*Antiviral Research*, 16 (1991) 227 232


*ADONIS* 016635429100271F

AVR 00505

# Suppression of ocular herpes recurrences by a thymidine kinase inhibitor in squirrel monkeys

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(Received 15 January 1991; revision accepted 26 March 1991)

**Summary**

5'-Ethynylthymidine, an inhibitor of viral thymidine kinase (TK), was given intraperitoneally to squirrel monkeys previously infected by the ocular route with Rodanus strain herpes simplex virus. Spontaneous ocular recurrences were reduced during therapy, compared to saline-treated controls. This is the first in vivo demonstration that a viral TK inhibitor can reduce recurrences of HSV-1. Similar benefit would be expected for HSV-2 and perhaps VZV (varicella zoster virus).

Dendritic keratitis; Herpes simplex virus; Recurrent ocular herpetic disease; Thymidine kinase; Virus inhibitor

## Introduction

It has been well established that systemic ACV (acyclovir) can prevent recurrences of genital herpes (Straus et al., 1984; Douglas et al., 1984). In contrast, we have not been able to prevent naturally occurring recurrences of corneal epithelial herpes in the rabbit either with intense topical antiviral therapy or with oral administration of ACV or BVDU (bromvinyl deoxyuridine) even though adequate blood levels of ACV were maintained

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and BVDU was given to the point of toxicity (Kaufman et al., 1983). We believe that the reason for this disparity may be that in genital herpes, the virus must multiply in the end organ for clinical disease to be apparent, and ACV effectively blocks this multiplication. In ocular herpes, ganglionic reactivation and transmission of virus to the corneal epithelium may be sufficient to produce a lesion without significant end organ multiplication; in this case, antiviral agents that do not prevent reactivation itself may be ineffective in preventing recurrent disease. On this basis, we looked for compounds that might effectively prevent ganglionic reactivation in order to prevent recurrent ocular epithelial herpetic disease.

It is known that TK (thymidine kinase)-deficient HSV-1 (herpes simplex virus type 1) cannot reactivate from the ganglion without some rescue mechanism such as superinfection. The ganglion and central nervous system appear to contain little or no thymidine kinase, and it is possible that de novo synthesis and the rescue pathway provide too little of the phosphorylated metabolite for reactivation of virus without the TK gene.

Although some in vitro studies (Nutter et al., 1987) have shown that selective viral TK inhibitors have an effect on virus reactivation, the conditions are very different from the in vivo situation. Because of this, we elected to study naturally recurring ocular herpes in squirrel monkeys in a model previously described by us (Varnell et al., 1987), to determine whether a selective viral TK inhibitor would prevent these recurrences. Such a drug would have little antiviral effect in peripheral cells where TK is available but, although it is not directly antiviral, might prevent viral reactivation where cellular TK is not available.

We evaluated the thymidine analog, 1-(2,5,6-trideoxy-β-D-*erythro*-hex-5-ynofuranosyl)-5-methyluracil (5′-ethynylthymidine) (Sharma and Bobek, 1978), for the ability to prevent recurrent ocular herpes in squirrel monkeys infected with HSV-1 (strain Rodanus). Viral shedding occurs in man and animals, but does not correlate with the presence or absence of disease (Kaufman et al., 1967). It can be found in the eye socket with undiminished frequency, even after the globe is removed. In the small monkeys with tight lids used in this study, it is not possible to swab for virus without damaging the cornea. Therefore we elected to study recurrences of disease rather than viral shedding.

## Materials and Methods

The drug 5′-ethynylthymidine was originally synthesized by Sharma and Bobek (1978) and was prepared for this study by an improved procedure. In vitro studies have shown that 5′-ethynylthymidine is competitive with thymidine as an inhibitor of HSV (strain KOS)-induced thymidine kinase (Nutter et al., 1987); inhibition of HSV (strain Rodanus), the strain we used in this study, is similar at the 50% point (Cheng and Prusoff, 1974; Cheng and Ostrander, 1976) (Fig. 1).

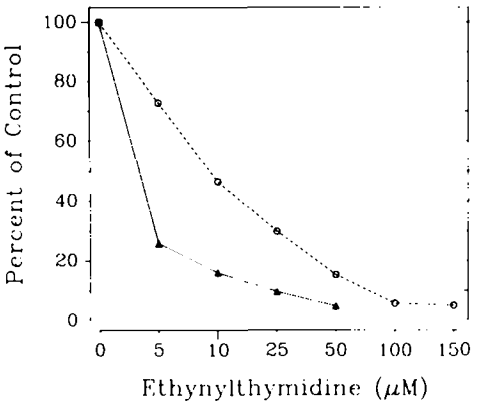


Fig. 1. Effect of 5'-ethynylthymidine on HSV strain Rodanus thymidine kinase. ▲, 10 μM thymidine substrate; ○, 50 μM thymidine substrate.

In pharmacokinetic studies, we measured blood levels of 5'-ethynylthymidine in four monkeys over a 6-h period to provide a guide for dosage and frequency of administration for the recurrence studies. Adult squirrel monkeys (*Saimiri sciureus*), 400–500 g body weight, were purchased from World Wide Primates (Miami, FL). All of the monkeys used in these studies were cared for in accordance with the rules and policies established by the L.S.U. Medical Center Institutional Animal Care and Use Committee. A suspension of 50 mg of 5'-ethynylthymidine in 1 ml of balanced salt solution was prepared and injected intraperitoneally in two monkeys and intravenously in the femoral vein in two monkeys. The animals were tranquilized with an intramuscular injection of ketamine (0.1 ml; 100 mg/ml) before injection of the drug and again at 1, 4, and 6 h after injection to obtain blood samples from the femoral vein. Serum samples were frozen at −81°C and sent to Dr. Cheng's laboratory for determination of drug concentrations (unpublished method, Dutschman) (Fig. 2). The amount of 5'-ethynylthymidine used was limited by low solubility but the efficacy of the competitive inhibitor should be greater in a tissue with a low thymidine pool and it was anticipated that the levels achieved would be therapeutically effective.

To test the ability of the drug to prevent recurrent disease, we infected the corneas of 20 squirrel monkeys with HSV-1 (strain Rodanus) as previously described (Varnell et al., 1987). All corneas showed typical herpetic dendritic lesions 3 or 4 days after infection. Fifteen days after infection, all corneas were clear. The monkeys were divided into two groups of 10 each. The experimental group received 50 mg 5'-ethynylthymidine in 1 ml balanced salt solution injected intraperitoneally; the control group received 1 ml balanced salt solution only, by the same route. Treatments were administered every 4 h for 25 days in both groups. After 10 days, the drug dosage was reduced to 25 mg 5'-ethynylthymidine in 0.5 ml balanced salt solution and the control treatment to

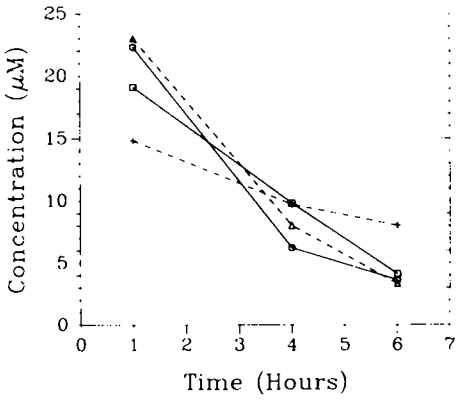


Fig 2. Serum concentrations of 5'-ethynylthymidine in four monkeys. Two monkeys were injected intravenously (○,□) and two were injected intraperitoneally (△, + ) with 50 mg of drug suspended in 1 ml balanced salt solution.

0.5 ml balanced salt solution alone. The animals were neither tranquilized nor anesthetized for the treatments, but only hand restrained.

All corneas were stained with sodium fluorescein (Flur-i-strip, Ayerst Laboratories, New York, NY) and examined daily each weekday over the 25-day treatment period (total examination days = 18) and for 25 days after cessation of treatment (total additional examination days = 18) by an observer masked as to the treatment group. Observed lesions were true recurrences in that the earlier lesions had completely healed and the new lesions developed in slightly different areas.

## Results and Discussion

Treatment with 5'-ethynylthymidine reduced the number of monkeys, as well as the number of eyes, that showed recurrences of herpetic keratitis, compared to the saline-treated controls (Table 1).

During the 25 days after cessation of treatment, three monkeys that had been

TABLE 1

Recurrent epithelial herpetic keratitis[a] in squirrel monkeys

| Treatment | Eyes | | Animals | |
|---|---|---|---|---|
| | Recurrent lesions | No recurrent lesions | Recurrent lesions | No recurrent lesions |
| Saline | 8:20 | 12:20 | 7:10 | 3:10 |
| 5'-ethynylthymidine | 3:20 | 17:20 | 3:10 | 7:10 |

[a]During 25-day treatment period. Eyes, $P = 0.077$; animals, $P = 0.074$; determined by one-tailed chi-square analysis.

treated with saline and five monkeys that had been treated with 5'-ethynylthymidine had recurrent herpetic lesions, suggesting some rebound after therapy was stopped (Kaufman et al., 1962).

The model we chose for recurrence is a naturally recurring model. We do not, however, know the penetration of the drug into the ganglion. In that these studies were not lethal to the animals and we did not believe it justifiable to sacrifice them, we were not able to remove the ganglia to analyze thymidine kinase or ethynylthymidine concentrations. Although there may be better thymidine kinase inhibitors (Ashton et al., 1989; Martin et al., 1989a,b; Leib et al., 1990; Nsaih et al., 1990), this study does represent the first demonstration that a selective viral TK inhibitor can prevent recurrences of herpes.

Although ACV can prevent recurrences of genital herpes, it does not appear to prevent ocular recurrences (Kaufman et al., 1983). Even in the case of genital herpes, ACV depends for its selectivity on the phosphorylation of ACV by viral TK rather than cellular TK, but this distinction is not absolute. The possibility that, if ACV were given chronically, small amounts of phosphorylated ACV could be incorporated into normal cells and interfere with host cellular DNA provided an impetus to search for drugs that have no mechanism for affecting normal cells.

Other simpler models are available to study recurrences but are more or less artificial. We elected to use a primate model, which presumably would be similar, metabolically, to man. We chose not to use immunosuppression because it might greatly increase the susceptibility to even minimal amounts of viral multiplication and therefore mask a drug effect. Similarly we thought that synchronizing recurrent episodes with iontophoresis or other mechanisms that might cause local corneal trauma could distort the applicability of our findings.

It is tempting to think that a selective viral TK inhibitor which would have no effect on virus multiplication directly and no effect on normal cellular metabolism could safely prevent recurrences of herpes and might provide a safe way to inhibit both HSV-1 and HSV-2 recurrences, as well as offer some clue to the inhibition of VZV (varicella zoster virus). Similarly, it has been suggested that this class of compounds could be useful in increasing the efficacy of antiviral treatment in the central nervous system in that viral multiplication may be somewhat slowed by inhibiting viral TK; selective antiviral drugs such as foscarnet, PMEA [9-(2-phosphonylmethoxyethyl)adenine], or HPMPC (hydroxyphosphonylmethoxypropylcytosine), which do not depend on activation by viral TK, might be used in conjunction with TK inhibitors. Agents that do depend on the phosphorylation by viral TK, such as ACV, lose their activity in the presence of a viral TK inhibitor.

In summary, this is the first in vivo demonstration that a specific viral thymidine kinase inhibitor can reduce recurrences of ocular herpes simplex. It suggests that a similar approach to HSV-2 and, perhaps, to herpes zoster may offer an even safer and more effective approach to the prevention of recurrent viral disease.

## Acknowledgements

This work was supported in part by U.S. Public Health Service grants EY02672 and EY02377 from the National Eye Institute and CA44358 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland and a departmental grant from Research to Prevent Blindness, Inc., New York.

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