Effect of flurbiprofen on herpes simplex keratitis in rabbits

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The rabbit ocular model was used to determine if flurbiprofen, a new nonsteroidal anti-inflammatory agent, caused exacerbation of herpes simplex virus (HSV) infection. Acute ocular HSV infections treated with flurbiprofen (0.1%) or dexamethasone (0.1%) drops four times a day for 10 days were similar in severity and duration as measured by corneal lesions, conjunctivitis, and corneal clouding. Eyes receiving placebo healed more rapidly than eyes treated with either anti-inflammatory drug. This preliminary study suggests that flurbiprofen appears to be comparable with dexamethasone in clinical exacerbation of acute ocular HSV infection.

Key words: experimental herpes simplex, herpes simplex keratitis, steroidal anti-inflammatory drug, nonsteroidal anti-inflammatory drug, flurbiprofen, dexamethasone, rabbit

For years, corticosteroid-containing medications have been used to treat a wide range of ocular inflammations including uveitis, keratitis, and conjunctivitis. The therapeutic regimen preferred by many ophthalmologists for treating patients with severe herpes simplex keratouveitis is the concomitant use of prophylactic antiviral therapy and corticosteroids. Although topical, periocular, and systemic steroids provide many benefits, their deleterious effects are well known.

Numerous nonsteroidal anti-inflammatory compounds have been described, including a variety of aspirin-like drugs, phenylakanoic acids, and anthranilic acids. Of the many nonsteroidal anti-inflammatory agents studied so far, flurbiprofen 2-(3-fluoro-4-biphenyl)-propionic acid has proven to be one of the most potent. This drug is active when given orally and has prolonged duration of anti-inflammatory action with low toxicity in several different animal species.

Availability of an effective nonsteroidal anti-inflammatory compound would be highly desirable. For instance, in herpes simplex keratouveitis, a drug which could suppress inflammation without exacerbation of the active viral keratitis would be extremely useful. With this specific idea in mind the present study was conceived to determine whether topical treatment with flurbiprofen potentiates experimental acute HSV-1 ocular infection in rabbits. The effect of flurbiprofen was compared against dexamethasone, a known potentiator of HSV keratitis, and placebo in a controlled double-masked fashion.

Materials and methods

Albino New Zealand rabbits weighing 3 to 4 kg were used. Both eyes of 36 rabbits were stained with 5% methylene blue and examined by slit-lamp biomicroscopy to detect preinoculation corneal defects. Bilateral ocular inoculation without
scarification was performed by placing 0.2 ml of HSV-1 (2 × 10^5 pfu of McKrae strain) into the conjunctival cul-de-sac, closing the eye, and rubbing gently for 30 sec.

Three days after inoculation, each eye was examined as described above. Rabbits were divided into three groups of 12 animals equally matched on the basis of herpetic corneal epithelial involvement. Severity of herpetic lesions was estimated on a scale of 0 to 100% (0 if no lesions were present; 25% if corneal lesions ranged from distinct punctate lesions to dendritics involving up to 25% of the cornea; 50%, 75%, or 100% with comparable corneal involvement).

Treatment was initiated on day 3 postinoculation and consisted of bilateral topical applications of the appropriate drug or placebo every 6 hr for 10 consecutive days. Animals received the same treatment in both eyes in order to eliminate the influence of systemic absorption of topically administered drug. Treatment was as follows: group A received 0.1% flurbiprofen drops; group B received 0.1% dexamethasone drops; and group C received placebo drops. The placebo was the same vehicle used with dexamethasone and flurbiprofen. These solutions were provided in coded containers by Allergan Co., Irvine, Calif. Response to therapy was assessed daily until the placebo-treated eyes returned to normal. The code was not broken until the eyes of one group had returned to normal.

At the conclusion of treatment, the corneal epithelium was removed and examined for HSV. The entire corneal epithelial layer was aseptically scraped with a sterile No. 15 surgical blade. After weighing, the cells were suspended in MEM to give a 10% solution (weight/volume), freezethawed three times. Serial-tenfold dilution of the lysed cell suspension was inoculated on duplicate confluent secondary rabbit kidney cells in 60 mm plastic petri dishes. The growth medium for these cells was GIBCO's MEM with 10% NCTC 135, 2 mM L-glutamine, and 10% fetal bovine serum (FBS). Virus adsorption was carried out at 37°C with an overlay consisting of 1% methyl cellulose in MEM supplemented with 5% FBS. A 1:5,000 dilution of neutral red from GIBCO was added to the overlay after 3 days and plaques counted 24 hr later.

Results

Each group of rabbits was evaluated in a double-masked fashion. Severity of corneal ulceration for each day was then calculated by averaging the readings of rabbits in each group. Results of corneal ulceration following HSV ocular infection and a 10-day treatment period are presented in Fig. 1.

### Table I. Comparison of treatment groups during treatment period

<table>
<thead>
<tr>
<th>Treatment day</th>
<th>Corneal involvement</th>
<th>Conjunctivitis</th>
<th>Clouding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A=B=C</td>
<td>A=B=C</td>
<td>A=B=C</td>
</tr>
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<td>A=B=C</td>
<td>A=B=C</td>
</tr>
<tr>
<td>2</td>
<td>A=B=C</td>
<td>A=B=C</td>
<td>A=B=C</td>
</tr>
<tr>
<td>3</td>
<td>A=B=C</td>
<td>A=B=C</td>
<td>A=B=C</td>
</tr>
<tr>
<td>4</td>
<td>A=B=C</td>
<td>A=B=C</td>
<td>A=B=C</td>
</tr>
<tr>
<td>5</td>
<td>A=B</td>
<td>A#C (p=0.025)</td>
<td>A=B=C</td>
</tr>
<tr>
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<td>A=B</td>
<td>A=C</td>
<td>A=B=C</td>
</tr>
<tr>
<td>7</td>
<td>A=C</td>
<td>A=C</td>
<td>A=B=C</td>
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<tr>
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<td>A=B</td>
<td>A=B</td>
<td>A=C</td>
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<td>A=C</td>
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<td>A=B</td>
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</tr>
<tr>
<td></td>
<td>B#C</td>
<td>B#C</td>
<td>B#C</td>
</tr>
</tbody>
</table>

Groups (A, Flurbiprofen; B, Dexamethasone; C, Placebo) were treated every 6 hr for 10 days starting 72 hr after inoculation. Results are compared by one-way analysis of variance using the Scheffe test to compare groups. Except as indicated, # represents a statistically significant difference (p ≤ 0.01).
The estimated area of corneal ulceration was 20% for each of the groups when treatment was initiated (treatment day 0). Corneal ulceration for group C (placebo) peaked at about 45% on treatment day 3, showed regression on days 5 to 7, and returned to near normal by treatment day 9.

Corneal ulceration in the flurbiprofen- and dexamethasone-treated HSV-1-infected eyes (24 eyes per group) progressed to an average maximum involvement of approximately 70% by treatment day 5. Corneal involvement of these two drug-treated groups decreased gradually at about the same rate to a level of about 20% when treatment was terminated at day 10.

Comparison of groups A, B, and C by one-way analysis using corneal involvement on a daily basis is given in Table I. Pairwise statistical comparison by the Scheffe test, a conservative statistical test for comparing different populations, showed no significant differences between the groups during the first five treatment days (days 0 to 4). On treatment days 6 to 9, groups A (flurbiprofen) and B (dexamethasone) differed significantly (p < 0.01) from group C (placebo), showing more extensive corneal involvement than the controls. No significant differences were demonstrated in the severity of corneal involvement between groups A and B throughout the study.

Estimates of the severity of conjunctivitis were derived from the average scores of 24 eyes (12 animals) in each group (Fig. 2). Conjunctivitis was not observed in any of the groups on treatment day 0. However, conjunctivitis was evident in all three groups by day 1. Maximum conjunctivitis was observed on day 4 for each of the groups. The eyes of the placebo group returned to near normal by day 6. Conjunctivitis in the dexamethasone group varied in the near-maximum range on days 4 and 7 and was still present at the 10% level when treatment was terminated on day 10. From days 7 to 10 the conjunctivitis was significantly less in the flurbiprofen- and placebo-treated eyes than in the eyes receiving dexamethasone (Table I).

Estimates of corneal clouding were derived from the average scores of 24 eyes (12 animals) in each group and are presented in
Fig. 3. Corneal clouding ranged from 20% to 40% during days 1 through 10 for eyes treated with flurbiprofen or dexamethasone. Clouding was significantly less in the placebo group after 5 days of treatment.

For toxicity testing, each medication was administered to four uninfected rabbit eyes three times a day for 7 days. No toxic reaction was detected.

Two weeks postinoculation, infectious HSV was detectable in pooled corneal scrapings taken from two rabbits in each group. In this preliminary study, dexamethasone-treated eyes had more infectious HSV than flurbiprofen- or placebo-treated eyes.

Discussion

The course of experimental herpetic ocular infection in rabbits has been well documented. If the infection remains untreated, corneal lesions reach a maximum at 5 to 8 days after inoculation, with gradual and complete clearing in about 14 days.

For this study, McKrae strain of HSV-1 was used without scarification. Inoculation of rabbit eyes with this strain consistently results in the formation of pinpoint, punctate, or small dendritic figures by 3 days postinoculation and maximum corneal involvement by day 6. Recovery was generally complete by 14 days in animals receiving placebo treatment.

Both flurbiprofen and dexamethasone treatment appeared to cause deleterious effects in that the severity and duration of acute corneal ulcers, conjunctivitis, and corneal clouding were enhanced. The adverse effects of using steroidal antiinflammatory drugs such as dexamethasone have been recognized clinically and experimentally for several years.

We had hoped that flurbiprofen, a new nonsteroidal antiinflammatory drug, might not enhance ocular HSV infection; however, no major clinically detectable difference was noted when flurbiprofen was compared to dexamethasone. It is not known whether the mechanism of action of flurbiprofen for producing enhanced clinical ocular HSV disease is the same as that of steroids.

REFERENCES