Treatment of Fatal Disseminated Vaccinia Virus Infection in Immunosuppressed Mice

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SUMMARY

Studies were performed to compare the therapeutic effectiveness of three antiviral drugs (ARA-A, ARA-C and IDU) on the course of fatal disseminated vaccinia virus infection in immunosuppressed mice. Treatment with ARA-A begun as late as 7 days after virus infection was significantly effective in preventing death; no antiviral effect of the other two drugs was demonstrated.

The use of several antiviral compounds has been advocated in the therapy of severe virus infections in immunologically comprised patients. Although these candidate antiviral drugs have been shown to be effective in a variety of experimental virus infections in animals, to our knowledge no attempt has been made to systematically evaluate their possible effectiveness and toxicity in immunosuppressed hosts. This seemed a worthwhile study to us because: (1) it permits a more realistic evaluation of the effectiveness of a drug or lack of effectiveness in a situation where host defence mechanisms are not fully operative; (2) it allows subtle, but important, drug toxicity to become apparent by further suppression of an already deficient host immune response. We have therefore evaluated the relative effectiveness of several antiviral drugs of potential clinical importance in the therapy of fatal virus infections in immunosuppressed mice. In the present paper, we report the results of treatment of fatal disseminated vaccinia virus infection in immunosuppressed mice with adenine arabinoside (ARA-A), cytosine arabinoside (ARA-C) and idoxuridine (IDU).

Four-week-old Swiss mice were obtained from the NIH colonies. Vaccinia virus was obtained from Dr W. A. Casel, Emory University, Atlanta, Georgia. Vaccinia virus was initially grown on the chick embryo chorioallantoic membrane; a pool of virus was then prepared in HeLa cells. This pool had a titre of $10^6$ p.f.u./ml on Vero cells. Rabbit antiserum to murine thymocytes (ATS) was prepared in our laboratory by the method of Levey & Medawar (1966).

All three antiviral drugs were given intraperitoneally (i.p.). Drugs were given at multiples of 24 h after virus injection as indicated. ARA-A was obtained in powder form from the Parke Davis Company, Ann Arbor, Michigan, and was suspended in distilled water to a final concentration of 40 mg/ml. The ARA-A was insoluble in water, and therefore the fine particles of ARA-A were well suspended before the injection. Autopsies on ARA-A-treated mice did not demonstrate significant amounts of ARA-A still present in the peritoneal cavity, indicating that the drug was well absorbed when administered i.p. IDU was obtained from Calbiochem Company, San Diego, California, and was diluted with phosphate-buffered saline at pH 7.2 to a final concentration of 40 mg/ml. ARA-C was obtained from the Upjohn Company, Kalamazoo, Michigan and was diluted with sterile water to a concentration of 5 mg/ml.

In each experiment a large number of mice were inoculated i.p. with 0.25 ml of ATS on days $-3, 0, +3$ and $+6$; these mice were also inoculated intravenously (i.v.) on day 0.
Table 1. Therapy of vaccinia virus infection in immunosuppressed mice*

<table>
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<tr>
<th>Experiment</th>
<th>Drug</th>
<th>Daily dose (mg/kg)</th>
<th>Day therapy initiated</th>
<th>% mortality</th>
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<tr>
<td>1</td>
<td>ARA-A</td>
<td>250</td>
<td>+1</td>
<td>13†</td>
</tr>
<tr>
<td></td>
<td>ARA-A</td>
<td>500</td>
<td>+1</td>
<td>6‡</td>
</tr>
<tr>
<td></td>
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<td>93</td>
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<td>12·5</td>
<td>+1</td>
<td>87§</td>
</tr>
<tr>
<td></td>
<td>ARA-C</td>
<td>25</td>
<td>+1</td>
<td>73</td>
</tr>
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</tr>
<tr>
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<td>—</td>
<td>—</td>
<td>64</td>
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<tr>
<td>3</td>
<td>IDU</td>
<td>125</td>
<td>+1</td>
<td>73</td>
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<td>500</td>
<td>+3</td>
<td>53</td>
</tr>
<tr>
<td></td>
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<td>—</td>
<td>—</td>
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* All mice received $10^4$ p.f.u. of vaccinia virus IV on day 0 and 0·25 ml of ATS i.p. on days −3, 0, +3 and +6.
† Treatment for a total of five daily i.p. doses.
‡ $P < 0·01$.
§ $P < 0·05$.

...with $10^4$ p.f.u. of vaccinia virus in 0·1 ml of Eagle's medium with 2% foetal calf serum. A group of 45 mice which received both ATS and vaccinia virus were kept as controls, while groups of 15 similar mice were treated with five daily i.p. doses of each of the antiviral drugs evaluated, beginning 1 to 7 days after virus infection. On various days after virus injection, mice were bled individually and their sera assayed for levels of virus on Vero cells. Mice were observed for a minimum of 3 weeks and in some experiments for 8 weeks after virus inoculation. As previously described, vaccinia virus-infected mice treated with ATS develop a fatal disseminated infection, with moderately severe bronchopneumonia and many areas of hepatic necrosis (Worthington, 1973).

The highest dose level of each of the three antiviral drugs evaluated was non-toxic, as determined by mortality, when combined with ATS treatment. Therapy with ARA-A at any of the three dosage levels of the drug evaluated (1000, 500 and 250 mg/kg/dose) was highly effective at reducing mortality when initiated 1 day after virus infection (Table 1). Neither ARA-C nor IDU at any of the three dose levels of each drug evaluated had any significant effect when begun either on day 1 or day 3 after infection (Table 1). In other experiments lower dose levels of ARA-C (5 mg/kg/dose) and IDU (25 mg/kg/dose) were also found to be ineffective in reducing or delaying mortality from this infection. Higher dose levels of ARA-C (100 mg/kg/day or greater) and IDU (750 mg/kg/day or greater) were toxic; use of these higher doses of ARA-C or IDU resulted in a higher mortality among these vaccinia virus-infected, immunosuppressed mice. These results indicate that ARA-A was highly effective at reducing mortality in vaccinia virus infection in immunosuppressed mice, while ARA-C and IDU were ineffective over a wide range of non-toxic dose levels under similar experimental conditions.

Experiments were performed to determine how late in the course of this experimental
Fig. 1. Effect of late therapy with ARA-A on mortality of immunosuppressed mice infected with vaccinia virus. (a) Mice received 250 mg/kg/day of ARA-A. ●—●, Vaccinia + ATS (90%); ▲—▲, day 1 ARA-A (27%); ■—■, day 3 ARA-A (20%); □—□, day 5 ARA-A (13%); ○—○, vaccinia (7%); △—△, ATS (6%). (b) Mice received 500 mg/kg/day of ARA-A. ●—●, Vaccinia + ATS (90%); □—□, day 1 ARA-A (60%); ▲—▲, day 7 ARA-A (53%); ■—■, day 5 ARA-A (27%); ▼—▼, day 3 ARA-A (7%); ○—○, Vaccinia (7%); △—△, ATS (0%). The numbers in parentheses indicate the % mortality at the plateau level for each treatment.

infection therapy with ARA-A could be started and still be effective (Fig. 1). Each of the three dose levels of ARA-A evaluated was highly effective at reducing mortality when begun as late as day 5 or 7 after infection (250 mg/kg/dose was only evaluated as late as day 5 after infection). By day 5 after infection vaccinia virus-infected mice had multiple discrete 'pock-like' tail lesions; thus therapy with ARA-A was effective when begun after the appearance of definite signs of disseminated vaccinia infection.

As previously described, vaccinia virus-infected mice treated with ATS developed viraemia which persisted until the time of death, with a peak viraemia of $10^4$ p.f.u./ml of serum reached between days 5 and 9 after infection. Mice which received their first dose of ARA-A (1000 mg/kg/dose) on day 4 after infection had levels of viraemia 24 h after this first dose of ARA-A that were about one-tenth the levels of viraemia in control mice not treated with ARA-A. By days 7 and 9 after infection (the last dose of ARA-A was given on day 7 after infection) the ARA-A-treated mice had levels of viraemia that were about one-fiftieth the levels of viraemia in control mice. Thus, treatment with ARA-A rapidly suppressed the level of viraemia in immunosuppressed mice.
Short communications

Immunosuppressed and immunologically deficient persons have been noted to be particularly susceptible to fatal virus infections (St Geme et al. 1965; Fulginiti et al. 1968; Montgomerie et al. 1969; Levine et al. 1974). Progressive systemic vaccinia virus infection has been a particularly severe problem in individuals with depression of both antibody-mediated and cellular immunity (Fulginiti et al. 1968). In numerous studies ATS has been demonstrated to be a potent suppressor of cell-mediated immunity (Levey, 1970), and as we have previously reported, this dose schedule of ATS dramatically inhibited the formation of neutralizing antibody to vaccinia virus in mice (Worthington, 1973). Thus the fatal systemic vaccinia virus infection which ATS-treated mice develop may be a model of the same infection in immunologically deficient patients.

The doses of ARA-A used in this study are significantly higher than the doses which have been used in patients. Previous work has demonstrated that the present dose levels were optimal in the therapy of herpes simplex virus and vaccinia virus infections in mice (Dixon et al. 1968; Sloan et al. 1968). Lower daily doses of ARA-A, which were very similar to the doses used clinically, were found to be less effective in these previous experiments; these lower daily doses of ARA-A did, however, still significantly prolong survival time in non-immunosuppressed mice infected with herpes simplex virus or vaccinia virus. In the present experiments we were unable to demonstrate any protective effect of either ARA-C or IDU in immunosuppressed mice infected with vaccinia virus, even though both drugs were evaluated over a very wide range of non-toxic and toxic dose schedules. It is therefore quite impressive that optimal doses of ARA-A were highly effective in reducing mortality from this infection when begun as late as day 7 after infection.

We are not aware of any previous report in which therapy with ARA-A was effective when begun as late as day 7 after experimental virus infection. In unpublished studies of type 1 herpes simplex virus infection in immunosuppressed mice, we have also found ARA-A to be effective therapeutically while the other four antiviral drugs evaluated (ARA-C, IDU, interferon and the interferon inducer, polyinosinic-polycytidylic acid) were ineffective. Taken together, these studies provide evidence that ARA-A can be a highly effective antiviral drug in a severely immunosuppressed experimental animal. This seems of great importance because most of the candidate antiviral drugs which have been used in man have significant immunosuppressive effects themselves. In the largest controlled study of ARA-C therapy of disseminated herpes zoster in cancer patients, the ARA-C therapy appeared to have a deleterious rather than a therapeutic effect, and this was thought by the authors to be related to the further suppression by ARA-C of already deficient host defence mechanisms (Stevens et al. 1973). It is therefore particularly encouraging that a recent controlled study demonstrated a significant therapeutic effect of ARA-A on the course of herpes zoster in immunosuppressed patients (Whitley et al. 1976). Clearly the role, if any, of ARA-A in the therapy of virus infections in immunosuppressed patients will be determined by such carefully performed controlled clinical studies.

Department of Medicine
St Elizabeth’s Hospital of Boston
Brighton
Massachusetts 02135
Food and Drug Administration
Bureau of Biologics, Division of Virology
Bethesda
Maryland 20014, U.S.A.

M. Worthington

Food and Drug Administration
Bureau of Biologics, Division of Virology
Bethesda
Maryland 20014, U.S.A.

M. Conliffe
REFERENCES


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