

The In Vitro and In Vivo Evaluation of ddC as a Topical Antiviral for Ocular Adenovirus Infections

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PURPOSE. To evaluate the antiviral activity of 2', 3'-dideoxycytidine (ddC) in vitro against a panel of ocular adenovirus serotypes and in vivo in the ocular Ad5/NZW rabbit replication model.

METHODS. In vitro, the 50% inhibitory concentrations (IC₅₀) of ddC and cidofovir were determined using standard plaque-reduction assays. In vivo, 40 rabbits were topically inoculated in both eyes with Ad5 after corneal scarification. On day 1, the rabbits were equally divided into four topical treatment groups: 3% ddC; 2% ddC; 0.5% cidofovir; and saline. ddC and saline eyes were treated four times daily for 7 days, and cidofovir-treated eyes were treated twice daily for 7 days. Eyes were cultured for virus a multiple times over 2 weeks.

RESULTS. The in vitro IC₅₀ for ddC ranged from 0.18 to 1.85 µg/mL, whereas those for cidofovir ranged from 0.018 to 5.47 µg/mL. ddC was more potent than cidofovir for seven of nine serotypes. In vivo, 3% ddC, 2% ddC, and 0.5% cidofovir significantly reduced the number of Ad5-positive cultures per total (days 1-14), mean Ad5 ocular titer (days 1-5), and duration of shedding (among other outcome measures) compared with the saline control. The 3% and 2% ddC treatments were significantly more efficacious than the 0.5% cidofovir treatment in the parameters listed above.

CONCLUSIONS. ddC demonstrated potent antiadenovirus activity in vitro and in vivo. Systemic safety studies after topical ocular administration are needed to evaluate ddC as a topical antiviral treatment for adenoviral ocular infections in the target population. (*Invest Ophthalmol Vis Sci.* 2009;50:5295-5299) DOI:10.1167/iovs.08-3286

Adenovirus (Ad) is the etiologic agent of the most commonly occurring ocular viral infections worldwide.¹ These ocular infections, in the forms of epidemic keratoconjunctivitis (EKC), follicular conjunctivitis (FC), and pharyngeal conjunctival fever (PCF), produce significant patient morbidity that results in lost time from school and work, as well as possible vision-altering infiltrates in the cornea (EKC).¹ At present, there is no FDA-approved antiviral treatment for these infections. Cidofovir, a nucleoside analogue antiviral that inhibits adenovirus DNA polymerase, was successfully tested in pre-clinical studies²⁻⁵ and in phase 1 and 2 clinical trials in the

United States for the treatment of adenoviral ocular infections.⁶ However, toxicity (epiphora due to secondary lacrimal canalicular blockade) and marketing issues led to the discontinuation of the development of topical cidofovir in the United States.⁷ While several experimental in vitro and in vivo studies have been reported recently with promising antiviral agents, none has demonstrated greater in vivo potency than the gold standard, cidofovir.^{8,9} An antiviral that is more potent and less toxic than cidofovir would be a welcome addition to ophthalmic treatments.

2', 3'-Dideoxycytidine (ddC) is a new candidate antiviral for the treatment of adenoviral ocular infections. It was approved by the U.S. Food and Drug Administration for the treatment of HIV (zalcitabine [Hivid]; Roche Laboratories, Indianapolis, IN), but Roche discontinued production and distribution of ddC as of December 31, 2006. Similar to cidofovir, ddC is a nucleoside analogue of cytosine (Fig. 1). It has been shown to inhibit viral replication after it is phosphorylated to form ddCTP, which competes with dCTP for incorporation into viral DNA. Previous reports showed that ddC demonstrates both in vitro and in vivo antiviral inhibitory activity against adenovirus.¹⁰⁻¹² Recently, Eiichi et al.¹³ showed that ddC possesses in vitro antiviral activity against several ocular serotypes of adenovirus. This finding led us to the present study for which the goals were to determine the in vitro potency of ddC in comparison with cidofovir against a panel of ocular adenovirus serotypes as well as adenovirus serotypes that will replicate in the rabbit ocular model and determine the in vivo efficacy of topical ddC, compared with cidofovir in the ocular Ad5/NZW rabbit replication model.³ These studies will determine whether ddC warrants further development as an antiviral agent for the treatment of adenoviral ocular infections.

METHODS

Viruses and Cells

For the in vitro antiviral testing, clinical adenovirus isolates of serotypes 1, 2, 3, 4, 5, 7a, 8, and 19 were collected anonymously from patients presenting with typical adenovirus ocular disease at the Charles T. Campbell Ophthalmic Microbiology Laboratory at the UPMC Eye Center over a 15-year period beginning in 1989 and were managed in accordance with the guideline for privacy of human donors in the Declaration of Helsinki. The isolates were retrieved from a frozen -70°C retrospective clinical collection that was deidentified and stored for diagnostic test validations. The serotypes of the isolates were determined using serum neutralization. No clinical isolates of Ad37 were identified, therefore the ATCC (American Type Culture Collection, Manassas, VA) reference strain of Ad37 was used. The clinical isolates along with the ATCC Ad37 reference strain were grown in A549 cell monolayers and stocks were prepared, aliquoted, and frozen at -70°C. The serotypes tested represent the most common adenovirus serotypes that cause ocular infections (Ad8, Ad19, and Ad37 [EKC]; Ad3, Ad4, and Ad7a [FC]) and serotypes that can replicate in the rabbit ocular model (Ad1, Ad2, and Ad5).

The same clinical isolate of Ad5 used for the in vitro antiviral testing was also used for the in vivo antiviral evaluation in the ocular Ad5/NZW rabbit replication model.³ A549 human lung carcinoma cells (CCL-185; ATCC) were grown and maintained in Eagle's MEM supple-

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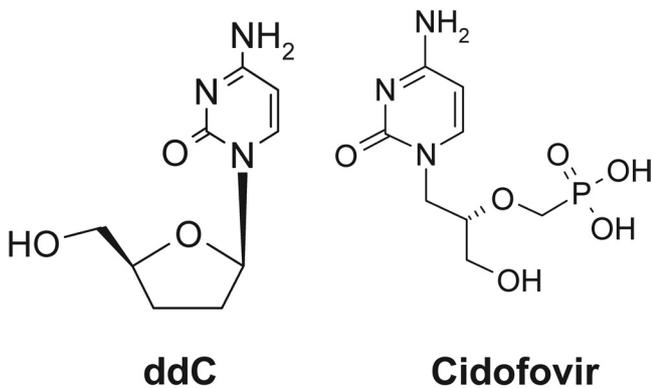


FIGURE 1. Chemical structures of ddC and cidofovir. Both are nucleoside analogues of cytosine.

mented with 10% fetal bovine serum (Sigma Cell Culture Reagents, St. Louis, MO).

Antivirals

ddC was purchased from the Sigma Chemical Co. ddC was dissolved in sterile water, then filter sterilized for use in both the in vitro and in vivo studies. Preliminary experiments in the ocular Ad5/NZW rabbit replication model demonstrated that 1% ddC provided limited viral inhibition (data not shown). Therefore, in the present study, we tested higher concentrations of ddC (2% and 3%) to determine whether there would be a dose-response inhibitory effect relative to the positive (cidofovir) and negative (saline) controls. Gilead Sciences (Foster City, CA) kindly provided cidofovir powder that was used in the in vitro studies. For the in vivo studies, 0.5% cidofovir was prepared in IV saline from the 7.5% injectable form of cidofovir (Vistide; Gilead Sciences) and was purchased from the University of Pittsburgh Medical Center pharmacy. IV saline (Baxter, Deerfield, IL) served as control drops.

Animals

Two to 3-1b female New Zealand White (NZW) rabbits were purchased from Myrtle's Rabbitry (Thompson Station, TN). All animal studies conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. University of Pittsburgh Institutional Animal Care and Use Committee (IACUC) approval was obtained and institutional and federal guidelines regarding animal experimentation were followed.

In Vitro Cytotoxicity Assay

A fluorometric viability stain (AlamarBlue, product number DAL1025; Invitrogen, Carlsbad, CA) was used to compare the in vitro cytotoxicity of ddC with cidofovir. The stain acts as a redox indicator that is reduced to a fluorescent form by metabolically active living cells. Cytotoxicity is determined by the percentage of residual viable cells after exposure to the test drugs at different concentrations. ddC and cidofovir were tested at concentrations of 30,000 (3%), 20,000 (2%), 10,000 (1%), 5,000 (0.5%), and 1,000 (0.1%) $\mu\text{g}/\text{mL}$ on A549 epithelial monolayers grown in 96-well plates. In each experiment, all concentrations were tested in triplicate. A detergent solution (G182A 26704601 Lysis Solution 10 \times ; Promega, Madison, WI) served as a positive cytotoxicity control. The test drugs were incubated on the A549 monolayers for 24 hours at 37°C with 5% CO_2 in a total volume of 100 μL . A 20- μL aliquot of the fluorometric stain was then added, and the cells were incubated for three additional hours at 37°C with 5% CO_2 . Fluorescence was then read with a plate reader (Biotek Synergy 2; Biotek), with a 500/27-nm excitation filter and a 620/40-nm emission filter, at a sensitivity of 35. The results were obtained by calculating the mean percentage of cytotoxicity of each of the triplicate samples for each drug at each concentration. The experiment was repeated a second time to confirm the results. The results were graphed as percentage of toxicity versus drug concentration for ddC and cidofovir

(Fig. 2). The observed differences were evaluated statistically using the paired *t*-test and significance was established at the $P \leq 0.05$ confidence level.

In Vitro Antiviral Testing: IC₅₀ Determinations

Plaque reduction assays were performed in duplicate using 24-well multiplates containing A549 monolayers. One plate per virus isolate per drug was used. The 24-well multiplates were inoculated with ~ 100 plaque-forming units (PFU) of Ad per well. After a 3-hour adsorption period, the inocula were removed from all wells. One milliliter of overlay medium containing 100, 10, 1.0, 0.1, 0.01 or 0.001 $\mu\text{g}/\text{mL}$ of test drug was added to three wells each. To the remaining six wells, 1 mL of overlay medium without test drug was added. The plates were incubated at 37°C in 5% CO_2 until plaque formation was visible. At that time, the cells were stained with 0.5% gentian violet, and the number of plaques per well counted. The IC₅₀ (concentration that inhibits plaque formation by 50%) for each virus isolate and drug were determined graphically from a plot of the average number of plaques versus drug concentration. The mean IC₅₀ of the duplicate trials was then determined.

Generally, antiviral agents with an IC₅₀ less than 10 $\mu\text{g}/\text{mL}$ are considered to be potent antivirals. Antivirals demonstrating an IC₅₀ between 10 and 50 $\mu\text{g}/\text{mL}$ are considered to be moderately potent, whereas agents that have an IC₅₀ between 50 and 100 $\mu\text{g}/\text{mL}$ are considered minimally potent. An IC₅₀ of greater than 100 $\mu\text{g}/\text{mL}$ signifies little or no antiviral activity.

Rabbit Model for In Vivo Antiviral Testing

Since the original rabbit model first reported in 1992,¹⁴ we have developed two rabbit models currently used for drug testing based on the drug class. The original adenovirus rabbit model evaluated clinical outcome parameters after intrastromal injection of virus. However, by 1994, we reported the major limitations of this model were (1) intrastromal virus injection proved to be a confounding variable that interfered with the evaluation of clinical signs as a response for antiviral efficacy, and (2) the duration of viral shedding was limited.³ In its place, we developed an ocular Ad5/NZW rabbit replication model based on topical inoculation after corneal scarification. This newer model proved to be superior to the original model for antiviral testing because it optimized viral replication for a longer time course of the infection. Reduction in viral titers proved to be an excellent outcome parameter for antiviral evaluation. However, clinical signs were also inconstant in this model and regrettably, they could not be reliably used to evaluate

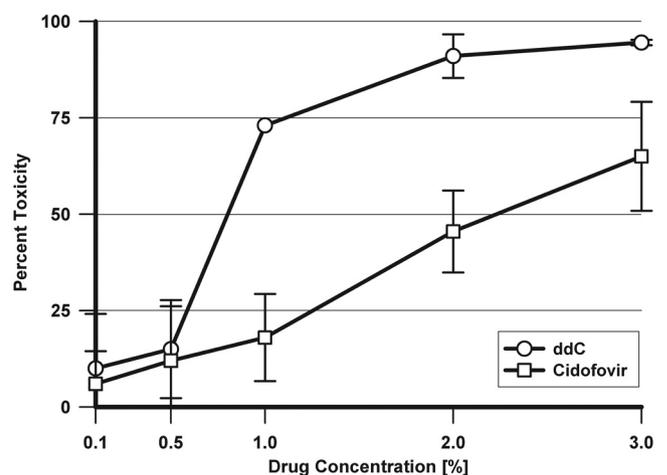


FIGURE 2. Determination of in vitro cytotoxic effects of ddC and cidofovir on A549 cells. A fluorometric viability stain was used to compare the in vitro cytotoxicity of ddC with that of cidofovir. In general, ddC appeared to be more cytotoxic than cidofovir at 1%, 2%, and 3%. The observed differences were statistically significant at 2% concentration (paired *t*-test, $P = 0.049$) based on two independent experiments.

TABLE 1. IC₅₀ of ddC and Cidofovir against Ocular Adenovirus Isolates

Antiviral	Ad1	Ad2	Ad3	Ad4	Ad5	Ad7a	Ad8	Ad19	Ad37
ddC	0.32	0.32	0.32	0.51	1.85	0.18	0.33	0.32	1.01
Cidofovir	2.25	2.55	0.18	1.33	3.10	5.47	0.018	4.30	1.15

Data are the mean IC₅₀ expressed in micrograms per milliliter.

antiviral efficacy.³ This improved replication model has been used for all antiviral studies since 1994,^{4,5,7-9} and was used in the present study.

A second rabbit ocular model, the subepithelial infiltrate (SEI) model, is immune-based after intrastromal viral inoculation. It was developed to test the effect of other classes of drugs (not antivirals) on adenoviral pathogenesis (e.g., subepithelial immune infiltrate formation). The drug classes tested in this model included: anti-inflammatory and immunosuppressive drugs (corticosteroids,¹⁵ NSAIDs¹⁶) and cytotoxic (cyclosporine) drugs.¹⁷

Using the Ad5/NZW rabbit replication model, the present study was performed in duplicate using a total of 40 rabbits (20 per trial). After systemic and topical anesthesia, the rabbits were topically inoculated with 50 μ L (1.5×10^6 pfu/eye) of Ad5 in both eyes after corneal epithelial scarification (12 cross-hatched strokes of a 25 sterile needle).^{3,8,9} Inoculation of both eyes of the rabbits allowed us to reduce the number of animals needed without jeopardizing statistical validity in accordance with the Animal Welfare Act Policy 12 (Consideration of Alternatives to Painful/Distressful Procedures, June 21, 2000). Twenty-four hours later, rabbits were randomly assigned to one of four topical treatment groups: (I) 3% ddC ($n = 10$), (II) 2% ddC ($n = 10$), (III) 0.5% cidofovir ($n = 10$), and (IV) control (saline) ($n = 10$). ddC and control rabbits were treated in both eyes four times daily for 7 days, whereas cidofovir rabbits were treated in both eyes twice daily for 7 days. All topical solutions (37 μ L drops) were instilled with an electronic pipette (EDP; Rainin, Oakland, CA) set in the multidispense mode. Ocular swabbing to recover adenovirus from the tear film and corneal and conjunctival surfaces, after topical anesthesia with proparacaine, was performed on days 0, 1, 3, 4, 5, 7, 9, 11, and 14 after inoculation. The swabs from each eye were placed individually into tubes containing 1 mL of medium and were frozen at -70°C pending viral plaque assay.

Determination of Viral Titers Using the Viral Plaque Assay

The samples to be assayed were diluted 1:10 for several dilutions. One-tenth milliliter of both the undiluted sample and the dilutions were inoculated onto duplicate wells of a 24 well multiplate containing A549 monolayers. The virus was adsorbed for 3 hours at 37°C in a 5% CO₂-water vapor atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 mL of medium plus 0.5% methylcellulose was added to each well, and the plates were incubated at 37°C in a 5% CO₂-water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.5% gentian violet, and the number of plaques counted. The viral titers were then calculated, and expressed as plaque-forming units per milliliter (PFU/mL).

Statistical Analysis of In Vivo Studies

After the completion of both trials from each study, the data from each trial was analyzed (Minitab software; Minitab Inc., State College, PA) statistical software. As comparable results were obtained in both trials of each study, the data were then pooled to obtain a larger subject number and analyzed using analysis of variance (ANOVA) with Fisher's pair-wise comparisons and χ^2 analyses (Minitab). Significance was established at the $P \leq 0.05$ confidence level.

RESULTS

In Vitro Cytotoxicity Assay

In general, ddC appeared to be more cytotoxic than cidofovir at 1%, 2%, and 3% (Fig. 2). The observed differences were

statistically significant at 2% concentration (paired *t*-test, $P = 0.049$) based on results in two independent experiments.

In Vitro Antiviral Testing: IC₅₀ Determinations

The mean IC₅₀s are presented in Table 1. The IC₅₀s for cidofovir ranged from 0.018 μ g/mL for Ad8 to 5.47 μ g/mL for Ad7a, with most of the isolates demonstrating an IC₅₀ of ~ 2 μ g/mL. Cidofovir demonstrated a 3030-fold difference in potency across the range of serotypes. ddC produced a lower IC₅₀ (range, 0.18–1.85 μ g/mL) than did cidofovir for the isolates tested, except for Ad3 and Ad8, and exhibited a narrower range of IC₅₀s across the serotypes tested (10.3-fold difference among serotypes) compared with cidofovir. These IC₅₀s suggest that both cidofovir and ddC are potent inhibitors of a range of adenovirus serotypes in vitro.

In Vivo Antiviral Testing

The results of the in vivo antiviral testing in the rabbit replication model are presented in Table 2 and Figure 3. ddC (3% and 2%), along with 0.5% cidofovir, demonstrated significant decreases in the number of Ad5-positive cultures per total over the entire course of the study (days 1–14; Table 2) compared with the saline control (χ^2). When these data were broken down into the early (days 1–5) and late (days 7–14) phases of infection (Table 2), 3% or 2% ddC and 0.5% cidofovir demonstrated fewer Ad5-positive cultures per total compared with the control during both the early and late phases of infection. Furthermore, 3% ddC and 2% ddC significantly reduced the number of Ad5-positive cultures per total, overall and during the early phase of infection compared with 0.5% cidofovir. The potent antiviral activity of ddC and cidofovir during both the early and late phases of infection is reinforced using the second outcome measure of mean Ad5 titer days 1 to 5 and 7 to 14 (Table 2). Both ddC concentrations and 0.5% cidofovir demonstrated significant decreases in titers compared with the saline control in both phases of the infection (ANOVA). In addition 3% and 2% ddC significantly reduced titers compared with 0.5% cidofovir in the early phase of infection (days 1–5). The daily mean Ad5 titers for all treatment groups are presented graphically in Figure 3. 3% ddC and 2% ddC demonstrated significantly lower mean ocular titers on days 3, 4, 5, and 7 compared with the saline control group, whereas 0.5% cidofovir demonstrated significantly lower mean ocular titers only on days 5 and 7 compared to the control group (ANOVA). In addition, 3% ddC and 2% ddC demonstrated significantly lower mean ocular titers on day 3 than did 0.5% cidofovir. There were no differences between 3% ddC and 2% ddC on any of the days. The potent antiviral activity of 3% ddC, 2% ddC, and 0.5% cidofovir also resulted in significant reductions in the duration of the infections. The mean duration of shedding (mean of the last day on which a positive culture was obtained) is also presented in Table 1. ddC (3% and 2%), and 0.5% cidofovir significantly reduced the duration of shedding compared with the control and 3% ddC and 2% ddC were more effective than was 0.5% cidofovir (ANOVA). Overall, the efficacy of 3% and 2% ddC was similar except for the number of Ad5-positive cultures per total during the late phase of infection for which 3% ddC was more effective than 2% ddC (χ^2).

TABLE 2. The Effects of ddC and Cidofovir on Adenovirus Replication

	3% ddC	2% ddC	0.5% Cidofovir	Control
Ad5-positive eye cultures/total				
Overall (days 1-14)	17/160 (11%)*†	17/152 (11%)*†	51/160 (32%)*	82/160 (51%)
Early phase (days 1-5)	17/80 (21%)*†	13/76 (17%)*†	48/80 (60%)*	64/80 (80%)
Late phase (days 7-14)	0/80 (0%)*‡	4/76 (5%)*	3/80 (4%)*	19/80 (24%)
Mean Ad5 ocular titers (pfu/mL)				
Early phase (days 1-5)	8.1 ± 31.0 × 10 ⁰ *†	1.7 ± 8.0 × 10 ¹ *†	7.4 ± 19.1 × 10 ¹ *	1.0 ± 2.2 × 10 ²
mean ± SD (<i>n</i> = 80§)				
(power ≥ 0.9769)				
Late phase (days 7-14)	0.1 ± 0.6 × 10 ⁰ *	0.2 ± 1.0 × 10 ⁰ *	1.4 ± 9.3 × 10 ⁰ *	2.5 ± 12.1 × 10 ¹
mean ± SD (<i>n</i> = 80§)				
(power ≥ 0.7184)				
Duration of Ad5 shedding (days)	1.7 ± 2.2*†	2.2 ± 3.0*†	4.2 ± 1.7*	6.9 ± 2.50
mean ± SD (<i>n</i> = 20)				
(power = 1.0000)				

* $P \leq 0.023$ when compared to the control group.

† $P \leq 0.001$ when compared to the 0.5% cidofovir group.

‡ $P \leq 0.05$ when compared to the 2% ddC group.

§ *n* = 76 for the 2% ddC group.

|| *n* = 19 for the 2% ddC group.

DISCUSSION

Currently, there is no clinically proven antiviral drug available for the treatment of adenoviral ocular infections worldwide. A recent review summarized candidate antivirals for topical administration currently under development.¹⁸ These drugs of interest include *N*-chlorotaurine (weak halogen-based oxidant),^{8,19,20} doxorvir (CTC-96, a cobalt-chelating agent),^{21,22} ionic contraviral therapy (a combination of digoxin and furosemide),²³ Iv-IgG (human immunoglobulin G),⁹ and cyclopentenylcytosine (CPE-C, a nucleoside analogue).^{24,25}

In a previous publication, we suggested three criteria that antiviral agents should meet to be considered for clinical development.⁸ First, the antiviral must possess in vitro antiviral activity against adenovirus serotypes (Ad3, Ad4, Ad7a, Ad8, Ad19, and Ad37) that commonly infect the eye and its antiviral activity against species C serotypes (Ad1, Ad2, and Ad5) that

replicate in the rabbit model must be comparable to species D serotypes (Ad8, Ad19, and Ad37) which do not replicate in the rabbit model.⁸ Second, the antiviral must possess potent antiviral activity in the Ad5/NZW rabbit replication model, which serves as a surrogate for clinical infections.⁸ Results from cidofovir in this model²⁻⁵ have proven to be predictive of those seen in the phase II clinical trial.⁶ Third, the antiviral must be safe to treat everyone, including children and uninfected, at-risk individuals (prophylaxis indication).⁸

Recently, we determined that two topical antiviral agents meet the criteria: *N*-chlorotaurine (NCT)⁸ and intravenous immunoglobulin (Iv-IgG).⁹ Both of these agents proved to be comparable in antiviral efficacy to cidofovir in our in vivo experimental evaluations.^{8,9} Although comparable in vivo efficacy with cidofovir is desirable, superior efficacy is always the goal.

We achieved that goal in the present study with topical ddC. 3% and 2% ddC administered four times daily for 7 days. At these concentrations, ddC was significantly more potent than topical 0.5% cidofovir administered twice daily for 7 days. This topical antiviral agent is the first that, in our hands, has demonstrated significantly greater efficacy than cidofovir in the rabbit replication model. One reason for the increased efficacy of ddC over cidofovir could be the relative potency that the compounds demonstrated in vitro. ddC was more potent than cidofovir for seven of the nine adenovirus serotypes tested, including the Ad5 isolate used in both the in vitro and in vivo studies. ddC was more than 1.67-fold more potent in vitro than cidofovir against Ad5. Another reason for the increased efficacy of ddC may be that greater corneal concentrations of ddC were achieved compared with cidofovir because of the dosage regimen and concentrations of topical ddC used. In a preliminary study, we previously demonstrated limited in vivo antiviral inhibition with 1% ddC (data not shown). In this study, we chose to test ddC at higher concentrations (2% and 3%) to evaluate the dose response. The cidofovir dosage regimen (0.5% twice daily for 7 days) had previously been optimized in a series of experiments (Gordon YJ, Romanowski EG, unpublished studies, 1990-1998) which was then used in the successful phase II U.S. clinical trial.⁶

The present study achieved two of the three criteria that we proposed to qualify for further development of a candidate antiviral against adenovirus (in vitro efficacy against a panel of serotypes and efficacy in the ocular Ad5/NZW rabbit replication model). The third criterion, safety, was not completely evaluated in this study. Our in vitro toxicity studies demonstrated that ddC

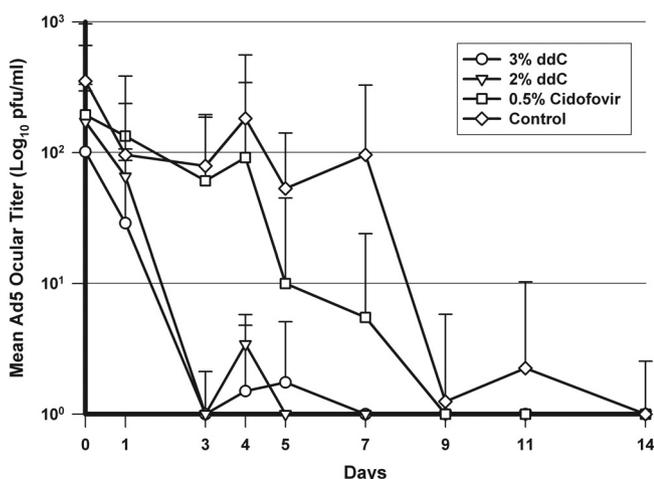


FIGURE 3. The effects of ddC and cidofovir on in vivo adenovirus replication. ddC (3% and 2%) demonstrated significantly lower mean ocular titers on days 3, 4, 5, and 7 compared with the PBS control group (ANOVA). Cidofovir (0.5%) demonstrated significantly lower mean ocular titers on days 5 and 7 compared with the control group (ANOVA). In addition, 3% ddC and 2% ddC demonstrated significantly lower mean ocular titers on day 3 than did 0.5% cidofovir (ANOVA). There were no differences between 3% ddC and 2% ddC on any day.

did appear to be more cytotoxic than cidofovir at concentrations of 1%, 2%, and 3% (Fig. 2). Despite these observations in vitro, the topical treatment dose regimens used in vivo for each drug appeared to be acceptable from a safety point of view in our rabbit ocular model of adenovirus infection.

The clinical data on safety (local ocular toxicity) of candidate antiviral drugs has been historically evaluated in uninfected rabbit eyes,³ but clinical safety may be more predictable when evaluated in virus-infected eyes. For example, we have tested antimicrobial peptides and other novel classes of antivirals that demonstrated no toxicity in uninfected rabbit eyes but were severely toxic after topical administration in virus-infected rabbit eyes.

In the present study, we report the absence of any local ocular toxicity (discharge; increased tearing; conjunctival erythema; and chemosis, corneal edema, or iritis) in the highest dose administered (3% ddC 4×/d × 7 days) to adenovirus-infected eyes. Furthermore, there were no behavioral signs of local toxicity or irritation (eye rubbing, vocalization, and avoidance behavior) during or after topical instillation at the 3% ddC dose regimen.

Systemic safety after topical ocular administration of ddC remains the most important issue to be formally addressed if commercial development is to occur. However, in the present study, there were no obvious signs of systemic toxicity (decreased appetite, weight loss, wasting, and death) after topical administration.

The use of systemic ddC to treat sick, debilitated patients with AIDS in the early 1990s was associated with very serious side effects and the drug required a "black box warning" in the *Physicians Desk Reference* (PDR). These side effects included severe peripheral neuropathy (22%–35% of patients in clinical studies²⁶), and reported fatal cases of pancreatitis, lactic acidosis and severe hepatomegaly with steatosis, and hepatic failure.²⁷ The challenge for pharmaceutical development will be to establish the safety of topical use in healthy children and adults. The margin of safety will have to be very high and convincing to obtain governmental approval for treatment of an ocular disease that is both self-limiting and nonblinding, although a recognized cause of patient morbidity and public health concerns.

In the present study, we report that ddC is the most potent inhibitor of adenovirus in vitro and in the rabbit replication model that we have tested to date. It is the first antiviral to demonstrate greater efficacy than our gold standard, cidofovir, in the rabbit model. Further development of ddC as a topical treatment for adenoviral ocular infections will require a major commitment to demonstrate systemic safety after topical administration in the proposed treatment population.

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