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The activity of (S)-1-[(3-hydroxy-2-phosphonyl methoxy) propyl] cytosine (HPMPC) against equine herpesvirus-1 (EHV-1) in cell cultures, mice and horses

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Summary

The activity of the nucleotide analogue, (S)-1-[(3-hydroxy-2-phosphonyl methoxy) propyl] cytosine (HPMPC), against equine herpesvirus-1 (EHV-1) was tested in cell culture, mice and foals. The ED_{50} for plaque reduction was found to be 0.07 and 0.03 μg/ml in RK-13 and EEL cells respectively. In mice, a single administration of HPMPC (20 mg/kg, s.c.) was very effective at reducing clinical signs and virus replication if given on the day before intranasal inoculation with EHV-1. Treatment on the day of infection or day 1 p.i. was less effective, but still significantly reduced clinical signs and virus titres in the target organs (lungs and nasal tissue). Furthermore, HPMPC was found to protect mice partially from an intracerebral inoculation with EHV-1. Experiments in the horse suggested that HPMPC was also very active against EHV-1 in the natural host. Thus a single administration of HPMPC at 20 mg/kg, s.c., on the day of infection, markedly reduced clinical signs and nasal excretion of virus following intranasal inoculation with EHV-1. HPMPC given as a divided dose of 1 mg/kg on day 0 and day 3 p.i. had no effect on clinical signs but did reduce nasal excretion of virus. The significance of these results is discussed.

(S)-1-[(3-hydroxy-2-phosphonyl methoxy) propyl] cytosine; Equine herpesvirus-1; Mouse; Foal

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Introduction

Equine herpesvirus-1 is a major pathogen of horses which, in addition to causing respiratory disease, can also result in abortion and/or neurological signs in infected animals (Allen and Bryans, 1986). Notwithstanding its considerable veterinary and economic importance, however, the pathogenesis and epidemiology of EHV-1 remain unclear. Control measures are inadequate and, although vaccines are available, they are not fully protective and outbreaks of disease still occur (Dutta and Shipley, 1975; Burrows et al., 1984; Allen and Bryans, 1986; Burki et al., 1990).

Better immunoprophylaxis is dependent upon a fuller understanding of the pathogenesis and epidemiology of the virus. In the meantime, another approach to controlling infection would be the provision of effective chemotherapy or chemoprophylaxis, and, to date, several antiviral compounds against EHV-1 have been investigated (De Clercq et al., 1986, 1987; Field and Awan, 1990; Fuente et al., 1992).

De Clercq and colleagues have demonstrated that a novel class of acyclic phosphonates are highly active against a range of viruses, including herpes viruses (De Clercq et al., 1986, 1987). Unlike drugs such as acyclovir and bromovinyl deoxyuridine, their action does not depend on phosphorylation by virus-induced thymidine kinase (De Clercq, 1991). One of these compounds, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA), is effective against EHV-1 in vitro at very low doses. Furthermore, we have observed that HPMPA is very active against EHV-1 in vivo: thus twice daily dosing with HPMPA was highly effective in ameliorating clinical signs and reducing virus replication following intranasal infection of mice with EHV-1 (Field and Awan, 1990).

Latterly, a related compound, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine, HPMPC, has been investigated (De Clercq et al., 1987; Snoeck et al., 1988; Bronson et al., 1989; De Clercq and Holy, 1991; Neyts et al., 1991). In addition to sharing many of the advantages of the other acyclic phosphonates, it has been shown that the compound retains its antiviral activity in vivo when given at infrequent intervals, and even following single administration (De Clercq and Holy, 1991).

This paper describes the use of HPMPC against EHV-1 infection in vitro and in vivo following infection of mice and also horses (i.e. the natural host). We show that HPMPC is an effective chemotherapeutic agent against EHV-1 in all three systems. The importance of these findings is discussed.

Materials and Methods

Antiviral agent: (S)-1-[(3-hydroxy-2-phosphonyl methoxy) propyl] cytosine (HPMPC)

HPMPC was a gift from Dr. E. De Clercq, Rega Institute, Leuven, Belgium.

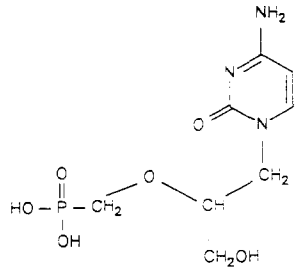

Fig. 1. Structural formula of (S)-1-[(3-hydroxy-2-phosphonyl methoxy)propyl] cytosine (HPMPC).

Further amounts of the drug was supplied by Dr. K. Field, formally of Bristol-Meyers-Squibb, USA. The compound was originally described by Holy and Rosenberg (1987) and the structural formula is shown in Fig. 1. HPMPC was received in powder form and kept frozen at −20°C, either dry or in a 1 mg/ml solution in distilled water until use.

For mice experiments, HPMPC was dissolved in sterile PBS at 5 mg/ml and administered to mice by subcutaneous (s.c.) injection at a dose of 20 mg/kg (approximately 100 μl/mouse). Mice not treated with HPMPC were given a similar volume of PBS to act as placebo controls: in the results section, these mice are referred to as untreated.

In foal experiments, both animals received different treatment regimes. One foal was given two separate s.c. injections at a dose of 1 mg/kg using a solution of HPMPC (1 mg/ml) in PBS. The second foal was given a single dose of 20 mg/kg s.c., for which 1.5 g HPMPC was first dissolved in 7 ml warm DMSO and then added to 120 ml PBS. The doses given were limited by availability of the compound.

Virus strain

EHV-1 strain Ab4 (Gibson et al., 1992) was used throughout this study. Virus working stock was prepared by infecting monolayers of RK-13 cells (see below) at low multiplicity of infection. The working stock, passage 11, had an infectivity of 4×10^8 plaque-forming units/ml (pfu/ml).

Tissue culture

All tissue culture was carried out in Eagle's minimum essential medium (EMEM) to which bicarbonate buffer and antibiotics had been added and supplemented with fetal or neonatal calf serum (FCS or NCS) as appropriate. Two cell-lines were used: a rabbit kidney line, RK-13 cells, and an equine embryonic lung cell-line, EEL's. Cell cultures were maintained at 37°C in a humidified incubator with an atmosphere of air plus 5% CO_2.

Animals

Female Balb/c mice, aged 3–4 weeks, were obtained from Bantam and Kingman (Hull, UK). Mice were kept for one week before infection. Specific

pathogen-free foals (SPF foals) were produced and reared as described previously (Chong et al., 1991). They were infected when 3–4 months old (Gibson et al., 1992) with weights between 70 and 90 kg. At the start of the experiment, these animals were tested serologically (complement fixation, virus neutralisation and fluorescent antibody tests) and by virus isolation methods to establish that they were free from EHV-1, EHV-2 and EHV-4 and also maternally-derived antibodies. The uncertain EHV status of naturally reared horses makes it very difficult to compare EHV-1 pathogenesis and the effect of prophylaxis in a small number of animals. By contrast, pathogenesis and immune responses in SPF foals is very reproducible (Gibson et al., 1992) and this allows meaningful experiments to be carried out in only a small number of animals.

In vitro assays

Antiviral assays were carried out in the rabbit kidney cell line (RK-13) and equine embryonic lung cells (EEL). Monolayers were grown in 5-cm Petri dishes and then infected with 200 pfu EHV-1. Virus was allowed to adsorb for 60 min at 37°C, after which the monolayers were overlaid with EMEM with 1% FCS and 1% carboxymethylcellulose (CMC medium) to which appropriate concentrations of HPMPC had been added. Plaques were allowed to develop over 2 days and were then stained with crystal violet prior to counting.

Infection of mice and foals

Intranasal (i.n.) inoculation of mice was carried out following light anaesthesia with ether. Mice were held ventrodorsally and the inoculum (50 μl) was pipetted slowly onto the nares until all the inoculum was inspired.

For intracerebral (i.c.) inoculation, mice were again anaesthetised with ether and the inoculum (20μl) introduced through a 25-gauge hypodermic needle into the left cerebral hemisphere.

Foal inoculations were carried out while animals were restrained manually. The inoculum (in 4 ml EMEM) was then administered intranasally into both nares via a Pasteur pipette.

All mice and foal infections were carried out using virus working stock suspended in EMEM, the precise dose being established by titration of surplus inoculum. Control animals received a similar sonicated, cell suspension of uninfected RK-13 cells.

Virus isolation from mice and foals

Lungs and turbinates were taken from mice post mortem and stored briefly on ice in 1 ml virus isolation medium (EMEM supplemented with additional antibiotics). Tissues were homogenised, sonicated at 4°C, and then centrifuged at 3000 rpm for 10 min. Supernatants were diluted in further aliquots of virus isolation medium and then plated onto RK-13 monolayers. Virus was allowed to adsorb for 60 min at 37°C, after which CMC medium was added. When viral CPE were visible, usually after 2–3 days, plates were stained with crystal violet and plaques enumerated.

Mucus samples from foals were obtained from nasal cavity and nasopharynx by gentle suction using a foot-operated vacuum pump connected to mucus extractors (Unoplast, Hundestund, Denmark). Samples were diluted into 1 ml virus isolation medium, mixed thoroughly on a vortex, sonicated at 4°C and centrifuged at 3000 rpm for 10 min. Supernatants were titrated as for mice tissues.

Viraemia was assessed in mice and foals by means of infectious centre assay. Blood was collected from foals by jugular venopuncture and from mice by direct cardiac puncture post mortem using 2 mg/ml EDTA as an anticoagulant. Samples were centrifuged in microfuge tubes and the buffy coats were isolated. These were treated with distilled water to lyse the red blood cells, after which isotonicity was restored using 10 × normal strength PBS. White blood cells were washed to remove cell debris, cells were counted in an haemocytometer and known numbers were then plated out onto RK-13 monolayers. Thereafter, samples were treated as for virus isolation from other samples as above, except that plaques usually took longer to develop.

No virus was recovered from any samples taken from uninfected, control mice.

Results

I. The effect of HPMPC against EHV-1 in tissue culture

The activity of HPMPC against EHV-1 was tested in rabbit and equine cell-lines (RK-13 and EEL cells) by plaque reduction assay. In three determinations, the ED_{50} for plaque reduction were found to 0.02–0.20 and 0.01–0.05 μg/ml in RK-13 and EEL cells respectively.

II. The effect of HPMPC against intranasal infection with EHV-1 in mice

In a preliminary experiment, 6 mice were inoculated intranasally with EHV-1 at 4×10^6pfu/mouse. Three mice were given a single administration of HPMPC s.c. at 20 mg/kg on the day of infection; the other 3 mice were given a similar volume of PBS s.c. and are referred to as untreated. All mice developed clinical signs typical of EHV-1 infection: huddling, ruffled fur and dyspnoea. Virus titres were measured in lung and turbinates on day 3 p.i. Mice treated with HPMPC had significantly lower virus titres in the organs compared with untreated animals. Geometric mean titres ± standard error of the mean in lung and turbinates from untreated mice were 4.26 ± 0.83 ($n = 3$) and 3.30 ± 0.24 (3) $\log_{10}$ pfu/organ respectively; those for HPMPC-treated animals were 3.17 ± 0.13 (3) and 1.62 ± 0.70 (3).

On the basis of this preliminary experiment, a fuller study was planned to test the antiviral effects of HPMPC in more detail.

III. The effect of HPMPC against intranasal infection with EHV-1 in mice: different dose regimes

In the second experiment, infected mice were divided into 3 groups of 20. Group 1 (untreated mice) was given an injection of sterile PBS s.c. on the day before infection (day −1); group 2 (HPMPC day −1) received a single administration of HPMPC (20 mg/ml, s.c.) on the same day (day −1), and group 3 (HPMPC day +1) was treated with HPMPC (20 mg/kg, s.c.) on day 1 p.i. All mice were inoculated intranasally with EHV-1 and titration of surplus inoculum showed the exact dose to be 4×10^6 pfu/mouse. Two sets of uninfected, control mice (2 mice in each group) were also kept: one was given HPMPC (20 mg/kg, s.c., day −1) and the other was injected with PBS (s.c., day −1).

Clinical signs

All infected mice showed similar clinical signs on day 1 p.i. Mice were huddled together, reluctant to move and had ruffled fur.

Clinical signs in the untreated mice increased markedly over the next few days. Mice were obviously dyspnoeic and tachypnoeic by day 3 p.i. and signs were exacerbated if the mice were disturbed. Slight amelioration of clinical signs was apparent after day 8 p.i. but all mice were dead by day 11 p.i. Deaths were observed, starting day 3 p.i. and continuing up to day 9 p.i. (Fig. 2).

Mice in group 2 treated with HPMPC on day −1 showed minimal clinical signs. On day 2 p.i. slightly ruffled fur was still apparent, but this had disappeared by day 3 p.i., after which the mice appeared clinically normal. No deaths were seen in this group (Fig. 2).

Mice in group 3 (HPMPC day +1) behaved in a similar fashion to untreated mice until day 6 p.i. Thus clinical signs were severe up to day 6 with high

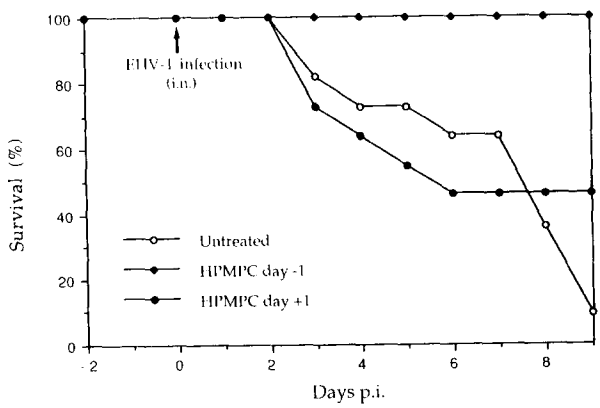

Fig. 2. Effect of administration of HPMPC (20 mg/kg, s.c.) on percentage of mice surviving an intranasal inoculation of EHV-1: untreated mice (PBS, s.c., day −1); HPMPC given on day −1; and HPMPC given on day +1.

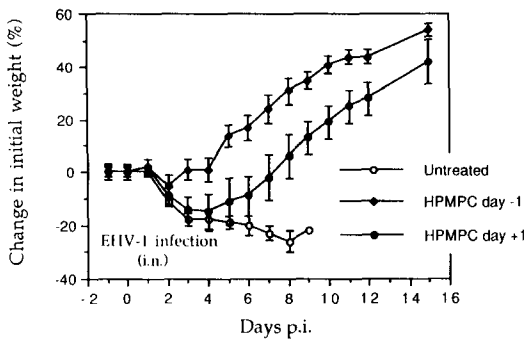

Fig. 3. Effect of administration of HPMPC (20 mg/kg, s.c.) on the weight of mice, expressed as a percentage of their initial weight, following intranasal infection with EHV-1: untreated, control mice, HPMPC given on day −1; and HPMPC given on day +1. Each point represents the mean ± S.E.M. of <20 mice.

mortality (Fig. 2). Thereafter, clinical signs gradually abated: surviving mice showed slight dyspnoea which gradually improved and by day 9 p.i. mice were normal. No deaths were seen after day 6 p.i.

Mice were weighed daily up to day 12 p.i. and intermittently thereafter (Fig. 3). All infected mice showed a weight loss on day 2 p.i. This loss was transient for the mice in group 2 (HPMPC day −1), which regained their pre-infection weight by day 3 p.i. and began to show weight gain from day 5 p.i. Untreated mice showed a sustained fall in weight throughout the experiment. Mice in group 3 (HPMPC day +1) followed the weight curve shown by untreated mice to day 4 p.i. but thereafter showed continual weight gain which paralleled that for mice in group 2 (HPMPC day −1).

No clinical signs were apparent in the uninfected, control mice, with or without administration of HPMPC.

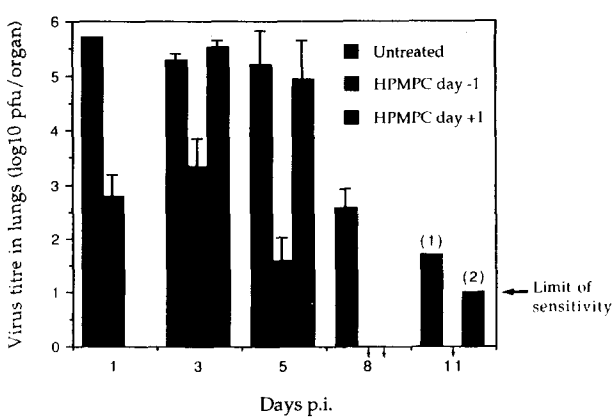

Fig. 4. Effect of administration of HPMPC (20 mg/kg, s.c.) on virus titres in lungs of mice infected intranasally with EHV-1. Mice were untreated, or given HPMPC on day −1 or day +1. Histograms represent the geometric mean ± S.E.M. for 3 mice on each day, unless indicated otherwise.

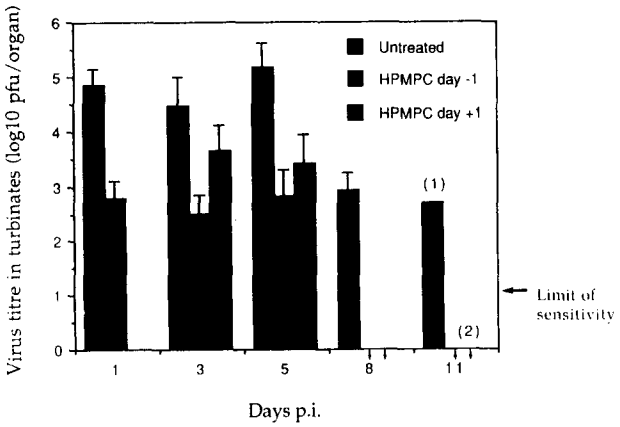

Fig. 5. Effect of administration of HPMPC (20 mg/kg, s.c.) on virus titres in turbinates of mice infected intranasally with EHV-1. Mice were untreated, or given HPMPC on day −1 or day +1. Histograms represent the geometric mean ± S.E.M. for 3 mice on each day, unless indicated otherwise.

Virus isolation

Virus isolation was attempted from lungs, turbinates and buffy coats of infected mice. Tissues from 3 mice in each group were processed on days 1, 3, 5 and 8 p.i. Samples were taken at less frequent intervals thereafter and, because of mortality in groups 1 and 3, from a smaller number of mice on each occasion.

Virus titres in lungs and turbinates are shown in Figs. 4 and 5 respectively. Virus titres in lungs and turbinates were consistently lower in both tissues (by about 2 to 4 $\log_{10}$ units) in mice given HPMPC on day −1 compared to

TABLE 1

Viraemia in mice infected intranasally with EHV-1, measured by means of infectious centre assay and expressed as number of infected cells per 10^6 white blood cells. Mice in group 1 (numbers 1–3) were untreated; mice in group 2 were given a single administration of HPMPC (20 mg/kg, s.c.) on the day before virus inoculation, and mice in group 3 (numbers 7–9) were treated with a single administration of HPMPC (20 mg/kg, s.c.) on the day after infection.

Group	Mouse number	Days p.i.	
		1	5
1	1	67	0
Untreated	2	36	50
	3	13	N.D.
2	4	0	0
HPMPC	5	0	0
Day-1	6	0	0
3	7	N.D.	1
HPMPC	8	N.D.	50
Day+1	9	N.D.	1

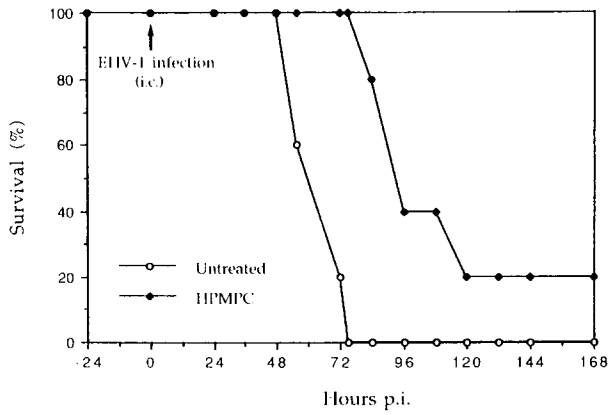

Fig. 6. Effect of administration of HPMPC on percentage of mice surviving an intracerebral inoculation of EHV-1. Mice were either untreated or given HPMPC, 20 mg/kg, s.c., on day −1 and day +1.

untreated controls. Also, mice treated on day −1 cleared virus from all tissues by day 8 p.i., in contrast to untreated mice which continued to show virus replication in lung and turbinates throughout the experiment, the last surviving mouse in this group being killed on day 11 p.i. Mice given HPMPC on day +1 showed no significant reduction in lung titres until day 8 p.i., compared to untreated controls. In turbinates, however, titres were reduced by 1 to 2 log units on days 3 and 5 p.i. Mice treated on day +1 were free from virus on day 8 p.i. but virus was found in the lungs of one mouse out of two on day 11 p.i.

Analysis of buffy coat samples by means of infectious centre assay showed that untreated mice and those treated with HPMPC on day +1 were viraemic on days 1 and 5 p.i. Virus was found in the blood of mice treated on day −1 only on the day after infection (day 1 p.i.), and this was at a very low levels (1 infectious centre per 10^6cells) in one mouse out of three (Table 1).

IV. The effect of HPMPC on intracerebral infection of mice with EHV-1

Although EHV-1 is not thought to be highly neuropathogenic for adult mice, previous work has shown that it is able to establish a productive infection in mice following intracerebral (i.c.) inoculation (Slater, unpublished observations). Mice given $>10^5$ pfu i.c. show almost 100% mortality after about 3 days, with histological evidence of encephalitis and meningitis. We therefore tested the effect of administration of HPMPC on mortality of mice following intracerebral inoculation of EHV-1.

Ten mice were divided into two groups of 5. HPMPC was administered to one group on two occasions, the day before and the day after infection, at a dose of 20 mg/kg s.c.; the other group received PBS s.c. Both groups of mice were then inoculated with EHV-1 i.c., and titration of surplus inoculum confirmed the dose as 4×10^5 pfu/mouse. Three control mice were given an intracerebral injection of uninfected, sonicated RK-13 cells suspended in

EMEM: no clinical signs were apparent in these mice.

Following infection, all infected mice developed clinical signs, including signs of neurological disease, such as inco-ordination and ataxia. Untreated mice began to die 56 h following infection, with 100% mortality by 75 h p.i. A different pattern was observed in mice treated with HPMPC. No deaths were seen before 84 h p.i. and one mouse survived the infection. Cumulative mortality for both groups is shown in Fig. 6.

V. The effect of HPMPC against intranasal infection with EHV-1 in SPF foals

The efficacy of HPMPC against EHV-1 in the natural host was tested using two SPF foals. Both foals were treated with HPMPC; one foal, F3, was given the drug in a single dose at 20 mg/kg s.c. on the day of infection, the other foal, F7, received a divided dose, 1 mg/kg s.c. on the day of infection and again on day 3 p.i.; 3 control foals, F1, F11 and F13, were untreated. All 5 animals were infected intranasally with EHV-1; surplus inoculum was titrated and the dose was determined to be 10^7 pfu/foal.

Clinical signs

All 3 untreated foals, F1, F11 and F13, developed typical signs of EHV-1 infection. These comprised a biphasic pyrexia, ocular and nasal discharge, conjunctivitis, lethargy, depression and enlarged submandibular lymph nodes. Similar signs were observed in the foal (F7) treated with the low dose of HPMPC. Clinical signs, other than the rise in temperature, were considerably less in the foal, F3, receiving 20 mg/kg HPMPC. By day 5 p.i., foal F3 was clinically normal, apart from an elevated rectal temperature. The other foals at this time continued to show depression, enlarged submandibular lymph nodes and profuse mucopurulent oculonasal discharges.

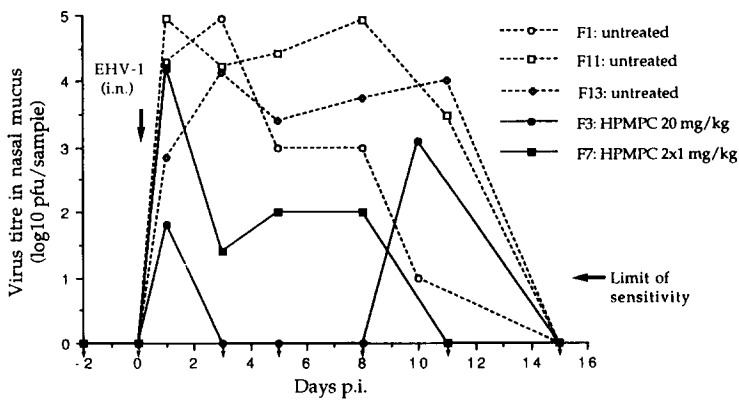

Fig. 7. Virus excretion in nasal mucus of five SPF foals infected intranasally with EHV-1. Three foals, F1, F11 and F13, untreated. F3 was given a single administration of HPMPC at 20 mg/kg on the day of infection; F7 received two doses of HPMPC at 1 mg/kg on the day 0 of infection and day 3 p.i.

Virus isolation

Virus isolation was monitored in both nasal mucus samples and buffy coat cells. Nasal excretion of virus is shown in Fig. 7. Untreated foals (F1, F11 and F13) showed peak virus titres of 10^5 pfu, with excretion continuing over at least 10 days, and up to 11 days in the case of two of the foals, F11 and F13. By contrast, in the foal, F1, given HPMPC at 20 mg/kg virus could be detected only on days 1 and 10 p.i. and not on any of the intermediate days. Furthermore, the titre on day 1 p.i. was 3 $\log_{10}$ units lower than that seen in the untreated foals. Virus excretion in the foal (F7) given two doses of HPMPC at 1 mg/kg was considerably higher than for the foal treated with 20 mg/kg but titres were less than those observed in the untreated animals (Fig. 7). Both foals treated with HPMPC and two out of the three untreated foals became viraemic on at least one day between days 3 and 11 p.i., at titres of between 1 infected cell per 10^5 to 10^6 white blood cells.

Discussion

The present findings demonstrate that HPMPC is a very effective antiviral agent against EHV-1 in vitro. In addition, we show that the drug is also highly active against EHV-1 in vivo in mice and, on the basis of two animals, in the natural host. Activity in both mice and horses could be seen following a single administration of the compound.

In tissue culture, HPMPC was found to have an ED_{50} against EHV-1 of approx. 0.07 and 0.03 μg/ml in RK-13 and EEL cells, respectively. Thus its activity against EHV-1 is comparable to that against African swine fever virus and Epstein-Barr virus, and two orders of magnitude greater than against HSV-1 and BHV-1 (De Clercq et al., 1987). The activity of HPMPC against EHV-1 compares favourably with that of other antiviral agents: reported ED_{50} values (all μg/ml) obtained in tissue culture are 0.01 for DHPG (9-[1,3-dihydroxy-2-propoxymethyl] guanine; Rollinson and White, 1983); 0.1 for (S)-9-[(3-hydroxy-2-phosphonyl methoxy) propyl] adenine (HPMPA; Field and Awan, 1990); 4.0 for PMEA (9-[2-phosphonyl-methoxyethyl] adenine; Field and Awan, 1990) and 0.4–7.0 for ACV (acyclovir; Rollinson and White, 1983; De Clercq et al., 1986).

To date, the acyclic phosphonates have proved extremely effective against a wide range of viruses (see De Clercq, 1991). The activity of HPMPC against viruses in vivo has been demonstrated in several species and against several viruses, including murine cytomegalovirus in mice (Kern et al., 1989); guinea pig cytomegalovirus in guinea pigs (Li et al., 1990); HSV-1 in rabbits and mice (Maudgal and De Clercq, 1991; De Clercq, 1991; De Clercq and Holy, 1991); simian varicella in African Green monkeys (Soike et al., 1991); and bovine herpesvirus-1 in calves (Gilliam and Field, 1992).

Our results extend these findings to EHV-1. We have demonstrated that HPMPC reduces clinical signs and virus replication in mice following

intranasal infection with EHV-1 (Figs. 2, 3, 4 and 5). At a dose of 20 mg/kg given on the day before infection in mice, HPMPC markedly reduces virus yields and almost abolishes clinical signs following intranasal infection. Significant virus titres were observed, however, for 5 days p.i., but viraemia, which is thought to be central in mediating the abortigenic and neurological effects of EHV-1, was very much reduced. By contrast, treatment on the day of infection (Results, section II) was less effective, whilst administration of a single dose on the day after infection appeared to reduce the effectiveness of HPMPC. Thus in mice treated with HPMPC on day +1, it required 4–6 days before full antiviral effect was seen (Figs. 2, 3, 4 and 5). A possible reason for this may have been the high dose of virus inoculated resulting in the rapid establishment of an overwhelming respiratory infection and untreated mice showed relatively high levels of virus replication in the target tissues (cf. Awan et al., 1990; Field and Awan, 1990).

Recently, we have observed that EHV-1 is also pathogenic following intracerebral inoculation in mice. Although EHV-1 is not generally considered to be neuropathogenic to adult mice, it is able to establish a productive infection following inoculation via this route. All mice infected with a dose of $>10^5$ pfu show neurological signs with 100% mortality. Histologically, focal encephalitis and meningitis are observed. In the present paper, we show that administration of HPMPC was also able to protect mice partially against intracerebral infection with EHV-1 (Fig. 6), both reducing and delaying mortality.

The chemotherapeutic effects of HPMPC against EHV-1 in mouse experiments encouraged us to investigate its efficacy in the natural host, the horse. It is difficult, however, to perform experiments with EHV-1 in naturally reared horses because their immune status is very variable. Even seronegative animals can show considerable variation in their response to the virus (personal observations). It is necessary, therefore, to make use of SPF foals. These animals show highly reproducible responses to EHV-1 (Gibson et al., 1992). Because of the cost and difficulty of producing SPF foals, only a small number of animals were available for the present study. We have demonstrated, however, that HPMPC is highly active against nasal excretion of EHV-1 in the natural host when given as a single dose of 20 mg/kg s.c. on the day of infection, and even had some activity when given in two doses of 1 mg/kg (Fig. 8). We have observed consistent patterns of clinical signs and virus excretion in all previous EHV-1 infections in SPF foals (14 animals, to date), and thus although only two foals were treated with HPMPC in the present experiment, we regard the results as highly indicative of effective antiviral action.

In the foal given 20 mg/kg, EHV-1 was isolated on the day following infection (at very low titre), and then no virus was recovered for the next 9 days. Mucus samples on day 10 p.i., however, did contain virus. The present work does not allow us to establish the reason for this. It is possible, however, that EHV-1 was able to establish latency and virus recovery on day 10 p.i. resulted from reactivation (Edington et al., 1985; Gibson et al., 1992). This

question, and an investigation of the effects of HPMPC on the immune response to EHV-1, will be addressed in further experiments.

Finally, although no specific tests were carried out to address the possible toxicity of HPMPC in mice or foals, no ill-effects were apparent other than a slight growth retardation in uninfected mice given a single administration of HPMPC at 20 mg/kg (cf. guinea pigs, Li et al., 1990). No clinical signs of toxic effects were seen in the two foals.

In summary, the present findings show that HPMPC is an effective antiviral agent against EHV-1 in vitro. In addition, the drug is active against EHV-1 in vivo in both mice and foals, even following a single administration. These results suggest that, providing serious toxicity is not encountered, HPMPC has potential as a chemotherapeutic and/or chemoprophylactic agent against EHV-1.

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