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Combination therapy with brincidofovir and valganciclovir against species C adenovirus infection in the immunosuppressed Syrian hamster model allows for substantial reduction of dose for both compounds

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ABSTRACT

Adenovirus infections of immunocompetent adults are usually mild and resolve without serious sequelae. However, adenovirus infections of immunocompromised patients often develop into life-threatening multi-organ disease. Pediatric hematopoietic transplant patients are especially threatened, with high incidence of infection and high mortality rates. Presently, there is no drug specifically approved by the FDA to treat adenovirus infections; thus there is an urgent need to develop effective antivirals against the virus. Previously, we demonstrated that brincidofovir and valganciclovir were efficacious against lethal intravenous challenge with human type 5 adenovirus in the Syrian hamster model. Here, we tested the *in vivo* efficacy of the combination of these two drugs and showed that the combination of brincidofovir and valganciclovir is more efficacious than either drug alone, thus potentially allowing decreased patient exposure to the drugs while maintaining antiviral efficacy. As antiviral compounds often have toxic side effects, a decrease in dose or duration of therapy allowed by the combination could also improve tolerability.

1. Introduction

While adenovirus (Ad) infections are usually self-limiting and cause only mild symptoms with healthy adults, disseminated Ad infections of immunocompromised humans can cause serious, often life-threatening disease (Wold and Ison, 2013). The most threatened population is pediatric hematopoietic stem cell transplant patients. A significant proportion of these patients develop disseminated Ad infections, with mortality approaching 100% (Echavarria, 2008; Ison, 2006; Ison and Hayden, 2016; Lenaerts et al., 2008; Lindemans et al., 2010; Lion, 2014; Matthes-Martin et al., 2013; Stercz et al., 2012; Wold and Ison, 2013). At the present there is no FDA-approved drug indicated for treatment of Ad infections.

To facilitate the testing of anti-adenoviral compounds, we developed an animal model based on the Syrian hamster. These animals, unlike mice and rats, are permissive for the replication of human Ads (Lichtenstein et al., 2009; Tollefson et al., 2017; Ying et al., 2009). When infected intravenously (i.v.) with a species C Ad, Syrian hamsters immunosuppressed with cyclophosphamide (CP) develop symptoms similar to disseminated Ad infection in immunocompromised patients. In this model, species C Ads infect various organs, most prominently the liver, replicate to high titers, and cause organ damage. This organ damage results in quantifiable pathology, which can be mitigated by treatment with antiviral drugs (Diaconu et al., 2010; Tollefson et al., 2014; Toth et al., 2008, 2015; Ying et al., 2014). Presently, the immunosuppressed Syrian hamster is the only available animal model to evaluate the efficacy of antiviral drugs against systemic Ad infection.

Currently, to treat Ad infection, clinicians most frequently use cidofovir (CDV), an acyclic nucleoside phosphonate analog of deoxycytidine monophosphate. CDV is approved to treat AIDS-related human cytomegalovirus (HCMV) retinitis, and has good efficacy against many DNA viruses, including Ad, in cell culture (De Clercq, 2011). According to case studies, CDV does appear to have anti-Ad activity in human patients (Matthes-Martin et al., 2013). Unfortunately, CDV can cause severe kidney toxicity (Izzedine et al.,

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2005). To counter the nephrotoxicity problem with CDV, brincidofovir (BCV), an alkoxyalkyl ester prodrug derivative of CDV with improved absorption in the small intestine was developed (Reviewed in Hostetler, (2009)). There are ongoing clinical trials with BCV, which has proven activity against human Ad *in vitro* (Hartline et al., 2005), in animal models (Tollefson et al., 2014; Toth et al., 2008), and in the clinic (Camargo et al., 2016; Florescu et al., 2012; Grimley et al., 2015b,a; Grimley et al., 2017; Hiwarkar et al., 2017; Voigt et al., 2016). However, preliminary data also indicate that BCV can cause significant gastrointestinal side effects when used for prolonged periods.

Ganciclovir (GCV), an analog of 2-deoxyguanosine, is another small molecule inhibitor that has been reported to have some clinical efficacy against Ad infections (Bruno et al., 2003). GCV, like CDV, is approved to treat HCMV retinitis, and it is used to combat HCMV infections in immunosuppressed transplant patients and AIDS patients. That GCV may be efficacious against Ad infections seems counterintuitive at first because it needs to be phosphorylated by a viral kinase to transform it to its active form, and Ads do not encode a viral kinase. However, we have demonstrated that both GCV and valganciclovir (VGCV), a valyl-ester prodrug of GCV, is able to inhibit the replication of Ad5 and mitigate the pathology caused by systemic Ad infection in the Syrian hamster model (Toth et al., 2015; Ying et al., 2014). VGCV is the drug of choice to prevent CMV infection after solid organ transplantation (Levitsky et al., 2008) because it has much better oral availability than GCV, reaching 60% (Sugawara et al., 2000). GCV does not appear to be phosphorylated in Ad5-infected cells; preliminary data suggest that GCV (and VGCV) may directly inhibit the Ad5 DNA polymerase (Toth et al., 2015; Ying et al., 2014). Unfortunately, one of GCV's (and VGCV's) side effects is myelosuppression, which may cause complications with already immunocompromised patients (Spivey et al., 2007).

As two of the drugs that may be used in the clinic to treat Ad infections have considerable side effects, this limits their utility in the target population. Thus, a reduction in the dose or duration of therapy required to fight the infection could improve tolerability of these compounds. To address this problem, we conducted a combination treatment experiment with BCV and VGCV in the Syrian hamster model, and demonstrated that the two drugs can be used at lower doses when combined.

2. Materials and methods

2.1. Cells and viruses

HEK293 human embryonic kidney cells were purchased from Microbix (Mississauga, Ontario, Canada), and A549 human lung adenocarcinoma cells were purchased from ATCC (Manassas, VA). Both cell lines were 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. The virus was purified and titered as described (Tollefson et al., 2007).

2.2. Antiviral compounds

BCV (hexadecyloxypropyl-cidofovir) was obtained from Chimerix, Inc. (Durham, NC), and was dissolved in PBS at 1 or 0.1 mg/ml for the different doses. VGCV (batch 20130625) was purchased from 2A Pharmachem (Lisle, IL), and dissolved in water at 10 mg/ml.

2.3. In vitro assay

A549 cells were plated into 96-well plates, incubated overnight, and were infected with Ad5 at 10 plaque forming units (PFU) per cell. The BCV and VGCV dilution series were done on separate dilution plates; the drugs were then combined into a new dilution plate prior to addition to the infection plates. Drugs were added to the infection plate wells at 90 min post-infection. The final concentrations of BCV were 233, 280, 335, 402, 482, 579, 694, 833, and 1000 nM (1.2-fold serial dilution), and the final concentrations of VGCV were 44, 66, 99, 148, 222, 333, and 500 μM (1.5-fold serial dilution). Each plate included the following controls: uninfected cells (9 wells), Ad5 infection with no drug (9 wells) and controls for each drug alone. The infected cells were incubated at 37 °C for 5 days, after which the cytopathic effect was quantified as described in Toth et al. (2015).

2.4. Animals

Female Syrian hamsters (*Mesocricetus auratus*) were purchased from Envigo (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.

2.5. Infection of hamsters with adenovirus; treatment with drugs

The hamsters were immunosuppressed by intraperitoneal (i.p.) administration of CP (Sigma, St. Louis, MO) at a starting dose of 140 mg/kg followed by twice weekly injections at a dose of 100 mg/kg for the duration of the study (Dhar et al., 2014; Thomas et al., 2008; Toth et al., 2008). Seven days after the first injection of CP, ketamine/xylazine anesthetized animals were injected i.v. (via the jugular vein) with Ad5 at a dose of 2×10^{11} PFU/kg body weight. Control animals were injected with PBS.

BCV and VGCV were administered through oral (p.o.) gavage at the doses indicated for each experiment. For both drugs, treatment started one day before challenge, and continued throughout the study according to the schedule indicated. Control animals were gavaged with vehicle (PBS).

The animals were randomized into groups of 15 by weight before virus challenge. After challenge, all hamsters were observed and weighed daily. Five previously designated hamsters of each group were sacrificed at 5 days post challenge, and serum and liver samples were collected. Virus burden in the liver was determined using a 50% Tissue Culture Infectious Dose ($TCID_{50}$) assay (Toth et al., 2008). 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 “Not detectable (ND)”. Serum was assayed for alanine aminotransferase (ALT) levels. The 10 hamsters remaining in each group were sacrificed at the conclusion of the study, or when they became moribund. Body weight gain/loss and survival data were collected from these animals. In these experiments, we deliberately opted to use a sublethal challenge dose. We believe that the incremental changes observed with body weight loss, serum transaminase levels, and virus burden allowed us to better document subtle differences in the efficacies of drugs.

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.03 (GraphPad Software). Column mean comparisons by two-way ANOVA were used to compare body weight changes. For serum transaminase levels and virus burden in the liver, the Kruskall-Wallis test was used to calculate the overall effect, and group-wise comparison was performed using two-tailed Mann-Whitney *U* test. $P \leq 0.05$ was considered significant.