but only delayed excystation of A. polyphaga organisms. The reason for the apparent greater effectiveness of this system over the other two chemical disinfection systems is not clear. It is also unclear whether different strains of Acanthamoeba species would be variably susceptible to these disinfection systems. However, results of this study indicate that heat disinfection was more effective overall in killing Acanthamoeba trophozoites and cysts as compared to cold disinfection systems.

Key words: Acanthamoeba, keratitis, soft contact lens, disinfection

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References


Bioavailability and Corneal Anti-Inflammatory Effect of Topical Suprofen

Howard M. Leibowitz,* William J. Ryan,* Allan Kupferman,**† and Louis DeSantis†‡

The bioavailability in rabbit cornea and aqueous humor of an ophthalmic formulation of suprofen, a nonsteroidal anti-inflammatory drug, was evaluated following topical administration of a single dose to the eye. The drug penetrated rapidly into the uninflamed cornea with intact epithelium; highest levels occurred during the first 30 to 45 min after instillation and decreased thereafter. The bioavailability of suprofen in cornea and aqueous humor following administration of a 1.0% concentration was twice that produced by a 0.5% concentration of the drug. Topical application of multiple doses of suprofen failed to suppress polymorphonuclear leukocyte invasion of the cornea if treatment was started after the induction of inflammation. Suprofen therapy initiated prior to the induction of corneal inflammation and maintained into the post-inflammation period did produce a significant (P < 0.01) decrease in the numbers of PMNs that invaded the inflamed cornea. There was no significant difference (P > 0.05) in the corneal anti-inflammatory effect achieved by the 0.5% and 1.0% concentrations of suprofen when administered according to this regimen. Invest Ophthalmol Vis Sci 27:628–631, 1986

Locally administered corticosteroids effectively suppress corneal inflammation, but their use carries the risk of several ocular complications, including cataract, glaucoma, and the enhancement of actively replicating herpes simplex virus. This has prompted the search for other effective but potentially less toxic compounds. We have studied suprofen, a nonsteroidal anti-inflammatory agent, and report here our data on its bioavailability and anti-inflammatory effectiveness in the cornea following topical administration to the rabbit eye.

Materials and Methods. Bioavailability studies: Sodium thiamylal was administered intravenously to New Zealand albino rabbits (1.8–2.4 kg), producing light...
anesthesia for approximately 10 min and allowing optimal control of the administration of radiolabeled drug. A single 0.05-ml dose of 3H-suprofen 0.5% (17.6 µCi/mg) or 1.0% (16.0 µCi/mg) was placed on the central corneal surface (epithelium intact) of both noninflamed eyes using a microsyringe. The 0.05-ml dose was contained within the conjunctival sac while the lids were manually blinked three times and taped closed. At fixed times thereafter animals were killed by intracardiac pentobarbital sodium; three saline-soaked, cotton-tipped applicators, followed by a dry, cotton-tipped applicator, were gently rolled over the corneal surface to remove radiolabeled surface contaminants. This procedure did not disrupt the epithelium, as determined by biomicroscopic observation and fluorescein staining.

An aqueous humor sample was aspirated from the anterior chamber with a 27-gauge needle attached to a tuberculin syringe, and an 8-mm full thickness central corneal specimen was obtained by trephination. The corneal samples were weighed and dissolved in 1.5 ml of 0.5N quaternary ammonium hydroxide in toluene (Soluene 100, Packard Instruments; Downers Grove, IL) at 37°C over a 24-hr period. Each sample was acidified with 0.2 ml concentrated HCl to eliminate chemiluminescence produced by the Soluene. Thereafter the corneal and aqueous samples were handled identically. Each was diluted with 15 ml of scintillation counting solution (Ultrafluor, National Diagnostics; Somerville, NJ) and counted for a minimum of 10 min in a Packard 460C scintillation spectrometer. The soluble samples were counted for a minimum of 10 min, quantitatively measuring the amount of radioactivity in the cornea. The specific calculations above. The soluble samples were counted for a minimum of 10 min, quantitatively measuring the amount of radioactivity in the cornea. The specific calculations used to determine the data have been reported.1 Statistical evaluation of the data was performed primarily with analysis of variance using the Scheffé test. This investigation conformed to the ARVO Resolution on the Use of Animals in Research.

**Results.** Bioavailability studies: Measurable quantities of suprofen were detected in both cornea and aqueous humor following topical application of 0.05-ml suprofen both when treatment was initiated after induction of inflammation and when treatment was initiated prior to inflammation and continued after induction of the inflammatory event. Each regimen is outlined in Table 4. A control group treated with suprofen vehicle (a proprietary buffered ophthalmic vehicle containing preservative and xanthine derivatives) was run with each experimental trial. One hour after completion of each treatment protocol a 10-mm full thickness corneal button was removed by trephination, and the tissue samples were solubilized with a commercially available solubilizing agent (Soluene 100, Packard), as described above. The soluble samples were counted for a minimum of 10 min, quantitatively measuring the amount of radioactivity in the cornea. The specific calculations used to determine the data have been reported.1 Statistical evaluation of the data was performed primarily with analysis of variance using the Scheffé test. This investigation conformed to the ARVO Resolution on the Use of Animals in Research.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cornea (µg-min/gm)</th>
<th>Aqueous humor (µg-min/ml)</th>
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</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>1571</td>
<td>119</td>
</tr>
<tr>
<td>1.0%</td>
<td>3732</td>
<td>255</td>
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* Table entries are derived from data obtained from uninflamed eyes with intact epithelium following topical application of a single 0.05-ml dose, as reported in Table 1.
The aqueous humor level decreased each of the values measured during this interval. (P < 0.05). The area under the time-concentration curve, an expression of the total quantity of drug in cornea and aqueous humor, is presented in Table 2. The data show that 1.0% suprofen produces twice the drug bioavailability in both ocular locations than does the 0.5% concentration. Mean values for the half life (T½) and the “elimination constant” (Ke) of topically administered suprofen are presented in Table 3.

Anti-inflammatory effectiveness of suprofen: The anti-inflammatory effectiveness in the cornea of suprofen was directly dependent on whether treatment was started before or after the induction of inflammation. Treatment started either immediately after induction of inflammation and continued on the day of injection and during the two subsequent days did result in a significant decrease (P < 0.01) in the numbers of radiolabeled polymorphonuclear leukocytes that invaded the cornea in comparison to simultaneously run, vehicle treated controls. There was no significant difference (P > 0.05) between the anti-inflammatory effect achieved by the 0.5% and 1.0% concentrations of the drug. Moreover, a less aggressive pretreatment regimen (ie, administration of suprofen only 1 day prior to induction of inflammation and elimination of its use on the day inflammation was induced) was ineffective. Specific values are presented in Table 4.

Discussion. The accumulation of polymorphonuclear leukocytes (PMNs) is a prominent event in acute inflammation of the cornea. A number of substances present in the inflammatory locus exert PMN chemotactic activity; these include denatured and degraded proteins, fibrin degradation products, leukotriene B₄, complement fragments, and bacterial factors. Presumably the primary role of the PMN in the inflamed cornea is to phagocytize such material as tissue debris, foreign particles, immune complexes, and microorganisms. Unfortunately, in this process lysosomal neutral proteases, oxygen-derived free radicals, and metabolites of arachidonic acid are liberated into the extracellular environment. These factors exert a pro-inflammatory effect that on balance may be detrimental to the optical integrity of the cornea.

Suprofen, currently under clinical investigation as a

<table>
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<tr>
<th>Table 3. Half life and “elimination constant” of topically applied suprofen in rabbit cornea and aqueous humor</th>
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</thead>
<tbody>
<tr>
<td><strong>Cornea</strong></td>
</tr>
<tr>
<td><strong>Half life (T½)</strong></td>
</tr>
<tr>
<td>Suprofen 0.5%</td>
</tr>
<tr>
<td>Suprofen 1.0%</td>
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</tbody>
</table>

<table>
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<tr>
<th>Table 4. Mean decrease in corneal inflammatory activity*</th>
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<tbody>
<tr>
<td><strong>Day 1†</strong></td>
</tr>
<tr>
<td>IV thymidine</td>
</tr>
<tr>
<td>no treatment</td>
</tr>
<tr>
<td>suprofen vehicle</td>
</tr>
<tr>
<td>no treatment</td>
</tr>
<tr>
<td>no treatment</td>
</tr>
<tr>
<td>no treatment</td>
</tr>
<tr>
<td>suprofen 0.5%</td>
</tr>
<tr>
<td>suprofen 1.0%</td>
</tr>
<tr>
<td>no treatment</td>
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</tbody>
</table>

* Table entries are the arithmetic mean ± standard error of the mean of data derived from the study of 12 eyes (six animals). Values are expressed as percent difference from the mean of 12 simultaneously run, untreated control eyes (six animals).
† All treatment regimens = 0.5 ml qih X 8.
‡ All treatment regimens = 0.5 ml qih X 6.
§ Values are significantly different (P < 0.01) from vehicle controls but are not significantly different (P > 0.05) from each other.
topical ophthalmic drug, reaches measurable levels in the cornea following a single topical instillation. Moreover, it does so in the noninflamed eye with intact corneal epithelium, a circumstance usually associated with comparatively low drug bioavailability. Higher corneal levels of suprofen can be anticipated in the inflamed eye, a situation in which there is a partial breakdown in the epithelial lipophilic barrier to drug penetration, and in the absence of an intact epithelial layer. Nonetheless, suprofen did not produce a significant \((P > 0.05)\) reduction in neutrophils invading the inflamed cornea unless therapy was initiated prior to the induction of inflammation. Although it is known that most nonsteroidal anti-inflammatory drugs inhibit the production of prostaglandins and thromboxanes,\(^5\) it must be emphasized that there is no unifying hypothesis that explains adequately how and why NSAIDs act to suppress inflammation. Even the mode of action of aspirin, the oldest member of the group, is obscure.

The effect of NSAIDs on leukocyte migration is controversial. Several investigators studying indomethacin have reported a suppressive effect on PMN accumulation,\(^6\)\(^7\) while others have found that this drug causes an increase in PMNs.\(^8\)\(^9\) There appears to be no satisfactory explanation for these contradictory results. Similarly, the mechanism of action for the effects of suprofen in the inflamed cornea is not known.

The present findings suggest that optimal use of suprofen may be in situations in which the ophthalmologist induces the inflammation and so controls its onset. In contrast to corticosteroids, suprofen does not inhibit the healing of stromal wounds.\(^10\) Thus, this drug may be especially useful for treatment of milder forms of inflammation associated with ocular surgery and laser therapy, particularly if it proves to be free from other toxic side effects that accompany corticosteroid therapy.

Key words: suprofen, nonsteroidal anti-inflammatory agent, corneal inflammation, bioavailability, polymorphonuclear leukocyte

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