

Oral Treatment of Murine Cytomegalovirus Infections with Ether Lipid Esters of Cidofovir

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To improve the oral bioavailability of cidofovir (CDV), a series of ether lipid ester prodrugs were synthesized and evaluated for activity against murine cytomegalovirus (MCMV) infection. Four of these analogs, hexadecyloxypropyl (HDP)-CDV, octadecyloxyethyl (ODE)-CDV, oleyloxyethyl (OLE)-CDV, and oleyloxypropyl (OLP)-CDV, were found to have greater activity than CDV against human CMV and MCMV in vitro. The efficacy of oral treatment with these compounds against MCMV infections in BALB/c mice was then determined. Treatment with HDP-CDV, ODE-CDV, OLE-CDV, or OLP-CDV at 2.0 to 6.7 mg/kg of body weight provided significant protection when daily treatments were initiated 24 to 48 h after viral inoculation. Additionally, HDP-CDV or ODE-CDV administered twice weekly or as a single dose of 1.25 to 10 mg/kg was effective in reducing mortality when treatment was initiated at 24 h, 48 h, or, in some cases, 72 h after viral inoculation. In animals treated daily with HDP-CDV or ODE-CDV, virus titers in lung, liver, spleen, kidney, pancreas, salivary gland, and blood were reduced 3 to 5 log₁₀-fold, which was comparable to CDV given intraperitoneally. These results indicated that HDP-CDV or ODE-CDV given orally was as effective as parenteral CDV for the treatment of experimental MCMV infection and suggest that further evaluation for use in CMV infections in humans is warranted.

Cytomegalovirus (CMV) infections continue to be a major cause of morbidity in solid organ (35) and stem cell recipients (10, 12). The management of these infections has generally been through the use of prophylaxis or prevention therapy with ganciclovir (GCV) (15) or acyclovir (ACV) (3); however, the lack of activity when they are administered orally has limited their use. While the availability of oral ACV (valacyclovir) (29) and GCV (valganciclovir) (32) has broadened the range of choices for treatment or prevention of CMV infections, a number of problems, including neutropenia, nephrotoxicity, and the selection of drug-resistant mutants, remain (6, 8, 13, 27, 28, 39). Cidofovir (CDV) has greater activity against CMV in vitro and in vivo than any of the other licensed drugs (16, 22, 23, 40) and retains activity against many of the GCV- and foscarnet (PFA)-resistant mutants (4). However, CDV does not have activity when given orally and may also cause severe nephrotoxicity (25, 37). One approach to overcoming the limitation of the lack of activity of CDV given orally has been to prepare prodrugs of CDV that are bioavailable when administered orally. The synthesis of alkylglycerol phosphate or alkylpropyl phosphate esters of ACV, penciclovir, or GCV provided prodrugs that were active when given orally in animal models of herpes simplex virus, murine CMV (MCMV), and woodchuck hepatitis virus infections (17–19). Using similar methodology, a series of ether lipid ester analogs of CDV or cyclic CDV were prepared and evaluated for activity against CMV and poxviruses (4, 24).

It has been reported previously that this series of ether lipid

ester prodrugs of CDV, particularly the hexadecyloxypropyl (HDP)-CDV and octadecyloxyethyl (ODE)-CDV analogs, exhibited potency that was multiple-log-enhanced compared with that of GCV and CDV for a variety of clinical and laboratory isolates of CMV (4). In addition, the analogs retained their activity against drug-resistant isolates with mutations in the UL97 or UL54 gene. It has also been reported that HDP-CDV and ODE-CDV had markedly enhanced activity in vitro against vaccinia and cowpox viruses (24) and were shown to have activity when administered orally in pharmacokinetic (9) and efficacy studies in mice (34).

Since there are no animal models that closely approximate human CMV (HCMV) disease, the surrogate viruses, MCMV, rat CMV (RCMV), and guinea pig CMV (GPCMV), have been used for preclinical evaluation of potential therapies for CMV infections (14, 22, 26, 41). We have used the murine model extensively for evaluation of new compounds and have reported previously that CDV has more activity than GCV (20, 21, 22).

The purpose of the experiments reported here was to compare the efficacies of HDP-CDV and ODE-CDV given orally with that of CDV given intraperitoneally (i.p.) for their ability to alter mortality and virus replication in target organs of mice infected with MCMV. Since CDV has been reported to have a long intracellular half-life (11) and can be efficacious when administered one to three times weekly (22, 33), we also determined the activities of these ether lipid esters when administered either once daily, twice weekly, or as a single dose.

MATERIALS AND METHODS

In vitro efficacy and toxicity. The activities and toxicities of the four ether lipid esters of CDV in vitro were determined in primary murine embryo fibroblast (MEF) cells using standard plaque assay methods described previously (36).

Virus. MCMV, strain Smith, was used both in vitro and in vivo.

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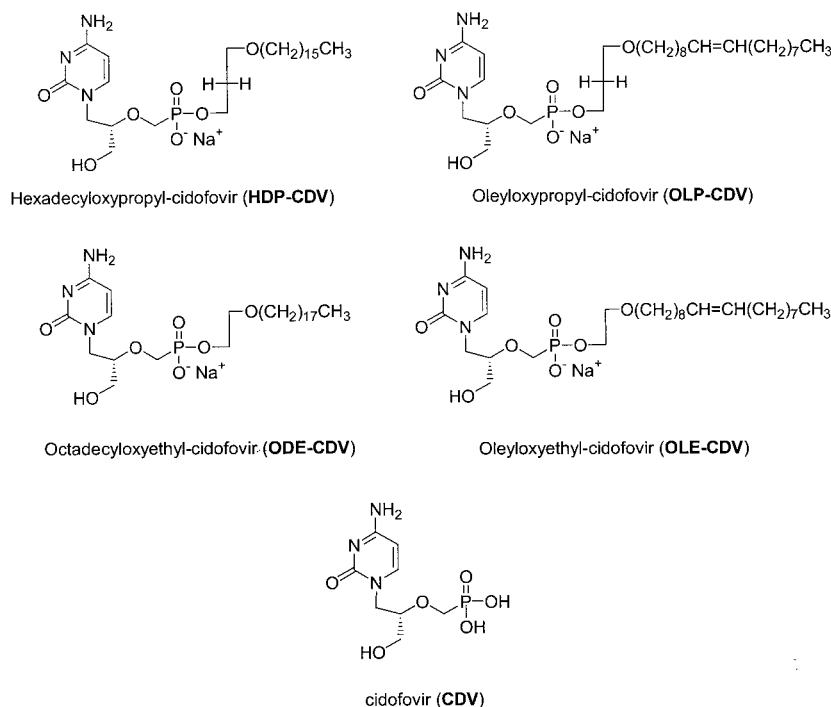


FIG. 1. Structures of CDV, HDP-CDV, ODE-CDV, OLP-CDV, and OLE-CDV.

Mice. Female BALB/c mice, 3 weeks of age, were obtained from Charles River Laboratories, Raleigh, N.C. Mice were group housed in microisolator cages and utilized at a quantity of 15 mice per treatment group for statistical analysis. Mice were obtained, housed, utilized, and euthanized according to USDA and Association for Assessment and Accreditation of Laboratory Care regulatory policies. All animal procedures were approved by The University of Alabama at Birmingham Institutional Animal Care and Use Committee prior to initiation of the studies.

Antiviral compounds. Cidofovir (Vistide; Gilead Pharmaceuticals, Foster City, Calif.) was diluted in sterile saline to yield the desired doses in 0.1-ml volumes. It was administered i.p. once daily for 5 days, two times weekly, or as a single dose, depending on the experimental protocol. HDP-CDV and ODE-CDV were synthesized, purified, and characterized as reported previously (4, 24) and provided as dry powders. OLE-CDV and OLP-CDV were synthesized by a similar methodology using oleyl bromide instead of hexadecyl bromide or octadecyl bromide. The structures of the compounds are shown in Fig. 1. The degree of purity was similar to that reported for HDP-CDV and ODE-CDV. The dry powders were weighed and dissolved in deionized water to yield the desired doses within 0.2-ml volumes for oral gavage. Each was administered orally as a single dose, twice weekly, or once daily for 5 days, depending on the experimental protocol. Uninfected mice served as toxicity controls for each compound and were treated similarly.

Experimental infections and viral pathogenesis. MCMV infections were initiated by i.p. inoculation of BALB/c mice with an approximate 90% lethal dose and observed daily for 21 days. In certain experiments, samples of lung, liver, spleen, kidney, pancreas, salivary gland, and blood were obtained from three mice per treatment group or from control mice on day 1, 3, 5, 7, 10, 12, or 15 after MCMV infection. The samples were homogenized in medium (10%, wt/vol) and frozen at -70°C until they were assayed for virus (22).

Virus quantitation. Tissue samples were thawed and assayed on MEF cells by plaque assay to determine MCMV titers (22). Briefly, samples of organ homogenates were diluted serially, and 0.2-ml volumes were placed into duplicate wells of 12-well plates containing MEF cell monolayers and incubated for 1 h. Medium containing 2% fetal bovine serum was added to each well, and the cultures were incubated for 7 days. The cultures were stained with neutral red (Gibco, Rockland, Md.) for approximately 6 h prior to enumeration of viral plaques.

Statistical evaluations. Mortality rates were analyzed by Fisher's exact test, and mean days of death and virus titers in tissues were analyzed using the Mann-Whitney U rank sum test. A *P* value of ≤ 0.05 was considered significant.

RESULTS

In vitro activity of ether lipid esters of CDV. It was reported previously that HDP-CDV and ODE-CDV were at least 100-fold more active than CDV against MCMV, RCMV, and GPCMV (4). For comparison purposes, these results, along with those for GCV and CDV, are included with those for the new analogs, oleyloxyethyl (OLE)-CDV and oleyloxypropyl (OLP)-CDV, in Table 1. In these studies, all four of the analogs were approximately 2- to 10-fold more active against MCMV than unmodified CDV. With the exception of OLP-CDV against RCMV, HDP-CDV, ODE-CDV, OLE-CDV, and OLP-CDV were 30- to 300-fold more active against RCMV and GPCMV than CDV.

Effect of daily treatment with HDP-CDV, ODE-CDV, OLE-CDV, or OLP-CDV on MCMV infections of mice. To determine the abilities of these compounds to influence mortality rates in mice infected with MCMV, the compounds were dis-

TABLE 1. In vitro activity of HDP-CDV, ODE-CDV, OLE-CDV or OLP-CDV against MCMV, RCMV, or GPCMV replication

Compound	50% Inhibitory concentration (μM) ^a against:		
	MCMV	RCMV	GPCMV
GCV	4.3 ± 1.7	48.2 ± 1.1	239.0 ± 10.6
CDV	0.01 ± 0.01	0.3 ± 0.03	0.31 ± 0.003
HDP-CDV	0.0009 ± 0	0.004 ± 0.001	0.0009 ± 0.00007
ODE-CDV	0.001 ± 0	0.005 ± 0	0.0009 ± 0.00007
OLE-CDV	0.004 ± 0.004	0.008 ± 0.003	0.0013 ± 0.0003
OLP-CDV	0.004 ± 0.003	0.12 ± 0.13	0.007 ± 0.001

^a Values are expressed as means \pm standard deviation from at least two plaque reduction assays.

TABLE 2. Effect of daily oral treatment with HDP-CDV, ODE-CDV, OLE-CDV, or OLP-CDV on mortality of BALB/c mice inoculated i.p. with MCMV

Time of initiation and treatment (mg/kg) ^a	No. of mice died/total no. (%)	Mortality result <i>P</i> value ^b	MDD ^c	MDD <i>P</i> value ^b
24 h				
i.p. saline placebo	12/15 (80)		6.3	
Oral water placebo	8/15 (53)		5.6	
CDV, 6.7	0/15 (0)	<0.001		
CDV, 2.0	0/15 (0)	<0.001		
HDP-CDV, 6.7	2/15 (13)	0.02	3.5	<0.05
HDP-CDV, 2.0	3/15 (20)	NS	4.0	0.07
ODE-CDV, 6.7	0/15 (0)	<0.001		
ODE-CDV, 2.0	1/15 (7)	0.01	5.0	NS
OLE-CDV, 6.7	1/15 (7)	0.01	6.0	NS
OLE-CDV, 2.0	0/15 (0)	<0.001		
OLP-CDV, 6.7	0/15 (0)	<0.001		
OLP-CDV, 2.0	1/15 (7)	0.01	4.0	NS
48 h				
i.p. saline placebo	15/15 (100)		5.1	
Oral water placebo	15/15 (100)	<0.001	5.3	
CDV, 6.7	0/15 (0)	<0.001		
CDV, 2.0	0/15 (0)	<0.001		
HDP-CDV, 6.7	0/15 (0)	<0.001		
HDP-CDV, 2.0	2/15 (13)	<0.001	6.0	NS
ODE-CDV, 6.7	5/15 (33)	<0.001	10.2	<0.001
ODE-CDV, 2.0	0/15 (0)	<0.001		
OLE-CDV, 6.7	0/15 (0)	<0.001		
OLE-CDV, 2.0	3/15 (20)	<0.001	8.0	0.09
OLP-CDV, 6.7	1/15 (7)	<0.001	8.0	0.06
OLP-CDV, 2.0	2/16 (13)	<0.001	7.5	NS

^a Compounds were prepared daily in water and delivered orally in 0.2-ml doses, except CDV, which was prepared in sterile saline and delivered i.p. in 0.1-ml doses. Animals were treated once daily for 5 days beginning 24 or 48 h after viral inoculation.

^b *P* values were determined by comparing results for drug treatment groups with results for placebo control groups. NS, not significant compared to placebo controls.

^c MDD, mean day of death.

solved in deionized water at doses of 20, 6.7, or 2 mg/kg of body weight and administered once daily for 5 consecutive days by oral gavage beginning 24, 48, or 72 h after viral inoculation. CDV was administered i.p. in similar doses for comparison, although it should be noted that the molecular weight of CDV is roughly 50% of that of the ether lipid conjugates of CDV. Toxicity was associated with the 20 mg/kg dose of HDP-CDV, ODE-CDV, OLE-CDV, or OLP-CDV, and some mortality and weight loss were observed (data not shown). At the 6.7 or 2.0 mg/kg dose, no toxicity was observed, and each compound significantly reduced final mortality rates when treatment was initiated at 24 to 48 h postinfection (Table 2). Only CDV at 6.7 mg/kg significantly reduced mortality rates when treatment was initiated at 72 h after viral inoculation (data not shown). Based on these data and other characteristics of the four analogs, HDP-CDV and ODE-CDV were selected for additional evaluation.

Effect of twice-weekly treatment with HDP-CDV or ODE-CDV on MCMV infections of mice. CDV has also been reported to significantly reduce mortality of cowpox or vaccinia virus-infected mice with only one or two doses due to the long intracellular half-life of this drug (33). CDV, HDP-CDV, or ODE-CDV was given at 10, 5 (results not presented), 2.5, or

1.25 mg/kg twice weekly, 3 days apart beginning 24, 48, or 72 h after viral inoculation. Oral treatment with HDP-CDV or ODE-CDV provided significant protection from mortality when initiation of treatment at concentrations as low as 1.25 mg/kg was delayed until 24 to 48 h postinoculation (Table 3). Again, CDV given i.p. was the most active drug when treatment was delayed until 72 h postinfection.

Effect of single-dose treatment with HDP-CDV or ODE-CDV on MCMV infections of mice. In the next experiment, CDV was given i.p., and HDP-CDV or ODE-CDV was given orally as a single dose of 30, 10, or 3 mg/kg beginning 24, 48, or 72 h after viral inoculation. Most of the treatment regimens used provided significant protection from mortality when initiated up to 48 h postinfection. When treatment was initiated at 72 h, little protection was observed. CDV significantly inhibited mortality rates when 30 mg/kg was given at 72 h after infection (Table 4).

Effect of CDV, HDP-CDV, or ODE-CDV on the pathogenesis of MCMV infections of mice. To compare the effect of treatment with oral HDP-CDV or ODE-CDV on the replication of MCMV in target organs of mice to that of treatment with i.p.

TABLE 3. Effect of twice-weekly oral treatment with HDP-CDV or ODE-CDV on mortality of BALB/c mice inoculated i.p. with MCMV

Time of initiation and treatment (mg/kg) ^a	No. of mice died/total no. (%)	Mortality result <i>P</i> value ^b	MDD ^c	MDD <i>P</i> value ^b
24 h				
Oral water placebo	15/15 (100)		4.5	
i.p. saline placebo	15/15 (100)		5.4	
CDV, 2.5	0/15 (0)	<0.001		
CDV, 1.25	1/15 (7)	<0.001	10.0	0.08
HDP-CDV, 2.5	0/15 (0)	<0.001		
HDP-CDV, 1.25	3/15 (20)	<0.001	4.0	NS
ODE-CDV, 2.5	4/15 (27)	<0.001	4.8	NS
ODE-CDV, 1.25	1/15 (7)	<0.001	12.0	0.07
48 h				
Oral water placebo	14/14 (100)		4.8	
i.p. saline placebo	15/15 (100)		5.5	
CDV, 2.5	3/15 (20)	<0.001	4.7	0.07
CDV, 1.25	1/15 (7)	<0.001	7.0	0.07
HDP-CDV, 2.5	4/13 (31)	<0.001	4.8	NS
HDP-CDV, 1.25	12/15 (80)	NS	4.3	NS
ODE-CDV, 2.5	3/14 (21)	<0.001	3.0	<0.05
ODE-CDV, 1.25	3/15 (20)	<0.001	4.0	NS
72 h				
Oral water placebo	14/15 (93)		5.1	
i.p. saline placebo	15/15 (100)		4.9	
CDV, 2.5	1/15 (7)	<0.001	7.0	0.06
CDV, 1.25	4/15 (27)	<0.001	6.5	<0.01
HDP-CDV, 2.5	12/15 (80)	NS	6.0	<0.05
HDP-CDV, 1.25	8/15 (53)	<0.01	6.8	<0.05
ODE-CDV, 2.5	11/15 (73)	<0.05	4.3	NS
ODE-CDV, 1.25	15/15 (100)	NS	6.5	0.001

^a CDV was prepared in sterile saline and delivered i.p. in 0.1-ml doses. HDP-CDV and ODE-CDV were prepared in deionized water and delivered orally in 0.2-ml doses. Animals were treated twice weekly beginning 24, 48, or 72 h after viral inoculation.

^b *P* values were determined by comparing results for drug treatment groups with results for placebo control groups. NS, not significant compared to placebo controls.

^c MDD, mean day of death.

TABLE 4. Effect of single-dose oral treatment with HDP-CDV or ODE-CDV on mortality of BALB/c mice inoculated i.p. with MCMV

Expt and time of initiation	Treatment (mg/kg) ^a	No. of mice died/total no. (%)	Mortality result <i>P</i> value ^b	MDD ^c	MDD <i>P</i> value ^b	
1	24 h	Placebo	14/15 (93)		5.5	
		HDP-CDV, 30	0/15 (0)	<0.001		
		HDP-CDV, 10	0/15 (0)	<0.001		
		HDP-CDV, 3	0/15 (0)	<0.001		
	48 h	Placebo	12/15 (80)		4.9	
		HDP-CDV, 30	1/15 (7)	<0.001	2.0	0.05
		HDP-CDV, 10	2/15 (13)	0.001	3.0	0.01
		HDP-CDV, 3	0/15 (0)	<0.001		
	72 h	Placebo	14/15 (93)		5.1	
		HDP-CDV, 30	7/15 (47)	0.01	5.9	NS
		HDP-CDV, 10	10/15 (67)	NS	6.8	NS
		HDP-CDV, 3	13/15 (87)	NS	5.6	NS
2	24 h	Placebo	12/15 (80)		5.1	
		CDV, 30	0/15 (0)	<0.001		
		ODE-CDV, 30	1/15 (7)	<0.01	2.0	NS
		ODE-CDV, 10	0/15 (0)	<0.001		
	48 h	ODE-CDV, 3	1/15 (7)	<0.001	3.0	NS
		Placebo	15/15 (100)		5.0	NS
		CDV, 30	2/15 (13)	0.001	9.5	NS
		ODE-CDV, 30	15/15 (100)	NS	7.0	<0.001
	72 h	ODE-CDV, 10	4/15 (27)	<0.001	3.8	<0.01
		ODE-CDV, 3	1/15 (7)	<0.001	5.0	NS
		Placebo	8/12 (67)		5.0	
		CDV, 30	0/15 (0)	<0.001		
	72 h	ODE-CDV, 30	11/14 (79)	NS	7.2	0.01
		ODE-CDV, 10	5/13 (38)	NS	5.2	NS
		ODE-CDV, 3	11/15 (73)	NS	5.0	NS

^a CDV was prepared in sterile saline and delivered i.p. in 0.1-ml doses. HDP-CDV or ODE-CDV was prepared in deionized water and delivered orally in 0.2-ml doses. Animals were treated once beginning 24, 48, or 72 h after viral inoculation.

^b *P* values were determined by comparing results for drug treatment groups with results for placebo control groups. NS, not significant compared to placebo controls.

^c MDD, mean day of death.

CDV, animals were inoculated with MCMV and treated with CDV, HDP-CDV, or ODE-CDV at 5 mg/kg once daily for 5 days beginning 24 h after infection. On various days postinfection, animals were euthanized, and tissues were removed and assayed for MCMV. All of the treatment regimens resulted in significant reduction in mortality ($P < 0.001$) (data not presented) and 3 to 5 log₁₀ decreases in virus titers in lung, liver, spleen, kidney, pancreas, salivary gland, and blood. The results observed with oral HDP-CDV or ODE-CDV indicated that they were at least as effective as i.p. CDV (Fig. 2).

DISCUSSION

Since HCMV does not generally replicate in tissues of non-human origin, there are few animal models for evaluation of new antivirals for CMV infections. There have been a number of models used in which immunocompromised mice have been implanted with CMV-infected cells (2, 31) or human tissue implants that could subsequently be infected with HCMV (5, 7, 23, 42). With the models in which human fetal tissue is implanted either into the eye or under the kidney capsule of SCID mice, the implants infected with HCMV and animals treated with experimental compounds have been particularly useful and appear to be predictive of the outcome in humans

(23). However, the models are too expensive and labor intensive to use for screening new agents.

There are a number of natural CMV infections in animals, including mice (14), rats (40), and guinea pigs (26), that have been utilized for antiviral evaluation. We have utilized both acute lethal and chronic nonlethal MCMV-infected mice for evaluation of a number of antiviral agents, including ACV, GCV, PFA, and CDV (14, 20, 21, 22, 30, 36). In MCMV-infected mice, the virus replicates in lung, liver, spleen, kidney, intestine, pancreas, adrenals, and salivary glands and persists in most tissues for 1 to 2 months and in salivary glands for more than 3 months. This infection has many similarities to chronic CMV infection in humans and is an ideal model for evaluation of new antiviral agents. The approved drugs PFA, GCV, and CDV all had good activity in this infection model (22).

CDV is very active in this infection model and is approved for use in CMV retinitis in AIDS patients. The drug, however, has the major limitation of nephrotoxicity and is not bioavailable when administered orally, which makes long-term administration difficult. It was reported previously that a series of ether lipid esters of CDV had multiple-log increases in activity in vitro against MCMV and HCMV compared with the parent CDV or cyclic CDV (4). Similarly enhanced activities were also

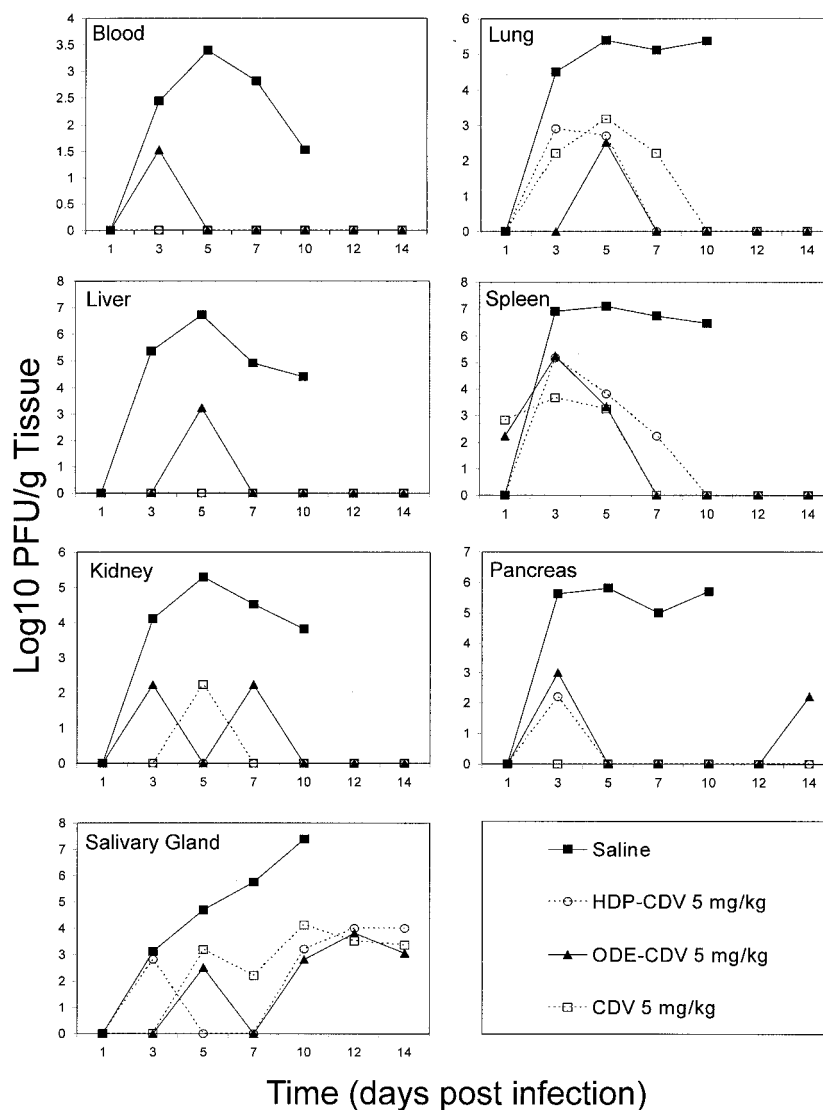


FIG. 2. Effect of daily oral treatment with HDP-CDV, ODE-CDV, or CDV on the pathogenesis of MCMV infection in mice. Treatment was initiated 24 h after viral inoculation and continued once daily for 5 consecutive days. Points represent the mean numbers of log₁₀ PFU per gram of tissue.

reported for vaccinia virus and cowpox virus (24), herpes simplex virus, Epstein-Barr virus, and human herpesvirus type 6 (43). The enhanced activity *in vitro* appears to be due to increased cellular uptake due to the lipid moiety. Intracellular levels of CDV are increased about 16-fold, and the long intracellular half-life of CDV is also manifested by HDP-CDV (1).

In pharmacokinetic studies in mice, HDP-CDV and ODE-CDV were shown to be bioavailable after oral administration. After oral administration of these prodrugs, plasma and tissue levels of CDV were higher and persisted longer than subcutaneously administered CDV. Of particular interest was the fact that the highest drug levels were in lung, liver, and spleen, but levels in kidney were considerably lower (9). These observations suggested that the ester analogs may be less nephrotoxic than parenteral CDV, since there is less drug accumulation in kidneys. In addition, the higher drug levels in lung and liver are encouraging, as these are tissues in which high levels of HCMV

are found in infection in humans. However, it is possible that toxicity manifestations in these tissues may result from the increased drug levels.

A very similar set of results with these compounds against vaccinia and cowpox viruses has been reported previously, showing activity that is highly increased *in vitro* (24) and good when delivered orally in mice but not highly superior to that of CDV (34). Although in the poxvirus models it was possible to significantly reduce virus replication in liver, spleen, and kidney, virus replication was not reduced in lung. In contrast, in the MCMV infection, treatments with these same compounds at similar doses were highly effective in reducing replication in all tissues, including the lung. The difference seen in the lung between the two models is apparently due to the increased susceptibility of MCMV to CDV *in vitro* over that seen with vaccinia and cowpox virus (4, 24) and indicates that drug levels in tissues and virus susceptibility are both important variables

that determine *in vivo* efficacy. Although it was not part of these studies, it would be of interest to determine if HDP-CDV and ODE-CDV have enhanced activity against CDV-resistant MCMV (38) in tissue culture cells and to correlate the *in vitro* activity with alteration of virus titers in tissues of mice infected with the resistant virus.

In the present studies, HDP-CDV and ODE-CDV given orally to mice infected with MCMV were highly effective in preventing mortality and reducing virus replication in all target organs tested. Administration of the drugs once daily, twice weekly, or as a single dose resulted in significant protection. Additionally, administration of the drugs could be delayed until 48 to 72 h postinfection and still significantly reduce mortality. It is interesting that, although the analogs are 10- to 100-fold more active than CDV *in vitro*, the level of protection obtained *in vivo* was very similar to that obtained with CDV given *i.p.* It should be pointed out, however, that when drug concentrations were compared on a molar basis, the ester analogs were found to be four to eight times as active as CDV due to the increased formula weight of the lipid. The possibility of being able to dose compounds such as these orally and only once or twice weekly would be a tremendous advantage in treatment of chronic viral infections such as CMV. This would greatly increase the flexibility of prophylactic, preemptive, or treatment regimens for these infections, particularly in transplant patients. Taking all of the experiments into account, there was no clear difference in the efficacies of HDP-CDV and ODE-CDV based on their ability to protect mice from mortality or reduce viral replication in target organs, and the two compounds were equally effective when treatment frequency was reduced or onset was delayed. The selection of one of these compounds to be developed for use in clinical studies will undoubtedly be made using additional criteria such as drug adsorption, distribution, metabolism, excretion, and toxicity.

We have also extended these studies to evaluation of the efficacies of HDP-CDV and ODE-CDV in two models of HCMV using SCID mice implanted either with human retinal tissue in the eye or with thymus and liver tissue under the kidney capsule. In both models, HDP-CDV and ODE-CDV given orally significantly reduced HCMV replication in HCMV-infected human implant tissue (5). The results obtained for mice infected with MCMV along with those obtained for both the HCMV-SCID-hu and orthopoxvirus-infected BALB/c mice indicate that these ether lipid ester analogs of CDV are extremely potent *in vitro*, have excellent bioavailability when given orally to mice, and are highly effective in these animal models. Oral administration of these analogs was at least as protective as parenteral CDV. These studies indicate that these new analogs need to be evaluated further to determine if they have a better toxicological profile than parenteral CDV, and if so, they need to be evaluated for use in CMV infections in humans.

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