Glycosaminoglycans of the Trabecular Meshwork of the Eye in Primary Juvenile Glaucoma

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Significant changes in the qualitative and quantitative composition of glycosaminoglycans (decreased content of sulfated glycosaminoglycans and increased content of collagen-bound proteoglycanes) in the trabecular meshwork of the eye in primary juvenile glaucoma indicate fibrosis of the juxtacanalicular tissue, which was detected in pathomorphological examination of the operation material.

Key Words: *primary juvenile glaucoma; trabecular system of the eye; proteoglycanes; glycosaminoglycans*

Primary juvenile glaucoma (PJG) develops at the age of 11-35 years, has a long latency, and is associated with myopia. Specific features of PJG are its congenital nature, absence of pathological enlargement of the eye and involution of the structures of the anterior eye compartments, and lack of visually discernible pronounced developmental abnormalities in the draining zone [3,4]. The disease clinically manifests, when visual functions are irreversibly lost, and in some cases by the moment of diagnosis one eye is already blind or loss of vision developed or far progressed in one or both eyes, as a result of which young people are disabled [1].

There is still no common concept on the causes of trabecular meshwork pathology in PJG, specific features of the pathological process, and sequence of morphological changes in the trabecular system. It was hypothesized that changes in the trabecular meshwork structure are essential for the development of the glaucoma symptom complex in young patients [5,9,11].

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The biochemical characteristics and physiological functions of the trabecular meshwork of the eye depend on the unique structure and specific interactions between the main components of the cell-cell matrix (collagen and proteoglycanes) [8]. Proteoglycanes (PG) are complex molecules consisting of covalently bound central protein and polysaccharide chains (glycosaminoglycans; GAG) [7]. Changes in the qualitative composition of GAG were detected in PJG; no relationships between these changes and structural transformations in ocular tissues were detected [6,11].

We analyzed quantitative and qualitative composition of GAG and morphological changes in the juxtacanalicular tissue in patients with PJG.

MATERIALS AND METHODS

Fragments of the sclera, trabecular meshwork, and juxtacanalicular tissue (posterior wall of the scleral sinus) were obtained at surgical intervention on 14 eyes of 10 patients aged 22-35 years with stages I, II, and III PJG. The aim of the intervention was to reduce intraocular pressure. Autopsied eyes of patients of the same age served as the control. The tissues were fixed in 10% neutral formalin. Histological sections were stained with hematoxylin and eosin, after Van Gieson, with toluidine blue at dif-

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ferent pH, with alcian blue, after Hayle, and periodic acid Schiff (PAS) reaction was carried out. Acid phosphatase was detected by the azo coupling reaction.

In order to detect PG, crushed samples were poured over with 4 M solution with inhibitors (0.1 M aminohexenoic acid, 0.05 M EDTA, 0.01 M N-ethylenamide, 0.01 M benzamidine chloride), the solution buffered with 0.05 M sodium acetate to a final concentration of 2 M (10 ml/g tissue). Incubation was carried out for 72 h at 4°C, after which the solution was dialyzed against 50 mM Na acetate buffer (pH 5.8) for 18 h at 4°C. Proteins were precipitated by adding 100% trichloroacetic acid to a final concentration of 5% to samples, centrifuged, and the solution was dialyzed against 50 mM Na acetate buffer (pH 5.8) for 18 h at 4°C. PG was precipitated with 3 volumes of 96% ethanol with 4% potassium acetate with subsequent centrifugation; the precipitate was dissolved in deionized water [2].

GAG were isolated from the tissue with papain solution in 0.2 M Na acetate buffer (pH 5.8) with 0.01 M EDTA and 0.01 M cysteine for 18 h at 60°C [10]. GAG specimens were purified according to the same protocol as PG. The content of GAG was evaluated by the content of uronic acids and sulfated GAG.

The results are presented in μg/ml dry tissue. Chondroitin sulfate C (ICN) served as the standard. The results were statistically processed using Student's *t* test.

RESULTS

Biochemical analysis of specimens of the trabecular meshwork of the eye in PJG showed that the content of sulfated GAG in the easily available first pool of PG (PG_1) virtually did not differ from the control (Table 1), while the difference in the content of uronic acids was significant. The difference in the content of sulfated GAG was significant in the not easily available PG pool (PG_2); the content of uronic acids in PJG surpassed the control values significantly (Table 1). The ratio of sulfated GAG and uronic acids in PG_1 in PJG was 0.53 *vs.* 1.57 in the control; 0.43 in PG_2 *vs.* 0.34, respectively.

Water content in the trabecular meshwork, scleral sinus wall, and sclera in PJG decreased by 20%, the content of dry substance being 72% (52% in the control).

Pathomorphological study of specimens of the juxtacanalicular tissue in stage II (developed) PJG showed decomplectation of the trabecular meshwork, fragmentation of collagen fibers, and changed tinctorial properties of the cells and extracellular matrix (as shown by the alcian blue test). Degeneration, apoptotic transformation, desquamation (Fig. 1, *a*), and compensatory proliferation (Fig. 1, *b*) of the trabecular endothelium are worthy of note.

More deep structural changes were observed in stage III (far advanced) PJG: loss of fibers and hyalinization of collagen structures (Fig. 1, *c*), sharply positive reaction to acid phosphatase in endotheliocytes. Hyalinization was paralleled by the formation of cell-free fields and focal proliferation of fibroblasts (Fig. 1, *d*).

Sulfated GAG in the control samples were located in the strata between fibrous structures and constituted an appreciable part of all diffuse PG. The content of PG_1, realizing diffusion of metabolites, and of sulfated GAG decreased in PJG, while the content of collagen-bound minor PG_2, characteristic of fibrotic tissues, increased.

The detected increased content of collagen-bound PG_2 was confirmed by morphological findings. The metabolism in vessel-free structures depends on the content of hyaluronic acid and chondroitin sulfate in loose connective tissue. This explains structural changes in the functionally most active tissues of the eye.

Changes in the biomechanical loading of tissue exposed to compression forces modulate the type of GAG synthesized by the cells. Reduction of the hydrodynamic properties of the trabecular meshwork in PJG patients leads to an increase in the compression loading of the scleral sinus wall, which modifies the volume and quality of matrix produced by the cells. Moreover, the biomechanical forces modulate the flows of nutritives to the trabecular meshwork, which are commonly determined by dynamic forces, acting as a pump, and by passive diffusion. These flows originate from the iridal capillary system, ciliary body, and vascular membrane. Alteration of the trabecular meshwork structure deteriorates the conditions of blood supply to the cells and their trophics, which, eventually, modifies tissue metabolism. These changes can be compared with those observed in elderly people. It can be expected that changes in cell metabolism in the trabecular system resulting from increased intra-

TABLE 1. Content of GAG (μg/mg) in Specimens of Trabecular Meshwork of the Eye ($M \pm m$; $n=14$)

Parameter		Control	PJG
PG_1	sulfated GAG	0.286±0.026	0.319±0.290
	uronic acids	0.203±0.012	0.539±0.032*
PG_2	sulfated GAG	51.777±4.660	86.750±7.808*
	uronic acids	151.652±9.100	201.000±12.060*

Note. *$p<0.001$ compared to control.

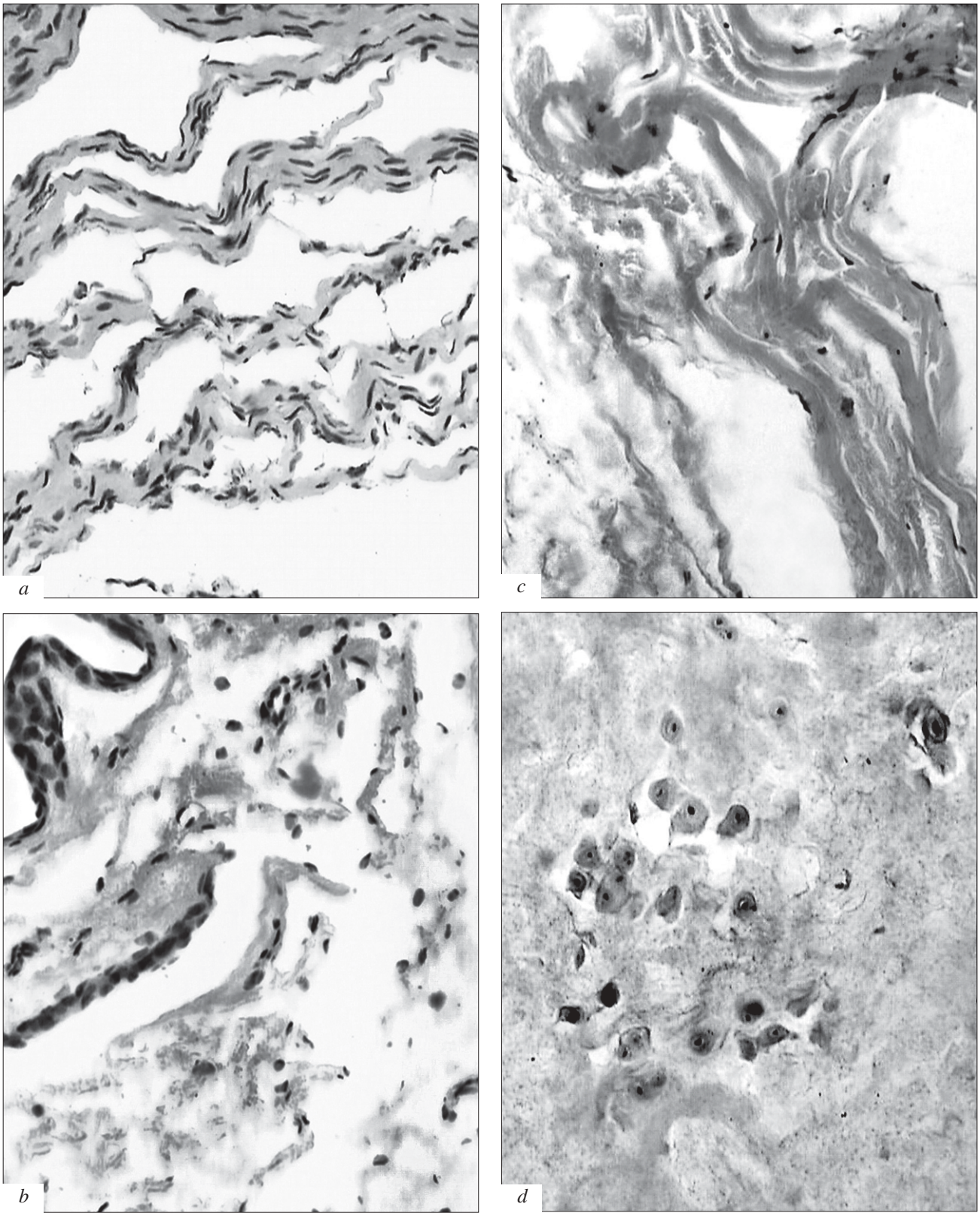

Fig. 1. Light microscopy of the juxtacanalicular tissue in developed PJG. *a*) degeneration and desquamation of endothelium; *b*) compensatory proliferation of trabecular endotheliocytes; *c*) loss of fibers and homogenization of collagen structures; *d*) fibrosis of trabecular structures, accumulation of proliferating fibroblasts. Hematoxylin and eosin (*a-c*) and alcian blue (*d*) staining, ×200.

ocular pressure, lead to restructuring with GAG synthesis type intrinsic of this tissue.

These characteristics of GAG in the trabecular meshwork and sclera cannot result from just the hydrodynamic effect of intraocular pressure and decreased intensity of metabolic processes in the tissues and age-associated changes. They are secondary and are determined by genetic disorders; this is confirmed by the analysis of medical histories of 42 families with cases of identified PJG, inherited by the autosomal dominant type with incomplete genotype penetration, leading to changes in the gene regulation of PG synthesis.

REFERENCES

1. E. A. Egorov, V. N. Alekseyev, E. B. Martynova, and A. O. Khar'kovskii, *Pathogenetic Aspects in the Treatment of Primary Open-Angle Glaucoma* [in Russian], Moscow (2001).
2. T. V. Rusova, V. I. Rykova, A. V. Korel', *et al.*, *Byull. Eksp. Biol. Med.*, **139**, No. 6, 738-740 (2005).
3. E. G. Sidorov and M. G. Mirzayants, *Congenital Glaucoma and Its Treatment* [in Russian], Moscow (1991).
4. L. E. Teplinskaya and E. V. Mazanova, *Vestn. Oftalmol.*, No. 5, 39-42 (1999).
5. A. Bakunowicz-Lazarczyk, M. Sulkowska, S. Sulkowski, and B. Urban, *J. Submicrosc. Cytol. Pathol.*, **33**, Nos. 1-2, 17-22 (2001).
6. N. Furuyoshi, M. Furuyoshi, R. Futa, *et al.*, *Ophthalmologica*, **211**, No. 3, 140-146 (1997).
7. R. V. Iozzo, *Annu. Rev. Biochem.*, **67**, 609-653 (1998).
8. A. Martos-Rodriguez, I. Santos-Alvarez, V. Campo-Ruiz, *et al.*, *J. Bone Joint Surg.*, **85**, No. 18, 1190-1195 (2003).
9. K. Segawa, *Nippon Ganka Gakkai Zasshi*, **99**, No. 12, 1291-1302 (1995).
10. A. D. Theocharis, M. E. Tsara, N. Papageorgakopoulou, *et al.*, *Biochem. Biophys. Acta*, **1528**, No. 2-3, 81-88 (2001).
11. J. Umihira, S. Nagata, M. Nohara, *et al.*, *Invest. Ophthalmol. Vis. Sci.*, **35**, No. 2, 486-494 (1994).