Effect of Acyclovir on Recurrence of Herpes Simplex Skin Lesions in Mice

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SUMMARY

Acyclovir (ACV) was effective in preventing recurrence of herpes simplex in mice whose skin was stripped with cellophane tape. Treatment with ACV did not eliminate latent herpes simplex virus from the cervical ganglia.

The nucleoside analogue 9-(2-hydroxyethoxymethyl) guanine (acyclovir; ACV) has been shown to possess substantial antiviral activity when tested against members of the herpes virus group in vitro or in vivo (Elion et al. 1977; Schaeffer et al. 1978; Crumpacker et al. 1979; Field et al. 1979; Klein et al. 1979). In animals, the incidence of latent infection with herpes simplex virus (HSV) in the ganglia is reduced or prevented by treatment of the primary infection (Field et al. 1979; Klein et al. 1979). Established latent infection was not affected by ACV in one study (Field et al. 1979), but in another its incidence was decreased (Pavan-Langston et al. 1979).

We have studied the effect of ACV on recurrent herpes simplex induced in mice by stripping the skin with cellophane tape (Hill et al. 1978). Four week-old female outbred Swiss white mice were injected subcutaneously (s.c.) in the skin of the ear with $6 \times 10^4$ p.f.u. HSV-1 strain SC16 (Hill et al. 1975). Mice were used at least 4 weeks after primary infection. They were then examined, and those with lesions persisting after the primary infection or spontaneous recurrent lesions were excluded from the experiments. Groups of mice were injected s.c. in the flank with 100 mg/kg ACV suspended in PBSA containing 0.5% methyl cellulose. As controls, other groups of mice were injected s.c. with the suspending medium. The drug was given twice daily, at 9:00 am and 5:00 p.m., for 8 days. On the second day, the ears of mice were stripped six times on the upper surface with cellophane tape (Hill et al. 1978) and then were examined daily for 7 days. Recurrent clinical disease was defined by the criteria of Hill et al. (1978). On any occasion when ears were stripped, only those mice with clinically normal ears were used. The ears of mice were stripped on five occasions at monthly intervals. In the first experiment, one group was treated with ACV as above, on each of the first four occasions. The control group, and on the fifth occasion the test group as well, did not receive the drug. In the second experiment the test group received drug only on the first and third occasions of stripping (Table 1). Of 62 mice never given AVC, 29 did not contract recurrent disease, 21 had a single recurrence, 11 had two recurrences and 1 had three recurrences. In the second experiment, mice developed recurrent disease only on those occasions when they were not treated. Of these 30 animals, 15 did not develop recurrent disease, 10 had a single recurrence and 5 had two recurrences. In the first experiment, of the 28 mice given drug on each occasion, 26 did not have recurrent disease. The other 2 each had a single recurrence. Three weeks after the fifth stripping, the right second, third and fourth cervical ganglia were removed from all remaining mice. They were cultured for 4 days in 0.5 ml growth medium, ground with a tissue grinder and two 50 µl samples were put on to Vero cell monolayers to isolate virus (Table 1). The frequency of isolation was similar in the test and control groups.
Short communications

Table 1. Recurrent herpes simplex in the ears of mice stripped with cellophane tape on five occasions at monthly intervals

<table>
<thead>
<tr>
<th>Stripping</th>
<th>Treatment*</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>Control</td>
<td>Drug</td>
</tr>
<tr>
<td>1st</td>
<td>1/28†</td>
<td>14/32</td>
<td>0/30</td>
</tr>
<tr>
<td>2nd</td>
<td>0/25</td>
<td>1/27</td>
<td>(7/28)‡</td>
</tr>
<tr>
<td>3rd</td>
<td>1/22</td>
<td>5/26</td>
<td>0/25</td>
</tr>
<tr>
<td>4th</td>
<td>0/20</td>
<td>1/27</td>
<td>(3/27)</td>
</tr>
<tr>
<td>5th</td>
<td>(0/19)</td>
<td>3/24</td>
<td>(10/23)</td>
</tr>
</tbody>
</table>

Incidence of latent infection in ganglia 15/24§ 16/30 15/24 16/26

* Drug, 100 mg/kg ACV s.c. twice daily for 8 days; control, suspending medium s.c. twice daily for 8 days.
† All figures represent clinical recurrences/mice tested.
‡ Figures in parentheses are results for mice previously treated with ACV and stripped while not receiving treatment.
§ Virus isolated/mice tested.

Table 2. Isolation of HSV from the skin and ganglia of mice after stripping with cellophane tape

<table>
<thead>
<tr>
<th>Treatment sampled</th>
<th>Days after stripping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>100 mg/kg ACV</td>
<td>Skin</td>
</tr>
<tr>
<td></td>
<td>Ganglia</td>
</tr>
<tr>
<td>Suspending medium</td>
<td>Skin</td>
</tr>
<tr>
<td></td>
<td>Ganglia</td>
</tr>
</tbody>
</table>

* Virus isolated/mice tested.

In further experiments, attempts were made to isolate virus from the skin and ganglia after stripping the ear. Latently infected mice were treated with ACV or diluent as above and their right ears were stripped with cellophane tape. Two to 5 days later, the skin of the ears was scraped off, ground in 0.4 ml maintenance medium and the suspension was put on to Vero cell monolayers. At the same time, the right second, third and fourth cervical ganglia were removed, ground in 0.4 ml maintenance medium and subjected to three cycles of freezing and thawing to kill all cells so that only infectious (and not latent) virus could be isolated. The suspensions were then put on to monolayers of Vero cells. All cultures were examined daily for a week for c.p.e. characteristic of HSV (Table 2). In the group treated with ACV, virus was isolated from the ears of 4 of the 11 animals tested 4 days after stripping but not at other times. In the control group, virus was isolated from the ears of 30 to 40% of animals on each of the 3rd, 4th and 5th days after stripping. Clinical disease could be assessed only in animals killed 4 or 5 days after stripping. Seven of the 20 in the control group showed recurrent disease and of these, five yielded virus. Only one of the animals treated with ACV showed clinical signs and virus was isolated from the skin of its ear. In both groups of animals, ganglia yielded virus sporadically.

To test whether ACV was anti-inflammatory, and thereby might disguise clinical recurrences, its effect on delayed hypersensitivity to HSV was studied. Injection of heat-killed HSV into the right ear of latently infected mice produced a delayed hypersensitivity response specific to the virus. This was assayed 24 h after injection of the virus by observation of erythema and by measuring the thickness of the ear with a dial caliper gauge (Pocotest
A-o2T, Carobronze Ltd., Belmont Road, London, U.K.). Treatment with ACV (commencing 1 day before injecting the virus and continuing throughout the experiment) did not affect the intensity of the reaction. It is probable, therefore, that the reduction in the incidence of recurrent clinical disease in mice following stripping was not due merely to a depression of the inflammatory response by the drug.

The results show that ACV almost completely prevents recurrence of herpes simplex provoked by stripping the skin with cellophane tape. Only two mice had clinical recurrence of disease while being treated with the drug in a total of 150 tests, whereas in mice never given the drug, there were 46 recurrences in 268 tests (Table I). These results were significantly different ($\chi^2$: $P < 0.001$). In mice treated with ACV, virus could still be isolated from the skin, but the incidence was less than in untreated animals. Since ACV is excreted rapidly (Schaeffer et al. 1978) it is likely that an effective antiviral level of drug was not maintained over the whole period of treatment, but the twice-daily dosage still suppressed clinical disease.

The proportion of mice with latent infection in the ganglia was not affected by treatment with the drug, a finding similar to that of Field et al. (1979). Moreover, the proportion of mice which developed one or more bouts of recurrent clinical disease was the same for both mice previously treated with drug and for untreated mice. This suggests that periodic treatment with ACV did not affect the likelihood of development of recurrent disease on later occasions when animals were not treated.

It seems likely that ACV will prove to be of value in the treatment of recurrent herpes simplex in man. However, further work is required to determine whether its use can eradicate latent infection from the nervous system.

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REFERENCES


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