharmacokinetic studies at an early, preclinical level to enable the rational and efficient development of topical antiviral agents for cutaneous HSV infections.

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