ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1975, p. 468-473

Vol. 8, No. 4
Printed in U.S.A.

Inhibition of Experimental Deoxyribonucleic Acid Virus-Induced Encephalitis by 9-β-D-Arabinofuranosylhypoxanthine 5'-Monophosphate

LOIS B. ALLEN,* JODIA M. THOMPSON, JOHN H. HUFFMAN, GANAPATHI R. REVANKAR, RICHARD L. TOLMAN, LIONEL N. SIMON, ROLAND K. ROBINS, AND ROBERT W. SIDWELL

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92664

Received for publication 27 June 1975

9-β-D-Arabinofuranosylhypoxanthine 5'-monophosphate (ara-HxMP) significantly controlled the development of encephalitis produced by deoxyribonucleic acid viruses in mice. In most experiments the activities of ara-HxMP and 9-β-D-arabinofuranosyladenine (ara-A) were determined simultaneously. In the intracerebral (target organ) and intravenous therapy experiments, ara-HxMP had a pronounced advantage over ara-A since the water solubility of ara-HxMP enabled it to be used in much higher concentrations. In experiments where the two drugs were administered intraperitoneally or orally they exhibited similar activity. In several intraperitoneal therapy experiments ara-HxMP was tested alone, using various treatment schedules and dosages. In these experiments, efficacy was observed in groups that had treatments initiated as late as 72 h after virus inoculation.

Drugs that may have potential for treating deoxyribonucleic acid (DNA) virus-induced central nervous system diseases in man include idoxuridine, cytosine arabinoside and adenine arabinoside (ara-A). Both idoxuridine and cytosine arabinoside have failed to significantly influence encephalitis in humans (5, 8, 10, 13, 15), and trials with ara-A are not completed. In experimental animal models, ara-A has demonstrated the greatest potential for treatment of herpesvirus-induced encephalitis (16-18, 20, 21), although the relative insolubility of this nucleoside has hampered its intravenous (i.v.) administration.

Among the compounds we have studied that may offer advantages over the presently known anti-DNA virus drugs, 9-β-D-arabinofuranosylhypoxanthine 5'-monophosphate (ara-HxMP; ICN 3952) has reproducible activity against DNA viruses (L. B. Allen, J. H. Huffman, R. L. Tolman, G. R. Revankar, L. N. Simon, R. K. Robins, and R. W. Sidwell, Abstr. 232, J. H. Huffman, L. B. Allen, R. L. Tolman, G. R. Revankar, L. N. Simon, R. K. Robins, and R. W. Sidwell, Abstr. 233, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., 1974), is minimally toxic to cultured cells and laboratory animals, and is water soluble. In this report, we describe experiments on the efficacy of this purine nucleotide on DNA virus-induced encephalitis in laboratory animals.

MATERIALS AND METHODS

Compounds. Ara-A was obtained from ICN Life Sciences, Cleveland, Ohio, and ara-HxMP was synthesized at this Institute by the method of Revankar et al. (14).

Viruses. Type 1 herpes simplex virus (HSV/1), strain 123, and vaccinia virus (VV), strain WR, were obtained from F. M. Schabel, Jr., Southern Research Institute, Birmingham, Ala. Type 2 HSV (HSV/2), strain MS, was purchased from the American Type Culture Collection, Rockville, Md.

Animals. Male Swiss Webster mice were purchased from Hilltop Lab Animals, Chatsworth, Calif., for use in encephalits experiments.

Mouse encephalitis experiments. Mice weighing approximately 20 g were infected intracerebrally (i.c.) with between 2 and 10 50% lethal doses of virus and were treated by one of four routes: i.c., i.v., intraperitoneal (i.p.), or per os (p.o.). In the i.c. (target organ) studies, each drug in saline (0.03 ml) was injected 6 h after virus inoculation (2). In these studies the maximum tolerated dose (MTD) or maximum obtainable dose (MOD), or a fraction thereof, was tested. The insolubility of ara-A necessitated the use of a saturated solution (2), which resulted in an MOD of ~2.5 mg/kg. Virus control mice received an i.c. saline treatment.

In the i.v. treatment experiments the drugs were administered 3 h after virus inoculation and were dissolved or suspended in saline or carboxymethylcellulose.

The i.p. and p.o. experiments utilized multiple treatment schedules for both drugs.

Evaluation of antiviral activity. The antiviral effect of the drugs was determined by comparing the

number of survivors and the mean day of death of the drug-treated and placebo-treated animals.

RESULTS

Initial experiments involved the target organ system. In a series of experiments, ara-HxMP (MTD 40 mg/kg) and ara-A (MOD 2.5 mg/kg) were tested in parallel to determine their relative effectiveness against HSV/1, HSV/2, and VV. Both drugs had comparable activity against each virus (Table 1).

To determine the efficacy of the drugs using i.p. therapy in an HSV/1 infection, ara-HxMP and ara-A were administered twice daily for 8.5 days, beginning 4 h after i.c. virus inoculation. both compounds were quite effective (Table 2), with prolongation of life being seen at the lowest doses administered.

Klein et al. (7) speculated that the ara-A therapeutic effect was more dependent on total dose of drug than on treatment route or schedule. They and Sloan et al. (20) found that a total dose of ~3,000 mg/kg produced significant antiviral activity. We therefore evaluated ara-HxMP in total doses of ≥3,000 mg/kg, using the i.p. route while varying the treatment schedule. In the first experiment of the series (Table 3), ara-HxMP (250 mg/kg) was given twice daily for 9 days, beginning at various times relative to i.c. virus inoculation. Treatments starting 2 days pre-virus to 1 day post-virus were effective in increasing the percentage of survivors to a significant degree. Increases in mean day of death were seen when treatment was initiated as late as 3 days after virus inoculation.

In another study, 1,000 mg of ara-HxMP per kg was administered i.p. on 3 consecutive days beginning at different times after virus inoculation. Using this schedule, significant increases in survivor numbers occurred when treatment was begun as late as 2 days post-virus (Table 4). In the next two experiments, a total of 3,000 mg of the drug per kg was given on a single day either as three separate 1,000-mg/kg injections or in a single 3,000-mg/kg injection. In the experiment where the drug was given in three divided injections (Table 5), a significant survivor number increase was seen only when treatment was on day 0, but significant increases in mean day of death were seen as late as 2 days after virus inoculation. When the drug was given as a single injection (Table 6), significant increases in survivor numbers were seen on days 0 and 1, whereas a significant increase in mean day of death was seen on day 2 after virus inoculation.

Before use of the drugs by i.v. therapy, the MTD for ara-HxMP by the i.v. route was

TABLE 1. *Effect of target organ treatment with ara-HxMP and ara-A on DNA virus encephalitis in mice*

Drug	Drug dosage (MTD or MOD)[a]	Infected, treated survivors/total	Survivor increase (P)[b]	Infected, treated mean day of death[c]	Mean day of death increase (P)[d]
HSV/1					
Saline		3/20		7	
Ara-HxMP	1	5/9	<0.003	8	>0.05
	1/4	5/10	<0.049	7.4	>0.05
	1/16	3/10	>0.1	8.6	>0.05
Ara-A	1	5/10	<0.049	7.8	>0.05
	1/4	3/10	>0.1	8.6	<0.01
HSV/2					
Saline		1/20		6.2	
Ara-HxMP	1	3/10	>0.05	8.8	<0.001
	1/4	1/10	>0.1	9.1	<0.001
	1/16	0/10		9.5	<0.001
Ara-A	1	2/10	>0.1	8.4	<0.001
	1/4	0/10		8.0	<0.001
	1/16	0/10		7.0	>0.05
VV					
Saline		0/21		6.1	
Ara-HxMP	1	3/10	<0.026	10.1	<0.001
	1/16	1/10	>0.1	8.0	<0.02
Ara-A	1	2/10	>0.05	7.4	<0.01
	1/4	1/10	>0.1	8.3	<0.001

[a] Ara-HxMP MTD, 40 mg/kg; ara-A MOD, 2.5 mg/kg.
[b] Probability (Fisher's exact test).
[c] Animals dying on or before day 21.
[d] Probability (Student's t test).

TABLE 2. *Effect of i.p. treatment[a] with ara-HxMP and ara-A on HSV/1-induced encephalitis in mice*

Drug	Drug dosage (mg/kg)	Toxicity control survivors/ total	Infected, treated survivors/total	Survivor increase (*P*)[b]	Infected, treated mean day of death[c]	Mean day of death increase (*P*)[d]
Saline	10 ml/kg		0/20		5.7	
Ara-HxMP	250	5/5	5/10	<0.002	7.6	<0.05
	125		4/10	<0.008	7.7	<0.02
	62.5		2/10	>0.1	8.6	<0.001
	31.3		0/9		7.1	<0.01
	15.7		0/10		7.1	<0.05
Ara-A	125	5/5	4/10	<0.008	9.0	<0.001
	62.5		3/10	<0.030	6.4	>0.05
	31.3		0/10		6.9	<0.05
	15.7		1/10	>0.1	7.6	<0.01

[a] Twice daily for 8.5 days (17 treatments), starting 4 h after virus inoculation.
[b-d] See Table 1.

TABLE 3. *Effect of time of initiation of i.p. treatments[a] with 250 mg of ara-HxMP per kg on HSV/1-induced encephalitis in mice*

Time of treatment relative to virus inoculation (days)	Infected, treated survivors/total	Survivor increase (*P*)[b]	Infected, treated mean day of death[c]	Mean day of death increase (*P*)[d]
Virus controls[e]	3/30		7.1	
−2–6	7/10	<0.001	10.7	—[f]
−1–7	7/10	<0.001	7.3	—
0–8	7/10	<0.001	10.0	—
1–9	6/10	<0.003	9.5	<0.02
2–10	1/10	>0.1	8.1	>0.05
3–11	2/10	>0.1	10.1	<0.001

[a] Total of 18 treatments (4,500 mg/kg, total dose).
[b-d] See Table 1.
[e] Ten animals were treated with saline on days −2 to 6; 10 were treated on days 1 through 9, and 10 were treated on days 3 through 11. Since no statistical differences were observed between the groups, data from all were combined.
[f] Too few animals for statistical analysis.

TABLE 4. *Effect of initiation time of once daily i.p. treatments[a] with ara-HxMP on HSV/1-induced encephalitis in mice*

Time of treatment relative to virus inoculation (days)	Infected, treated survivors/total	Survivor increase (*P*)[b]	Infected, treated mean day of death[c]	Mean day of death increase (*P*)[d]
Virus controls[e]	4/40		7.6	
1, 2, 3	6/10	<0.002	8.3	>0.05
2, 3, 4	4/10	<0.036	7.2	
3, 4, 5	2/10	>0.1	8.9	>0.05
4, 5, 6	0/10	>0.1	8.3	>0.05

[a] Three treatments totaling 3000 mg/kg.
[b-d] See Table 1.
[e] Four groups of 10 animals each were treated with saline, one group for each period of drug therapy. No statistical differences were observed between groups; therefore data from all groups were combined.

established at ~2,000 mg/kg; however, it was necessary to do the injection slowly (~60 s for total amount). As a result of the particulate nature of ara-A, doses greater than 100 mg/kg could not be given because of death, possibly from mechanical heart blockage. Ara-HxMP appeared to be more effective overall (Table 7) since its greater water solubility enabled it to be given at much higher doses than ara-A.

The effect of p.o. administration of ara-

TABLE 5. *Effect of initiation time of one-day i.p. treatments[a] with ara-HxMP on HSV/1-induced encephalitis in mice*

Time of treatment relative to virus inoculation (h)	Infected, treated survivors/total	Survivor increase (P)[b]	Infected, treated mean day of death[c]	Mean day of death increase (P)[d]
Virus controls[e]	5/30		7.3	
−4, 0, 4	5/10	<0.043	7.8	>0.05
24, 28, 32	2/10	>0.1	9.8	<0.02
48, 52, 56	4/10	>0.1	9.2	<0.05

[a] Three treatments totaling 3,000 mg/kg.
[b-d] See Table 1.
[e] Groups of 10 mice served as virus controls by receiving saline on day 1, 2, or 3. Since there was no statistical difference between the groups, the data from all were combined.

TABLE 6. *Effect of time of single i.p. treatments[a] with ara-HxMP on HSV/1-induced encephalitis in mice*

Time of treatment relative to virus inoculation (h)	Infected, treated survivors/total	Survivor increase (P)[b]	Infected, treated mean day of death[c]	Mean day of death increase (P)[d]
Virus controls[e]	4/30		7.4	
−2	6/10	<0.007	7.8	>0.05
24	5/10	<0.026	9.2	>0.05
48	4/10	>0.1	9.8	<0.05

[a] 3000 mg/kg.
[b-d] See Table 1.
[e] Three groups of 10 mice served as virus controls by receiving saline on day 1, 2, or 3. Since there was no statistical difference between the groups, the data from all were combined.

TABLE 7. *Effect of i.v. treatment with ara-HxMP and ara-A on HSV/1-induced encephalitis in mice*

Drug	Drug dosage (mg/kg)	Infected, treated survivors/total	Survivor increase (P)[a]	Infected, treated mean day of death[b]	Mean day of death increase (P)[c]
Saline	10 ml/kg	0/20		6.3	
Ara-HxMP	2,000	4/10	<0.008	8.7	<0.02
	1,000	3/10	<0.03	7.1	>0.05
	100	1/10	>0.1	8.1	<0.05
Ara-A	100	1/10	>0.1	8.0	<0.02
	50	2/10	>0.1	6.6	>0.05

[a] Probability (Fisher's exact test).
[b] Animals dying on or before day 21.
[c] Probability (*t* test).

HxMP and ara-A was determined against both HSV/1 and HSV/2, with each drug given twice daily for 8.5 days, starting 4 h after virus inoculation. Results of these experiments (Table 8) indicated that both compounds had comparable activity.

DISCUSSION

This report has presented evidence that a new, water-soluble, purine nucleotide, ara-HxMP, is capable of modifying the course of DNA virus-induced encephalitis in mice when given directly in the brain or systemically. The activity when given systemically (i.v., i.p., p.o.) suggests that ara-HxMP or some active metabolic product may be able to cross the blood-brain barrier.

Our initial experiments utilizing target organ therapy of HSV/1- or HSV/2-induced encephalitis indicate that the system is predictive of subsequent activity seen when the drugs are given peripherally, as noted previously (18).

The initial i.p. therapy experiment (Table 2) utilized a treatment schedule found satisfactory for ara-A and its derivatives (17, 20). This experiment demonstrated that ara-HxMP had antiviral activity similar to that of ara-A. Mul-

TABLE 8. *Effect of p.o. treatment[a] with ara-HxMP or ara-A on HSV-induced encephalitis in mice*

Drug	Drug dosage (mg/kg)	Toxicity control survivors/ total	Infected, treated survivors/ total	Survivor increase (*P*)[b]	Infected, treated mean day of death[c]	Mean day of death increase (*P*)[d]
HSV/1						
Saline	10 ml/kg		0/20		5.9	
Ara-HxMP	250	5/5	3/10	<0.03	8.1	<0.02
	125		4/10	<0.008	7.8	<0.01
	62.5		1/10	>0.1	7.4	<0.01
Ara-A	250	5/5	7/10	<0.001	8.0	<0.02
	125		4/10	<0.005	8.6	<0.001
	62.5		1/10	>0.1	7.3	<0.05
HSV/2						
Saline	10 ml/kg		0/20		6.3	
Ara-HxMP	250	5/5	8/10	<0.001	11.3	—[e]
	125		3/7	<0.012	11.8	<0.001
Ara-A	250	5/5	2/10	>0.1	11.8	<0.001
	125		4/10	<0.008	12.7	<0.001

[a] Twice daily for 8.5 days (17 treatments), starting 4 h after virus inoculation.
[b-d] See Table 1.
[e] Too few animals for statistical analysis.

tiple doses of both compounds as low as 15.7 mg/kg twice daily, totaling 266.9 mg/kg, were sufficient to prolong the survival of the mice. Significant survivor numbers were seen with doses as low as 125 and 62.5 mg/kg for ara-HxMP and ara-A, respectively. These would correspond to total doses of 2,125 mg/kg for ara-HxMP and 1,062.5 mg/kg for ara-A, which is lower than that (~3,000 mg/kg) seen by Sloan et al. for ara-A. This range of effectiveness may result from a lower dose of challenge virus used in the present study than that used by Sloan et al. (21).

Using a dose (250 mg/kg per injection) found effective by Sloan et al. (20) for ara-A, we administered multiple twice daily treatments of ara-HxMP, beginning at various times with respect to virus inoculation. It appears from these studies that the drug was less effective when administered late in the infection, despite the large total dose administered (4,500 mg/kg). Other experiments in which a total dose of 3,000 mg of ara-HxMP per kg was administered in three equally divided doses or a single dose also reveal that treatments initiated later than 24 to 48 h after virus inoculation were less effective than those administered earlier. Thus, with ara-HxMP the time of treatment initiation seems more important than the total drug dose. This differs from the findings of Klein et al. (7), who found that the ara-A therapeutic effect was less dependent on the treatment time than on the total dose of drug. In our studies the virus was inoculated directly into the brain, whereas Klein et al. (7) inoculated the virus onto the skin, although the virus eventually reached the brain after replication in the skin. We have previously found high concentrations of virus in the brain by 2 days after i.c. inoculation, with the maximum or peak concentration occurring by day 4 (2). In the work of Klein et al. (7), the virus control animals survived 3 to 4 days longer than animals in the present study, which may indicate that the virus reaches the brain 3 to 4 days after skin inoculation. In either of these systems, effective drug therapy probably results only from treatment initiated before the time that a critical concentration of virus is attained in the brain.

The i.v. treatment data again indicate a versatility of ara-HxMP, due to its water solubility, that cannot be achieved with ara-A, that is, delivery of a large dose of the compound at a single time or during a short period of time. This would permit patient treatment with desired concentrations of drug reached earlier in the infection, as well as greatly reduce the volume of infused vehicle used to attain effective concentrations of drug. In the case of ara-A the patient must be infused with large volumes of drug vehicle over long periods of time in order to deliver a desired amount of the drug.

Of the treatment routes tested in mice, the i.v. and p.o. routes are most appropriate for human administration of the drug.

Although the magnitude of DNA viruses in central nervous system disease has not been defined (10), the following information illustrates the extent to which HSV may be involved. Recent reviews (5, 6) have identified

HSV as a common cause of sporadic fatal encephalitis and acute necrotizing encephalitis (1). HSV infections of the brain can also be presented as temporal lobe syndrome (4) or as an intracranial mass lesion (13). Both types 1 and 2 HSV have been implicated as causative agents in aseptic meningitis (12, 19, 20). Also, the suggestion has been made that HSV is responsible for the chronic and paroxysmal headache syndromes (3) and Bell's palsy (9). Consideration of these various diseases reveals that drugs for treatment are greatly needed. The data presented in this paper suggest that ara-HxMP has potential for treatment of such DNA virus-induced central nervous system infections.

ACKNOWLEDGMENTS

Appreciation is expressed to Anita H. Beck, Carol J. Hintz, Ana M. Shuman, Lonna L. Smith, and Paulette G. Suddarth for expert technical assistance.

LITERATURE CITED

1. Adam, J. H., and W. B. Jenett. 1967. Acute necrotizing encephalitis: a problem in diagnosis. J. Neurol. Neurosurg. Psychiat. **30**:248-260.
2. Allen, L. B., and R. W. Sidwell. 1972. Target-organ treatment of neurotropic virus diseases: efficacy as a chemotherapy tool and comparison of activity of adenine arabinoside, cytosine arabinoside, idoxuridine, and trifluorothymidine. Antimicrob. Agents Chemother. **2**:229-233.
3. Coddon, D. R. 1974. A new viral hematologic etiology hypothesis for chronic and paroxysmol headache syndromes. Mt. Sinai J. Med. N.Y. **41**:64-65.
4. Drachman, D. A., and R. D. Adams. 1962. Herpes simplex and acute inclusion-body encephalitis. Arch. Neurol. **7**:45-63.
5. Gurwith, M. J., C. E. Harman, and T. C. Merigan. 1971. Approach to diagnosis and treatment of herpes simplex encephalitis. Calif. Med. **115**:63-71.
6. Hanshaw, J. B. 1970. Idoxuridine in herpes simplex virus encephalitis. N. Engl. J. Med. **282**:47.
7. Klein, R. J., A. E. Friedman-Kien, and E. Brady. 1974. Herpes simplex virus skin infections in hairless mice: treatment with antiviral compounds. Antimicrob. Agents Chemother. **5**:318-322.
8. Luby, J. P., M. T. Johnson, and S. R. Jones. 1974. Antiviral chemotherapy. Am. Rev. Med. **25**:251-267.
9. McCormic, D. P. 1972. Herpes simplex virus as a cause of Bell's palsy. Lancet **1**:937-939.
10. Miller, J. R., and D. H. Harter. 1972. Acute viral encephalitis. Med. Clin. N. Am. **56**:1393-1404.
11. Olsen, L. C., E. L. Buescher, M. S. Atenstein, and P. P. Parkman. 1967. Herpes virus infections of the human central nervous system. N. Engl. J. Med. **277**:1271-1277.
12. Pierce, N. F., B. Portnoy, N. E. Leeds, R. L. Morrison, and P. F. Wehrle. 1964. Encephalitis associated with herpes simplex infection presenting as a temporal-lobe mass. Neurology **14**:708-713.
13. Rappel, M. 1973. The management of acute necrotizing encephalitis: a review of 369 cases. Postgrad. Med. J. **49**:419-427.
14. Revankar, G. R., J. H. Huffman, L. B. Allen, R. W. Sidwell, R. K. Robins, and R. L. Tolman. 1975. Synthesis and antiviral activity of certain 5'-monophosphates of 9-D-arabinofuranosyladenine and 9-D-arabinofuranosylhypoxanthine. J. Med. Chem. **18**:721-726.
15. Rosenthal, M. S. 1974. Viral infections of the central nervous system. Med. Clin. N. Am. **58**:593-603.
16. Schabel, F. M., Jr. 1968. The antiviral activity of 9-β-D-arabinofuranosyladenine (ara-A). Chemotherapy **13**:321-338.
17. Sidwell, R. W., L. B. Allen, J. H. Huffman, T. A. Khwaja, R. L. Tolman, and R. K. Robins. 1973. Anti-DNA virus activity of the 5'-nucleotide and 3',5'-cyclic nucleotide of 9-β-D-arabinofuranosyladenine. Chemotherapy **19**:325-340.
18. Sidwell, R. W., G. J. Dixon, F. M. Schabel, Jr., and D. H. Kaump. 1969. Antiviral activity of 9-β-D-arabinofuranosyladenine. II. Activity against herpes simplex keratitis in hamsters, p. 148-152. Antimicrob. Agents Chemother. 1968.
19. Skoldenberg, B. 1972. On the role of viruses in acute diseases of the central nervous system: clinical and laboratory studies in hospitalized patients. Scand. J. Infect. Dis. **3**(Suppl):1-96.
20. Sloan, B. J., F. A. Miller, J. Erhlich, I. W. McLean, Jr., and H. E. Machamer. 1969. Antiviral activity of 9-β-D-arabinofuranosyladenine. IV. Activity against intracerebral herpes simplex virus infections in mice, p. 161-171. Antimicrob. Agents Chemother. 1968.
21. Sloan, B. J., F. A. Miller, and I. W. McLean, Jr. 1973. Treatment of herpes simplex virus types 1 and 2 encephalitis in mice with 9-β-D-arabinofuranosyladenine. Antimicrob. Agents Chemother. **3**:74-80.
22. Stadler, H., M. N. Oxman, D. M. Dawson, and M. J. Levin. 1973. Herpes simplex meningitis: isolation of herpes simplex virus type 2 from cerebrospinal fluid. N. Engl. J. Med. **289**:1296-1298.