Antischistosomal Activity of Hexadecyloxypropyl Cyclic 9-(S)-[3-Hydroxy-2-(Phosphonomethoxy)Propyl]Adenine and Other Alkoxyalkyl Esters of Acyclic Nucleoside Phosphonates Assessed by Schistosome Worm Killing In Vitro

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9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]adenine [(S)-HPMPA] has been reported to have antischistosomal activity. Ether lipid esters of (S)-HPMPA and cidofovir (CDV) have greatly increased activities in antiviral assays and in lethal animal models of poxvirus diseases. To see if ether lipid esters of CDV and (S)-HPMPA enhance antischistosomal activity, we tested their alkoxyalkyl esters using Schistosoma mansoni worm killing in vitro. Hexadecyloxypropyl (HDP)-cyclic-(S)-HPMPA and HDP-cyclic-CDV exhibited significant in vitro antischistosomal activities and may offer promise alone or in combination with praziquantel.

Schistosomiasis is the second most prevalent parasitic disease worldwide after malaria, with about 200 million human beings infected in 74 countries. It is estimated that 779 million people are at risk of contracting schistosomiasis and more than 200 million individuals are infected, with more than half of them suffering from disease-associated symptoms (18, 29, 34). Severe disease manifestations are exhibited in about 20 million individuals (30). The annual mortality rate due to schistosomiasis in sub-Saharan Africa might be as high as 280,000 (31). Chemotherapeutic measures have been the mainstay for control of schistosomiasis (12), and since the 1970s, praziquantel (PZQ) has become the drug of choice against the three major species of schistosomes, Schistosoma mansoni, Schistosoma haematobium, and Schistosoma japonicum (10, 13). PZQ is a relatively safe, orally administered drug that leads to reduction in the prevalence of schistosomiasis (3, 28). Mass drug administration programs currently rely heavily on PZQ for the control of schistosome-induced morbidity. However, with only one drug of choice for treatment and with the possibility of development of parasite resistance, the present situation is dangerous. There is a real and pressing need for discovering alternatives to the only available antischistosomal drug worldwide (5).

Acyclic nucleoside phosphonates are a group of biologically active compounds which have been developed primarily as antivirals (15). The S-enantiomer of 9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] is of particular interest because it has a broad spectrum of antiviral activity (8) as well as in vivo activity against Plasmodium falciparum and Plasmodium berghei (murine models for human malaria) (27). The compound showed a trypanocidal activity against all extracellular trypanosomes and some of the intracellular hemoflagellates (9). We previously reported antischistosomal activity for (S)-HPMPA (4), as it caused significant reductions in vivo in worm loads, tissue egg loads, and the frequency of egg developmental stages. Most prominently, (S)-HPMPA treatment resulted in the nearly complete disappearance of mature and immature eggs (4). In vitro, (S)-HPMPA did not significantly affect the muscle tension of S. mansoni worms regardless of the concentration tested (4). In this report we have evaluated the antischistosomal activity of various alkoxyalkyl esters of (S)-HPMPA, CDV, and other acyclic nucleoside phosphonate compounds to assess their potential antischistosomal activities.

Chemistry. Analogs of (S)-HPMPA, including the 3-(hexadecyloxy)propyl 9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (HDP) and 2-(octadecyloxy)ethyl 9-(S)-[3-hydroxy-2-(phosphono-methoxy)propyl]adenine (ODE) esters as well as the HDP ester of cyclic-(S)-HPMPA were tested in vitro for their potential antischistosomal activity based on adult Schistosoma mansoni worm killing. Also tested were HDP-cyclic CDV, ODE-HPMPG, ODE-HPMP-diaminopurine (ODE-HPMPDP), praziquantel (PZQ), and dimethyl sulfoxide (DMSO) controls. HDP-(S)-HPMPA and ODE-(S)-HPMPA were synthesized as described previously (2). HDP-(S)-HPMPA was cyclized by reaction with N,N-dicyclohexylcarbodiimide and purified by silica gel column chromatography. HDP-cyclic-CDV was synthesized as described by Beadle et al. (1), and the ODE esters of diaminopurine and guanosine were synthesized and purified as described by Valiava et al. (30a). All compounds were greater than 98% pure as determined by 1H nuclear magnetic resonance, 31P nuclear magnetic resonance, elemental combustion analysis, and liquid chromatography/mass spectrometry.
Antischistosomal activities of test compounds based on in vitro schistosome worm killing. Syrian golden hamsters (Mesocricetus auratus) weighing 100 to 120 g were obtained from the Schistosome Biological Supply Center, Theodor Bilharz Research Institute. Schistosoma mansoni cercariae (Egyptian strain CD) were used to infect the hamsters with 350 cercariae each by abdominal skin exposure (21). Praziquantel (Shin Poong Pharmaceutical Co., South Korea) and the respective acyclic nucleoside phosphonate analogs were prepared as 5 mM stock solutions in aqueous DMSO. Immediately before use, the stock solutions were diluted with complete medium to the concentrations indicated.

S. mansoni-infected hamsters were sacrificed and worms harvested from the portomesenteric vessels (11). Twelve to 16 worms were placed in duplicate petri dishes, and fresh RPMI 1640 medium (glutamine, 20% fetal calf serum, and antibiotics [streptomycin, penicillin, and gentamicin]) containing the indicated concentration of test compounds was added. The worms were incubated overnight in a CO₂ incubator, washed thrice with saline, and fresh medium without drug was added and the incubation was continued overnight in the CO₂ incubator. On the second day, worm motility was observed and the medium was again changed and the incubation continued. On day 5 the numbers of living and dead worms were recorded. Negative controls using pure medium alone or medium with DMSO and positive control media containing various concentrations of praziquantel were similarly evaluated. At the end of the observation period worms were examined in a laminar flow hood for their motility and appearance by using a stereomicroscope, and the final recording of percent worm mortality was assessed (the number of dead worms [contracted and opaque] relative to the total number of worms).

S. mansoni killing results. The percentages of Schistosoma mansoni worm killing in vitro under the influences of different test compounds at different concentrations versus untreated and DMSO negative controls and positive controls treated with PZQ were determined (Table 1 and Fig. 1). Controls and DMSO-treated controls had no observed mortality. PZQ was the most effective compound studied, with 100% worm mortality found between 5 and 100 μM drug. (S)-HPMPA was the least active compound, with a 50% effective concentration
(EC \(_{50}\)) of >100 \(\mu\text{M}\). Covalent addition of an HDP ester group increased the antischistosomal activity somewhat to a mortality of 30.8% at 100 \(\mu\text{M}\), the highest concentration tested. The most active compound was the HDP ester of cyclic-(S)-HPMPA, which had an \(EC_{50}\) of 5.0 \(\mu\text{M}\) and achieved 93.3% mortality at 100 \(\mu\text{M}\). The progressive increases in antischistosomal activity caused by successive modifications of (S)-HPMPA by HDP esterification and cyclization can be appreciated best by examination of the left panel of Fig. 2 and Table 2.

Ode esters of (S)-HPMPA, (S)-HPMPG, and (S)-HPMPDAP had moderate activities similar to that of HDP-(S)-HPMPA, with worm mortality of 33% to 47% at the highest concentration tested. HDP-cyclic-CDV showed substantial antischistosomal activity, with an \(EC_{50}\) of 28 \(\mu\text{M}\) and 100% mortality at 100 \(\mu\text{M}\) (Table 2 and Fig. 2, right panel). All acyclic nucleoside phosphonate analogs were less active than PZQ, which had an \(EC_{50}\) of 0.22 \(\mu\text{M}\).

Discovery of new antischistosomal drugs depends on both in vitro whole parasite screens and \textit{S. mansoni}-infected animal models of disease. The in vitro worm killing screen is advantageous because it allows rapid screening of many compounds at several drug concentrations. ODE-(S)-HPMPA and ODE-CDV were previously shown to have greatly increased antiviral activities versus unmodified (S)-HPMPA and CDV (2, 6, 14, 16, 17, 19, 20, 22–26, 32, 35) primarily due to greatly increased cell uptake and conversion to the active metabolite (7). In the \textit{S. mansoni} worm killing assay, HDP-(S)-HPMPA and ODE-(S)-HPMPA were marginally more active than unmodified (S)-HPMPA, but the increase in activity was only two- to fourfold instead of the multiple log_{10} increases in antiviral activity noted against human immunodeficiency virus type 1, vaccinia virus, cowpox virus, and human cytomegalovirus (1, 2, 16, 17, 32).

The order of antischistosomal activity appears to be related to the negative charges on the phosphonate moiety (Table 2). (S)-HPMPA (two negative charges) and HDP-(S)-HPMPA (one negative charge) have \(EC_{50}\) of >100, while HDP-cyclic-(S)-HPMPA (neutral) has an \(EC_{50}\) of 5 \(\mu\text{M}\). If one compares the percent mortality at 100 \(\mu\text{M}\) drug, the values are as follows: (S)-HPMPA, 13.3%; HDP-(S)-HPMPA, 30.8%; HDP-cyclic-(S)-HPMPA, 93.3% (Table 2). This is in contrast with the antiviral activity of this type of analog where the open form, i.e., HDP-CDV, is more active than the cyclic compound (1, 32).

In conclusion, HDP-cyclic-(S)-HPMPA and HDP-cyclic-CDV exhibit substantial antischistosomal activities as judged by in vitro worm killing. It would be of interest to examine the in vivo effects of these compounds in \textit{S. mansoni}-infected animals, because a previous study with (S)-HPMPA found promising in vivo activity (4).

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**REFERENCES**


**TABLE 2.** Effects of phosphonate negative charge on antischistosomal activities of (S)-HPMPA and two analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Charge</th>
<th>(EC_{50}) ((\mu\text{M}))</th>
<th>Max. % worm mortality</th>
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<tbody>
<tr>
<td>(S)-HPMPA</td>
<td>-2</td>
<td>&gt;100</td>
<td>13.3</td>
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<tr>
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<td>&gt;100</td>
<td>30.8</td>
</tr>
<tr>
<td>HDP-cyclic-(S)-HPMPA</td>
<td>0</td>
<td>5.0</td>
<td>93.3</td>
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