Glycosaminoglycans of the Vertebral Body Growth Plate in Patients with Idiopathic Scoliosis

**T. V. Rusova, V. I. Rykova*, A. V. Korel',
A. M. Zaidman, and D. S. Tkachev**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 711-713, June, 2005
Original article submitted June 7, 2004

The concentrations of keratan sulfates and unmodified keratan sulfates increased in the vertebral body growth plate in patients with idiopathic scoliosis. Sulfation and acetylation of total glycosaminoglycans decreased by 50 and 30%, respectively. These changes reflect the decrease in biological activity of molecules that modulate function of the growth plate.

Key Words: *idiopathic scoliosis; growth plate; glycosaminoglycans*

Idiopathic scoliosis (IS) is a severe lateral deformation of the vertebral column that causes disability in children and adolescents. IS is a familial and genetic disease [1]. Growth asymmetry can serve as a pathogenetic factor for deformation of the vertebral column. However, fine biochemical mechanisms of this disorder remain unclear. The vertebral body growth plate is an integral structure of the intervertebral disc, which provides growth of the vertebral body. It belongs to cartilaginous tissue, whose matrix mainly consists of proteoglycans (PG) [4]. Molecules of PG have complex structure and consist of protein cortex and covalently bound linear chains of glycosaminoglycans (GAG). GAG carry high negative charge and provide biological function of these molecules. PG are polyfunctional molecules. They play an important role in the growth and proliferation of cells and morphogenesis of tissues [3]. Each tissue has specific GAG that differ in the fine structure of disaccharides. These structural units undergo modification depending on tissue function or type of dysfunction. The composition and properties of GAG in cartilaginous tissue of intervertebral discs during IS were evaluated [2,5]. Little is known about biochemical changes in GAG of the vertebral growth plate in patients with IS.

Novosibirsk Institute of Traumatology and Orthopedics, Russian Ministry of Health; *Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences. ***Address for correspondence:*** trusova@niito.ru. T. V. Rusova

Here we studied structural characteristics of GAG in the vertebral body growth plate in patients with IS and healthy children.

MATERIALS AND METHODS

The growth plates were obtained from the upper part of thoracic spinal deformation during surgical correction of vertebral deformation in 16 patients with IS (degree IV, 14-16 years). The autopsy material obtained during forensic medical examination of 5 cadavers (12-14 years, 2 samples from the thoracic spine) served as the control.

The samples were stored at -20°C. The growth plate was prepared. To isolate GAG the tissue was hydrolyzed with papain (0.2 mg/g wet tissue weight) in 0.2 M sodium acetate buffer (pH 5.8) containing 0.01 M ethylenediaminetetraacetic acid (EDTA) and 0.005 M cysteine at 60°C for 18 h. Proteins were precipitated with 5% trichloroacetic acid and removed by centrifugation. The solution was dialyzed against 50 mM sodium acetate with 0.01 M EDTA (pH 5.8) for 4 and 24 h. After dialysis GAG were precipitated from the solution with a 3-fold volume of ethanol in the presence of 4% potassium acetate at -18°C for 24 h. The precipitate was separated by centrifugation, washed with ethanol, and dissolved in 0.4 M guanidine tetrachloride and 50 mM sodium acetate buffer (pH 5.8) [6]. The concentration of monosaccharides was

measured in this solution. The content of uronic acids was estimated by the standard method with carbazole. The amount of sulfated GAG was determined using 1,9-dimethyl methylene blue. The concentration of hexoses and acetylated or nonacetylated hexosamines was measured after hydrolysis of samples in 0.5 N hydrochloric acid at 100°C for 30 min. The results were analyzed by Student's *t* test.

GAG were identified by horizontal electrophoresis in 1% agarose gel (width 2 mm).The reaction was performed on a 5×10-cm plate using 50 mM barium acetate buffer (pH 5.0, 4°C, 1 h, 10 V/cm^2) and specific enzymes. The samples of GAG were pretreated with chondroitin-ABC and chondroitin-AC lyases (20 mM Tris buffer, pH 7.5), keratanase (20 mM Tris buffer, pH 7.4), and heparin lyase I (50 mM sodium acetate and 50 mM calcium acetate, pH 7.0) at 37°C for 18 h and applied to the gel in parallel to the source samples. After electrophoresis the gel was stained with 0.1% azure A in 10 mM sodium formate/10 mM magnesium chloride. An excess of the staining agent was washed out with 10 mM sodium acetate (pH 5.8). Chondroitin sulfates A, B, and C, keratan sulfate, and heparan sulfate served as the markers. The type of GAG was estimated by the disappearance of a zone or spot in the gel (depending on the enzyme used) [7]. Preparative electrophoresis was performed under the same conditions. The width of gel was increased from 2 to 5 mm. After electrophoresis a GAG-containing zone was cut from the gel, extracted with electrophoresis buffer, dialyzed against water, and used for analysis.

RESULTS

The total yield of GAG estimated by the total concentration of glucuronic acid and galactose or total hexosamine content did not differ in healthy children and patients with IS (26.15 and 24.09 μg/mg, respectively). However, we revealed changes in the ratio between the contents of monosaccharides. The relative content of galactose and glucosamine increased, while the relative content of glucuronic acid and galactosamine decreased (Table 1). These changes reflect an increase in the content of keratan sulfate-typical monosaccharides and decrease in the content of chondroitin sulfate-typical monosaccharides. Quantitative changes in monosaccharides were accompanied by profound structural variations. In patients with IS the amount of nonacetylated glucosamines increased compared to normal (40 and 9%, respectively). Galactosamines were unidentified in healthy children, but revealed in patients with IS (30% of total monosaccharide concentration). The number of sulfate groups in total GAG decreased by 2 times in patients with IS.

GAG identification by electrophoresis in 1% agarose gel and GAG-specific enzymes confirmed analytical data. GAG of the vertebral body growth plate in healthy children and patients with IS included chondroitin sulfates AC and keratan sulfate (Fig. 1). We

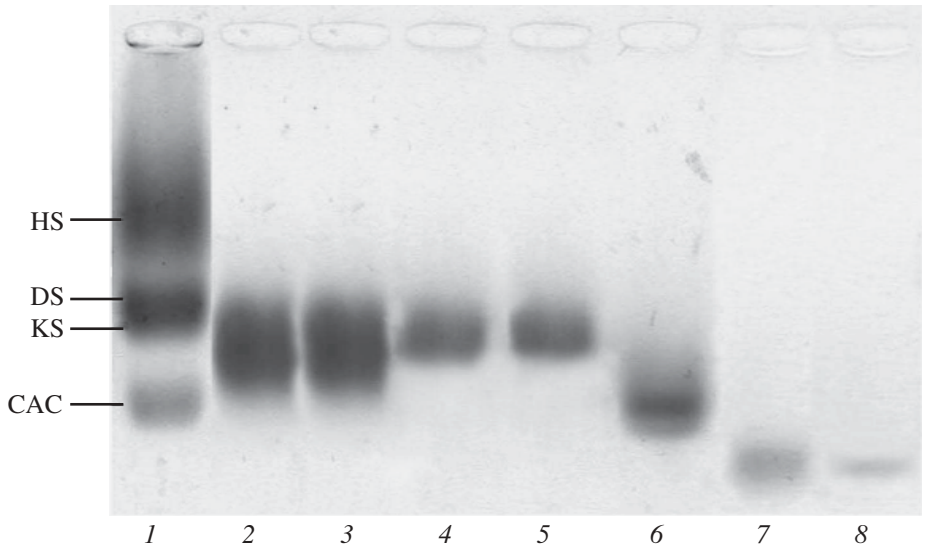

Fig. 1. Electrophoresis of glycosaminoglycans (GAG) from the vertebral body growth plate (1% agarose gel in 50 mM sodium acetate buffer, pH 5.0). Mixture of standards (HS, heparan sulfate; DS, dermatan sulfate; KS, keratan sulfate; CAC, chondroitin sulfate AC; *1*); source preparation of IS GAG (*2*); source preparation of control GAG (*3*); IS GAG after treatment with chondroitin-ABC lyase (*4*); IS GAG after treatment with chondroitin-AC lyase (no changes in spot *4* reflects the absence of dermatan sulfate, *5*); keratanase (hydrolysis of the upper part in the spot reflects the presence of keratan sulfate, *6*); fraction with greater mobility compared to CAC (appearance after subsequent hydrolysis of source GAG with specific enzymes, *7*); source preparation of control GAG after consecutive treatment with specific enzymes (concentration of mobile GAG is 2-3-fold lower compared to the control).

TABLE 1. Concentration of GAG Monosaccharides (μg/mg wet tissue weight) in the Vertebral Body Growth Plate of Healthy Children and Patients with IS (*M*±*m*)

GAG components	Control (*n*=10)	IS (*n*=16)
Glucuronic acid	14.50±1.02	5.48±0.47*
Galactose	11.70±1.39	17.70±1.62*
N-Acetylgalactosamine	15.2±1.5	6.20±0.67*
N-Acetylglucosamine	10.9±1.1	18.10±1.42*
Nonacetylated galactosamine, %		29.80±2.43*
Nonacetylated glucosamine, %	8.55±0.69	38.80±3.91*
Sulfated GAG	17.80±1.56	8.90±0.76*

Note. *$p<0.05$ compared to the control.

also revealed a fraction of GAG not hydrolyzed with GAG-specific enzymes (chondroitin-ABC and chondroitin-AC lyases, keratanase, and heparin lyase I). The content of this GAG fraction in patients with IS was 2-3-fold higher compared to normal (Fig. 1, fragments *6* and *7*, treatment of the gel plate with a similar amount of experimental and control samples). Isolation of this fraction by preparative electrophoresis and study of the monosaccharide composition showed that it includes galactose and glucosamine. These monosaccharides enter the composition of disaccharide units in keratan sulfate. Staining of the gel with azure A turned this fraction to a rose color (similarly to keratan sulfate). It should be emphasized that chondroitin sulfates changed to a blue color. Monosaccharides of this fraction did not have acetyl and sulfate groups. Due to the absence of sulfate groups, this fraction could not be disintegrated with keratanase that hydrolyzes only sulfated GAG [8].

Our results show that IS is accompanied by profound changes in the composition and structure of GAG in the vertebral body growth plate, which reflects activation of keratan sulfate synthesis. A decrease in the concentration of sulfated GAG and appearance of a considerable amount of unmodified keratan can be associated with inactivation of acetylases and sulfatases. It cannot be excluded that molecular changes in GAG during IS modulate function of the vertebral body growth plate.

REFERENCES

1. T. I. Axenovich, A. M. Zaidman, I. V. Zorkoltseva, *et al*., *Am. J. Med. Genet.*, **86**, No. 4, 389-394 (1999).
2. J. Antoniou, V. Arlet, T. Goswami, *et al*., *Spine*, **26**, No. 10, E198-E206 (2001).
3. R. V. Iozzo, *Annu. Rev. Biochem*., **67**, 609-652 (1998).
4. A. Martos-Rodriguez, I. Santos-Alvarez, V. Campo-Ruiz, *et al*., *J. Bone Joint Br.*, **85**, No. 18, 1190-1195 (2003).
5. J. Melrose, K. R. Gurr, T. C. Cole, *et al*., *J. Orthop. Res*., **9**, No. 1, 68-77 (1991).
6. A. D. Theocharis, M. Tsara, N. Papageorgakopoulou, *et al*., *Biochim. Biophys. Acta*, **1528**, Nos. 2-3, 81-88 (2001).
7. C. H. van de Lest, E. M. Versteeg, J. H. Veerkamp, and T. H. van Kuppevelt, *Anal. Biochem*., **221**, No. 2, 356-361 (1994).
8. K. Yamagishi, K. Suzuki, K. Imai, *et al*., *J. Biol. Chem*., **278**, 25,766-25,772 (2003).