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Treatment of Fatal Disseminated Herpes Simplex Virus, Type 1, Infection in Immunosuppressed Mice (39899)

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The use of several antiviral compounds has been suggested in the therapy of several viral infections in immunologically compromised patients (1, 2). While these candidate antiviral drugs have been shown to be effective in a variety of experimental viral infections in animals, to our knowledge, no attempt has been made to systematically evaluate their possible effectiveness and toxicity in an immunosuppressed host. This seemed a worthwhile undertaking to us because: (i) It permits a more realistic evaluation of a drug's effectiveness or lack of effectiveness in a situation where host defense mechanisms are not fully operative; (ii) it allows subtle, but important, drug toxicity to become apparent by further suppression of an already deficient host immune response.

The present study was undertaken to compare therapy of a fatal disseminated herpes simplex virus, type I (HSV) infection in immunosuppressed mice by adenine arabinoside (ARA-A), cytosine arabinoside (ARA-C), idoxuridine (IDU), interferon, and the interferon inducer, polyinosinic-polycytidylic acid (In.Cn).

Materials and methods. Mice and virus. HSV, type I, strain VR_3, was originally obtained from Dr. A. J. Nahmias and passaged in primary rabbit kidney cells. The virus infectivity was assayed by a microplaque assay in BHK-21 cells. Six-week-old male CFW mice from Carworth Farms, Wilmington, Massachusetts, were used.

Drugs. All drugs were given intraperitoneally (ip). Drugs given on the same day as virus were given 2 hr after the virus injection. ARA-A was obtained in powder form from the Parke Davis Company, Ann Arbor, Michigan, and suspended in distilled water to a final concentration of 40 mg/ml. The ARA-A was not soluble in water, and therefore the fine particles of ARA-A were well suspended before the injection. IDU was obtained from Calbiochem Company, San Diego, California, and 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 diluted with sterile water to a concentration of 5 mg/ml. Double-stranded In.Cn was obtained from P. L. Biochemicals, Milwaukee, Wisconsin, and prepared as a 1.0 mg/ml solution in phosphate-buffered saline, pH 7.6. Interferon was prepared in mouse C-243 cells using Newcastle disease virus, as previously described (3). The final concentration was $10^{5.5}$ units/ml. Interferon titers were determined on the pooled sera of five mice at various times after ip injection of interferon or In.Cn, using GD-7 virus in mouse L cells (4).

Antithymocyte sera (ATS). Rabbit antiserum to murine thymocytes (ATS) was prepared in our laboratory by the method of Levey and Medawar (5). Another pool of rabbit antiserum to murine thymocytes was obtained from Microbiological Associates, Bethesda, Maryland. Both pools of ATS were heated at 56° for 1 hr and absorbed three times with murine red blood cells; both pools of ATS were highly effective in protecting mice injected ic with 10^3 LD_{50} of lymphocytic choriomeningitis (LCM) virus in our laboratory. This indicates that the cell-mediated immune response to LCM virus was suppressed by these sera, since most of the available evidence indicates that acute LCM disease is a cell-mediated immunopathological process (6, 7).

Experimental design. A large number of mice were inoculated ip with 0.25 ml of ATS on days −3, −1, 0, and +1; they also were inoculated ip with 10^3 plaque-forming

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units (pfu) of HSV in 0.2 ml of Eagle's medium with 10% fetal calf serum (FCS) on day 0. A group of 45 of these ATS-treated and virus-infected mice was kept as controls, while groups of 15 similar mice were treated with five daily ip doses of each of the antiviral drugs evaluated, beginning each day after virus injection. Mice were observed for a minimum of 3 weeks and in some experiments for 8 weeks. Treatment with ATS potentiated this primary infection with HSV, as has been previously reported (8).

The ATS-treated mice were found on pathological examination on days 7 and 9 after virus infection to have many areas of focal necrosis in the liver, with some areas of more extensive hepatic necrosis and polymorphonuclear cell infiltration. The ATS-treated mice also had occasional areas of focal necrosis in brain, while the other organs examined (heart, lung, spleen and kidney) appeared normal. Nonimmunosuppressed mice had only very rare areas of focal necrosis of the liver on days 7 and 9 after HSV infection.

Results. Effect of early therapy with ARA-A, ARA-C, or IDU on HSV infection in immunosuppressed mice (Table I). The first experiments were designed to determine if any of these three drugs was effective when begun on day 1 or 3 after HSV infection in ATS-treated mice. HSV-infected mice which did not receive ATS had a mortality from 0 to 20% in different experiments. Preliminary experiments indicated that all dose schedules used of these drugs were nontoxic (as determined by mortality) when combined with ATS treatment.

All three dose schedules of ARA-A used (1000, 500, and 250 mg/kg/day) were highly effective in reducing mortality from HSV infection in immunosuppressed mice ($P < 0.001$). Neither ARA-C nor IDU was protective when begun either 1 or 3 days after HSV infection. In other experiments not shown here, lower doses of ARA-C (down to 5 mg/kg/day) were used and were also found not to be protective. Thus ARA-A was highly effective when begun either 1 or 3 days after HSV infection in immunosuppressed mice, while ARA-C and IDU over a wide range of nontoxic doses were not effective under similar experimental conditions.

Effect of late therapy with ARA-A on HSV

TABLE I. THERAPY OF HERPES SIMPLEX VIRUS INFECTION IN IMMUNOSUPPRESSED MICE[a]

Experiment number	Drug	Daily dose (mg/kg)[b]	Day therapy initiated	Mortality (%)
1	ARA-A	250	+1	13[c]
	ARA-A	500	+1	6[c]
	ARA-A	1000	+1	10[c]
	ARA-A	1000	+3	27[c]
	None			93
2	ARA-C	12.5	+1	73
	ARA-C	25	+1	87
	ARA-C	50	+1	93[d]
	ARA-C	12.5	+3	67
	ARA-C	25	+3	80
	ARA-C	50	+3	73
	None			73
3	IDU	125	+1	67
	IDU	250	+1	73
	IDU	500	+1	73
	IDU	125	+3	93[d]
	IDU	250	+3	80
	IDU	500	+3	80
	None			70

[a] All mice received 10^3 pfu of herpes simplex virus ip on day 0 and 0.25 ml of ATS ip on days −3, −1, 0, and +1.

[b] Therapy for a total of five daily ip doses.

[c] $P < 0.001$.

[d] $P < 0.05$.

infection in immunosuppressed mice (Fig. 1). Experiments were performed to determine how late in the course of HSV infection therapy with ARA-A could be initiated and still be effective. Treatment with 1000 mg/kg/day of ARA-A was still effective in preventing deaths when begun as late as day 6 after virus infection (Fig. 1). A lower dose of 500 mg/kg was somewhat less effective, but a significant reduction in mortality was still observed when this dose was begun on day 4 after infection. In addition, a significant delay in death was observed when therapy with this lower dose of ARA-A (500 mg/kg/day) was begun on day 8 after HSV inoculation. Thus, therapy with ARA-A was effective when begun as late as 6 days after this experimental HSV infection in immunosuppressed mice.

Virus content in the organs of immunosuppressed mice. Three mice from each experimental group were sacrificed on days 4, 6, and 8 after HSV infection and a 10% weight/volume suspension of each individual brain, liver, lung, heart, and spleen was prepared in Eagle's medium with 5% FCS. Mice from each group were also bled daily in an attempt to detect viremia.

No virus was isolated from any of the organs from nonimmunosuppressed control mice or from immunosuppressed mice treated with ARA-A (1000 mg/kg/day) beginning on day 4 after infection. Immunosuppressed mice not treated with ARA-A had 10^6 pfu of HSV/g of tissue in their brains by day 8 after infection, but virus could not be isolated from any other site, including liver and blood.

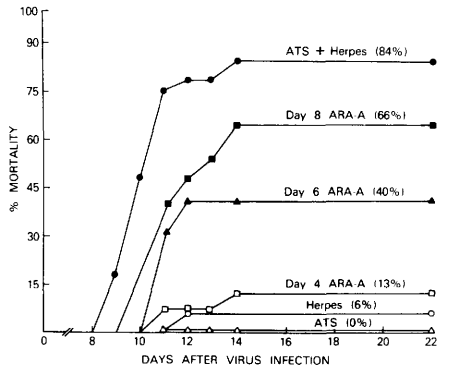

FIG. 1. Effect of late therapy with ARA-A on HSV infection in immunosuppressed mice. Mice were injected ip with 0.25 ml of ATS on days −3, −1, 0 and +1; they were also inoculated ip with 10^3 pfu of HSV on day 0. Mice which received ARA-A were treated with five daily ip doses of 1000 mg/kg/day of ARA-A beginning on the day indicated.

These results indicate that therapy with ARA-A begun 4 days after HSV infection prevented the development of detectable levels of virus in the brains of immunosuppressed mice.

Effect of therapy with interferon or In.Cn on HSV infection in immunosuppressed mice (Table II). Mice were treated with either interferon (10^5 units/day ip for five daily injections) or In.Cn (100 μg/day for 5 days) beginning 1 or 3 days after HSV infection.

Mice treated with this dose of interferon had serum titers of interferon of 10^3 units/ml 1 hr after an ip injection of interferon, falling to 10^2 units/ml 24 hr after injection. The ATS-treated mice had serum interferon titers of $10^{3.5}$, $10^{4.2}$, and $10^{2.6}$ units/ml at 1, 6, and 24 hr respectively, after one ip dose of 100 μg of In.Cn. Serum interferon titers from nonimmunosuppressed mice after 100 μg of In.Cn ip were essentially identical. Thus, this dose of ATS did not influence the interferon response to a single ip dose of In.Cn.

These doses of In.Cn and interferon were completely nontoxic when combined with ATS; in addition, at similar dose levels, both drugs have been reported to have significant antiviral effects against a number of viruses, including HSV, in nonimmunosuppressed mice (9, 10). However, in the present experiments immunosuppressed mice were not protected from death in this experimental infection by either drug. Thus, nei-

TABLE II. EFFECT OF INTERFERON AND In.Cn ON HERPES SIMPLEX VIRUS INFECTION IN IMMUNOSUPPRESSED MICE[a]

Drug	Daily dose[b]	Day Therapy Initiated	Mortality (%)
Interferon	100,000 units	+1	87
Interferon	100,000 units	+3	80
In.Cn	100 μg	+1	80
In.Cn	100 μg	+3	73
None			83

[a] All mice received 10^3 pfu of herpes simplex virus ip on day 0 and 0.25 ml of ATS on days −3, −1, 0 and +1.

[b] Therapy for a total of five daily doses.

ther interferon nor In.Cn was effective under conditions in which ARA-A was highly effective.

Discussion. In these studies, treatment with ARA-A begun as late as 6 days after experimental HSV infection in immunosuppressed mice was effective in preventing death. In similar experiments no antiviral effect of either IDU or ARA-C could be demonstrated; furthermore, both IDU and ARA-C significantly potentiated this experimental infection at one of the dose levels of each drug which was evaluated. A similar lack of effectiveness was noted with early therapy with interferon or In.Cn. Thus, in these experiments, of the five drugs evaluated, only ARA-A was a highly effective therapeutic agent in a severely immunosuppressed host.

In recent years viral infections, particularly herpes group virus infections, have become an increasingly important problem in patients who are immunologically compromised by their underlying disease or by treatment with immunosuppressive agents (11–13). These include patients on immunosuppressive agents, such as azathioprine and cyclophosphamide (11), patients with certain cancers, such as Hodgkins disease (12), and those with inborn disorders of thymus-dependent immune function (14, 15). Although the basic defect common to all of these conditions has not been clearly defined, it is highly likely that the common variable is a defect in function of thymus-dependent lymphocytes (T cells). T-Cell dysfunction has, in fact, been implicated in virtually all the situations associated with disseminated HSV infection: infancy (16), malnutrition and kwashiorkor (17), burns (18), lymphoma (19), Wiskott–Aldrich syndrome (20), and immunosuppressive therapy (21). Antithymocyte serum has been well documented to be a very potent suppressor of T-cell immunity in a variety of experimental animals, including mice (22). Therefore, the disseminated HSV infection which ATS-treated mice developed in these experiments may well be a reasonable model for severe HSV infection in patients with defective T-cell-dependent immunity. It is also of interest that renal and heart transplant patients treated with ATS have been noted to have increased susceptibility to HSV infection (23, 24).

Immunosuppressed patients with severe viral infections clearly are candidates for therapy with an effective antiviral drug, and a variety of experimental antiviral drugs have been used in this clinical situation. It would thus appear worthwhile to evaluate the effectiveness and toxicity of candidate antiviral drugs in models in which the experimental animal is to some extent immunologically deficient. To our knowledge, no studies have been reported on the comparative effectiveness of various antiviral drugs in experimental viral infections in immunologically compromised hosts. Several previous reports have evaluated specific drugs in this setting; we have previously reported that In.Cn protected Cytoxan-treated mice from fatal disseminated vaccinia virus infection, even when therapy with In.Cn was begun 4 days after virus infection (4). Another interferon stimulator, pyran copolymer, has been found to exert an antiviral effect against Rauscher virus in mice immunosuppressed by adult thymectomy and treatment with ATS (25). Newborn mice might be considered to be immunologically deficient hosts, and both In.Cn and IDU have recently been evaluated in newborn mice with a disseminated infection with herpes simplex virus, type II (26, 27). In this model, both of these drugs were found to prolong survival of the newborn mice by 1 or 2 days, but not to significantly reduce mortality. Finally, in a controlled study in cancer patients, ARA-C was noted to have a deleterious effect on disseminated herpes zoster infection of man (28). This report emphasizes the necessity of a very careful evaluation of antiviral drugs which have significant side effects, particularly immunosuppressive effects.

In the present studies, as well as in unpublished studies of these drugs in fatal disseminated vaccinia virus infection in immunosuppressed mice, ARA-A has been shown to be dramatically more effective therapeutically than ARA-C, IDU, interferon, or In.Cn. Several previous studies have suggested that ARA-A was more effective than IDU in the therapy of HSV and vaccinia infections in nonimmunosuppressed mice

when the two drugs were evaluated under similar experimental conditions (29–31). Taken together, these reports indicate that ARA-A can be highly effective in immunologically compromised as well as immunologically intact hosts. Particularly encouraging is the recent report of a controlled clinical study in which therapy with ARA-A appeared to have a statistically significant therapeutic effect on herpes zoster infection in immunosuppressed patients (32). Clearly, the exact role, if any, of systemic ARA-A in therapy of viral infections in man will be based on the results of further carefully conducted controlled studies in man.

Summary. Studies were performed to compare the therapeutic effectiveness of five antiviral drugs (ARA-A, ARA-C, IDU, interferon, and In.Cn) on the course of a disseminated herpes simplex virus, type I, infection in immunosuppressed mice. Treatment with ARA-A begun as late as 6 days after virus infection was effective in preventing death; no antiviral effect of the other four drugs was demonstrated.

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