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Effect of Treatment with 1-β-D-Arabinofuranosylthymine of Experimental Encephalitis Induced by Herpes Simplex Virus in Mice

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1-β-D-Arabinofuranosylthymine (ara-T) was examined for its therapeutic efficacy against encephalitis in mice inoculated intracerebrally with herpes simplex virus. Intraperitoneal treatment with 100 mg of ara-T per kg twice daily for 4.5 days was as effective as treatment with 50 mg of arabinosyladenine 5'-monophosphate per kg. Under the same conditions, doses of 5-iododeoxyuridine or arabinosylcytosine (50 mg/kg each) were not effective. Even when the virus inoculum was as high as 320 or 3,200 50% lethal doses, ara-T increased the life span significantly. Oral treatment with 27 mg of ara-T per kg produced a modest increase in the mean survival time, equal to that of 50 mg of ara-T per kg administered intraperitoneally or subcutaneously. A single dose of ara-T, 800 mg/kg intraperitoneally or 400 mg/kg orally, was effective. The 50% lethal dose of ara-T administered intraperitoneally and that administered orally were more than 10 and 15 g/kg, respectively. The therapeutic indexes (maximal tolerated dose divided by minimal effective dose) in multiple intraperitoneal treatments and in multiple oral treatments were estimated to be more than 25 and 100, respectively.

Herpes simplex virus (HSV) is a ubiquitous virus which causes a variety of diseases in humans, including localized vesicular and ulcerative lesions of oral or genital regions and encephalitis (17, 19). A number of antiherpesvirus compounds have been tested for therapeutic effectiveness in animals and in humans. Among experimental animal models of HSV infection used for the evaluation of new drugs, HSV-induced encephalitis in mice is the one employed most frequently (5). Still, only a small number of effective antiherpetic drugs have emerged (20). The most effective of these, 9-β-D-arabinofuranosyladenine (ara-A), has high antiviral activity in vitro and in vivo (25, 27) and has been approved by the U.S. Food and Drug Administration for the treatment of herpes encephalitis in humans. Undesirable reactions to ara-A have been reported (11, 13, 18, 23), although overall this compound is well tolerated.

Several thymidine analogs have been developed. According to Aswell et al. (2), Cheng (4), and Schaeffer et al. (24), these analogs are phosphorylated selectively by virus-induced thymidine kinase but not by normal cellular thymidine kinase. Although these derivatives selectively inhibit viral multiplication in vitro, their therapeutic effect in animal models has not been clearly proved. More recently, 5-ethyl-2'-deoxyuridine (5), 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine) (9, 10, 24), 5-iodo-5'-amino-2',5'-dideoxyuridine (21), and (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (6) were reported effective in the treatment of HSV-infected animals.

In the previous search for a well-tolerated anti-DNA virus agent, we found that 5-ethyl-1-β-D-arabinofuranosyluracil, as well as 1-β-D-arabinofuranosylthymine (ara-T), had significant activity against herpesviruses in vitro at concentrations well below those that were inhibitory to cell growth (13). Ara-T has also shown striking activity in vitro against HSV (2, 8) and varicella-zoster virus (14). Although Müller et al. (15, 16) reported that ara-T was phosphorylated by both HSV-infected and noninfected cells, its inhibitory action on cell growth was known to be lower than those of other antiviral substances such as 1-β-D-arabinofuranosylcytosine (ara-C), ara-A, 5-iododeoxyuridine (IUdR), and acycloguanosine (6, 13). In vivo antiviral efficacy of ara-T, however, was reported only by Aswell et al. (2) and by Aswell and Gentry (3) in the treatment of equine abortion virus-infected hamsters. We have evaluated the activity of ara-T and of 5-ethyl-1-β-D-arabinofuranosyluracil against infection with herpesvirus in mice. Since 5-ethyl-1-β-D-arabinofuranosyluracil had little, if any, activity, the present report is limited to the

description of the efficacy of ara-T against experimental encephalitis induced by HSV types 1 (HSV-1) and 2 (HSV-2) and its toxicity for mice.

MATERIALS AND METHODS

Viruses. The source and history of strain VR-3, prototype of HSV-1, and strain MS, prototype of HSV-2, have been described previously (7). The viruses, kindly supplied by S. Yamazaki, Central Virus Diagnostic Laboratory, National Institute of Health of Japan, had been propagated five times in human embryonic lung fibroblasts. The culture fluids of the cells infected with HSV were titrated and stored at −80°C until use.

Mice. Four-week-old random-bred albino ICR-JCL Swiss mice were used in the encephalitis experiments. Six-week-old mice of the same strain were used in the drug toxicity experiments.

Encephalitis experiments. Male or female mice, weighing about 20 g, in groups of 9 or 10 for each drug dose, were inoculated intracerebrally (i.c.) with 0.01 or 0.02 ml of various doses of HSV (usually 3.2 or 10 50% lethal doses [LD_{50}]). Infected mice were treated i.c., intraperitoneally (i.p.), orally (p.o.), or subcutaneously (s.c.). A total of 18 or 20 mice infected and treated with phosphate-buffered saline (PBS) instead of with a drug were used as controls. In the i.c. (target-organ) treatment studies, the initial dose of drug in PBS was injected with the virus and was followed by i.c. administration daily for an additional 2 days. When drugs were administered by other routes, treatment was started at 4 h postinfection and repeated twice daily for 4.5 days for a total of nine treatments. In some experiments, schedules of drug treatment were modified as indicated.

Mice were observed daily for 21 days to identify death and other reactions. Data on the comparative mortalities of control and drug-treated groups were evaluated by the Fisher Exact test. Student's t test was used to evaluate the differences in mean survival times between the two groups. Only animals that died on or before day 21 were included in calculations of mean survival times.

In vivo toxicity experiments. Groups of 10 uninfected mice, 6 weeks old, weighing about 30 g, were injected with single doses of ara-T in the amounts indicated and observed for 1 week for death and adverse reactions. Similar groups, 4 weeks old, were treated with drugs as in the therapeutic experiments described above.

Antiviral agents. Ara-T was prepared and identified by S. Sakata in this laboratory as described previously (13). Ara-C and IUdR were commercial products of the Yamasa Shoyu Company, Ltd. Ara-A was purchased from P-L Biochemicals, Inc. Ara-A 5′-monophosphate (ara-AMP), used in this study instead of ara-A for the purpose of increased water solubility, was prepared from ara-A by K. Fujiyama in this laboratory by the method of Revankar et al. (22). Measurements in a Hitachi high-speed liquid chromatograph type 635 with an ultraviolet detector showed that the purities of ara-T, IUdR, and ara-C were more than 99% and that of ara-AMP was about 95%. No ultraviolet-absorbing contaminants were detected. Drugs were usually dissolved in PBS. When the size of the dose of ara-T or of IUdR precluded solution of PBS, these agents were suspended in PBS containing 0.5% carboxymethyl cellulose.

RESULTS

Comparison of ara-T and other antiviral agents for therapeutic effects on encephalitis induced by HSV in mice. In the initial experiments, ara-T was compared with IUdR, ara-C, and ara-AMP for efficacy against HSV-1- and HSV-2-induced encephalitis. When administered i.c., all of the above but IUdR were effective in reducing final mortality or in increasing the mean survival time of mice infected with both HSV-1 and HSV-2 or both (Table 1). Doses of 2.5 mg of ara-AMP per kg daily for 3 days were more effective against HSV-1 infection

TABLE 1. *Comparison of effects of i.c. treatment with IUdR, ara-C, ara-AMP, and ara-T on HSV-induced encephalitis in mice*

Challenge virus	Treatment[a]	Survivors/total	Mean survival time (days)
HSV-1 (3.2 LD_{50})	PBS	4/18	6.7 ± 0.60[b]
	IUdR, 5	4/9	8.2 ± 1.24
	Ara-C, 10	6/9[c]	7.0 ± 1.35
	Ara-AMP, 2.5	8/9[d]	6
	Ara-AMP, 10	2/9	7.0 ± 1.25
	Ara-T, 10	6/9[c]	8.0 ± 1.15
HSV-2 (10 LD_{50})	PBS	1/18	6.5 ± 0.63
	IUdR, 5	0/9	6.8 ± 0.76
	Ara-C, 10	4/9[c]	7.3 ± 1.05
	Ara-AMP, 2.5	6/9[d]	10.0 ± 2.31[c]
	Ara-T, 10	5/9[d]	10.8 ± 1.93[c]

[a] Numbers represent milligrams per kilogram for 3 days.
[b] Standard error.
[c] $P < 0.05$.
[d] $P < 0.01$.

than doses of 10 mg of either ara-T or ara-C per kg. Thus, on a milligram-per-kilogram basis, ara-AMP appeared more effective than ara-T. However, treatment with 10 mg of ara-AMP per kg for 3 days was excessively toxic, even though Sidwell et al. (26) reported that ara-AMP could be administered i.c. to mice in single doses of up to 35 mg/kg without producing toxicity-related deaths. Therefore, ara-AMP was tested for its efficacy against HSV-2-induced encephalitis only at a dose of 2.5 mg/kg. Doses of 10 mg of ara-T per kg were as effective against HSV-2 infection as doses of 2.5 mg of ara-AMP per kg and more effective than doses of 10 mg of ara-C per kg.

As shown in Table 2, i.p. administration of 100 mg of ara-T per kg twice daily for 4.5 days was as effective in reducing mortality (HSV-1) or in increasing the mean survival time (HSV-2) as dosage with 50 mg of ara-AMP. Treatment with ara-C or with IUdR was not effective. Administration of 50 or 100 mg of ara-C per kg or 150 or 300 mg of IUdR per kg was also not effective (data not shown).

Effects of dose and duration of therapy on the effectiveness of ara-T. As shown in Table 3, the i.p. administration of 100 mg of ara-T per kg twice daily for 2.5 days (five doses) was moderately effective against HSV-1 infection. Extending the treatment with this dose to 5.5 days did not increase effectiveness. As shown in Table 4, treatment with 50 mg of ara-T per kg for 4.5 days (total dose, 450 mg/kg) and with 60 mg of ara-T per kg for 2.5 days (total dose, 300 mg/kg) increased the mean survival time. Therapy with 200 mg of ara-T per kg for 4.5 days was effective in reducing final mortality and in increasing the mean survival time. Treatment with 25 mg/kg or less for 4.5 days and with 45 mg/kg or less for 2.5 days was not effective against HSV-1-induced encephalitis.

Influence of inoculum size on the efficacy of ara-T. In this appraisal, groups of mice were inoculated i.c. with 32 to 3,200 LD_{50} of virus (in log_{10} increments) and treated with ara-T twice daily for 4.5 days. As shown in Table 5, treatment with 100 mg of ara-T per kg was as effective in increasing survival rate and survival time in mice infected with 32 LD_{50} of HSV-1 as it was in those infected with 3.2 LD_{50} (Table 2). When the size of the challenge dose was increased to 320 and 3,200 LD_{50}, treatment with 100 or 200 mg of ara-T per kg led to an increase in the survival time, although it did not increase the number of survivors.

Effectiveness of s.c. and p.o. dosage with ara-T against HSV-1-induced encephalitis in mice. The benefits of p.o. and s.c. dosage were compared in a regimen in which ara-T was administered twice daily for 4.5 days beginning

TABLE 3. *Effect of duration of therapy with ara-T on HSV-1-induced encephalitis in mice*[a]

Duration of therapy	Survivors/total	Mean survival time (days)
Control	0/18	5.9 ± 0.38[b]
1.5 days	0/9	5.9 ± 0.58
2.5 days	3/9[c]	7.8 ± 0.91[c]
3.5 days	2/9	7.6 ± 0.68[c]
4.5 days	3/9[c]	6.7 ± 0.52
5.5 days	3/9[c]	6.9 ± 0.74

[a] Mice were infected with 10 LD_{50} of HSV-1 and treated i.p. with 100 mg of ara-T per kg twice daily for the indicated days.
[b] Standard error.
[c] $P < 0.05$.

TABLE 2. *Comparison of effects of i.p. treatment with IUdR, ara-C, ara-AMP, and ara-T on HSV-induced encephalitis in mice*

Challenge virus	Treatment[a]	Survivors/total	Mean survival time (days)
HSV-1 (3.2 LD_{50})	PBS	4/20	6.6 ± 0.48[b]
	IUdR, 50	1/10	6.7 ± 0.47
	Ara-C, 25	1/10	6.0 ± 0.70
	Ara-AMP, 50	6/10[c]	7.5 ± 1.32
	Ara-T, 100	7/10[d]	9.3 ± 1.45[c]
HSV-2 (10 LD_{50})	PBS	2/18	6.4 ± 0.51
	IUdR, 50	0/9	6.2 ± 0.32
	Ara-C, 25	0/9	5.0 ± 0.52
	Ara-C, 50	0/9	4.6 ± 0.18[e]
	Ara-AMP, 50	4/9	10.3 ± 2.38[c]
	Ara-T, 100	4/9	8.8 ± 0.91[c]

[a] Numbers represent milligrams per kilogram administered twice daily for 4.5 days.
[b] Standard error.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] Decrease ($P < 0.05$).

TABLE 4. *Effect of treatment with ara-T on HSV-1-induced encephalitis in mice*[a]

Expt	Ara-T (mg/kg)	Survivors/total	Mean survival time (days)
1	0	3/20	5.6 ± 0.32[b]
	12.5	3/10	6.1 ± 0.46
	25	2/10	5.9 ± 0.48
	50	4/10	8.7 ± 0.92[c]
	100	6/10[d]	7.0 ± 0.58
	200	7/10[c]	10.7 ± 4.17[c]
2	0	1/18	5.1 ± 0.30
	35	2/9	5.6 ± 0.37
	45	1/9	5.4 ± 0.73
	60	1/9	6.2 ± 0.40[d]
	77	2/9	7.1 ± 0.85[d]
	100	2/9	7.0 ± 0.53[c]

[a] Mice were infected with 10 LD_{50} of HSV-1 and treated i.p. with ara-T twice daily for 4.5 and 2.5 days in experiments 1 and 2, respectively.
[b] Standard error.
[c] $P < 0.01$.
[d] $P < 0.05$.

TABLE 5. *Influence of size of challenge dose on the efficacy of ara-T against HSV-1-induced encephalitis in mice*[a]

Dose of virus (LD_{50})	Ara-T (mg/kg)	Survivors/total	Mean survival time (days)
32	0	0/20	4.6 ± 0.22[b]
	50	1/10	5.8 ± 0.47[c]
	100	3/10[c]	6.9 ± 0.51[d]
320[e]	0	0/10	2.8 ± 0.21
	100	0/10	4.1 ± 0.16[f]
	200	0/10	5.5 ± 0.27[f]
3,200[e]	0	0/10	1.85 ± 0.13
	100	0/10	2.44 ± 0.21[c]
	200	0/10	3.70 ± 0.48[d]

[a] Mice were infected with the indicated dose of virus and treated i.p. with ara-T twice daily for 4.5 days.
[b] Standard error.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] Survivors were observed every 12 h.
[f] $P < 0.001$.

4 h after i.c. virus inoculation (Table 6). Whereas s.c. treatment with ara-T was as effective against HSV-1-induced encephalitis as i.p. treatment, p.o. administration was somewhat more effective. As the results in Table 7 show, minimal doses of ara-T, at which increases in survivors or in the mean survival time with statistical significance ($P < 0.05$) or both were observed, were 40 and 27 mg/kg or less for the i.p. and p.o. routes, respectively.

Activity of a single dose of ara-T. As shown in Table 8, a single dose of 800 mg of ara-T per kg administered i.p. at 12 h post-inoculation effected an increase in the mean survival time. A single i.p. dose of 1,600 or 3,200 mg/kg effected increases both in the mean survival time and in the number of survivors. Single p.o. doses of 800 or 1,600 mg/kg administered at 12 h post-inoculation were also effective in increasing the mean survival time. Thus, even a single large dose of ara-T was effective against HSV-induced encephalitis in mice.

Toxicity of ara-T for mice. As shown in Table 9, various doses of ara-T were adminis-

TABLE 6. *Effects of s.c. and p.o. treatments with ara-T on HSV-1-induced encephalitis in mice*[a]

Administration route	Ara-T[b]	Survivors/total	Mean survival time (days)
s.c.	0	0/20	3.2 ± 0.39[c]
	25	0/10	3.4 ± 0.22
	50	0/10	4.6 ± 0.45[d]
	100	0/10	5.2 ± 0.13[e]
p.o.	0	1/18	4.0 ± 0.13
	100	3/9	8.0 ± 0.71[f]
	200	5/9[e]	7.5 ± 0.65[f]
	400	5/9[e]	6.0 ± 1.08[e]
	800	7/9[f]	9.5 ± 0.50[f]

[a] Mice were infected with 100 and 32 LD_{50} of HSV-1 in s.c. and p.o. treatment experiments, respectively. Ara-T was administered twice daily for 4.5 days as in i.p. treatment experiments.
[b] Numbers represent milligrams per kilogram administered twice daily for 4.5 days.
[c] Standard error.
[d] $P < 0.05$.
[e] $P < 0.01$.
[f] $P < 0.001$.

TABLE 7. *Effects of i.p. and p.o. treatments with ara-T on HSV-1-induced encephalitis in mice*[a]

Administration route	Ara-T[b]	Survivors/total	Mean survival time (days)
i.p.	0	0/10	4.7 ± 0.37[c]
	27	0/10	5.7 ± 0.45
	40	0/10	5.8 ± 0.33[d]
	60	2/10	6.8 ± 0.56[e]
	90	2/10	6.8 ± 0.75[d]
p.o.	0	0/9	4.3 ± 0.26
	27	2/9	6.6 ± 0.74[e]
	40	2/9	7.6 ± 0.57[f]
	60	1/9	6.8 ± 0.48[f]
	90	3/9	7.4 ± 0.24[f]

[a] Mice were infected with 10 LD_{50} of HSV-1 and injected i.p. or p.o. with ara-T.
[b] Numbers represent milligrams per kilogram administered twice daily for 4.5 days.
[c] Standard error.
[d] $P < 0.05$.
[e] $P < 0.01$.
[f] $P < 0.001$.

TABLE 8. *Effect of single doses of ara-T on HSV-1-induced encephalitis in mice*[a]

Administration route	Ara-T (mg/kg)	Survivors/total	Mean survival time (days)
i.p.	0	0/20	5.1 ± 0.23[b]
	200	0/10	5.9 ± 0.44
	400	0/10	5.7 ± 0.33
	800	0/10	6.3 ± 0.50[c]
	1,600	3/10[c]	7.9 ± 0.86[e]
	3,200	8/10[e]	7.5 ± 0.50[d]
p.o.	0	0/20	4.5 ± 0.22
	800	0/10	7.5 ± 0.52[e]
	1,600	2/10	6.7 ± 0.16[e]

[a] Mice were infected with 10 LD_{50} of HSV-1 and injected i.p. or p.o. with ara-T at 12 h postinfection.
[b] Standard error.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.001$.

TABLE 9. *Tolerated doses of ara-T and ara-C in mice*[a]

Drug	Administration route	Dose[b]	Survivor rate (%)
Ara-T	i.p.	1	100
	i.p.	2	40
	i.p.	4	0
	p.o.	1.5	100
	p.o.	3	100
	p.o.	6	80
Ara-C	i.p.	0.05	100
	i.p.	0.1	80

[a] Groups of five male mice, 4 weeks old, were treated with drugs as in the therapeutic experiments and observed for 3 weeks for death.
[b] Numbers represent grams per kilogram administered twice daily for 4.5 days.

tered i.p. or p.o. to normal mice twice daily for 4.5 days. All recipients of p.o. doses as high as 3 g/kg (total course dose, 27 g/kg) and of i.p. doses as high as 1 g/kg (total course dose, 9 g/kg) survived. In the same dosage schedule, 100 mg of ara-C per kg administered i.p. was lethal to 20% of the mice. The therapeutic indexes (maximal tolerated dose divided by minimal effective dose) in i.p. treatments and in p.o. treatments were estimated to be more than 25 and 100, respectively.

The evaluation of the single-dose toxicity showed that ara-T had an LD_{50} of more than 10 g/kg, regardless of the route of administration or of the sex of the mice used, except when administered s.c. to male mice (Table 10). Treatment either i.p. or s.c. with doses of 7.5 or 10 g/kg produced at most a 10 to 15% loss of weight in both male and female mice, except when

TABLE 10. *Acute toxicity of ara-T in mice*

Administration route	Sex	LD_{50} (g/kg)	Mortality (%) at a dose of 10 g/kg
i.p.	Male	>10	20
	Female	>10	20
s.c.	Male	7.5	70
	Female	>10	0
p.o.	Male	>15	0
	Female	>15	0
i.v.[a]	Male	>0.2	
	Female	>0.2	

[a] Maximal injection dose in the intravenous route (i.v.) was 0.2 g/kg as a result of the relatively low solubility of ara-T.

administered s.c. to male mice (about 25% loss of weight). At these doses, thymic atrophy was noted in male mice but not in females. The weights of other organs were not affected by treatment. There were no other significant illnesses or pathological abnormalities in mice treated with ara-T at any doses employed, regardless of the injection route. In contrast, mortalities in male mice injected i.p. with 1.5 and 3 g of IUdR per kg were 20 and 100%, respectively (data not shown).

DISCUSSION

This report has shown that both systemic and p.o. treatments with ara-T protected mice against otherwise fatal HSV-induced encephalitis resulting from challenge with from 3.2 to 3,2000 LD_{50} of virus. Thus, the activity of ara-T against herpesvirus encountered in vitro in earlier studies (13) has been reproduced in vivo. Aswell and Gentry (3) suggested that the effectiveness of ara-T would depend upon the frequency of dosage because of rapid urinary excretion. However, in this study, even a single dose of a rather large amount of ara-T was effective. Because ara-T has been reported to be effective in inhibiting the replication of varicella-zoster virus in HEL-F cells (14), it should also have activity against varicella-zoster virus infections in vivo. The activity of ara-T via the p.o. route (Tables 6 and 7) should be advantageous in clinical applications.

As Sidwell et al. (26) showed earlier, ara-A and ara-AMP administered either i.c. or i.p. were also effective against HSV-induced encephalitis in mice. However, the LD_{50} of ara-A or of ara-AMP was much lower (11) than that of ara-T presented here (Table 10). IUdR and ara-C were not effective when the drugs were administered i.p. These findings agree with those reported by

Lefkowitz et al. (12). Although ara-C was effective in target-organ treatment, agreeing with the result reported by Allen and Sidwell (1), i.c. administration was not practical for clinical use. It is of special interest that ara-T was more effective against encephalitis and less toxic when administered p.o. than when administered i.p. It is noteworthy that p.o. treatment with acycloguanosine was also highly active against encephalitis in mice inoculated i.c. with HSV and that toxicity was correspondingly low (24). However, the LD_{50} of acycloguanosine for mice by the i.p. route was reported to be 1,000 mg/kg (24), much lower than that of ara-T.

To establish firmly the safety and efficacy of ara-T, further experiments in animal models other than HSV-induced encephalitis in mice and more elaborate toxicological studies are needed.

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