Topical Treatment of Acute Adenoviral Keratoconjunctivitis With 0.2% Cidofovir and 1% Cyclosporine

A Controlled Clinical Pilot Study

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Objective: To evaluate the efficacy of 0.2% cidofovir eyedrops and 1% cyclosporine eyedrops administered 4 times daily (qid) to treat acute adenoviral keratoconjunctivitis.

Methods: A randomized, controlled, double-masked study was conducted on 39 patients with acute adenoviral keratoconjunctivitis of recent onset. Patients were divided into 4 treatment groups: (1) cidofovir qid, (2) cyclosporine qid, (3) cidofovir+cyclosporine qid, and (4) sodium chloride qid (control). The diagnosis was confirmed using adenoviral polymerase chain reaction from conjunctival swabs. Duration of treatment was 21 days.

Main Outcome Measures: Severity of conjunctival hyperemia, conjunctival chemosis, superficial punctate keratitis during treatment, and presence and severity of corneal subepithelial infiltrates were evaluated using a clinical score. Duration until subjective improvement of symptoms was recorded.

Results: Subjective improvement of local symptoms was accelerated in the cyclosporine group. All other clinically relevant variables showed no statistically significant difference among the 4 treatment groups. Particularly, we did not find a difference in the frequency of corneal subepithelial infiltrates at the end of treatment.

Conclusions: Use of cidofovir, cyclosporine, or both did not accelerate the improvement of clinical symptoms of acute adenoviral keratoconjunctivitis compared with the natural course of the infection as demonstrated by this pilot study. This might be because of the wide spectrum of the clinical course of the infection, low sensitivity to cidofovir, too low of a concentration of cidofovir, or early cessation of viral replication in the course of the infection. The effect of a higher concentration of topical cidofovir with and without cyclosporine requires investigation in a larger group of patients.

Arch Ophthamol. 2001;119:1487-1491
PATIENTS AND METHODS

We designed this randomized controlled study according to the CONSORT statement. Approval was obtained from the ethics committee of the Heinrich-Heine-University, Dusseldorf, Germany.

CLINICAL INVESTIGATION

A total of 39 patients with acute, previously untreated AKC of recent onset were randomly divided into 4 treatment groups of 9 or 10 patients each: (1) 0.2% cidofovir eye-drops 4 times daily to both eyes; (2) 1% cyclosporine eye-drops 4 times daily to both eyes; (3) 0.2% cidofovir eye-drops and 1% cyclosporine eye-drops 4 times daily to both eyes; and (4) sodium chloride eye-drops 4 times daily to both eyes as a control group (Figure). The allocation sequence was generated by chance and was concealed until the patient was randomized. Patients of either sex who were 18 years or older were eligible. Patients with any other ocular anterior segment morbidity, pregnant women, and patients taking any other topical ocular medication were excluded from the trial. We admitted 17 women and 22 men (range, 23-85 years; average age, 44.9 years).

POLYMERASE CHAIN REACTION

The diagnosis of AKC was made by clinical examination (J.H.) and confirmed by adenoviral polymerase chain reaction (PCR) from ocular swabs (R.S.R. and M.R.). Confirmation by PCR usually required 4 to 7 days. We initially admitted all eligible patients with a clinical diagnosis of AKC to the study, but only patients with a positive PCR result remained in the study for continued treatment with study medication and statistical evaluation. Patients with a negative PCR result withdrew from the study and were seen for clinical reevaluation of the diagnosis. Polymerase chain reaction has been described as a highly suitable method for the detection of adenovirus in ocular swabs. For detection of adenovirus DNA by PCR, DNA was extracted using a viral DNA kit (Qiagen, Hilden, Germany). For nested PCR, general primers derived from the well-conserved hexon gene sequence were used that could detect all adenovirus types: HexA 5'-CGG TGC GTA GTC TTA CAT GCA CAT C-3' (PCR 1, nt 18858 to 18882, numbering according to human adenovirus type 2 GenBank sequence J01917); HexB 5'-CAG CAC GCC GCG GAT GTG AAA GT-3' (PCR 1, nt 19136 to 19138); ADP-1 5'-CCA AGA CGC CTC GGA GTA CCT-3' (PCR 2, nt 18888 to 18908); and ADP-2 5'-GGA AGC CAT ATC AAG CAC AC-3' (PCR 2, nt 19112 to 19131). Amplified DNA from the second PCR was fractionated using 1% agarose gel electrophoresis, and the 243-base pair fragment was visualized using UV fluorescence after staining with ethidium bromide.

CIDOFOVIR AND CYCLOSPORINE EYEDROPS

Cidofovir and cyclosporine eyedrops were obtained from the pharmacy of Heinrich-Heine-University. Cidofovir was prepared as a 0.2% solution in a sodium borate buffer (pH 8.2) with 0.01% thiomersal preservative as previously described by Gordon et al. The uncompromised antiviral potency of cidofovir eyedrops for 12 months after preparation was confirmed at the Rega Institute for Medical Research, Catholic University, Leuven, Belgium (E.D.). Cyclosporine eyedrops were prepared in peanut oil as a 1% solution. All patients were also treated with preservative-free topical lubrication.

Duration of treatment was 21 days. Patients gave informed consent and were examined on admission to the study and 3, 6, 12, and 21 days after the onset of treatment.

At each visit, local ocular inflammation was photodocumented to evaluate the severity of inflammation using a clinical score. After randomization, both patient and physician (J.H.) knew the treatment, but the photodocumentation of each patient visit was evaluated by an experienced ophthalmologist (T.R.) in a masked fashion.

STATISTICAL EVALUATION

We evaluated and recorded the length of time until subjective improvement of the typical distressing symptoms. In addition, the following objective variables were evaluated using a clinical score: (1) conjunctival hyperemia (0 indicates no; 1, mild; and 2, severe); (2) conjunctival chemosis (0 indicates no; 1, mild; and 2, severe); (3) superficial punctate keratitis (0 indicates no; 1, mild; and 2, severe); (4) corneal subepithelial infiltrates (0 indicates no; 1, few [<10]; and 2, many [≥10]); and (5) the Schirmer test (with topical anesthesia) (0 indicates >15 mm; 1, 5-15 mm; and 2, <5 mm).

The subjective variable and objective variables 1 through 3 and 5 were statistically evaluated (by E.G.) using 2-factorial analysis of variance (general linear model, Duncan multiple range test). Variable 4 was statistically evaluated using the χ2 test (Pearson test). We used SPSS version 8.1 for MS-Windows NT 4.0 software (SPSS Inc, Chicago, Ill).

treatment but recur when corticosteroid use is discontinued. Conjunctival adenovirus infection is a highly contagious disease that occurs worldwide sporadically and epidemically. Although not blinding, AKC remains the most common external ocular viral infection. The economic and social price of this community epidemic also remains high.

Currently, no specific antiviral therapy is available to shorten the course of the infection, to improve the distressful clinical symptoms, to stop the viral replication, and to avoid the development of corneal opacities. Cidofovir or HPMPC, (S)-1-(3-hydroxy-2-phosphonomethoxypropyl)cytosine, a broad-spectrum antiviral agent, has demonstrated significant antiviral activity in the adenovirus type 5 McEwen/New Zealand rabbit ocular model. In 1996, Gordon et al first reported the clinical efficacy and safety of topical cidofovir in the treatment of a patient with proven AKC. Cyclosporine is a well-established immunosuppressant that has been used in the prevention of transplant rejection for 20 years. Topical cyclosporine was used effectively in the treatment of Mooren ulcers, vernal keratoconjunctivitis, ulcerative keratitis associated with...
rheumatoid arthritis,\textsuperscript{15} anterior uveitis,\textsuperscript{16} and Thygeson punctate keratitis.\textsuperscript{17,18}

Furthermore, topical cyclosporine was reported to be valuable in the treatment of patients with AKC-related corneal opacities.\textsuperscript{4} To our knowledge, the effect of cyclosporine eyedrop administration on the course of the acute phase of AKC has not yet been investigated. Topical administration of cyclosporine might alter the host’s local immune response, conserving the ability of virus elimination, while ameliorating the distressing symptoms of the acute phase of AKC. The development of corneal opacities in the late phase of AKC might be inhibited by early use of topical cyclosporine.

From our long-standing positive clinical experiences in the standard treatment of deep forms of herpetic anterior segment infection with a combination of virustatic agents and corticosteroids,\textsuperscript{19} we suspected that treatment with cidofovir combined with cyclosporine might be superior to the respective monotherapies.

The aim of this study was to investigate the efficacy of cidofovir eyedrops with and without cyclosporine eyedrops in the treatment of the acute phase of AKC and to evaluate the ability of both agents as monotherapy and combined therapy to shorten the course of the acute phase of AKC and to prevent the development of corneal opacities in the late phase of the infection.

### RESULTS

No significant ocular toxic effects from using either topical cidofovir or cyclosporine were observed. Four patients complained of mild local burning associated with administration of cyclosporine eyedrops, but no patient decided to withdraw from the study.

The duration of complaints (interval between the first onset of symptoms and admission to the study) ranged from 1 to 7 days for all patients except 2 (10 and 14 days), who were both randomized to the cidofovir + cyclosporine group, thus leading to a statistically significantly (\(P = .03\)) longer duration of symptoms in that group. We therefore reevaluated all variables without taking into account patients with a duration of symp-

toms longer than 6 days. Thirty-three patients remained, and there was no statistically significant difference in duration of symptoms among the 4 treatment groups. We also did not find a statistically significant difference for the subjective variable and objective variables 1 through 4. The difference in the measurements of the Schirmer test remained unchanged. In this evaluation, length of time until subjective improvement of symptoms was not statistically significantly different among the 4 treatment groups.

Use of cyclosporine, but not cidofovir, accelerated the subjective improvement of clinical symptoms related to the acute phase of AKC (\(P = .046\)) (Table 1).

For variables 1 through 3, we were unable to prove a statistically significant difference using 2-factorial analysis of variance among the 4 treatment groups for conjunctival hyperemia, conjunctival chemosis, and superficial punctate keratitis (Table 1).

According to the clinical score, we compared the number of patients with severe corneal infiltrates with patients either mild or no corneal infiltrates (variable 4). As evaluated using the \(\chi^2\) test (Pearson test), the incidence of corneal infiltrates was not affected by application of cidofovir. There might have been a weak trend toward a lower incidence of corneal infiltrates in the cyclosporine group (Table 2).

Evaluation of the Schirmer test showed that eyes treated with cidofovir were statistically significantly less dry, as evaluated using 2-factorial analysis of variance (general linear model, Duncan multiple range test) \(P = .048\). There might have been a weak tendency toward dryer eyes in the cyclosporine group (Table 1).

### COMMENT

The inhibitory effect of cidofovir in vitro on adenovirus types 1, 5, 8, and 19 isolated from patients with AKC has been described.\textsuperscript{22} The efficacy of cidofovir has also been documented in vivo. Cidofovir demonstrated significant antiviral activity in the adenovirus type 5 McEwen/New Zealand rabbit ocular model.\textsuperscript{10,23}
However, to our knowledge, there is to date only one case report providing information on the safety and possible efficacy of topical 0.2% cidofovir administration. Gordon et al11 treated a 31-year-old patient with proven adenoviral conjunctivitis. Symptoms improved markedly within 4 days, and all clinical findings completely resolved within 7 days; the cornea remained clear, and the other eye, which had received prophylactic treatment, remained free of symptoms.11

To our knowledge, the present study provides the first data on the treatment of AKC with topical cidofovir in a controlled clinical trial. Our observations indicate, in accordence with those of Gordon et al,11 that topical 0.2% cidofovir is a well-tolerated drug without evidence of local or systemic adverse effects. Our pilot study, however, did not demonstrate a statistically significant effect of the tested treatment regimens on the natural course of the acute phase of AKC. Only the subjective improvement of the distressing local symptoms was accelerated by cyclosporine application. We did not find a statistically significant difference among the 4 treatment groups in the frequency of corneal infiltrates at the end of 21 days of treatment.

Keratoconjunctival infection is usually associated with intense tearing during the first days of the acute phase of the disease. During the further course of the infection, the intense tearing subsides and patients often develop a dry eye syndrome.14 Eyes treated with topical cidofovir were not as dry as those treated with either sodium chloride or cyclosporine, as indicated by the Schirmer test (P = .048). This result might indicate a therapeutic effect of cidofovir.

The observed weak tendency toward dryer eyes in the cyclosporine group might be secondary to the immunosuppressant properties of cyclosporine. This theory would be in accordance with the findings of Trauzettel-Klosinski et al24 of a significant incidence of severe dry eyes in patients with AKC treated with topical corticosteroids.

There are several possible explanations for the failure of cidofovir to show the clinical efficacy that might have been expected from the antiviral activity demonstrated in vitro22 and in the rabbit ocular model10,23 (see the following paragraphs).

**CONCENTRATION OF CIDOFOVIR**

We administered 0.2% cidofovir 4 times daily. Gordon et al12 showed that 0.2% cidofovir administered 4 to 5 times daily limited adenoviral replication in the adenovirus type 5/New Zealand rabbit ocular model. Cidofovir, 0.5% and 1%, administered only twice daily was not superior but equally effective.

**SEROTYPE DEPENDENCY**

Adenovirus demonstrated serotype-dependent differences in in vitro infectivity titers and clinical course. Adenovirus type 8 was the most frequent serotype in 106 positive adenoviral cultures, causing significantly more often a severe clinical course with marked eyelid edema than other serotypes. Adenovirus types 3 and 4 were associated with higher infectivity titers than other sero-

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### Table 1. Two-Factorial Analysis of Variance*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Duration of Symptoms at Study Admission, d</th>
<th>Chemosis, d</th>
<th>Hyperemia, d</th>
<th>Superficial Punctate Keratitis, d</th>
<th>Schirmer Test, mm</th>
<th>Duration to Subjective Improvement of Symptoms, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>Mean (SD)</td>
<td>3.9 (1.37)</td>
<td>11.0 (6.6)</td>
<td>15.2 (6.75)</td>
<td>11.9 (7.58)</td>
<td>10.63 (5.78)</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>0.2% Cidofovir</td>
<td>Mean (SD)</td>
<td>2.56 (1.01)</td>
<td>14.0 (10.4)</td>
<td>19.0 (8.56)</td>
<td>13.67 (6.3)</td>
<td>13.71 (5.91)</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>P</td>
<td>.08</td>
<td>.31</td>
<td>.18</td>
<td>.72</td>
<td>.048</td>
<td>.24</td>
</tr>
<tr>
<td>1% Cyclosporine</td>
<td>Mean (SD)</td>
<td>3.0 (1.15)</td>
<td>9.0 (8.2)</td>
<td>16.3 (6.70)</td>
<td>10.7 (5.81)</td>
<td>6.63 (3.74)</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>P</td>
<td>.06</td>
<td>.51</td>
<td>.77</td>
<td>.28</td>
<td>.07</td>
<td>.046</td>
</tr>
<tr>
<td>0.2% Cidofovir + 1% cyclosporine</td>
<td>Mean (SD)</td>
<td>6.3 (3.95)</td>
<td>12.1 (9.0)</td>
<td>19.4 (9.02)</td>
<td>10.4 (5.64)</td>
<td>11.0 (5.03)</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>P</td>
<td>.03</td>
<td>.88</td>
<td>.89</td>
<td>.82</td>
<td>.73</td>
<td>.56</td>
</tr>
</tbody>
</table>

*General linear model, Duncan multiple range test. Boldface P values indicate statistically significant.

### Table 2. Corneal Opacities*

<table>
<thead>
<tr>
<th>Corneal Opacity Score</th>
<th>Sodium Chloride</th>
<th>0.2% Cidofovir</th>
<th>1% Cyclosporine</th>
<th>0.2% Cidofovir + 1% Cyclosporine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 or 1</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>&gt;1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>38</td>
</tr>
</tbody>
</table>

*Evaluated by x² test (Pearson test), P = .73.
types. Infectivity and the clinical course of AKC are serotype dependent.\textsuperscript{25}

Cidofovir proved to be effective against adenovirus types 1, 5, and 6 in the rabbit model.\textsuperscript{10,26} but a relative resistance of adenovirus type 19 to cidofovir application was reported.\textsuperscript{22} Variants of adenovirus type 5 with different sensitivity to topical treatment with 0.5\% cidofovir in the rabbit ocular model have been described by Araullo-Cruz et al.\textsuperscript{27} Consequently, the efficacy of cidofovir in patients might also be serotype dependent. Patients enrolled in our study were probably infected with various adenovirus types. Because we were unable to statistically prove a therapeutic effect of either cidofovir or cyclosporine treatment in this pilot study, we did not further extend this investigation by identifying adenoviral serotypes. Regarding statistical analysis, dividing our data according to adenoviral serotypes would have further decreased the number of patients in each treatment group.

PHARMACOKINETICS

Eyedrops can be washed out by intense tearing; in addition, regular conjunctival absorption of eyedrops can be impaired in patients with severe conjunctival chemosis and swelling of the conjunctival plica.

VIRAL REPLICATION AND ONSET OF TREATMENT

The New Zealand rabbit ocular model of adenovirus type 5 infection showed a duration of viral replication of 9 days, with a peak on day 3. The symptoms of AKC after the phase of viral replication are thought to be caused by the host’s immune response.\textsuperscript{26} Treatment with cidofovir in the rabbit model began 24 hours after inoculation.\textsuperscript{26} Treatment of our patients began 1 to 7 days (mean, 3.5 days) after the onset of symptoms, when patients first came to our clinic. Cidofovir might have been effective in the rabbit ocular model because treatment began early in the course of the infection, whereas treatment of our patients might have failed because it did not start until the phase of viral replication had almost been completed. On the other hand, cyclosporine use might be effective because of its suppressant effect on the local symptoms caused by the host’s immune response.

The trend toward earlier improvement of local symptoms in the acute phase and a lesser incidence of corneal infiltrates with topical cyclosporine treatment warrants further investigation in a greater number of patients. Furthermore, the effect of using a higher concentration of topical cidofovir, with and without cyclosporine, requires investigation in a greater number of patients.

Because of the wide spectrum of duration and intensity of local symptoms of the natural course of the acute phase of AKC, a possible topical treatment will ultimately have to be investigated in a multicenter trial once the necessary pilot studies have been completed.

Accepted for publication February 12, 2001.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Fla, April 30 to May 3, 2000.

We thank Gerlinde Westphal, Helen Basler, and Kerstin Bisgeli for their expert technical assistance.

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