Effects of chronic intracameral injections of chondroitin sulfate on cat eyes

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Abstract. The effect of chondroitin sulfate on the metabolism of trabecular meshwork was studied by repeatedly injecting a chondroitin sulfate solution into the anterior chamber of cat (experimental) eyes for 28–29 weeks. The aqueous humor of the opposite (control) eyes were similarly replaced with a phosphate-buffered saline-glucose solution. The intraocular pressure of the experimental eyes was increased 4–7 mm Hg above that of the control eyes for a prolonged period of time. In addition, collagen fibers, elastic fibers, and basement membrane-like material were moderately increased in the trabecular meshwork of experimental eyes. Three forms of thickened basement membranes of endothelial cells were observed. The compact multilaminated basement membrane was formed concentrically around some of the trabecular beams. The discontinuous, thin basement membrane was seen among bundles of coarse collagen in association with long spacing collagen fibers. Also, fine filaments were noted streaming from the plasma membrane and basement membrane of endothelial cells of angular aqueous veins. Chondroitinase ABC was able to remove ground substance in the elastic fiber of the trabecular meshwork. Biochemically, the chamber angle tissues from these eyes showed a higher synthetic activity of glycosaminoglycans and proteins. Chondroitin sulfate appears to have a direct effect on trabecular meshwork and can influence the physiologic function of these cells.

Introduction

The trabecular meshwork in the chamber angle is thought to play a key role in the regulation of aqueous outflow and intraocular pressure (IOP). The presence of glycosaminoglycans in the outflow apparatus has been demonstrated previously by histochemical, enzymatic, and biochemical methods (Armaly and Wang 1975; Barany 1956; Barany and Scotchbrook 1954; Berggren and Vrabec 1957; Knepper et al. 1981; Richardson 1982; Segawa 1975, 1979; Zimmerman 1957). This class of substances is one component of extracellular matrices that forms the ground substance in which collagen fibers and other connective tissue materials are embedded. These extracellular matrix components, singly or in combination, may be related to cell behavior and metabolism (Toole 1981). In trabecular meshwork, glycosaminoglycans particularly have been implicated in modulations of outflow resistance and the development of glaucoma (Bill and Svedbergh 1972; Richardson 1982; Rohen and Witter 1972).

Recently, we have shown that after repeatedly replacing the aqueous humor of rabbit eyes with a chondroitin sulfate solution, the IOP in these eyes could be induced to rise (Yue et al. 1984). Accumulations of extracellular matrix material and abnormal basement membranes were also observed in the trabecular meshwork (Fei et al., unpublished work). It seems that chondroitin sulfate has a direct effect on the metabolism of trabecular meshwork cells and can in turn influence the physiologic functions of these cells.

In the present study, we used the cat to study further the effects of chondroitin sulfate on the trabecular meshwork. Unlike that in rabbits, the outflow system in cats has anatomic features very similar to those of humans and primates (Richardson 1982). The purpose of this experiment was to show the pathologic and biochemical changes of the trabecular meshwork in cats after repeated exposure to chondroitin sulfate.

Materials and methods

Four cats, weighing 8–10 lbs each, were anesthetized with intramuscular injections of ketamine hydrochloride (10 mg/lb). Using a 30-gauge needle, the aqueous humor (approximately 0.7–0.8 ml) was tapped from the anterior chamber, and 0.9 ml of 10 mg/ml chondroitin 4-sulfate solution (Yue et al. 1984) was immediately introduced into the right (experimental) eye of each cat to reform the anterior chamber. An equal volume of phosphate-buffered saline (PBS) with supplemental glucose was injected into the left (control) eye after the aqueous humor had been withdrawn in a similar manner.

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Injections were performed twice a week for 4 weeks (initial injection period), discontinued for 4 weeks (resting period), and then resumed once a week for several weeks or even less frequently. The IOP was measured with the Alcon Applanation Pneumotonograph at least once a week following anesthesia.

**Morphologic studies**

The eyes of the animals were enucleated after receiving two courses of injections with a resting period in between. Both eyes of one normal cat were also obtained for comparison. Freshly enucleated experimental, control, and normal cat eyes were opened at the equatorial region. The anterior segments were hemisected vertically and the lenses removed. Portions of trabecular meshwork were dissected from each eye and used for biochemical studies. The remaining tissues from the iridocorneal angle of each eye were excised with the aid of a dissecting microscope and cut into sections 0.5–1.0 mm thick. These sections of chamber angle tissue were then divided into three groups. Sections in group 1 were placed immediately in fixative consisting of 3% glutaraldehyde, 0.1% cetylpyridinium chloride, 0.05% ruthenium red, and 0.1 M sodium cacodylate buffer, pH 7.4, for 7 days. Sections in group 2 were placed in a 0.1 M Tris acetate-buffered enzyme solution, pH 8.0, and immersed in 3% glutaraldehyde, 0.1% chondroitinase ABC, 0.05% ruthenium red, and 0.1 M sodium cacodylate for 7 days.

After fixation, all tissues were washed in 0.1 M sodium cacodylate containing 0.05% ruthenium red and postfixed for 3 h at room temperature in 1% osmium tetroxide buffered with 0.1 M sodium cacodylate, pH 7.4. Tissues were then dehydrated in the ethanol solution and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate for electron microscopy.

**Biochemical studies**

Chamber angle tissues dissected from experimental and control cat eyes were incubated for 48 h at 37°C in a CO₂ incubator with Eagle’s minimum essential medium (MEM) containing 10% fetal calf serum (Flow Laboratories, McLean, Va), 1 mCi/ml of (35S)sulfate (New England Nuclear Corp., Boston, Mass) or 50 µCi/ml of (3H)proline (New England Nuclear Corp., Boston, Mass). In the last case, 50 µg/ml of ascorbic acid and B-aminopropionitrile were also included in the media. At the end of incuba-