Prospects for adenovirus antivirals

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Adenoviruses cause a number of self-limiting but often highly infectious diseases that affect multiple organs, most commonly those associated with respiratory, genitourinary and gastrointestinal tracts and the ocular surface. Many factors have driven a search for effective topical and systemic antivirals to adenoviruses. These include patient morbidity, economic losses and chronic visual disturbances associated with epidemic keratoconjunctivitis; and the startling recent trend of high morbidity and rising mortality associated with systemic adenoviral infections in the immunosuppressed, particularly paediatric bone marrow transplant recipients. The development of effective antivirals has proven to be a complex task, owing to the fact that multiple and often genetically divergent adenovirus serotypes can cause similar diseases. Currently, there remains no licensed systemic or topical treatment in the USA or Europe. However, many compounds have been explored for activity against adenoviruses, and some have been evaluated clinically in either a topical setting for ocular disease or in the setting of systemic treatment in the face of life-threatening adenovirus infections. This article outlines such compounds, discusses the potential for their clinical development, and highlights some problems that may be faced in evaluating their efficacy clinically.

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Adenoviruses and the target diseases for antiviral development

There are at least 51 distinct human adenovirus serotypes (Ad1 – Ad51 inclusive) identified to date, which are subdivided into PCR-distinguishable1,2 species (groups A–F) based on DNA homology, RFLP analyses, haemagglutination grouping, pathogenesis in rodents and differential ability to transform murine primary cells.3 In addition to humans, a number of mammalian species harbour adenoviruses, but these are unable to infect humans. By electron microscopy, all adenoviruses appear structurally identical and have a distinct, non-enveloped icosahedral infectious particle with projecting spikes at the 12 vertices that, together with the hypervariable regions of the hexon capsid, confer serotype specificity and mediate attachment and entry to specific receptors. Receptors for adenovirus appear to vary with serotype grouping, and may, in part, dictate the host and tissue range of each group. The non-enveloped nature of the particle enhances transmission and communicability by conferring prolonged survival after desiccation. The viral double-stranded DNA genome of ~34 000 base pairs encodes a large number of structural, regulatory and host cell modifying proteins, which have been discussed at length elsewhere.4 Among the important encoded viral proteins in consideration of antivirals, adenoviruses encode their own viral DNA polymerase, as well as a single-stranded DNA binding protein and a pre-terminal/terminal DNA binding protein. Together with host factors, which have been partly characterized, these act preferentially to replicate viral DNA over that of the host. Adenoviruses do not encode any of the multiple nucleotide pool-modifying enzymes found in the herpesviruses (e.g. dUTPase, thymidine kinase, ribonucleotide reductase), and as such, most adenoviruses are not responsive to anti-herpesvirus agents activated by such enzymes.

Adenovirus infections have many clinical presentations in the immunocompetent individual, with the most common type of infection being subclinical. However, over half of adenovirus serotypes can cause clinical disease associated with one or multiple organs, of which the most common are the respiratory, gastrointestinal and genitourinary tracts, the liver and the eye. A thorough treatise on clinical diseases associated with adenoviruses has been recently presented elsewhere.5 For example, it is estimated that some 5% of acute respiratory illnesses in children under 5 are due to adenovirus infection, and gastrointestinal disease induced by adenovirus is considered a major contributing factor to childhood diarrhoea in underdeveloped and high population density areas. By and large, these diseases are of short duration with few long-term consequences and do not warrant the development of antivirals. Infection generally induces a strong
adaptive and protective immunity that usually limits disease instigated by the same or closely related serotypes. There has been some vaccine development, and live vaccines have been used in both the USA and Canada to protect military recruits against adenovirus-induced respiratory disease. However, vaccination is not routinely carried out in the general population and the vaccine serotypes (Ad4 and Ad7) are not normally associated with epidemic keratoconjunctivitis (EKC), one of the target diseases for antiviral development. There is a low prevalence of antibody against the most common serotypes of adenovirus that cause EKC (Ad8, Ad19 and Ad37).

There are two specific clinical circumstances in which an effective adenovirus antiviral would have a considerable impact on morbidity associated with adenovirus infections. The first concerns the immunocompromised individual, where increased morbidity and mortality associated with adenovirus infections occurs, predominantly as a result of the increased severity of adenoviral infection and the inability of adaptive immunity to limit disease.

Incidence of up to 10% occur in liver transplant recipients, and >60% of immunocompromised adenovirus-infected individuals develop clinical symptoms. Disseminated adenovirus disease with multiple organ involvement is much more frequent, severe and has increased mortality in the immunocompromised. In particular, there has been an emergence of fatal disseminated adenovirus infections, with up to 21%–80% mortality, in paediatric and bone marrow transplant patients. This has dramatically framed the urgent need for an effective systemic antiviral agent.

The second circumstance in which antivirals would have an impact is in occurrences of disease of the ocular surface. Ocular adenovirus infections are common, although there is no accurate incidence and epidemiological data in the USA or Europe. However, adenovirus ocular infections are particularly rife in Japan, where over 1 million cases are reported to a National Epidemiological Surveillance of Infectious Agents registry. There are three clinical presentations of ocular disease: follicular conjunctivitis (FC), which is relatively mild and usually lasts for 3–5 days; pharyngeal conjunctival fever (PCF), which is associated with cold-like symptoms and conjunctivitis lasting 5–7 days; and EKC, a more serious condition involving the cornea and conjunctiva, which may have long-term consequences on visual acuity. EKC is accompanied with some or all of several distressing symptoms (photophobia, moderate to severe irritation, foreign body sensation, increased tearing, lid swelling and sometimes conjunctival haemorrhages) and usually spreads to the second eye in a milder form. The acute phase involving viral replication usually lasts up to 2 weeks. However, following the acute phase of EKC, there is often an immune T cell mediated infiltration of the corneal stroma, leading to the formation of multiple small, white dots (sub-epithelial infiltrates) that can cause disturbances in vision (e.g. decreased visual acuity, photophobia) for periods of months and sometimes years. The majority of cases of EKC are believed to be caused by selected species D viruses, including Ad8, Ad19 and Ad37, whereas FC and PCF are more commonly associated with serotypes Ad3, Ad4 and Ad7. However, over half of 51 adenovirus serotypes have been associated with ocular diseases. In an environment with a lack of effective antivirals, treatment is currently limited to symptomatic therapy and physician-recommended epidemiological control measures to reduce transmission; or to topical corticosteroid treatment to alleviate the immune infiltration.

The latter can be difficult to manage by the physician, owing to rebound effects of these infiltrates following steroid withdrawal, and the adverse consequences of chronic topical steroid therapy (glaucoma, cataracts and microbial superinfection).

Development of animal models for antiviral testing

Most human adenoviruses do not productively replicate in cells or animals of non-human origin, although they do often infect with variable efficiency and may produce a few virus proteins. Some specific serotypes demonstrate extended host range, enabling animal model development for antiviral testing. In particular, human type C adenoviruses can replicate in the eyes and lungs of the outbred cotton rat, resulting in seroconversion. However, the cotton rat has not gained wide acceptance as an animal model as the animals are wild, highly active and aggressive, and can be physically challenging to handle and manage in captivity. Over the last 10 years, a rabbit eye model of adenovirus replication was developed using adenovirus serotype Ad5, which was subsequently extended to include other species C adenoviruses (Ad1, Ad2 and Ad6). In this model, inoculation into scardified corneas of New Zealand white rabbits results in multiple cycles of virus replication, peaking at days 3–5 post-infection with subsequent waning, leading to clearance of virus by days 10–14, presumably by natural immune clearance mechanisms. In this model, the time course of viral shedding from the eye mimics the course of a typical case of human ocular adenoviral infection and the ocular adenoviral titles are achieved similarly. Whereas the model does not support replication of common serotypes that cause EKC in humans (Ad8, Ad19 and Ad37), and offered only limited reproducibility of clinical conjunctivitis, it enabled antiviral testing and assessment in the light of the confounding variables associated with topical antiviral application to the eye. In addition, topical administration largely bypassed many toxicity issues associated with systemic delivery.

The promise and problems of cidofovir

The most developed group of compounds evaluated for activity to adenoviruses are the phosphonyl acyclic nucleotides, which include (S)-9-(3-hydroxy-2-phosphonomethoxy propyl)cytosine dehydrate (also known as S-HPMPC, cidofovir), 2-nor-cyclic-GMP and (S)-9-(3-hydroxy-2-phosphonomethoxy propyl)adenine dehydrate (also known as S-HPMPA). Mechanistically, these compounds mimic the monophosphate form of nucleotides and can pass through the cell membrane. They target the viral DNA polymerase directly. Confirmation of the viral target for cidofovir was achieved by deriving adenovirus mutants, which were resistant to cidofovir, and demonstrating that resistance was associated with changes in the viral polymerase near conserved structural regions involved in nucleotide binding. Cidofovir was chosen for further development, based on its favourable efficacy/toxicity ratio in culture. It is approved for treatment of human cytomegalovirus (hCMV) retinitis in AIDS patients in the USA and in the EU.

In the rabbit model, cidofovir successfully and significantly reduced adenovirus titres, even with infrequent topical dosing, in both therapeutic and prophylactic applications. The effectiveness of cidofovir was presumably a result of rapid corneal penetration, coupled with a documented prolonged intracellular
half life.\(^\text{19,26}\) Interestingly, cidofovir demonstrated high efficacy against herpes simplex virus type 1 (HSV-1) replication and HSV-1-induced keratitis in the rabbit model, with dosing regimens of much lower frequency than current topical antiviral therapy.\(^\text{27}\) The success of cidofovir in numerous studies using the rabbit model ultimately resulted in a large, multicentre, randomized and controlled clinical trial carried out in the USA to evaluate cidofovir against human adenovirus ocular infections. Results from the trial (unpublished results) indicated that there was significant efficacy of cidofovir in the rapid clearance of adenovirus in the presenting eye, in the protection of the fellow eye from infection and in the reduction in the number of subepithelial infiltrates. However, recent off-label usage in Europe and Hawaii of higher doses of topical cidofovir for \(>1\) week was associated with rare cases of lachrymal canalicular blockage.\(^\text{31,44–46}\) The laboratory isolation of adenoviruses resistant to cidofovir with apparently normal pathogenesis in animal models\(^\text{20,28}\) also added to concerns regarding the possible emergence of clinical resistance following widespread usage. Despite the failure of the USA trials, several small clinical trials in Europe continue to evaluate cidofovir for the treatment of human adenovirus ocular infections.\(^\text{29,30}\)

Whereas topical cidofovir has not undergone further clinical development, there has been broader parenteral application beyond its approved use for treatment of hCMV in AIDS patients to control adenoviral disease in life-threatening conditions. Several case reports, as well as some larger retrospective studies, indicate that cidofovir appears to be at least partially effective in limiting systemic adenoviral complications and reducing blood adenovirus DNA loads in adult and paediatric bone marrow transplant patients.\(^\text{31–36}\) The high morbidity and mortality associated with systemic adenoviral infections in this patient population appear to warrant the risks, side effects and nephrotoxicity of cidofovir, which are well documented from its clinical use in the treatment of severe hCMV disease in AIDS patients.\(^\text{21,37}\)

**Ribavirin**

Whereas there is no approved antiviral treatment options for systemic adenovirus infections, several agents have been explored clinically in life-threatening disease, and ribavirin is one such compound. Ribavirin is approved in the USA for inhalation treatment of lower respiratory tract respiratory syncytial virus infections in children, and is approved in both the USA and EU for the systemic treatment (in combination with interferon \(\alpha–2b\)) of hepatitis C virus infections. Mechanistically, ribavirin passes the cell membrane and is phosphorylated intracellularly, where it inhibits inosine monophosphate dehydrogenase. The inhibition leads to: (1) reduced levels of GTP pools; (2) inhibition of initiation and elongation by viral RNA dependent polymerases; and (3) interference with mRNA capping. There are several case reports of successful ribavirin treatment of systemic adenovirus infection.\(^\text{38–44}\) However, others have reported failure of ribavirin therapy in the treatment of adenovirus disease.\(^\text{10,31,44–46}\) Our *in vitro* studies suggest ribavirin efficacy is serotype specific in culture tests, and lacks efficacy against the three predominant adenovirus serotypes associated with EKC (Ad8, Ad19 and Ad37). As such, it is unlikely to have much value in the topical treatment of ocular disease.\(^\text{45}\) Given that there is serotype specificity, which is the most likely reason for the variable clinical efficacy of ribavirin, its general use in treating life-threatening systemic infections should be cautious and accompanied with adenovirus serotyping.

**Additional compounds with potential for development**

The hCMV drug ganciclovir, a nucleoside analogue of guanine, has shown some promise against specific adenovirus serotypes *in vitro*. Ganciclovir is approved in both the USA and the EU for the treatment of hCMV infections in AIDS and other immunocompromised individuals, and is licensed in several European communities for the topical treatment of herpetic keratitis. Whereas topical ganciclovir has been advocated by some for topical ‘off label’ usage against EKC, there was found to be only limited efficacy against common ocular adenovirus serotypes *in vitro*\(^\text{46}\) and in the cotton rat ocular model with serotype Ad5.\(^\text{14}\) A few case reports exist of pilot clinical studies,\(^\text{59,60}\) but there are no controlled trials indicating efficacy and clinical activity. It is notable that ganciclovir, coupled with replication-defective and competent adenoviruses expressing the herpes simplex virus thymidine kinase, are being extensively explored as suicide gene therapy systems for elimination of tumours and specific tissues.

Other nucleosides with anti-adenoviral activity include the antiretroviral nucleoside analogue 2',3'-dideoxycytidine (ddC, also known as zalcitabine), which has efficacy against multiple adenovirus serotypes *in vitro*\(^\text{47,51,52}\) as well as against Ad2 in the mouse pneumonia model.\(^\text{15}\) The HIV-effective antiviral 6-azacytidine also appears to inhibit adenovirus replication *in vitro*\(^\text{57,58}\) and is effective against multiple adenovirus serotypes, with IC\(_{50}\) concentrations from 3.5–48.7 \(\mu\)g/mL.\(^\text{47}\)

Several non-nucleoside agents have antiviral activity to adenovirus, including the sulfated sialyl lipid NMSO3.\(^\text{55,56}\) The mechanism of antiviral activity of NMSO3 appears to be the inhibition of virus adsorption. A second agent is the endogenous microbicidal N-chlorotaurine, which is found in the supernatant of stimulated granulocytes. Its mechanism is thought to be through the oxidation of thiols and amines, and its antimicrobial activity is immediate following contact. This has demonstrated antimicrobial activity against adenoviruses as well as bacteria fungi and HSV-1.\(^\text{55,58}\) N-chlorotaurine was safe when administered topically to eyes of both rabbits and humans and in guinea-pig ears,\(^\text{59,60}\) and demonstrated efficacy compared with gentamicin in the improvement of clinical signs of viral conjunctivitis in a small clinical trial performed in Austria.\(^\text{51}\) This antiseptic-like agent may have much broader applications for conjunctivitis as it may also be effective against other viral and bacterial causes.

The non-nucleoside doxovir (also known as CTC-96), represents a new class of antiviral agent, the cobalt chelates, with activity against adenoviruses. Although incompletely characterized, the mechanism of action appears to be related to strong binding to histidine and is an imidazole derivative. In the Ad5
rabbit replication model, doxovir at 50 μg/mL eliminated virus by day 10 compared with day 21 in placebo-treated controls, and resolution of clinical conjunctivitis was fastest in the doxovir 50 μg/mL treatment group, compared with lower doxovir concentrations and placebo-treated controls.62 The agent had activity against several herpesviruses, including HSV-1 and-2, hCMV, Epstein–Barr virus, varicella-zoster virus and human herpesvirus 6 (HHV-6). Topical doxovir was as effective as Vir- optic (trifluridine) in diminishing HSV-1-induced corneal disease and ocular surface titres of HSV-1 in the rabbit keratitis model.63 Doxovir may target a maturational protease in HSV-1, which is required late in the replication cycle. Its activity against both adenovirus and herpesviruses increases its value as a potential ocular antiviral.

A number of compounds have been noted to have anti-adenoviral activity in vitro, but most remain to be evaluated in animal models. These include traditional plant-derived compounds,44 plant green tea catechins,35 cycloferon,66 lactoferrin,67 heterocyclic Schiff bases of aminohydroxyguanidine tosylate,68,69 a topoisomerase inhibitor,70 and papain and protease inhibitors.71,72 Furthermore, peptidomimetic integrin-binding antagonists block adenoviruses by interfering with receptor binding.73 Finally, it has been noted that human α-defensin peptides are effective against adenoviruses in vitro.74

**Future issues in the development of adenovirus antivirals**

The typical issues faced in all antiviral development strategies (such as production costs, safety, efficacy/toxicity ratios and practical dosing regimens) also apply to adenovirus antiviral development. However, there are some specific considerations that complicate the development of effective anti-adenovirus drugs.

**Breadth of serotype efficacy**

It is clear that optimal antivirals to adenoviruses must have broad serotype specificity. Multiple serotypes can cause similar diseases, despite some often extensive primary protein sequence and structural divergence. By and large, serotyping of the causative adenovirus is not routinely carried out in a diagnostic setting, and so compounds with wider serotype breadth offer distinct advantages over those with narrower specificity. A topical antiviral for ocular disease would hopefully target at least the most common pathogenic serotypes that cause EKC (Group D Ad8, Ad19, Ad37), PCF and FC (Group B Ad3, Ad7; Group E Ad4; and Group C Ad1, Ad2, Ad5, Ad6). Targeting of respiratory diseases would also require an extensive serotype breadth to be effective.

**Antiviral targeting**

The ideal antiviral would target a virus-specific process that does not have homologous processes in the host. Whereas adenoviruses encode their own DNA polymerases that are structurally divergent from host polymerases, both utilize the same nucleotide substrates, and the basic replication machinery is similar. As adenoviruses lack nucleotide metabolism enzymes found in herpesviruses, the ‘nucleoside activation’ principle using nucleotide pool modifying enzymes cannot be applied to adenoviruses and antiviral effectiveness must rely on preferential activity on the viral polymerase over the host’s polymerases. However, there are multiple, highly specific virus processes for which antiviral strategies could be developed, including attachment, uncoating, virion assembly and targeting of key viral protein:protein interactions. High throughput screens to identify lead compounds acting on such processes for adenoviruses largely remain to be developed. However, it is encouraging that several antitherpesvirus antiviral strategies have recently been developed that target key protein:protein interactions.75

**Clinical trial challenges**

The design and execution of large, controlled, clinical trials required prior to government approval for any antiviral has some critical hurdles to overcome, particularly with regard to adenovirus ocular diseases. Patient capture for enrolment may be a problem, as the milder ocular diseases—PCF and FC—are seasonal, self-limited, of short duration and may not be seen or referred to a physician. EKC occurs in sporadic epidemics in the western world, and as such, patient enrolment will require multiple centres in geographically diverse sites in the USA or Europe to capture sufficient patients for EKC trials. An alternative would be to evaluate such antiviral candidates in clinical trials in Asia (Japan, China, Korea), where adenoviral infections are endemic. Accurate diagnosis may also be a key issue, as many adenoviral ocular and respiratory infections mimic clinical symptoms caused by many infectious and non-infectious agents. Rapid diagnostic serotyping may also be important if an antiviral with limited serotype breadth of efficacy is to be evaluated in clinical trials. A successful clinical trial must address the logistics and costs of rapid laboratory diagnosis (real-time PCR, enzyme immunoassay, culture) for timely enrolment. Our experience from the cidofovir trials indicated difficulty in demonstrating antiviral efficacy in cases where correct diagnosis was delayed.

**Conclusion**

Despite these challenges unique to adenovirus, successful clinical trials for an ophthalmic antiviral can be completed, as demonstrated during the evaluation of topical cidofovir in the USA by Bausch & Lomb Inc. There still remains a continuing worldwide need to develop effective topical therapy to treat community and epidemic adenoviral ocular infections, and the immunocompromised population will probably not decrease in the near future, but rather expand. Given the high level of patient morbidity, and significant economic societal losses, we remain optimistic that a suitable antiviral will soon be identified and developed.

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