Regional and zonal variations in the sulfation patterns of chondroitin sulfate in normal equine corneal stroma

Daniel J. Biros, DVM; Dennis E. Brooks, DVM, PhD; Murray P. Brown, DVM, MSc; Kelly A. Merritt, BS; Paul S. Kubilis, MS

Objective—To determine regional and zonal variation in sulfation patterns of chondroitin sulfate in normal equine corneal stroma.

Sample Population—22 normal eyes from 11 horses.

Procedure—Corneas were collected within 24 hours of death from equine necropsy specimens. After papain-chondroitinase digestion of corneal tissue, disaccharides ΔDi4S and ΔDi6S were quantified by use of capillary zone electrophoresis in the superficial, middle, and deep zones of central and peripheral regions of the cornea.

Results—For the 2 regions combined,ΔDi6S/ΔDi4S values were significantly lower in the deep and middle zones, compared with that of the superficial zone. In the central region, deep and middle zones had significantly lower ΔDi6S/ΔDi4S values than the superficial zone did. In the peripheral region, the deep zone had significantly lower ΔDi6S/ΔDi4S values, compared with superficial and middle zones. In the deep zone, the peripheral region had significantly lower ΔDi6S/ΔDi4S values than the central region did.

Conclusions and Clinical Relevance—Distribution of ΔDi6S/ΔDi4S values follows a gradient across the healthy equine cornea, being smallest in the deep and middle zones of the central region and the deep zone of the peripheral region. Regional and zonal differences in the distribution of stromal ΔDi6S and ΔDi4S may influence the role of glycosaminoglycans in health, disease, and wound repair of the equine cornea. (*Am J Vet Res* 2002;63:143–147)

The equine cornea is composed of 3 distinct layers: the superficial stratified epithelium, the middle layer of corneal stroma, and the deep single layer of endothelium with Descemet's membrane adjacent to the anterior chamber.[1] Similar to corneas in all species, the equine cornea is able to absorb nutrients and eliminate metabolic waste through the precorneal tear film and the internal aqueous humor.[1]

Healthy corneal stroma consists of a few cells and a precise arrangement of collagen fibers and proteoglycans in the stromal extracellular matrix. Highly specific and regulated configuration of the collagen and the various proteoglycans is responsible for the transparency of the cornea and its ability to transmit light to the retina.[1] Any substantial derangement of the extracellular matrix and stromal collagen fibers during corneal inflammatory disease results in corneal opacification. It is possible that during corneal wound healing, treatment directed at influencing proteoglycan concentrations may minimize corneal scarring.

Glycosaminoglycans (GAG) are components of proteoglycans that consist of polyanionic chains of disaccharides with various degrees of sulfation. Previously called mucopolysaccharides, they are present in all connective tissues including muscle, blood vessels, cartilage, tendon, and bone.[2-4] It has been proposed that corneal stromal GAG influence the regulation of cell growth and development, the assembly of extracellular matrix components, and the regulation of cell to cell interactions.[5-7] Many GAG have been isolated and identified, but GAG of interest in the cornea are chondroitin sulfate, dermatan sulfate, and keratan sulfate.[8-10] The distribution of chondroitin sulfate, dermatan sulfate, and keratan sulfate in ocular tissue is well described in humans and domestic and laboratory animals.[11-14] Sulfation patterns of GAG have regional variations within the corneal stroma.[15-17] In bovine eyes, chondroitin sulfate and dermatan sulfate are predominantly in the superficial peripheral corneal stroma, whereas keratan sulfate is located in the deep corneal stroma.[16,17] There are several recent reports on the effect of GAG composition and ionic interactions in the extracellular matrix influencing oxidative stress, wound modeling, and binding of inflammatory cytokines.[18-21] Further-more, ratios in GAG sulfation are now being used to measure anabolic and catabolic events in tissue for prognosis and therapeutic monitoring.[22,23]

During the past 20 years, great advances have been made in the treatment of corneal disease in horses, with a keen interest in vision preservation in performance horses. Corneal transplants are now performed on many horses with aggressive keratopathies including fungal and bacterial keratitis, stromal abscesses, and acute severe corneal trauma.[24,25] Severe equine keratitis, for example, induces a rapid and fulminant inflammatory response with substantial keratomalacia and edema.[26-28] Although there are a number of medical and surgical procedures described for the equine cornea, there is a lack of knowledge about its normal biochemical composition. Therefore, knowledge of the

Received Jan 8, 2001.
Accepted Mar 23, 2001.
From the Department of Large and Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610. Dr. Biros' present address is The Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, 20 Staniford St, Boston MA 02114.
Published as part of the University of Florida College of Veterinary Medicine, Journal Series No. 577.
This study was presented at the American College of Veterinary Ophthalmologists Annual Meeting, Oct 13, 2000.

Unauthenticated | Downloaded 01/29/23 10:28 PM UTC

GAG composition of the normal equine cornea will provide important baseline information for the study of biochemical changes in equine corneal disease. To the authors' knowledge, data on GAG in equine corneas has not been published. The purpose of the study reported here was to determine regional and zonal variation in sulfation patterns of chondroitin sulfate in normal equine corneal stroma and to provide insight toward understanding its biochemical structure.

Materials and Methods

Horse corneas—Twenty-two normal eyes from 11 horses were obtained from the University of Florida Veterinary Medical Teaching Hospital necropsy service. All horses died or were euthanatized for reasons unrelated to the eyes. Collection of corneas was performed within 24 hours of death from cadavers that had been placed in the necropsy refrigerator immediately after death. Eyes were evaluated for lesions such as corneal edema, hyphema, anterior uveitis, or subconjunctival hemorrhage, and only those considered normal were used. Corneas were obtained from 4 Thoroughbreds, 1 Standardbred, and 6 ponies. Ages of horses ranged from 2 to 14 years (mean, 5.6 years). Six horses were geldings, and 5 were mares.

Tissue preparation—One hundred thirty-two corneal samples were analyzed; these were obtained from 44 corneal tissue core samples from 22 corneas. Two core samples were taken from each region of the cornea (1 from the center and 1 from the periphery). Within each core, the superficial third, middle third, and deep third zones of the cornea were separately assayed for chondroitin sulfate disaccharide. Assays were performed in 3 batches: 14 corneas assayed in 2 batches, each containing 7 corneas, and 8 corneas assayed in 1 batch. Corneal samples were obtained from eyes immediately after enucleation. Using an operating microscope, the corneal epithelium was removed with a No. 64 blade.[a] With the same blade, a 12 × 15-mm rectangle was carved in the axial cornea to the level of Descemet's membrane. Using a Martinez corneal dissector,[b] the superficial and middle thirds of the cornea were removed and individually placed in microassay tubes. An equal area of cornea was dissected on the medial and lateral sides of the central core down to Descemet's membrane. These peripheral cores extended to the limbus and were divided into superficial, middle, and deep thirds. The most superficial layers were collected with a Martinez corneal dissector, as described. The deep corneal samples, including Descemet's membrane and endothelium, were collected with corneal scissors and individually placed in microassay tubes. All corneal slices were frozen at –70 C until GAG analysis.

Isolation of the GAG disaccharides was performed by adding the corneal slice to 5 µl of papain mixed with 295 µl of 0.05*M* sodium acetate for overnight digestion at 60 C. After incubation, the supernatant was removed, and 10 µl of 1N NaOH and 110 µl of 10% cetylpyridinyl chloride was added. The sample was incubated for 15 minutes at 20 C and centrifuged at 13,000 × g for 15 minutes. The supernatant was discarded, and the precipitate was washed by adding 1 ml of a saturated sodium acetate-ethanol solution. After gentle rotation, tubes were centrifuged again, and the wash was repeated. Samples were dried for 15 minutes, using a vacuum concentrator. The dried pellet was mixed with 50 µl of 4*M* guanidine hydrochloride and 1 ml of cold ethanol. Samples were incubated for 1 hour at 4 C. After incubation, samples were centrifuged, supernatant was discarded, and incubation with guanidine hydrochloride was repeated. Samples were dried overnight on the vacuum concentrator. The isolated GAG pellet was resuspended in 90 µl of 0.05*M* sodium acetate (pH, 7.3), digested with 10 µl of chondroitinase AC II[c] for 2 hours at 37 C, and assayed by use of capillary zone electrophoresis immediately.

Determination of Δ-disaccharides—The digestion product was analyzed with an automated capillary zone electrophoresis system.[c] Samples were vacuum-loaded onto a 75-µm × 50-cm capillary cartridge and subjected to electrophoresis at 30,000 V in 50 m*M* sodium borate. Disaccharides ΔDi6S and ΔDi4S were detected at 230 nm and quantified by use of peak area after calibration with known amounts of disaccharide standards.[d] Pecentage recovery was not determined, but there was no evidence of chondroitin sulfate in discard supernatants collected during extraction. The area measurements for standards were directly and linearly related to the amount of standard. All area measurements for test samples were within the range of the standards. Coefficient of variation of repeated measures at the low end of the standard curve was +5.3% and at the high end was +3.6%. R values from curve fits of standard curves were > 0.9999. Unsulfated disaccharide (ΔDi0S) was not quantitated because of comigration with hyaluronan in the sample.

Data analysis—A general mixed-effects linear model[e] was used to compare mean ΔDi6S/ΔDi4S values observed at the 6 locations from which the zones were sampled from each cornea. Region (central or peripheral), zone (superficial, middle, or deep), and the interaction between region and zone were modeled as within-cornea fixed effects, whereas cornea and its interactions with the fixed effects were modeled as random effects. The Satterthwaite approximation was used to estimate the denominator *df* for the fixed-effect F tests and comparison of cell means. Assessment of the distributional properties of the residuals obtained from an analysis of the untransformed ΔDi6S/ΔDi4S values indicated that logarithmic transformation of the ratios would adequately improve model fit and thus was used in the definitive analysis. It should be noted that log transformation of the ΔDi6S/ΔDi4S values may also be interpreted as the difference obtained by subtracting log(ΔDi4S) from log(ΔDi6S). Processing batch was also considered as an additional source of random variation (random effect) in the experimental design. However, variance components estimated for batch-related random effects were either negative or negligible and thus dropped from the definitive analysis. A value of $P < 0.05$ indicated significance.

Results

There was a significant ($P < 0.001$) within-core location main effect and a significant ($P = 0.046$) between-core location main effect. Comparison of mean response (mean values of center and peripheral regions) among the 3 within-core locations revealed that the deep ($P < 0.001$) and middle zones ($P = 0.001$) had significantly lower ΔDi6S/ΔDi4S values than the superficial zone did but did not differ significantly ($P = 0.177$) from each other (**Fig 1**). Within the central region, the deep ($P < 0.001$) and middle zones ($P = < 0.001$) had significantly lower ΔDi6S/ΔDi4S values than the superficial zone did but did not differ significantly ($P = 0.809$) from each other. Within the peripheral region, the deep zone had significantly lower ΔDi6S/ΔDi4S values than did the middle ($P = 0.023$) and superficial zones ($P < 0.001$), whereas the ΔDi6S/ΔDi4S values between the middle and superficial zones did not differ significantly ($P = 0.068$). Within the deep zone, the peripheral region had sig-

Unauthenticated | Downloaded 01/29/23 10:28 PM UTC

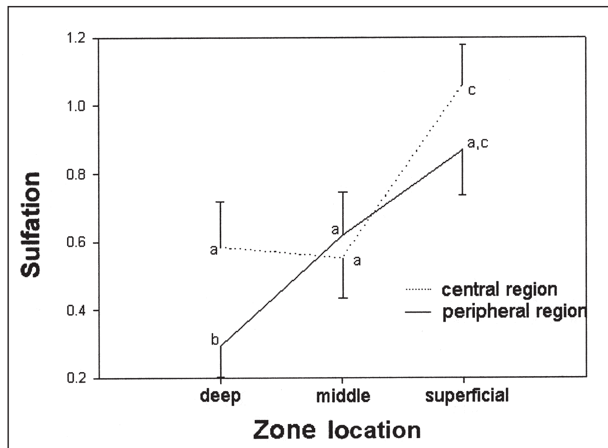

Figure 1—Sulfation of chondroitin sulfate in deep, middle, and superficial zones of the central and peripheral regions of the cornea in horses. Sulfation was estimated by measurement of the ratio of 2 disaccharides, ΔDi6S and ΔDi4S (mean ± SD, log values). [a-c]Values with different superscripts are significantly ($P < 0.05$) different.

nificantly ($P = 0.025$) lower ΔDi6S/ΔDi4S values than the central region mean did. Central and peripheral regions did not differ significantly within the middle and superficial zones. No differences in ΔDi6S/ΔDi4S values were detected with regard to sex, breed, or age; however, the number of horses in the population sample was too small to draw any definitive conclusions from these comparisons.

Discussion

Chondroitin sulfate is located throughout the corneal stroma of several species. In horses, it has been studied in normal and degenerative articular cartilage. Variations in sulfation patterns of chondroitin sulfate in cartilage have been associated with aging and osteoarthritis.[22,23,29]

Regional and zonal differences in ΔDi6S and ΔDi4S in the equine cornea were expected on the basis of reports in other species and may be a result of embryologic development or as a change in the stromal GAG content over time attributable to biological, environmental, nutritional, or species-specific factors. Results of studies[1,30-31] in avian and canine species indicate that the corneal stroma is produced from 2 embryologic sources. The corneal epithelium develops from the surface ectoderm and is an extension of the conjunctival epithelium. The basement membrane of the corneal epithelium is believed to lay down the anterior stroma in much the same way as the conjunctival epithelium lays down the substantia propria on the epibulbar surface. The posterior corneal stroma comes from the neural crest cells of the mesenchyme and is associated with the formation of the anterior chamber, Descemet's membrane, and the corneal endothelium.[1,30] Transmission electron micrographs of corneas from young dogs illustrate regional differences in the appearance of the anterior and posterior stroma that may reflect the embryologic tissue origins.[30] Although, to the authors' knowledge, no specific embryologic studies of the equine cornea have been undertaken, the conserved prenatal origins of corneal stroma across species may suggest that the development of the equine corneal stroma, including regional differences in GAG sulfation, may be inherent to the embryologic origin of the tissue.

Growth, maintenance, and repair of the avascular corneal stroma is provided by nutrient and oxygen diffusion from the aqueous humor, the limbal blood vessels, and the tear film.[1] These 3 energy sources vary in the quality of delivery of nutrients and elimination of wastes, which over time may influence the composition of GAG in the corneal stroma. A marked microenvironmental difference is observed across the corneal tissue.[1] The contaminated external surface is on 1 side of the corneal stroma and is protected by the eyelids, the tear film, and an active epithelial turnover. The sterile aqueous microenvironment bordered by the relatively stable endothelium and associated basement membrane rests on the other side of the corneal stroma. This marked gradation in environments is separated by the 800-mm-thick stroma[32]; variation in GAG sulfation patterns from superficial to deep corneal layers may represent differences in function throughout the depth of the healthy cornea. Regional and zonal variation in GAG composition in healthy corneal tissues may result in different responses to corneal disease, depending on the location of the injury.

Corneal injury specifically requires a high level of nutritive repair with a tremendous influx of leukocytes that are not normally present in the tissue. There is subsequent production of free radicals, collagenases, and proteases that need to be neutralized to prevent irreversible damage ranging from corneal scarring to loss of vision from corneal perforation.[33] Glycosaminoglycan content in corneas of horses has not been studied after inflammation has occurred, but alterations in the corneal extracellular matrix as a result of wound repair are suspected. The severity of focal corneal disease, or a predilection of certain regions of the equine cornea to harbor infection, such as the tropism of fungi for the deep region of the cornea in stromal abscesses, may be related to differences in GAG sulfation within the cornea. Frozen or freshly harvested corneas used in corneal transplant surgeries may undergo postmortem alterations in the extracellular matrix and GAG, which in turn affect the long-term functional clarity of the allograft.

Increasing age has been associated with changes in composition of GAG in degenerative connective tissues of horses. The population sample in our study was not large enough to draw any conclusions regarding the influence of age, but 6-sulfation of terminal and internal chondroitin sulfate residues in GAG chains in articular cartilage increases with advancing age.[29] Altered chondroitin sulfate sulfation ratios also occur in human osteoarthritic cartilage in response to anabolic and catabolic changes.[22,23]

By establishing baseline information regarding chondroitin sulfate composition of the normal equine cornea, future comparisons may be made with diseased corneas. Alterations in distribution of GAG because of tissue injury have been associated with loss of corneal transparency.[34-38] Glycosaminoglycan disorders have been recently implicated in congenital eye anomalies.[39]

Unauthenticated | Downloaded 01/29/23 10:28 PM UTC

Models of corneal wounding reveal a substantial change in synthesis and deposition of GAG.[19-20,40-41] In addition, some drugs and corneal preservation media interfere with GAG metabolism.[42-44] Humoral and cell-mediated mechanisms are likely to be involved in corneal disease associated with abnormalities of GAG.[45-46]

Because the corneal slices used in our study had a mass < 10 mg when dehydrated, absolute amounts of chondroitin sulfate could not be measured accurately. In a number of recent studies on chondroitin sulfate sulfation, important findings in ΔDi6S/ΔDi4S values have been reported, whereas similar findings in terms of concentration of chondroitin sulfate per mg of tissue have not been reported[22-23]; for this reason, significant differences in ΔDi6S/ΔDi4S values were reported for our study.

---

[a]No. 64 blade, Rudolph Beaver Inc, Waltham, Mass.
[b]Martinez corneal dissector, Storz Ophthalmics, St Louis, Mo.
[c]Beckman P/ACE 5510, Beckman Instruments Inc, Brea, Calif.
[d]Seikegaku America Inc, Rockville, Md.
[e]Littell RC, Milliken GA, Stroup WW, et al. *SAS system for mixed models*. Cary, NC: SAS Institute Inc, 1996.

References

1. Gelatt KN, ed. *Veterinary ophthalmology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 1999;3–181.
2. Scott JE. Supramolecular organization of extracellular matrix glycosaminoglycans, in vitro and in the tissues. *FASEB J* 1992; 6:2639–2645.
3. Scott JE. Proteoglycan: collagen interactions in connective tissues. Ultrastructural, biochemical, functional and evolutionary aspects. *Int J Biol Macromol* 1991;13:157–161.
4. Schittny JC, Kresse H, Burri PH. Immunostaining of a heterodimeric dermatan sulfate proteoglycan is correlated with smooth muscles and some basement membranes. *Histochem Cell Biol* 1995;103:271–279.
5. Buddecke E, Wollensack J. Biochemistry and pathobiochemistry of proteoglycans of the eye. *Fortschr Ophthalmol* 1989;86: 502–508.
6. Nakamura M, Kobayashi M, Hirano K, et al. Glycosaminoglycan and collagen interactions in the mouse corneal stroma. *Matrix Biol* 1994;14:283–286.
7. Axelsson I. Heterogeneity, polydispersity, and physiologic role of corneal proteoglycans. *Acta Ophthalmol* 1984;62:25–38.
8. Li W, Vergnes JP, Cornuet PK, et al. cDNA clone to chick corneal chondroitin/dermatan sulfate proteoglycan reveals identity to decorin. *Arch Biochem Biophys* 1992;296:190–197.
9. Midura RJ, Hascall VC. Analysis of the proteoglycans synthesized by corneal explants from embryonic chicken. Structural characterization of the keratan sulfate and dermatan sulfate proteoglycans from corneal stroma. *J Biol Chem* 1989;264:1423–1430.
10. Takahashi T, Cho HI, Kublin CL, et al. Keratan sulfate and dermatan sulfate proteoglycans associate with type IV collagen in fetal rabbit cornea. *J Histochem Cytochem* 1993;41:1447–1457.
11. Rada JA, Fini ME, Hassel JR. Regionalized growth patterns of young chicken corneas. *Invest Ophthalmol Vis Sci* 1996;37: 2060–2067.
12. Cintron C, Covington HI. Proteoglycan distribution in developing rabbit cornea. *J Histochem Cytochem* 1990;38:675–684.
13. Meek KM, Leonard DW. Ultrastructure of the corneal stroma: a comparative study. *Biophys J* 1993;64:273–280.
14. Nakazawa K, Isomura T, Shimoeda S. Proteoglycan synthesis by chicken corneal explants. *J Biochem* 1995;117:697–718.
15. Coster L, Rosenberg LC, van der Rest M, et al. The dermatan sulfate proteoglycans of bovine sclera and their relationship to those of articular cartilage. *J Biol Chem* 1987;262:3809–3812.
16. Bettleheim FA, Goetz D. Distribution of hexosamines in bovine cornea. *Invest Ophthalmol Vis Sci* 1976;15:301–304.
17. Castoro JA, Bettelheim AA, Bettelheim FA. Water gradients across bovine cornea. *Invest Ophthalmol Vis Sci* 1988;29:963–968.
18. Arai H, Kashiwagi S, Nagasaka Y, et al. Oxidative modification of apolipoprotein E in human very-low-density lipoprotein and its inhibition by glycosaminoglycans. *Arch Biochem Biophys* 1999; 367:1–8.
19. Kato T, Nakayasu K, Ikegami K, et al. Analysis of glycosaminoglycans in rabbit cornea after laser excimer keratectomy. *Br J Ophthalmol* 1999;83:609–612.
20. Brown CT, Applebaum E, Banwatt R, et al. Synthesis of stromal glycosaminoglycans in response to injury. *J Cell Biochem* 1995; 59:57–68.
21. Fernandez-Botran R, Yan J, Justus DE. Binding of interferon γ by glycosaminoglycans: a strategy for localization and/or inhibition of its activity. *Cytokine* 2001;11:313–325.
22. Lewis S, Crossman M, Flannelly J, et al. Chondroitin sulfate patterns in synovial fluid in osteoarthritis subsets. *Ann Rheum Dis* 1999;58:441–445.
23. Shibata T, Murakami K, Kubota E, et al. Glycosaminoglycan components in temporomandibular joint synovial fluid as markers of joint pathology. *J Oral Maxillofac Surg* 1998;56:209–213.
24. Hendrix DVH, Brooks DE, Smith PJ, et al. Corneal stromal abscesses in the horse: a review of 24 cases. *Equine Vet J* 1995;27: 440–447.
25. Whittaker CJG, Smith PJ, Brooks DE, et al. Therapeutic penetrating keratoplasty for deep corneal stromal abscesses in eight horses. *Vet Comp Ophthalmol* 1997;7:19–28.
26. Hamilton HL, McLaughlin SA, Whitely EM, et al. Histological findings in corneal stromal abscesses of 11 horses: correlation with cultures and cytology. *Equine Vet J* 1994;26:448–453.
27. Nassise MP, Nelms S. Equine ulcerative keratitis. *Vet Clin North Am Equine Pract* 1992;8:537–555.
28. Neaderland MH, Riis RC, Rebhun WC, et al. Healing of experimentally induced corneal ulcers in horses. *Am J Vet Res* 1987; 48:427–430.
29. Brown MP, West LA, Merritt KA, et al. Changes in sulfation patterns of chondroitin sulfate in equine articular cartilage and synovial fluid in response to aging and osteoarthritis. *Am J Vet Res* 1998; 59:786–791.
30. Shively JN, Epling GP. Fine structure of the canine eye: cornea. *Am J Vet Res* 1970;31:713–722.
31. Johnston MC, Noden DM, Hazelton RD, et al. Origins of avian ocular and periocular tissues. *Exp Eye Res* 1979;29:27–43.
32. Van der Woerdt A, Gilger BC, Wilkie DA, et al. Effect of auriculopalpebral nerve block and intravenous administration of xylazine on intraocular pressure and corneal thickness in horses. *Am J Vet Res* 1995;56:155–158.
33. Stern GA, Schultz GS. The pathogenesis of bacterial infections of the cornea. In: Tasman W, Jeager EA, eds. *Duane's foundations of clinical ophthalmology* [Book on CD Rom]. Philadelphia: Lippincott Williams & Wilkins, 1996;42:1–11.
34. Constantanopoulos G, Scott JA, Shull RM. Corneal opacity in canine MPS I. Changes after bone marrow transplantation. *Invest Ophthalmol Vis Sci* 1989;30:1802–1807.
35. Hodson S, Kaila D, Hammond S, et al. Transient chloride binding as a contributory factor to corneal stromal swelling in the ox. *J Physiol (Lond)* 1992;450:89–103.
36. Kangas TA, Edelhauser HF, Twining SS, et al. Loss of stromal glycosaminoglycans during corneal edema. *Invest Ophthalmol Vis Sci* 1990;31:1994–2002.
37. Menasche M, Savoldelli M, Pouliquen Y. The keratocyte or fibroblast of the cornea: morphological and biochemical characteristics in normal stroma and a few cases of corneal dystrophy. *Pathol Biol (Paris)* 1992;40:871–878.
38. Quantock AJ, Meek KM, Brittain P, et al. Alteration of the stromal architecture and depletion of keratan sulphate proteoglycans in oedematous human cornea: histological, immunochemical and X-ray diffraction evidence. *Tissue Cell* 1991;23:593–606.
39. Ozeki H, Shirai S, Ikeda K, et al. Histochemical studies on two cases of Peter's anomaly. *Nippon Ganka Gakkai Zasshi* 1996;100: 471–477.

Unauthenticated | Downloaded 01/29/23 10:28 PM UTC

40. Cintron C, Gregory JD, Damle SP, et al. Biochemical analyses of proteoglycans in rabbit corneal scars. *Invest Ophthalmol Vis Sci* 1990;31:1975–1981.

41. Saika S, Uenoyama K, Hiroi K, et al. Ascorbic acid phosphate ester and wound healing in rabbit corneal alkali burns: epithelial basement membrane and stroma. *Graefes Arch Clin Exp Ophthalmol* 1993;231:221–227.

42. Fischer J, Hein L, Lullmann-Rauch R, et al. Tilorone-induced lysosomal lesions: the biphasic character of the drug is essential for its high potency to cause storage of sulphated glycosaminoglycans. *Biochem J* 1996;315:369–375.

43. Fischer J. Drug-induced glycosaminoglycan storage: dose-dependent changes in the pattern of accumulated glycosaminoglycans in cultured bovine and human fibroblasts. *Biochem Pharmacol* 1996;52:1331–1337.

44. Slack JW, Kangas TA, Edelhauser HF, et al. Comparison of corneal preservation media for corneal hydration and stromal proteoglycan loss. *Cornea* 1992;11:204–210.

45. Gottsch JD, Liu SH, Minkovitz JB, et al. Autoimmunity to a cornea-associated stromal antigen in patients with Mooren's ulcer. *Invest Ophthalmol Vis Sci* 1995;36:1541–1547.

46. Gottsch JD, Liu SH, Stark WJ. Mooren's ulcer and evidence of stromal graft rejection after penetrating keratoplasty. *Am J Ophthalmol* 1992;113:412–417.

Unauthenticated | Downloaded 01/29/23 10:28 PM UTC