Diabetic Complications in Lens and Nerve and their Prevention by Sulindac or Sorbinil: Two Novel Aldose Reductase Inhibitors

Michael Jacobson, Yog Raj Sharma, Edward Cotlier, and Jan Den Hollander

Sorbitol, resulting from glucose metabolism through aldose reductase, may play a role in diabetic complications such as cataracts, neuropathy, and vasculopathy. Sulindac (Clinoril®) and sorbinil, two inhibitors of aldose reductase, decreased sorbitol formation in cataract or nerve tissue incubated in high glucose TC-199 media. Sulindac, a widely used antirheumatic drug, may have clinical applications in preventing diabetic complications. Invest Ophthalmol Vis Sci 24:1426-1429, 1983

The presence of the polyol pathway in animal lens and nerve and the role of sorbitol in formation of diabetic cataracts and neuropathy is well established. In the diabetic rat lens, the accumulation of non-diffusible sorbitol creates a hyperosmotic state leading to lens swelling and increased permeability to cations, sodium, and potassium. Compensatory pump activity occurs, until the pump is overwhelmed and intracellular cations and water increase. Eventually, the clarity of the lens is lost and a cataract is formed. In the rat lens, aldose reductase (AR) inhibition prevented sorbitol production with its subsequent pathologic steps. However, in human diabetic cataracts, sorbitol increases are not uniform and its role in cataract formation requires further investigation. Similarly, the polyol pathway in other tissues has obvious implications for the etiology of diabetic vasculopathy or neuropathy.

Fatty acids, flavonoids, 3,3-tetramethylene glutaric acid, and Alrestatin, have been shown, in vitro, to effectively inhibit AR of animal and human lenses. Lens incubation with high glucose allowed evaluation of AR inhibition in vitro. Both in vitro and in animal experiments, Alrestatin inhibited AR and decreased sorbitol formation. However, drug toxicity, including hepatotoxicity, in human clinical trials obviated its usefulness. More potent, less toxic AR inhibitors are being sought for therapy of diabetic neuropathy, cataracts, and retinopathy. Recently, we found that the antirheumatic drugs, salicylate, indomethacin, oxyphenbutazone, and sulindac, inhibit AR of animal and human lenses. Now we report that sulindac [cis-5-fluoro-2-methyl-1-(methylsulfinyl) benzylindene indene-3 acetic acid] (Fig. 1), is an inhibitor of sorbitol formation in human cataracts, rat lens, and sciatic nerve. The inhibitory effect of sulindac was compared with that of sorbinil (d-6-fluoro-spirochroman-4'-imidazolidine-2'-5' dione), another potentially clinically useful AR inhibitor.

Materials and Methods. From 250-g albino rats, the lenses were removed after enucleation with ether anesthesia and then weighted. Rat lenses were placed in 1 ml of TC 199 bicarbonate media containing 5.5 mM glucose (low) or 35.5 mM glucose (high glucose) and incubated at 37°C for 24 hours in round-bottom, Kjeldahl flasks. Sulindac or sorbinil were both tested in 250-g albino rabbits immediately after death. The nerves were dissected longitudinally into pieces of equal size (ap-
proximately 100 mg) and weighed. Each nerve segment was incubated in 10 ml of TC 199 media for 48 hours at 37°C. Again, sulindac or sorbinil were added to the media. Cataracts obtained immediately after intracapsular cryoprobe extraction at Yale-New Haven Hospital (New Haven, CT) were placed in Tyrode's solution, classified, and then incubated in 10 ml of TC 199 media for 24 hours at 37°C. Glucose (35.5 mM) was added to test the effect of high glucose. Similarly, inhibitors were added directly to the media. Forty-six cataractous lenses were obtained. The mean age of the patients was 70 ± 2 years. The mean (±SD) net weight of nonincubated cataracts was 221 ± 10 mg (n = 16), and of incubated cataracts was 269 ± 8 mg (n = 40), indicating lens water gains after incubation in high glucose media as found in other animal species.3,4

All incubated tissues were processed as follows. After weighing, they were homogenized in a glass homogenizer in 0.3 N ZnSO₄-0.3 N Ba(OH)₂ and the clear supernatant was obtained after 2,500 RPM centrifugation for 15 minutes at room temperature. In the supernatants, sorbitol was determined with sorbitol dehydrogenase according to Clements et al.8 Sulindac powder (Clinoril®) was provided by Dr. Duggan of Merck Sharp, and Dohme (West Point, PA). Sorbinil (CP 45,634) was provided by Dr. Peterson of Pfizer and Co. (Groton, CT).

13C NMR spectra were obtained of rabbit lenses that had been incubated overnight in 35.5 mM (1-13C) glucose, and then changed to a fresh medium, without 13C-enriched glucose before starting the experiment. Nuclear magnetic resonance (NMR) spectra were obtained by a Bruker® WH360 wide-bore NMR spectrometer. 13C NMR spectra were measured at 90.55 MHz, using 75° pulse-angles and 1.0-second pulse-intervals. Two level broad band proton decoupling was used to avoid excessive heating of the sample. The decoupling power during the acquisition time of 0.1 second was 10 watts, while during the pulse-delay period of 0.9 second, it was 0.5 watt. Standard 10 mm OD NMR tubes were used, into which four intact rabbit lenses were introduced. During the NMR experiments, the temperature was maintained at 37°C.

Statistical analysis was performed on a TI-35 calculator (Dallas, TX). Data was subjected to a one-tailed t test to determine levels of significance.

Results. The sorbitol content of rat lenses was 0.62 ± 0.14 μM/g, (n = 16). After incubation in high glucose TC-199 media, lens sorbitol increases more than 10-fold (7.05 ± 0.61 μM/g, n = 20). In rat lens experiments, media containing 10 nM of either sulindac or sorbinil inhibited sorbitol formation by 36% and 76%, respectively (Table 1). The sorbitol content of sciatic nerve incubated in high glucose TC-199 media was 1.04 ± 0.26 (n = 4) μM/g as compared with 0.28 ± 0.12 μM/g (n = 4) for nerves incubated in TC-199 media with 5.5 mM glucose. The sorbitol accumulation in rabbit sciatic nerve was inhibited by 79% and 73% at the 100 nM concentrations of sorbinil and sulindac, respectively (Table 1). The sorbitol content of human cataracts incubated in high glucose TC-199 media increased sixfold to 1.90 ± 0.27 μM/g (n = 5) from 0.32 ± 0.09 μM/g (n = 16) in nonincubated cataracts. In human cataracts, sorbinil or sulindac (100 μM) inhibited sorbitol accumulation by 47% and 48%, respectively (Table 1).

The specific accumulation of sorbitol in lenses incubated in high glucose TC 199 media was established

Table 1. Sorbitol content in tissues incubated in TC-199 media with high glucose and aldose reductase inhibitors

<table>
<thead>
<tr>
<th>Inhibitor conc. (μM)</th>
<th>Sorbitol (μM/g) (mean ± SEM)</th>
<th>Inhibition %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>1.90 ± 0.27 (5)</td>
<td>47 &lt;0.025</td>
<td></td>
</tr>
<tr>
<td>Sorbinil 100</td>
<td>0.80 ± 0.30 (6)</td>
<td>7.05 ± 0.61 (16)</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Sorbinil 10</td>
<td>1.46 ± 0.56 (6)</td>
<td>1.36 ± 0.24 (10)</td>
<td>82 &lt;0.0005</td>
</tr>
<tr>
<td>Sulindac 100</td>
<td>0.79 ± 0.11 (6)</td>
<td>1.61 ± 0.62 (10)</td>
<td>76 &lt;0.0005</td>
</tr>
<tr>
<td>Sulindac 10</td>
<td>1.91 ± 0.40 (6)</td>
<td>1.56 ± 0.38 (10)</td>
<td>77 &lt;0.0005</td>
</tr>
</tbody>
</table>

NS = not significant.
in NMR spectroscopy. In the past, sorbitol was identified by sorbitol dehydrogenase and gas liquid chromatography.7 Rabbit lenses incubated in TC-199 media with 35.5 mM glucose containing high (1-13C) glucose demonstrate high (1-13C) sorbitol levels and smaller lactate levels in NMR spectra (Fig. 2). In addition, minute quantities of residual (1-13C) glucose also were present.

Discussion. Cataract, neuropathy, and capillary microangiopathy are major complications of long-standing diabetes mellitus. Uncontrolled plasma glucose levels and duration of disease may be partially responsible for such complications, as nerve, lens, and vascular endothelium are not among insulin-responsive tissues.18 The following two mechanisms have been postulated in which elevated blood glucose levels cause damage to tissues: (1) increased glucosylation of membrane proteins that induces permeability or metabolic abnormality9; and (2) accumulation of sorbitol via the enzyme aldose reductase that results in increased water content or inhibition of enzyme systems inducing leak out of essential metabolites (K+, amino acids, inositol) from cells.1 Inhibition of aldose reductase prevents sorbitol build up and may be of value in delaying or preventing complications of long standing diabetes mellitus. Flavonoids, long in use as an adjuvent to prevent deterioration of microangiopathy and arteriosclerotic disease, inhibit aldose reductase.6 Furthermore, aspirin and salicylate, by their recently uncovered effect as aldose reductase inhibitors,6 may retard the progression of diabetic and cerebrovascular ischemic disease. Similarly, sulindac or sorbinil, effective in preventing sorbitol build up in diabetic nerve or lens, appear of potential usefulness for human use. Sulindac (Clinoril) has received ample clinical use as an anti-inflammatory drug in patients with rheumatoid arthritis and osteoarthritis. Sorbinil, currently under investigation, improves nerve conduction time in humans with diabetes10 and prevents cataracts in streptozotocin diabetic rat.7 Penetration into target tissue may, however, limit the usefulness of these drugs. Whereas sulindac inhibits human cataract aldose reductase by 50% at 1 X 10^-7 M concentrations,6 much higher levels are needed to prevent sorbitol build up by cataracts incubated in high glucose media. In rat lenses, sorbinil is more effective than sulindac in preventing sorbitol build up. It is conceivable that repeated administration of sulindac could result in build up in tissues with a high protein content such as lens, as 95% or more sulindac binds to plasma protein or to lens protein.6 Sulindac sulfide, the active metabolite of sulindac, penetrates rapidly into the diabetic rat eye. According to Duggan (personal communication) administration of sulindac sulfide in three doses of 100 fig applied topically to the eye during a 24-hour period decreased by 38% the sorbitol build up in the lens of the streptozotocin diabetic rat.

Testing inhibitors of AR by incubation of lenses, cataracts, or nerve, in vitro, appears, however, a practical method for determining the potential usefulness of drugs in target tissues. We found that in addition to providing an alternate means to evaluate sorbitol accumulation, 13C glucose NMR spectroscopy, uniquely, permits determination of sorbitol production which could be of use for monitoring humans with diabetes.

Key words: Aldose reductase inhibition, diabetic cataract, lens, nerve, sorbitol, sulindac, sorbinil
From the Department of Ophthalmology and Visual Science (Michael Jacobson, Yog Raj Sharma, and Edward Cotlier) and the Department of Molecular Biophysics and Biochemistry (Jan Den Hollander), Yale University, New Haven, Connecticut. Supported by NIH Grants EY02490 and AM27121. Submitted for publication: July 29, 1982. Reprint requests: Dr. Edward Cotlier, Department of Ophthalmology, The New York Hospital, Cornell University Medical Center, 515 E. 71 Street, New York, NY 10021.

References

Multiple Optic Fiber Patterns in the Catfish Retina
Beryn L. Frank and Stephen Goldberg

The retinas of certain catfish contain multiple optic discs. This report describes the patterns of optic nerve fibers and optic discs as seen in silver-stained flat mounts, in 11 different families of catfish. As many as 50 optic discs may exist in a single retina, in paired and unpaired combinations, and in slit and ring-like arrays. Invest Ophthalmol Vis Sci 24:1429-1432, 1983

Multiple optic discs have been found in the retinas of a variety of species of fish, amphibians, and certain members of the deer family. The retinal fiber patterns of these animals do not appear to have been described.

In our studies of the retinas of a variety of fishes, we found that the feature of multiple optic discs is a striking peculiarity of the catfish retina. The present study is an analysis of the variety of retinal fiber patterns in a broad range of catfish families, as seen with silver-stained flat mounts.

Materials and Methods. Forty-six species of catfish, representing 11 families, were examined for retinal fiber pattern. These included fish native to North, Central, and South America, Europe, Asia, India, Thailand, and Africa.

Enucleated eyes were fixed in 50% pyridine:50% Carnoy's solution overnight, and then washed in tap water. Pigmented eyes then were bleached for 1-2 days in 3% hydrogen peroxide prior to partial dehydration in 70% ethanol (overnight). After a 3- to 4-day incubation in 1.5% AgNO3 (in distilled water) at 37°C, optic axons were stained by pyrogallic acid reduction. Retinal whole mounts were prepared and examined for optic discs and organization of optic axons (Fig. 1). Phase contrast microscopy confirmed the staining of all or most optic axons and the essential correctness of the patterns seen in silver stains.

Results. We found the following 11 distinct patterns of retinal fibers and optic nerve forms among the catfish examined (see Table 1 and Figs. 2A-K): (A) central optic nerve head and a radial pattern of relatively evenly spaced fascicles of fibers, as in most mammals; (B) ventral fissure with fibers entering in a smooth layer along the entire fissure as is common in birds; (C) radial pattern with six evenly spaced, well-isolated bundles; (D) radial pattern with 10 to 14 well-separated, but smaller, radial fascicles; (E) fibers enter the fissure in discreet fascicles, rather than in a smooth layer; (F) at least 25 separate and distinct optic discs on each side of the fissure, through which fibers exited; (G) a straight ventral line of four to six optic discs that differed in size; (H) a single large optic disc on each end of a straight row of paired exit holes, located ventrally at the site of the fissure; (I) a single large exit centrally, a row of paired exits, and then a single row of one to five discs toward the retinal periphery. Slight variations were seen (in different species) that lacked the more peripheral singles; (J) forms that lacked the central large disc, but did include a row of paired exit points of varying size, and then one to five discs in a row...