Efficacy of (S)-HPMPA Against Thymidine Kinase-Deficient Herpes Simplex Virus-Keratitis

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A new acyclic adenosine analogue, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA], was evaluated for its efficacy in the topical treatment of experimental keratitis caused by the thymidine kinase-positive (TK+) and thymidine kinase-deficient (TK-) herpes simplex virus type 1 (HSV-1) strains. In the treatment of TK+ HSV-1 keratitis, 0.2% (S)-HPMPA eyedrops were as effective as the reference compounds, 0.2% (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and 0.2% 5-(2-chloroethyl)-2'-deoxyuridine (CEDU) eyedrops. The three compounds produced a statistically significant healing effect, as compared with placebo eyedrops. In the treatment of keratitis caused by the TK- HSV-1 strain, 0.2%BVDU and 0.2% CEDU eyedrops did not differ from placebo eyedrops, whereas 0.2% (S)-HPMPA eyedrops exerted a highly significant healing effect. Invest Ophthalmol Vis Sci 28:243-248, 1987

During the past decade, potent and selective anti-herpes compounds have been developed for the treatment of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. The new compounds acyclovir [9-(2-hydroxyethoxymethyl)guanine] and BVDU [bromovinyldeoxyuridine, (E)-5-(2-bromovinyl)-2'-deoxyuridine] owe their selective antiherpes activity to the preferential phosphorylation by the virus-encoded thymidine kinase. Once converted to their triphosphate form, these drugs effectively inhibit the viral DNA polymerase leading to viral DNA synthesis inhibition, and consequently shut off virus replication.1-5

HSV type 1 (HSV-1) and HSV type 2 (HSV-2) infections are particularly amenable to acyclovir treatment. Although bromovinyldeoxyuridine is slightly more effective than acyclovir against HSV-1 and markedly more effective than acyclovir against VZV, it is definitely less active against HSV-2, because the HSV-2-induced thymidine kinase does not phosphorylate bromovinyldeoxyuridine as efficiently as does the HSV-1-induced thymidine kinase.6

Other herpes viruses, such as cytomegalovirus (CMV), do not induce a specific viral thymidine kinase, and therefore are not markedly affected by acyclovir or bromovinyldeoxyuridine. Similarly, the mutant strains of HSV and VZV, which either lack thymidine kinase-inducing ability or induce a thymidine kinase with diminished or altered substrate affinity, are relatively insensitive to the compounds that utilize the thymidine kinase phosphorylation pathway for their mechanism of action.1 These mutant virus strains may develop during treatment with antiviral compounds, and acyclovir-resistant HSV mutants have indeed been isolated from immunosuppressed patients treated with acyclovir.8-11

Recently, a new acyclic adenosine analogue, (S)-HPMPA [(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] (Fig. 1), has been reported to inhibit a broad spectrum of DNA viruses, ie, HSV-1, HSV-2, VZV, thymidine kinase-deficient (TK-) mutants of HSV-1, TK- VZV, human CMV, vaccinia virus, and retroviruses (ie, murine sarcoma virus).* The present study was designed to investigate whether (S)-HPMPA has a therapeutic potential for the treatment of TK- HSV-1 keratitis, as well as thymidine kinase-positive (TK+) HSV-1 keratitis, in an experimental rabbit model.

Materials and Methods

Animal Model

Holland rabbits weighing between 1.8 and 2.4 kg were used. In a first experiment, 40 rabbits were inoculated in both eyes, at a single sitting, by instilling 20 μL of the inoculum containing 104 plaque-forming units (PFU) of the virus. The rabbits were divided into two groups: the control group received placebo solution, and the treated group received 0.2% (S)-HPMPA solution. The rabbits were observed daily for the development of keratitis, and the healing time was recorded.

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units of the (TK+) McIntyre strain of HSV-1 per milliliter. The eyes were gently massaged before instilling the virus inoculum.

In a second experiment, another 40 rabbits were infected by instilling 20 μL of virus inoculum containing 10^4 plaque-forming units per 0.1 mL of a TK− HSV-1 mutant isolated from an immunosuppressed patient who had been treated with acyclovir for an orofacial HSV-1 infection, and who later developed a relapse of herpetic gingivostomatitis that no longer responded to acyclovir or bromovinyldeoxyuridine. A large number of HSV-1 isolates were obtained from this patient. One of the posttreatment isolates, designated VMW 1837, was grown in primary rabbit kidney cells. When evaluated in TK− HeLa cells, it induced about 1/30 of the TK activity found with a pretreatment isolate (designated VMW 841): 2.8 and 84 mmoles thymidine phosphorylated per hr per mg protein with VMW 1837 and VMW 841, respectively. The VMW 1837 isolate was therefore considered as virtually TK−. It was resistant to acyclovir, bromovinyldeoxyuridine, and other 5-substituted 2′-deoxyuridines such as 5-(2-chloroethyl)-2′-deoxyuridine (chloroethyldeoxyuridine, CEDU) and 5-ethyl-2′-deoxyuridine, when evaluated in human diploid (E6SM) cell cultures.

For inoculation of the TK− HSV-1 mutant, the corneas were gently stroked with the blunt end of a sterile glass pipette after instilling the virus inoculum. Gentle stroking of the corneas was necessary, as previous attempts to inoculate the eyes by closed-lid massage had failed to produce keratitis.

The use of rabbits in this investigation conformed to the ARVO Resolution on the Use of Animals in Research.

Drugs

Bromovinyldeoxyuridine (BVDU), chloroethyldeoxyuridine (CEDU), and (S)-HPMPA eyedrops were prepared at a concentration of 0.2% (weight per volume) in an isotonic borate buffer containing 1.52 g of boric acid, 0.008 g of sodium borate, and 0.01 g of benzalkonium chloride in 1 dL of distilled water. The pH of the buffer solution was 5.7. The borate buffer alone, without any antiviral ingredients, was used as placebo eyedrops. The eyedrops were dispensed in coded amber bottles. The code was known neither to the person who administered the eyedrops nor to the person who evaluated the severity of keratitis.

Experiment 1

Fifty HSV-1 (McIntyre strain)-infected rabbits were numbered serially and allocated at random to four treatment groups of 10 rabbits each. Each group was treated by 0.2% eyedrops of either BVDU, CEDU, (S)-HPMPA or placebo eyedrops. The allocation of rabbits to treatment groups was not revealed to the person who evaluated keratitis. In each group, both eyes of the same rabbit received the same medication. Treatment was started on day 4 after virus inoculation, when epithelial keratitis had appeared in all eyes, and continued for 5 consecutive days. One drop (20 μL) of the drug was instilled nine times a day at hourly intervals.

Experiment 2

Another series of 40 rabbits were infected with the TK− HSV-1 mutant that was resistant to acyclovir, bromovinyldeoxyuridine, and chloroethyldeoxyuridine in vitro. The allocation of rabbits to the different treatment groups, the drugs used, the duration of treatment, and the treatment regimen were as described for experiment 1.

Keratitis Evaluation

The rabbits were examined daily with a slit lamp by the same observer who used 1% fluorescein sodium eyedrops and a cobalt blue filter. The severity of epithelial disease was scored on a scale from 0 to 5, where grade 0 denoted a normal transparent cornea; grades 0.1 to 0.9, one to nine punctate lesions on the cornea; grade 1, more than ten punctate lesions, dendrites, or small epithelial ulcers, involving less than one-third of the corneal surface; grade 2, dendrites or small ulcers involving one-third of the cornea; grade 3, more than one-third but less than two-thirds corneal involvement; grade 4, more than two-thirds but not total corneal surface affected; and grade 5, total corneal ulceration.

Statistical Analysis

Keratitis scores of different treatment groups in each experiment were analyzed by a nonparametric two-way analysis of variance, comparing treatment vs time and the difference between the treatments. Probability values less than 0.05 were considered significant.
Antiviral Activity In Vitro

Antiviral activity was measured in primary rabbit kidney cell cultures inoculated with TK⁺ HSV-1 (McIntyre strain) or TK⁻ HSV-1 (VMW-1837 strain) and was based on a reduction in viral cytopathogenicity. The details of this procedure have been described previously.13,14

Results

Experiment 1

The severity of keratitis caused by TK⁺ HSV-1 (McIntyre strain) was almost equal for the four different groups when the treatment procedure was started. In the placebo group the corneal involvement steadily progressed (Fig. 2) during the treatment period, whereas the severity of keratitis continuously decreased in rabbits treated with either BVDU, CEDU or (S)-HPMPA. The three compounds exerted a similar healing effect that was significantly different from the effect produced by the placebo treatment (\( P < 0.01 \)).

Experiment 2

All eyes infected with the TK⁻ HSV-1 mutant (VMW 1837 strain) showed severe keratitis when the treatment procedure was started. Administration of BVDU and CEDU did not affect the severity of the corneal infection. The mean keratitis scores for these groups were similar to that of the placebo group throughout the entire treatment period. None of the eyes of the BVDU-, CEDU-, or placebo-treated rabbits healed or showed any signs of improvement (Fig. 3). However, (S)-HPMPA, promoted a prompt healing of epithelial ulceration (Fig. 3). On the last (fifth) day of treatment with (S)-HPMPA, nine eyes had healed (grade 0 keratitis), nine eyes had from grade 0.1 to 0.5 keratitis, and two eyes had grade 1 keratitis. The healing effect produced by (S)-HPMPA was highly significant as compared to the effect of either BVDU, CEDU, or placebo eyedrops (\( P < 0.005 \)).

In neither experiment 1 nor experiment 2 did we observe any sign of ocular irritation (ie, redness, tearing, photophobia, discharge), punctate epitheliopathy, follicular conjunctivitis, corneal edema or opacification, erythema, induration or ulceration of the eyelids, periorbital skin reactions, or any other signs suggestive of local drug toxicity. Nor were there any signs of systemic toxicity (ie, restlessness, irritability, paralysis, convulsions, skin eruptions; changes in behavior, food or water intake, appearance of the fur or excretions).

Antiviral Activity In Vitro

(S)-HPMPA, BVDU, CEDU, and acyclovir were all effective against TK⁺ HSV-1 (McIntyre strain) in vitro, whereas only (S)-HPMPA was effective against the TK⁻ HSV-1 (VMW-1837) strain (Table 1). None of the compounds caused a microscopically detectable toxicity for the host cells (change in cell morphology) at concentrations up to 200 \( \mu \)g/mL (the highest concentration tested).

(S)-HPMPA did not affect normal host cell metabolism, ie, DNA synthesis, as monitored by the incorporation of [methyl-\(^{3}H\)]2'-deoxythyimidine or \([^{32}P]\)orthophosphate (and subsequent analysis of the DNA by CsCl gradient ultracentrifugation),\(^{\dagger}\) unless

Table 1. Antiviral activity of (S)-HPMPA, BVDU, CEDU and acyclovir (ACV) in primary rabbit kidney cell cultures

<table>
<thead>
<tr>
<th>Compounds</th>
<th>TK⁺ HSV-1 (McIntyre strain) (µg/mL)</th>
<th>TK⁻ HSV-1 (VMW-1837 strain) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-HPMPA</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>BVDU</td>
<td>0.02</td>
<td>100</td>
</tr>
<tr>
<td>CEDU</td>
<td>0.2</td>
<td>&gt;200</td>
</tr>
<tr>
<td>ACV</td>
<td>0.1</td>
<td>70</td>
</tr>
</tbody>
</table>

* Required to reduce virus-induced cytopathogenicity by 50%.

The present experiments demonstrate that (S)-HPMPA has a highly significant healing effect on experimental keratitis caused by either TK⁺ HSV-1 or TK⁻ HSV-1.

The healing effect of (S)-HPMPA eyedrops on epithelial disease caused by TK⁺ HSV-1 was similar to that of BVDU and CEDU eyedrops. Bromovinyldeoxyuridine is more potent and selective against HSV-1 and VZV than various other antiviral drugs, including acyclovir, bromovinyldeoxyuridine, chloroethyldeoxyuridine, trifluridine, vidarabine, idoxuridine (5'-iodo-2'-deoxyuridine), trifluridine, 5-trifluoromethyl-2'-deoxyuridine), vidarabine (9-β-D-arabinofuranosyldadenine), acyclovir (9-(2-hydroxyethoxymethyl)guanine), foscarnet (phosphonoformate), trifluridine, and TFT (tetrafiuridyl) eyedrops. Bromovinyldeoxyuridine needs to be phosphorylated by the virus-encoded thymidine kinase, a function that cannot be accomplished by TK⁻ HSV-1 mutant strains. The mechanism of action of chloroethyldoxyuridine is not fully known at present, but it is thought to be similar to that of bromovinyldeoxyuridine.

Additional experiments were carried out to assess the effect of trifluridine (TFT) on TK⁻ HSV-1 keratitis. Although TFT 0.2% eyedrops were equally effective as BVDU 0.2% eyedrops in suppressing TK⁺ HSV-1 keratitis, neither TFT or BVDU exhibited any healing effect on TK⁻ HSV-1 keratitis (data not shown). These data further attest to the unique usefulness of (S)-HPMPA for the treatment of TK⁻ HSV-1 infections. They also suggest that the antiviral activity of TFT, although nonspecific, at least partially depends on phosphorylation by the virus-encoded thymidine kinase.

To inhibit viral DNA synthesis, bromovinyldeoxyuridine needs to be phosphorylated by the virus-encoded thymidine kinase, a function that cannot be accomplished by TK⁻ HSV-1 mutant strains. The mechanism of action of chloroethyldoxyuridine is not fully known at present, but it is thought to be similar to that of bromovinyldeoxyuridine.

In immunocompromised patients following treatment with antiviral drugs such as acyclovir, fulminant HSV-1 infections are treated with acyclovir, chloroethyldoxyuridine, and other 5-substituted 2'-deoxyuridines in cell culture. This observation suggests that, when clinical HSV-1 infections are treated with acyclovir, cross-resistance may develop to other antiviral compounds.

Recently, new acyclic guanosine analogues have been developed: ie, dihydroxypropoxymethylguanine [9-(1,3-dihydroxy-2-propoxymethyl)guanine; also referred to as DHPG, BIOLF-62, BW B759U, NDG or 2'-nor-2'-deoxyguanosine], dihydroxybutylguanine [DHBG, 9-(3,4-dihydroxybutyl)guanine], and iso-dihydroxypropoxymethylguanine [iNDG, 9-(2,3-dihydroxy-1-propoxymethyl)guanine]. Dihydroxypropoxymethylguanine may be of some use against human CMV infection and is currently being evaluated for this purpose in immunosuppressed patients.

(S)-HPMPA exhibits potent antiviral activity against a broad spectrum of DNA viruses. It appears quite selective in its antiviral activity, as it is inhibitory to HSV, VZV, CMV, and various other herpesviruses, including TK⁻ strains of HSV and VZV, at a concentration of 1 µg/mL or lower, whereas normal cell metabolism (DNA, RNA or protein synthesis) is not affected by the compound up to a concentration of 100 µg/mL or higher.* The activity of (S)-HPMPA against CMV has been demonstrated with several virus strains (ie Davis,


AD 169) and assay methods (i.e., plaque reduction, cytotoxicity inhibition), and, recently,§ (S)-HPMPA has also proven to be a selective inhibitor of adenovirus replication in vitro (minimum inhibitory concentration: 1 μg/mL; selectivity index: 50).

The mechanism of action of (S)-HPMPA remains subject of further study. Being a phosphonyl analog, it cannot be enzymatically dephosphorylated. (S)-HPMPA is probably taken up as such by the cells. Its metabolic fate within the cell is under investigation. There is no evidence that (S)-HPMPA would be phosphorylated differently by virus-infected and uninfected cells. However, within the virus-infected cells, (S)-HPMPA preferentially inhibits viral DNA synthesis over cellular DNA synthesis, as demonstrated recently† for Vero cells infected with the KOS strain of HSV-1. This differential inhibitory effect of (S)-HPMPA on viral and cellular DNA polymerization processes must clearly contribute to the selectivity of the compound as an anti-DNA virus agent.

Topical treatment for 5 days with 0.2% (S)-HPMPA eyedrops did not produce any clinically detectable signs of local or systemic toxicity in rabbits. Judging from its broad anti-DNA virus spectrum and its selective and potent antiviral activity, (S)-HPMPA deserves to be further explored for its clinical usefulness. Should it prove safe for human use, it might turn out to be a life-saving drug, especially in immunosuppressed patients, in infections caused by cytomegalovirus, adenovirus, and TK− mutant strains of HSV and VZV which are resistant to other antivirals compounds.

Key words: new antiviral drug, (S)-HPMPA, CEDU, BVDU, thymidine kinase-deficient (TK−), herpes simplex virus type 1 (HSV-1), keratitis

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