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# Effect of a thymidine kinase inhibitor (L-653,180) on antiviral treatment of experimental herpes simplex virus infection in mice

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## Summary

Thymidine kinase (TK) inhibitors can block the activity of TK-dependent antiviral drugs in vitro. We have examined the ability of the TK inhibitor (±)-9-{[(Z)-2-(hydroxymethyl)cyclohexyl] methyl}guanine (L-653,180) to prevent the therapeutic effect of acyclovir (ACV) in experimental herpes simplex virus type 1 (HSV) skin infections of mice. The results showed that ACV given in the drinking water prevents, in a dose-dependent way, the evolution of the viral infection, and that L-653,180 can reverse some of the therapeutic effects of the antiviral drug. Among the parameters used to evaluate the effect of the TK inhibitor mortality was increased compared to ACV treatment alone, only in the presence of low doses of ACV, whereas the establishment of latent infections in sensory ganglia was significantly increased compared to ACV treatment alone, even when high doses of ACV were administered together with L-653,180.

Herpes simplex virus; Thymidine kinase inhibitor; Acyclovir; Experimental treatment

## Introduction

Herpes simplex viruses types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV) induce in infected cells a virus-specified thymidine kinase (TK) (Kit and Dubbs, 1963; Klemperer et al., 1967; Cheng, 1976) which is not essential for replication in cell cultures (Dubbs and Kit, 1964). It is known that TK negative mutants have a reduced pathogenicity and it appears that the enzyme plays a role in HSV latency (Field and Wildy, 1978; Tenser and Dunstan, 1979; Tenser et al.,

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1979; Klein et al., 1980, 1981; Field and Darby, 1980). Recent results suggest that TK-negative HSV mutants can establish latent infections, but fail to reactivate from explant cultures of ganglia (Coen et al., 1989).

The antiviral compound acyclovir (ACV) is a selective substrate of HSV TK and is dependent on phosphorylation by the enzyme to exert its inhibitory action (Elion et al., 1977; Fyfe et al., 1978). It has been shown that HSV mutants deficient in TK synthesis are resistant to ACV (Coen and Schaffer, 1980; Schnipper and Crumpacker, 1980). Recently, several thymidine kinase inhibitors have been described (Focher et al., 1988; Sim et al., 1988; Martin et al., 1989). The thymidine analog 5′-ethynylthymidine exhibited no antiviral activity in vitro, but prevented inhibition of HSV by five compounds requiring phosphorylation by TK (Nutter et al., 1987). Another group of TK inhibitors are the 9-{[(Z-2-(hydroxymethyl) cycloalkyl]methyl}guanines. They also had no effect on virus replication in cell culture, but are able to block the antiviral effect of the TK-dependent compound ganciclovir. The most potent compound in this group is the the cyclohexyl derivative (L-653,180) (Ashton et al., 1989). The observation that L-653,180 delays the reactivation of HSV from explant cultures of latently infected ganglia (Ashton et al., 1989) and that it may suppress the reactivation of the virus in latently infected human embryo lung cells (Nsiah et al., 1990) supports the idea that TK may be necessary not for the establishment, but for the reactivation of latent virus (Efstathiou et al., 1989; Coen et al., 1989).

We report here that the TK inhibitor L-653,180 can block or reduce the antiviral efficacy of ACV in acute cutaneous HSV infection of mice, and that it affects in a differential manner the various parameters used to evaluate the severity of the infection.

## Materials and Methods

### *Inhibitors and antiviral compounds*

TK inhibitor, L-653,180 (Ashton et al., 1989) was obtained from Dr R.L. Tolman of the Merck Sharp & Dohme Research Laboratories, Rahway, NJ and was prepared as an aqueous solution at a concentration of 0.33 mg/ml. ACV (Zovirax, sterile injectable powder, Burroughs Wellcome) was dissolved at various concentrations (3, 10, 30 and 100 mg/ml) either in water or in L-653,180 solution. The structural formula of the TK inhibitor is shown in Fig. 1.

### *Virus*

HSV-1, strain S, was used in all experiments. The maintenance of the virus, the preparation of stock virus, and the quantification of inocula have been described in a previous publication (Klein et al., 1977).

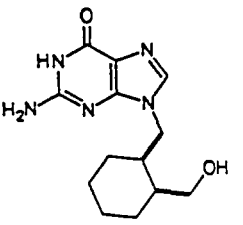

Fig. 1. The structural formula of the thymidine kinase inhibitor (±)-9-{[(Z)-2-(hydroxymethyl)cycloalkyl] methyl}guanine (L-653,180). (Ashton et al., 1989).

*Inoculation of mice*

Female hairless mice of the fully immunocompetent HRS/J strain were obtained from Jackson Laboratories, Bar Harbor, ME, and used in the experiments at the age of 8 to 9 weeks. The mice were inoculated percutaneously on a triangular skin area of the snout cross-hatched six times with a needle. Approximately $10^4$ PFU of a virus preparation were applied with a cotton-tipped applicator and rubbed into the scarified skin of the mouse.

*Scoring skin lesions*

The development of lesions was recorded daily until healing was observed in surviving mice. The intensity of lesions was graded on a scale from 0 to 4 as described elsewhere (Klein et al., 1977). Average daily lesion scores were calculated for each treatment group. The maximum lesion score for each treatment was calculated from the highest score of each mouse irrespective of the day on which it was observed.

*Monitoring latent virus infections in sensory ganglia*

At 4 weeks after virus inoculation, the surviving mice were exsanguinated by heart puncture under complete sodium pentobarbital anesthesia. The trigeminal ganglia were removed and each ganglion was maintained for 7 days in explant culture. After 7 days the medium from each culture was removed and assayed for the presence of reactivated infectious virus. After adding fresh medium, the ganglia were maintained for another 7 days in culture. Ganglia which did not release detectable infectious virus in the 7-day culture medium were homogenized by sonication and assayed for the presence of reactivated virus. All assays for infectious virus were done in monolayers of human fibroblasts (FS7 cells).

*Treatment of mice*

All compounds were administered in the drinking water of the mice for 10 days. The amount of water intake was monitored over two periods of 4 days in groups

of 4 to 6 mice. Water consumption did not show significant variations in time, and was not dependent on the drug dose. Mice given ACV at concentrations of 3, 10, 33 or 100 $\mu$g/ml consumed on the average per day 7.3 ± 0.5, 7.2 ± 0.4, 7.5 ± 0.5 and 7.8 ± 0.6 ml fluid, respectively. These volumes correspond to a daily dose expressed as mg per kg, of 1.4 ± 0.1, 4.2 ± 0.2, 14.6 ± 0.9 and 46 ± 4 for the treatment groups given ACV. The daily dose of L-653,180 ranged between 139 and 151 mg per kg in the various treatment groups (mean 145 mg/kg). The daily dose of drug per weight of mice was calculated from average daily consumption of water containing the various drug concentrations. Treatments were started 24 h after virus inoculation. One series of mice was pretreated for 48 h with L-653,180 before the initiation of ACV treatment.

## Results

*Effect of TK inhibitor and ACV on evolution of HSV-induced skin infection*

Untreated mice developed severe skin lesions attaining the highest score on day 7 post infection (p.i.) (Fig. 2A). Mice treated with the inhibitor alone 24 h before or after virus inoculation had a similar evolution of their skin lesions. The evolution of skin lesion in ACV treated mice was dose dependent starting with a dose of 4.2 mg/kg (no differences were seen between a dose of 1.4 and 4.2 mg/kg). A dose of 14.5 mg/kg reduced slightly the intensity of lesions and a dose of 46 mg/kg suppressed the development of lesions almost completely. In mice treated with ACV the development of lesions was delayed; the highest score was observed on day 8 p.i. (Fig. 2A).

When L-653,180 was added to the antiviral treatment the suppressive effect of ACV was reversed and severe lesions were observed in mice treated with doses up to 14.5 mg/kg of ACV. Only a dose of 46 mg/kg of ACV was able to prevent to a significant extent the enhancing effect the TK inhibitor. Pretreatment with the inhibitor did not enhance the severity of lesions compared to treatment started after virus inoculation (Fig. 2B, C).

*Effect of TK inhibitor and ACV on mortality rate in HSV-induced skin infections (Table 1)*

The mortality rate in untreated mice and in mice treated with L-653,180 alone was of the same order of magnitude varying between 60 and 80%. In mice treated with ACV alone, even the lowest dose (1.4 mg/kg) was able to reduce the mortality rate to a statistically significant extent (2 out of 11 or 18%, $P<0.0025$). The mortality in mice treated with 1.4 mg/kg ACV and L-653,180 was similar to that observed in control groups. A daily dose of 4.2 mg/kg ACV with L-653,180 given before the antiviral treatment enhanced the mortality rate but did not prevent the therapeutic effect of ACV when given 24 h after virus inoculation. In the presence of daily doses of 14.5 or 46 mg/kg ACV the TK inhibitor had no enhancing effect on the mortality rate of mice, whether given before or after infection.

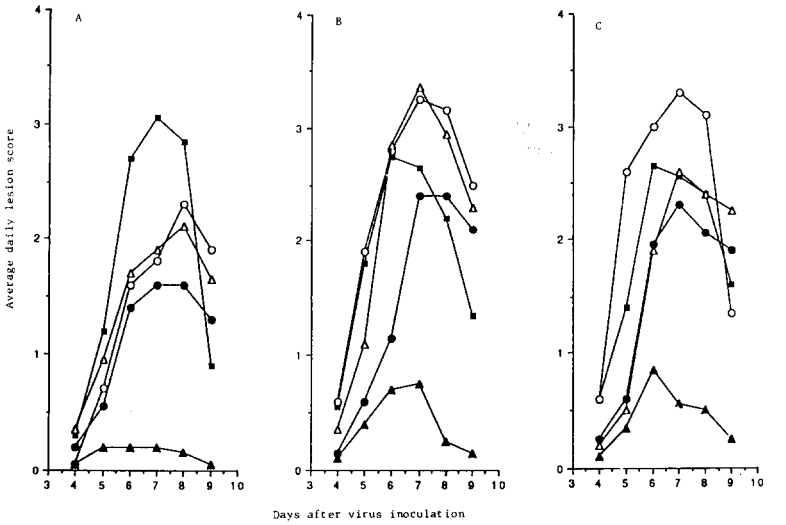


Fig. 2. Average daily lesion scores and the trend of evolution of HSV infection in mice treated with ACV or ACV and L-653,180 (145 mg/kg). The drugs were given in the drinking water and treatment with ACV was started 24 h after infection. Treatment with L-653,180 was started either at 24 h before or after infection. Panel A: mice treated only with ACV. Panel B: mice treated with ACV and L-653,180 given 24 h before infection. Panel C: mice treated with ACV and L-653,180 given 24 h after infection. Untreated mice (■), ACV 1.4 mg/kg (○), 4.2 mg/kg (△), 14.6 mg/kg (•), and 46 mg/kg (▲).

*Effect of TK inhibitor and ACV on the establishment of latency in sensory ganglia*

Latent infections were detected in the majority of surviving untreated mice, of mice treated with L-653,180 or mice treated with doses of 1.4 or 4.2 mg/kg of ACV with or without the addition of the inhibitor. Daily doses of 14.5 or 46 mg/kg of ACV were able to prevent the establishment of latency in surviving mice at a statistically significant level. However, even the highest dose of ACV used (46 mg/kg) was not sufficient to prevent the establishment of latency when L-653,180 was added to the antiviral treatment. (Table 1).

## Discussion

Since ACV is dependent on phosphorylation by the HSV-induced TK to exert its antiviral action (Elion et al., 1977; Fyfe et al., 1978), it is not surprising that various TKIs were able, in the presence of TK-dependent antiviral drugs, to sustain virus replication in cell cultures (Nutter et al., 1987; Ashton et al., 1989). The experiments described in this report showed that the TK inhibitor L-653,180 also counteracts the proven excellent antiviral properties of ACV in experimental HSV infections of mice (Schaeffer et al., 1978; Field et al. 1979; Klein et al., 1979).

It is interesting that the inhibition of the viral TK and the consequent failure of

TABLE 1
Effect of a thymidine kinase inhibitor on acyclovir treatment of herpes simplex virus infection in hairless mice

| ACV[a] (mg/kg) | TKI (145 mg/kg) | Average max. lesion score | Mortality rate | $P^b$ | Frequency of latent infections | $P^b$ |
|---|---|---|---|---|---|---|
| | none | 3.1 ± 1.7 | 9/12 | | 2/3 | |
| none | 24 h a.i. | 2.8 ± 1.8 | 10/12 | NS | 1/2 | NS |
| | 24 h p.i. | 2.8 ± 1.8 | 7/12 | NS | 3/5 | NS |
| | none | 2.1 ± 1.9 | 2/11 | | 8/9 | |
| 1.4 | 24 h a.i. | 3.4 ± 1.5 | 10/12 | <0.03 | 2/2 | NS |
| | 24 h p.i. | 3.4 ± 1.5 | 10/12 | <0.03 | 2/2 | NS |
| | none | 2.2 ± 1.7 | 1/10 | | 6/9 | |
| 4.2 | 24 h a.i. | 3.3 ± 1.4 | 7/12 | <0.025 | 5/5 | NS |
| | 24 h p.i. | 2.7 ± 1.6 | 4/12 | NS | 7/8 | NS |
| | none | 1.8 ± 1.7 | 2/10 | | 3/8 | |
| 14.5 | 24 h a.i. | 2.4 ± 0.9 | 1/12 | NS | 11/11 | <0.005 |
| | 24 h p.i. | 2.4 ± 1.5 | 1/12 | NS | 9/11 | NS |
| | none | 0.3 ± 0.4 | 0/8 | | 1/8 | |
| 46 | 24 h a.i. | 0.8 ± 0.4 | 0/12 | NS | 8/12 | <0.025 |
| | 24 h p.i. | 0.8 ± 0.5 | 0/12 | NS | 10/12 | <0.005 |

[a]ACV treatment was started in all mice 24 h post infection (p.i.). TK inhibitor treatment started either 24 h before infection (a.i.) or 24 h p.i.
[b]Probability that the mortality rate and the frequency of latent infections in mice treated with the TK inhibitor is different from that observed in mice not treated with the inhibitor (Fisher's Exact Test).

ACV treatment has a differential effect on the various parameters by which the severity of experimental HSV infections are evaluated. Although the intensity of skin lesions was reduced by ACV in a dose-dependent manner, a constant dose of L-653,180 increased the lesion scores of mice from each treatment group. On the other hand, the mortality of mice which was reduced significantly even by lowest dose of ACV (1.5 mg/kg), was increased to the level of control group when L-653,180 was included in the treatment. However, at the higher doses of ACV (14 and 46 mg/kg) the addition of the inhibitor to the treatment did not result in an increase of the mortality of mice. In ACV-treated mice the strongest effect of the TK inhibitor was exerted on the establishment of latency: mice treated with a dose of 46 mg/kg ACV and L-653,180, which developed only minor skin lesions and showed a 100% survival rate, developed latent infections to the same extent as untreated mice or mice treated with low doses of ACV.

The enhancing effect of L-653,180 in ACV-treated HSV-infected mice on the evolution of skin lesions, on the mortality rate and on the establishment of latent infections was not different in mice treated with the inhibitor 24 h before or after virus inoculation.

As already mentioned TK negative HSV mutants have a reduced pathogenicity and probably a defect in their ability to reactivate from latently infected ganglia. The

fact that mice treated with an inhibitor of the enzyme developed latent infections at the same rate as untreated mice or mice treated with low doses of ACV could be the result of a partial inhibition of ACV phosphorylation reducing the effective antiviral drug dose to levels which are sufficiently high for the prevention of severe skin lesions and of mortality, but not high enough to protect against the establishment of latency.

In addition, the observation that ACV (14 and 46 mg/kg) was able to reduce significantly the number of latently infected mice shows that oral treatment, even when started 24 h after virus inoculation, is superior to topical administrations, or to injecting the drug subcutaneously or intraperitoneally, which had little effect in reducing the establishment of latency when treatments were delayed by 24 h (Field et al., 1979; Klein et al., 1979).

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