Cidofovir and Experimental Herpetic Stromal Disease

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Objective: To compare topical cidofovir with topical trifluridine for the prevention and treatment of herpes simplex type 1 stromal keratitis in rabbits.

Methods: The RE strain of herpes simplex virus 1 was injected into the central stroma of both eyes of New Zealand white rabbits. Two to 3 days after virus inoculation, the rabbits were randomized to treatment groups of 10 each and treated with 1% trifluridine administered 5 or 7 times a day, 1%, 0.5%, or 0.2% cidofovir administered twice a day, fluorometholone administered twice a day, or balanced salt solution (BSS) administered twice a day (control) until day 21 after injection. The treated corneas were examined 3 times a week and the severity of stromal keratitis was graded in a masked fashion. To evaluate the ability of cidofovir to treat established stromal disease, groups of 10 rabbits each were inoculated with herpes simplex virus and treated with 1% cidofovir twice a day, 1% trifluridine 5 times a day, fluorometholone twice a day, or BSS twice a day beginning on day 7 after virus inoculation through day 21.

Results: Treatment with 0.2% cidofovir twice a day was not effective in preventing the appearance of stromal disease (P = .89), whereas treatment with 0.5% (P < .001) or 1% (P < .001) cidofovir twice a day or 1% trifluridine 5 times a day (P < .001) or 7 times a day (P = .006) significantly reduced the appearance of stromal keratitis on the 8 evaluation days, compared with BSS treatment (F test analysis of variance). There was no difference between the eyes treated with 0.5% cidofovir twice a day and those treated with 1% trifluridine 5 times a day. Treatment with 1% cidofovir was not effective in treating established stromal disease.

Conclusions: Cidofovir and trifluridine are highly effective in preventing the appearance of herpetic stromal disease. Cidofovir is as effective as, but no more effective than, trifluridine in this model. Neither cidofovir nor trifluridine benefits established stromal disease, however.

Clinical Relevance: Cidofovir is a new, potent antiviral that seems similar in efficacy to trifluridine and is effective in the prevention of the development of stromal herpes, but is not effective in the treatment of established stromal disease in which hypersensitivity predominates.


IN 1979, TRIFLURIDINE eye drops were shown to prevent the development of herpes simplex virus (HSV) stromal keratitis in rabbit eyes injected intrastromally with the RE strain of herpes simplex virus type 1 (HSV-1). Since that time, there have been, to our knowledge, no published reports of another antiviral with this capability.

Cidofovir (1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) is a phosphonate with a broad antiviral spectrum. It is highly effective against HSV epithelial keratitis when administered twice a day as 0.2% to 1% eye drops. Cidofovir can produce adverse events when administered systemically to humans, and topical application can cause decreased intraocular pressure and morphologic changes in the corneas of guinea pigs; however, the eye drops do not affect intraocular pressure or appear to be toxic when applied to the rabbit cornea. Cidofovir has been reported to persist in tissues for a long time. We evaluated the ability of this compound to prevent the appearance of herpetic stromal disease in the New Zealand white (NZW) rabbit and compared its efficacy with that of 1% trifluridine. We also tested cidofovir for the ability to treat established stromal disease in the rabbit.

RESULTS

Treatment of rabbit corneas with 0.2% cidofovir eye drops twice a day was not...
MATERIALS AND METHODS

MATERIALS

New Zealand white rabbits (weighing 2-3 kg) of both sexes were used. All animals were handled in accordance with the National Institutes of Health guidelines on the care and use of animals in research, the Louisiana State University Medical Center, New Orleans, Institutional Animal Care and Use Committee guidelines and approvals, and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Drugs used in this study included 1% trifluridine drops (Viroptic; Glaxo Wellcome Inc, Research Triangle Park, NC) and 0.2%, 0.5%, and 1% cidofovir (Gilead Sciences, Foster City, Calif). Cidofovir drops were formulated from cidofovir powder in a 1% carboxymethyl cellulose solution in our laboratory. Trifluridine at a concentration of 1% was chosen because our experience shows that 1% trifluridine administered 2, 4, or 7 times a day in the herpetic epithelial disease model provides an effect that is similar to that of cidofovir given 2 times a day. There are, to our knowledge, no reports that twice-a-day treatment with trifluridine is effective against herpetic stromal disease; thus, we chose to administer 1% trifluridine 5 to 7 times a day, a dosage schedule that has been shown to have such an effect.

Eyes were also treated with fluorometholone (FML ophthalmic suspension; Allergan Pharmaceuticals, Horsham, PA) or balanced salt solution (BSS; Alcon Surgical Inc, Fort Worth, Tex). Although dexamethasone sodium phosphate is very effective in this model, it can be administered only a few times (≤6) before the rabbits develop gastrointestinal ulcers. Therefore, fluorometholone was used to provide a mild steroid effect while avoiding the adverse effects of dexamethasone.

METHODS

Both corneas of anesthetized NZW rabbits were injected with 10 µL of the RE strain of HSV-1 (1 × 10⁶ plaque-forming units per milliliter). To determine the efficacy of cidofovir in preventing stromal disease, the rabbits were randomized to groups of 10 each on day 2 or 3 after virus injection; treatment was begun on that day and continued through day 21. Both eyes of each rabbit received the same treatment. In 3 separate studies, groups of eyes were treated with (1) 0.2% cidofovir twice a day, 1% trifluridine 7 times a day, or BSS twice a day; (2) 0.5% cidofovir twice a day, 1% trifluridine 5 times a day, or BSS twice a day; and (3) 1% cidofovir twice a day, 1% trifluridine 5 times a day, fluorometholone twice a day, or BSS twice a day.

In another study undertaken to test the efficacy of cidofovir in treating established stromal disease, both eyes of 4 groups of 10 rabbits each were inoculated as described above. The severity of stromal keratitis was evaluated 7 days after virus injection and the rabbits were randomized to 4 groups of comparable severity prior to the start of treatment. Treatment was begun 7 days after virus injection and continued through day 21. Treatments consisted of 1% cidofovir twice a day, 1% trifluridine 3 times a day, fluorometholone twice a day, or BSS twice a day. Because of variability in the severity of stromal keratitis in individual experiments, a concurrent control group was included with each study.

Corneas were evaluated 3 times a week in a masked fashion. Stromal disease was graded as follows: 0 = normal, thin cornea; 1 = detectable edema, iris details clearly visible; 2 = gross edema with stromal swelling, iris details still distinct; 3 = pupillary border no longer distinct; 4 = opaque cornea, anterior chamber structures not visible.

STATISTICAL ANALYSES

The outcome variable was the grade of severity of stromal disease. The drug treatment regimens were the dependent variable. Variability due to rabbit differences observed over time within treatments was controlled for by application of a repeated-measures design in the analysis of variance (ANOVA). Comparisons between the stromal disease severity score means for each drug regimen were conducted by protected t tests on least-square means derived from the ANOVA. All P values were derived from these tests; the ANOVA and all subsequent comparisons of treatment means were conducted using programs and procedures from the Statistical Analysis System language.

Predictably, treatment with fluorometholone beginning 3 days after virus injection led to very severe stromal disease.

Treatment of established HSV stromal disease with either cidofovir or trifluridine had no beneficial effect; scores in both the trifluridine- and cidofovir-treated groups were significantly worse than scores in the BSS-treated group (P = .02 and P = .003, respectively) (Figure 4). In the calculation of overall effect, fluorometholone treatment twice a day was not significantly different from BSS treatment (P = .58) (Figure 4).

Punctate keratitis was noted in the eyes of animals that received trifluridine 7 times a day and in those that received 1% cidofovir. The punctate keratitis appeared 4 to 5 days after the start of treatment and remained mild to moderate.
Cidofovir at concentrations of 0.5% and 1% and 1% trifluridine are highly and equally effective in preventing the development of herpetic stromal disease in the NZW rabbit injected with the RE strain of HSV-1. Neither cidofovir nor trifluridine is effective for treating established stromal disease in this model. Although corticosteroids are effective in treating established herpetic stromal disease, in this study treatment with the mild corticosteroid fluorometholone was significantly better than BSS treatment only at 9 days after virus injection.

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REFERENCES