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Mini-review

Antiviral therapy for adenovirus infections

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

The treatment of severe adenovirus keratoconjunctivitis and life-threatening adenovirus infections in immunocompromised patients is still unsatisfactory. We here review the mode of action and antiviral data for cidofovir and ribavirin, obtained in cell culture, animal models or patients. Several nucleoside or nucleotide analogues have been described that target the adenovirus polymerase, whereas other antiviral targets have been poorly investigated. Furthermore, optimal therapeutic response may be achieved by combining antiviral therapy with immunotherapeutic approaches, as currently being explored.

Keywords: Adenovirus; Immunocompromised host; Antiviral; Cidofovir; Ribavirin; Immunotherapy

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1. Introduction

Adenoviruses (Ads) are non-enveloped, lytic DNA viruses with a linear double-stranded genome and icosahedral symmetry. To date, 51 human adenovirus serotypes have been described, grouped into six species (A–F) based on genome size, composition and organization, DNA homology, hemagglutinating properties, and oncogenicity in rodents. This subdivision has some clinical relevance as distinct adenovirus species show a preference for specific organs: C, E, and some B species typically infect the respiratory tract, other B species the urinary tract; species A and F target the gastrointestinal tract and species D the eyes (Kojaoghlanian et al., 2003). The receptor usage, which differs among adenovirus subgroups, may in part determine the tissue tropism of different adenoviruses (Mei et al., 1998; Segerman et al., 2003; Xiao et al., 2005).

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Primary adenovirus infections usually occur in young children; approximately 5% of the acute respiratory illnesses in children up to 5 years is due to an adenovirus infection (Brandt et al., 1969) and enteric adenoviruses are a major cause of viral gastroenteritis in infants. In immunocompetent individuals, infections can manifest in diverse clinical syndromes, such as upper and lower respiratory tract disease, (kerato)conjunctivitis, gastroenteritis and hemorrhagic cystitis. In rare cases, hepatitis, myocarditis, meningoencephalitits or nephritis are encountered (Straussberg et al., 2001; Chuang et al., 2003). Adenovirus disease in immunocompetent individuals is mostly mild and self-limiting with few long-term consequences, and therefore does not warrant antiviral therapy. However, the availability of an antiviral therapy for ocular adenovirus infections may be of socio-economic value.

In individuals with an impaired immune response, life-threatening adenovirus infections are common. Among these are patients with hereditary immunodeficiencies [such as severe combined immunodeficiency patients], transplant recipients receiving immunosuppressive therapy and patients with acquired immunodeficiency syndrome (AIDS) (reviewed in Kojaoghlanian et al., 2003). In these patients, adenovirus infections may remain asymptomatic; however, infection often results in severe manifestations such as hemorrhagic cystitis, enteritis, hepatitis, encephalitis, pneumonitis, and multiple-organ failure. Disseminated infections frequently show a fatal outcome (Chakrabarti et al., 2002; Schilham et al., 2002). Pediatric transplantation patients are at three times higher risk for adenovirus infection than adult patients and are particularly prone to disseminated disease, with mortality rates up to 83% (Munoz et al., 1998; Howard et al., 1999; Baldwin et al., 2000). Whether adenovirus disease in the immunocompromised host results from a primary infection or from reactivation of latent virus is still unclear. Adenoviruses can establish an asymptomatic persistent infection with intermittent viral shedding, the probable source of persistent virus being mucosa-associated lymphoid tissue (Garnett et al., 2002).

Due to the growing number of transplant recipients and AIDS patients, the impact of severe adenovirus infections, and, concurrently, the need for effective antiviral therapy, is increasing. Unfortunately, no formally approved drugs are yet available. Two antiviral compounds, i.e., cidofovir and ribavirin, have been used in clinical studies, with variable outcome. In addition, a number of investigational compounds have been reported to have activity against adenoviruses in cell culture, but have not yet been evaluated in patients.

2. Anti-adenovirus therapy with cidofovir and ribavirin

2.1. Mode of action

Most compounds reported to have anti-adenovirus activity are nucleoside or nucleotide analogues, such as cidofovir [(*S*)-HPMPC; (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine], (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] and 2′-nor-cyclic GMP (Baba et al., 1987; De Clercq, 2003). In general, cidofovir displays a higher antiviral selectivity index (efficacy/toxicity ratio) compared to (*S*)-HPMPA. The acyclic nucleoside phosphonates are taken up by the cells by endocytosis, followed by conversion to their active metabolite through two consecutive phosphorylation steps by cellular enzymes. Their diphosphate forms then act as an analogue of the normal deoxyribonucleotide triphosphate substrate and are used as an alternative substrate by the adenovirus DNA polymerase. The antiviral selectivity of the acyclic nucleoside phosphonates is based on their higher affinity for the viral DNA polymerase compared to cellular DNA polymerases. The inhibitory effect of (*S*)-HPMPA diphosphate on adenovirus DNA chain elongation has been demonstrated using a reconstituted in vitro DNA replication system (Mul et al., 1989). Enzyme assays that mimic adenovirus DNA replication are rather complicated since the reaction requires the association of a multi-protein complex consisting of viral and cellular host factors (van der Vliet et al., 1984). The precise effects of cidofovir diphosphate on adenovirus DNA polymerase are unknown since mechanistic studies with cidofovir have not yet been performed. Cidofovir-resistant adenovirus mutants, isolated after virus passage in vitro, have been found to contain distinct sequence changes in the adenovirus DNA polymerase, some being located close to a conserved region implicated in nucleotide binding (Gordon et al., 1996a; Kinchington et al., 2002).

Since adenoviruses, unlike herpesviruses, do not encode a thymidine kinase (TK), they are relatively insensitive to classical acyclic nucleoside analogues, such as ganciclovir and acyclovir, which depend on a specific virus-encoded TK for their first phosphorylation (De Clercq, 2003). In vitro, acyclovir and ganciclovir are active against adenovirus vectors carrying the herpes simplex virus type 1 (HSV-1) TK, hence, their triphosphate metabolites should be efficient inhibitors of the adenovirus DNA polymerase (Wildner et al., 2003). This has, however, not been studied at the enzymatic level.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a purine nucleoside analogue, that is converted to its triphosphate form by cellular enzymes. Five different mechanisms have been proposed to explain its broad-spectrum antiviral activity (Graci and Cameron, 2006). Indirect mechanisms include reduction in cellular guanosine triphosphate pools via inhibition of inosine monophosphate dehydrogenase, and an immunomodulatory effect based on enhanced T-cell response. Direct mechanisms include inhibition of RNA capping activity, direct inhibition of viral polymerases, and increased mutation frequency via incorporation of ribavirin into newly synthesized genomes leading to error catastrophe. No information is available concerning the possible mechanism of action of ribavirin against adenoviruses.

2.2. Antiviral assays for adenovirus

The in vitro methodologies used to determine the activity of antiviral compounds against human adenoviruses are not standardized, making a comparison of available in vitro data very difficult. Accurate estimation of antiviral activity obviously depends on factors such as viral load (‘multiplicity of infection’), host cell line, adenovirus serotype and the assay

used. Indirect tests to measure adenovirus replication include classical assays that are based on evaluation of the cytopathic effect (CPE), plaque formation and virus yield (Baba et al., 1987; Gordon et al., 1991). Though relatively easy and inexpensive, microscopic examination of CPE (characterized by rounding and clumping of cells) may be somewhat subjective. Alternatively, the viability of the infected cells can be measured with a spectrophotometric formazan-based method (Kodama et al., 1996). Direct quantification using immunofluorescence with antibodies directed against an adenovirus protein have been used (Mentel et al., 1997), yet microscopic quantification of fluorescent cells is relatively time consuming. More recently, real-time PCR methodology has been applied to evaluate antiviral compounds, based on the quantitative detection of human adenovirus DNA in infected cells (Wildner et al., 2003; Naesens et al., 2005). This real-time PCR technique is sensitive and reproducible and can replace classical and more labor-intensive techniques such as virus yield assays.

2.3. In vitro data

Several groups have determined the anti-adenovirus activity of cidofovir in cell culture. This compound proved to be a potent and selective inhibitor of adenovirus serotypes from all species (Morfin et al., 2005). In general, the reported EC_{50}-values of cidofovir against adenoviruses fall in the range of 4.6–17 μg/ml (Gordon et al., 1991; Kodama et al., 1996; de Oliveira et al., 1996; Kaneko et al., 2001). In our experience, the activity of cidofovir is slightly higher ($EC_{50} \approx 0.8$ μg/ml) (Naesens et al., 2005). This broad anti-adenovirus activity is in agreement with the observation that one mutation in the adenovirus polymerase gene that is associated with cidofovir resistance (as demonstrated by marker rescue) is located close to a highly conserved region (Kinchington et al., 2002).

Concerning the anti-adenovirus activity of ribavirin in cell culture, the reports are much more divergent. Allen et al. (1978) and Sidwell et al. (1972) were the first to report inhibition of the CPE of Ad3 by ribavirin. The efficacy was confirmed for Ad5 (EC_{50}: 1.7 μg/ml) (Wildner et al., 2003) and Ad2 (EC_{50}: 20 μg/ml) (Kirsi et al., 1983). On the contrary, we found ribavirin to be inactive against Ad2 (Naesens et al., 2005), in line with the antiviral data from Baba et al. (1987), who studied different adenovirus serotypes from species B–E. The controversy on the activity of ribavirin has been adequately addressed by Morfin et al. (2005), who recently demonstrated that its in vitro activity is restricted to group C adenoviruses (EC_{50}: 11.7–26.4 μg/ml) and is dependent on the cell line.

2.4. In vivo data

A number of animal models have been developed for the in vivo evaluation of human adenovirus infection. In mice and cotton rats, inoculation of human type B or C species in the respiratory tract produces inflammatory pneumonia, but virus replication is abortive and restricted to the synthesis of early adenovirus proteins (Ginsberg et al., 1991; Kajon et al., 2003). On the other hand, after ocular inoculation, human type C adenoviruses are able to replicate in the eyes of cotton rats and New Zealand rabbits (Gordon et al., 1992; Tsai et al., 1992; Kaneko et al., 2004). Whereas no symptoms are seen in cotton rat eyes, some ocular inflammation can be observed in New Zealand rabbits. The ocular titers and the time course for viral shedding from the eye are similar to what is seen in human ocular adenovirus infections (Kinchington et al., 2005), thus making these animal models suitable for evaluating antiviral drugs against adenovirus-induced ocular diseases. Topical therapy with cidofovir (administered as eye drops containing 0.5–1% cidofovir) was found to significantly reduce the ocular adenovirus titers, in both therapeutic and prophylactic regimens (Gordon et al., 1994; de Oliveira et al., 1996; Romanowski et al., 2001; Kaneko et al., 2004).

Since adenoviruses are species-specific, in vivo models for disseminated adenovirus infections require the use of a non-human adenovirus. Inoculation of adult C57BL/6 mice with mouse adenovirus type 1 (MAV-1) results in a fatal hemorrhagic encephalomyelitis, whereas adult mice with a BALB/c background are resistant. Immunodeficient BALB/c mice are highly susceptible to MAV-1 infection and die from a non-neurological disseminated disease, characterized by hemorrhagic enteritis (Guida et al., 1995; Charles et al., 1998; Moore et al., 2004). We recently established a mouse model for anti-adenovirus therapy, based on MAV-1 infection of BALB/c-derived 'severe combined immunodeficient' (SCID) mice (Lenaerts et al., 2005). This fatal disseminated adenovirus infection in SCID mice is reminiscent of the clinical situation of adenovirus infection in humans in several aspects: (i) human adenovirus infections are mostly mild and self-limiting in healthy individuals, but can cause severe or lethal illness in immunocompromised patients; (ii) in these patients, a disseminated infection is common; (iii) enteritis, hemorrhagic cystitis, and hepatitis are common manifestations, while adenovirus encephalitis is relatively rare in humans. When cidofovir was evaluated in this MAV-1/SCID model, it caused a marked delay in MAV-1-induced disease. However, cidofovir was unable to completely suppress virus replication despite continued drug treatment, suggesting that complete virus clearance during antiviral therapy for disseminated adenovirus infection requires an efficient adaptive immune response from the host.

2.5. Clinical data

Antiviral treatment for adenovirus infections is warranted in two particular clinical settings: immunocompromised persons with life-threatening complications from adenovirus infections and cases of ocular adenovirus disease. In pilot studies, 1% cidofovir eyedrops for the treatment of adenoviral keratoconjunctivitis, were found to prevent severe corneal opacities, although frequent administration was associated with dose-dependent and potentially severe local toxicity (Gordon et al., 1996b; Hillenkamp et al., 2001, 2002). Moreover, the outcome of antiviral therapy requires early intervention during the viral replicative phase, while medical consultation for severe keratoconjunctivitis usually occurs later, when pathology is mainly inflammatory. On the other hand, antiviral prophylaxis would

Table 1
Clinical studies with cidofovir and/or ribavirin for the treatment of severe adenovirus disease in immunocompromised patients

Patient type[a]	No. of patients receiving therapy[b]	Ad subgroup[c]	Therapy[d]	Outcome	Reference
BMT	2	NA	Ribavirin	2 died	Hale et al. (1999)
	9	NA	Ribavirin (8, 15, 16 mg/kg)	3 completely recovered; 6 had transient and partial recoveries but eventually died; efficacy of ribavirin was related to the donor type	Miyamura et al. (2000)
	12	NA	Ribavirin	2 showed clinical improvement	La Rosa et al. (2001)
	6	A, B, C	Cidofovir (5 mg/kg)	5 recovered; 1 died of disseminated adenovirus after interruption of cidofovir therapy	Legrand et al. (2001)
	2	NA	Cidofovir + ribavirin	2 died	Walls et al. (2005)
	4	A, B	Ribavirin (10 → 5 mg/kg) + IVIG	3 patients died; no patient cleared the adenovirus infection	Hromas et al. (1994)
	2	A or NA	Ribavirin (10 mg/kg), IVIG	1 receiving IVIG plus ribavirin died; 1 receiving IVIG survived	Crooks et al. (2000)
HSCT	8	NA	Cidofovir (1 mg/kg)	8 showed clinical improvement	Hoffman et al. (2001)
	41	A, B, C or NA	Cidofovir (1, 3, 4 or 5 mg/kg)	31 were successfully treated; 10 died	Ljungman et al. (2003)
	14	B or NA	Cidofovir (1 mg/kg)	10 showed clinical improvement	Nagafuji et al. (2004)
	2	NA	Cidofovir (5 mg/kg)	1 showed clinical improvement; 1 died	Gorczynska et al. (2005)
	10	NA	Cidofovir (5 mg/kg)	9 clinical improvement; 1 died	Muller et al. (2005)
	4	A, C	Ribavirin (30 → 60 mg/kg), cidofovir (5 mg/kg)	2 receiving ribavirin died; 2 receiving ribavirin, followed by cidofovir, died	Lankester et al. (2004)
	21	A, B, C, F or NA	Ribavirin (8 → 5 mg/kg), cidofovir (5 mg/kg)	16 receiving ribavirin or ribavirin plus cidofovir achieved resolution of adenoviraemia; 5 died	Kampmann et al. (2005)
	21	A, B, C, D	Ribavirin (35 → 25 mg/kg), cidofovir (5 mg/kg), DLI, vidarabine (10 mg/kg)	7 survived: 3 with ribavirin, 2 with cidofovir, 1 with ribavirin plus DLI and cidofovir, and 1 with ribavirin plus cidofovir	Bordigoni et al. (2001)
	4	C	Ribavirin (15 mg/kg), cidofovir (5 mg/kg), IVIG	3 receiving ribavirin died; 1 receiving cidofovir plus IVIG cleared adenovirus	Chakrabarti et al. (2002)

[a] BMT, bone marrow transplantation; HSCT, hematopoietic stem cell transplantation.
[b] Patients whose death was not related to adenovirus infection were excluded from the analysis.
[c] NA, data not available.
[d] IVIG, intravenous immunoglobulin; DLI, donor leukocyte infusion. When available, the dose is indicated between brackets.

be particularly useful in limiting adenovirus transmission to the adjacent eye as well as to relatives.

Clinical studies in immunocompromised patients have thus far focused on cidofovir or ribavirin (Table 1), except for one report showing that patients receiving ganciclovir for CMV prophylaxis have a lower risk of developing adenovirus infections (Bruno et al., 2003). Unfortunately, no placebo-controlled, randomized trials have yet been conducted. Also, interpretation and comparison of different studies is hampered by the heterogeneous background of the patients (i.e., pediatric versus adult patients; bone marrow, stem cell, or solid-organ transplant recipients and AIDS patients), by differences in the drug dosing schedules for antiviral and immunosuppressive drugs, and in the definitions of adenovirus disease and diagnostic procedures.

Most reports are from young children undergoing bone marrow or stem cell transplantation and manifesting severe adenovirus disease. Although failures with cidofovir have been described, most studies demonstrated that cidofovir is effective against adenovirus infections (Table 1). However, thus far cidofovir (Vistide®) has only been formally approved for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients. Since cidofovir can be nephrotoxic, dose reductions are recommended in transplant recipients receiving cidofovir in combination with other nephrotoxic drugs. The renal toxicity of cidofovir is counteracted by concomitant use of hydration and probenecid.

The efficacy of ribavirin in the treatment of adenovirus infections is more controversial. In the clinical studies of Miyamura et al. (2000) and La Rosa et al. (2001), ribavirin appears rather ineffective against severe adenovirus diseases. Yet a number of case reports mention successful therapy with ribavirin (Cassano, 1991; Arav-Boger et al., 2000). These inconsistencies may be partially explained by serotype-related differences in the anti-adenovirus activity of ribavirin (Morfin et al., 2005).

In general, neither cidofovir nor ribavirin has been found to be particularly effective against established adenovirus disease. Their success rate is higher when treatment is initiated early after diagnosis of adenovirus infection and before it has progressed into disease. This favors the possible use of these antivirals for the pre-emptive therapy or prophylaxis of adenovirus infections, which would require prospective and sensitive virus monitoring.

A factor that is often overlooked in the clinical studies on anti-adenovirus therapy is related to the patient's immune status. In several retrospective studies, failure of anti-adenovirus therapy was observed in heavily immunosuppressed patients who had received a T-cell-depleted graft or who suffered from severe graft-versus-host-disease. Conversely, there is a strong correlation between a positive outcome of adenovirus disease and immunological recovery, achieved by reduction or withdrawal of immunosuppressive therapy (Chakrabarti et al., 2002; van Tol et al., 2005). The importance of the immune response

was emphasized in a case report on an AIDS patient with disseminated adenovirus disease, who did not respond to antiviral therapy and died (Nebbia et al., 2005).

3. New nucleoside/nucleotide analogues

The clinical success of antiviral nucleoside analogues has paved the way for the synthesis and antiviral evaluation of new derivatives. Although a high-throughput screening system for adenoviruses remains to be established, small-scale antiviral studies have revealed a number of experimental compounds that are worth mentioning here.

An interesting class of new antivirals is represented by (*S*)-HPMPO-DAPy [2,4-diamino-6-[3-hydroxy-2-(phosphonomethoxy)-propoxy]pyrimidine], an acyclic nucleoside phosphonate derivative of 2,4-diaminopyrimidine. The marked anti-adenovirus activity of this compound is only slightly inferior to that of cidofovir and its adenine analogue (*S*)-HPMPA (Naesens et al., 2005). A related compound, PMEO-DAPy, is devoid of anti-adenovirus activity. PMEO-DAPy is identical to HPMPO-DAPy except for the hydroxymethyl group, that is missing in the acyclic chain of PMEO-DAPy (Holy et al., 2002). This hydroxymethyl group is present in cidofovir, (*S*)-HPMPA and (*S*)-HPMPO-DAPy, and appears to be a prerequisite for anti-adenovirus activity of the acyclic nucleoside phosphonates.

To overcome the low oral bioavailability of cidofovir, several lipophilic ester prodrugs of cidofovir have been synthesized. In adenovirus-infected cells, some of these prodrugs appeared to be 5- to 300-fold more active than cidofovir, with excellent selectivity (Hartline et al., 2005). These esters were shown to have an enhanced oral activity in mouse models for CMV and orthopoxvirus infections. In addition, these cidofovir prodrugs appear to have a lower potential for renal toxicity than cidofovir (Quenelle et al., 2004).

S-2242 [2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine] is an N_7-substituted acyclic purine derivative with broad-spectrum anti-DNA virus activity due to its high phosphorylation efficiency by cellular kinases (Neyts et al., 1998). In cell culture, S-2242 has emerged as a potent inhibitor of adenovirus replication with a selectivity index that exceeds that of cidofovir (de Oliveira et al., 1996; Naesens et al., 2005).

An intriguing observation is the inhibition of adenoviruses by the antiretroviral 2′,3′-dideoxynucleoside analogues. The marked in vitro activity of zalcitabine (ddC) and alovudine (FddT) was documented earlier by Mentel et al. (1997), and the efficacy of ddC was confirmed in a mouse model for adenovirus pneumonia (Mentel and Wegner, 2000). In our own in vitro experiments, ddC and FddT displayed EC_{50}-values in the same range as obtained for cidofovir (0.2–0.7 μg/ml) (Naesens et al., 2005). Recently, the 5′-triphosphates of ddC and FddT, which are typical chain-terminating inhibitors of HIV reverse transcriptase, have been shown to inhibit the adenovirus DNA polymerase by competitive inhibition with the natural substrate (Mentel et al., 2000). Uckun et al. (2004) developed novel aryl phosphate derivatives of the antiretroviral 2′,3′-dideonucleoside analogue stavudine (d4T). Besides their inhibitory effect on HIV, these derivatives exhibit potent and selective anti-adenovirus activity with EC_{50}-values in the nanomolar range. Unlike other broad-acting nucleoside/nucleotide analogs, such as ribavirin, cidofovir, (*S*)-HPMPO-DAPy or S-2242, the antiviral activity of these 2′,3′-dideonucleoside analogues is restricted to HIV and adenoviruses. In one anecdotal report, the nucleoside analogue 6-azacytidine was observed to have an inhibitory effect on adenovirus replication in vitro (Alexeeva et al., 2001). Unfortunately, for most compounds summarized here, no biochemical data are available on their mode of action at the level of the adenovirus DNA polymerase complex.

4. New targets for anti-adenovirus therapy

The number of adenovirus proteins that have been exploited as potential target for antiviral therapy is limited. As described above, most compounds with reported anti-adenovirus activity are nucleoside or nucleotide analogues that act by inhibition of the adenovirus DNA polymerase.

The adenovirus adsorption process appears to be an attractive target, since the sulfated sialic acid derivative $NMSO_3$ was found to inhibit cellular binding of several human adenovirus serotypes at compound concentrations that did not affect cell viability (Kaneko et al., 2001). This virus adsorption inhibitor competes with the sialylated cell receptors for the binding of adenovirus particles. Since human adenoviruses with ocular tropism bind to a sialic acid-containing receptor (Arnberg et al., 2000a,b), $NMSO_3$ may be useful for the topical treatment of ocular adenovirus infections.

Inhibition of the adenovirus cysteine protease may be another antiviral approach, since this enzyme is indispensable for the production of infectious viral particles (Mangel et al., 2003). The adenovirus protease was shown to be susceptible to miscellaneous protease inhibitors, including papain inhibitors and green tea catechins (Sircar et al., 1998; Weber et al., 2003). Unfortunately, these inhibitors have a low specificity for the adenovirus protease which makes them unsuited as therapeutics. In order to identify highly specific adenovirus protease inhibitors, high-throughput screening along with computer modeling, as performed for HIV and hepatitis C virus, is needed. Recently, Pang et al. (2001) performed an in silico screening of a chemical library and identified 2,4,5,7-tetranitro-9-fluorenone as a new lead compound that selectively and irreversibly inhibits the adenovirus cysteine protease.

In a completely different approach, broad-spectrum antimicrobial compounds with an intrinsic role in innate immunity may have potential as therapeutic agents. The endogenous microbicide *N*-chlorotaurine is a weak oxidant produced by granulocytes and monocytes during inflammatory reactions. Because of its unspecific reaction mechanism (i.e., oxidation of amino-, thio-, and aromatic groups), it has demonstrated activity against bacteria, fungi, HSV and adenovirus (Nagl et al., 1998). *N*-chlorotaurine proved to be well tolerated when administered topically to rabbit and human eyes and in guinea pig ears (Neher et al., 2004), and was shown to be effective in phase II clinical trials with viral conjunctivitis (Teuchner et al., 2005). Likewise, activity against a broad range of pathogens (including aden-

ovirus) has been described for defensins, cathelicidins and other antimicrobial peptides (Gordon et al., 2005).

In addition, a number of miscellaneous compounds have been identified as inhibitors of adenovirus infection in cell culture. Among these are cyclic D,L-α-peptides that target the uncoating process of adenoviruses (Horne et al., 2005); cycloferon (Zarubaev et al., 2003); lactoferrin (Arnold et al., 2002); medical plant compounds (Chiang et al., 2003); nitric oxide (Cao et al., 2003); the anti-herpes cobalt chelate doxovir (Kinchington et al., 2005); heterocyclic Schiff bases of aminohydroxyguanidine tosylate (Das et al., 1999) and RGD peptidomimetic molecules (Hippenmeyer et al., 2002).

5. Immunotherapy for adenovirus infections

The clinical association between severe adenovirus infection and immunosuppression is a proof for the importance of the cellular and/or humoral immune response in the control of adenovirus infections. Since tapering of the immunosuppressive therapy is not always feasible, reconstitution of the host's immune system by immunotherapy could be an effective approach to prevent and treat adenovirus disease. However, the relative contribution of virus-neutralizing antibodies and virus-specific T-cells in the clearance of adenoviruses is still unclear.

Intravenous immunoglobulin (IVIG) has been used in a few cases with variable results (Crooks et al., 2000; Emovon et al., 2003). Since IVIG preparations contain neutralizing antibodies against common adenovirus serotypes, they cannot confer protection against certain serotypes that are less prevalent in the general population, yet are often encountered in immunocompromised patients. IVIG could be effective against exogenous or primary adenovirus infections, but is unlikely to provide protection against reactivating virus. Likewise, in CMV therapy, IVIG has shown efficacy as a prophylactic agent, but not as a therapeutic modality. The fact that viral clearance requires functional T-cells, has led to the experimental use of donor leukocyte infusions in adenovirus-infected transplant recipients. This therapy is not feasible in cases of graft-versus-host-disease, unless prior selective depletion of alloreactive T-cells is performed. Alternatively, there may be a therapeutic benefit with ex vivo generated donor-derived adenovirus-specific T-cells, similarly as has been described for Epstein–Barr virus and CMV. Studies in healthy individuals demonstrated both CD4+ and CD8+ response to adenovirus, with the viral capsid antigens as the target for the cytotoxic T-cell (CTL) response (Leen and Rooney, 2005). Due to extensive cross-reactivity of adenovirus-specific T-cells across serotypes, adoptive transfer of CTLs may protect immunocompromised patients from infections by adenoviruses of all serotypes. To date, there have been no clinical trials using adenovirus-specific CTLs. As the CTLs must be generated for all individual patients, this process will be extremely costly, time-consuming and labor-intensive.

6. Perspectives

As reviewed here, current therapeutic modalities for adenovirus infections are limited. With the population of immunocompromised patients growing, severe adenovirus infections will increase in frequency, underlining the urgent need for effective and safe anti-adenovirus therapy. The optimal approach would consist of prospective virus monitoring and pre-emptive suppression of adenovirus replication using antiviral and/or immunotherapy, in combination with tapering of the immunosuppression to allow immune recovery. To realize this, more investigations will be needed to discover new anti-adenovirus compounds, preferably targeting new viral targets, or to develop immunotherapeutic strategies by an improved understanding of the cellular and humoral immune response to adenoviruses.

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