Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model

R. Mark Buller, a,∗ Gelita Owens, a Jill Schriewer, a Lora Melman, a James R. Beadle, b and Karl Y. Hostetlerb

a Department of Molecular Microbiology and Immunology, Saint Louis University Health Sciences Center, St. Louis, MO 63104, USA
b Department of Medicine, VA San Diego Healthcare System and the University of California, La Jolla, San Diego, CA 92093-0676, USA

Received 29 September 2003; received in revised form 16 November 2003; accepted 16 November 2003

Abstract

Cidofovir (CDV) is a highly effective inhibitor of orthopoxvirus replication and may be used intravenously to treat smallpox or complications arising from the smallpox vaccine under an investigational new drug application (IND). However, CDV is absorbed poorly following oral administration and is inactive orally. To improve the bioavailability of CDV, others synthesized alkoxyalkanol esters of CDV and observed >100-fold more activity than unmodified CDV against cowpox, vaccinia, and variola virus (VARV) replication. These ether lipid analogs of CDV have high oral bioavailability in mice. In this study, we compared the oral activity of CDV with the hexadecyloxypropyl (HDP)-, octadecyloxyethyl-, oleyloxypropyl-, and oleyloxyethyl-esters of CDV in a lethal, aerosol ectromelia virus (ECTV) challenge model in A/NCR mice. Octadecyloxyethyl-CDV appeared to be the most potent CDV analog as a dose regimen of 5 mg/kg started 4 h following challenge completely blocked virus replication in spleen and liver, and protected 100% of A/NCR mice, although oral, unmodified CDV was inactive. These results suggest that this family of compounds deserves further evaluation as poxvirus antiviral.

Keywords: Ether lipid; Cidofovir; Mousepox

Introduction

With the heightened potential for bioterrorism, there is a need for an efficacious antiviral for the treatment of smallpox or human monkeypox and the complications that arise from the smallpox vaccine. Cidofovir (CDV) is effective against a wide-range of DNA virus and retrovirus infections probably by interfering with viral DNA synthesis (De Clercq, 1998). CDV was first shown active in vitro against vaccinia virus (VACV) by De Clercq et al. (1987), although others demonstrated 50% effective concentration (EC_{50}) values in the 10–50 μM range against several other orthopoxviruses including variola virus (VARV), monkeypox virus, and cowpox virus (CPXV) (Baker et al., 2003; Kern, 2003). Furthermore, parenterally administered CDV was active against lethal VACV and CPXV infections in mice (Bray et al., 2000; Neyts and De Clercq, 1993; Smee et al., 2001), but was poorly absorbed when administered orally (Wachsman et al., 1996). Oral bioavailability is a desirable property of drugs proposed for emergency use in an outbreak of smallpox or monkeypox. Previous studies by Hostetler et al. (1997) showed that derivatization of acyclovir with 1-O-hexadecyl-glycerol-3-phosphate provided a compound that was 100% orally bioavailable in mice. Using this approach, several ether lipid esters of CDV and cyclic CDV were synthesized and tested in vitro against VACV and CPXV (Kern et al., 2002). The hexadecyloxypropyl (HDP)- and octadecyloxyethyl (ODE)-esters of CDV had EC_{50} values of 0.2–1.1 and 0.1–0.4 μM, respectively, against five strains of VACV versus 10.1–46.2 μM for CDV; however, no in vivo experiments have yet been reported. In this study, we evaluate the antiviral activity of this class of compounds in vitro and in vivo against a natural mouse pathogen, ectromelia virus (ECTV), the causative agent of mousepox (Buller and Palumbo, 1991).

Natural infections with ECTV occur through microscopic abrasions, which allow access of the virus to the epidermal or dermal layers of the cornified mouse skin. ECTV replicates locally at the site of infection, and reaches internal organs via the afferent lymphatics, draining lymph node,
and bloodstream (primary viremia). Extensive replication in major organs, especially spleen and liver, results in detection of virus in blood by 4 or 5 days p.i. (secondary viremia). Virus replication in skin produces the typical exanthem, which can be seen as early as 6 days p.i., and is dependent on the mouse strain. Virus is transmitted from the primary and secondary lesions. With the A/NCR strain of mouse used in this study, death is observed 7–10 days p.i. before the development of an exanthem, and is a consequence of extensive liver necrosis. The mousepox model differs from smallpox in the short course of disease, and severe pulmonary pneumonia associated with local replication following an aerosol infection. The major advantage of using ECTV for the evaluation of antiviral compounds lies in its severe disease course in adult, immunocompetent mice, which results in uniform mortality and LD_{50} values of less than 1 plaque forming unit (PFU) by the footpad route of infection (Buller and Palumbo, 1991; Chen et al., 1992).

Results

In vitro sensitivity of ectromelia virus to alkoxyalkyl esters of cidofovir

A series of analogs of CDV were synthesized having alkoxyalkanols of varying structure as shown in Fig. 1. (Beadle et al., 2002). As a first step in testing these compounds in an ECTV mouse model, we determined their EC_{50} values in a CV-1 cell plaque reduction assay with VACV strain WR (VACV-WR), ECTV, and a second pool of ECTV prepared with an ECTV recombinant expressing murine IL-4. VACV-WR was included as its sensitivity to CDV and analogs had previously been reported. The EC_{50} for HDP-CDV, ODE-CDV, oleyloxyethyl-CDV (OLE-CDV), and oleyloxypropyl-CDV (OLP-CDV) against the two distinct pools of ECTV were 0.2–1.1 \mu M, which represented an 11- and 60-fold enhanced antiviral activity as compared to CDV (Table 1). The tested compounds had similar EC_{50} values against ECTV and VACV-WR.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ECTV (\mu M)</th>
<th>ECTV-7.5 (\mu M)</th>
<th>VACV-WR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDV</td>
<td>12 \pm 2.8</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>HDP-CDV</td>
<td>0.5 \pm 0.1</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>ODE-CDV</td>
<td>0.2 \pm 0.0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>OLE-CDV</td>
<td>0.2</td>
<td>0.2</td>
<td>NA</td>
</tr>
<tr>
<td>OLP-CDV</td>
<td>1.1 \pm 0.2</td>
<td>NA</td>
<td>1.1 \pm 0.0</td>
</tr>
</tbody>
</table>

a Plaque reduction assay in CV-1 cells.

b An ECTV recombinant expressing murine IL-4 from the 7.5 early promoter.

c No IC_{50} value available.

Efficacy of four alkoxyalkyl esters of cidofovir in an ectromelia virus aerosol challenge model

Because ECTV has an LD_{50} of less than 1 PFU in the A/NCR strain of mouse following footpad inoculation, this strain was chosen to evaluate the efficacy of CDV analogs (Chen et al., 1992). Preliminary experiments demonstrated that adult A/NCR mice were also highly sensitive by the aerosol route. Doses of 20,000, 1,000, 63, and 6 PFU per mouse gave mean time to death (MTD) values of 8.1, 9.3, 10.2, and 14 days, respectively. The calculated LD_{50} value was 32 PFU. For comparison, the commonly used CPXV mouse model required an aerosol exposure dose of 5 \times 10^6 PFU of CPXV to obtain 100% mortality with ~3-week-old

![Fig. 1. Structure of cidofovir and ether lipid esters.](image-url)
BALB/c mice, and lowering the exposure dose to $5 \times 10^4$ PFU caused only transient, mild illness, and weight loss, but no deaths (Bray et al., 2000). Thus, the ECTV aerosol model provides a much greater dynamic range for evaluating antivirals. One can choose a low lethal dose of 100 PFU, which is approximately threefold greater than the LD$_{50}$ or use a high dose of 1000–10000 times the LD$_{50}$ to fully test the robustness of the antiviral.

The goal of our initial in vivo assessment was to select the two best compounds for a more in depth analysis. A 3 mg/kg dose was chosen for the evaluation as preliminary studies suggested this dose would permit discrimination among the activities of closely related compounds. This drug dose was not toxic as the maximal tolerated dose for HDP-CDV was >30 mg/kg and >10 mg/kg for ODE-, OLP-, and OLE-CDV when administered as five consecutive daily doses to uninfected mice. A/NCR mice were treated by gavage with 3 mg/kg of test compounds, exposed to an aerosol dose of $1.7 \times 10^5$ PFU of ECTV, and were monitored for mortality, day of death, and lung, spleen, and liver infectivity levels (Table 2). As expected from its low bioavailability, CDV failed to protect any mice from a lethal infection, and gave the shortest MTD (9.8 $\pm$ 3.5 days) of any of the compounds. ODE-CDV showed the greatest promise among the tested compounds as only 10% of the treated mice died following aerosol challenge, significantly lower virus titers were detected in spleen tissue as compared to the other compounds, and no detectable virus infectivity was found in liver tissue. Careful examination of the survival percentages, MTD values, and virus titers in tissues indicated that HDP-CDV, OLP-CDV, and OLE-CDV had similar activities. Because the double bond contained in the lipid moiety of OLP-CDV and OLE-CDV could potentially affect long-term drug stability, and because these compounds were not demonstrably better than HDP-CDV, we chose not to study them further in vivo.

### Dose response evaluation of HDP-CDV and ODE-CDV in an ectromelia virus aerosol challenge model

Groups of A/NCr mice were treated by gavage with 10, 5, 2.5, or 1.25 mg/kg of CDV, HDP-CDV, or ODE-CDV, exposed to an aerosol dose of $2.3 \times 10^4$ PFU of ECTV, and monitored for mortality, day of death, and lung, spleen, and liver infectivity levels (Fig. 2). As expected, oral CDV failed to provide any protection against a lethal ECTV infection over the range of doses tested. ODE-CDV was clearly more potent than HDP-CDV for protection against a lethal aerosol ECTV challenge as it protected more mice against death at the lower doses (5, 2.5, and 1.25 mg/kg), and also extended the MTD values (Fig. 2, Panel A). Virus titers in lung tissue of HDP-CDV and ODE-CDV-treated mice were similar at all tested doses, and only differed significantly from phosphate-buffered saline or CDV-treated mice at the 10 mg/kg dose (Fig. 2, panel B). Virus titers in spleen tissue of HDP-CDV and ODE-CDV-treated mice differed significantly from phosphate buffered saline or CDV-treated mice at all tested doses (Fig. 2, panel C). Although the mean virus titers in spleen tissue of HDP-CDV and ODE-CDV-treated mice were similar at doses of 1.25 and 2.5 mg/kg, three ODE-treated mice lacked detectable virus infectivity as compared to none for the HDP-CDV-treated mice. The greatest difference in tissue virus infectivity between HDP-CDV and ODE-CDV-treated mice was observed in the liver, which shows severe pathology in fatal cases of mousepox (Buller and Palumbo, 1991). At the lowest drug dose employed (1.25 mg/kg), virus titers in liver were almost 100-fold lower in ODE-CDV-treated mice as compared to the HDP-CDV treatment group (Fig. 2, panel D). In addition, two ODE-CDV-treated mice lacked detectable virus infectivity. The apparent difference in liver titers between HDP-CDV and ODE-CDV-treated mice at 2.5 mg/kg is not considered significant as each data point contained

### Table 2

Effect of oral gavage treatment with cidofovir and alkoxyalkyl esters on an infection induced in mice by aerosolized ectromelia virus

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Day of death mean ± SD</th>
<th>Mortality at 21 days post-aerosol challenge</th>
<th>Virus infectivity (PFU/ml ± SD)$^b$</th>
<th>[\text{Spleen}]</th>
<th>[\text{Liver}]</th>
<th>[\text{Lung}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDV</td>
<td>9.8 ± 3.5</td>
<td>11/11 (100%)</td>
<td>$1.1 \times 10^5$ ± 1.5</td>
<td>$1.2 \times 10^5$ ± 1.6</td>
<td>$3.8 \times 10^6$ ± 1.0</td>
<td></td>
</tr>
<tr>
<td>HDP-CDV</td>
<td>12.8 ± 1.3***</td>
<td>8/11 (72%)</td>
<td>$2.0 \times 10^5$ ± 2.4</td>
<td>$3.3 \times 10^4$ ± 2.9 (2)$^c$</td>
<td>$2.0 \times 10^5$ ± 1.7</td>
<td></td>
</tr>
<tr>
<td>ODE-CDV$^d$</td>
<td>NA****</td>
<td>1/10 (10%$^*$)</td>
<td>$6.4 \times 10^3$ (2)</td>
<td>$&lt;1 \times 10^3$ (3)</td>
<td>$1.6 \times 10^4$ ± 1.0</td>
<td></td>
</tr>
<tr>
<td>OLP-CDV$^e$</td>
<td>14.4 ± 4.4***</td>
<td>9/11 (81%)</td>
<td>$7.8 \times 10^5$ ± 15</td>
<td>$1.3 \times 10^6$ ± 2.1 (1)</td>
<td>$5.0 \times 10^4$ ± 4.7</td>
<td></td>
</tr>
<tr>
<td>OLE-CDV$^f$</td>
<td>13.4 ± 3.4***</td>
<td>6/11 (54%$^*$)</td>
<td>$2.1 \times 10^6$ ± 2.6</td>
<td>$2.3 \times 10^5$ ± 3.2</td>
<td>$2.4 \times 10^5$ ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.3 ± 0.4</td>
<td>11/11 (100%)</td>
<td>$5.0 \times 10^4$ ± 1.0</td>
<td>$9.6 \times 10^5$ ± 14</td>
<td>$3.0 \times 10^4$ ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

Arithmetic means and standard deviations from the mean are provided. Statistical significance from control was indicated by *, **, and *** for $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

- Mice were treated daily with 3 mg/kg of drug by gavage on days 0–4. Infection by aerosol occurred 4 h before the first drug treatment. The presented aerosol dose was $1.7 \times 10^5$ PFU.
- Only three mice were available for necropsy.
- Only two mice were available for necropsy.
- No deaths (Bray et al., 2000). Thus, the ECTV aerosol model.

$^a$ Only two mice were available for necropsy.
$^b$ Only three mice were available for necropsy.
$^c$ The value in ( ) indicates the number of samples in which infectivity was below the limit of detection ($<10^2$).
$^d$ Five mice from each treatment group were sacrificed at 7 days post-aerosol challenge. Lung, spleen, and liver tissues were isolated and tested for virus infectivity.
$^e$ Not applicable.
$^f$ Arithmetic means and standard deviations from the mean are provided. Statistical significance from control was indicated by *, **, and *** for $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
only one mouse with the remaining four mice of each group lacking detectable virus infectivity. When surviving animals were sacrificed at 21 days postinfection at the completion of the experiment, no residual virus infectivity was detected in spleens and livers (data not shown).

Discussion

CDV (Vistide) is licensed for intravenous use in the treatment of human cytomegalovirus retinitis (HCMV) in HIV-infected patients, and is the subject of a treatment investigational new drug application (IND) for smallpox and complications arising from the smallpox vaccine. It also has broad spectrum antiviral activity against a wide-range of DNA virus and retrovirus infections, but oral bioavailability of <5% hinders its development as an efficacious oral antiviral drug (De Clercq, 1998; Wachsman et al., 1996). Oral bioavailability can be significantly enhanced by derivatization of CDV with ether lipid residues of various chain lengths (Ciesla et al., 2003). Radioactive versions of HDP-, ODE-, and OLP-CDV orally administered to mice achieved plasma bioavailability levels of 88–97%, and concentrations of 1.4–3.4 µM (Ciesla et al., 2003). This enhanced bioavailability appeared because of the general resemblance of alkoxyalkyl esters of CDV to a naturally occurring compound, lysophosphatidylcholine, most of which is absorbed intact from the small intestine (Scow et al., 1967). Unexpectedly, the alkoxyalkyl esters of CDV also showed a dramatic decrease in the EC₅₀ values against VACV, CPXV, and HCMV (Beadle et al., 2002; Kern et al., 2002). Using VACV-WR in human foreskin fibroblasts,
Kern et al. (2002) obtained EC_{50} values for HDP-CDV and ODE-CDV of 1.1 ± 1.0 and 0.2 ± 0.2 μM, respectively, which compared favorably with the values summarized in Table 1 (HDP-CDV, 0.5 ± 0.1 μM, and ODE-CDV, 0.2 ± 0.0 μM). Importantly, the Kern et al. (2002) study determined the selectivity indices (SI) of HDP-CDV and ODE-CDV to be 6- and 11-fold, respectively, greater than CDV using CPXV and VACV. The SI value relates the toxicity and efficacy of a compound by dividing the drug’s 50% cytotoxic concentration by the 50% effective concentration. As compared to CDV, the larger SI values for analogs HDP-CDV and ODE-CDV indicate that these compound are excellent candidates for in vivo efficacy testing.

This marked increase in antiviral activity in vitro was due to greatly increased cellular uptake of HDP-CDV relative to CDV. Intracellular levels of the activated antiviral compound, CDV-diphosphate, were 100 times greater after exposure of cells to HDP-CDV than CDV (Aldern et al., 2003). Furthermore, the intracellular half-life for CDV-DP was 10 and <2.7 days following exposure of cells to HDP-CDV and CDV, respectively (Aduma et al., 1995; Aldern et al., 2003). The efficient uptake into cells of alkoxyalkyl esters of CDV such as HDP-CDV is thought to occur via rapid association with cellular membrane phospholipids, whereas the uptake of CDV is slow, and has been shown to occur by fluid phase endocytosis (Connelly et al., 1993). Although this increased cellular uptake of alkoxyalkyl esters of CDV was associated with increased toxicity, the SI index was still significantly better than CDV (Kern et al., 2002). Furthermore, CDV is highly concentrated in the kidney and its dose-limiting toxicity is renal; however, the peak kidney levels of HDP-CDV and ODE-CDV are only 3.3% of CDV (Ciesla et al., 2003). Thus, this class of CDV analogs is worthy of further investigation as possible oral therapies for diseases caused by viruses including HCMV, herpes simplex viruses, and poxviruses.

In this study, we investigated the efficacy of alkoxyalkyl esters of CDV in a mouse model for smallpox. We
compared the oral activity of CDV and CDV analogs in
an aerosol ECTV challenge model in A/NCR mice. ODE-
CDV appeared to be the most potent CDV analog as a 5-
mg/kg, five dose regimen protected 100% of A/NCR
mice from a lethal aerosol challenge dose of ~1000
times the LD50. Significant protection was also observed
at 1.25 mg/kg dose. Virus replication in spleen and liver
was not detected when mice were treated with oral doses
of ODE-CDV at 5 mg/kg or greater; however, virus titers
in lung tissue were reduced from control only when the
dose was at least 10 mg/kg. The relative level of virus
infectivity in tissues did not strongly correlate with drug
tissue concentration. Peak HDP-CDV and ODE-CDV
concentrations, respectively, were 1.00 and 1.19 nmol/g
in lung, 0.56 and 0.79 nmol/g in spleen, and 43.7 and
25.7 nmol/g in liver (Ciesla et al., 2003). The high levels
of HDP-CDV and ODE-CDV in liver tissue may favor
antiviral efficacy against ECTV as compared to VACV
and VARV because ECTV replicates to far higher levels
in liver tissue than does VACV or VARV. The synthesis
of an alkoxyalkyl ester of CDV that exerts greater
antiviral activity in lung tissue than ODE-CDV, or the
administration of aerosolized CDV, may reduce ECTV
virus replication in the lung following an aerosolized
challenge (Bray et al., 2002; Smee et al., 2000). A
strategy of adjusting the character of the lipophilic adduct
to increase the concentration of lung CDV-diphosphate is
based on the observation that 14C-labeled ODE-CDV
given orally provides substantially higher drug concentra-
tion in lung tissue than the slightly less lipophilic HDP-
CDV (Ciesla et al., 2003). These approaches may not be
necessary, however, as pulmonary disease and presumably
extensive variola virus replication in lung was not an
early finding in smallpox, rather bronchopneumonia when
observed was usually associated with late stage disease
(Fenner et al., 1988). A strategy to optimize the pharma-
cokinetics of alkoxyalkyl esters of CDV would be deper-
dent on a thorough understanding of the pathogenesis of
smallpox. This knowledge is currently lacking. The results
presented here suggest that the ODE-CDV and
similar compounds should be further examined in oral
dose optimization and therapeutic intervention studies as
potential antivirals against human orthopoxvirus systemic
disease caused by VACV, monkeypox virus, and VARV.

Materials and methods

Cells and virus

BS-C-1 cells (ATCC CCL 26) were grown in Dulbec-
co’s modified Eagle’s medium (DMEM) containing 10% bovine serum fetal clone III (HyClone, Logan, UT), 2
mM l-glutamine (GIBCO, Grand Island, NY), 100 U/ml penicillin (GIBCO), and 100 μg/ml streptomycin
(GIBCO). A plaque-purified isolate of the MOS strain
of ECTV (ATCC VR-1374) designated MOS-3-P2 was
propagated in an African green monkey kidney cell line,
BS-C-1 (Chen et al., 1992). Virus was purified through a
sucrose cushion as described elsewhere (Moss and Earl,
1998). The VACV-WR (ATCC VR-119) was propagated
as described elsewhere (Moss and Earl, 1998). Virus
infectivity was estimated as described previously (Wallace
and Buller, 1985). Briefly, virus suspensions were serially
diluted in PBS +1% sera, absorbed to monolayers for 1
h at 37 °C, and overlayed with a suspension of 1%
carboxyl methyl cellulose in DMEM +5% Fetal clone III.
After 4 days at 37 °C, virus plaques were visualized and
virus inactivated by the addition to each well of 0.5 ml
of a 0.3% crystal violet/10% formalin solution.

Animals

Four- to six-week-old female A/NCR 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 an
animal biosafety level 3 containment area. Animal husband-
ry and experimental procedures were in accordance with
PHS policy, and approved by the Institutional Animal Care
and Use Committee.

Antiviral compounds

Cidofovir ([S]-1-[3-hydroxy-2-phosphonylmethoxypro-
pyl]cytosine, HPMPC, Vistide) was purchased from a retail
pharmacy or was provided by Gilead Sciences, Inc. (Foster
City, CA). Ether lipid analogs of CDV (HDP-CDV, ODE-
CDV, and OLP-CDV) were prepared as described previously
(Beadle et al., 2002). CDV and analogs were prepared fresh for each experiment by dissolving the compounds in
sterile, distilled water. Solutions were stored at 4 °C over
the course of the experiment.

Plaque reduction assay

CV-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
+5% Fetal clone III 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 for ECTV and 2 days for
VACV-WR, monolayers were stained, and plaques counted
using a stereomicroscope. The EC50 concentration for each
drug was calculated.

In vivo drug evaluation

Mice were exposed to aerosolized ECTV suspended in
MEM 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 1.5 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 approximately –0.5 in of vacuum relative to the outside atmospheric pressure. The NOIES delivered a predicted median particle diameter of 0.54 ± 1.2 μm (Dr. Chad Roy, personal 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 (Cₐ) in PFU by the total volume (Vₐ) 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 the virus was optimally aerosolized and completely taken up on inhalation. Four hours following aerosol exposure to ECTV, groups of 16 mice were treated by gavage with 0.1 ml of sterile, distilled water alone or water containing the test compound. This treatment was repeated on days 1, 2, 3, and 4 for a total of five doses. Five mice from each treatment group were sacrificed at 7 days post-aerosol challenge, and lung, spleen, and liver tissues were isolated. Tissue was ground in PBS (10%w/v), frozen, and thawed three times, and sonicated.

Acknowledgments

R.M.B. was supported by a NIAID contract NO1-AI-15436 and K.Y.H. was supported by NEI grant EY11834, NIAID grant AI29164, and Department of the Army grant DAMD17-01-2-007. The U.S. Army Medical Research Acquisition Activity, Fort Detrick, MD, was the awarding acquisition office. The content of this article does not necessarily reflect the position of policy of the government, and no official endorsement should be inferred. We would like to thank Dawn Schwartz for editorial assistance and Meribeth Broadway for technical expertise.

References


