

Efficacy of 9- β -D-Arabinofuranosylhypoxanthine 5'-Monophosphate in Therapy of Equine Abortion Virus-Induced Hepatitis in Hamsters¹

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Equine abortion virus (EAV)-induced hepatitis in hamsters presents an interesting animal model for the evaluation of drugs possessing anti-deoxyribonucleic acid virus activity. These experiments demonstrate that 9- β -D-arabinofuranosylhypoxanthine 5'-monophosphate (ara-HxMP), a new synthetic, water-soluble, antiviral agent, effectively controls this disease in hamsters with a therapeutic index of ~60. Ara-HxMP prevented hepatitis-associated deaths in hamsters, reduced the titer of EAV developing in hamsters, and inhibited the increase of serum glutamic pyruvic transaminase in EAV-infected hamsters.

Human hepatitis continues to present a major challenge to chemotherapy (2), with supportive therapy as the present method of treatment. Recent findings indicate that the agent causing human type B hepatitis is probably a deoxyribonucleic acid (DNA) virus (6). It would seem reasonable, therefore, that the quest for chemotherapeutic agents suitable for treating type B hepatitis should be for drugs that have anti-DNA virus activity. An animal system that provides a reasonable model for DNA virus-induced hepatitis was first described by Doll et al. (1) in 1956 and was further developed by Randall and Bracken in 1957 (4). In this system, hamsters infected parenterally by equine abortion virus (EAV), a member of the herpesvirus group, develop a rapidly progressing, fatal hepatitis with death occurring 3 to 6 days after virus inoculation. Lieberman et al. (3) have recently used this hamster model to evaluate the effectiveness of several anti-DNA agents and interferon inducers. In their study, 9- β -D-arabinofuranosyladenine (ara-A) was highly effective. In previous reports we have described 9- β -D-arabinofuranosylhypoxanthine 5'-monophosphate (ara-HxMP) (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), a new, water-soluble nucleotide chemically related to ara-A,

which has significant efficacy against viruses of the herpesvirus and poxvirus groups. The present report describes studies with ara-HxMP and ara-A on EAV-induced hepatitis in hamsters.

MATERIALS AND METHODS

Virus. The EAV used in this study was obtained from P. E. Came, Schering Corp., Bloomfield, N.J. The virus used in these experiments was prepared as the second hamster liver passage in our laboratory.

Hamsters. Female golden Syrian hamsters weighing 40 to 50 g were obtained from Lakeview Hamster Colony, Newfield, N.J.

Drugs. Ara-HxMP was synthesized at this institute by the method of Revankar et al. (5). Ara-A was obtained from ICN Life Sciences, Cleveland, Ohio.

Experimental method. Hamsters weighing 40 to 50 g were inoculated intraperitoneally (i.p.) with a 90% lethal dose of virus and observed for 21 days. Animals were treated i.p. twice daily for 5 days, beginning 1 h before virus inoculation. At various intervals, samples of blood and liver were obtained and pools from three animals were stored for later assay.

Therapeutic efficacy was determined by comparing the survivor numbers and mean day of death of drug-treated groups with those of saline-treated virus control groups. Survivor increase significance was evaluated by the *t* test. In one experiment, animals surviving on day 21 were reinoculated i.p. with three times the original virus challenge dose in order to determine whether resistance to infection had developed. In all experiments, the hamsters were caged individually to prevent possible reinfection resulting from cannibalism during the experiment.

SGPT assay. This serum enzyme was measured using the serum glutamic pyruvic transaminase (SGPT) kit commercially available from Sigma Chemical Co., St. Louis, Mo. (7). This kit provides the substrate alanine and the amine 2-ketoglutaric

¹ Drug number ICN 3952.

acid. In the reaction GPT deaminates alanine to pyruvic acid, which is detected by a Sigma color reagent. The pyruvate reacts with the color reagent to produce a hydrazone that is highly colored and easily measured colorimetrically.

Titration of EAV from liver. Twenty percent suspensions were prepared by homogenizing the livers in minimum essential medium, 0.25% NaHCO₃, and 5% fetal bovine serum. The homogenates were clarified by centrifugation at 2,800 rpm at 4 C for 20 min. Aliquots of the resulting supernatants were stored in sterile plastic tubes at -70 C until they were titered in hamsters by i.p. injection of serial dilutions of the supernatants. Four hamsters were used for each virus dilution, with deaths occurring up to day 21 considered to indicate infectious virus.

RESULTS

In two initial experiments of this series, ara-HxMP and ara-A were compared on the basis of milligrams per kilogram of body weight for their respective effects on the EAV infection of hamsters (Table 1). Ara-HxMP was protective and nontoxic at doses ranging from 125 to 31.3 mg/kg. A later toxicity experiment revealed that hamsters could also tolerate a dose of at least 500 mg of ara-HxMP per kg and up to 100 mg of ara-A per kg, using this treatment schedule. Ara-A was quite effective at doses of 62.5 and 31.3 mg/kg but was not tolerated well at 125 mg/kg. It was considered that the best comparison of the two compounds would be on a molar basis. Therefore, in the last experiment of this series (Table 2), equimolar solutions were pre-

pared for treatments, beginning with a solution (10 mM) that would approximate the lowest effective dose previously tested (31.3 mg/kg). The lowest dose of ara-HxMP producing a significant increase in survivor numbers was 2.5 mM (8.7 mg/kg). Using 500 mg/kg as a maximum tolerated dose (MTD) without knowing the true MTD, and using 8.7 mg/kg as the minimum inhibitory concentration, the therapeutic index is approximately 60. With ara-A, the lowest dose producing significant protection was 0.625 mM (1.7 mg/kg). If 100 mg/kg is considered the MTD, the therapeutic index is also approximately 60.

In the last study the animals surviving on day 21 were inoculated a second time with EAV at a dose three times greater than the original (Table 3). For both compounds most of the animals surviving rechallenge were from groups that previously had received the lowest significantly protective dose, 8.7 mg/kg for ara-HxMP and 1.7 mg/kg for ara-A.

In this same experiment both compounds were observed at the highest doses tested (10 mM) for ability to control production of EAV in the liver and for control of changes of SGPT. Both were quite effective in controlling virus development (Fig. 1), with ara-HxMP-infected animals having an early day 1 peak that was 4.5 log₁₀ lower than the peak virus titer (which occurred on day 3) of the virus control group. Ara-A strongly suppressed virus replication, with detectable virus first being observed on

TABLE 1. Effect of i.p. ara-HxMP or ara-A treatment^a on survival of EAV-infected hamsters

Compound	Dosage (mg/kg/day)	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
Expt 1						
Saline	5 ml/kg/injection		2/20		4.1	
Ara-HxMP	125	3/3	10/10	<0.001	>21	
	62.5	3/3	10/10	<0.001	>21	
Ara-A	125	2/3	0/8	>0.05	9.2	<0.001
	62.5	2/2	8/9	<0.001	13.0	— ^e
Expt 2						
Saline	5 ml/kg/injection		1/20		3.6	
Ara-HxMP	125	3/3	10/10	<0.001	>21	
	31.3	ND ^f	9/10	<0.001	8.0	—
Ara-A	125	1/2	3/10	>0.05	12.0	<0.001
	31.3	ND	10/10	<0.001		

^a Twice daily dose × 5, beginning 1 h before virus inoculation.

^b Probability (Fisher exact test).

^c Animals dying on or before day 21.

^d Probability (*t* test).

^e Too few animals for statistical evaluation.

^f ND, Not determined.

TABLE 2. Effect of equimolar doses of ara-HxMP or ara-A applied *i.p.*^a to EAV-infected hamsters

Compound	Dosage		Infected, treated survivors/total	Survivor increase (P) ^b	Infected, treated mean day of death ^c	Mean day of death increase (P) ^d
	Treatment solution (mM)	Approx mg/kg/day				
Saline			1/20		6.4	
Ara-HxMP	10	34.8 ^e	7/10	<0.001	9.0	— ^f
	5	17.4	10/10	<0.001	>21	
	2.5	8.7	8/10	<0.001	7.0	—
	1.25	9.4	3/10	>0.05	6.1	>0.05
	0.625	2.2	1/10	>0.05	6.7	>0.05
Ara-A	10	26.6 ^e	10/10	<0.001	>21	
	5	13.3	10/10	<0.001	>21	
	2.5	6.7	8/10	<0.001	6.5	—
	1.25	3.3	8/10	<0.001	8.5	—
	0.625	1.7	5/10	<0.01	6.6	>0.05

^{a-d} See Table 1.^e Of toxicity control hamsters, five of five survived at this dose.^f Too few animals for statistical analysis.

TABLE 3. Effect of initial infection and treatment with ara-HxMP or ara-A on immunity as evidenced by susceptibility to rechallenge with EAV in hamsters

Compound	Concn of treatment solution (mM)	Initial, treated survivors/total	Rechallenge survivors/total	Survivor increase (P) ^a	Mean day of death	Mean day of death increase (P) ^b
(Normal controls)		Not initially infected	0/7		3.7	
Saline		1/20	0/1		6	
Ara-HxMP	10	7/10	0/7		4.7	<0.02
	5	10/10	1/10	>0.05	4.8	<0.05
	2.5	8/10	4/8	0.052	4.3	>0.05
	1.25	3/10	0/3		4.0	>0.05
	0.625	1/10	1/1	— ^c		
Ara-A	10	10/10	0/10		6.5	<0.05
	5	10/10	0/10		5.1	<0.02
	2.5	8/10	0/8		4.6	>0.05
	1.25	8/10	1/8	>0.05	3.7	
	0.625	5/10	2/5	0.152	3.7	

^a Probability (Fisher's exact test).^b Probability (*t* test).^c Too few animals for statistical evaluation.

day 3. By days 3 and 5 livers from animals treated with both compounds had comparable levels of virus. Samples from virus control animals on day 5 represented a select population since most animals in that group had died; therefore surviving animals may have been recovering or representative of those initially more resistant.

Analysis of the serum samples for SGPT (Fig. 2) indicated that this enzyme reflected the development of virus in the liver, with SGPT curves corresponding to EAV curves. Both ara-HxMP and ara-A at 10 mM markedly inhibited the enzyme increase, with ara-A exhibiting a moderately stronger effect.

DISCUSSION

In these experiments two antiviral compounds of possible clinical promise, ara-HxMP and ara-A, were evaluated for ability to modify EAV-induced hepatitis in hamsters. The data reveal that both compounds were quite effective in controlling the infection. Of the two, ara-A was the more potent, having protective effects at very low doses (1.7 mg/kg), but was considerably more toxic to hamsters when administered *i.p.*; hence the therapeutic indexes were approximately the same. The greater water solubility (≥ 50 -fold) of ara-HxMP over ara-A renders it a more versatile therapeutic agent. Therefore, a

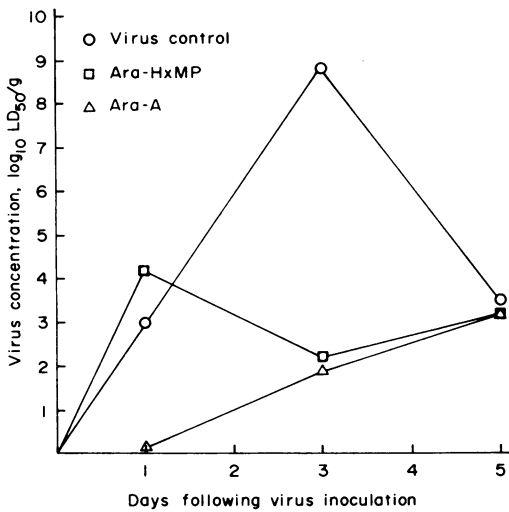


FIG. 1. Effect of *i.p.* ara-HxMP (10 mM) or ara-A (10 mM) treatment on EAV concentration in the liver.

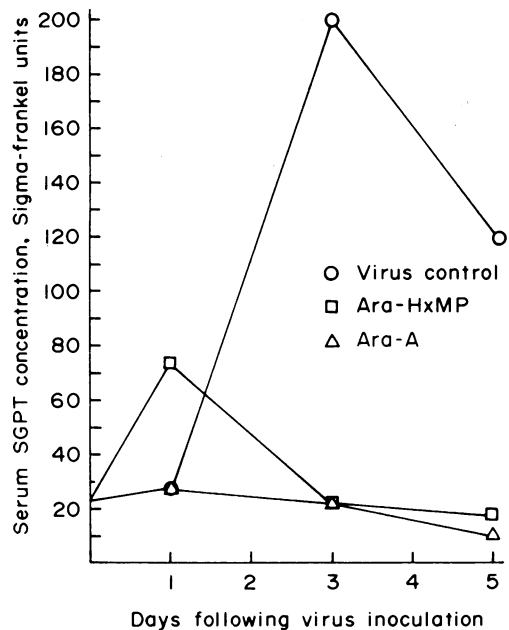


FIG. 2. Effect of *i.p.* ara-HxMP (10 mM) or ara-A (10 mM) treatment on SGPT concentrations of hamsters with EAV-induced hepatitis.

wide range of doses could possibly be administered to human patients by various routes without stressing the fluid balance.

The data obtained on the rechallenge of hamsters surviving the original infection probably illustrate the commonly occurring problem of limited immunity resulting from herpesvirus infections. In animals that had originally been treated with the higher doses of compounds, none survived the second infection, but the animals did exhibit a prolonged survival time, suggesting that a partial immunity had developed. At the lowest dose of each compound that originally produced significant survivor increase, 8.7 and 1.7 mg/kg for ara-HxMP and ara-A, respectively, a greater proportion of animals survived the second infection. It is likely that higher doses of the compounds suppressed virus replication to a greater extent than did lower doses; therefore, at lower doses more virus may have been available for stimulation of resistance in the animals. It is, of course, possible that at the higher doses (34.8 mg/kg for ara-HxMP and 26.6 mg/kg for ara-A) the drugs were immunosuppressive. However, the virus production data (Fig. 1) support the contention that reduced immunity was a result of low production of virus. We have no other information suggesting that ara-A is an immunosuppressant.

It is interesting to note that Sloan et al. (8) found that type 1 herpesvirus-infected mice that survived as a result of ara-A treatment also survived rechallenge with the virus, suggesting development of long-term immunity. This rechallenge was given 60 days after the original

virus inoculation, rather than 21 days as given in the present study with EAV. Of course, the two models differ greatly and comparison is of questionable value. Such data may indicate that the EAV hamster system resembles human infections that repeatedly occur.

In these experiments SGPT appeared to be a satisfactory indicator of liver virus development and probably of liver damage, since its changes corresponded to changes in concentration of virus in the liver. Therefore, as in man, this enzyme level in the serum is a very useful diagnostic tool in the study of EAV-induced hepatitis in hamsters.

Although EAV is not found to naturally infect hamsters, it appears to be a satisfactory model for evaluation of chemotherapeutic agents against DNA viruses causing severe liver disease. The results of the present study would therefore suggest consideration of the use of either ara-HxMP or ara-A for systemic human diseases such as type B hepatitis.

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