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Short communication

The cotton rat model for adenovirus ocular infection: antiviral activity of cidofovir

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Abstract

To determine the antiviral effects of compounds against ocular adenovirus (AdV) infection, we established an animal model of AdV infection in cotton rat eyes. Cotton rat eyes were inoculated intrastromally and topically with four AdV serotypes 4, 5, 8, and 37, and treated topically with 1% HPMPC (cidofovir) eye drops twice a day. The infected corneas were extracted and homogenized, and virus titers in the cornea specimens were determined by a plaque assay. The virus titer in AdV type 5-inoculated eyes peaked on days 0 through 3 after inoculation and virus shedding was detected for 18.0 ± 2.8 days. AdV 5 antigen in the infected corneas was demonstrated in the corneal epithelial cells by immunofluorescence stain. However, for AdV serotypes 4, 8, and 37, no evidence of continued virus replication in cotton rat eyes was noted. Specimens from cidofovir-treated eyes infected with AdV 5 demonstrated a statistically significant reduction in the mean virus titer (days 3–15) ($P = 0.028$) and virus shedding duration ($P = 0.0014$), as compared with those of the control group.

Keywords: Adenovirus; Adenoviral conjunctivitis; Cidofovir; Cotton rat

Adenovirus (AdV) is the most common cause of viral conjunctivitis and acute contagious infections associated with community and nosocomial epidemics (Ford et al., 1987). In general, adenoviral conjunctivitis presents as mild clinical symptoms and is a self-limiting disease without specific therapy, although it may on occasion exhibit serious symptoms, such as ocular irritation, visual disturbance, photophobia, and eye pain. Currently, there is no effective antiviral chemotherapy for the reduction of the symptoms or duration of the AdV infection, but many investigators have reported several agents with antiviral activity against AdV in vitro (Gordon et al., 1991; Kodama et al., 1996; Mentel et al., 1997; Nagl et al., 1998; Kaneko et al., 2001).

To develop an antiviral drug for adenoviral conjunctivitis, it is necessary to show anti-AdV activity in vitro and in vivo, and for this purpose, ocular AdV infection models in the cotton rat (Tsai et al., 1992; Trousdale et al., 1994) and New Zealand rabbit (Gordon et al., 1992, 1994; Trousdale et al., 1995; de Oliveira et al., 1996) have been reported and successfully applied to antiviral studies. In these models, the antiviral effect was evaluated by virus titer in ocular swab specimens. However, we suspected that daily swabbing in these tests would modify the course of the disease and variations in individual swabbing techniques variation would influence the results. To minimize these technical errors, we established an improved animal model using the cotton rat.

The viruses used were clinical isolates of AdV type 4 (AdV 4), type 8 (AdV 8) and type 37 (AdV 37) obtained from a patient with conjunctivitis, and a prototype strain of AdV type 5 (AdV 5). AdV 5 prototype strain was provided by the National Institute of Infectious Diseases (Tokyo, Japan). Human lung cancer cell line, A549 cells, were grown and maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, and were used for virus isolation and titration.

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The viruses were inoculated into the bilateral eyes of 10-week old female cotton rats as previously described (Gordon et al., 1992). In brief, cotton rats were anesthetized with a peritoneal injection of sodium pentobarbital and topical 0.4% oxybuprocaine hydrochloride eye drops were added to each eye. Thirty microliters of AdV at 4×10^5 plaque formation units (pfu)/ml was inoculated into the central cornea intrastromally with a 30G needle to form two focal blebs (dice pattern). The cornea was then scarified with a 27G needle superficially and inoculated topically with 20 μl of virus suspension. The lids were closed, and the eye was massaged through the lids for 30 s. Groups of 6 eyes (3 rats) or 16 eyes (8 rats) were inoculated with AdV 4, AdV 8, and AdV 37, or AdV 5, respectively. Following viral adsorption for 2 h, all inoculated eyes were irrigated with a balanced salt solution to wash out unadsorbed virus. Virus titer in the cornea was estimated on days 0 (3 h), 1, 3, 5, 7, 9, 12, 15, 18, 21, after inoculation as follows. The cornea was cut circularly along with the limbus, extracted with the surgical scissors for an eye operation, and placed in 0.2 ml MEM. After homogenization for 30 s on ice, the specimens were centrifuged at 3000 rpm for 10 min and virus in the supernatant was titrated on A549 cells by a plaque assay. In this AdV infection model, the AdV 5 titer did not increase, but infectious virus was detected for 18.0 ± 2.8 days in the eyes. In contrast, the titer of the other serotypes, 4, 8, and 37, decreased rapidly and the viruses were detected for only 3.3 ± 1.4 days (Fig. 1).

To clarify whether AdV 5 can establish an infection and replicate in a cotton rat eye, AdV antigen in the infected corneas was visualized and observed by immunofluorescence staining using an anti-AdV monoclonal antibody. Formalin-fixed, paraffin-embedded sections of mouse cornea were prepared by standard procedures. Briefly, sections were deparaffinized in xylene and rehydrated with graded ethanols. After eliminating endogenous peroxidase activity by treatment with 0.3% H_2O_2 in methanol, the sections were incubated with mouse anti-adenovirus (AdV 3) monoclonal antibody, clone 2/6, (CHEMICON International, Temecula, CA) at 4 °C overnight and then with biotinylated anti-mouse immunoglobulin antibody (DAKO, Tokyo, Japan) at room temperature for 20 min. The reacted antibody was visualized with an Avidin-Biotin peroxidase complex kit (DAKO, Tokyo, Japan) with a diaminobenzidine (DAB)-H_2O_2 solution (60 g DAB in PBS, 100 ml) as the substrate. The sections were counterstained with hematoxylin. The virus antigen was detected in the corneal epithelial cells, indicating that AdV 5 can replicate in a cotton rat eye (Fig. 2). Viral antigens were not detected in the corneal stroma.

In past reports (Tsai et al., 1992; Gordon et al., 1992; Trousdale et al., 1995), AdV 5 was often used in ocular animal infection models. Gordon's group reported

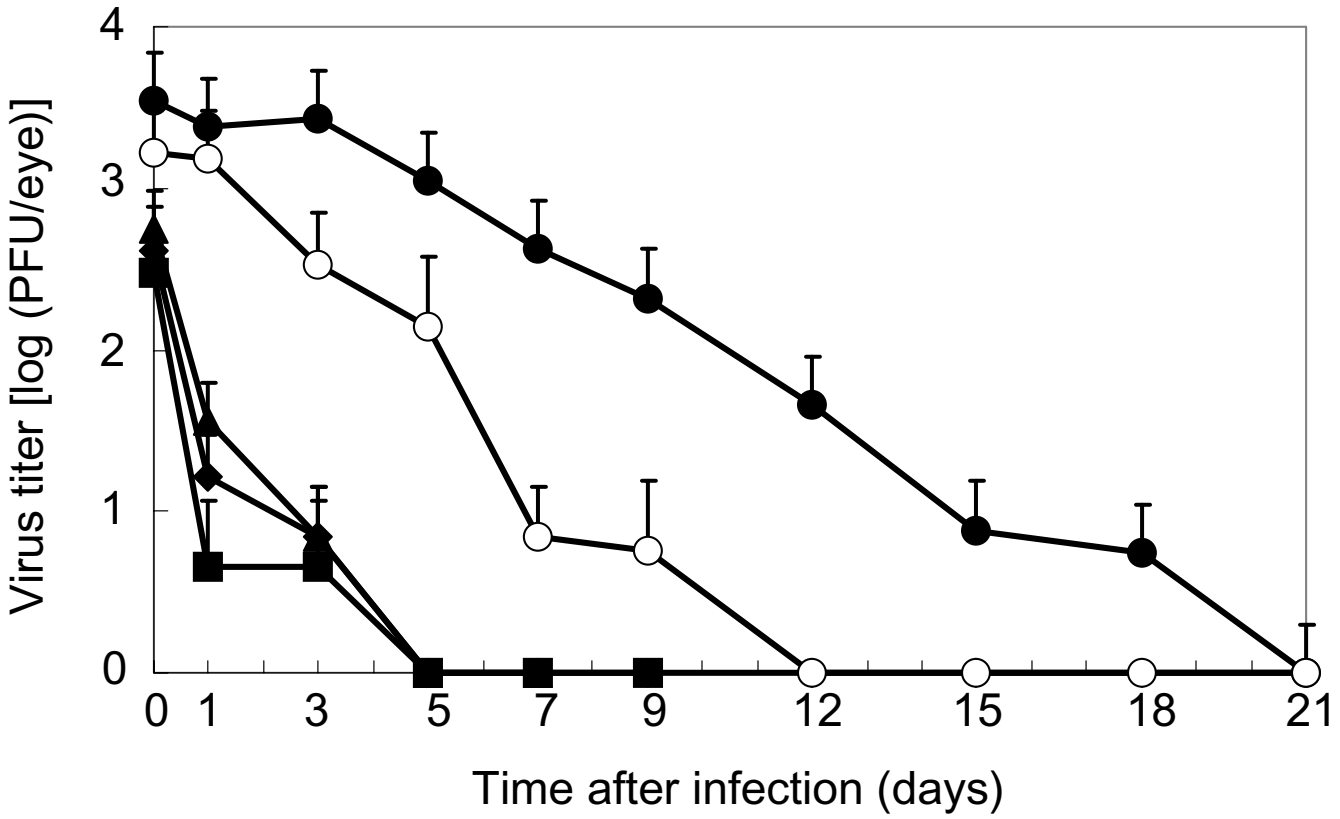

Fig. 1. AdV titers in the cornea specimens. Virus titers are expressed as means ± standard error (error bars) calculated from the results of 16 eyes for all the groups. Symbols: AdV 4 (◆), AdV 5 (●), AdV 8 (▲), AdV 37 (■), swabbing specimens of AdV 5 (○).

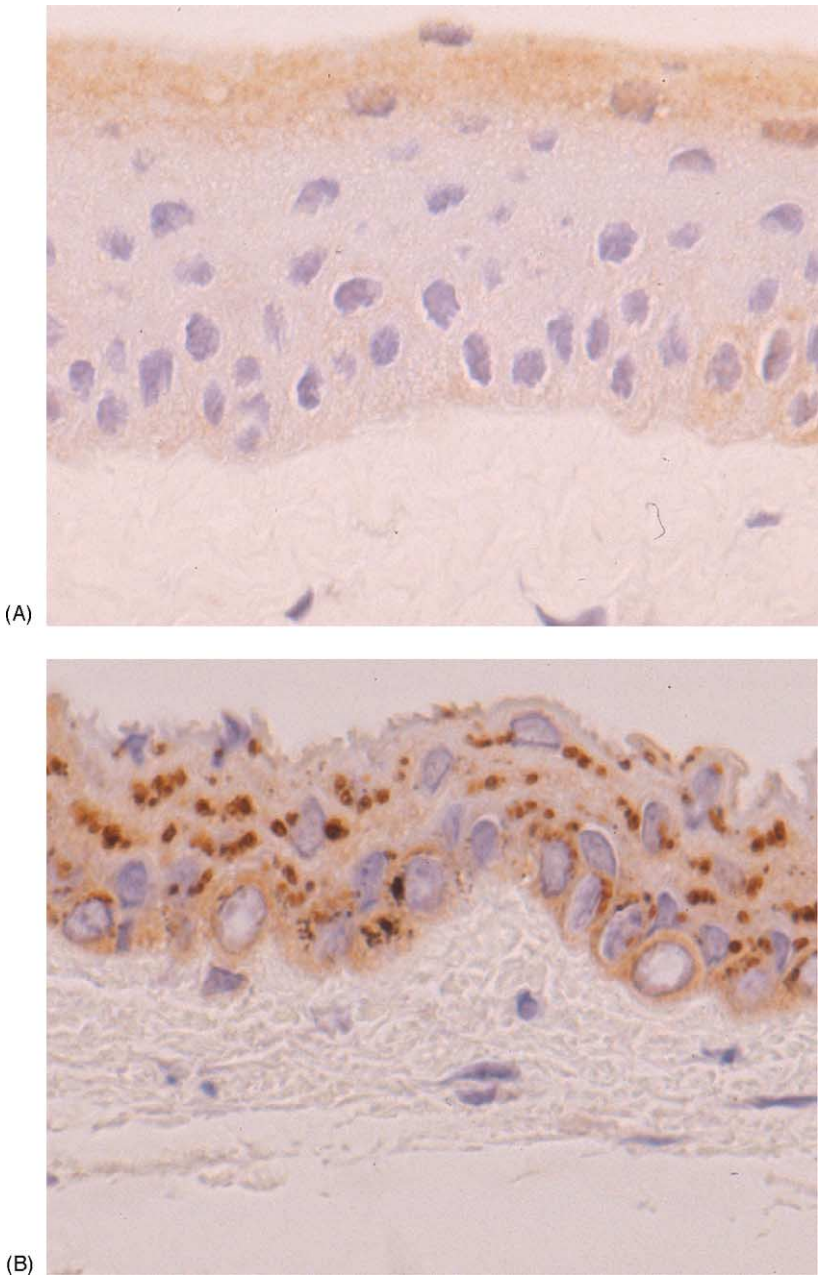

Fig. 2. AdV 5 antigens in cotton rats, as determined by immunochemical staining in a non-infected control cornea (A) and an AdV 5-infected cornea at 3 days post infection (B) (original magnification 200×). Viral antigens are visualized as brown spots.

(Romanowski et al., 1998) that animal eyes are easily infected with subgroup C AdVs including AdV 5, although the reason for this is unclear. Our results confirmed these studies.

In previous animal models, the antiviral activity of compounds was evaluated by the ocular virus titer from swabbing specimens (Tsai et al., 1992; Gordon et al., 1992, 1994; Trousdale et al., 1994, 1995; de Oliveira et al., 1996). For comparison with the efficacy of the previous methods, the virus titer of swabbing specimens from six eyes was also evaluated. The upper and lower fornices of each eye were swabbed with a cotton applicator. The sample was then placed in 0.2 ml MEM and stored at −70 °C until titration. Our results showed that the virus titers from swabbing specimens varied and the values were obtained inconsistent with the virus titers from the extracted cornea (Fig. 1). This indicates that the method reported allows for a more quantitative AdV infection model than past models.

To confirm the applicability of our model to the evaluation of antiviral chemotherapy, the effect of topical treatment with (*S*)-1-[3-hydroxy-(2-phosphonylmethoxypropyl) cytosine] (cidofovir, HPMPC) was evaluated along with the AdV 5 infection model. HPMPC was purchased from Gilead Sciences (Foster City, CA). HPMPC powder was

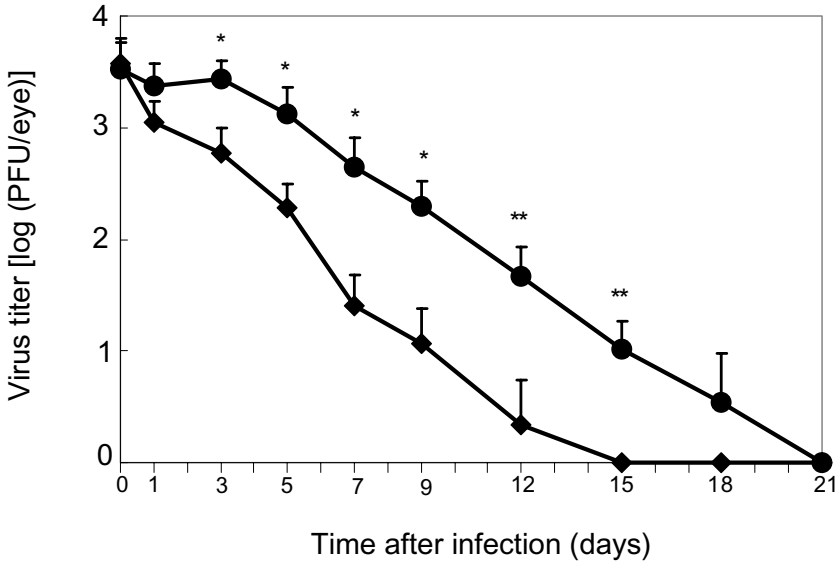

Fig. 3. Anti-AdV effect of HPMPC (cidofovir) treatment in the cotton rat model. The results are expressed as mean standard error (error bars) calculated from the results of six eyes. Symbols: HPMPC + AdV (◆), AdV 5 control (●); $^{*}P < 0.03$; $^{**}P < 0.05$.

prepared for topical use as a 1% aqueous formulation in phosphate-buffered saline. Twenty-four hours after inoculation, eyes were treated topically with one drop of 1% HPMPC or vehicle as a control in each eye. This treatment was continued twice a day until cornea extraction. The virus titer in the cornea was assayed based upon virus extracted from homogenized corneas. The total number of eyes in both the control and HPMPC-treated groups was six eyes (three cotton rats). The HPMPC-treatment group demonstrated a statistically significant reduction in the mean ocular virus titer (day 3–15) for AdV 5 (8.0 ± 1.5 days versus 18.0 ± 2.8 days ($P = 0.028$)) and duration of ocular shedding (8.0 ± 1.5 days versus 18.0 ± 2.8 days ($P = 0.0014$)), as compared with the control group (Fig. 3). These results indicate that the model permits a sensitive, reproducible and quantitative analysis of antiviral activity.

In conclusion, an AdV 5 ocular infection model for antiviral efficacy studies has been developed in the cotton rat. This model requires the use of many cotton rats, which is not a problem as large numbers of cotton rats can be easily bred in a small area. The major problem with the model is that AdV does not induce any symptoms in cotton rats, although it is known to cause several ocular symptoms in the rabbit model (Gordon et al., 1992; Trousdale et al., 1994). However, the model is suitable for evaluating the antiviral effect of antiviral agents in a quantitative manner by the titration of AdV in the cornea.

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