Site-Dependent Differences in Collagen Lamellae in the Corneal Substantia Propria of Beagle Dogs

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ABSTRACT. The fine structure in the center and periphery of the cornea of 16 beagle dogs were characterized and compared. The central cornea (about 540 μm) was apparently thinner than the peripheral cornea (about 720 μm). Thickness ratios of the corneal substantia propria to the entire cornea were approximately 86% in both portions. In addition, number of collagen lamellae, collagen fibril diameter, and collagen fibril index of the central substantia propria are different from those of the periphery (253 vs 236 lamellae, 29.1 vs 32.0 nm, and 39.0 vs 41.6%, respectively). These differences are thought to be due to site-dependent accumulation of proteoglycans (decorin and lumican) which are responsible for production of thin fibrils. The central portion with higher proteoglycans would have abundant thin fibrils with less slippage but better elasticity to buffer against the direct impact of intraocular pressure on the cornea. In contrast, thick fibrils in the peripheral substantia propria would contribute to the maintenance of tensile strength acting on the transition zone between the cornea and sclera.

KEY WORDS: canine, collagen fibrils, collagen lamellae, cornea, corneal substantia propria.

The general structure of the cornea has been described in many textbooks [1, 6, 17, 18]. With the recent development of technology in regenerative medicine, biomedical studies on the cornea of dogs and other animals have been increasing [11, 19, 21]. An understanding of the fine structure of the normal canine cornea is essential for the development of corneal tissue engineering for treatment of corneal diseases. The fine structure of the corneal substantia propria, which occupies approximately 90% of the entire thickness of the cornea, has not yet been completely determined. The objective of this study was to characterize and compare the fine structures in the central portion and peripheral portion of the normal canine substantia propria of beagle dogs.

The use of experimental animals was approved by the Ethics Committee of Rakuno Gakuen University, Japan, and compliance with the NIH Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892]. Sixteen male beagles at the age of 16.8 ± 7.0 months with body weight of 10.7 ± 1.1 kg were used. After anesthesia with 25 mg/kg of pentobarbital sodium, IV (Kyoritsu Seiyaku Corporation, Tokyo, Japan), these animals were euthanized by exsanguination. Then, the left eyeball was surgically removed from each dog. Twelve eyeballs were perfused with 10% formalin, and bisected in the median plane by using a razor blade for measurements of central and peripheral corneal thicknesses at ten sites for each sample. Four eyeballs were fixed in 3.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hr at room temperature. Then, 2-mm 2 corneal samples were excised from the center and periphery of the eyeballs and post-fixed in 1.0% osmium tetroxide in 0.1 M phosphate buffer for 1 hr at room temperature. Thereafter, the samples were washed with 0.1 M phosphate buffer (pH 7.4), dehydrated in a graded ethanol series, and embedded in Quetol 812 (Nissin EM, Tokyo, Japan). Semithin sections were stained with 2.0% toluidine blue and examined by a light microscope. Thickness of the whole cornea and corneal substantia propria was measured at ten sites for each sample using Image J software (version 1.30). Thickness ratio of the substantia propria to the whole cornea was calculated. Ultrathin sections were stained with 1.0% uranyl acetate and 1.0% lead citrate and examined by a transmission electron microscope (JEM-1220; JEOL, Tokyo, Japan). The averages of thickness of collagen lamellae were calculated and measurement of collagen fibril index CFI were calculated. CFI is the percentage of area covered by collagen and represents a collagen-to-non collagen ratio in the extracellular matrix [5]. Student’s t-test was used for data analysis and comparison between the central and peripheral portions of the cornea. Statistical significance was considered at the level of p=0.05.

Thickness of the central cornea (540.8 ± 205.5 μm) was significantly lower than that of the peripheral cornea (724.6 ± 178.3 μm) (Fig. 1). However, thickness ratio of the corneal substantia propria to the whole cornea was approximately 86% in both portions. There was no significant
difference between the number of collagen lamellae in the central portion (253 ± 49 lamellae) and that in the peripheral portion (236 ± 22 lamellae). Collagen lamellae in the central portion (796.2 ± 426.9 nm) tended to be thinner than those in the peripheral portion (1,113.5 ± 562.9 nm) (Fig. 2). CFI of the central portion (29.1 ± 3.8 nm and 39.0 ± 4.9%) were significantly lower than those of the peripheral portion (32.6 ± 3.5 nm and 41.6 ± 4.8%) (Fig. 2). These results were summarized in Table 1.

The difference between thickness of the central portion and that of the peripheral portion of the cornea in the beagle dog is somewhat consistent with that found in other species [1, 17, 18]. Such a site-dependent difference appears to be attributable to the difference in thickness of the substantia propria, which occupies a large ratio of the cornea. The substantia propria is an important area for light passing through in the cornea. In the corneal substantia propria, collagen fibril diameter at the central portion was approximately 10% smaller than that at the peripheral portion, and CFI was also significantly lower at the central portion than at the peripheral portion by approximately 5%. These factors appeared to be responsible for the difference in thickness of the collagen lamellae, which reflected the difference in corneal thickness.

The difference in collagen fibril diameter would reflect difference in the composition ratio of collagen molecular species and proteoglycans [2, 3, 7, 15, 20]. Furthermore, the amount and types of proteoglycans could affect the arrangement and density of collagen fibrils [8, 9]. Collagen from both corneal portions was therefore extracted and site-dependent difference in the composition ratio of collagen molecular species was determined. As a result, type I (\(\alpha_2\)) to type V (\(\alpha_1\)) collagen ratios in both portions were approximately 75:25. In addition, ratios of decorin to lumican in the central portion and the peripheral portion were 1:0.64 and 1:0.74, respectively (unpublished data). Although there was no apparent site-dependent difference in the composition ratio of collagen molecular species, the amount of decorin and lumican, the two major proteoglycans accumulated in the corneal substantia propria, were found to be larger at the central portion than at the peripheral portion. Both proteoglycans have been shown to inhibit an increase in collagen fibril diameter and regulate fibrillar spacing [4, 9, 15]. Therefore, the larger accumulation of proteoglycans in the central portion may be important for the production of many small diameter collagen fibrils, resulting in thinness of the
entire cornea. The central portion of the cornea was abundant in the collagen fibrils of small diameter, which have been shown to prevent slippage between collagen fibrils and thereby impart their elasticity [10, 12–14, 16]. Since the cornea covers the anterior part of the eyeball and constitutes the outmost layer of the eyeball, these fibrils may also buffer the direct impact of intraocular pressure on the central portion of the cornea. The peripheral portion of the cornea substantia propria was abundant in the collagen fibrils of large diameter, which are characterized by high-density intermolecular crosslink to provide a strong resistance to tensile force [10, 12–14, 16]. Because the periphery of the cornea continues with the sclera [1, 18], the abundance of collagen fibrils with large diameter in the substantia propria of this portion would also provide a strong resistance to tensile force from the adjacent sclera. The present study has revealed that the site-dependent difference in the cornea is closely associated with function and maintenance of the unique shape of the eyeball.

REFERENCES


