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Cidofovir and brincidofovir reduce the pathology caused by systemic infection with human type 5 adenovirus in immunosuppressed Syrian hamsters, while ribavirin is largely ineffective in this model

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ABSTRACT

There are no drugs approved specifically to treat disseminated adenovirus (Ad) infections in humans. Cidofovir is active against Ad in cell culture, and it is used frequently in the clinic with disseminated infection in pediatric transplant patients; however, controlled clinical studies have not been conducted to prove the anti-Ad efficacy of cidofovir. Brincidofovir, a lipid-linked derivative of cidofovir, which has strong activity against Ad in cell culture and in animal models, is a promising new drug currently in clinical trials. Ribavirin, which has modest activity against some Ad types in cell culture, has been used in the clinic against disseminated Ad, but the efficacy of ribavirin is unknown. In the current study, we have examined the activity of cidofovir, brincidofovir, and ribavirin against disseminated Ad5 infection in the immunosuppressed Syrian hamster model. Hamsters are immunosuppressed by treatment with cyclophosphamide, then infected intravenously with Ad5, leading to disseminated Ad5 infection, especially in the liver. We found that cidofovir and brincidofovir have excellent activity against Ad5 pathology and replication in the liver, even when administered therapeutically starting at 3 days post-challenge with Ad5. Ribavirin did not have anti-Ad5 activity in our model. Our data support the use of cidofovir and brincidofovir in humans for the treatment of disseminated Ad infections in humans.

1. Introduction

Disseminated adenovirus (Ad) infections of immunocompromised humans can cause serious, often life-threatening disease (Wold and Ison, 2013). The situation is particularly dire in the case of pediatric hematopoietic stem cell transplant patients (Ison, 2006). At present, no drug is approved specifically to treat Ad infections. The most frequent treatment option is off-label use of cidofovir (CDV), an acyclic nucleoside phosphonate analog of deoxycytidine monophosphate that is approved to treat human cytomegalovirus (HCMV) retinitis, and which has good efficacy against many DNA viruses, including Ad, in cell culture (De Clercq, 2011). CDV appears to have anti-Ad activity in humans, especially pediatric hematopoietic stem cell transplant patients (Matthes-Martin et al., 2013); however, controlled clinical trials with CDV have not been completed. In the rabbit and cotton rat eye models, CDV has antiviral activity against human Ad infections when administered topically (Kaneko et al., 2004; Romanowski and Gordon, 2000), and against systemic mouse adenovirus type 1 infection of SCID mice (Lenaerts et al., 2005). Unfortunately, systemically administered CDV can cause severe nephrotoxicity (Izzedine et al., 2005), and therefore its prolonged use is to be avoided.

Another drug used occasionally to treat severe Ad infection is ribavirin, a guanosine analog that is approved for the treatment of hepatitis C virus and respiratory syncytial virus infections and is used off-label to treat other virus infections. While ribavirin was shown to have activity against certain Ad types in cell culture, especially Species C types (Morfin et al., 2009), as of now the efficacy of ribavirin to treat Ad infections in humans or animal models has not been established (Shah et al., 2012).

The need for effective and safe anti-Ad drugs prompted research on new compounds to battle the virus. With CDV, esterifying the compound with an alkoxyalkyl group improves the oral absorption in the small intestine and cellular uptake and prevents nephrotoxicity (reviewed by Hostetler, 2009). The lipid moiety is removed in cells by phospholipase C, leaving behind CDV which is charged and cannot exit cells easily. There are ongoing clinical trials with a

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promising lipid-linked derivative of CDV, brincidofovir (BCV, formerly named CMX001), which has proven activity against type 5 human Ad (Ad5) in vitro (Hartline et al., 2005), in animal models (Toth et al., 2008), and in the clinic (Florescu et al., 2012).

We have compared the anti-Ad5 activity of CDV, ribavirin, and BCV in an animal model. In order to model disseminated Ad infection in immunocompromised patients, we used Ad5 to intravenously (i.v.) challenge Syrian hamsters immunosuppressed with cyclophosphamide (CP). In this animal model, Ad5 infects most organs, most significantly the liver, and replicates to high titers (Lichtenstein et al., 2009; Ying et al., 2009). Immunocompetent hamsters clear the virus by approximately 7 days post challenge. However, with immunosuppressed animals, the virus replication is unchecked, and it causes organ damage. Relatively high doses of Ad5 are necessary to induce pathogenicity, because Kupffer cells, which are refractory to Ad replication, have very high affinity for Ad5, and take up the majority of intravenously injected virus. The pathology caused by Ad challenge is quantifiable, and it can be alleviated by treatment with BCV (Toth et al., 2008). To our knowledge, the immunosuppressed Syrian hamster is the only animal model currently used to evaluate the efficacy of antiviral drugs against systemic Ad infection. The clinical relevance of the findings obtained using the hamster model was confirmed by the successful use of BCV to treat human immunocompromised patients with disseminated Ad infections; BCV has entered a Phase 3 clinical trial for this purpose (trial ID: NCT02087306).

Here we report that CDV and BCV are efficacious against i.v. challenge with Ad5 in immunosuppressed Syrian hamsters. CDV and BCV inhibit Ad5 replication in the liver and reduce liver damage, morbidity, and mortality when administered either prophylactically or therapeutically. In contrast to these findings, ribavirin demonstrated no significant anti-Ad activity in our animal model.

Our study is the first to examine the activity of CDV and ribavirin against Ad in the immunosuppressed Syrian hamster model which is permissive for replication of Ad5. Further, to our knowledge, there have been no reported studies on the anti-Ad activity of CDV or ribavirin in any immunosuppressed, permissive animal model.

2. Materials and methods

2.1. Cells and viruses

HEK293 human embryonic kidney cells were purchased from Microbix (Mississauga, Ontario, Canada) and cultured in Dulbecco's modified Eagle's medium (Sigma–Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (FBS) at 37 °C. Ad5 *wt500*, a wild-type human Ad5, was isolated by our laboratory from an Ad5 stock purchased from the ATCC (Manassas, VA). The virus was purified by isopycnic gradient centrifugation, as described in (Tollefson et al., 2007). The titer of the virus was determined by plaque assay on A549 cells (ATCC).

2.2. Antiviral compounds

CDV was obtained from the National Institute of Allergy and Infectious Diseases (NIAID) and dissolved in phosphate buffered saline (PBS) at 3.7 mg/ml and 2 mg/ml for the 37 mg/kg and 20 mg/kg doses, respectively. Ribavirin was purchased from Spectrum Chemical Manufacturing Corp. (New Brunswick, NJ), and dissolved in PBS at 20, 15, 10, or 6 mg/ml, for the 200, 150, 100, and 60 mg/kg doses, respectively. BCV (hexadecyloxypropyl-cidofovir) was obtained from Chimerix, Inc. (Durham, NC), and was dissolved in PBS at 1 mg/ml.

2.3. Animals

Female Syrian hamsters (*Mesocricetus auratus*) were purchased from Harlan Laboratories (Indianapolis, IN) at approximately 100 g body weight. All studies were approved by the Institutional Animal Care and Use Committee of Saint Louis University and were conducted according to federal and institutional regulations.

2.4. Infection of hamsters with adenovirus; treatment with drugs

The hamsters were immunosuppressed using CP (Toth et al., 2008). CP was administered intraperitoneally (i.p.) at a dose of 140 mg/kg, and then twice weekly at a dose of 100 mg/kg for the duration of the study. CP used in this manner reduced nearly all leukocyte types more than 7-fold within a few days (Dhar et al., 2014; Thomas et al., 2008; Toth et al., 2008). Five to seven days after the first administration of CP, the animals were anesthetized with a ketamine/xylazine mixture, and Ad5 was injected i.v. (via the jugular vein) at a dose ranging from 1.2×10^{11} (ca. the LD_{50}) to 2×10^{11} (ca. the LD_{90}) plaque forming units (PFU)/kg body weight (Thomas et al., 2007). Control animals were injected with PBS.

CDV was injected i.p. at an initial dose of 37 mg/kg, and then continued three times a week at the single dose of 20 mg/kg. These doses correspond to 5 and 2.7 mg/kg Human Equivalent Doses (HED) (CDER, 2005). These doses and the dosing schedule are close to the doses and schedule recommended to treat disseminated Ad infections in human patients (Hatakeyama et al., 2003; Refaat et al., 2008), and these doses were found to be non-toxic in previous experiments (data not shown). Control animals received vehicle (PBS) injections.

BCV was administered through oral (p.o.) gavage at a dose of 2.5 mg/kg once daily throughout the study. This dose was proved to be non-toxic in previous studies (data not shown). Control animals were gavaged with vehicle (PBS).

Ribavirin was injected i.p. at doses of 30 or 100 mg/kg twice daily (b.i.d.) (Freiberg et al., 2010), starting a day before Ad5 challenge and continuing daily throughout the study, or given p.o. at doses of 50 or 75 mg/kg b.i.d., starting at 12 h before Ad5 challenge and continuing daily throughout the study. The 75 mg/kg b.i.d. p.o. dose was established as the maximum tolerated dose for ribavirin with CP-treated hamsters (data not shown). For both the i.v. and the p.o. experiments, control animals were administered with vehicle (PBS) via the appropriate route.

For all studies presented here, there were 15 animals in each treatment group. All hamsters were observed and weighed daily. Five hamsters of each group were sacrificed at 5 days post Ad5 challenge. At necropsy, blood and liver were collected. Infectious virus was extracted from the liver and virus burden was determined using a 50% Tissue Culture Infectious Dose ($TCID_{50}$) assay. Samples in which no infectious virus was detected (i.e. the level of virus load was under the detection limit of the assay, ca. 10^4 - $TCID_{50}$/g tissue) were marked "Nondetectable", while samples in which virus was detected but for which no quantitative assessment could be made (i.e. the virus load was at or barely above the detection limit) were labeled "Nonquantifiable". Serum was assayed for liver transaminase levels, namely alanine aminotransferase (ALT) and aspartate aminotransferase. The results obtained for these two enzymes were very similar, so for all studies only the ALT data are shown. The 10 hamsters remaining in each group were sacrificed at the conclusion of the study, or when they became moribund. The same data were collected from these animals as for the 5 day groups, with the addition of a survival study.