(S)-1-(3-Hydroxy-2-Phosphonyl-Methoxypropyl)Cytosine in the Therapy of Thymidine Kinase-Positive and -Deficient Herpes Simplex Virus Experimental Keratitis

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The phosphonylmethoxyalkyl derivative, (S)-1-(3-hydroxy-2-phosphonyl methoxypropyl)cytosine (HPMPC), was evaluated for its efficacy in the topical treatment of experimental keratitis caused by thymidine kinase-positive (TK⁺) or thymidine kinase-deficient (TK⁻) herpes simplex virus type 1 (HSV-1) strains. The HPMPC 0.2% eyedrops were as effective as the reference compound, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) 0.2% eyedrops in stimulating the healing of epithelial disease caused by the HSV-1 TK⁺ strain. Both drugs achieved a significant (P < 0.005) healing effect compared with placebo eyedrops. No significant differences were noted in the efficacy of HPMPC 0.2% eyedrops when instilled one, three, or nine times a day. In the treatment of keratitis caused by the HSV-1 TK⁻ strain, 0.2% BVDU eyedrops were similar to placebo; 0.2% HPMPC eyedrops again had a brisk and significant healing effect (P < 0.005). Invest Ophthalmol Vis Sci 32:1816-1820, 1991

The established antiviral compounds, bromovinyldeoxy-uridine or (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and acyclovir (ACV) owe their selective antiviral activity to a preferential phosphorylation by the virus-encoded thymidine kinase (TK). In their triphosphate forms, both drugs then inhibit the viral DNA polymerase, blocking viral DNA synthesis and, consequently, virus replication.1-5 Both herpes simplex virus (HSV) type 1 (HSV-1) and HSV type 2 (HSV-2) are sensitive to ACV. Although BVDU is less active than ACV against HSV-2, it is markedly more effective than ACV against HSV-1 and varicella-zoster virus (VZV).5 Other herpes viruses, ie, cytomegalovirus (CMV), that do not encode a virus-specific TK, are not particularly sensitive to the inhibitory action of ACV and BVDU. Similarly, mutant strains of HSV and VZV, that either do not induce an active viral TK or induce a viral TK with diminished or altered substrate affinity, are insensitive to BVDU and ACV.7

In recent years we showed that phosphonylmethoxyalkyl derivatives of purines and pyrimidines possess potent activity against a broad-spectrum of DNA viruses, including adeno-, herpes-, irido-, pox- and hepadnaviruses, and retroviruses, including human immunodeficiency virus.8-12 In a previous report we described the efficacy of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) in the treatment of experimental keratitis caused by either TK⁺ or TK⁻ strains of HSV-1.13 In this article we report on the efficacy of the phosphonylmethoxypropyl pyrimidine analogue, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine (HPMPC) (Fig. 1), in the therapy of experimental keratitis caused by TK⁺ or TK⁻ HSV-1. The HPMPC does not differ markedly in anti-HSV potency from HPMPA, but it is about ten times more selective than HPMPA in anti-CMV activity.11 In fact, HPMPC exerts a long-lasting inhibitory effect on CMV infection in cell cultures.14 This contrasts with the rapidly vanishing anti-CMV activity of ganciclovir, the drug that is commonly used for the treatment of CMV infections in immunosuppressed patients. Because of the unusually prolonged antiviral activity shown by HPMPC in vitro, we also investigated whether the compound may be effective against HSV-1 keratitis after infre-
Materials and Methods

Drugs and Animals

The BVDU and HPMPC eyedrops were prepared at a concentration of 0.2% (w/v) in a vehicle consisting of an isotonic borate buffer containing 1.52 g boric acid, 0.008 g sodium borate, and 0.01 g benzalkonium chloride in 100 ml of distilled water. The vehicle alone, devoid of any antiviral constituent, was used as placebo eyedrops. The eyedrops were dispensed in coded, similar-appearing ophthalmic eye drop dispensing bottles. The code was not revealed to the person who administered the eyedrops or the person who evaluated the severity of keratitis. The animals were treated conform to the ARVO Resolution on the Use of Animals in Research. Male and female Dutch rabbits, weighing about 2 kg each, and approximately 3 months of age, were used.

Experiment 1: TK+ HSV-1 Keratitis

At a single setting, both eyes of 30 rabbits were inoculated by instilling one drop (20 μl) of the virus inoculum containing 10^4.5/0.1 ml plaque-forming units of TK+ HSV-1 (Mclntyre strain). The eyes were gently massaged before virus inoculation. The animals were numbered serially and randomly allocated to three treatment groups of ten rabbits each. Treatment was started on day 4 postinoculation, when epithelial lesions had appeared in all eyes, and continued for 5 consecutive days. In each group, both eyes of the rabbit received the same drug. One drop (20 μl) of the drug was instilled into the eyes nine times a day at 1-hr intervals.

Experiment 2: TK− HSV-1 Keratitis

Another series of 30 rabbits were infected by instilling one drop (20 μl) of the virus inoculum containing 10^4/0.1 ml plaque-forming units of an HSV-1 TK− mutant (VMW-1837) isolated from an immunosuppressed patient who had been treated with ACV. For inoculation of the HSV-1 TK− mutant, the corneas were traumatized gently with the blunt end of a sterile glass pipette after the virus suspension had been instilled into the eyes.

The allocation of the rabbits to the three treatment groups, the drugs used, the duration of treatment, and the drug treatment regimen (nine times a day at 1-hr intervals) were as in Experiment 1.

Experiment 3: TK+ HSV-1 Keratitis—Influence of Varying Frequency of Drug Instillation

Forty more rabbits were infected with TK+ HSV-1 (Mclntyre strain) as in Experiment 1 and allocated randomly to four treatment groups of ten rabbits each. The HPMPC eyedrops were administered nine times a day in one group, three times a day in a second group, and once a day in the third group. Placebo eyedrops were instilled nine times a day. The duration of treatment was as in Experiment 1.

Keratitis Evaluation

The same observer evaluated the severity of keratitis, on a daily basis, using 1% fluorescein sodium eye drops and a slit lamp fitted with a cobalt blue filter. Keratitis was scored on a scale from 0–5, where 0 denoted a normal transparent cornea; 0.1–0.9, one to nine punctate epithelial lesions; 1, more than ten punctate lesions, dendrites, or small epithelial ulcers involving less than one third of the corneal surface; 2, dendrites or small ulcers involving one third of the cornea; 3, more than one third but less than two thirds corneal involvement; 4, more than two thirds but not total corneal surface involved; and 5, total corneal ulceration.

Statistical Analysis

Mean keratitis scores were obtained by averaging the daily keratitis scores of all eyes treated with the
same drug, ie, 20 eyes in each group. Keratitis scores of different treatment groups in each experiment were analyzed further by a nonparametric two-way analysis of variance, comparing treatment versus time and the difference between the treatment groups. Probability values less than 0.05 were considered significant.

Results

Experiment 1

When treatment began, there was no difference in the severity of keratitis in the three groups. Treatment with either HPMPC or BVDU eyedrops led to a brisk reduction in the severity of keratitis, whereas the severity of keratitis progressively increased in the placebo-treated eyes (Fig. 2). Statistical analysis of the data revealed that the healing effect caused by both drugs was highly significant compared with placebo eyedrops \( (P < 0.005) \). Differences between the healing effects of HPMPC and BVDU were not significant.

Experiment 2

Although there was only mild keratitis in the three groups when treatment began, the severity of keratitis gradually increased in both the BVDU and placebo treatment groups. There were no significant differences in keratitis scores between the BVDU and placebo groups. In contrast, HPMPC eyedrops showed a consistent and highly significant healing effect on the TK- HSV-1 keratitis compared with treatment by either BVDU or placebo eyedrops \( (P < 0.005, \text{ Fig. 3}) \).

Experiment 3

In the third experiment again we observed a progressive worsening of the severity of keratitis in the eyes treated with placebo eyedrops (Fig. 4). In contrast, the disease resolved completely in the eyes that received the HPMPC eyedrops, irrespective of the frequency at which the eyedrops were instilled. When applied nine times a day, HPMPC 0.2% eyedrops caused a more rapid healing effect than when treatment was given only three times or once a day. However, the differences between these three treatment schedules were not statistically significant. When the keratitis scores of each of the three HPMPC treatment group were compared with the keratitis scores of the placebo treatment group, significant differences \( (P < 0.005) \) were denoted for all three HPMPC treatment groups.

We did not observe any signs of ocular irritation (ie, redness, tearing, photophobia, or 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. Likewise, there were no signs of systemic toxicity (ie, restlessness, irritability, paralysis, convulsions, skin eruptions, changes in behavior, food or water intake, or appearance of the fur or excretions).

Discussion

The HPMPC and BVDU eyedrops achieved an equally efficient healing effect on TK+ HSV-1 keratitis in rabbits. The agent BVDU is a more potent and selective inhibitor of TK+ HSV-1 and VZV than various other established antiviral compounds, such as idoxuridine, trifluridine, vidarabine, ACV, and foscarnet. It has been shown to be effective in the treatment of experimental HSV-1 epithelial keratitis, stromal disease, and clinical disease in patients.

However BVDU did not influence the course of TK- HSV-1 keratitis. This agrees with the results of our previous experiments, where keratitis was induced by using the same virus mutant. Like HPMPA, HPMPC had a marked healing effect on keratitis caused by the TK- HSV-1. This is not surprising in view of the in vitro data obtained for HPMPC. The compound caused a 50% reduction in viral cytopathogenicity in cell culture at a concentration of 4 μg/ml (TK+ HSV-1), 2 μg/ml (TK- HSV-2), 10 μg/ml (HSV-2), 0.25 μg/ml (VZV), or 0.08 μg/ml (CMV), whereas host cell metabolism or proliferation was affected only at a concentration 100- to 1000-fold higher than the antivirally active concentration. The fact that HPMPC and HPMPA are active against TK- HSV and CMV indicates that these compounds do not depend on phosphorylation by the viral TK. Instead, they are taken up by the cells and then phosphorylated by cellular enzymes to their monophosphorylated and diphosphorylated derivatives. The latter may be considered the active forms in which the compounds inhibit viral DNA synthesis.

The HPMPA and HPMPC have been shown to discriminate between viral and cellular DNA synthesis, and this may contribute to their selective inhibitory activity against HSV, CMV, and other herpesviruses, ie, Epstein-Barr virus. The selective viral DNA synthesis inhibition appears to be based on a specific interaction of diphosphorylated forms of these compounds with the viral DNA polymerase; HPMPC and HPMPA are able to inhibit viral DNA synthesis at concentrations that are 103-fold lower than the drug concentrations required to inhibit cellular DNA synthesis. Furthermore, the diphosphorylated derivative of HPMPC persists for a long time in the HPMPC-treated cells. The long intracellular persistence of HPMPC derivatives may be related to the fact that the phosphonate linkage of HPMPC, unlike phosphate bonds, is not hydrolyzed by esterases. The prolonged presence of diphosphorylated drug in the cells may explain why a pulse HPMPC treatment of only 6 hr postinfection is able to suppress CMV replication for several days in cell culture. Also HPMPC has been found effective against murine CMV and simian VZV when administered systemically at infrequent doses to mice and monkeys, respectively.

In Experiment 3, described in this article, we found that HPMPC was equally effective in stimulating the healing of herpetic keratitis whether administered topically (as eyedrops) one, three, or nine times a day. Thus, one application of HPMPC eyedrops per day caused complete healing of herpetic keratitis in 4-5 days (Fig. 4). Infrequent instillation of eyedrops (ie, once a day) to treat ocular virus infections would give HPMPC a great advantage over the antiviral agents which are currently used in ophthalmologic practice and which have to be applied either six times a day as an eye ointment (ACV or vidarabine) or every hour as eyedrops (idoxuridine, trifluridine, or BVDU) by the patient.

Another advantage of HPMPC is that, because of its broad-spectrum anti-DNA virus activity, it could be used in the treatment of various DNA virus infections, including adenovirus, TK+ HSV-1, TK- HSV-1, HSV-2, VZV, and CMV infections of the eye. This again gives HPMPC a therapeutic edge over the classic antiviral agents such as ACV and ganciclovir which are much more limited in the range of therapeutic applications (TK+ HSV and VZV for ACV and CMV for ganciclovir).

Topical administration of HPMPC 0.2% eyedrops for 5 days, at a frequency of once, three, or nine times a day, did not produce any clinically detectable signs of local or systemic toxicity in rabbits. Because of its broad-spectrum anti-DNA virus activity, its selectivity, and unusually long-lasting antiviral activity, HPMPC is a prime candidate to be explored further for its clinical usefulness in both the topical and systemic treatment of herpesvirus and other DNA virus infections.

Key words: HPMPC, TK+ HSV, TK- HSV, keratitis, antiviral drugs

References

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