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Therapy and short-term prophylaxis of poxvirus infections: historical background and perspectives

Johan Neyts*, Erik De Clercq

Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium

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Abstract

The era of antiviral chemotherapy started more than 50 years with the findings by Domagk and his colleagues that thiosemicarbazones showed activity against vaccinia virus. One of the derivatives, methisazone, was even investigated in the prophylaxis of smallpox. With the successful implementation of the smallpox vaccine, the use of methisazone was not further pursued. Should there be a threat of smallpox or other poxvirus infections, that could not be immediately controlled by vaccination, a therapeutic intervention could be envisaged based on several therapeutic strategies targeted at such cellular enzymes as IMP dehydrogenase, SAH hydrolase, OMP decarboxylase and CTP synthetase, as well as viral enzymes such as the DNA polymerase. Most advanced as a therapeutic or early prophylactic modality to tackle poxvirus infection is cidofovir, which was found active (i) in vitro against all poxviruses studied so far; (ii) in vivo, against vaccinia and cowpox virus infections in experimental animal models; as well as (iii) some human poxvirus infections, such as molluscum contagiosum. In case of an inadvertent poxvirus epidemic, antiviral therapy (i.e. with cidofovir) will offer the possibility to provide short-term prophylaxis, or therapy. Cidofovir should also allow to treat severe complications of vaccination as may happen in for example immunosuppressed patients.

Keywords: Smallpox; Cidofovir; Antiviral; Vaccinia

1. Introduction

The last case of naturally occurring smallpox was in 1977 in Somalia (the last laboratory infection in 1978) and the World Health Organization (WHO) declared global eradication of smallpox in 1980. The subsequent discontinuation of vaccination against smallpox has rendered most humans vulnerable to smallpox infection. Virtually all children and many adults, are now fully susceptible to smallpox. Following eradication of smallpox, every country that held variola, the causative agent, should have destroyed the virus or transferred the stock(s) to two central repositories, i.e. the Centers for Disease Control in Atlanta (USA) or the State Research Center of Virology and Biotechnology (VECTOR labs) in Koltsovo (Russia). Since variola is very stable and easy to hold, even at −20 °C, it may be well possible that certain countries have kept secret stocks of the virus. Before the eradication, the virus was studied in many countries. In case that illegally preserved stocks of smallpox virus would be used for biological or terroristic purposes, it could, in a highly mobile and susceptible population, cause a real catastrophe. A WHO advisory committee on variola defined in 1999 that it is important to develop drugs to treat human smallpox (or other poxvirus) infections should they reappear (press release WHO/77, 10 December 1999).

There is only a very limited stock of vaccine available, which may not at all have been properly stored or monitored for potency. Recent studies suggest that the diluted (1/5 or 1/10) vaccine retains its immunogenicity, which thus allows to increase the supply of vaccines available. Novel stocks of vaccine are being prepared (Enserink and Stone, 2001; Enserink, 2002). Two vaccines will be tested, one, known as Dryvax, of which 15 million doses are available and 70 million doses of the other vaccine, which were donated by Aventis Pasteur Inc. to the US government. A mathematical model was used to compare four different vaccination strategies against smallpox (Kaplan et al., 2002). The least effective method was found to be ring vaccination around any outbreak (110,000 expected deaths in the USA); the most effective method would be mass vaccination before an attack would occur (440 deaths in the USA). Even waiting to switch from ring vaccination to mass vacci-

* Corresponding author. Tel.: +32-16-337-367; fax: +32-16-337-340.
E-mail address: johan.neyts@rega.kuleuven.ac.be (J. Neyts).

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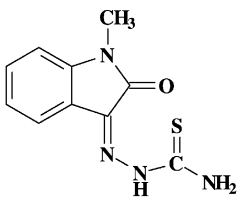
nation until day 33 of a crisis, would in the USA, according to the model study, result in 15,570 cases and 4680 deaths (Kaplan et al., 2002).

Human infection with monkeypox occurs sporadically in parts of Western and Central Africa. In 1996 and 1997 an important outbreak of monkeypox occurred in humans in the Democratic Republic of Congo (Anonymous, 1997; Heymann et al., 1998; Mukinda et al., 1997; Hutin et al., 2001). Also more recently there have been outbreaks of monkeypox disease in humans. Thus, also for the treatment of monkeypox disease in humans it would be important to have an effective treatment at hand. Inhibitors of orthopoxviruses are also be of interest for the treatment of molluscum contagiosum and importantly, disseminated vaccinia as well as other complications of vaccination.

2. The search for anti-poxvirus agents

Interestingly, the search for antivirals agents started more than 50 years ago with poxviruses as target. The thiosemicarbazones that had been introduced by Domagk and colleagues as tuberculostatic agents, were found to be active against vaccinia virus as well (Domagk et al., 1946). This work was continued by Bauer who demonstrated activity of the compound against vaccinia virus infections in mice (Bauer, 1955). In 1963, Bauer et al. (1963) had shown that the thiosemicarbazone derivative methisazone (Marboran, *N*-methylisatin 3-thiosemicarbozone) (Fig. 1) was effective in the prophylaxis of smallpox. Methisazone was found to reduce the smallpox attack rate by 75–95% in several trials in India. The drug proved also effective for the treatment of complications of smallpox vaccination, i.e. vaccinia gangrenosa and eczema vaccinatum (Fenner and White, 1970). In a double-blind field trial, however, others did not find an effect of prophylactic activity of methisazone against smallpox (Heiner et al., 1971). The principal side effect of methisazone was vomiting, which, although severe, was considered justifiable in the face of the life-threatening smallpox infection. Following successful implementation of the smallpox vaccine, the use of methisazone was not further pursued. Another tuberculostatic agent that was found to inhibit the replication of poxviruses is rifampin (Subak-Sharpe et al., 1969). However, rather high concentrations of this compound were needed to inhibit viral replication, which made its clinical use in the treatment of orthopoxvirus infections rather impractical (Müller, 1979). It may perhaps be of interest to study what the mechanism of action of methisazone was against poxviruses and to directly compare the efficacy of the compound with that of for example cidofovir. If methisazone would, from such studies, indeed appear to be a selective anti-poxvirus agent, it may perhaps be worth to prepare novel analogs. We are, however, not aware that such work is currently being carried out.



N-methylisatin 3-thiosemicarbazone

Fig. 1. Methisazone (Marboran).

3. Molecular targets for antiviral therapy

Poxviruses are the largest viruses on earth, having the largest viral genome and encoding for the largest number of specific viral proteins that could be envisaged as targets for antiviral intervention. Such targets may include viral proteins that are essential for the viral replication cycle. Several of such virus-encoded enzymes and factors are packaged in the infectious virion and are directly involved in the synthesis and modification of mRNA (e.g. RNA polymerases and an RNA polymerase-associated protein (RAP94), capping and methylation enzymes (RNA triphosphatase, guanylyltransferase, methyltransferase), poly A polymerase) (Moss, 2001). Many viral proteins are involved in processes required for virus replication, such as viral entry, uncoating, viral gene expression, DNA replication, virion assembly, maturation and release. Each of these steps may provide a valuable target for antiviral intervention. The few selective anti-poxvirus agents that have been discovered so far are assumed to target the viral DNA polymerase. An exhaustive review of compounds that have been shown to inhibit the replication of poxviruses has been published (De Clercq, 2001).

4. Compounds targeted at cellular enzymes

Many compounds that have been discovered to inhibit the replication of poxviruses do not inhibit a specific viral process or protein but, instead, are targeted at cellular enzymes. These compounds include inhibitors of inosine 5′-monophosphate (IMP) dehydrogenase, *S*-adenosylhomo-cysteine (SAH) hydrolase, orotidine 5′-monophosphate (OMP) decarboxylase, CTP synthetase or thymidilate synthetase.

4.1. Inhibitors of IMP dehydrogenase

IMP dehydrogenase converts IMP to xanthine 5′-monophospohate (XMP), a crucial step in the biosynthesis of the purine mononucleotides GMP, GDP, GTP, dGDP and dGTP. Inhibition of IMP dehydrogenase leads to a depletion of GMP, GDP, dGDP, GTP and dGTP pools and, hence, inhibition of both RNA and DNA synthesis. Ribavirin (virazole,

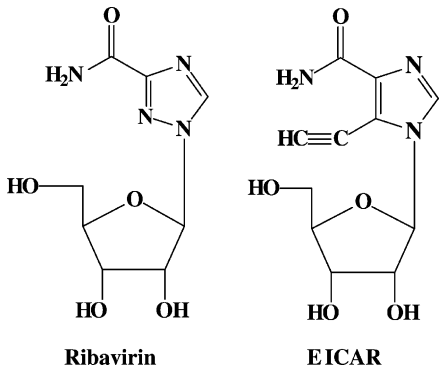


Fig. 2. IMP dehydrogenase inhibitors ribavirin and EICAR.

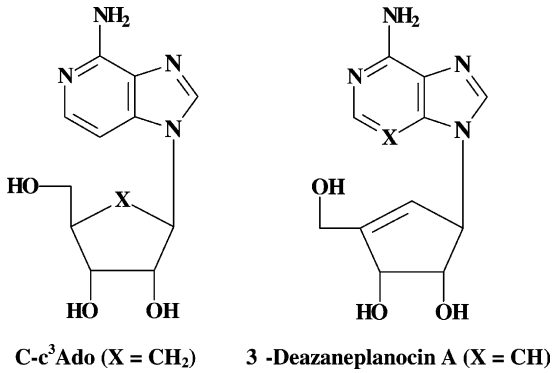


Fig. 3. SAH hydrolase inhibitors carbocyclic 3-deazaadenosine (C-c^{3}Ado) and 3-deazaneplanocin A.

1-β-ribofuranosyl-1,2,4-triazole-3-carboxamide) (Fig. 2), through its 5′-monophosphate metabolite, inhibits IMP dehydrogenase. Ribavirin was one of the first compounds shown to inhibit vaccinia virus replication in vitro and in vivo (Sidwell et al., 1972, 1973). Ribavrin was used to treat progressive vaccinia in a patient with metastatic melanoma and chronic lymphocytic leukemia that was inadvertently given a vaccinia melanoma oncolysate vaccination. The lesion started to heal apparently only when the patient was treated with ribavirin (Kesson et al., 1997). EICAR, (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide) (Fig. 2), the 5-ethynyl derivative of ribavirin is significantly more potent than ribavirin: it inhibits vaccinia virus replication in vitro with a 50% inhibitory concentration (IC_{50}) of 0.2 μg/ml. Importantly, EICAR was found to inhibit vaccinia virus-induced pox tail lesion formation in mice at doses that were not toxic to the host (De Clercq et al., 1991a).

4.2. SAH hydrolase inhibitors

SAH is a product/inhibitor of the SAM (*S*-adenosylmethionine)-dependent methyltransferase reactions; it should be removed by SAH hydrolase for the methylation to proceed unabatedly. SAH is normally cleaved by the hydrolase into two components, homocysteine and adenosine. If this hydrolysis is suppressed by SAH hydrolase inhibitors, SAH accumulates and negatively affects the methyltransfer reactions.

A wide variety of carbocyclic adenosine analogues that are potent inhibitors of SAH hydrolase have been found to selectively inhibit vaccinia virus replication in vitro. The replication of vaccinia, and other viruses that are strongly inhibited by SAH hydrolase inhibitors, strongly depends on methylations for 5′-cap formation requiring SAM as the methyl donor. As mentioned before, poxviruses encode for their own methyltransferase (mRNA capping enzyme). SAM-dependent methyltransferases play an important role in the 5′-cap formation and, hence, the maturation of vaccinia mRNA (Borchardt, 1980; Borchardt et al., 1984). Viruses, in their replicative cycle, are apparently more sensitive to the action of the SAH hydrolase inhibitors than uninfected non-dividing cell. Carbocyclic 3-deazaadenosine (De Clercq and Montgomery, 1983) (Fig. 3), neplanocins A and C (De Clercq et al., 1984), 3-deazaneplanocin A (De Clercq et al., 1989; Tseng et al., 1989), 9-(*trans*-2′, *trans*-3′-dihydrocyclopent-4′-enyl)adenine (DHCeA) and 9-(*trans*-2′, *trans*-3′-dihydrocyclopent-4′-enyl)-3-deazaadenine (C3DHCeA) (De Clercq et al., 1989; Hasobe et al., 1987) and the saturated derivatives thereof (DHCaA, C3DHCaA) (De Clercq et al., 1989) (±)-6′-β-fluoro-aristeromycin (F-C-Ado) (Cools et al., 1991), 5′-noraristeromycin (Patil et al., 1992; Siddiqi et al., 1994a,b), (−)-3-deaza-5′-noraristeromycin (Siddiqi et al., 1995), (*R*)-6′-C-methylneplanocin A (Shuto et al., 1992), 6′-homoneplanocin A (Shuto et al., 1996), 2-fluoroneplanocin A (Obara et al., 1996) and 6′-iodo acetylenic Ado (Robins et al., 1998) all inhibit vaccinia replication in cell culture at an IC_{50} of less than 1 μg/ml. C-c^{3}Ado (De Clercq et al., 1984) and 3-deazaneplanocin A (Tseng et al., 1989) were shown to inhibit vaccinia virus-induced pox tail lesion formation in mice.

4.3. OMP decarboxylase and CTP synthetase inhibitors

OMP decarboxylase inhibitors prevent the conversion of OMP to UMP and thus lead to an inhibition of UTP and CTP pools. CTP synthetase inhibitors block the convertion of UTP to CTP and hence deplete CTP pools. As can be deduced from their target of action, inhibitors of both OMP decarboxylase and CTP synthetase should suppress RNA synthesis, which in non-dividing cells may result in a distinct antiviral effect. Pyrazofurin, the prototype of the OMP decarboxylase inhibitors, is a potent inhibitor of vaccinia virus replication and exceeds ribavirin and several other compounds in both potency and selectivity (Descamps and De Clercq, 1978). Among the CTP synthetase inhibitors, cyclopentyl cytosine (C-Cyd, carbodine) and cyclopentenyl cytosine (Ce-Cyd) result in stationary (non-dividing) cells in potent and selective anti-vaccinia virus activity (De Clercq et al., 1990, 1991b).

4.4. Thymidylate synthase inhibitors

Thymidylate synthase converts dUMP to dTMP and inhibition of the enzyme causes depletion of dTTP pools that are required for efficient viral (and cellular) DNA synthesis. Several inhibitors of thymidylate synthase (TS) were shown to elicit anti-vaccinia virus activity in vitro. Some of the dUrd derivatives, namely 5-trifluoromethyl-dUrd, 5-nitro-dUrd, 5-formyl-dUrd, 5-ethynyl-dUrd and 5-amino-dUrd, displayed even more potent activity against vaccinia than against herpes simplex virus (De Clercq, 1980).

5. Nucleoside analogues targeted at viral DNA synthesis

There are a number of nucleoside analogues that may be postulated to target viral DNA synthesis. Adenine arabinoside (Ara-A) is in vitro about 10 times more potent against vaccinia virus than against HSV-1 or HSV-2 (Hasobe et al., 1987) and its 5′-triphosphate is believed to enter into competition with dATP, the natural substrate for DNA synthesis. Some branched-chain sugar nucleosides (Nutt et al., 1968) such as 3′-C-methyladenosine and 3′-C-methylcytidine (Fig. 4) have been accredited with activity against vaccinia virus. They completely inhibited vaccinia tail lesion formation in mice (Walton et al., 1969). However, the therapeutic potential of these nucleoside analogues has not been further explored.

Of a series of 2-, 6-, and 8-alkylated adenosine analogues, 8-methyladenosine (Fig. 4) emerged as a potent and selective inhibitor of vaccinia virus (Van Aerschot et al., 1993). Given its remarkable anti-vaccinia virus activity, 8-methyladenosine should be further explored from both a mechanistic and therapeutic viewpoint.

Of particular interest is 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine (or S2242) (Fig. 4). This compound is a potent and selective inhibitor of virtually all herpesviruses and is an efficient inhibitor of vaccinia virus replication (Neyts and De Clercq, 2001). Although the mode of anti-vaccinia virus activity of S2242 has not been established, it can be surmised that the compound is phosphorylated intracellularly to its triphosphate (Neyts et al., 1998) before it blocks viral DNA synthesis. The diacetate ester of S2242, an oral prodrug form, was shown to be highly protective against vaccinia virus infection in both immunocompetent and SCID mice (Neyts and De Clercq, 2001). The protective effect of S2242 and its prodrug was confirmed in mice that had been lethally infected intranasally with cowpox virus (Smee et al., 2002a). A compound that could be given orally would certainly provide an advantage over compounds that must be administered intravenously, particularly when many people would have to be treated.

We have recently demonstrated that 5′-iodo-2′-deoxyuridine (IDU) (Herpid®, Stoxil®, Idoxene®, Virudox®, a well know inhibitor of herpesvirus replication (Fig. 4)) is able to markedly delay vaccinia virus-induced mortality in SCID

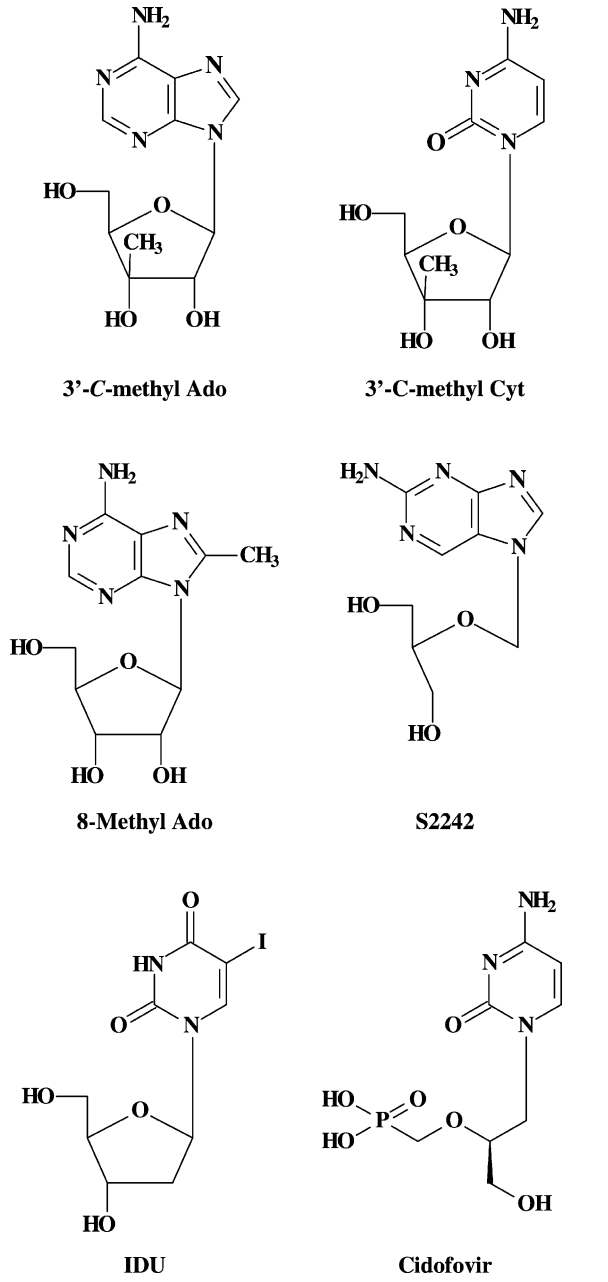


Fig. 4. Presumed inhibitors of the viral DNA polymerase, 3′-C-methyladenosine, 3′-C-methylcytidine, 8-methyladenosine, S2242, IDU and cidofovir.

mice, even when treatment with IDU was postponed until 2 or 4 days after infection (Neyts and De Clercq, 2002). The protective activity of IDU in a non-lethal vaccinia pox tail lesion model was already reported a long time ago (De Clercq et al., 1975). Other compounds, such as trifluorothymidine (TFT) and arabinofuranosyl cytosine (Ara-C) that were also reported to cause protection in the vaccinia tail lesion model (De Clercq et al., 1975) did not prove effective in the lethal vaccinia virus infection model in SCID mice (Neyts and De Clercq, 2002). The use of IDU for the treatment of herpesvirus infections is restricted to topical use because the

compound was found to be too toxic for intravenous use (Alford and Whitley, 1976). In such cases where cidofovir would not be an option (e.g. in case of deliberate release of cidofovir-resistant strains of variola), another drug would be needed. The fatality rate associated with smallpox may possibly justify the administration of a compound such as IDU for a short period of time during the acute phase of the infection.

5.1. Cidofovir

The antiviral activity spectrum of the acyclic nucleoside phosphonate analogue (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, cidofovir) (Fig. 5) encompasses all DNA viruses, in particular papillomaviruses, polyomaviruses, adenoviruses, herpesviruses and poxviruses (De Clercq et al., 1987; Naesens et al., 1997). Cidofovir has been licensed (as Vistide®) for the treatment of CMV retinitis in AIDS patients, but it also has therapeutic potential, upon either systemic or topical administration, for the treatment of various other herpesvirus, polyomavirus, papillomavirus, adenovirus and poxvirus infections, as reviewed previously (De Clercq, 1996; De Clercq, 1998; De Clercq, 2002). Cidofovir is available as an aqueous solution of 375 mg/5 ml, intended for intravenous infusion at a dose of (maximally) 5 mg/kg (in the treatment of CMV retinitis, once weekly for the first 2 weeks, and thereafter once every other week).

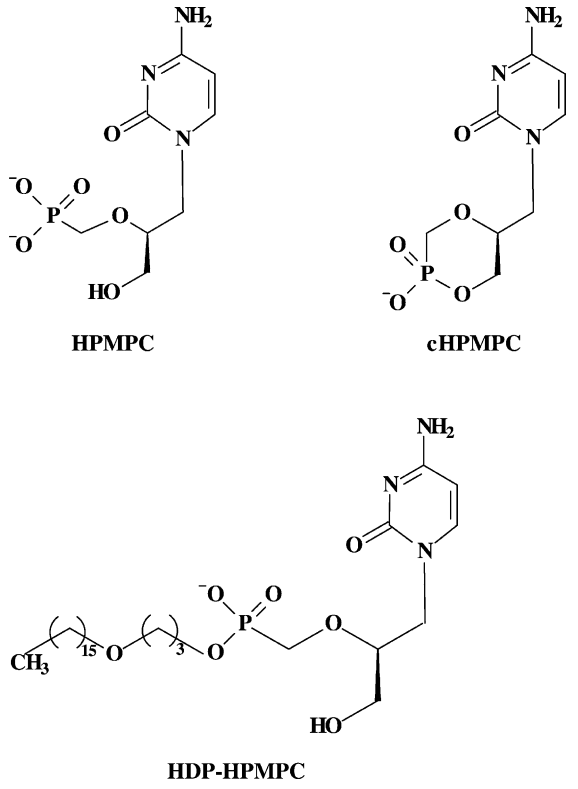


Fig. 5. Cidofovir (HPMPC) and its derivatives cHPMPC and HDP-HPMPC.

In 1993, we were the first to report on the successful use of cidofovir in the prevention and therapy of a lethal vaccinia virus infection in immunosuppressed (SCID) mice (Neyts and De Clercq, 1993). These data have been corroborated in more recent studies (Smee et al., 2001a,b). Even when given as a single dose of 100 mg/kg at 7 days before vaccinia virus infection, cidofovir was able to delay virus-induced mortality by 6 days, and a single dose of 100 mg/kg of cidofovir on the day before the infection delayed virus-induced mortality by about 20 days. (Neyts and De Clercq, 1993). Moreover, even if the start of treatment with cidofovir (25 mg/kg per day, for 5 consecutive days) was delayed until day 6 post-infection, virus mortality was markedly delayed with about a week (Neyts and De Clercq, 1993). Cidofovir, when given subcutaneously as one dosis of 100 mg/kg on day 0, 2 or 4 after infection, was found to be able to protect mice (90–100% survival) that had been exposed to the cowpox virus by aerosol or by the intranasal route (Bray et al., 2000). When cidofovir was given as an aerosol (0.5–5 mg/kg) to mice that had been infected via aerosol with cowpox, the compound was always more effective than 25 mg/kg of the compound given subcutaneously (Bray et al., 2002). Even if administered as a single intranasal dose (at 10, 20 or 40 mg/kg) at 24 h after intranasal challenge with cowpox virus, cidofovir conferred up to 100% protection against mortality (Smee et al., 2000). That aerosolized cidofovir would be efficacious in both pre- and post-exposure prophylaxis of an aerosolized cowpox virus infection, has, again, be confirmed in a recent study (Bray et al., 2002). In this study, aerosol doses of 0.5–5 mg/kg of cidofovir were invariably more effective than subcutaneous doses of 25–100 mg/kg, as monitored by a series of parameters (body and lung weight, lung virus titers, lung pathology and survival). Thus, the efficacy of cidofovir has been unequivocally demonstrated in a number of animal models for poxvirus infections (Table 1).

Cidofovir has also demonstrated its efficacy in monkeys against an experimental infection with monkeypox virus, a virus that, although less virulent and less contagious than variola virus, may have the potential for rapid spread in populous areas and could also be used as a bioterrorist weapon. Monkeys exposed to large quantities (as much as 1000 LD_{50}) of aerosolized monkeypox could be rescued from this overwhelming viral challenge, provided treatment with cidofovir was started shortly after exposure (Huggins, unpublished data, quoted by Bray et al., 2000). Recently, it was shown that cidofovir-resistant strains can be generated in vitro (Smee et al., 2002b). Deliberate release of cidofovir-resistant variola strains, although less virulent than the wild-type virus strains, would implicate that therapeutic or prophylactic agents other than cidofovir may have to be used.

Cidofovir as such penetrates only poorly and slowly into cells and is virtually not taken up by the oral route (Cundy et al., 1999). In attempts to circumvent these problems, several prodrugs of cidofovir have been synthesized such as its cyclic form (cHPMPC) and the alkyloxyalkyl

Table 1
Animal model poxvirus infections in which the efficacy of cidofovir has been demonstrated

Virus	Route of virus infection	Route of drug administration	Reference
Vaccinia	Intravenous	Subcutaneous	Neyts and De Clercq (1993)
	Intranasal	Subcutaneous or intraperitoneal	Smee et al. (2001a,b)
Cowpox	Intranasal	Subcutaneous	Bray et al. (2000), Smee et al. (2002a,b)
	Intranasal (aerosolized)	Intranasal (aerosolized)	Smee et al. (2000), Bray et al. (2002)
	Aerosolized	Oral HDP-HPMPC	Winegarden et al. (2002)
Monkeypox	Aerosolized		Huggins, quoted in Bray et al. (2000)

(1-*O*-hexadecyloxypropyl (HDP) ester (HDP-HPMPC)) (Fig. 5). Linking cidofovir onto a lipid tail such as HDP enhanced the in vitro potency against several poxviruses, including variola virus, by one to three orders of magnitude (Kern et al., 2002; Huggins et al., 2002). HDP-HPMPC also showed markedly increased oral bioavailability (93% as compared to 0.6% for cidofovir itself) and provided 100% protection against aerosolized cowpox virus infection in mice when administered orally at 5, 10 or 20 mg/kg, once daily for 5 days (Winegarden et al., 2002). This may make it feasible to use cidofovir for the oral treatment of smallpox and all other virus infections that are sensitive to the parent compound including disseminated vaccinia.

In humans, cidofovir has so far been used only in the treatment of two types of poxvirus infections, namely molluscum contagiosum and orf (ecthyma infectiosum): in molluscum contagiosum, by both the parenteral (intravenous) and local route (Meadows, 1997; Davies, 1999; Zabawski and Cockerell, 1999; Ibarra, 2000; Toro et al., 2000) and in orf, by topical application (Geerinck, 2001). In all these cases, cidofovir proved highly effective in curbing the infection and the therewith associated symptoms; in the case of orf, the ecthyma lesion completely disappeared following topical application. Obviously, this has not been, and could not be, proven for smallpox, as the disease was officially declared eradicated before cidofovir was discovered. However, there is compelling evidence to assume that cidofovir should be efficacious in the therapy and short-term prophylaxis of smallpox and complications of vaccination: it has proven effective, whether administered intravenously, subcutaneously, topically, intranasally (aerosolized), or perorally (the latter as a lipid prodrug) in a number of experimental infections in animal models, caused by poxviruses that in vitro are even less sensitive to the compound than variola virus, and furthermore, it has already been used, with success, in humans, against a wide variety of infections due not only to pox-, but also herpes- and papillomaviruses.

It should be pointed out that cidofovir is approved, and available, only for parenteral (i.e. intravenous) administration. This may limit its practical use in case of a massive outbreak of smallpox. However, results obtained in experimental animal model infections with aerosolized cidofovir (Bray et al., 2002) and its oral lipid (HDP) prodrug (Winegarden et al., 2002) clearly indicate that the drug could be useful by different delivery routes. Moreover, repeated observations have pointed to the efficacy of cidofovir, when applied topically, in the treatment of pox-, herpes- and papillomavirus infections.

The modalities of treatment may differ, however, with the indication, i.e. the treatment of progressive vaccinia as a complication of vaccination versus the treatment or prophylaxis of smallpox/monkeypox in case of an outbreak. If treatment of complications of vaccination were to be treated this could likely be done best by intravenous administration of cidofovir (at the doses and schedules that are being used for the treatment of CMV retinitis). Patients should be well hydrated, treatment being combined with probenicid and renal functions should be monitored. The situation may be very different in case of an outbreak of smallpox. Possibly (very) large numbers of patients would need treatment or would have to be treated prophylactically. Such high numbers of individuals that would need intravenous treatment may exceed the capacity of the facilities where these people would receive medical care. The ability to use an oral prodrug form or an aerosolized formulation of cidofovir would of course mean a significant improvement.

6. Interferon and interferon inducers

Interferon and the interferon inducers polyacrylic acid and poly(inosinic acid), poly(cytidylic acid) (poly IC) have since long been recognized as being exquisitely active against vaccinia virus. Interferon was shown to be effective in mice if administered within 24 h prior to infection with vaccinia (De Clercq and De Somer, 1968). Poly IC and polyacrylic acid were shown to protect mice and rabbits against local vaccinia virus infection (De Clercq and De Somer, 1968, 1971, 1973; Korngold and Doherty, 1985). The prophylactic protection afforded in the mouse vaccinia tail lesion model by polyacrylic acid was remarkable in that the protective effect of a single injection of polyacrylic acid lasted for 4 weeks (De Clercq and De Somer, 1968). An ointment containing poly (ICLC) (a complex of poly IC with poly-L-lysine and carboxymethylcellulose) was effective both prophylactically and therapeutically against vaccinia virus infection in rabbit skin (Levy and Lvovsky, 1978). We corroborated the pronounced protective effect of poly IC in the mouse vaccinia virus pox tail lesion model. Three doses of poly IC at 20 mg/kg per day (on day −1, 0 and

+1 relative to the infection) virtually completed prevented the development of pox tail lesions. Moreover, in a lethal model for vaccinia virus infections in SCID mice, treatment with poly IC resulted in a delay of virus-induced mortality of almost 2 weeks (our unpublished data). Also, Ampligen, (an analogue of poly IC with a U mismatch at every 12th base of the C strand), a compound that has been evaluated clinically for a variety of conditions, including Myalgic Encephalomyelitis (ME) and Chronic Fatigue Syndrome (CFS), provided marked protection in the vaccinia virus pox tail lesion model (our unpublished data).

Interferon inducers (and/or interferon) may represent interesting therapeutic and/or prophylactic agents for the treatment or prevention of poxvirus infections. Therapy with interferon (inducers) should protect persons (in case of an outbreak of smallpox) for at least several days, that is the time needed for a vaccine to induce a protective response.

7. Perspectives

In a recent feature article on biodefense, Henderson was quoted as having said to be doubtful that an antiviral drug would prove effective against symptomatic smallpox or would be preferably to a vaccine during the window of time between infection and disease (Cohen and Marshall, 2001). There is, however, as we review in the present paper, a preponderance of evidence that an effective therapeutic strategy or (short-term) prophylaxis against smallpox may well be feasible.

Selective antiviral therapy against smallpox may offer an important alternative to vaccination for short-term prophylaxis against smallpox should the smallpox vaccine not be available or provide insufficient protection (either prophylactically or therapeutically) during the period (~4 days) that is needed before the vaccine offers any chance of protection. Also, antiviral therapy may be a supplement to vaccination, should the vaccine not completely prevent breakthrough of the viral infection, or should vaccination by itself lead to severe complications, as may happen in immunosuppressed patients (Redfield et al., 1987). A consequence of the use of an antiviral such as cidofovir could be a reduced efficacy of the vaccine, as the drug will inhibit the replication of the vaccine virus. Given the increasing number of immunodeficient people, i.e. transplant recipients and AIDS patients, this latter group may be an important target population for treatment. Another benefit of antiviral therapy for smallpox may be the reduction in disease transmission that might accompany a reduction in viral titers.

Although one should aim at using for the treatment of variola virus infections antiviral compounds that cause as little adverse effects as possible, the fact that the fatality rate associated with smallpox may be estimated at 10–40% (or even higher in immunocompromised patients), the administration of relatively toxic compounds for a short period of time during the acute phase of the infection seems justified. Indeed, the fatality rate of smallpox is much higher than that of many oncological diseases for which patients receive treatment with (relatively) toxic substances. Antiviral treatment for a relative short period of time, during the acute phase of the infection, may reduce the viral load sufficiently to allow the infected individual to survive the infection. Cidofovir, as well as IDU and S2242 should thus be considered as potential agents for the treatment of poxvirus infections. Also, interferons, and interferon inducers, should be envisaged as potential therapeutic or prophylactic means for the prophylaxis and therapy of poxvirus infections. Obviously, however, cidofovir (either as such or as its lipid prodrug) would seem the current drug of choice in case of an outbreak of smallpox, monkeypox as any other threatening poxvirus infection.

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