its slow release, possibly increasing exposure due to length of contact time.

It is noteworthy that BAC is used in concentrations between 0.004% and 0.02% commercially. The lower concentrations used show far less damage to the epithelial surface than the higher concentrations still present in many ophthalmic solutions. Where possible, the formulation of these solutions should be limited to the minimum compatible with sterility.

Extrapolation of these data to the human eye must await a nondestructive test of damage in response to preservative agents, such as the use of fluorescein to measure changed corneal permeability.9

Specific proprietary formulations should be evaluated by the increasingly sensitive tests of corneal side effects now available, and consideration must be given to the addition of the minimal concentration of preservatives compatible with bacterial prophylaxis.

From the Division of Ophthalmology, Stanford University Medical Center, Stanford, Calif. Supported by NIH grant ET 07053. Submitted for publication May 21, 1979. Reprint requests: Dr. Neal L. Burstein, Division of Ophthalmology, Stanford University Medical Center, Stanford, Calif. 94305.

Key words: toxicity, cytotoxicity, benzalkonium chloride, chlorhexidine digluconate, preservatives, corneal epithelium, scanning electron microscopy, membrane effects

REFERENCES


Altering the course of cataracts in diabetic rats. S. FUKUSHI, L. O. MEROLA, AND JIN H. KINOSHITA.

A potent new aldose reductase (AR) inhibitor was effective in preventing cataractous changes in diabetic rats. Untreated diabetic rats developed early lens changes by 3 weeks and dense nuclear opacities by 6 to 9 weeks. In contrast, diabetic rats treated with the AR inhibitor showed no lens changes during the 5-month period of the experiment.

Aldose reductase (AR) has been implicated in the initiation of sugar cataracts.1 The most convincing support for this thesis has come from the use of AR inhibitors which were shown to delay the onset of lens changes in this type of cataracts in animals.1,2 In the case of galactose cataract, the evidence is substantial in that several AR inhibitors, structurally different, were found to retard cataracts in rats fed galactose.1

Galactose feeding in young rats leads to dense nuclear opacities in 2 to 3 weeks. In contrast, a much longer and variable period of 6 to 9 weeks is required for the frank opacity to appear in diabetic rats. Thus, in diabetic rats, it has been difficult to assess the effectiveness of AR inhibitors which are only marginally active and capable of delaying the onset of the nuclear opacity by only a few weeks. For this reason, the South American rodent degu has been used, which when made diabetic, develops cataracts by 2 weeks. With these diabetic degus it was possible to show that treatment with the AR inhibitor quercitrin could delay the formation of nuclear opacity. However, since so much information about diabetic cataracts was previously derived from studies with rats, it was considered important to demonstrate that an AR inhibitor could alter lens opacification in diabetic rats. This has now been accomplished with the use of the very potent AR inhibitor CP-45,634 (d-6-fluoro-spiro (chroman-4,4'-imidazolidine-2',5' dione) developed by the Pfizer, Inc. Previously, Peterson et al.2 have demonstrated this inhibitor to be effective in retarding cataracts in galactosemic rats.

Methods. Rats of the Charles River strain weighing approximately 80 gm were made diabetic by an intravenous injection of streptozotocin (100 gm/kg body weight). Lens polyols were analyzed by the gas-liquid chromatographic method as previously
Fig. 1. Stages of cataract in diabetic rats. Frontal and sagittal views are given for the earliest change, stage 1, which occurs by 3 weeks; stage 2, by 5 weeks; and the dense nuclear opacity, stage 3, which appears after 6 weeks.

Table I. Sorbitol content in the lenses of diabetic rats

<table>
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<tr>
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<th>Sorbitol (μmol/gm)</th>
<th>Fructose (μmol/gm)</th>
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</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>27.70 ± 2.40</td>
<td>8.90 ± 1.50</td>
</tr>
<tr>
<td>Treated</td>
<td>0.90 ± .29</td>
<td>0.88 ± .30</td>
</tr>
</tbody>
</table>

The blood sugar levels of the two groups of rats diabetic for 2 weeks were statistically the same (534 ± 42 mg/dl and 508 ± 54, untreated). The treated rats were administered 60 mg/kg/day of CP-45,634 by stomach tube. The values are given per gram of wet lens as the mean ± S.D. for 8 lenses from 4 rats.

Results. The effectiveness of the Pfizer compound to inhibit rat lens aldose reductase was first established. In vitro, against rat lens homogenate, CP-45,634 had a 50% inhibitory concentration (IC50) of $7 \times 10^{-8}$M, indicating that it is the most potent AR inhibitor reported to date. We next determined the ability of this inhibitor to reduce the accumulation of sorbitol in a lens of a diabetic rat. The results in Table I show that rats diabetic for 2 weeks with blood sugar in excess of 500 mg/dl had a lens sorbitol level of 27.7 μmol/gm and a lens fructose level of 8.9 μmol/gm. The results indicated that in the diabetic rat lens the enzymes of the polyol pathway are functioning, leading to the accumulation of sorbitol and fructose. In those diabetic rats treated with CP-45,634 the polyol pathway appeared completely blocked. The sorbitol level was essentially the same as the level found in normal lens and the fructose level was slightly higher than the normal level of 0.4 μmol/gm. Furthermore, the wet weights of the lenses of untreated diabetic rats were 15% higher than those of the treated rats, indicating that osmotic swelling along with polyol accumulation was diminished by treatment with AR inhibitor. Thus systemic treatment with this AR inhibitor appears to block effectively the synthesis of sorbitol.
The results of the study on the efficacy of CP-45,634 in delaying cataract development in diabetic rats are depicted in Fig. 1. Within 3 weeks after the onset of diabetes, small vacuoles in the equatorial region appeared in the lens of a diabetic rat. This change was followed by a diffuse but uneven cloudiness in the anterior subcapsular region. In some cases the posterior subcapsular region was involved, but the anterior region was much more severely affected. As the cataract progressed, there was considerable disruption of the cortical fibers, leading to diffuse but marked clouding of this region. In 6 to 9 weeks a dense nuclear opacity appeared. However, the striking fact was that in diabetic rats treated with CP-45,634, none of these lens changes occurred. Even after 5 months, the early lens changes were not detected by slit-lamp examination in any of the 36 diabetic rats treated with CP-45,634.

Discussion. The AR inhibitor CP-45,634 is highly potent and effective in animals. Previously, Peterson et al.2 found that CP-45,634 administered orally at a dose of 5 to 10 mg/kg/day to galactose-fed rats delayed lens changes so that only 10% of treated rats developed any lens opacities after 29 days on the galactose diet. At the same time untreated galactose-fed rats all developed lens opacities after 15 days on the diet. In our study with diabetic rats where we used a higher daily dose of 60 mg/kg, cataract formation appeared to be prevented. During the 5-month period no discernible lens changes were observed by slit-lamp examination of the diabetic rats treated with the AR inhibitor, whereas lens changes in the untreated diabetic rats were detected within 3 weeks after onset of diabetes. In addition, the sorbitol level in the lenses of treated diabetic rats was not significantly elevated.

In contrast to the low dose of the Pfizer inhibitor necessary to alter the course of cataract formation in diabetic rats, a much higher dose of quercitrin was used in delaying cataracts in diabetic degus.4 In those studies quercitrin at a level of approximately 700 mg/kg was administered orally to retard the development of the nuclear opacity.4 In the quercitrin-treated diabetic degus, lens changes in the form of vacuoles did appear, indicating that the cataractous process was not completely abolished.

The effectiveness of CP-45,634 in retarding the cataractous process in diabetic rats and the delaying of cataract formation in diabetic degus by quercitrin treatment strongly indicate that AR is involved in the initiation of diabetes cataracts. The study also suggests the possibility that AR inhibitors may be useful clinically in altering the course of human diabetes cataracts.

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Key words: diabetes cataracts, aldose reductase, sorbitol

REFERENCES

ERG lens with built-in Ganzfeld light source for stimulation and adaptation. AART C. KOOIJMAN AND ALBERT DAMHOF.

A contact lens for routine electroretinography which contains a Ganzfeld light source is described. The light source consists of six light-emitting diodes and serves both as a stimulus source and a background illumination. The response characteristics with this source are comparable with those of an integrating sphere stimulator.

For clinical electroretinography generally a Ganzfeld stimulus is required. This is done to avoid indirect stimulation of a part of the retina through stray light and to obtain maximal responses. Usually an integrating sphere is used with a xenon flash tube attached to it, and, if an adapting background is required, a second light source is also incorporated. In this report we describe an electroretinogram (ERG) contact lens with a built-in Ganzfeld light source which serves both for stimulation and for adaptation.

It proved possible to obtain identical ERGs with this light-emitting diode (LED) stimulator and an integrating sphere stimulator.

Methods. The design of our contact lens with...