Activities of Arabinosyladenine Monophosphate and 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine Against Ground Squirrel Hepatitis Virus In Vivo as Determined by Reduction in Serum Virion-Associated DNA Polymerase

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Treatment of chronic ground squirrel hepatitis virus infection with arabinosyladenine monophosphate at 20 mg/kg per day for 3 weeks caused marked decreases in serum virion-associated DNA polymerase concentrations in three of five squirrels. Statistically significant but less dramatic decreases in enzymatic activity were noted in two of six squirrels treated with 50 mg of 9-(1,3-dihydroxy-2-propoxymethyl)guanine per kg per day. After therapy, DNA polymerase activities rose to pretreatment levels.

An antiviral treatment for chronic carriers of hepatitis B virus is being sought by many investigators. The most promising treatment thus far has involved a combination therapy with human leukocyte interferon and arabinosyladenine (Ara-A) (9-11). This treatment, however, has failed to eradicate infection in most carriers. Until recently, all testing of new drugs and drug regimens has been in humans, who have suffered neurotoxicity as well as other side effects from the various drugs (8). With the discovery of animal virus counterparts of hepatitis B virus that infect woodchucks, ground squirrels, and domestic ducks (4, 6, 14), potential therapeutic compounds can now be tested in nonhuman subjects. A report of one such study has appeared in the literature, in which viremia in chronically infected woodchucks was unaffected by treatment with phosphonoformate (7). In a recent publication, several nucleoside triphosphates were shown to be inhibitors of woodchuck hepatitis virus DNA polymerase (1) and thus might be effective in vivo. In this report we describe the effects of two antiviral drugs, Ara-A monophosphate (Ara-AMP) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), on concentrations of virion-associated DNA polymerase in the sera of chronically infected Beechey polymerase squirrels. Ara-AMP is a water soluble form of Ara-A which has already shown promise in treating hepatitis B virus infection (2, 16). DHPG has not yet been evaluated in patients with liver disease although a related compound, acyclovir, does reduce clinical signs of hepatitis B virus replication (17). DHPG has been found to be highly inhibitory to other DNA viruses (13).

Ara-AMP was obtained from Warner-Lambert Co., and DHPG and DHPG triphosphate were obtained from Syntex (USA) Inc. Ara-A triphosphate was purchased from Sigma Chemical Co. Chronically infected squirrels were trapped locally. The nature of their infection and the assay of serum virion-associated DNA polymerase activity were described previously (3, 4). The polymerase procedure uses virus pelleted by ultracentrifugation, so no residual drug is present

in the assay. We chose to use the DNA polymerase method to assess drug effect since the enzymatic activity is proportional to circulating infectious virus titers. In the animal experiments, chronically infected squirrels served both as drug treatment subjects and as their own placebo control. The DNA polymerase activity of each squirrel was monitored starting 1 week pretherapy. Additionally, five squirrels, four of which were Ara-AMP treated at a separate time, were injected daily with saline to assess any placebo effect on polymerase activity.

Of five animals treated with Ara-AMP, three showed dramatic declines in viral DNA polymerase levels (Table 1). Of six DHPG-treated squirrels, two responded to therapy but to a much lesser degree than those treated with Ara-AMP. Drops in hybridizable viral DNA (5, 12) paralleled reduction in DNA polymerase activities for each compound (data not shown). Both of these parameters correlate to a decrease in viremia. DNA polymerase levels quickly returned to pretreatment levels after the drug was discontinued, an effect which often occurs with Ara-A therapy in humans (15). Animals injected daily with saline over a 3-week period showed a $\pm 25\%$ or less fluctuation in DNA polymerase levels (Table 1), indicating that the effect described above was a true drug effect rather than an effect of stress caused by the injection procedure or blood sampling.

There were six animals that did not have statistically significant reductions in DNA polymerase activities while under treatment, possibly because the administered dose was too low or due to other factors. Higher doses of each compound were not given because they were found to induce rapid weight loss in the animals. In hepatitis B infections, those patients who respond best to Ara-A therapy have low levels of serum DNA polymerase levels (11). Infected ground squirrels, as a rule, have much higher levels of circulating virus with associated DNA polymerase activity (4) as was the case here, so they may be more difficult to treat than humans.

Presumably, the mode of action of each compound involves inhibition of viral DNA synthesis in liver cells by the triphosphate of each species, which would decrease virion

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TABLE 1. Effects of Ara-AMP and DHPG on chronic infection of squirrels by ground squirrel hepatitis virus

Animal	Compound ^a	DNA polymerase activity (cpm $\times 10^{-3}$) on day ^b :							P value ^c
		-1	5	10	15	20	25	30	
1	Ara-AMP	18.9	2.4	1.0	4.4	1.4	14.5	17.0	<0.001
2	Ara-AMP	23.2	3.0	2.2	3.6	4.8	19.7	19.5	<0.002
3	Ara-AMP	18.4	6.8	8.2	7.5	4.3	20.1	6.8	<0.003
4	Ara-AMP	13.8	14.3	13.3	4.7	5.1	17.1	13.5	NS
5	Ara-AMP	1.5	0.9	1.7	1.5	0.8			NS
6	DHPG	32.9	9.2	10.0	7.7	18.8	15.9	19.0	<0.025
7	DHPG	2.8	0.3	1.1	0.9	3.9	4.3	3.9	<0.05
8	DHPG	18.9	7.7	3.4	3.8	14.9	11.9		NS
9	DHPG	31.6	18.1	21.2	12.4	27.3	29.6	36.9	NS
10	DHPG	19.7	10.5	13.2	11.2	18.4	24.0	16.9	NS
11	DHPG	11.4	8.1	12.3	7.7	8.8	15.3	17.8	NS
1	Saline	6.2	6.7	7.5	10.8	7.7	7.7	NT	
2	Saline	9.4	7.6	7.7	7.9	10.1	10.4	NT	
3	Saline	11.5	10.9	12.3	11.7	10.8	11.4	NT	
4	Saline	8.7	9.2	7.4	11.3	12.4	13.3	NT	
12	Saline	1.9	1.9	2.0	1.9	2.7	2.8	NT	

^a Ara-AMP was administered in subcutaneous doses of 20 mg/kg once a day for 21 days; DHPG was administered in subcutaneous doses of 25 mg/kg twice a day for 21 days. Animals 1 through 4 were used for both the Ara-A treatment and saline controls, but at separate times. DNA polymerase counts from these controls and from animal 12 were lower due to a lower specific radiolabel used in the assay.

^b Day relative to initiation of treatment: samples were taken before, during, and after the 21-day treatment period. NT, Not tested.

^c Significance was evaluated by two-tailed Mann-Whitney U-test. NS, Not statistically significant ($P > 0.05$).

production and the presence of virus in serum. Consistent with this hypothesis, we have found Ara-A triphosphate and DHPG triphosphate to inhibit ground squirrel virion-associated DNA polymerase, with K_i values of 3 and 15 μ M, respectively. The weak effect of DHPG observed in vivo compared with that of Ara-AMP may be related to the lower potency of DHPG as a DNA polymerase inhibitor. It should be stated, however, that since the role of the virion DNA polymerase in virus replication has not been firmly established, we cannot conclude at this point that the activities observed in vivo can be correlated to the inhibition of that enzyme.

In these tests, Ara-AMP was judged to be superior to DHPG in its activity against ground squirrel hepatitis virus infection in chronically infected animals. The inhibition of virus concentrations in serum, as indirectly measured by reduction in virion-associated DNA polymerase activity, was only manifested during therapy. Drug effects on other parameters of the disease, such as on viral antigen and antibody levels and on liver histopathology, should be the subject of additional research.

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