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Short communication

Effect of oral treatment with (*S*)-HPMPA, HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on replication of murine cytomegalovirus (MCMV) or human cytomegalovirus (HCMV) in animal models

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Abstract

We utilized BALB/c mice infected with murine CMV (MCMV) or severe combined immunodeficient (SCID) mice implanted with human fetal tissue and infected with HCMV to determine the efficacy of (*S*)-9-[3-hydroxy-2-(phophonomethoxy)propyl]adenine ((*S*)-HPMPA), hexadecyloxypropyl-(*S*)-HPMPA (HDP-(*S*)-HPMPA) or octadecyloxyethyl-(*S*)-HPMPA (ODE-(*S*)-HPMPA). In MCMV-infected BALB/c mice, oral HDP-(*S*)-HPMPA at 30 mg/kg significantly reduced mortality when started 24–48 h post inoculation. In the experimental HCMV infection, oral administration of vehicle or 10 mg/kg of (*S*)-HPMPA, HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA was initiated 24 h after infection and continued for 28 consecutive days. Cidofovir (CDV), at 20 mg/kg given i.p., was used as a positive control. HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA significantly reduced viral replication compared to vehicle-treated mice, while oral (*S*)-HPMPA was ineffective.

Keywords: Cytomegalovirus; Antiviral; Animal models; Phosphonate nucleotides

Synthesis of orally active alkoxyalkyl esters of cidofovir (CDV) and (*S*)-9-[3-hydroxy-2-(phophonomethoxy)propyl]adenine ((*S*)-HPMPA) have resulted in modified compounds that are highly effective in vitro against both orthopoxviruses and cytomegalovirus (CMV) (Beadle et al., 2006; Keith et al., 2004). The EC_{50} values of (*S*)-HPMPA, hexadecyloxypropyl-(S)-HPMPA (HDP-(*S*)-HPMPA), octadecyloxyethyl-(*S*)-HPMPA (ODE-(S)-HPMPA) and CDV against murine CMV (MCMV) and HCMV, respectively, were 0.16, 0.002, 0.003 and 0.04 for MCMV and 0.82, 0.003, 0.003 and 1.2 for HCMV (Beadle et al., 2006). (*S*)-HPMPA, HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA were synthesized as reported previously (Quenelle et al., 2007). These compounds were given orally to mice by gavage once daily using a 0.2 ml volume. CDV was given once daily i.p. as a positive control in a 0.1 ml volume.

MCMV infections were initiated by i.p. inoculation of BALB/c mice with 6×10^4 PFU/mouse, approximately the 90% lethal dose (Kern et al., 2004a,b), and HDP-(*S*)-HPMPA at doses of 30, 10 or 3 mg/kg was administered beginning 24, 48 or 72 h post viral inoculation and the results are summarized in Table 1. When HDP-(*S*)-HPMPA was administered to BALB/c mice for 5 days using 30, 10 or 3 mg/kg once daily there was no observed toxicity as indicated by mortality or clinical signs (data not shown). When HDP-(*S*)-HPMPA was given at 30 mg/kg, mortality due to MCMV was reduced significantly ($p < 0.001$) even when initiation of treatment was delayed until 48 h post viral inoculation. There appears to be a reasonable dose–response with HDP-(*S*)-HPMPA. However, CDV, the positive control, was more effective in preventing mortality.

HCMV infections were initiated by direct intra-xenograft inoculation of human fetal tissues implanted beneath the renal capsule of SCID mice (Bidanset et al., 2004; Kern et al., 2004a,b,c) using 4700 PFU of HCMV (strain Toledo). Six to 12 implants were sampled on days 14, 21, 28 and 35, homogenized, and frozen at −70 °C until assayed for HCMV using a plaque assay in human foreskin fibroblasts (Bidanset et al., 2004; Kern

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Table 1
Effect of oral treatment with HDP-(S)-HPMPA on the mortality of BALB/c mice inoculated intraperitoneally with MCMV

Treatment[a]	Mortality		*p*-Value[b]	MDD ± S.D.[c]	*p*-Value[d]
	Number	Percent			
Vehicle-deionized water + 24 h	14/15	93		5.6 ± 0.5	
CDV 10 mg/kg, i.p.	0/15	0	<0.001		
HDP-(*S*)-HPMPA 30 mg/kg	1/15	7	<0.001	6.0	NS[e]
HDP-(*S*)-HPMPA 10 mg/kg	2/15	13	<0.001	6.0 ± 0	NS
HDP-(*S*)-HPMPA 3 mg/kg	15/15	100	NS	6.3 ± 0.6	<0.01
Vehicle-deionized water + 48 h	15/15	100		5.7 ± 0.6	
CDV 10 mg/kg, i.p.	0/15	0	<0.001		
HDP-(*S*)-HPMPA 30 mg/kg	5/15	33	<0.001	9.0 ± 3.1	<0.05
HDP-(*S*)-HPMPA 10 mg/kg	14/15	93	NS	6.4 ± 0.5	<0.01
HDP-(*S*)-HPMPA 3 mg/kg	10/15	67	<0.05	6.6 ± 1.1	<0.05
Vehicle-deionized water + 72 h	15/15	0		5.2 ± 0.6	
CDV 10 mg/kg, i.p.	5/15	33	<0.001	7.4 ± 3.0	0.08
HDP-(*S*)-HPMPA 30 mg/kg	15/15	100	NS	6.0 ± 0.8	<0.01
HDP-(*S*)-HPMPA 10 mg/kg	15/15	100	NS	5.7 ± 0.8	0.04
HDP-(*S*)-HPMPA 3 mg/kg	15/15	100	NS	5.5 ± 0.5	NS

[a] CDV was prepared in sterile saline and delivered i.p. in 0.1 ml doses. HDP-(*S*)-HPMPA was prepared in deionized water and delivered p.o. in 0.2 ml doses. Animals were treated once daily for 5 days beginning 24, 48 or 72 h after viral inoculation.
[b] Statistical significance determined for mortality rates by two-tailed Fisher's exact test with $p < 0.05$ considered significant when compared to vehicle-treated controls.
[c] MDD, Mean day of death.
[d] Statistical significance determined for mean day of death by Mann-Whitney *U* rank sum with $p < 0.05$ considered significant when compared to vehicle-treated controls.
[e] NS, Not significant.

et al., 2004a,b,c). When HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA was given orally once daily for 28 days at 10 mg/kg to SCID mice inoculated with HCMV, a significantly reduced number of positive biopsy samples were found on day 28 and days 14–35, respectively, and HCMV titers in biopsy samples was reduced significantly ($p < 0.05$) by 1.4–2.8 logs (Table 2). The (*S*)-HPMPA-treated group had reduced numbers of positive implants on day 14 and CDV was active at all time points. Overall, the comparable order of efficacy of compounds in these studies was CDV > ODE-(*S*)-HPMPA > HDP-(*S*)-HPMPA > (*S*)-HPMPA.

Previous work with HDP-cidofovir (HDP-CDV) has shown that the addition of the ether lipid side chains improved oral bioavailability and also altered the cellular and systemic distribution in a way which markedly reduced the renal toxicity of CDV (Ciesla et al., 2003). Esterification of (*S*)-HPMPA with an alkoxyalkyl group alters drug metabolism, distribution and

Table 2
Effect of oral treatment with (*S*)-HPMPA, HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on infection rates and viral titers in SCID-hu/thymus liver implant tissues inoculated with HCMV

Treatment[a]	Parameter	Days post infection			
		14	21	28	35
Vehicle-water	% Positive (#)[b]	83 (10/12)	75 (9/12)	45 (5/11)	36 (4/11)
	$\log_{10}$ PFU/g[c]	5.6 ± 5.9	4.9 ± 5.1	4.8 ± 5.1	4.7 ± 4.9
CDV (i.p.) 20 mg/kg	% Positive (#)	0 (0/6)[d]	0 (0/6)[d]	0 (0/2)	0 (0/2)
	$\log_{10}$ PFU/g	0 ± 0	0 ± 0	0 ± 0	0 ± 0
(*S*)-HPMPA 10 mg/kg	% Positive (#)	33 (4/12)[d]	42 (5/12)	50 (5/10)	50 (4/8)
	$\log_{10}$ PFU/g	4.8 ± 5.3	4.7 ± 5.3	4.5 ± 4.8	4.8 ± 4.9
HDP-(*S*)-HPMPA 10 mg/kg	% Positive (#)	42 (5/12)	70 (7/10)	9 (1/11)[d]	40 (4/10)
	$\log_{10}$ PFU/g	4.8 ± 5.1	3.5 ± 3.8[d]	3.4 ± 3.9[d]	4.3 ± 4.6
ODE-(*S*)-HPMPA 10 mg/kg	% Positive (#)	0 (0/12)[d]	33 (4/12)[d]	11 (1/9)[d]	13 (1/8)[d]
	$\log_{10}$ PFU/g	0 ± 0	4.2 ± 4.6	2.1 ± 2.6[d]	4.4 ± 4.9

[a] Treatments were administered once daily for 28 consecutive days by oral gavage beginning 24 h post viral inoculation. CDV was administered i.p. as the positive control.
[b] Number of positives evaluated by two-tailed Fisher's Exact test with $p < 0.05$ considered significant.
[c] Titers are shown as $\log_{10}$ plaque forming units per gram of biopsy tissue and evaluated by stratified Wilcoxon rank sum test with $p < 0.05$ considered significant. The results obtained throughout the entire 28-day treatment period were used to calculate significance.
[d] Significant ($p < 0.05$).

toxicity profile. The hexadecyloxypropyl esters of CDV and (*S*)-HPMPA show gastrointestinal toxicity at high oral doses but no evidence of kidney or liver toxicity (Hostetler, unpublished observations, 2007). The leading active compound identified in these studies was ODE-(*S*)-HPMPA, and additional work is clearly needed to further investigate the toxicity and the potential efficacy of these acyclic nucleoside phosphonate analogues against CMV and other herpesvirus infections.

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