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Efficacy of Multiple- or Single-Dose Cidofovir against Vaccinia and Cowpox Virus Infections in Mice

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Orthopoxviruses, including variola and monkeypox, pose risks to human health through natural transmission or potential bioterrorist activities. Since vaccination has not recently been utilized for control of these infections, there is renewed effort in the development of antiviral agents not only for postexposure smallpox therapy but also for treatment of adverse reactions following vaccination. The objectives of this study were to expand on the results of others that cidofovir (CDV) is effective in mice inoculated with cowpox virus (CV) or vaccinia virus (VV) and to document the efficacy of single and interval dosing beginning prior to or after infection, particularly including evaluations using suboptimal doses of CDV. We utilized BALB/c or SCID mice inoculated with CV or VV as models for systemic poxvirus infections. BALB/c mice were inoculated intranasally with CV or VV and treated with CDV prior to or after virus inoculation. CDV, at concentrations as low as 0.7 to 6.7 mg/kg of body weight/day for 5 days, conferred significant protection when treatment was initiated as late as 72 to 96 h postinfection. A single-dose pretreatment or posttreatment with CDV at 3 to 100 mg/kg was effective when given as early as 5 days prior to infection or as late as 3 days after infection with either VV or CV. Interval treatments given every third day beginning 72 h postinfection using 6.7 or 2 mg of CDV/kg also proved effective against CV infections. When SCID mice were inoculated intraperitoneally with CV or VV and treated for 7 to 30 days with CDV, all the mice eventually died during or after cessation of treatment; however, significant delays in time to death and reduction of virus replication in organs occurred in most treated groups, and no resistance to CDV was detected.

The search for new antiviral agents that are effective against orthopoxvirus infections has escalated recently due to concerns about potential bioterrorist use of smallpox (4, 14, 15), especially in light of recent activities using anthrax (10) and the growing number of cases of human monkeypox in recent years (11). Variola virus, the causative agent for smallpox disease, poses a real threat to human health through potential bioterrorist activities, since vaccination programs ended during the 1970s. A growing percentage of the world's population, now susceptible to infection, is at risk to an intentional release and subsequent aerosol exposure. Variola virus was accidentally aerosolized in a German hospital in 1966, and the resulting human cases documented the ease of transmitting the virus by the aerosol route (27). It is also known that human-to-human transmission of monkeypox occurs by an aerosol route (11). Variola virus does not cause disease in rodents, and research involving this agent is restricted to one site in Russia and one in the United States. It is therefore necessary to develop closely related orthopoxvirus infections in small animal models that simulate the disease in humans in order to evaluate new antiviral agents for these diseases.

There are only a few experimental animal infections available for evaluation of antiviral compounds against orthopoxviruses. Mims (19) first described the pathology associated with intravenous inoculation of cowpox virus in BALB/c mice, and later, Buller (5) proposed BALB/c mice as a model for the study of poxviruses. Miller et al. (17, 18) examined the immunological parameters of poxvirus infections and used an alternative route, footpad inoculation of cowpox virus, and described a prolonged inflammatory local reaction of C5-deficient mice to the virus. De Clercq and coworkers, (6–9), Neyts and De Clercq (20, 21), and Boyle et al. (1) have used models where vaccinia virus (VV) was given systemically, either intraperitoneally (i.p.) or intravenously, to mice and mortality or tail skin lesions were quantified for evaluation of antiviral activity. A more relevant evaluation of antiviral efficacy in animal models that simulates a bioterrorist release would require initiation of infections by the respiratory route, either by intranasal (i.n.) inoculation or exposure via an aerosol chamber, and a delayed treatment of at least 48 h. Martinez et al. (16) described in detail the pathology associated with aerosol exposures of mice to cowpox virus. The distal bronchoalveolar junctions of the respiratory tracts of mice were infected by aerosolization of cowpox virus (CV), while only half of i.n. infected mice had pulmonary lesions in the central hilar regions. Intranasally infected mice also had tracheal, bronchial, and nasal lesions of a hemorrhagic nature, which may, in fact, be more similar to the hemorrhagic type of smallpox in humans. Bray et al. (2, 3) and Smee et al. (23–26) have evaluated cidofovir (CDV) in mice infected with CV or VV by i.n. instillation or aerosol exposure and found CDV to be highly effective.

In our studies, we have documented the in vitro efficacy of CDV (13) and utilized CV and VV infections in normal and immunocompromised mice to determine the efficacy of CDV. Viruses were administered systemically by i.p. inoculation or by the respiratory route by using i.n. instillation. Treatments were administered 24 to 96 h after virus inoculation by using several dosage levels of CDV in order to determine its effec-

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tiveness under suboptimal conditions. To determine if CDV could be utilized prophylactically or as therapy postexposure, we evaluated single-dose administration given at various time intervals either prior to infection or postinfection and also determined the efficacy of multiple interval dose administration.

MATERIALS AND METHODS

Mice. Female BALB/c mice, 3 weeks of age, and male SCID mice, 5 to 10 weeks of age, were obtained commercially (Charles River Laboratories, Raleigh, N.C., and Wilmington, Va., respectively). The mice were group-housed in microisolator cages, and 15 mice per treatment group were used. The mice were obtained, housed, utilized, and euthanized according to U.S. Department of Agriculture and Association for Assessment and Accreditation of Laboratory Animal Care International regulatory policies. All animal procedures were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee prior to the initiation of studies.

Virus. Cowpox virus, strain Brighton (CV-BR), was kindly provided by John W. Huggins (U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md.). Vaccinia virus, strain WR (VV-WR), was obtained from the American Type Tissue Collection, Washington, D.C.

Antiviral compounds. Cidofovir (Gilead Pharmaceuticals, Foster City, Calif.) was weighed and dissolved in sterile saline to yield the desired dosages within a 0.1-ml volume. It was administered i.p. once daily for 7 days, three to four times weekly for periods of 7 to 30 days, or as a single dose, depending on the experimental protocol.

Experimental infections and viral pathogenesis. Systemic infections were initiated either by i.n. inoculation (BALB/c mice) or by i.p. injection (SCID mice). For i.n. inoculations, mice were anesthetized with ketamine-xylazine and infected with CV-BR or VV-WR by using a micropipetor at a total volume of 40 μl per animal. For i.p. inoculations, mice were injected with CV-BR or VV-WR at a total volume of 0.1 ml.

To determine the extent of viral replication in tissues from SCID mice inoculated i.p. with CV-BR, three animals from both the untreated and CDV-treated groups were euthanized on days 3, 5, 7, 10, 14, 18, 24, 28, 32, and 37. Lung, liver, spleen, and kidney samples were aseptically removed, weighed, homogenized in minimal essential medium (10% wt/vol), and frozen at −70°C as described previously (22) until assayed for virus. To determine if failure of therapy in this model was due in part to the development of resistance to CDV, virus isolated from tissue homogenates from both control and treated mice was analyzed for susceptibility to CDV using a plaque reduction assay as described previously (13).

Virus quantitation. Samples were thawed and assayed on Vero cells by using an agarose overlay plaque assay to determine CV-BR titers (13). Briefly, samples of organ homogenates were diluted serially, and a 0.2-ml volume was placed into each of 12 wells of Vero cell monolayers and incubated for 1 h. A solution containing 0.5% agar in minimal essential medium (Seakem ME agarose; FMC BioProducts, Rockland, Md.) was added to each well, and the cultures were incubated for 3 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 the mean day of death (MDD) was determined by using the Mann-Whitney U rank sum test. A P value of ≤ 0.05 was considered significant.

RESULTS

Effect of multiple daily dose treatment with CDV on mortality of mice inoculated with CV or VV. Three-week-old BALB/c mice were inoculated i.n. with about 5×10^5 PFU of CV-BR and treated i.p. once daily for 7 days with 60, 20, or 6.7 mg of CDV/kg of body weight beginning 24, 48, or 72 h after infection. The effect of treatment on the mortality of these mice is summarized in Table 1 as experiment A. Although the placebo-treated animals had only 47% mortality, all three concentrations of CDV significantly reduced mortality even if treatment was delayed until 72 h postinfection. In a second experiment designed to achieve a higher mortality rate (Table 1, experiment B), mice were inoculated as above with 1.6×10^6 PFU of CV-BR and treated once daily for 7 days with 6.7, 2.2, or 0.7 mg of CDV/kg beginning 48, 72, or 96 h postinfection. Protection was obtained only with the 6.7-mg/kg dose, but treatment could be delayed until 72 or 96 h postinfection.

TABLE 1. Effect of treatment with CDV on mortality of BALB/c mice inoculated i.n. with CV-BR

Treatment	Mortality rate (%)	P	MDD	P
Expt A				
Placebo-saline	7/15 (47)		14	
CDV				
60 mg/kg +24 h	0/15 (0)	<0.01		
20 mg/kg +24 h	0/15 (0)	<0.01		
6.7 mg/kg +24 h	1/14 (7)	<0.05	10	NS[a]
60 mg/kg +48 h	0/11 (0)	<0.01		
20 mg/kg +48 h	0/15 (0)	<0.01		
6.7 mg/kg +48 h	0/15 (0)	<0.01		
60 mg/kg +72 h	0/15 (0)	<0.01		
20 mg/kg +72 h	0/15 (0)	<0.01		
6.7 mg/kg +72 h	0/15 (0)	<0.01		
Expt B				
Placebo-saline	14/15 (93)		10.3	
CDV				
6.7 mg/kg +48 h	6/15 (40)	<0.01	12.3	NS
2.2 mg/kg +48 h	14/15 (93)	NS	11.2	NS
0.7 mg/kg +48 h	14/15 (93)	NS	9.5	NS
6.7 mg/kg +72 h	8/15 (53)	<0.05	10.8	NS
2.2 mg/kg +72 h	10/15 (67)	NS	12.2	<0.05
0.7 mg/kg +72 h	15/15 (100)	NS	10.7	NS

[a] NS, result not significant compared to that of the placebo control.

To determine the activity of CDV against VV-WR infection, mice were inoculated i.n. with 4×10^4 PFU of VV-WR and treated i.p. once daily for 7 days with 6.7, 2.2, or 0.7 mg of

TABLE 2. Effect of treatment with CDV on mortality of BALB/c mice inoculated i.n. with VV-WR

Treatment	Mortality rate (%)	P	MDD	P
Placebo-saline	15/15 (100)		6.7	
CDV				
+48 h				
6.7 mg/kg	0/15 (0)	<0.001		
2.2 mg/kg	1/15 (7)	<0.001	7	NS[a]
0.7 mg/kg	6/15 (40)	<0.001	8.3	NS
+72 h				
6.7 mg/kg	0/15 (0)	0.001		
2.2 mg/kg	6/15 (40)	<0.01	7.3	NS
0.7 mg/kg	10/15 (67)	0.05	7.7	NS
+96 h				
6.7 mg/kg	6/15 (40)	<0.01	8.5	NS
2.2 mg/kg	15/15 (100)	NS	8.3	NS
0.7 mg/kg	15/15 (100)	NS	7.5	0.03

[a] NS, result not significant compared to that of the placebo control.

TABLE 3. Effect of interval treatment with CDV on mortality of BALB/c mice inoculated i.n. with CV-BR

Treatment[a]	Mortality rate (%)	*P*	MDD	*P*
Placebo				
+24 h	16/16 (100)		9.2	
+48 h	14/15 (93)		10.1	
+72 h	14/14 (100)		9.3	
CDV once daily				
6.7 mg/kg +24 h	1/15 (7)	<0.001	9.0	NS[b]
6.7 mg/kg +48 h	0/15 (0)	<0.001		
6.7 mg/kg +72 h	1/15 (7)	<0.001	11.0	0.07
2 mg/kg +24 h	1/16 (6)	<0.001	3.0	0.07
2 mg/kg +48 h	5/15 (33)	<0.01	14.2	NS
2 mg/kg +72 h	5/15 (33)	<0.001	15.4	0.001
CDV every 48 h				
6.7 mg/kg +24 h	0/15 (0)	<0.001		
6.7 mg/kg +48 h	1/15 (7)	<0.001	12.0	0.09
6.7 mg/kg +72 h	1/15 (7)	<0.001	15.0	0.07
2 mg/kg +24 h	4/15 (27)	<0.001	12.8	<0.001
2 mg/kg +48 h	7/15 (47)	<0.01	13.0	<0.01
2 mg/kg +72 h	8/15 (53)	<0.01	11.4	<0.01
CDV every 72 h				
6.7 mg/kg +24 h	0/15 (0)	<0.001		
6.7 mg/kg +48 h	5/15 (33)	<0.01	14.6	<0.01
6.7 mg/kg +72 h	0/15 (0)	<0.001		
2 mg/kg +24 h	1/15 (7)	<0.001	21.0	0.07
2 mg/kg +48 h	14/15 (93)	NS	11.8	NS
2 mg/kg +72 h	11/15 (79)	NS	12.8	<0.001

[a] CDV was prepared in sterile saline and delivered i.p. in 0.1-ml doses. The animals were treated once daily every other day or every third day for 7 days beginning at 24, 48, or 72 h after viral inoculation.
[b] NS, result not significant compared to that of the placebo control.

TABLE 4. Effect of single dose CDV on mortality of BALB/c mice inoculated i.n. with CV-BR

Treatment[a]	Mortality rate (%)	*P*	MDD	*P*
None	12/15 (80)		10.5	
Placebo, day +1	15/15 (100)		10.6	
CDV				
Day −5				
100 mg/kg	8/15 (53)	<0.01	12.6	<0.05
30 mg/kg	12/15 (80)	NS[b]	12.3	0.07
10 mg/kg	9/15 (60)	<0.05	11.0	NS
3 mg/kg	14/15 (93)	NS	11.6	NS
Day −3				
100 mg/kg	2/15 (13)	<0.001	10.5	NS
30 mg/kg	3/15 (20)	<0.001	11.3	NS
10 mg/kg	11/15 (73)	NS	11.5	NS
3 mg/kg	14/15 (100)	NS	10.6	NS
Day −1				
100 mg/kg	1/15 (7)	<0.001	10.0	NS
30 mg/kg	4/15 (27)	<0.001	12.3	NS
10 mg/kg	5/15 (33)	<0.001	12.0	NS
3 mg/kg	8/15 (53)	<0.01	10.6	NS
Day +1				
100 mg/kg	1/15 (7)	<0.001	9.0	
30 mg/kg	4/15 (27)	<0.001	10.8	NS
10 mg/kg	7/15 (47)	<0.01	10.6	NS
3 mg/kg	12/15 (80)	NS	10.1	NS
Day +3				
100 mg/kg	0/15 (0)	<0.001		
30 mg/kg	3/15 (20)	<0.001	13.0	NS
10 mg/kg	4/15 (27)	<0.001	15.5	<0.05
3 mg/kg	13/15 (87)	NS	11.2	NS

[a] CDV was prepared in sterile saline and delivered i.p. in 0.1-ml doses. The animals were treated one time only for each time period beginning on day −5, −3, or −1 before or day 1 or day 3 after viral inoculation.
[b] NS, result not significant compared to that of the placebo control.

CDV/kg beginning 48, 72, or 96 h after infection. Results similar to those described above for CV-BR were obtained; however, significant protection against mortality was observed at concentrations of CDV as low as 0.7 to 6.7 mg/kg. Significant protection could be obtained even if therapy was delayed until 72 to 96 h postinfection (Table 2).

Effect of interval dosing treatments with CDV on mortality of mice inoculated with CV. Since intravenous administration of CDV under emergency conditions would be logistically difficult, we also determined if dosing two to three times weekly would be effective. BALB/c mice were inoculated i.n. with 5.3×10^5 PFU of CV-BR and treated with CDV either once daily for 7 days, every other day, or every third day to determine the efficacy of interval treatments with lower dosages of CDV. CDV was given at 6.7 and 2 mg/kg i.p. beginning 24, 48, or 72 h after viral inoculation. The results in Table 3 clearly indicate the protective effects of interval dosing, even with suboptimal levels of CDV and even when delayed up to 72 h postinfection.

Effect of single-dose CDV given prophylactically or therapeutically to mice infected with CV or VV. Since CDV has the unique property of having a long intracellular half-life of about 15 to 65 h but is associated with severe nephrotoxicity after multiple dosing in humans, we next determined how long a single dose of CDV would retain efficacy when given at 100, 30, 10, or 3 mg/kg beginning either 5, 3, or 1 day prior to CV-BR infection (5×10^5 PFU/mouse) or if administered 1 or 3 days after infection. The results summarized in Table 4 clearly indicate that a single dose of 100 mg of CDV/kg provided significant protection when given any time from day −5 to day +3. The 30-mg/kg dose was highly effective when given at day −3 to day +3. The 10-mg/kg dose was most effective when given at day −1 to day +3, and the 3-mg/kg dose was most effective when given at day 1. Similar results were obtained when mice were infected with VV and treated as described above (Table 5). These results indicate that the effectiveness of CDV in these animal models is retained for at least 5 days after a single treatment and is dose dependent.

Effect of treatment of CV or VV infections in SCID mice with CDV. Acquisition of smallpox or vaccination with VV in an immunocompromised host can result in serious morbidity and even mortality. As a model for the immunocompromised host with disseminated orthopoxvirus disease, we have used SCID mice inoculated i.p. with either CV-BR or VV-WR. In the experiments summarized in Table 6, groups of 6- to 8-week-old SCID mice were inoculated with about 1×10^4 PFU of either CV-BR or VV-WR and treated once daily for 7 days with 20, 6.7, or 2.2 mg of CDV/kg beginning 48, 72, or 96 h after infection. In all the treatment groups, there was essentially 100% mortality; however, the MDD was significantly prolonged in most groups and particularly for mice infected with

TABLE 5. Effect of single-dose CDV on mortality of BALB/c mice inoculated i.n. with VV-WR

Treatment[a]	Mortality rate (%)	*P*	MDD	*P*
None	15/15 (100)		9.1	
Placebo, day +1	14/15 (93)		8.6	
CDV				
Day −5				
100 mg/kg	1/15 (7)	<0.001	10.0	0.7
30 mg/kg	9/15 (60)	0.08	9.0	NS[b]
10 mg/kg	8/15 (53)	<0.05	8.8	NS
3 mg/kg	14/15 (93)	NS	8.5	NS
Day −3				
100 mg/kg	2/15 (13)	<0.001	8.5	NS
30 mg/kg	7/15 (47)	0.01	9.1	NS
10 mg/kg	15/15 (100)	NS	8.4	NS
3 mg/kg	12/15 (80)	NS	8.3	NS
Day −1				
100 mg/kg	0/15 (0)	<0.001		
30 mg/kg	0/15 (0)	<0.001		
10 mg/kg	2/15 (13)	<0.001	12.0	0.01
3 mg/kg	12/15 (80)	NS	8.6	NS
Day +1				
100 mg/kg	0/15 (0)	<0.001		
30 mg/kg	0/15 (0)	<0.001		
10 mg/kg	0/15 (0)	<0.001		
3 mg/kg	4/15 (27)	<0.001	9.0	NS
Day +3				
100 mg/kg	2/15 (13)	<0.001	10.5	0.01
30 mg/kg	1/15 (7)	<0.001	8.0	NS
10 mg/kg	0/15 (0)	<0.001		
3 mg/kg	8/15 (53)	<0.05	8.6	NS

[a] CDV was prepared in sterile saline and delivered i.p. in 0.1-ml doses. The animals were treated one time only for each time period beginning day −5, −3, or −1 or day 1 or day 3 after viral inoculation.
[b] NS, result not significant compared to that of the placebo control.

TABLE 6. Effect of treatment with CDV on mortality of SCID mice inoculated i.p. with CV-BR or VV-WR

Virus	Treatment	Mortality rate (%)	*P*	MDD	*P*
CV-BR	Placebo-saline	15/15 (100)		8.3	
	CDV				
	+48 h				
	20 mg/kg	15/15 (100)	NS[a]	17.3	<0.001
	6.7 mg/kg	15/15 (100)	NS	10.7	<0.05
	2.2 mg/kg	15/15 (100)	NS	8.0	NS
	+72 h				
	20 mg/kg	14/15 (93)	NS	11.9	<0.01
	6.7 mg/kg	15/15 (100)	NS	10.0	NS
	2.2 mg/kg	15/15 (100)	NS	7.7	NS
VV-WR	Placebo-saline	10/10 (100)		12.7	
	CDV				
	+72 h				
	20 mg/kg	10/10 (100)	NS	22.7	<0.001
	6.7 mg/kg	10/10 (100)	NS	19.9	<0.001
	2.2 mg/kg	10/10 (100)	NS	18.3	<0.001
	+96 h				
	20 mg/kg	10/10 (100)	NS	19.6	0.001
	6.7 mg/kg	10/10 (100)	NS	17.9	<0.001
	2.2 mg/kg	10/10 (100)	NS	15.7	<0.05

[a] NS, result not significant compared to that of the placebo control.

VV-WR. To determine if the extended MDD was associated with reduced viral replication in critical target organs, SCID mice were inoculated i.p. with a lethal concentration of CV-BR and treated with 20 mg of CDV/kg three times weekly for 30 days. Treatment was started at 96 h after infection to ensure that viral replication in target organs was maximal prior to initiation of treatment. Groups of mice from placebo- and CDV-treated animals were euthanized, and the lung, liver, spleen, and kidney were harvested at various days during infection. Tissues were harvested and assayed for the presence of the virus. Although the data are not provided here, the MDD of CDV-treated animals was increased from 14 to 24 days ($P < 0.001$), which was similar to the results in Table 6. The titers of virus in the tissue homogenates are shown in Fig. 1. Although there were no alterations of final mortality rates, mice treated with CDV had significantly reduced titers of virus in the four tissues tested. In the lung, peak CV titers were the same in both placebo- and CDV-treated mice; however, virus replication was delayed by about 21 days. There was a dramatic reduction in liver, spleen, and kidney tissue to low but still detectable levels at 21 to 31 days in treated mice.

Since there was persistence of the virus in all organs and high levels of replication of CV in lung tissue while CDV was being administered, a similar experiment was conducted to determine if resistance to CDV was developing in these mice. Virus pools were prepared by using tissue homogenates from saline- or CDV-treated mice through 37 days after infection. Each tissue-derived viral pool was then evaluated in vitro for susceptibility to CDV. The CDV 50% effective concentration (EC_{50}) values for CV from lung and liver tissue in vehicle or CDV-treated animals were essentially identical through day 37 of the infection and were similar to the value for the stock virus pool (Table 7). These results indicate that resistance to CDV did not develop during the 30 days of CDV treatment and suggest that failure of CDV to protect against mortality in SCID mice was due to their inability to clear the virus because of their immunodeficient state.

DISCUSSION

Smallpox is caused by variola virus, and presently there are no validated animal models for this disease. The virus does not infect rodents, and the murine models of systemic orthopoxvirus disease using surrogate viruses, CV, VV, and ectromelia virus in normal or immunocompromised mice are presently the only small animal models available for evaluating new compounds for antiviral activity in vivo. Since correlations between in vitro activity and in vivo activity are not often found, it is important to follow up the results from screening new compounds in vitro with evaluation of active compounds in vivo against CV and VV. Using these models, one can determine efficacy, minimal effective dose, maximum tolerated dose, maximum time of delay for initiation of effective treatment, and the length of treatment necessary for either postexposure treatment or for the potential use of postexposure prophylaxis, as

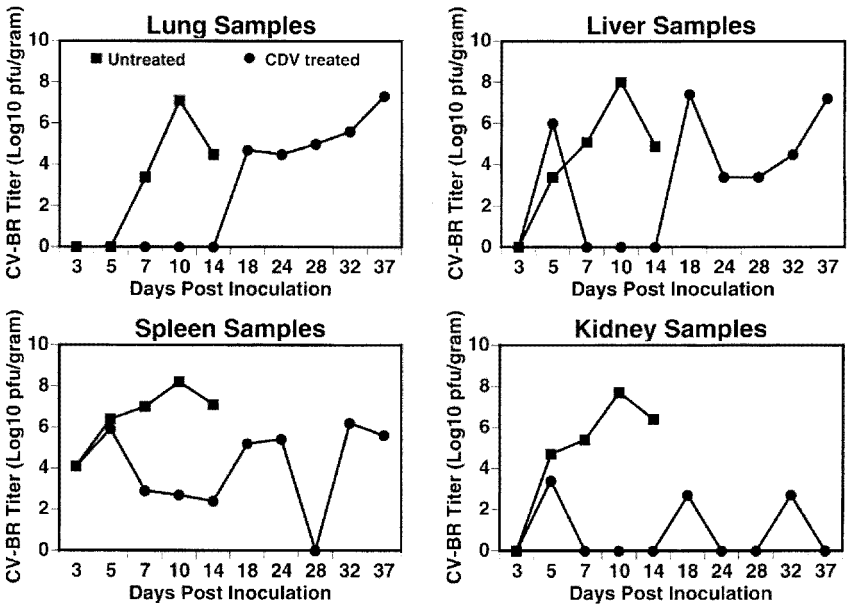

FIG. 1. Changes in CV-BR virus titers in organs of SCID mice treated three times weekly with 20 mg of CDV/kg of body weight beginning 24 h after infection and continuing through day 30.

well as for treatment of complications from vaccination. Infection of SCID mice simulates infection in severely immunocompromised individuals, such as AIDS patients, and is useful for evaluating new forms of treatment that might be utilized for these populations.

At present, CDV is the drug of choice for therapy of potential smallpox outbreaks and vaccination complications, and an investigational new drug protocol was approved recently for use in response to an actual smallpox outbreak. Although CDV is very active against all the orthopoxviruses (6), it has major limitations in its usefulness. CDV is toxic to kidney tubules and is not active orally, which necessitates intravenous administration. From a practical standpoint, it is anticipated that dosing will be limited to a single dose or to no more than two doses in a smallpox outbreak except for immunocompromised individuals who may require lengthy antiviral therapy.

TABLE 7. Lack of development of resistance to CDV in SCID mice infected with CV-BR and treated with CDV

Treatment	Sample type	CDV EC_{50} (μg/ml)	CDV EC_{50} mean ± SD
None	Virus stock	13.3 ± 1.7	
Saline			
Day 7	Lung	10.7	
Day 10	Lung	11.2	11.3 ± 0.71
Day 14	Lung	12.1	
Day 7	Liver	11.8	
Day 10	Liver	11.1	11.6 ± 0.44
Day 14	Liver	11.9	
CDV			
Day 18	Lung	11.9	
Day 24	Lung	12.3	
Day 28	Lung	12.1	12.0 ± 0.67
Day 32	Lung	12.7	
Day 37	Lung	10.9	
Day 18	Liver	11.1	
Day 24	Liver	12.1	
Day 28	Liver	11.5	12.0 ± 0.84
Day 32	Liver	11.8	
Day 37	Liver	13.3	

These studies confirm and expand upon previous reports of CDV efficacy in murine models of systemic orthopoxvirus infections (2, 3, 23–26). Our results indicated that CDV given systemically as late as 96 h after CV or VV inoculation can protect BALB/c mice from death or delay the time to death in a dose- and time-dependent manner. Since CDV is not effective when given orally, this delay in initiating therapy is necessary for attempting to plan postexposure intravenous treatments for potentially large numbers of people if an actual bioterrorist event occurred. Bray et al. (2) reported similarly that a single treatment with 100 mg of CDV/kg i.p. on day 0, 2, or 4 after aerosol exposure to cowpox virus increased survival of BALB/c mice to 90 to 100%. They also showed that aerosolization of CDV for inhalation therapy was effective at 0.5 to 5 mg/kg in CV-infected mice (3). Smee et al. (25) reported that a single dose of 10 mg of CDV/kg administered i.n. at +24 h in a 40-μl volume protected mice inoculated i.n. with CV-BR. In our studies, a single dose of 3 to 100 mg/kg provided protection if given 5 days prior to infection or up to 3 days after infection.

Bray et al. (2) also documented that CDV increased the MDD in SCID mice but did not protect them from mortality from CV infections. Our studies confirm these observations and further indicate that viral replication is significantly reduced in target organs of SCID mice. Our results with CV mirror those of Neyts and De Clercq (21), indicating reduced viral titers in lung, kidney, and liver of VV-infected SCID mice treated with CDV. Collectively, these data indicate that CDV is able to significantly reduce mortality in mice exposed to VV or CV when given as late as 96 h postinoculation. For protec-

tion of normal mice from death, CDV can be reduced to one single dose or one to three smaller doses. Protection from infection can also be conferred by pretreatment with CDV as early as 5 days prior to exposure. The results obtained for SCID mice suggest that long-term treatments may be necessary for protecting immunocompromised individuals.

These results have major implications, as they suggest that CDV, in addition to being effective as a treatment for smallpox or vaccine complications, can be used for pre-or postexposure prophylaxis of smallpox contacts, i.e., ring treatment, and that a single dose may provide significant protection. We have obtained similar results for a murine cytomegalovirus infection, where a single dose of CDV provided protection for 5 to 6 days (Kern, unpublished results). It was subsequently shown that once-a-week dosing in CMV retinitis in humans was also efficacious (12), thereby validating the results from the animal model. From these data it is clear that the effect of CDV is long lasting and may translate to short-term or single-dose treatment for smallpox or other orthopoxvirus infections.

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