Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 73 (2007) 212–218
Antiviral Research
www.elsevier.com/locate/antiviral

Oral 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycero-3-cidofovir targets the lung and is effective against a lethal respiratory challenge with ectromelia virus in mice☆

Karl Y. Hostetler[a,b,*], James R. Beadle[a,b], Julissa Trahan[a,b], Kathy A. Aldern[a,b], Gelita Owens[c], Jill Schriewer[c], Lora Melman[c], R. Mark Buller[c]

[a] *Department of Medicine, Division of Infectious Disease, University of California, San Diego, La Jolla, CA 92093-0676, United States*
[b] *The Veterans Medical Research Foundation, San Diego, CA 92161, United States*
[c] *Department of Molecular Microbiology and Immunology, St. Louis University Health Sciences Center, St. Louis, MO 63104, United States*

Received 26 July 2006; accepted 15 October 2006

Abstract

Hexadecyloxypropyl-cidofovir (HDP-CDV) has been shown to be orally active against lethal infection with orthopoxviruses including, mousepox, cowpox, vaccinia and rabbitpox. The alkoxyalkyl group provides oral absorption and reduces greatly the amount of drug reaching the kidney, the site of CDV's dose limiting toxicity. However, the amount of HDP-CDV detected in lung, an important site of early poxvirus replication, is low and the reduction of viral titers in surviving animals is reduced moderately compared with the liver where poxvirus titers are virtually undetectable. We synthesized a novel glycerol ester of CDV, 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycero-3-CDV (ODBG-CDV), and compared its oral pharmacokinetics with that of HDP-CDV. Surprisingly, ODBG-CDV levels in lung are much higher and liver levels are reduced, suggesting that the compound is transported in small intestinal lymph instead the portal vein. ODBG-CDV has excellent in vitro activity in cells infected with ectromelia virus (ECTV). In mice infected with a lethal aerosol or intranasal challenge of ECTV, HDP-CDV and ODBG-CDV are equally effective in preventing death from disease. Other drugs esterified to 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol or 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol-3-phosphate may provide lung targeting for treatment of microbial or neoplastic diseases while reducing first pass removal by the liver during oral absorption.

Keywords: Ectromelia virus; Cidofovir; Prodrugs; Lung targeting

1. Introduction

It has been previously reported that alkoxyalkyl analogs of acyclic nucleoside phosphonates like hexadecyloxypropyl-cidofovir (HDP-CDV) are orally bioavailable and active in lethal orthopoxvirus challenge models (Buller et al., 2004; Quenelle et al., 2004). However, oral pharmacokinetics with radiolabeled HDP-CDV (Ciesla et al., 2003) show low levels of drug and metabolites in the lung, an important site of early poxvirus replication. We reported previously that 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycero-cidofovir (ODBG-CDV; Fig. 1) was active against cowpox and vaccinia virus strains with 50% effective concentration values (EC_{50}) ranging from 0.09 to 0.4 μM (Wan et al., 2005). ODBG-CDV was also highly active against HCMV, ganciclovir-resistant and phosphonformate-resistant isolates of HCMV, HSV-1, HSV-2, VZV, EBV, HHV-6A, HHV-6B and HHV-8 with EC_{50} values in the nanomolar range (Williams-Aziz et al., 2005). In vaccinia infection in organotypic epithelial raft cultures of primary human keratinocytes, ODBG-CDV was the most active analog of CDV with an EC_{90} value <0.04 μM (Lebeau et al., 2006).

We prepared ODBG-[2-^{14}C]-CDV to assess oral and parenteral pharmacokinetics. The compound was given orally and intraperitoneally to mice and tissue and plasma levels of radiolabeled drug and metabolites were measured at various times up to 72 h. Relative oral bioavailability was determined from the plasma area under curve (AUC) obtained with oral versus intraperitoneal administration. Tissue distribution of drug and metabolites was assessed in liver, kidney, and lung. Surprisingly,

☆ Portions of this paper were presented in abstract form at the International Conference on Antiviral Research, 11–14 April 2005, Barcelona, Spain.

* Corresponding author at: Department of Medicine (0676), University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0676, United States. Tel.: +1 858 552 8585x2616; fax: +1 858 534 6133.
E-mail address: khostetl@ucsd.edu (K.Y. Hostetler).

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.10.009

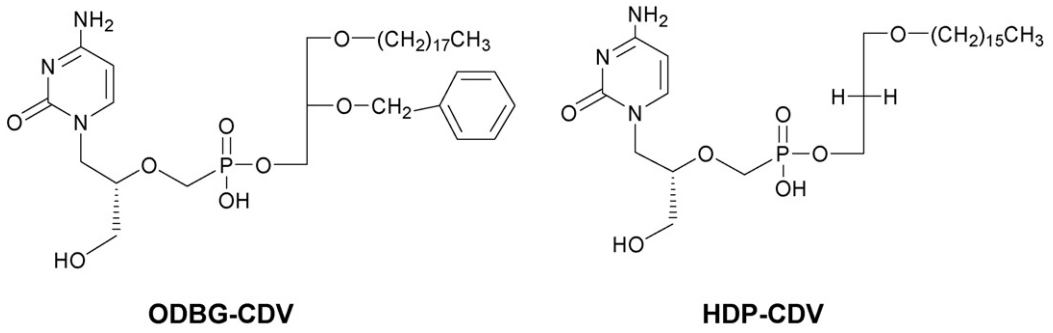

Fig. 1. Structures of 1-*O*-octadecyl-2-*O*-benzyl-glycero-cidofovir and hexadecyloxypropyl-cidofovir.

oral ODBG-[2-^{14}C]-CDV produced very high levels of drug and metabolites in lung compared with oral HDP-[2-^{14}C]CDV, indicating that oral ODBG-[2-^{14}C]-CDV targets the lung in mice. ODBG-CDV and HDP-CDV were evaluated orally in aerosol and intranasal ectromelia virus (ECTV) lethal challenge models. Both compounds were highly effective in preventing death from disease.

2. Methods

2.1. Chemistry

1-*O*-Octadecyl-2-*O*-benzyl-*sn*-glycerol was purchased from Bachem, Torrance, CA. 1-*O*-Octadecyl-2-*O*-benzyl-*sn*-glycero-3-cidofovir was synthesized as previously described (Wan et al., 2005). Briefly, anhydrous cCDV (1 equiv.), 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol (2 equiv.), and triphenylphosphine (2 equiv.) were dissolved/suspended in anhydrous *N*,*N*-DMF (6.5 ml/mmol of cCDV), and stirred vigorously under a nitrogen atmosphere. Diisopropyl azadicarboxylate (DIAD, 2 equiv.) was then added in three equal portions over 15 min before the mixture was allowed to stir overnight. The solvent was then evaporated under vacuum and the residue purified by column chromatography with silica gel and recrystallized from *p*-dioxane. The cyclic CDV ring was opened with base (Wan et al., 2005). Using this method, ODBG-[2-^{14}C]-CDV was prepared by Moravek Biochemicals, Inc. (Brea, CA). The structures of HDP-CDV and ODBG-CDV are shown in Fig. 1.

2.2. Animal pharmacokinetic studies

Female Swiss-Webster mice weighing approximately 25 g received a single dose of 10 mg/kg ODBG-[2-^{14}C]-CDV (specific activity, 53 mCi/mmol) in sterile 0.9% saline either by oral gavage or by intraperitoneal injection. Three animals were sacrificed at 1, 3, 6, 12, 24, 48, and 72 h and blood and tissue samples were obtained. Fifty microlitres of plasma was added to 10 ml of Ecolite cocktail and analyzed for drug and metabolite content by a scintillation counting. The kidney, lung and liver were also obtained from each mouse, washed with 0.9% saline, and weighed. The tissue was treated with 3 ml TS-2 tissue solubilizer and 0.5 ml of water and placed in a 50 °C water bath for 36–48 h. Glacial acetic acid was added to each vial to neutralize the TS-2 and Flo-Scint IV was added before counting. Plasma pharmacokinetic data was calculated as reported previously (Ciesla et al., 2003).

2.3. HPLC analysis of metabolites

2.3.1. Lung and liver tissue

Twenty percent homogenates of liver and lung tissue were made in ultrapure distilled water saline and aliquots were reserved frozen at −70 °C until analysis. A portion of the liver and lung homogenates was frozen and thawed two times in an isopropanol/dry ice bath and then sonicated on ice in a bath sonicator for 5 min. Trichloroacetic acid was added to a 7% final concentration and centrifuged at 4 °C for 10 min at 1000 rpm. The supernatant was removed and aliquots counted in a scintillation counter. An aliquot representing approximately 10,000 DPM was analyzed by HPLC (System Gold, Beckman Coulter, Fullerton, CA). The samples were injected into a Partisil 10 SAX column (Alltech, Deerfield, IL), 4.6 cm × 15 cm, and a SAX guard column. Metabolites were eluted at a flow rate of 1 ml/min using a potassium phosphate buffer gradient of 20–700 mM, pH 5.8, beginning at 9 min for 20 min followed by a 5 min terminal hold. One minute fractions were collected and Ultima Flo scintillation fluid added and their content of radioactivity was determined by liquid scintillation. The identity of the radioactive peaks was compared with the retention time of pure standards of CDV, CDV-monophosphate (CDVp) and CDV-diphosphate (CDVpp). CDVp and CDVpp were obtained from TriLink Biotechnologies Inc. (San Diego, CA).

2.4. Ectromelia virus studies

2.4.1. Plaque reduction assay

BSC-1 cells were plated in wells of a 24 well cluster plate. Each monolayer was infected with ∼75 plaque forming units (PFU) of indicator virus in 0.1 ml of DMEM +2% fetal clone II for 60 min at 37 °C. Media was removed by aspiration and standard virus overlay media containing no drug or the test drug at concentrations ranging from 0.05 to 50 μM was added. The plates were incubated at 37 °C for 3–4 days and monolayers were

stained and plaques counted using a stereomicroscope. The EC_{50} concentration for each drug was calculated.

2.4.2. Measurement of cytotoxicity

Cytotoxicity was determined using Promega Cell Titer 96* Aqueous Non-radioactive Cell Proliferation Assay kit (G5421). BSC-1 cells were plated at 4×10^3 cells per well in a 96-well flat bottom plate and treated with serial dilutions of the test compounds for 48–72 h then assayed using MTS dye. Four hours following the addition of the dye, the plates were read at 490 nm on an ELISA plate reader. The percent cytotoxicity was calculated and plotted against the concentration of the test compound to determine the 50% cytotoxic concentration (CC_{50}).

2.5. Animals

Four to six week old female A/NCR (A/JCr) mice were obtained from the National Cancer Institute, Frederick Md., housed in filter-top microisolator cages and fed commercial mouse chow and water, *ad libitum.* The mice were housed in a biosafety level 3 containment area. Animal husbandry and experimental procedures were in accordance with PHS policy, and approved by the Institutional Animal Care and Use Committee.

2.6. In vivo drug evaluation

Mice were exposed to aerosolized ECTV suspended in DMEM using a nose-only inhalation exposure system (NOIES; CH Technologies) equipped with a 1-jet BioAerosol Nebulizing Generator, and operated within a class II biological safety cabinet. The NOIES was operated with a primary air pressure of 20 psi giving 2 l/min flow rate to the aerosol chamber (without secondary air), a virus suspension flow rate of 0.5 ml/min, and a system operating pressure~ -0.5 in. of vacuum relative to the out-side atmospheric pressure. The NOIES delivered a predicted median particle diameter of 0.8 ± 1.2 μm (Dr. Chad Roy, personnel communication). The quantity of virus delivered to the mice over the course of exposure was not measured directly, but estimated by multiplying the concentration of virus in the aerosol (C_A) in PFU by the total volume (V_M) of air respired by a mouse of given body weight over the exposure time using Guyton's formula for minute volumes administered to rodents (Guyton, 1947). This presented virus dose is likely an upper limit as it assumes that all of the virus was optimally aerosolized and completely taken up on inhalation. In another experiment, ECTV was administered intranasally. Four hours following aerosol exposure to ECTV, groups of 10–13 mice were treated by gavage with 0.1 ml sterile, distilled water alone or water containing

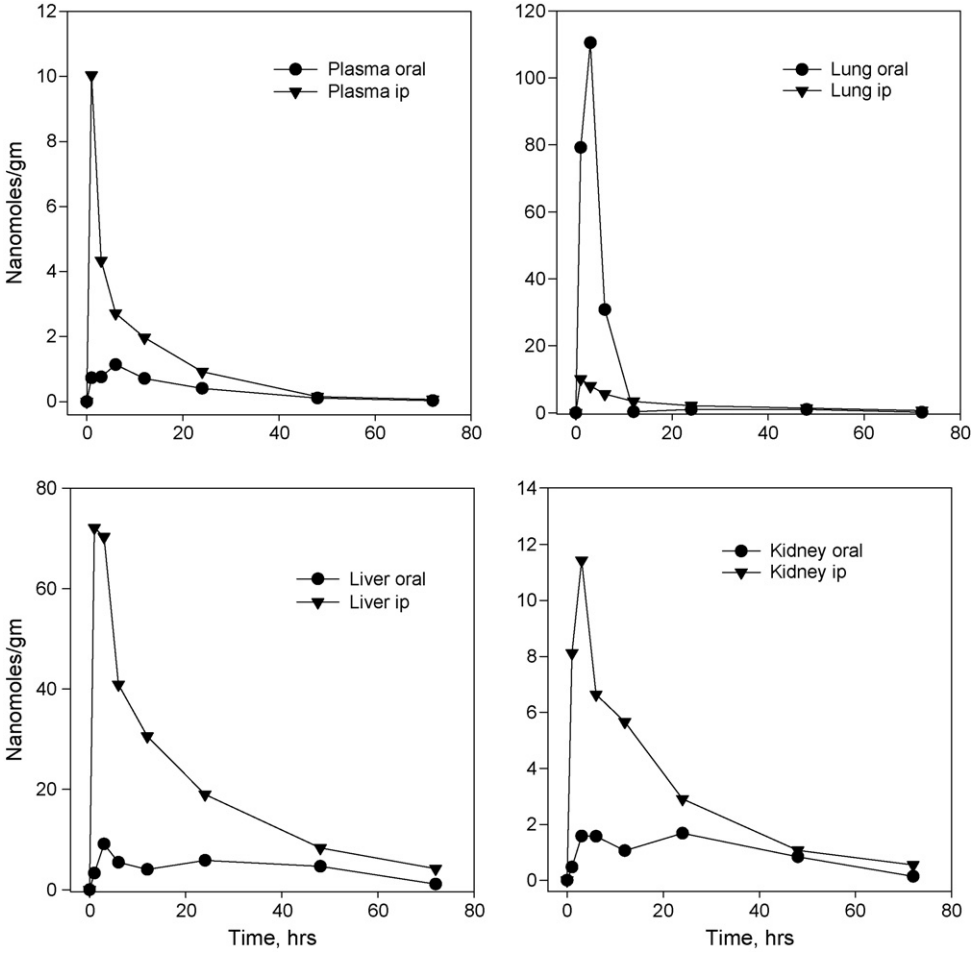

Fig. 2. Plasma and tissue distribution of oral and intraperitoneal administration of 10 mg/kg of ODBG-[2-^{14}C]-CDV to mice.

Table 1
Plasma pharmacokinetic comparison of ODBG-CDV and HDP-CDV

Compound	Plasma $T_{1/2}$ (h)	Plasma C_{max} (μM)	Plasma T_{max} (h)	Relative oral bioavailability (%)
ODBG-CDV	16.9	1.14	6	32
HDP-CDV[a]	14.9[a]	2.37[a]	12[a]	88[a]

Compounds were administered orally at a dose of 10 mg/kg. Relative oral bioavailability was estimated by comparing the plasma area under curve obtained with oral versus subcutaneous ODBG-CDV.
[a] Data adapted from Ciesla et al. (2003).

HDP-CDV or ODBG-CDV. This treatment was repeated on days 1, 2, 3, and 4 post-infection for a total of 5 doses. Mice were observed over 21 days for clinical signs of disease (morbidity) and mortality (mean time to death and number of mice killed). Mice showing conjunctivitis, little or no movement, or marked respiratory distress were euthanized as they were near death.

2.7. Statistical methods

Groups of mice treated with the test compounds were compared statistically to vehicle treated groups. Mortality rates were analyzed by Fisher's exact test. Day of death data were analyzed by Mann–Whitney *U*-rank sum test. A *p*-value of 0.05 or less was considered significant.

3. Results

3.1. Antiviral activity in vitro

The antiviral activity of HDP-CDV and ODBG-CDV was evaluated in vitro in BSC-1 cells infected with ECTV as reported previously (Buller et al., 2004). HDP-CDV had an EC_{50} value of 0.22 μM versus 0.01 μM for ODBG-CDV, and the CC_{50} values were 13.4 and 35 μM. The selectivity indexes for HDP-CDV and ODBG-CDV were 61 and 3500, respectively.

3.2. Pharmacokinetics of ODBG-CDV

Plasma drug levels following a 10 mg/kg dose of ODBG-[2-^{14}C]-CDV given orally and intraperitoneally were determined (Fig. 2). In the oral group, plasma drug levels and metabolites peaked at 1.1 μM at 6 h compared to 10 μM at 1 h in the intraperitoneal group. Plasma CDV equivalents declined, rapidly reaching 0.03 μM in the oral group and 0.07 μM in the intraperitoneal group at 72 h. The plasma AUC values for oral and intraperitoneal ODBG-[2-^{14}C]-CDV were compared. The intraperitoneal group had a larger area under the curve; based on the comparative AUC values for oral and intraperitoneal ODBG-CDV, we estimated a relative oral bioavailability of 32.3% for ODBG-CDV (Table 1). We previously used a similar approach to evaluate the oral absorption and tissue levels of HDP-[2-^{14}C]-CDV (Ciesla et al., 2003) and have included the data in Table 1 for comparison with ODBG-[2-^{14}C]-CDV. Oral ODGB-CDV shows a longer plasma half life ($T_{1/2}$) than HDP-CDV, but the plasma AUC with oral HDP-CDV is substantially greater than ODBG-CDV. The relative oral bioavailability of HDP-CDV was estimated to be 88% versus 32% for ODBG-CDV (Table 1).

Tissue drug levels were assessed following oral administration of ODBG-[2-^{14}C]-CDV (Fig. 2) and peak levels of radiolabeled CDV compared. Surprisingly, in the lung, oral ODBG-[2-^{14}C]-CDV gave peak levels of 111 nmol/g at 3 h versus only 10 nmol/g in lung at the 1 h peak in the intraperitoneal group (Fig. 2). Liver drug and metabolite levels, however, were higher with intraperitoneal administration than with oral. The peak level in liver was 72 nmol/g (1 h) in the intraperitoneal group compared to peak levels of 9.1 nmol/g in the oral group (3 h) (Fig. 2). Drug exposure to the liver was notably lower with oral ODBG-[2-^{14}C]-CDV than with intraperitoneal administration, suggesting that there is targeting of orally administered ODBG-

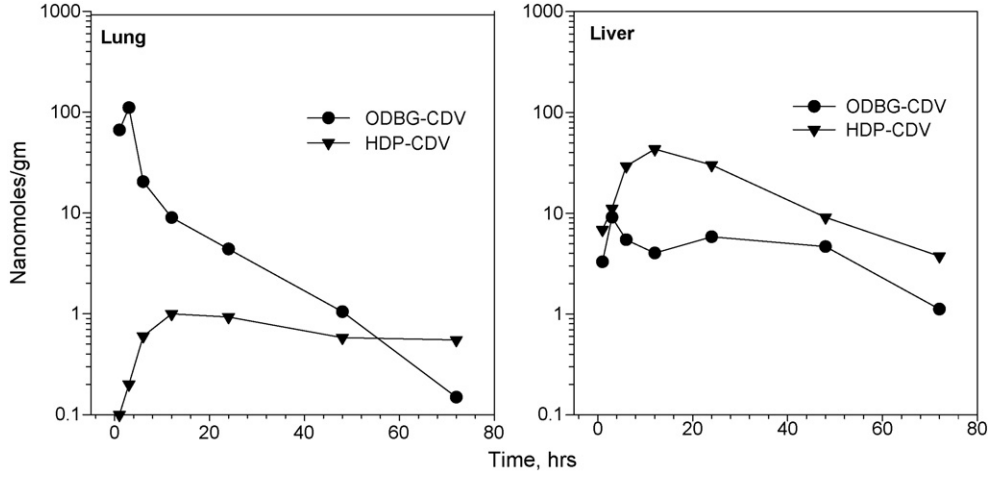

Fig. 3. Tissue levels of ODBG-[2-^{14}C]-CDV and HDP-[2-^{14}C]-CDV in lung and liver after oral administration of 10 mg/kg to Mice. The HDP-CDV data was adapted from Ciesla et al. (2003).

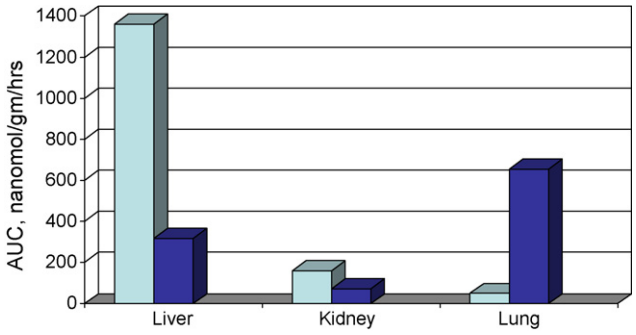

Fig. 4. Area under curve comparison: oral HDP-[2-^{14}C]-CDV vs. ODBG-[2-^{14}C]-CDV in liver, kidney and lung. A 10 mg/kg single oral dose of HDP-[2-^{14}C]-CDV or ODBG-[2-^{14}C]-CDV was administered to mice and the area under curve (AUC) was determined from 0 to 72 h in liver, kidney and lung. The HDP-CDV data was adapted from Ciesla et al. (2003). Light bars: HDP-CDV; dark bars: ODBG-CDV.

[2-^{14}C]-CDV to lung. Drug levels in kidney peaked at 11 nmol/g (3 h) in the intraperitoneal group compared to 1.7 nmol/g (24 h) for the oral group (Fig. 2).

3.3. Comparison of ODBG-CDV and HDP-CDV pharmacokinetics

The lung and liver levels of orally administered ODBG-[2-^{14}C]-CDV and HDP-[2-^{14}C]-CDV were compared (Fig. 3). With oral administration of ODBG-[2-^{14}C]-CDV, the lung C_{max} was 100-fold greater than oral HDP-[2-^{14}C]-CDV in spite of the fact that ODBG-CDV has a lower oral bioavailability. However, in liver, the C_{max} of ODBG-CDV was only 21% of HDP-CDV (Fig. 3). We calculated the $AUC_{0–72\,h}$ for the two compounds in liver, lung and kidney (Fig. 4). HDP-CDV given orally provides large AUC values in liver (1360 nmol/(g h)) and small AUC values in lung (50 nmol/(g h)). Liver AUC values are 25 times greater than that of lung with HDP-CDV. However, when ODBG-CDV is given orally, the AUC in lung is larger than that of the liver (654 nmol/g h versus 319 nmol/g h), representing a reversal of drug distribution from principally liver (HDP-CDV) to principally lung (ODBG-CDV). Kidney drug levels were low with both compounds.

Table 2
CDV and metabolite levels in liver and lung after oral administration of 10 mg/kg ODBG-CDV or HDP-CDV

Metabolite[a]	3 h liver		3 h lung	
	HDP-CDV[b]	ODBG-CDV	HDP-CDV[b]	ODBG-CDV
CDV	15.9	1.10	<0.05	1.10
CDVp	0.20	0.11	<0.05	0.26
CDVpp	<0.05	<0.05	<0.05	<0.05
HPMPU	0.90	<0.05	<0.05	<0.05

[a] For ODBG-CDV, extracts of liver and lung tissue were prepared and analyzed by HPLC as noted in Section 2.

[b] Frozen liver and lung tissues from HDP-CDV experiments reported by Ciesla et al. (2003), were stored at −70 °C and analyzed. Data are nmol/g tissue. The limit of detection under these experimental conditions was 0.05 nmol/g. *Abbreviations*: CDV: cidofovir; CDVp: cidofovir monophosphate; CDVpp: cidofovir diphosphate; HPMPU: (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]uridine.

To evaluate tissue levels of key drug metabolites, we prepared TCA extracts of liver and lung tissue from the single dose oral pharmacokinetic experiments and quantified liver and lung levels of CDV, CDVp and CDVpp at 3 h by ion exchange HPLC as previously described (Aldern et al., 2003). With HDP-[2-^{14}C]-CDV, liver tissue contained readily detectable CDV and CDVp at 15.9 and 0.20 nmol/g, respectively. In lung, these metabolites were below the level of detection (Table 2). However, with ODBG-[2-^{14}C]-CDV, lung tissue contained CDV and CDVp, 1.10 and 0.26 nmol/g, respectively, and liver tissue levels of CDV and CDVp were 1.10 and 0.11 nmol/g (lower than noted with HDP-[2-^{14}C]-CDV) (Ciesla et al., 2003). Levels of the active antiviral, CDVpp, were below the level of detection with both analogs of CDV in lung and liver. However, these results clearly show that the lung is able to cleave ODBG-[2-^{14}C]-CDV to CDV and carry out anabolic phosphorylation.

3.4. In vivo antiviral activity of HDP-CDV and ODBG-CDV in ECTV infected mice

We tested oral HDP-CDV and ODBG-CDV against high dose aerosol and intranasal lethal challenges of ECTV in A/NCR mice as reported previously (Buller et al., 2004). Mice were

Table 3
Efficacy of oral HDP-CDV and ODBG-CDV in a high dose intranasal challenge of A/NCR female mice with ectromelia virus

Drug[a]	Drug dose (mg/kg)	Intranasal virus challenge	Morbidity[b] on day 7 pi	Day of death, range	Mean time to death ± S.D.	Mortality at day 21 pi
HDP-CDV	2	+	10 = 0	14	14 ± 0*	2/10**
HDP-CDV	8	+	10 = 0	NA	NA	0/10**
ODBG-CDV	2	+	10 = 1	12–14	13 ± 1.4*	2/10**
ODBG-CDV	8	+	10 = 1	NA	NA	0/10**
Vehicle	None	+	5 = 2, 3 = 3	7–8	7.4 ± 0.5	10/10
Control	None	None	10 = 0	NA	NA	0/10**

[a] Mice were infected with 3.0×10^3 PFU ($60{,}000 \times LD_{50}$) (5 μl of virus suspension in one nare) of ECTV via intranasal route ~4 h prior to the first drug treatment. Treatment was repeated on days 1, 2, 3, and 4 with the indicated dose of drug in 100 μl of vehicle. Mice treated with placebo received only 100 μl of sterile water on the same days.

[b] Morbidity 0 = healthy, no signs of sickness; 1 = face fur ruffled, no conjunctivitis; 2 = face and body fur ruffled, hunched posture, eyes starting to swell; 3 = 2 plus conjunctivitis; 4 = 3 plus eyes swollen shut, near death, little or no movement, marked respiratory distress. *Abbreviations*: pi: postinfection; NA: not applicable. *p*-Values vs. vehicle treated animals: $^{*}p < 0.05$; $^{**}p < 0.001$.

Table 4
Efficacy of HDP-CDV and ODBG-CDV in a high dose aerosol challenge of A/NCR female mice with ectromelia virus

Drug[a]	Drug dose (mg/kg)	Aerosol virus challenge	Morbidity[b] on day 7 pi	Day of death, range	Mean time to death ± S.D.	Mortality at day 21 pi
HDP-CDV	2	+	8 = 0	NA	NA	0/8**
HDP-CDV	8	+	8 = 0	NA	NA	0/8**
ODBG-CDV	2	+	8 = 0	13	13*	1/8**
ODBG-CDV	8	+	8 = 0	NA	NA	0/8**
Vehicle	None	+	2 = 3	7–11	7.5 ± 1.4	8/8
Control	None	None	2 = 1	NA	NA	0/8**

[a] Mice were exposed to a presented dose of 2.5×10^5 PFU ($7000 \times LD_{50}$) of ECTV via aerosol route ~4 h prior to the first drug treatment. Mice were treated on day 0, 1, 2, 3, and 4 with the indicated dose of drug in 100 μl of vehicle. Mice treated with vehicle received only 100 μl of sterile water on the same days.
[b] Morbidity 0 = healthy, no signs of sickness; 1 = face fur ruffled, no conjunctivitis; 2 = face and body fur ruffled, hunched posture, eyes starting to swell; 3 = 2 plus conjunctivitis; 4 = 3 plus eyes swollen shut, near death, little or no movement, marked respiratory distress. *p*-Values vs. vehicle treated animals: *$p < 0.05$; **$p < 0.001$. Abbreviations as in Table 3.

challenged intranasally with 60,000 LD_{50}s of ECTV and 4 h after infection animals were treated orally with 2 or 8 mg/kg of HDP-CDV or ODGB-CDV (Table 3). Dosing was repeated daily for a total of five doses. Morbidity on day 7 was minimal or absent in treated animals, but was grade 2 or 3 in untreated, infected mice. At 2 mg/kg, 2 of 10 animals died with HDP-CDV and ODBG-CDV at days 13–14, while all 10 infected, untreated controls died with a mean day of death of 7.4 ± 0.5 days. All animals treated with HDP-CDV or ODBG-CDV at 8 mg/kg survived. Animals were also challenged with 7000 LD_{50}s of ECTV by the aerosol route and treated with 2 or 8 mg/kg of HDP-CDV of ODBG-CDV as before and the results are shown in Table 4. No treated animals showed morbidity at day 7. All untreated controls died with a mean day of death of 7.5 ± 1.4 days. With a dose of 8 mg/kg for 5 days, all animals treated with HDP-CDV and ODBG-CDV survived (8/8). At the lower dose of 2 mg/kg for 5 days, 8 of 8 mice survived with HDP-CDV and 7 of 8 with ODBG-CDV. From this data, we can conclude that ODBG-CDV is equivalent to HDP-CDV is its ability to protect against lethal ECTV challenge in spite of its lower oral bioavailability.

4. Discussion

CDV and other drugs can be directed to lung by making the alkoxyalkyl lipid ester more hydrophobic as we demonstrate here with ODBG-CDV. We hypothesize that this is because highly hydrophobic conjugates of CDV and other drugs are taken up by the enterocyte, exiting in the small intestinal lymphatics with the triglyceride-rich chylomicrons, instead of passing directly into the liver via the portal vein. Intestinal lymphatic lipids (chyle) drain via the thoracic duct successively into the left subclavian vein, innominate vein, superior vena cava, right side of the heart and hence, into the lung via the pulmonary artery (Gray, 1901). This may account for the very high levels of drug noted in the lung in the present studies. Levels of ODBG-CDV and its metabolites in the liver were reciprocally lower, consistent with this hypothesis.

Esterfication of CDV with 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol provides a compound which, when given orally, targets the lung and is orally bioavailable. Plasma levels show peak values at 6 h for mice treated orally and 1 h for mice treated by the intraperitoneal route. After 12 h, drug levels decline at a similar rate with either route of administration. The lung AUC of the oral administration group was much greater than with intraperitoneal administration (Fig. 2). Compared with HDP-CDV, the AUC of ODBG-CDV in lung is 13-fold greater (Fig. 4) and levels of CDV and CDVp are detectable, showing that the lung can metabolize OBDG-CDV toward its active metabolite. Both HDP-CDV and ODBG-CDV given orally were equally active in providing protection from lethal challenges with ECTV administered by the intranasal route or in a small droplet aerosol. While lung levels of drug were greater with ODBG-CDV, mortality was similar to that observed with HDP-CDV. Although it has been suggested that death in lethal poxvirus infection is due to a pneumonitis, the precise cause of death in ectromelia virus infection is not known with certainty. In our earlier study with HDP-CDV, mortality in lethal ectromelia virus infection correlated with reduction of viral titer in liver and spleen, but did not correlate with reduction of lung viral titers (Buller et al., 2004). This is consistent with the possibility that death in lethal ectromelia infection is not due to the lung infectious process. Although the in vivo efficacy studies were done in A/NCR mice and the pharmacokinetics in Swiss Webster mice, both are albino mice strains and we have not noted any differences in pharmacokinetic behavior in NIH Swiss, Swiss Webster and CD-1 mice (unpublished observation).

First pass removal of oral drugs by the liver is a problem commonly encountered in drug development. Esterification of CDV to 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol increases lung exposure to drug while reducing liver area under curve. ODBG-CDV is essentially equivalent to HDP-CDV in reducing mortality in lethal aerosol ectromelia infections in mice. Other small molecules having suitable functional groups for linking may also be candidates for this approach. Disposition of other drugs esterified to 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol or 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol-3-phosphate may provide targeting drugs to lung for microbial or neoplastic diseases while reducing drug taken up by the liver during oral absorption. A particularly good candidate for further study might be the ODBG-phosphate adduct of the non-orally active compound, zanamivir, for influenza.

Acknowledgements

The studies were supported by NIH grants AI-64615, AI-66499 and AI-29164 from NIAID (KYH) and NIAID contract NO1-AI-15436 (RMB). KYH has an equity interest and serves as a consultant to Chimerix Inc. The terms of the arrangements have been reviewed and approved by the University of California, San Diego in accordance with conflict of interest policies.

References

Aldern, K.A., Ciesla, S.L., Winegarden, K.L., Hostetler, K.Y., 2003. The increased antiviral activity of 1-*O*-hexadecyloxypropyl-cidofovir in MRC-5 human lung fibroblasts is explained by unique cellular uptake and metabolism. Mol. Pharmacol. 63, 678–681.

Buller, R.M., Owens, G., Schriewer, J., Melman, L., Beadle, J.R., Hostetler, K.Y., 2004. Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. Virology 318, 474–481.

Ciesla, S.L., Trahan, J., Wan, W.B., Beadle, J.R., Aldern, K.A., Painter, G.R., Hostelter, K.Y., 2003. Esterfication of cidofovir with alkoxyalkanols increases oral bioavailability and diminishes drug accumulation in kidney. Antiviral Res. 59, 163–171.

Gray, H., 1901. Gray's Anatomy. Bounty Books, New York, NY, p. 624.

Guyton, A.C., 1947. Measurement of the respiratory volumes of laboratory animals. Am. J. Physiol. 150, 70–77.

Lebeau, I., Andrei, G., Dal Pozzo, F., Beadle, J.R., Hostetler, K.Y., De Clercq, E., van den Oord, J., Snoeck, R., 2006. Activity of alkoxyalkyl esters of CDV, cCDV and HPMPA against orthopox- and parapoxviruses in cell monolayers and in organotypic cultures. Antimicrob. Agents Chemother. 50, 2525–2529.

Quenelle, D.C., Collins, D.J., Hostetler, K.Y., Beadle, J.R., Wan, W.B., Kern, E.R., 2004. Oral treatment of cowpox and vaccinia infections in mice with ether lipid esters of cidofovir. Antimicrob. Agents Chemother. 48, 404–412.

Wan, W.B., Beadle, J.R., Hartline, C.B., Kern, E.R., Ciesla, S.L., Valiaeva, N., Hostetler, K.Y., 2005. Comparison of the antiviral activity of alkoxyalkyl and alkyl esters of cidofovir against human and murine cytomegalovirus replication in vitro. Antimicrob. Agents Chemother. 49, 656–662.

Williams-Aziz, S.L., Hartline, C.B., Harden, E.A., Daily, S.M., Prichard, M.N., Kushner, N.L., Beadle, J.R., Wan, W.B., Hostetler, K.Y., Kern, E.R., 2005. Comparative activity of lipid esters of cidofovir and cyclic cidofovir against replication of herpesviruses in vitro. Antimicrob. Agents Chemother. 49, 3724–3733.