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Effects of cidofovir on the pathogenesis of a lethal vaccinia virus respiratory infection in mice

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Abstract

Intranasal infection of BALB/c mice with the WR strain of vaccinia virus leads to pneumonia, profound weight loss, and death. Although the major sites of virus replication are in the lungs and nasal tissue, dissemination of the virus to other visceral organs and brain occurs via the blood. In this report the effects of cidofovir on the pathogenesis of the infection was studied. Mice were infected intranasally with virus followed 1 day later by a single intraperitoneal treatment with cidofovir (100 mg/kg) or placebo. Placebo-treated mice were dead by day 8, whereas all cidofovir-treated animals survived through 21 days. Cidofovir treatment did not prevent profound weight loss from occurring during the acute phase of the infection, but the mice gained weight quickly after the 8th day. Significantly higher arterial oxygen saturation levels, as determined by pulse oximetry, were seen in cidofovir-treated animals compared to placebos on days 4–7. Cidofovir treatment markedly improved lung consolidation scores and prevented lung weights from increasing during the infection. Virus titers in lungs and nasal tissue were high starting from the first day of the infection, whereas the titers in liver, spleen, brain, and blood was low for 3 days then markedly rose between days 4 and 6. Lung and nasal virus titers were reduced 10–30-fold by cidofovir treatment on days 2, 4 and 6. Virus titers in the other tissues and blood at their peak (day 6) were 30- to >1000-fold less than in tissues of placebos. These results illustrate the ability of a single cidofovir treatment to control the pathogenesis of an acute lethal infection in various tissues during the vaccinia virus infection in mice.

Keywords: Vaccinia virus; Pathogenesis; Cidofovir; Antiviral

1. Introduction

Inhibitors of orthopoxviruses are being sought as treatments for the biowarfare agents variola (smallpox) and monkeypox viruses (Breman and Henderson, 1998; Hooper, 1998; Orent, 1998), or for infections such as molluscum contagiosum (Meadows et al., 1997; Davies et al., 1999; Ibarra et al., 2000; Toro et al., 2000) and disseminated vaccinia (Kesson et al., 1997). At the present time only a limited number of compounds appear to have potential in treating lethal orthopoxvirus

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infections, which include cidofovir (Neyts and De Clercq, 1993; Bray et al., 2000; Smee et al., 2000b), ribavirin (Smee et al., 2000a) and compound S2242 (2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine) (Neyts and De Clercq, 2001). In a preliminary report, Neyts et al. (2001) have indicated that 5-iodo-2′-deoxyuridine treatment will delay mortality in vaccinia virus infected immunocompromised mice. Other inhibitors have shown some efficacy in non-lethal models of vaccinia virus infection (as reviewed by De Clercq, 2001). We have tested many such compounds in the cowpox and vaccinia virus respiratory infection models and have found them to be ineffective (D.F. Smee et al., unpublished). Because cidofovir is so remarkably potent against respiratory infections induced by cowpox (Bray et al., 2000; Smee et al., 2000b) and vaccinia (Smee et al., 2001) viruses, it is currently the leading candidate for treatment of orthopoxvirus infections in humans. The drug was also recently reported to inhibit parapoxviruses in cell culture (Nettleton et al., 2000). Safety concerns coupled with the lack of oral bioavailability of cidofovir (Wachsman et al., 1996) may be impediments to its widespread use.

Small animal models are primarily used for studying potential antiviral drugs against cowpox and vaccinia viruses. Cowpox virus induces a fatal disease in mice when administered intranasally (i.n.) (Bray et al., 2000; Martinez et al., 2000). A commonly used vaccinia virus infection is the tail lesion model (De Clercq et al., 1989). Vaccinia virus causes a lethal disseminated infection in severe combined immunodeficient mice (Neyts and De Clercq, 1993). Other vaccinia virus models included intracerebral inoculation of mouse brain (Schabel, 1968), infection of rabbit eyes (Sidwell et al., 1973), and infection of scarified rabbit skin (Sloan, 1975).

We recently reported the treatment of a lethal respiratory infection in mice caused by the WR strain of vaccinia virus (Smee et al., 2001). This strain of virus was shown by others to induce lethal disease by the i.n. inoculation route (Nelson, 1938; Turner, 1967; Lin et al., 2000). In our studies we found that the virus replicated to high titers in lungs and nasal tissue, but was also present at reasonably high titers in many other organs, tissues, and blood near the time of death. Lung pathology in the mice was also very pronounced just before death. Treatment with cidofovir prevented mortality and reduced signs of the infection in mice exposed to this virus (Smee et al., 2001).

In the present report we examined the pathogenesis of the vaccinia virus (WR strain) infection in greater detail. The effect of cidofovir treatment on various disease parameters was studied over several days during the course of the infection. From these analyses we were able to determine time points useful for antiviral assessments as well as to understand the impact of cidofovir treatment on the replication of the virus in tissues besides the lungs and nasal region.

2. Materials and methods

2.1. Antiviral compound

Norbert Bischofberger of Gilead Sciences (Foster City, CA) kindly provided cidofovir. The compound was dissolved in sterile saline for injection into mice. Sterile saline served as the placebo control.

2.2. Viruses and cells

Vaccinia virus (WR strain) was purchased from the American Type Culture Collection (ATCC) (Manassas, VA). The virus was initially propagated in African green monkey kidney (Vero) cells (ATCC). Higher titer virus for infection of mice was prepared in a second line of African green monkey kidney (MA-104) cells, purchased from BioWhittaker (Walkersville, MD). Both types of cells were cultured in Eagle's medium (MEM) containing 10% fetal bovine serum. The serum concentration was reduced to 2% for viral propagation and plaque assays.

2.3. Mouse infection studies

BALB/c mice (13–15 g) were purchased from B & K Universal (Fremont, CA). They were quarantined 48 h before use. Experiments with the

mice were done using an infectious vaccinia virus challenge of 1×10^6 plaque forming units (10–20 50% lethal doses) per mouse (Smee et al., 2001), administered i.n. in a 50 μl volume following anesthesia with ketamine (100 mg/kg given by intraperitoneal [i.p.] injection). Infection under anesthesia (as opposed to application to non-narcotized animals) allowed the infectious fluid to penetrate deeper into the lungs, and less virus was required for achieving a 50% lethal dose. A single i.p. treatment with cidofovir (100 mg/kg) or placebo was given 24 h after virus exposure. The 100 mg/kg dose was found to be optimal against cowpox (Bray et al., 2000) and vaccinia (Smee et al., 2001) virus respiratory infections, particularly when given as a single injection. Animals were individually weighed every 2–3 days and deaths recorded for 21 days. There were ten mice per group held for death and five mice per group per day for virus titer determinations. Extra animals were included in the placebo group to allow enough mice to be alive for the necropsy on day 6.

Lung infection parameters were determined in a manner similar to those reported for influenza virus (Sidwell et al., 1998) in groups of vaccinia virus-infected mice. On days 1, 2, 4, and 6 of the infection, lungs from sacrificed mice (using cervical dislocation) were collected, given a severity score based upon lung discoloration ranging from 0 (normal) to 4 (100% of lung area exhibiting a plum discoloration), weighed, and frozen for later virus titration. Other tissues (nose/sinus, liver, spleen, kidney, and brain) and whole blood (placed in 0.5 ml of cell culture medium and vortexed) were taken for virus titer determinations. The samples were frozen at −80 °C prior to homogenization and titration. Virus titers from these samples were later determined by plaque assay in Vero cells as described previously (Smee et al., 2001).

Arterial oxygen saturation (SaO_2) levels (a measure of lung function) were determined on days 2–10 of the infection using a pulse oximetry method (Sidwell et al., 1992). Animals dead on the particular day of measurement were assigned an SaO_2 value of 75 for that day, since this value was the lowest that we have observed in mice near death.

2.4. Statistical methods

Statistical comparisons were made of the cidofovir-treated group to the placebo control by two-tailed analyses. The Fisher exact test was used to interpret differences in numbers of survivors. Mean day of death, mean body weight, mean tissue or blood virus titers, mean lung scores, mean lung weight comparisons, and mean SaO_2 level differences were statistically analyzed by the Mann–Whitney *U*-test. Calculations were made using the InStat computer program (GraphPad Software, San Diego, CA).

3. Results

3.1. Effect of cidofovir treatment on survival and mean body weight

Mice were infected i.n. with vaccinia virus and treated i.p. 1 day later with cidofovir (100 mg/kg) or placebo. All cidofovir-treated mice survived, whereas all placebo-treated animals were dead by day 8 after virus exposure (Fig. 1A). Prior to death, the drug-treated mice lost nearly the same amount of weight as the placebo-treated animals (Fig. 1B). Starting after day 8, the group of animals treated with cidofovir began to quickly regain weight, indicating recovery from the infection. However, there was a large mouse-to-mouse variation in weight during the recovery phase. For example, on day 13 the weight range for the cidofovir-treated group was 8.4–16.7 g, indicating that some of the mice barely survived the infection.

3.2. Effect of cidofovir treatment on lung infection parameters

The i.n. infection with vaccinia virus led to an increase in mean lung weights, which was detectable on days 4 and 6 after virus exposure (Fig. 2A). Placebo-treated animals had lung weights that were nearly triple those of mice at the onset of infection (day 0). Cidofovir treatment dramatically prevented lung weight increases compared to the placebo group. Infected lungs in the placebo

group exhibited a hemorrhagic appearance, which was characteristic of a diffuse, bilateral viral pneumonitis, as described by Martinez et al. (2000) for cowpox virus infections. Lung condition worsened in severity between days 4 and 6 (Fig. 2B). On day 6 nearly the entire lung area of each animal showed this consolidation. Lungs of mice treated with cidofovir remained relatively normal throughout the 6-day period.

Because of the severity of the lung infection, it was anticipated that lung function would be impaired, which would manifest itself as a decrease in arterial oxygen saturation. Indeed, placebo-treated mice showed decreases in SaO_2 levels that were significantly less than those of the cidofovir-treated group on days 3–7 of the infection (Fig. 3). The cidofovir group had lower SaO_2 levels compared to uninfected mice until day 10, indicating some impairment of lung function despite the treatment.

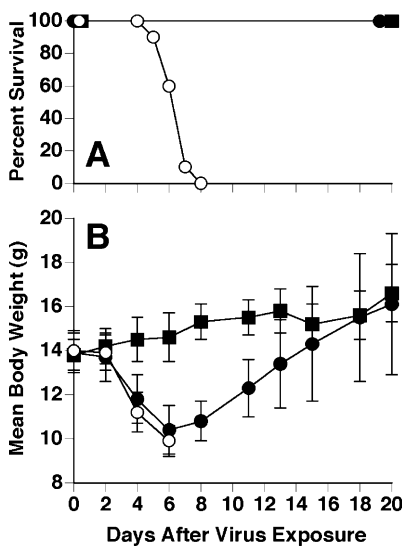

Fig. 1. Effect of cidofovir treatment on survival (A) and on mean body weight (B) during a vaccinia virus respiratory infection in mice. Data points in B represent means ± S.D. (10 mice/group initially). A single i.p. treatment was given 24 h after virus exposure. ■, uninfected; ●, cidofovir at 100 mg/kg; ○, placebo. Survival data comparing cidofovir to placebo treatments were statistically significant ($P < 0.001$); body weight differences were not significant on days 0–6 ($P > 0.05$).

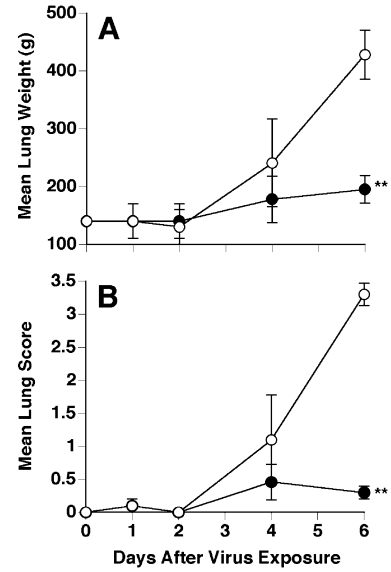

Fig. 2. Effect of cidofovir treatment on mean lung weights (A) and on lung consolidation scores (B) during a vaccinia virus respiratory infection in mice. Data points represent means ± S.D. (5 mice/group). A single i.p. treatment was given 24 h after virus exposure. ●, cidofovir at 100 mg/kg; ○, placebo. * $P < 0.05$, ** $P < 0.01$.

3.3. Effect of cidofovir treatment on virus titers in tissues and blood

Our previous observation was that the major sites of vaccinia virus infection were in the lungs

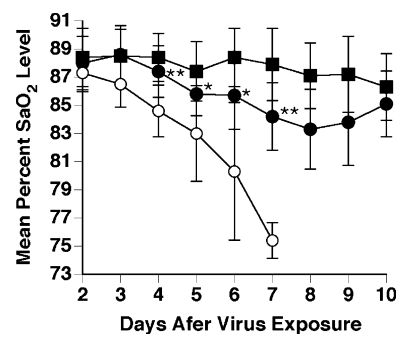

Fig. 3. Effect of cidofovir treatment on arterial oxygen saturation (SaO_2) levels during a vaccinia virus respiratory infection in mice. Data points represent means ± S.D. (10 mice/group). A single i.p. treatment was given 24 h after virus exposure. ■, uninfected; ●, cidofovir at 100 mg/kg; ○, placebo. * $P < 0.05$, ** $P < 0.01$.

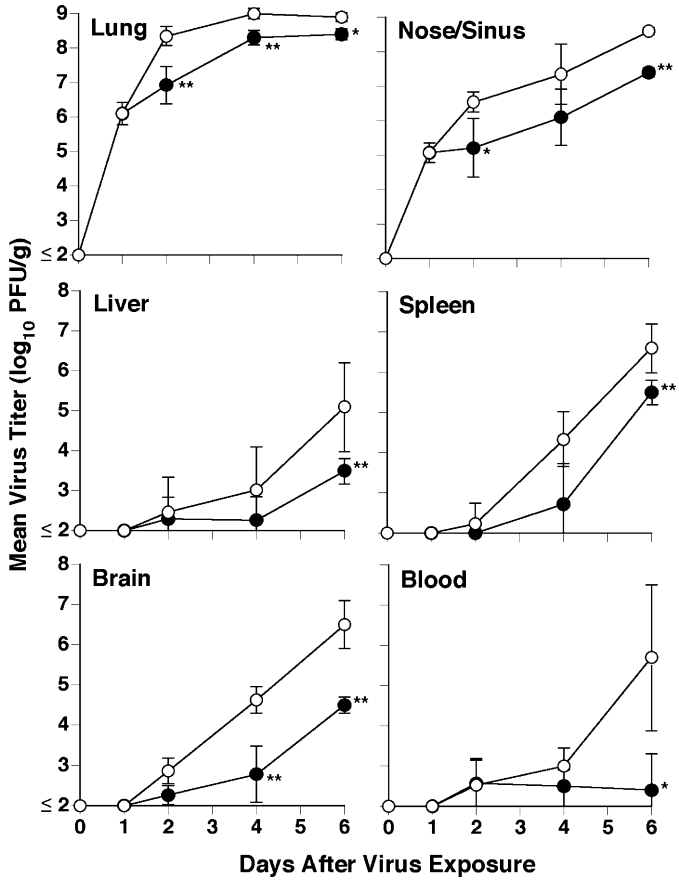

Fig. 4. Effect of cidofovir treatment on development of tissue and blood virus titers during a vaccinia virus respiratory infection in mice. Data points represent means ± S.D. (5 mice/group). A single i.p. treatment was given 24 h after virus exposure. ●, cidofovir at 100 mg/kg; ○, placebo. * $P < 0.05$, ** $P < 0.01$.

and nasal tissue, with virus at lower but appreciable levels in other tissues and blood near the time of death (Smee et al., 2001). We wanted to determine how rapidly virus titers developed in these other tissues in comparison to lung and nasal tissue, and to determine if a single cidofovir treatment could reduce virus production in these other sites. Virus titers in lungs and nose/sinus tissue were high starting on the first day, then increased 100–1000-fold after that (Fig. 4). In liver, spleen, brain, and blood, virus was at a low level during the first 3 days of the infection then started to climb rapidly between days 4 and 6. Treatment with cidofovir was effective in reducing virus titers in all tissues and blood. Reduction of virus in the blood and brain was impressive, being decreased approximately 1000- and 100-fold, respectively, on day 6. Reduction of virus in the blood was most likely due to decreased virus production in the various organs and tissues.

4. Discussion

Cidofovir was effective when given as a single intraperitoneal dose 24 h after virus exposure in this lethal model of vaccinia virus infection,. Previously we showed that treatment given on days 1 and 4, every other day, or every day for 5 days were also effective in preventing mortality in this model (Smee et al., 2001). Mice so infected and treated underwent extensive weight loss compara-

ble to that of the placebo control, except that the placebo animals died whereas all of the cidofovir-treated animals survived. We have investigated various methods to attempt to prevent this weight loss from occurring, primarily using the cowpox model. Prevention of weight loss during the first 7 days of the infection is an important indicator of antiviral protection. To date, marked prevention of weight loss during this time period has been best achieved by very early i.p. or subcutaneous (s.c.) treatment with cidofovir such as immediately after virus exposure (Bray et al., 2000), or by i.n. treatment (given 24 h after virus challenge; Smee et al., 2000b). Intranasal treatment concentrates more drug at the site of virus replication than does i.p. or s.c. treatment. By comparison, cidofovir treatment had a greater effect in controlling weight loss during cowpox virus infections than it did in these vaccinia virus infections. It may be important to understand why profound weight loss occurs in mice infected i.n. with cowpox or vaccinia virus.

Lung pathology in mice infected with the WR strain of vaccinia virus was more profound and consistent than was reported for cowpox virus (Bray et al., 2000; Martinez et al., 2000). The effect of excessive lung weight (with large fluid accumulation) and lung consolidation resulted in a reduction in lung function and in a decrease in SaO_2. The SaO_2 values in cowpox infected mice do not decline to the same extent that was shown here with vaccinia virus (D.F. Smee et al., unpublished data), thus SaO_2 determinations may only be a useful measure of lung function in the vaccinia virus model.

The major sites of infection in this model were in lungs and nasal tissue as indicated by the large amount of virus produced and the condition of the lungs (severe pneumonitis) near death. Toxemia is also a condition that develops during infection. Both lung pathology and toxemia are believed to lead to death of the animals. In the cowpox model, some animals do not develop pneumonitis but die anyway (Bray et al., 2000; Martinez et al., 2000), suggesting the importance of toxemia on mortality. In the vaccinia model described here, the extent of viral pneumonitis was consistent from animal to animal receiving the same virus challenge dose. Although some virus was found in the brain, the amount there was relatively small compared to the respiratory tract. The animals did not exhibit the characteristic signs (tremors, limb paralysis, leaning to one side) of encephalitis prior to death. Cidofovir prevented death in the animals by reducing the overall virus burden, particularly early in the infection in the lungs and nasal tissue. This resulted in quantifiably less lung pathology and reduced virus titers in all organs and tissues near the time of death of the placebo group.

Previously we demonstrated that vaccinia virus was detectable in relative high concentrations in the tissues and blood of mice just prior to death (Smee et al., 2001). The present report showed virus titers in liver, spleen, brain and blood to be relatively low for the first few days of the infection, then to increase dramatically between the 4th and 6th day. This is in contrast to the early appearance of virus in lungs and nasal tissue. Of course the inoculation route directly results in infection of the lungs and nasal area, whereas viral spread to the other tissues requires transport through the blood. It is an important observation that cidofovir given as a single treatment on day 1 was able to reduce virus titers in all of the tissues and blood assayed. The effect of cidofovir in reducing lung virus titer when given as a single treatment is similar to its efficacy when given at multiple times during the infection (Smee et al., 2001). Because cidofovir treatment caused a reduction of virus in the brain, this may indicate that the drug penetrates the blood–brain barrier. Alternatively, it may be the result of an indirect effect of reducing virus in other tissues, thus lowering the amount of virus able to seed the brain and cause infection.

These studies support the utility of the vaccinia virus (WR strain) infection model for antiviral studies (Smee et al., 2001). Various infection parameters can be ascertained to evaluate antiviral efficacy. A novel finding was the use of SaO_2 determinations to assess the effect of the infection on lung function. Although we have not examined lungs histologically from treated and untreated mice, it is anticipated that the pathological findings will be similar to those reported from cowpox

virus infections (Martinez et al., 2000) due to the close relatedness of the two viruses. The present results show that it is most useful to assess virus titers in tissues other than lungs and nose/sinus near the time of death, since tissues harvested at earlier time points contain considerably less virus. Virus titers in lungs and nasal tissue can be determined early in the infection, such as on day 3. A 10-fold or greater reduction in lung virus titer on day 3 is a good indicator of successful treatment outcome.

A single cidofovir treatment was able to control the pathogenesis of an acute lethal vaccinia virus infection in various tissues in mice. Cidofovir is the only compound thus far identified that may be potent enough in animal models to be effective against severe orthopoxvirus infections in humans (caused by monkeypox or variola viruses), although compound S2242 also looks promising (Neyts and De Clercq, 2001). Continued use of animal models to assess other potentially effective anti-orthopoxvirus agents is warranted.

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