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Effects of phosphonylmethoxyalkyl-purine and -pyrimidine derivatives on TK^+ and TK^- HSV-1 keratitis in rabbits

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Summary

The phosphonylmethoxyalkyl derivatives HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine], HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] and PMEA [9-(2-phosphonylmethoxyethyl)adenine] were evaluated as 0.2% eyedrops for their efficacy in the treatment of experimental herpes simplex virus type 1 (HSV-1) keratitis in the rabbit model. BVDU 0.2% eyedrops were used as the reference treatment. HPMPA, HPMPC, PMEA and BVDU eyedrops showed a rapid and highly significant healing effect ($P < 0.005$) on keratitis caused by TK^+ HSV-1 (McIntyre strain) when compared with placebo eyedrops, whereas BVDU treatment did not affect the course of TK^- HSV-1 (VMW-1837) keratitis. HPMPA and HPMPC treatment again caused a highly significant healing ($P < 0.005$, compared with placebo eyedrops). Although PMEA eyedrops were less effective than HPMPA or HPMPC eyedrops, the effect of PMEA eyedrops was significantly ($P < 0.05$) different from the effect of either BVDU or placebo eyedrops.

HSV-1 keratitis; TK^- HSV-1; BVDU; HPMPA; HPMPC; PMEA; Phosphonylmethoxyalkyl derivative

Introduction

Phosphonylmethoxyalkyl derivatives of purines and pyrimidines, or acyclic nucleoside phosphonate analogues, represent a new class of antiviral

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compounds. Various 3-hydroxy-2-phosphonylmethoxypropyl (HPMP) and 2-phosphonylmethoxyethyl (PME) derivatives of purine [adenine (A), guanine (G), 2,6-diaminopurine (DAP), 2-monoaminopurine (MAP), hypoxanthine (Hx)] and pyrimidine [cytosine (C), uracil (U), thymine (T)] have been synthesized (Holý and Rosenberg, 1987a,b) and evaluated for their antiviral properties. Their spectrum of antiviral activity includes adeno-, herpeto-, irido-, pox-, hepadnaviruses, and also retroviruses, including human immunodeficiency virus (HIV) (De Clercq et al., 1986,1987; Gil-Fernandez and De Clercq, 1987; Lin et al., 1987; Osterhaus et al., 1987; Votruba et al., 1987; Pauwels et al., 1988; Snoeck et al., 1988; Gangemi et al., 1989).

In contrast with the established selective anti-herpes compounds, i.e. bromovinyldeoxyuridine [BVDU, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine] and acyclovir [Zovirax, ACV, 9-(2-hydroxyethoxymethyl)guanine], acyclic nucleoside phosphonates do not require phosphorylation by the viral thymidine kinase (TK) to exert their inhibitory action on virus replication. Instead, these compounds are as such taken up by the cells and then phosphorylated by cellular enzymes to their diphosphoryl derivatives which, in turn, inhibit viral DNA synthesis (Votruba et al., 1987). HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine], HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] and PMEA [9-(2-phosphonylmethoxyethyl)adenine] not only inhibit the replication of herpes viruses that encode viral TK, i.e., herpes simplex virus (HSV) type 1 (HSV-1; De Clercq et al., 1986, 1987), herpes simplex virus type 2 (HSV-2; De Clercq et al., 1986, 1987), varicella-zoster virus (VZV; De Clercq et al., 1986, 1987), but also the replication of TK-deficient (TK^-) mutant strains of these viruses. Similarly, these compounds are markedly inhibitory to the replication of those herpesviruses that do not encode a specific viral TK, i.e., cytomegalovirus (CMV; De Clercq et al., 1986, 1987; Snoeck et al., 1988) and Epstein-Barr virus (EBV; Lin et al., 1987).

The efficacy of HPMPA and PMEA has been shown in the treatment of cutaneous HSV-1 or HSV-2 infection in hairless mice, TK^- HSV-1 infection in athymic nude mice and HSV-1, HSV-2 or TK^- HSV-1 encephalitis in NMRI mice (De Clercq et al., 1989). In addition, PMEA has proven to be an effective agent in the treatment of both retrovirus infection and HSV-1 superinfection in the murine AIDS-model (LP-BM5 virus infection; Gangemi et al., 1989). HPMPC has been found effective against murine CMV infection in mice (Kern and Vogt, 1989) and simian VZV infection in monkeys (Soike et al., 1990) when administered systemically at infrequent dosage regimens. It has been demonstrated that, inside the cells, HPMPC persists for a long time in its diphosphoryl form (Hitchcock et al., 1989; Kern and Vogt, 1989). Consequently, pulse treatment of CMV-infected cells for only six hours with HPMPC suffices to achieve suppression of CMV replication for several days (Neyts et al., 1990).

In this article, we review the effects obtained with HPMPA, HPMPC and PMEA in the treatment of experimental HSV-1 keratitis in the rabbit model. The experiments were conducted in a double-masked fashion, and BVDU

eyedrops were used as the reference treatment. BVDU is a potent and selective anti-herpes compound that surpasses various other antiviral compounds, e.g. idoxuridine (5-iodo-2′-deoxyuridine), trifluridine (5-trifluoromethyl-2′-deoxyuridine), vidarabine (9-β-D-arabinofuranosyladenine), acyclovir [9-(2-hydroxyethoxymethyl)guanine], and foscarnet (phosphonoformate) in their efficacy to inhibit HSV-1 or VZV replication in vitro (De Clercq et al., 1979, 1980a,b). BVDU 0.1% eyedrops have proven efficacious in the treatment of experimental HSV-1 epithelial (Maudgal et al., 1979) and stromal (Maudgal et al., 1982a) keratitis and iritis (Maudgal et al., 1982b) in rabbits, and dendritic and geographic corneal ulcers and stromal keratitis in patients (Maudgal et al., 1981, 1985).

Effects of HPMPA, HPMPC and PMEA on TK$^+$ HSV-1 keratitis

In different sets of experiments, keratitis was produced in normal rabbit eyes by instilling one drop of the virus inoculum containing $10^{4.5}$ plaque-forming units (PFU) of TK$^+$ HSV-1 (McIntyre strain). Eyes were gently massaged prior to instillation of the virus inoculum. The animals were numbered serially and allocated at random to treatment groups of ten rabbits each.

HPMPA, HPMPC, PMEA and BVDU eyedrops were prepared at a 0.2% (w/v) concentration in an isotonic borate buffer and dispensed in coded eyedrop-dispensing bottles. Treatment was started four days post-infection and continued for five consecutive days. One drop of the drug was instilled nine times a day at 1-h intervals. Both eyes of each rabbit of the same group were treated with the same medication.

The severity of keratitis was evaluated daily by using fluorescein sodium 1% eyedrops and a slitlamp fitted with a cobalt-blue filter. A scale from grade 0 to 5 was used to designate the severity of the disease: grade 0, a normal transparent cornea; grade 0.1 to 0.9, one to nine punctate epithelial lesions; grade 1, more than ten punctate lesions, dendrites, a small epithelial ulcer involving less than one-third of the cornea; grade 2, dendrites or small ulcer 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 involved; and grade 5, total corneal ulceration. In contrast to placebo eyedrops, treatment of rabbit eyes with HPMPA, HPMPC, PMEA or BVDU eyedrops caused a prompt healing of keratitis (Fig. 1). The effect of the antiviral drugs did not differ significantly from each other when subjected to a nonparametric analysis of variance (Armitage, 1971). However, the healing effect of each of the four drugs on keratitis was significantly different ($P < 0.005$) from that of placebo eyedrops.

Effect of HPMPA, HPMPC and PMEA on TK$^-$ HSV-1 keratitis

For the induction of TK$^-$ HSV-1 keratitis, we used an HSV-1 mutant (VMW-1837) that had been isolated from an immunosuppressed patient

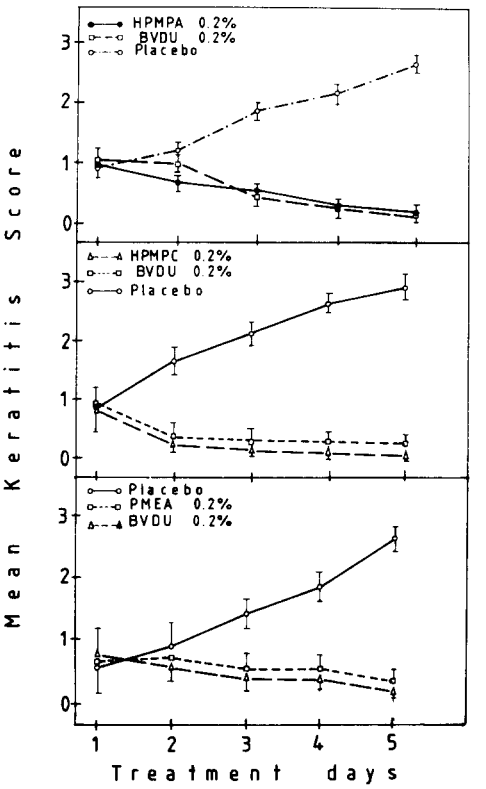

Fig. 1. Effect of HPMPA, HPMPC and PMEA eyedrops on TK^+ HSV-1 (McIntyre strain) mean keratitis scores (± S.E.M.). The three compounds were as efficient as BVDU eyedrops (reference treatment). All antiviral agents effected a highly significant healing, compared with placebo eyedrops.

treated with acyclovir (Vinckier et al., 1987). The design of the experiments and the drug treatment regimen used were the same as in the TK^+ HSV-1 keratitis experiments. The virus inoculum contained $10^{4.5}$ PFU per 0.1 ml. Treatment with BVDU eyedrops did not affect the severity of TK^- HSV-1 keratitis, which gradually increased during the treatment period (Fig. 2). PMEA eyedrops initially slowed down progression of the disease, and then tended to gradually decrease the daily keratitis scores. Statistically, the healing effect of PMEA eyedrops was significantly different ($P < 0.05$) from the placebo eyedrops. Both HPMPA and HPMPC eyedrops caused an healing effect on TK^- HSV-1 keratitis which was more pronounced than that of PMEA eyedrops, and highly significant ($P < 0.005$) compared with placebo eyedrops or BVDU eyedrops.

We did not observe any signs of local or systemic toxicity of HPMPA, HPMPC, PMEA or BVDU during the treatment (observation) period.

Discussion

The healing effects of HPMPA, HPMPC and PMEA eyedrops on TK^+

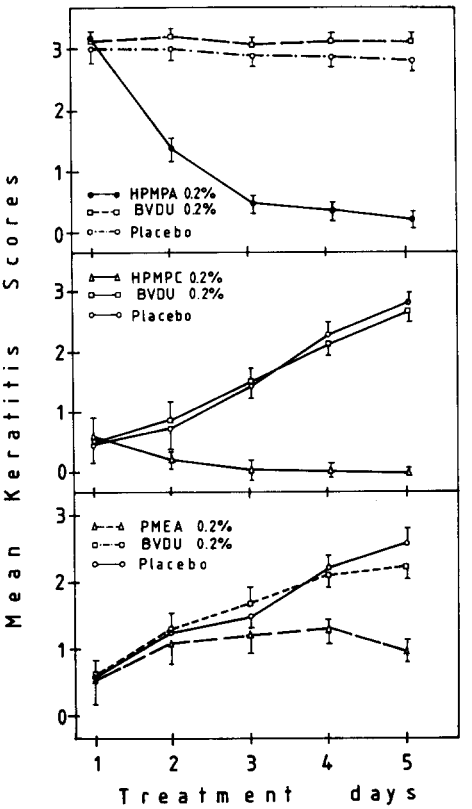

Fig. 2. BVDU eyedrops did not influence the course of TK$^-$ HSV-1 (VMW 1837) mean keratitis scores (± S.E.M.), compared with placebo eyedrops. HPMPA and HPMPC eyedrops caused a prompt and highly significant healing of the disease. Although less pronounced, the healing effect of PMEA eyedrops was also significantly different from that of placebo eyedrops.

HSV-1 keratitis in our experiments was equal to that of BVDU eyedrops. BVDU is a potent and selective antiherpes compound with established efficacy in the treatment of HSV-1 epithelial disease (Maudgal et al., 1979), stromal keratitis (Maudgal et al., 1982a) and iritis (Maudgal et al., 1982b), both in the rabbit model and in patients (Maudgal et al., 1981, 1985). It has also proven useful for the treatment of herpetic corneal ulcers clinically resistant to other antiviral drugs, i.e., IDU, TFT, Ara-A and/or Zovirax (Maudgal et al., 1981, 1985). However, BVDU is not very effective against HSV-2, for it is not an efficient substrate for the HSV-2-encoded TK (De Clercq et al., 1980a; Cheng et al., 1981). Similarly, TK$^-$ HSV-1 and TK$^-$ VZV mutant strains are not susceptible to BVDU, since BVDU needs to be phosphorylated by the viral TK before it can be further phosphorylated by the cellular enzymes to its 5'-triphosphate form which then inhibits the viral DNA polymerase (Allaudeen et al., 1981; Cheng et al., 1981; Descamps and De Clercq, 1981). Therefore, it is not surprising that BVDU eyedrops did not exhibit a beneficial effect on the

TK^- HSV-1 keratitis.

Both HPMPA and HPMPC eyedrops were very efficient in the treatment of TK^- HSV-1 keratitis. This is in agreement with their antiviral activity in vitro. HPMPA causes a 50% reduction in viral cytopathogenicity in cell culture at a concentration of 2 μg/ml (TK^+ or TK^- HSV-1), 4 μg/ml (HSV-2), 0.02 μg/ml (TK^+ or TK^- VZV), and 0.15 μg/ml (CMV). Similar data have been obtained for HPMPC: 4 μg/ml (TK^+ HSV-1), 10 μg/ml (HSV-2), 2 μg/ml (TK^- HSV-1), 0.25 μg/ml (TK^+ or TK^- VZV) and 0.08 μg/ml (CMV) (De Clercq et al., 1987). In inhibiting the viral replication, HPMPA and HPMPC discriminate between viral and cellular DNA synthesis (Lin et al., 1987; Votruba et al., 1987; Snoeck et al., 1988), and this obviously contributes to their selective inhibitory activity against HSV, CMV and other viruses. Moreover, the diphosphoryl derivative of HPMPC has been shown to persist for a long period inside the cells exposed to this compound (Hitchcock et al., 1989; Bronson et al., 1990), and this may explain why a 6-h pulse treatment with HPMPC suffices to suppress CMV replication for several days in cell culture (Neyts et al., 1990). Such long-lasting antiviral effect has also been observed with HPMPC in the TK^+ HSV-1 keratitis rabbit model, where no significant decrease in the healing effect of HPMPC 0.2% eyedrops was observed when the frequency of eyedrop instillation was reduced from nine times a day to only once a day (Maudgal and De Clercq, 1990). The fact that HPMPC could be administered infrequently (i.e. once a day) in the treatment of herpetic keratitis would give HPMPC a great advantage over the currently available antiviral compounds which have to be applied several times a day as eyedrops (IDU, TFT) or eye ointment (Ara-A, Zovirax).

In vitro PMEA effects a 50% reduction in the cytopathogenicity of both TK^+ and TK^- HSV-1 at a concentration of 1–10 μg/ml, whereas VZV and CMV cytopathogenicity are inhibited at a concentration of 10 μg/ml and 25 μg/ml, respectively (De Clercq et al., 1987). PMEA is also inhibitory to HIV (Pauwels et al., 1988). In the murine AIDS model (LP-BM5 virus infection), PMEA has proven efficacious in the treatment of both the retrovirus infection and HSV-1 superinfection (Gangemi et al., 1989).

The most important feature of the acyclic nucleoside phosphonate analogues is their broad-spectrum of antiviral activity, which permits their use in the treatment of a broad variety of DNA virus infections. In this regard, HPMPA and HPMPC may be particularly useful for the treatment of adenovirus, TK^+ HSV-1, TK^- HSV-1, HSV-2, VZV and CMV eye infections. In contrast, the 'classical' antiviral agents are limited in their therapeutic usefulness, i.e., TK^+ HSV and VZV infections for Zovirax, and CMV infections for ganciclovir. Whereas HPMPA and HPMPC are applicable in the treatment of various DNA virus infections, PMEA has the unique potential of being applicable in the treatment of both HSV and HIV infections.

In our experiments the drugs did not cause any local or systemic toxic side effects. However, the drugs were used only at a low concentration (0.2%) for only a short period (five days). Further pharmacological, pharmacokinetic and

toxicological studies are needed to ascertain the therapeutic potential of these drugs for human use.

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