The Effect of Dexamethasone on Glycosaminoglycans of Human Trabecular Meshwork in Perfusion Organ Culture

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The effect of dexamethasone treatment on glycosaminoglycans (GAG) in the human trabecular meshwork was studied by placing 20 pairs of eyes in perfusion organ culture. One eye received 550 nM dexamethasone in addition to culture medium; the fellow eye received culture medium only. 3H-glucosamine and 3SS-sulfate were added to the medium for the final 48 hr of culture. The meshwork was then dissected, and the GAGs were isolated and subjected to sequential enzymatic degradation. Active labeling of hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratan sulfate, and heparan sulfate was found in both control and steroid-treated eyes. Dexamethasone-treated eyes had an average 92% increase in the 3H-glucosamine incorporation rate in the undigestible GAG residue fraction after 14–21 days treatment (20.6% versus 10.7%, P = 0.03). This change was not apparent in eyes treated for 7 days. In this preliminary study, dexamethasone appeared to cause a time-dependent increase in the undigestible GAG incorporation profile in human trabecular meshwork.

Corticosteroids may increase intraocular pressure and decrease outflow facility in susceptible individuals. Although steroid-induced glaucoma has been recognized for over 30 yr, the pathogenesis of this change is unknown. Histologic studies of eyes with steroid-induced glaucoma describe variable findings: from normal ultrastructure to an accumulation of amorphous fibrillar material in the juxtacanalicular area.2–5 Biochemical alterations in the glycosaminoglycans (GAGs) and proteoglycans of the extracellular matrix have been found in studies of animals and in human trabecular cell monolayer and explant culture.6–17

The results of these studies of the effect of dexamethasone are inconsistent and do not readily explain steroid-induced glaucoma. Dexamethasone causes an increase in 3H-glucosamine incorporation in GAGs of the rabbit aqueous outflow pathway as determined biochemically,10 but a decrease in 3H-glucosamine radiolabeling as determined by light microscopic autoradiography in cats and monkeys.8 In vitro studies suggest that dexamethasone may decrease both degradation12 and synthesis of trabecular GAGs.9,12,16 Recent work indicates dexamethasone may alter GAGs in trabecular monolayer cultures only in the presence of fetal calf serum; cells grown in serum-free media did not have altered GAGs in response to dexamethasone.15 The difference in methods and in results between species8,10 and in vivo and in vitro work9,10 and the variability of results depending on the presence of fetal calf serum15 led to the current study using organ culture of human trabecular meshwork in a serum-free medium.

Materials and Methods

Twenty pairs of fresh (within 20 hr post mortem) normal human eyebank eyes were studied. The average age of the donor eyes was 73 ± 18 yr (range, 23–93 yr), although only two pair were from donors under 60 yr of age. No eyes had prior surgery, glaucoma, or known histories of uveitis. The culture technique was similar to that described previously.18 Eyes were bisected at the equator, and the iris, lens, and vitreous were removed. The anterior segment was clamped in a modified petri dish and the eye perfused with culture medium. The culture periods were 7, 14, or 21 days (Fig. 1).

The culture medium was Dulbecco's modified Eagle's medium with a mixture of penicillin (10,000 units), streptomycin (10 mg), amphotericin B (25 μg) (A9909; Sigma, St. Louis, MO), and gentamicin (17 mg, G0889; Sigma) in 100 ml of medium perfused at

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Fig. 1. Trabecular GAG incorporation profiles, ^3^H-glucosamine, control eyes, and dexamethasone-treated eyes, 7 days (n = 5 pairs) and 14–21 days (n = 11 pairs). Dexamethasone treatment caused an increase in the incorporation rate of the undigestible GAG residue (REM) in the 14–21-day group (P = 0.03) and a small decrease in the heparan sulfate rate at 7 days (P < 0.05). No significant dexamethasone effect was found in the ^35^S-sulfate data analysis. HA, hyaluronic acid; CS, chondroitin sulfate; DS, dermatan sulfate; KS keratan sulfate; HS, heparan sulfate; REM, remaining undigestible GAG residue after sequential enzyme degradation.

the normal human flow rate (2.5 µl/min). One eye received the standard culture medium, and the fellow eye received the culture medium with the addition of dexamethasone (5.5 × 10^-7 M). The eyes were cultured at 37°C in a 5% CO₂ atmosphere. Intraocular pressures were measured at least three times a week using a pressure transducer connected to a second cannula in the culture dish. Forty-eight hours before the end of the culture period, the medium was modified by the addition of ^3^H-glucosamine (7.5 µCi/ml) and ^35^S-sulfate (10 µCi/ml); dexamethasone was continued in the appropriate eyes. At the end of the culture period, the trabecular meshwork was dissected from the eye, frozen, and subsequently analyzed. In addition, a small section of the eye, including meshwork, was placed in glutaraldehyde and processed for light microscopy using 1-µm araldite sections. Two pairs of eyes did not have adequate tissue saved for microscopy; the remaining 18 pairs were examined.

The methods for isolation, partial purification, and sequential enzymatic degradation of the GAGs to determine relative incorporation profiles were similar to those described previously. Briefly, tissue lipids were extracted with chloroform/methanol; explant proteins were digested with papain; proteins were precipitated with trichloroacetic acid (TCA); small molecules, TCA, and free radiolabel were removed by molecular sieve chromatography on Bio-Gel P-6DG columns (Bio-Rad, Richmond, VA); and the GAGs were collected by precipitation from an ethanol/potassium acetate solution.

The enzyme sequence to degrade each respective GAG (given in parenthesis after the enzyme name) was as detailed previously, ie, streptomycetal hyaluronidase (HA), chondroitinase AC (CS), chondroitinase ABC (DS), keratanase (KS), and heparitinase (HS). The remaining unidentified material which was resistant to all enzymatic degradation was termed "REM." The supernatant from each alcohol precipitation step was dried in a scintillation vial, dissolved in 100 µl of water, and then counted in 10 ml of Atomlite (New England Nuclear, Boston, MA).

Statistical analysis was done using a paired, two-tailed t-test to compare mean values of the relative incorporation profiles of each type of GAG between dexamethasone and control eyes for each time period and for the 14- and 21-day eyes combined. This was done for both the ^3^H-glucosamine and the ^35^S-sulfate data. Potential dexamethasone-associated time-dependent changes in the incorporation profiles between the 7-day and 14–21-day groups were compared with an unpaired two-tailed t-test.

Results

Histologic examination revealed that most cultures were viable as viewed with the light microscope using previously established criteria. Twenty eyes were judged to have good-to-excellent preservation of trabecular morphology, nine eyes had fair preservation, and seven eyes had poor preservation, with necrotic cells. As mentioned previously, four eyes did not have adequate tissue saved for microscopy. The nonviable cultured eyes (poor preservation) and their fellow eyes were excluded from analysis (two pairs of eyes in the 7-day group and two pairs from the 21-day group). Eyes in the fair category and the four eyes without histologic examination were included in the biochemical analysis because incorporation profiles were similar to those in the good and excellent groups and the total amount of precursors incorporated was similar as determined by the absolute counts. In all three of the successful-culture categories, light-microscopic autoradiography showed similar cellular and extracellular matrix labeling.

Biochemical analysis revealed an increase in the relative incorporation profile of ^3^H-glucosamine in the undigestible GAG fraction (REM) in the 14–21-
day dexamethasone group when compared with fellow control eyes (Fig. 1). This difference represented a 92% increase over the control value, although the absolute magnitude was relatively small (20.6% ± 10.8% versus 10.7% ± 3.9%; \( P = 0.03 \)). This difference was found only in the \(^{3}H\)-glucosamine analysis and not in the \(^{35}S\)-sulfate analysis. It was not found in the 7-day group. All other GAG groups had dexamethasone values within 34% of control eye values for both \(^{3}H\)-glucosamine and \(^{35}S\)-sulfate analysis at all times. Because the multiple groups of GAGs studied necessitated multiple statistical comparisons, the significance level of \( P = 0.03 \) could have occurred by chance, and the result should be confirmed in future studies. A significant biologic variation was noted between pairs of eyes in the total incorporation of label, as noted previously.\(^{20}\)

No consistent dexamethasone-induced elevations of intraocular pressure were found at any time.

**Discussion**

Dexamethasone treatment caused an increase in the relative incorporation profile of the undigestible GAG fraction in the trabecular meshwork after 14–21 days, as determined by the incorporation of \(^{3}H\)-glucosamine during the final 48 hr of culture. Although the absolute magnitude of this increase is small, 20.6% versus 10.7%, it represents a 92% increase in the dexamethasone-treated eyes (\( P = 0.03 \)) and is several times larger than any changes in the other GAG groups. The significance of this increased REM fraction should be considered preliminary, however, because the multiple comparisons required in this experiment increase the possibility that such a \( P \) value could occur by chance. These results should be confirmed in future studies.

The differences in the REM fraction between the 7-day and 14–21-day group suggest a time-dependent change in GAG relative incorporation profiles caused by dexamethasone treatment. This time course is similar to that of the clinical development of steroid-induced glaucoma. Statistical analysis of this 7-day versus 14–21-day change in the undigestible GAG profile showed the increase with time to be significant at \( P = 0.06 \) (unpaired, two-tailed t-test). Although fewer eyes were in the 7-day group, the study had a reasonable chance of detecting an increase in the REM fraction (93% power of detecting a 50% difference in the REM fraction at \( \alpha = 0.05 \)).

An increased amount of GAG-enzyme resistant material has been found in the meshwork of eyes with primary open-angle glaucoma in recent preliminary reports.\(^{22,23}\) Increased amounts of GAG-enzyme resistant material have also been described in the meshwork of glaucomatous beagles.\(^{24,25}\) These studies used a different method, quantitating the total amount of GAGs present in the tissue and not the relative incorporation profile, as in our study. The similarity of an increase in undigestible GAG fraction in these reports and in our study may represent a potential link between steroid-induced glaucoma and primary open-angle glaucoma.

Although the findings are interesting, they do not indicate that GAGs or changes in the undigestible GAG fraction are responsible for elevated intraocular pressure. No consistent increase in intraocular pressure was observed in the dexamethasone-treated eyes in our study. This could be due to the perfusion culture technique itself or a lack of correlation between GAGs and intraocular pressure. In the perfusion culture technique, the intraocular pressures in some eyes may be high in the first few days of culture as the eyes adapt to culture, and this may mask any steroid effect. Future studies have been designed to overcome this potential masking effect.

The increase in undigestible GAG was present only in the \(^{3}H\)-glucosamine analysis and was not found in the \(^{35}S\)-sulfate data. This may be indicative of several factors. The excess undigestible GAG may be of a different type than usual, being less sulfated. Sulfation, a posttranslational step in the synthetic pathway, could also be rate limited for the undigestible GAG fraction, with the sulfation mechanism not able to keep up with the GAG synthetic mechanism, resulting in a compensatory increase of undersulfated GAGs. The undigested material could also be oligosaccharides instead of GAGs. Finally, dexamethasone could influence the synthetic pathway at different steps, affecting both synthesis and sulfation to different degrees.

Comparison of the relative incorporation values for the different GAG groups at 21 days in the control eyes of this study with values reported using a non-perfusion organ culture technique\(^{20}\) revealed them to be qualitatively similar, although minor quantitative differences were noted. Both techniques had chondroitin sulfate and dermatan sulfate as the major GAGs, followed by heparan sulfate, with smaller amount of keratan sulfate and hyaluronic acid. The use of paired eyes compensated for several of the drawbacks inherent in tissue culture studies, such as the biologic variability between patients and the effect of the culture technique itself on the GAG profiles.\(^{20,26}\)

This study indicates that human meshwork remains synthetically active for at least 21 days in perfusion organ culture. The relative incorporation pro-
files of GAGs may be influenced by dexamethasone; an increase in the undigestible GAG fraction was noted after 14–21 days of treatment.

Key words: dexamethasone, trabecular meshwork, organ culture, glycosaminoglycans, steroid glaucoma

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References