Efficacy of Topical Immunoglobulins against Experimental Adenoviral Ocular Infection

Edward C. Nwanegbo,[1] *Eric G. Romanowski,*[2] *Y. Jerold Gordon,*[2] *and Andrea Gambotto*[1]

Purpose. Presently, there is no U.S. Federal Drug Administration (FDA)–approved antiviral therapy for the treatment of adenoviral (Ad) ocular infections. The goal of the present study was to determine the antiviral efficacy of human immunoglobulin (Ig), a preparation of highly purified and concentrated immunoglobulin (IgG) antibodies isolated from a large pool of human plasma donors, in vitro and on acute Ad replication in the Ad5 New Zealand White (NZW) rabbit ocular model.

Methods. The antiviral activity of human Ig against multiple wild-type and human ocular isolates of adenovirus serotypes was investigated in vitro by using neutralizing assays in different human epithelial cell lines. In vivo bilateral topical ocular toxicity and antiviral efficacy were evaluated with established Ad5/NZW rabbit ocular models. In vivo Ig antiviral results were compared with those obtained with topical 0.5% cidofovir and saline.

Results. In three different epithelial cell lines, ≤6.25 mg/mL of the Ig neutralized several wild-type adenoviral serotypes that cause ocular infections. A dose of ≤10 mg/mL neutralized 88% of ocular isolates of the adenovirus serotypes. After treatment of infected animals, adenovirus-positive cultures per total cultures (days 1–14; $P = 0.021$), the duration of Ad5 shedding, ($P = 0.008$), and the mean combined ocular viral titer during the early (days 1–5; $P = 0.0001$) and the late (days 7–14; $P = 0.013$) phases of infection were significantly lower in Ig-treated animals than in saline-treated animals and were similar to those in cidofovir-treated animals.

Conclusions. Ig demonstrated antiviral properties against multiple adenoviral serotypes in vitro and in the Ad5/NZW rabbit ocular model. Further studies are needed to advance topical immunoglobulin for treatment and prophylaxis of ocular infections. (*Invest Ophthalmol Vis Sci.* 2007;48:4171–4176) DOI:10.1167/iovs.07-0491

Adenovirus serotypes are the most common etiologic agents of external ocular viral infection in many parts of the world.[1] Although follicular conjunctivitis is the most common, epidemic keratoconjunctivitis (EKC) is the most serious adenoviral ocular disease responsible for global and community epidemics.[1] Although many adenovirus serotypes have been implicated, the most common serotypes involved are types 3, 8, 19, and 37.[1,2] The disease is very contagious and can cause community and medical facility epidemics resulting in significant patient morbidity, societal losses from worker and student absenteeism,[3] and increased direct medical costs.[4] Acute adenoviral conjunctivitis accounts for 1% of consultation in primary care.[5,6] One in eight children experiences an attack of the disease every year.[5] EKC has a variable course, but the appearance of immune-based subepithelial infiltrates (SEIs) can impair visual acuity for months in some patients.[7,8] In previous studies in a rabbit model of adenovirus ocular infection, Trousdale et al.[9] found that adenoviral ocular infection stimulates infiltration of corneal epithelial cells by $CD4^+$, $CD8^+$, $CD18^+$, MHC class I, and MHC class II cells and also demonstrated the presence of serum-neutralizing antibody to the virus used in infecting the animals.[9] Although this has not been reported in human studies, similar features may be responsible for the development of SEI in human ocular infection.

As there is no currently effective antiviral treatment, nonsteroidal anti-inflammatory eye drops, steroid eye drops, and artificial tears have been used as supportive therapy. In many centers, the prescription of topical antibiotics to prevent secondary bacterial infections is a common practice. However, their benefits have not been fully established.[10,11] Antiviral drugs such as trifluorothymidine have not been effective,[12,13] whereas topical cidofovir was effective in preclinical and clinical trials, but toxicity and marketing problems led to the discontinuation of its development for human ocular use.[14,15] Studies are on-going on other investigational products such as *N*-chlorotaurine,[16] cobalt chelate CTC-96,[17] and povidone iodine.[18,19]

Gammagard S/D IgIV is a human intravenous immunoglobulin (IV-Ig) preparation that contains all classes of IgG, along with trace amounts of IgA and IgM. It is prepared from the pooled plasma of thousands of blood donors and contains antibodies against bacteria, viruses, proteins, glycosides, and numerous self-antigens.[20] IV-Ig preparations are currently used as a replacement therapy in both primary and secondary hypogammaglobulinemia, to protect patients from recurrent infection.[21,22] In combination with antibiotics, it reduced the mortality rate in neonates,[23] improved outcomes in premature infants with group B streptococcal infections,[24,25] and prevented infection in susceptible preterm infants.[26] There were also reported applications in the treatment of HIV[27] and Dengue fever.[28] Because of its immune modulating and anti-inflammatory properties that depend on its interaction with complement system, cytokines, and endothelial cells,[29] it has also been used in autoimmune diseases such as idiopathic thrombocytopenic purpura (ITP), Kawasaki disease, systemic vasculitis,[30–32] and Guillain-Barré syndrome.[33]

Previously Goosens et al.[34] demonstrated that anti-Ad5 IgG, present in synovial fluid, prevents Ad5-based gene transfer to synovial fluid. These antibodies have been shown to target the adenoviral capsid proteins[35] and prevent cell infection by the virus. Use of topical immunoglobulin (Ig) may therefore bind the viral capsid proteins and inhibit reinfection of conjunctival

From the [1]Department of Surgery, and the [2]The Charles T. Campbell Ophthalmic Microbiology Laboratory, UPMC Eye Center, Ophthalmology and Visual Sciences Research Center (OVSRC), Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Supported by National Eye Institute (NEI) Grant EY08227 (YJG), NEI Core Grant for Vision Research EY08098 (OVSRC), The Eye and Ear Foundation of Pittsburgh, Research to Prevent Blindness, and the University of Pittsburgh Office of Technology Management.

Submitted for publication April 23, 2007; revised June 1 and 12, 2007; accepted July 13, 2007.

Disclosure: **E.C. Nwanegbo**, None; **E.G. Romanowski**, None; **Y.J. Gordon**, None; **A. Gambotto**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Andrea Gambotto, Department of Surgery, University of Pittsburgh, 300 Technology Drive, Pittsburgh, PA 15219; agamb@pitt.edu.

TABLE 1. Neutralization of Adenoviral Serotypes in Three Epithelial Cell Lines by IG

Serotypes	HeLa Cell Line	A549 Cell Line	CJ Cell Line*	Mean IG ± SD
Ad3	1.56	0.1	0.4	0.69 ± 0.62
Ad4	1.56	0.1	0.4	0.69 ± 0.62
Ad8	0.1	6.25	0.1	2.15 ± 2.9
Ad11	6.25	6.25	6.25	6.25 ± 0
Ad19	6.25	6.25	0.03†	4.18 ± 2.93
Ad37	6.25	6.25	1.56	4.68 ± 2.21

Data are milligrams per milliliter.
* Conjunctival cell line.
† Infectivity of Ad19 in conjunctival cells was poor.

and corneal epithelial cells in the eyes, decrease viral replication, and prevent transmission to other people. Ig is therefore a promising candidate for bacterial and viral causes of conjunctivitis, especially when the etiology of the eye infection is unknown. The anti-inflammatory property may also prevent the formation of subepithelial infiltrates responsible for decreased visual acuity in many patients with EKC.[7,8] In theory, the combined antiviral and anti-immune properties of Ig make it attractive for topical treatment and prevention of EKC.[36]

In this study, we investigated the neutralizing activity of Ig against multiple wild-type and clinical ocular isolates of adenoviral serotypes in different human cell lines. In addition, we evaluated topical ocular toxicity and antiviral efficacy in the Ad5/New Zealand White (NZW) rabbit ocular model in which Ad5 replication takes place in the corneal epithelium.[37] Furthermore, we compared the antiviral result with the well-known antiadenoviral drug cidofovir. Apart from evaluating topical Ig's effect on viral shedding and its duration in infected eyes, we also compared the efficacy of Ig, an extracellular neutralizing antibody, and cidofovir, a potent intracellular acting adenoviral DNA polymerase inhibitor.[38]

METHODS

Experimental Drugs

Clinical grade Ig was purchased from Baxter (Westlake Village, CA). As required, lyophilized powder was aseptically reconstituted with water, filtered, and stored at 4°C until needed. Cidofovir (Vistide; Gilead Sciences, Foster City, CA) was purchased commercially. A 0.5% topical solution was prepared with 0.9% sodium chloride solution (Baxter Healthcare Corp., Deerfield, IL). The diluted drug was stored at 4°C until needed.

Adenoviruses

Wild-type adenovirus serotypes 3, 4, 5, 8, 11, 19, and 37 were obtained from American Type Culture Collection (ATCC, Manassas, VA). The clinical adenovirus isolates were cultured from patients who had typical adenoviral ocular disease, at the Eye and Ear Institute of the University of Pittsburgh. The isolates were serotyped by serum neutralization and found to be serotypes 1, 2, 3, 4, 5, 7, 8, and 19. Except for Ad8, all wild-type viruses were propagated with a human embryonic kidney cell line (HEK293). All human ocular isolates and wild-type Ad8 were propagated in a human bronchogenic carcinoma cell line (A549). Viruses were aliquoted and stored at −70°C until needed. Recombinant Ad5 encoding enhanced green florescent protein (Ad5EGFP) was generated in our laboratory, as described previously.[39]

Cell Culture

A549 cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM), as described previously.[39] A human cervical cancer cell line (HeLa; ATCC) was grown and maintained like the A549 cell line. A conjunctival cell line (CCL-20.2 [Wong-Kilbourne derivative (D) of Chang conjunctiva]; ATCC) was grown and maintained with M199 medium (Mediatech, Herndon, VA) enriched with 0.68 mM of L-glutamine 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin, 100 mg/mL streptomycin, and 2.5 mg/mL amphotericin B.

In Vitro Antiviral Studies

Flow Cytometric Neutralization. This assay, which is described elsewhere,[39] was designed to compare the neutralizing titer of two different lots of human IV-IgG solution. The test was conducted with serial final Ig concentrations of 50, 25, 6.25, 1.56, 0.39, 0.1, 0.02, and 0.01 mg/mL. Fifty microliters of the Ig dilutions were incubated in duplicate with 50 μL of 1×10^9 virions/mL of Ad5EGFP in 96-well flat-bottomed plates for 1 hour at 37°C. Freshly harvested A549 cells (1×10^5) were seeded on the plate and incubated overnight. The cells were harvested and analyzed by flow cytometery (FACScan and CellQuest software; BD Biosciences, Mountain View, CA). The neutralizing titer is the reciprocal of Ig dilution at which >50% cell transduction was inhibited.

Microneutralization. This assay, described previously,[40] was optimized to evaluate Ig-neutralizing activity against wild-type adenoviral serotypes 3, 4, 8, 11, 19, and 37. Fourfold duplicate serial dilutions corresponding to final concentrations of 25, 6.25, 1.56, 0.4, and 0.1 mg/mL of Ig (50 μL) were incubated with 50 μL of 1×10^{10} virons/mL of test virus in 96-well plates for 1 hour at 37°C. Freshly harvested cells (1×10^5 cells/100 μL; A549, HeLa, or conjunctival) were seeded into the wells and incubated for 3 days at 37°C and 5% CO_2. After incubation, the plates were stained with 0.5% crystal violet-formaldehyde solution and washed with distilled water. Complete neutralization of the test virus was associated with the persistence of cell adherence and staining of the cells. Infected cells in control wells with viral sample but without Ig did not stain, because of loss of cell adherence. The Ig concentration at which the cells were stained was taken as the titer. The mean Ig concentration titer in the three cell lines was calculated and plotted as shown in Table 1 and Figure 1.

Log Reduction Neutralization. This study was conducted with multiple human ocular isolates of adenoviral serotypes 1, 2, 3, 4, 5, 7, 8, 19, and ATCC type Ad37. The final viral concentration of 1×10^6 pfu/mL was incubated with an Ig final concentration of 1000, 500, 100, 10, and 1.0 μg/mL. Further experiments were conducted with Ig final concentrations of 50, 10, 5, 1, and 0.1 mg/mL and 80, 50, 10, 5, and 1 mg/mL. After the viral-Ig mixture was prepared, it was incubated for 1 hour at 37°C in a water bath, followed by 10-fold serial dilutions of each mixture. The samples were subjected to a standard plaque assay using A549 cells as described in the next section. An Ig concentration that demonstrated at least a 1 $\log_{10}$ decrease in Ad titer was considered to have significant antiviral activity.

In Vivo Antiviral Studies

Animals. All animals used for the study (NZW rabbits) were obtained from Myrtle's Rabbitry, Thompson Station, TN). Approval for the study was obtained from the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC; protocol number 0502953A-

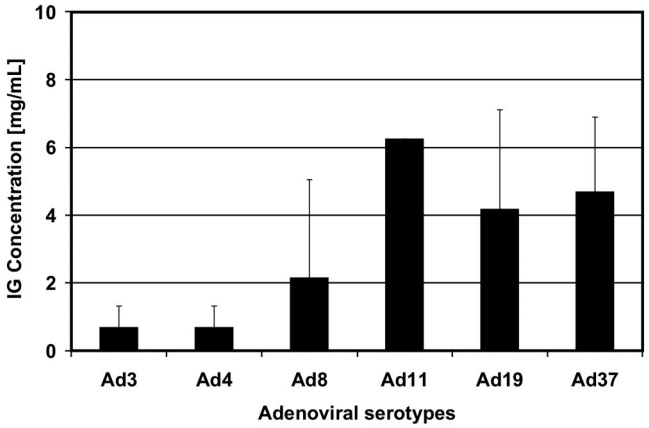

FIGURE 1. Neutralization of wild-type EKC adenoviral serotypes by Ig. In vitro microneutralization of wild-type adenoviral serotypes 3, 4, 8, 11, 19, and 37 at various concentrations of Ig was tested in A549, conjunctival, and HeLa epithelial cell lines. The mean Ig neutralizing titer concentration against each serotype in the three cell lines was plotted. Error bar, SEM of results in triplicate experiments.

1). Studies were conducted in accordance with institutional guidelines regarding animal experimentation and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Ocular Toxicity Study. Eight animals were divided into four groups on the basis of treatment with four masked solutions labeled NXER, NXTR, NXSR, and NXFR, containing 100 mg/mL Ig, 30 mg/mL Ig, 6 mg/mL Ig, and 5% albumin, respectively. Two animals in each group received four ocular drops of 37 μL of each solution daily for 5 days. Ocular toxicity was evaluated and scored on days 1, 3, and 5 by an ophthalmologist according to the Draize scoring system.[41] This method was used to classify the degree of eye irritation caused by the solutions, as described previously.[42]

Antiviral Efficacy Study. Two studies, designated R1 and R3, were similarly performed in 15 rabbits in each experiment. After general anesthesia with ketamine and xylazine and topical anesthesia with proparacaine, all 15 animals were inoculated in both eyes with 50 μL of 1.5×10^7 pfu/eye of a clinical isolate of Ad5 after 12 cross-hatched strokes of a no. 25 sterile needle. Twenty-four hours after infection, the 15 animals were divided into three topical treatment groups of 5 animals each, on the basis of treatment with 0.5% cidofovir, 100 mg/mL Ig, and saline. 0.5% Cidofovir drops were given in both eyes twice daily for 7 days during the normal working day. Ig and saline were masked and administered in both eyes four times daily for 10 days during the normal working day. Ocular swabbing (one culture per eye per day) was performed on days 0, 1, 3, 4, 5, 7, 9, 11, and 14 post infection (PI) and at least 1 hour after the last day's treatment. Swabs were taken from the cornea and the upper and lower fornices with a dry Dacron-tipped applicator. The swab from each eye was placed into tubes containing 1 mL of outgrowth media and frozen at −70°C until needed for plaque assay. A similar study was repeated with another set of 15 rabbits. In this study, the identity of Ig and saline were masked so that treatment, swabbing, and viral titer determination were masked to those performing the experiments.

Inoculation of both eyes of the rabbits allows us to minimize the number of animals killed without jeopardizing statistical validity in accordance with Animal Welfare Act Policy 12 (Consideration of Alternatives to Painful/Distressful Procedures, June 21, 2000). Symptomatically, we observe no behavioral signs of pain. Examinations of the rabbits during acute adenovirus corneal infection reveal no changes in their behavior, no swelling of the eyes, and no excessive discharge from the eyes. There was no diminished appetite or associated weight loss. The evolving infection did not cause any pain or distress to the animals, and therefore no analgesia was necessary during the course of the experiments.

Plaque Assay. Ocular swabs were taken at different time points from the animals, masked, and frozen at −70°C in growth medium. Ad5 titers were determined in A549 cell monolayers by using a standard plaque assay described elsewhere.[37] Briefly, the ocular swab cultures were thawed and 1:10 dilution made before inoculation of the A549 monolayer. The virus was adsorbed for 3 hours. Thereafter, 1 mL of medium and 0.5% methylcellulose was added to each well. The plates were incubated for 37°C in a 5% CO_2-water vapor atmosphere until visible plaques had formed. After incubation, the cells were stained with 0.5% gentian violet, and the number of plaques was counted under a dissecting microscope. The viral titers were calculated and expressed as plaque-forming units per milliliter.

Statistical Analysis

For the in vivo efficacy study, the masking codes were broken after each experiment was completed, and the data were calculated. Data from the two studies (R1 and R3) were combined, analyzed by analysis of variance (ANOVA; MiniTab Statistical Software; State College, PA) and χ^2 analyses. Significance was established at $P < 0.05$.

RESULTS

In Vitro Antiviral Efficacy

Ig Lots and Antiviral Activity. In this study we compared the antiviral properties of two different Ig lots (03I17AX21 and 02F30AX12). Each lot was serially diluted and incubated with Ad5 encoding green florescent protein. Inhibition of an A549 cell line infection was analyzed by flow cytometry, as described previously. The two solutions demonstrated similar neutralization of Ad5EGFP. In the two lots, the neutralizing titer concentration of Ig was 0.02 mg/mL.

Common EKC Serotypes. We investigated the viral-neutralizing activity against wild-type common EKC adenoviral serotypes in three cell lines. In the A549 and HeLa cell lines, 5×10^3 virons/cell of all test viruses produced maximum cellular infection. A similar rate of infection was seen with all test viruses in the conjunctival cell line, except Ad19. Despite using 5×10^4 virions/cell in the conjunctival cell line assay, Ad19 was neutralized at an even lower (0.03 ± 0 mg/mL) concentration of Ig. As shown in Table 1, the mean neutralizing Ig concentration was 0.1 ± 0 mg/mL against the Ad3 and Ad4 serotypes and 6.25 ± 0 mg/mL against Ad8, Ad11, Ad19, and Ad37, in an assay using the A549 cell line. Results from similar experiments conducted with HeLa and conjunctival cell lines are shown in Table 1. Ad11, Ad19, and Ad37 demonstrated similar results in both HeLa and A549 cell lines, whereas the same neutralizing titer was seen against Ad8 in HeLa and conjunctival cell lines. Ad11 neutralizing titers remained unchanged in the three cell lines. Less than 10 mg/mL of Ig neutralized all the wild-type, common EKC serotypes in the three cell lines (Fig. 1).

Human Ocular Isolates. We evaluated the in vitro direct antiviral inhibitory activity of different concentrations of Ig against human ocular isolates of adenoviruses. In this study, 0.1 mg/mL of Ig reproducibly produced >1 $\log_{10}$ decrease in titers of multiples isolates of Ad1, Ad2, Ad3, Ad4, and Ad5. Also, 0.1 to 1 mg/mL of Ig produced >1 $\log_{10}$ decrease in titers of multiple isolates of Ad7, Ad19, and ATCC Ad37. For three isolates of Ad8, 10 mg/mL of Ig demonstrated a similar result. However, for another two isolates of Ad8, 50 mg/mL of Ig was necessary to produce >1 $\log_{10}$ decreases in viral titers (Fig. 2). In all, 10 mg/mL or less of Ig demonstrated significant antiviral effect on more than 88% of all isolated ocular serotypes.

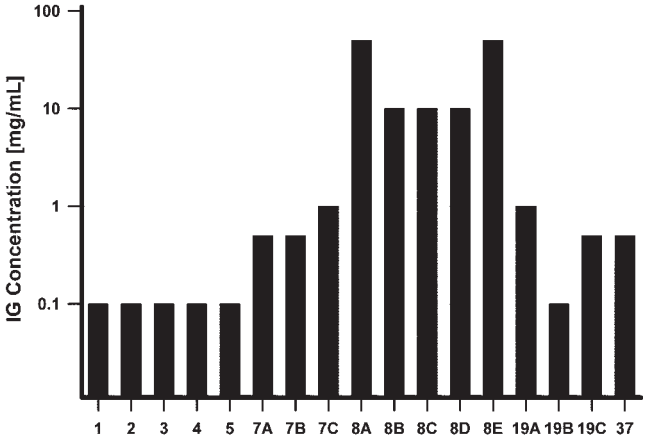

FIGURE 2. One $\log_{10}$ reduction in titer by neutralization of multiple clinical ocular isolates of adenoviral serotypes. Shown is the Ig concentration that decreased titers of multiple clinical ocular isolates of adenoviral serotypes and ATCC type Ad37 by 1 $\log_{10}$ pfu/mL. The numbers in the *x*-axis labels represent the adenovirus serotype, whereas the letters represent multiple isolates of the same serotypes.

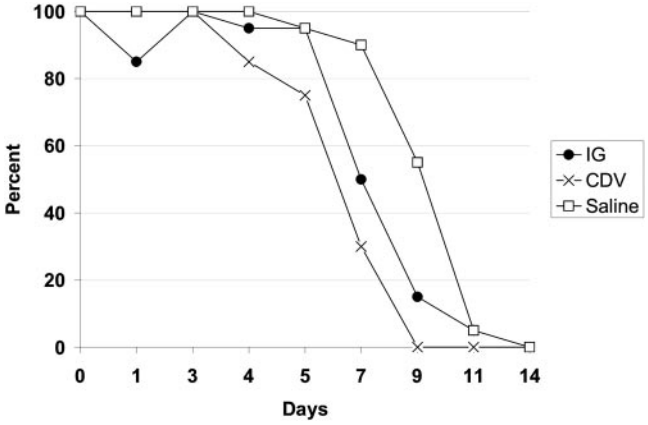

FIGURE 3. Percentage of Ad5-positive cultures in the total cultures for each culture day for eyes treated with 100 mg/mL Ig ($n = 20$), 0.5% cidofovir ($n = 20$), and saline ($n = 20$). Swabs were taken on days 0, 1, 3, 4, 5, 7, 9, 11, and 14 PI. Both 100 mg/mL or Ig and 0.5% cidofovir demonstrated significantly fewer Ad5-positive cultures compared with the saline control on days 7 and 9 (χ^2). There were no significant differences among the groups on any other day.

In Vivo Safety and Efficacy

Ocular Toxicity. Ocular toxicity studies were conducted in four groups of animals, as described earlier. All the animals tolerated the drugs at all concentrations. The maximum mean total scores (MMTSs) were 0 in all groups, and all Ig concentrations and the placebo (albumin) were considered nonirritating. Ophthalmic examinations during the study demonstrated no corneal involvement, conjunctival reddening, chemosis, discharge, or iritis.

Efficacy Studies. The results of the combined studies are summarized in Table 2 and Figures 3 and 4. The number of Ad5-positive cultures per total was determined for each treatment group by ascertaining the number of eye swabs that demonstrated a positive Ad5 culture per total number of cultures. These data were divided into the early phase (days 1–5) of infection, during which most of the adenovirus replication takes place, and the late phase (days 7–14) of infection, during which the normal immune and antiviral aided clearance of adenovirus occurs. A comparison of the total number of Ad5-positive cultures per total number of cultures taken per group over the entire course of the study (14 days) demonstrated that both Ig and 0.5% cidofovir significantly decreased the number of Ad5-positive cultures per total cultures (Table 2) compared with the control. Breaking these data down into the early and late phases of infection (Table 2) showed that Ig and cidofovir exerted significant antiviral activity compared with the control, but during only the late phase of infection. The daily reduction in percentage of Ad5-positive cultures in all treatment groups is presented graphically in Figure 4. Ig and cidofovir treatment resulted in significant decreases in the number of Ad5-positive cultures on days 7 and 9, compared with the saline-treated eyes.

The mean combined Ad5 ocular titers represent a global measure of adenovirus replication during the early and late phases of infection. These were determined by calculating the mean and SD of all ocular cultures from each treatment group during the early and late phases ($n = 80$ for all groups). The results are presented in Table 2. During the early phase of infection, the mean Ad5 ocular titers were significantly decreased when the eyes were treated with Ig and cidofovir ($P = 0.0001$, power 0.7599, ANOVA). Similar results were demonstrated during the late phase compared with the control ($P = 0.013$, power 0.9998, ANOVA).

TABLE 2. Viral Outcome Measures of 100 mg/mL IG in the Ad5/NZW Rabbit Ocular Model

	IG (100 mg/mL)	Cidofovir (0.5%)	Saline
Adenoviral positive culture/total (%)			
Overall (days 1–14)	89/160 (55.6)*	78/160 (48.75)*	109/160 (68.1)
Early phase (days 1–5)	75/80 (93.75)	72/80 (90.0)	79/80 (98.75)
Late phase (days 7–14)	14/80 (17.5)*	6/80 (7.5)*	30/80 (37.5)
Mean combined Ad5 titer (pfu/mL)			
Early phase (days 1–5)†			
Mean ± SD (power 0.7599)	$9.9 \pm 14.6 \times 10^{2*}$	$1.4 \pm 2.1 \times 10^{3*}$	$2.3 \pm 2.4 \times 10^{3}$
Late Phase (days 7–14)†			
Mean ± SD (power 0.9998)	$2.5 \pm 10.1 \times 10^{1*}$	$0.8 \pm 0.4 \times 10^{0*}$	$9.6 \pm 35.0 \times 10^{1}$
Duration of Ad5 shedding (days)‡			
Mean ± SD (power 0.9998)	6.4 ± 1.7*	5.3 ± 1.3*	8.1 ± 1.4

χ^2 was used for the analysis of Ad5-positive cultures/total. ANOVA was used for the analysis of mean combined Ad5 titer and duration of Ad5 shedding.

* $P \leq 0.05$ when compared with the control.

† $n = 80$.

‡ $n = 20$.

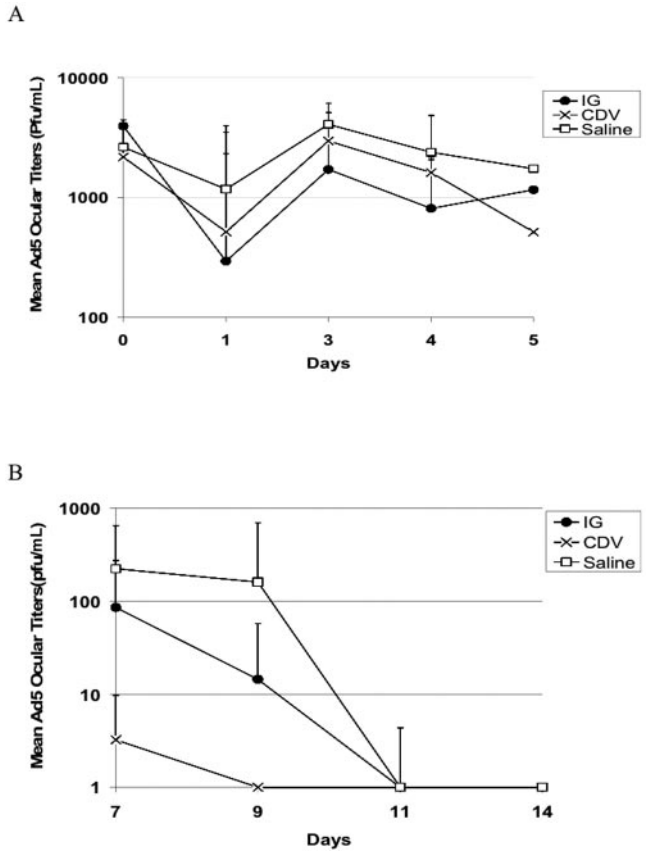

FIGURE 4. R1 and R3 mean Ad5 ocular daily titers. In two separate studies (R1 and R3), animals were infected with Ad5 and treated with the study drugs. Serial ocular viral cultures were performed, and mean daily viral ocular titers were calculated. The combined mean daily ocular titers of the two studies during early (days 1–5) and late (days 7–14) phases of infection in animals treated with topical Ig was compared with animals treated with saline and cidofovir (CDV). (**A**) Early-phase infection; (**B**) late-phase infection. Error bar, SEM of the two studies. *Topical Ig significantly reduced mean daily ocular titers on these days compared with the saline treatment.

The mean duration of shedding was estimated by determining the last day on which adenovirus-positive cultures were obtained and calculating the mean and SD. The results, shown in Table 2, demonstrate that both Ig and cidofovir significantly decreased the duration of shedding compared with the saline control ($P = 0.008$, power 0.9998, ANOVA). Furthermore, cidofovir significantly decreased the mean duration of shedding compared with Ig.

In general, Ig and cidofovir demonstrated equivalent antiviral inhibitory activity (except for the duration of Ad5 shedding for which cidofovir was superior), and each antiviral drug was significantly better than the control group, according to the outcome measures described earlier.

DISCUSSION

In this study, Ig met the previously suggested minimal criteria for development of an antiviral treatment for ocular adenoviral infections: (1) antiviral activity against a wide range of adenovirus serotypes that infect the eye, (2) antiviral efficacy in the Ad5/NZW rabbit ocular model, and (3) safety after topical administration.[16]

In general, Ig demonstrated antiviral activity that was equivalent to cidofovir despite major differences in their mechanisms of inhibitory action. Although cidofovir is a nucleoside analogue that works intracellularly to block DNA replication, Ig works by neutralization of free infectious virus on the ocular surface. Ig was remarkably effective during the critical early phase of infection (days 1–5) as demonstrated in the significant reduction of mean daily ocular titers on days 1, 3, and 4 (Fig. 4). Ig also reduced the combined ocular titers during the early phase of infection compared with both the cidofovir and saline treatments (Table 2). These findings support that IG acts rapidly through extracellular viral neutralization on the ocular surface. The clinical implications may be summarized as follows: First, topical Ig may accelerate clearance of the virus from infected eyes, leading to a more rapid cure. Second, because of rapid decreases in ocular titers in the early phase of the infection, transmission to susceptible hosts will be limited thereby, reducing local epidemics. Third, the prophylactic use of topical Ig in susceptible persons may prevent additional clinical infections. Although Ig and cidofovir were equivalent in most outcome parameters, cidofovir demonstrated a significantly shorter duration of viral shedding (Table 2), presumably because of its intracellular-mediated adenoviral DNA polymerase-blocking activities[38] and prolonged tissue half-life after rapid uptake into cells.

Because commercial Ig is produced from serum pooled from many donors, the problem of product consistency should be addressed during future development of an ophthalmic topical antiviral preparation. Data from the current in vitro studies indicate that different lots of Ig demonstrated similar antiviral features, indicating that antiadenoviral activity was consistent from lot to lot (data not shown). Future studies to test the antiviral activity of Ig from different manufacturers may also be informative.

In summary, the current experimental study represents the first successful evaluation of topical antiviral properties of Ig against etiologic agents of adenoviral ocular diseases, both in vitro and in vivo. Because of its many beneficial properties, a topical solution containing Ig may provide anti-inflammatory, anti-immune as well as antiviral activity against EKC. Furthermore, because of its broad-spectrum antimicrobial properties, topical ocular application of Ig may be effective against other viral and bacterial causes of conjunctivitis. The potential risk of transmission of infectious diseases has been minimized by current methods of producing Ig. Also the risk of anaphylaxis is minimal because of presumed very low levels of ocular absorption. The topical ophthalmic use of Ig may be of immense benefit in the ophthalmology units, pediatric units, community clinics, and global public health. These potential benefits and our preclinical data support further studies of topical Ig.

References

1. Gordon JS, Aoki K, Kinchington PR. Adenovirus keratoconjunctivitis. In: Pepose JS, Holland GN, Wilhelmus KR, eds. *Ocular Infection and Immunity*: St. Louis: Mosby; 1996;877–894.
2. Kinchington PR, Turse SE, Kowalski RP, Gordon YJ. Use of polymerase chain amplification reaction for detection of adenoviruses in ocular swab specimens. *Invest Ophthalmol Vis Sci*. 1994;35:4126–4134.
3. Gordon JS. Adenoviruses and other nonherpetic viral diseases. In: Smolin G, Thoft RA, eds. *The Cornea.* 3rd ed. Boston: Little, Brown and Co.; 1994;215–227.
4. Piednoir E, Bureau-Charlot F, Merle C, Gotzamanis A, Wuibout J, Bajolet O. Direct costs associated with a nosocomial outbreak of adenoviral conjunctivitis infection in a long-term care institution. *Am J Infec Control*. 2002;30:407–410.
5. Dart JK. Eye disease at a community health center. *BMJ.* 1986;293:1477–1480.

6. McDonnell PJ. How do general practitioners manage eye disease in the community? *Br J Ophthalmol*. 1988;72:733–736.
7. Barnard DL, Hart JCD, Marmion VJ, Clarke SKR. Outbreak in Bristol of conjunctivitis caused by adenovirus type 8, and its epidemiology and control. *BMJ*. 1973;2:165–169.
8. Kanski JJ. *Clinical Ophthalmology*. 3rd ed. Boston: Butterworth-Heinemann; 1998;86.
9. Trousdale MD, Nobrega R, Wood RL, et al. Studies of adenovirus-induced eye disease in the rabbit model. *Invest Ophthalmol Vis Sci.* 1995;36:2740–2748.
10. Everitt H, Little P. How do GPs diagnose and manage acute infective conjunctivitis?—a GP survey. *Fam Pract*. 2002;19:658–660.
11. Rose WP, Harnden A, Brueggemann AB, Perera R, Sheikh A, Crook D, Mant D. Chloramphenicol treatment for acute infective conjunctivitis in children in primary care: a randomized double-blind placebo-controlled trial. *Lancet*. 2005;366:37–43.
12. Ward JB, Siojo LG, Waller SG. A prospective, masked clinical trial of trifluridine, dexamethasone and artificial tears in the treatment of epidemic keratoconjunctivitis. *Cornea*. 1993;12:216–221.
13. Hutter H. Epidemic keratoconjunctivitis: treatment results during an epidemic (in German). *Klin Monatsbl Augenheilkd*. 1990;197:214–217.
14. Gordon YJ, Araullo-Cruz T, Romanowski EG. The effects of topical NSAIDs on adenoviral replication. *Arch Ophthalmol*. 1998;116:900–905.
15. Kaufman HE. Treatment of viral diseases of the cornea and external eye. *Prog Retin Eye Res*. 2000;19:69–85.
16. Romanowski EG, Yates KA, Teuchner B, Nagl M, Irschick EU, Gordon YJ. *N*-chlorotaurine is an effective antiviral agent against adenovirus in vitro and in the Ad5/NZW rabbit ocular model. *Invest Ophthalmol Vis Sci*. 2006;47:2021–2026.
17. Epstein SP, Pashinsky YY, Gershon D, et al. Efficacy of topical cobalt chelate CTC-96 against adenovirus in a cell culture model and against adenovirus keratoconjunctivitis in a rabbit model. *BMC Ophthalmol*. 2006;6:22.
18. Liesegang TJ. Use of antimicrobials to prevent postoperative infection in patients with cataracts. *Curr Opin Ophthalmol.* 2001;12:68–74.
19. Isenberg SJ, Apt L, Valenton M, et al. A controlled trial of povidone-iodine to treat infectious conjunctivitis in children, *Am J Ophthalmol.* 2002;134:681–688.
20. Martin T, Duffy SF, Carson DA, Kipps TJ. Evidence for somatic selection of natural autoantibodies. *J Exp Med*. 1992;175:983–991.
21. de Gracia J, Vendrell M, Guarner L, et al. The use of human gammaglobulin in the treatment of common variable immunodeficiency. *Med Clin Barc*. 1995;104:201–206.
22. Galli E, Barbieri C, Cantani A, Solano A, Longhi MA, Businco L. Treatment with gammaglobulin preparation for intravenous use in children with humoral immunodeficiency: clinical and immunologic follow-up. *Ann Allergy*. 1990;64:147–150.
23. Baker CJ, Rench MA, Noya FJ, Garcia PJ. Role of intravenous immunoglobulin in prevention of late-onset infection in low-birthweight neonates. The Neonatal IVIG Study Group. *Rev Infect Dis*. 1990;12:S463–S469.
27. Hague RA, Eden OB, Yap PL, Mok JYQ, Rae P. Hyperviscosity in HIV infected children: a potential hazard during intravenous immunoglobulin therapy (in German). *Blut*. 1990;61:66–67.
28. Ascher DP, Laws HF, Hayes CG. The use of intravenous gammaglobulin in dengue fever; a case report. *Southeast Asian J Trop Med Public Health*. 1989;20:549–554.
29. Bayry J, Misra J, Latry N, et al. Mechanisms of action of intravenous immunoglobulins in autoimmune and inflammatory diseases. *Transfusion Clin Biol*. 2003;10:165–169.
30. Schenkein DP. Intravenous IgG for treatment of autoimmune disease. *Hosp Pract Off Ed*. 1992;7:29–36.
31. Hurez V, Kaveri SV, Kazatchkine MD. Normal polyspecific immunoglobulin G (IVIg) in the treatment of autoimmune diseases. *J Autoimmun*. 1993;6:675–681.
32. Gold H, Hartung HP, Toyka KV. Therapy with immunoglobulins in neurologic autoimmune diseases: indications and mechanism of action (in German). *Fortschr Neurol Psychiatr*. 1995;63:17–29.
33. The Dutch Guillain-Barre Study Group. Treatment of Guillain-Barre syndrome with high-dose immune globulins combined with methylprednisolone: a pilot study. *Ann Neurol.* 1994;35:749–752.
34. Goossens PH, Vogels R, Piteterman E, et al. The influence of synovial fluid on adenovirus-mediated gene transfer to the synovial tissue. *Arthritis Rheum*. 2001;44:48–52.
35. Toogood C, Crompton J, Hay R. Antipeptide antisera define neutralizing epitopes on the adenovirus hexon. *J Gen Virol*. 1992;73:1429–1435.
36. Lenaerts L, Naesens L. Antiviral therapy for adenovirus infections. *Antiviral Res*. 2006;71:172–180.
37. Gordon YJ, Romanowski E, Araullo-Cruz T. An ocular model of adenovirus type-5 infection in the NZ rabbit. *Invest Ophthalmol Vis Sci*. 1992;33:574–580.
38. Romanowski EG, Gordon YJ, Araullo-Cruz T, Yates KA, Kinchington PR. The antiviral resistance and replication of cidofovir-resistant adenovirus variants in the New Zealand White rabbit ocular model. *Invest Ophthalmol Vis Sci*. 2001;42:1812–1815.
39. Nwanegbo E, Vardas E, Gao W, et al. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa, and the United States. *Clin Diagn Lab Immunol*. 2004;11:351–357.
40. Piedra PA, Poveda GA, Ramsey B, McCoy K, Hiatt PW. Incidence and prevalence of neutralizing antibodies to the common adenoviruses in children with cystic fibrosis. *Pediatrics*. 1998;101:1013–1019.
41. Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and toxicity of articles applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther*. 1944;82:377–390.
42. Kay JH, Calandra JC. Interpretation of eye irritation tests. *J Soc Cos Chem*. 1962;13:281–289.