

Antiviral prophylaxis of smallpox

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Proof-of-concept studies suggest that current defences against smallpox could be strengthened by supplementing vaccination with antiviral drug prophylaxis, based on aerosolized or orally available forms of the long-acting medication cidofovir. Delivery of aerosolized cidofovir to mice results in its prolonged retention in respiratory tissues and protection against lethal intranasal or aerosol poxviral challenge. Although cidofovir itself is not orally available, the addition of an alkoxyalkanol ether side-chain allows it to be absorbed from the gastrointestinal tract. This also markedly increases its antiviral activity and lengthens its intracellular half-life from roughly 3 to 8–10 days. Oral treatment also protected mice against lethal poxviral challenge. These results suggest that a single aerosol dose of cidofovir (or an alkoxyalkanol–ether derivative) could provide prolonged protection against initiation of smallpox infection, whereas oral treatment could prevent both initiation of infection and internal dissemination of virus. Both approaches may avoid the nephrotoxicity that occasionally results from intravenous cidofovir therapy.

Keywords: monkeypox, airborne infections, antiviral therapy, aerosol therapy, cidofovir, vaccination, biodefence

Introduction

The threat of a bioterrorist release of smallpox has stimulated research into the potential of antiviral drug prophylaxis as a supplement to vaccination. The ability of an antiviral medication to provide immediate protection against smallpox was first tested during the decade before global eradication, but the only compound then available (methisazone, Marboran) showed at best a minimal protective effect.^{1,2} The subsequent development of the long-acting antiviral drug cidofovir (HPMPC, Vistide), which has much stronger activity against poxviruses, may now make effective drug prophylaxis an attainable goal.^{3,4}

Several considerations indicate that antiviral prophylaxis would be a valuable addition to our defences against smallpox. Because the success of global eradication led to the end of universal vaccination, more than half the world's population is now unprotected against the disease. Large amounts of vaccine are being stockpiled, but current plans do not call for initiating mass vaccination until after an outbreak has been detected—meaning that some persons may already have been infected by the time they receive the vaccine, when immunization may be only partially protective.^{2,5} In addition, currently available vaccines pose a risk of serious complications in persons with atopic dermatitis or cell-mediated immunodeficiency, and may not induce protective responses in the latter.⁶ Concern that the virulence of variola

virus, the agent of smallpox, could be increased by genetic manipulation, enabling it to overcome vaccine-induced immunity, is also a driving force for developing effective drug prophylaxis.⁷

A two-pronged approach of vaccination and antiviral prophylaxis would resemble measures already in place for influenza. Vaccines are the basic means of limiting a flu epidemic, but protection is also available in the form of antiviral drugs: an aerosolized compound (zanamivir) delivered from a portable inhaler, or oral medications (oseltamivir and amantadine). Such treatment, begun either before or after exposure to an influenza patient, can be highly effective in preventing or mitigating illness and blocking disease transmission.^{8,9} The stockpiling of these drugs has been urged in preparation for a possible flu pandemic.⁹

As presently employed, cidofovir is an intravenous medication that requires increased hydration and other measures to prevent nephrotoxicity, so it would be difficult to administer the drug on a large scale during a smallpox epidemic. In this article, we describe recent proof-of-concept studies indicating that cidofovir could also be administered by aerosol or in an orally available form, that treatment would be effective in preventing disease if given before or after exposure to smallpox, and that these approaches would be safer than intravenous therapy. Our own studies have evaluated the protective efficacy of aerosolized

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cidofovir in mice,^{10,11} whereas others have synthesized orally available forms of the compound and given them to mice by gavage.^{12–16} After reviewing basic information on cidofovir and the pathogenesis of smallpox, we present the results of these proof-of-concept studies, then discuss whether cidofovir prophylaxis would interfere with simultaneous vaccination.

Cidofovir and poxviral infections

Cidofovir is a phosphonate analogue of deoxycytidine (Figure 1). The active form of the drug, cidofovir diphosphate, interferes with poxviral DNA replication at concentrations well below those that are toxic for human cells. Because the highly accurate viral DNA polymerase has conserved the sequence of its own gene among all poxviruses that infect vertebrates, cidofovir is active against vaccinia, cowpox, monkeypox and all isolates of variola virus so far tested, as well as against two distantly related poxviruses, molluscum contagiosum and orf.^{3,4,17–20} Although cidofovir-resistant poxviruses have been generated by cell-culture passage in the presence of the drug, such variants have shown a loss of virulence *in vivo*, suggesting that the sequence

of the enzyme's nucleotide binding site must be preserved for normal viral function.²¹

Cidofovir's remarkably long intracellular half-life, on the order of 3 days, would be a major advantage for prophylactic use during a smallpox outbreak, since a single dose could exert a prolonged protective effect (as maintenance therapy for cytomegalovirus retinitis, intravenous cidofovir is given once every 2 weeks). Persistence within cells results from the fact that cidofovir can replace cytidine as a carrier molecule in the synthetic pathway of the membrane lipids phosphatidylcholine and phosphatidylethanolamine.^{22,23} These covalent cidofovir–choline and –ethanolamine adducts (Figure 1c) serve as intracellular 'reservoirs' for the slow release of the phosphorylated drug into the cytoplasm. The molecule's intracellular half-life can be further extended through addition of an alkoxyalkanol ester group (see below).

Cidofovir is not toxic to cells at the low levels needed to block viral replication, but intravenous treatment can cause the rapid accumulation of damaging concentrations in the kidneys unless special precautions are taken.²⁴ Efforts to adapt cidofovir for smallpox prophylaxis aim to avoid this problem, either by delivering a low dose of aerosolized drug to the respiratory tract, or by taking advantage of the gradual absorption of a larger orally administered dose to provide sufficient time for renal excretion.

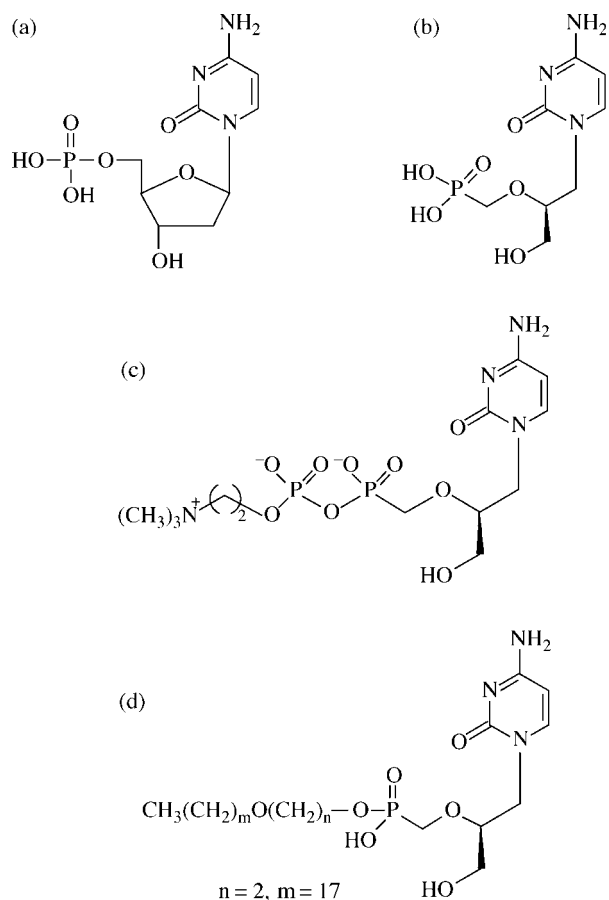


Figure 1. Chemical structures of (a) cytosine monophosphate; (b) its phosphonate analog (S-1-[3-hydroxy-2(phosphonylmethoxy) propyl] cytosine (HPMPC, cidofovir, Vistide); (c) cidofovir-choline adduct; (d) cidofovir modified to increase oral bioavailability: octadecyloxyethyl-cidofovir (ODE-cidofovir).

Smallpox pathogenesis: implications for antiviral prophylaxis

Both the initiation of smallpox infection and the early phase of disease development are potential targets for cidofovir intervention. Infection begins with the entry of variola virus into the respiratory tract. In a bioterror attack, the agent would probably arrive in the form of airborne particles small enough to be inhaled into the lungs, whereas during the resulting outbreak, transmission would occur through saliva droplets expelled from the oropharynx of smallpox patients. The subsequent sequence of events is not known for humans, but studies of ectromelia virus (mousepox) infection in mice suggest that infection results in the transfer of virus within macrophages (and possibly dendritic cells) to mediastinal lymph nodes, followed by further dissemination to similar cells in the spleen, liver and other tissues.^{2,5} A battery of virus-encoded immunomodulatory proteins may shield replication against innate antiviral responses during this silent phase of infection.⁵

The 10–12-day incubation period ends when the release of cytokines and virus from infected cells into the bloodstream causes fever, malaise and development of a vesiculopustular rash in the skin and oropharynx (lesion formation at these sites is favoured by a virus-encoded epidermal growth factor).^{2,5} In nearly half of cases, the circulating virus titre is low enough to produce discrete pocks separated by areas of normal skin, but in a small percentage of patients, much higher levels of viral replication result in a shortened incubation period and rapidly lethal haemorrhagic disease.

The ability of innate and adaptive immune responses to restrict viral replication during the incubation period appears to play the major role in determining the outcome of infection.⁵ If given early enough after exposure to smallpox, vaccination may stimulate the development of cross-protective immunity

and reduce the severity of subsequent illness. Aerosolized or oral cidofovir may provide an additional means of blocking the development of clinical smallpox, either by creating a barrier to infection in the respiratory tract, or by preventing the internal spread of virus.

Modelling antiviral prophylaxis in laboratory animals

Antiviral prophylaxis has so far been tested in three murine models of lethal poxviral infection. One involves intranasal challenge of mice with vaccinia virus, whereas the second employs cowpox virus delivered by the intranasal or aerosol route. In both cases, a large amount of virus is required to achieve lethality, and the resulting disease is largely confined to the respiratory tract. The third model uses ectromelia virus, which is a natural pathogen of mice, and produces a disease more closely resembling human smallpox, since challenge with a small dose of aerosolized virus is followed by viral dissemination to lymphoid tissues and lethal systemic infection.

Orally available cidofovir derivatives

The intestinal absorption of cidofovir can be greatly enhanced by attaching an ether lipid residue to the phosphonate group (Figure 1d).^{12–14} Not only is uptake increased from <1% for cidofovir to >98% for cidofovir-alkoxyalkanol esters such as octadecyloxyethyl (ODE)-cidofovir, but these compounds are also taken up much more efficiently into cells. They then remain in the cytoplasm for some time before the ester bond is cleaved, increasing the drug half-life to some 8–10 days. As a result, alkoxyalkanol esters of cidofovir are 50–100-fold more active against variola and other poxviruses than the parent compound.^{12–14}

Initial testing of orally available cidofovir derivatives in mice showed that a single dose, given as long as 5 days before infection, protected against lethal intranasal cowpox virus challenge.¹⁵ A low daily dose (5 mg/kg) of ODE-cidofovir, the most active compound, also protected mice against aerosolized ectromelia virus when treatment was begun on the day of infection.¹⁶ Treatment completely blocked viral replication in the liver and spleen.

Data from mice also indicate that treatment with orally available cidofovir derivatives would pose less risk of nephrotoxicity than intravenous cidofovir therapy. In the experiment shown in Figures 2(a and b), parenteral injection of cidofovir caused rapid drug accumulation in the kidneys, whereas a similar dose of orally administered ODE-cidofovir was absorbed much more gradually from the intestinal tract, resulting in a 30-fold lower peak renal concentration.¹³ Even though most of an oral dose is taken up by the liver, distribution of the remainder to the spleen, lungs and other organs should provide tissue levels capable of blocking variola replication. Initial studies have found no evidence of hepatotoxicity, but additional testing will be required.

Aerosolized cidofovir

Aerosol prophylaxis aims to block the initiation of smallpox infection by delivering cidofovir to cells lining the respiratory tract and to adjacent lymph nodes. Initial studies in mice showed that a single dose of 0.5–5 mg/kg of aerosolized cidofovir

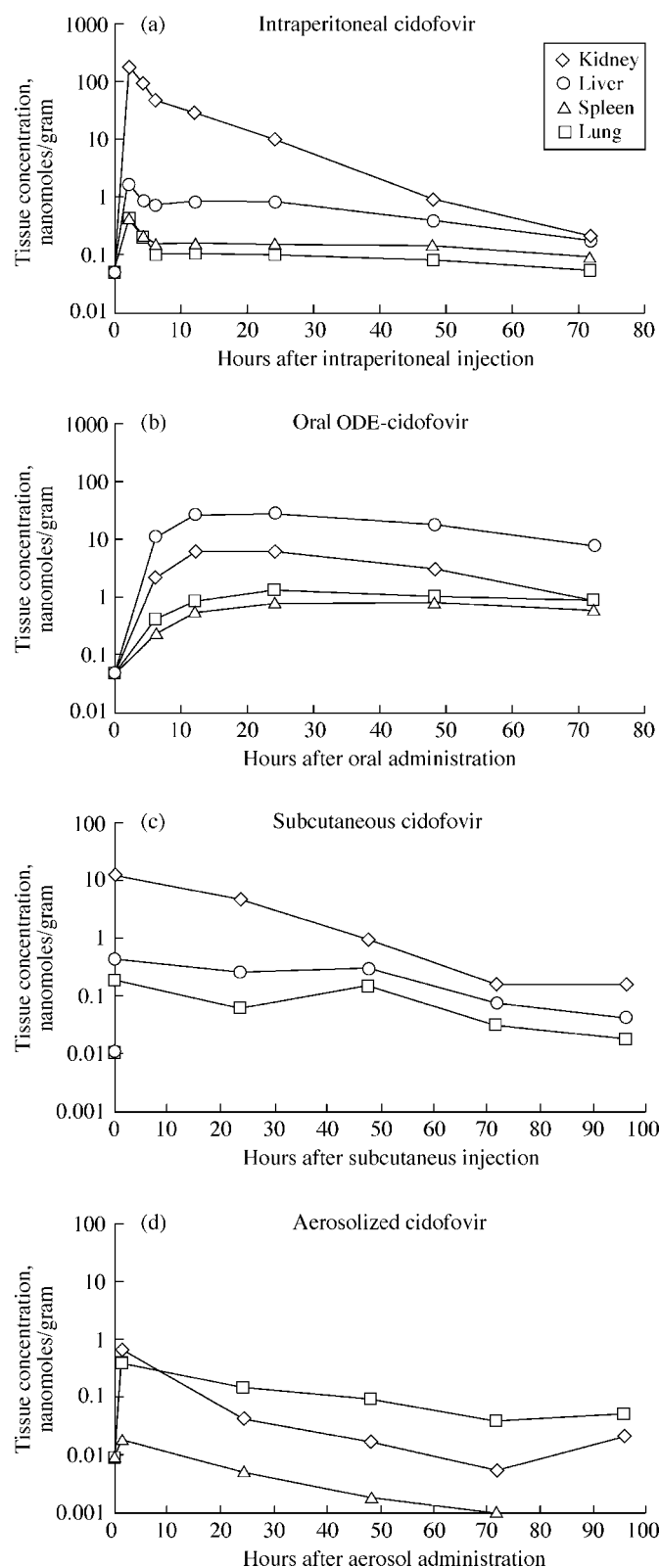


Figure 2. Tissue [¹⁴C]-cidofovir or ODE-cidofovir concentrations over time after (a) intraperitoneal injection of cidofovir (5.6 mg/kg); (b) oral treatment with ODE-cidofovir (10 mg/kg); (c) subcutaneous injection of cidofovir (1 mg/kg); (d) aerosol delivery of cidofovir (1 mg/kg).

prevented lethal intranasal or aerosol cowpox virus infection and gave equal or better protection than a subcutaneous dose of 100 mg/kg.¹⁰ Treatment was equally effective when given the day before or on the day of virus challenge. Subcutaneous injection of radiolabelled cidofovir caused rapid drug accumulation in the kidneys (Figure 2c), but aerosol delivery of a similar dose resulted in retention of a significant fraction in pulmonary tissues and a 15-fold lower peak renal concentration (Figure 2d).¹¹ One day after treatment, the level of cidofovir in the lungs of aerosol-treated mice was three times higher than in the kidneys, whereas in subcutaneously injected mice the kidney concentration was 75 times higher than in the lungs.

These findings suggest that a single dose of aerosolized cidofovir could protect the respiratory tract against poxviral infection for a number of days, without risk of renal injury. Aerosol delivery of a compound such as ODE-cidofovir might further extend protection for as long as several weeks. Our studies revealed no evidence of pulmonary toxicity, but further testing will be required. Experience with the use of cidofovir in the human respiratory tract is so far limited to the treatment of laryngeal papillomas by injection,²⁵ supplemented in one case by aerosol (R. Snoeck, personal communication).

Effect of cidofovir treatment on vaccination

At present, the only approved treatment for persons who have been exposed to smallpox is immediate vaccination. Would the addition of prophylactic cidofovir diminish vaccine efficacy? Limited data suggest that cidofovir would not interfere with a vaccine 'take', and suggest that the drug is much more active against poxviral replication in internal organs than in the skin. Cidofovir treatment of mice at the time of vaccinia scarification somewhat decreased the size of the resulting lesion, but the animals were still protected against later cowpox¹⁸ or ectromelia virus challenge (M. Buller, personal communication). Smee *et al.*²⁶ made similar observations, and also noted that systemic treatment with a high dose (100 mg/kg) of cidofovir did not reduce viral titres of vaccinia skin lesions in immunocompromised mice, and was much less effective than topical therapy. Even if cidofovir did interfere somewhat with vaccinia replication, the fact that highly attenuated poxviruses—such as modified vaccinia Ankara, that fail to replicate in human cells—still elicit strong immune responses indicates that full replication may not be required for protection.²⁷ These questions should be examined further by testing the effect of cidofovir treatment on vaccination of non-human primates.

Conclusion

Proof-of-concept studies suggest that cidofovir could provide immediate protection against smallpox in the setting of a bio-terror attack. A self-administered aerosolized dose could block the initiation of infection, whereas an oral dose would interfere with both initiation of infection and further viral dissemination. The drug's long intracellular half-life means that a single treatment might provide a prolonged effect. The protective efficacy of these approaches and their effect on simultaneous vaccination should be further evaluated in non-human primate models of lethal poxviral infection. The potential utility of oral or

aerosolized cidofovir for the treatment of other DNA viral infections may serve as a further stimulus for their development. If the threat of a more virulent, genetically modified form of variola virus ever becomes a reality, additional antiviral compounds with differing mechanisms of action may also be needed as supplements to vaccination.

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