

EXTRACELLULAR MATRIX CHANGES IN URETHRAL STRICTURE DISEASE

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ABSTRACT

Purpose: Glycosaminoglycans (GAGs) and collagen are major components of the extracellular matrix and they have key roles in fibrotic diseases. Little is known about the molecular environment in urethral stricture and the majority of the studies available focused on collagen analysis. However, to our knowledge there are no data on GAG composition in urethral stricture disease.

Materials and Methods: Bulbar urethral strictured segments were obtained from 10 patients 18 to 61 years old (mean age 41.8) who underwent end-to-end anastomotic urethroplasty. GAGs in dry tissue samples were extracted by papain digestion and cetylpyridinium chloride/ethanol precipitation. The concentration of total GAGs was assessed by hexuronic acid assay and expressed in μg . hexuronic acid per mg. dry tissue, while the proportion of sulfated GAGs was determined by agarose gel electrophoresis. The concentration of hyaluronic acid was determined by ion exchange chromatography and total tissue collagen was estimated as its hydroxyproline content. The control group consisted of 10 bulbar urethras obtained from fresh normal cadavers 22 to 53 years old (mean age 32.8).

Results: Mean total GAG concentration plus or minus standard deviation in the stricture group was 1.09 ± 0.13 , which was significantly lower than in controls ($p < 0.05$). While the predominant GAG in normal bulbar urethras was hyaluronic acid, dermatan sulfate predominated in strictured urethras (mean $44.1\% \pm 8.4$ and $45.6\% \pm 7.7\%$, respectively). Hyaluronic acid decreased 49.9% and dermatan sulfate increased 68.3%. There were no significant changes in the concentration of heparan sulfate or chondroitin sulfate in normal and strictured bulbar urethras. Mean total collagen significantly increased 32.3% ($p < 0.05$).

Conclusions: Composition changes in GAGs in strictured urethras could contribute to the noncompliant nature of urethral scar tissue and cause functional changes. These results may be useful for defining new targets for therapy for urethral stricture disease.

KEY WORDS: urethra, urethral stricture, extracellular matrix; glycosaminoglycans, collagen

Regardless of its etiology, the consequence of urethral injury is the formation of scar tissue that may decrease the caliber of the urethral lumen and result in stricture. However, the cascade of events that occur in human urethral wound healing has not been as carefully studied as in other tissues, for example skin.¹ Also, urethral healing has been characterized by modest histological techniques in the majority of studies available.^{2–4} Since pharmacological control of undesirable scar tissue is theoretically possible, a better understanding of the molecular and cell biology of urethral healing is necessary for defining new targets for therapy.

Molecular changes that occur in the urethral extracellular matrix (ECM) are still little understood. The ECM has an important role in the biophysical properties of tissues and far from being an inert scaffold around cells it actively orchestrates the key steps of wound healing.⁵ The regulation of ECM deposition is required for normal healing. Matrix molecules must be rapidly synthesized during the formation of granulation tissue and during the final replacement by mature connective tissue and tissue remodeling.⁶ An altered proteolytic balance favors the accumulation of ECM and can produce a decrease in tissue compliance. Excessive deposi-

tion of connective tissue is the pathological hallmark of fibrotic conditions, as in urethral stricture, and it can cause functional alterations and clinical problems.

Glycosaminoglycans (GAGs) are heteropolysaccharide chains composed of disaccharide repeating units, in which 1 sugar is a hexosamine and the other is a neutral sugar or uronic acid.⁷ Except for hyaluronic acid, which is not sulfated and occurs as free chains in tissues, each GAG is covalently linked to a protein, forming proteoglycans, which are important components of the cell surface and ECM. Individual proteoglycans interact specifically with other matrix components, such as collagen or growth factors.⁸ These interactions are responsible for structural organization of the ECM, regulation of cell-cell and cell-matrix interaction, and modulation of the cytokine effect. Therefore, GAGs have a key role in wound healing.⁹

However, little is known about GAG changes in the course of scarring tissues other than skin. Recently the composition of GAGs was described in different segments of the normal male urethra,¹⁰ although to our knowledge there are no data on GAG composition in urethral pathological conditions. In addition to determining total collagen content, in the current study we analyzed the composition changes of GAGs in urethral stricture disease.

MATERIALS AND METHODS

We used bulbar urethral strictured segments obtained from 10 patients 18 to 61 years old (mean age 41.8) who underwent

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end-to-end anastomotic urethroplasty. The local committee on human research approved the investigation. The etiology of stenosis was trauma in 6 cases, infection in 2 and idiopathic in 2. The control group consisted of 10 bulbar urethras obtained from fresh, macroscopically normal cadavers 22 to 53 years old (mean age 32.8). Death was due to causes not related to the urogenital tract. The time elapsed between death and specimen extraction was 4 to 10 hours (mean 6).

Blood residues and eventual secretions of intraurethral glands were removed with saline and the material was immediately immersed in 10 volumes of acetone. These samples were then finely minced and defatted in chloroform-methanol, 2:1 volume per volume. Total collagen in the tissue was estimated as its hydroxyproline content¹¹ and total GAGs were extracted by a previously described protocol.^{10, 12}

The concentration of total GAGs was assessed by hexuronic acid assay¹³ and expressed in μg . hexuronic acid per mg. dry tissue. Total GAGs were submitted to agarose gel electrophoresis in 0.05 M. 1,3-diaminopropane-acetate buffer, pH 9.0.¹⁴ The relative concentration of sulfated GAGs (chondroitin sulfate, dermatan sulfate and heparan sulfate) was determined by densitometry of toluidine blue stained gel using commercially available software. Total GAGs were fractionated by ion exchange chromatography on DEAE-Sephacel (Pharmacia, Piscataway, New Jersey) columns eluted with a linear gradient of 0.1 \rightarrow 0.9 M. NaCl. The 3 peaks obtained using previously described procedures¹² were identified as hyaluronic acid, heparan sulfate and chondroitin sulfate plus dermatan sulfate. The peak area corresponding to hyaluronic acid was calculated and the concentration was expressed as a percent of total GAGs. Between group differences were determined by the Mann-Whitney U test. All numerical data are expressed as the mean \pm 1 standard deviation (SD) and $p < 0.05$ was considered significant.

RESULTS

Mean total concentration of GAGs in strictured bulbar urethras was $1.09 \pm 0.13 \mu\text{g}$. hexuronic acid per mg. dry tissue, which was significantly lower than in the control group ($p < 0.05$), fig. 1. While the predominant GAG in normal bulbar urethras was hyaluronic acid, dermatan sulfate predominated in the urethral stricture group (mean $44.1\% \pm 8.4\%$ and $45.6\% \pm 7.7\%$, respectively, fig. 2). Hyaluronic acid

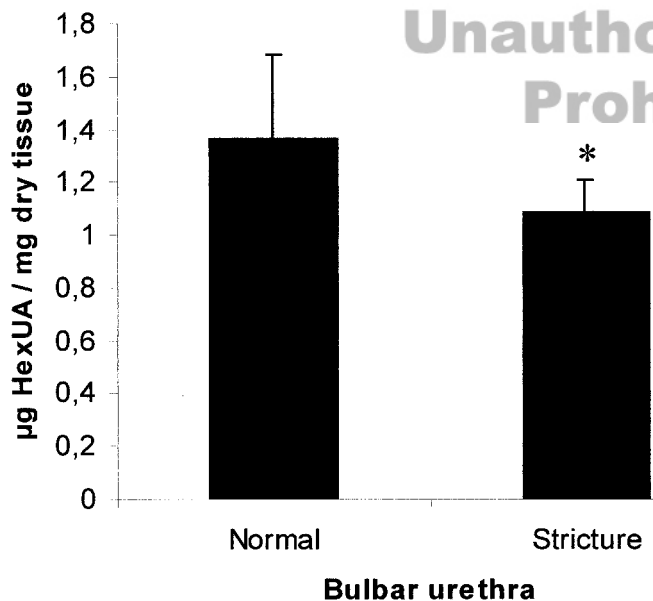


FIG. 1. Mean concentration of total GAGs in 10 normal and 10 strictured segments of bulbar urethra was significantly lower in stricture group. *HexUA*, hexuronic acid. Error bars represent 1 SD. Asterisk indicates $p < 0.05$.

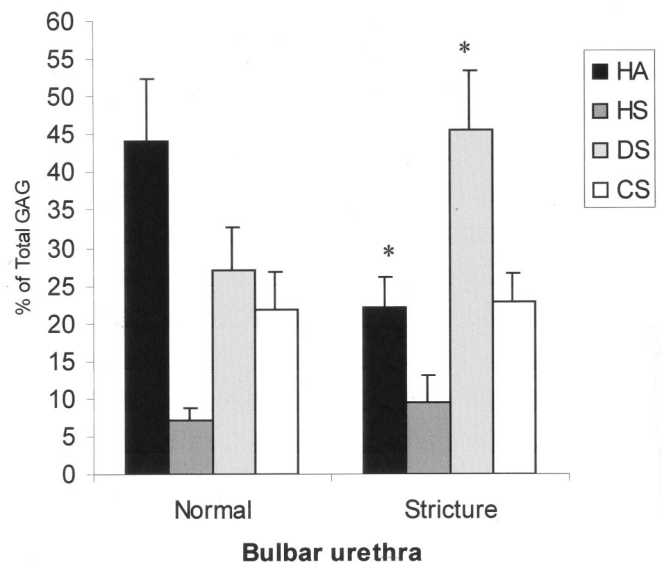


FIG. 2. Mean relative concentration of GAGs in 10 normal and 10 strictured segments of bulbar urethra. While predominant GAG in normal urethras was hyaluronic acid (HA), dermatan sulfate (DS) predominated in urethral stricture group. Note conspicuous increase in dermatan sulfate and decrease in hyaluronic acid in stricture group. HS, heparan sulfate. CS, chondroitin sulfate. Error bars represent 1 SD. Asterisk indicates $p < 0.001$.

in the stricture group significantly decreased 49.9% ($p < 0.001$) and the concentration of dermatan sulfate significantly increased 68.3% ($p < 0.001$). Despite the 35.7% increase in heparan sulfate concentration in strictured bulbar urethras it was not significant ($p = 0.162$). The concentration of chondroitin sulfate remained unchanged ($p = 0.734$). Mean total collagen concentration in strictured bulbar urethra significantly increased 32.3% ($p < 0.05$, fig. 3, A). The GAG-to-collagen ratio was significantly lower in the urethral stricture group ($p < 0.01$, fig. 3, B).

DISCUSSION

Wound healing is a complex process involving the ECM, cells and cytokines. Heterogeneity in GAG chains associated with different types of proteoglycans may provide sites for the interaction of growth factors with ECM components.⁷ In fact, heparan sulfate proteoglycans are important cell-surface receptor in wound healing and specific structural domains show anti-proliferative activity for smooth muscle cells.⁸ Urethral strictures are composed of dense fibrous tissue with high collagen content and scarce smooth muscle cells,² a characteristic that should be related to urethral heparan sulfate.^{15, 16} Heparan sulfate has a heterogeneous distribution along the spongy urethra and its highest concentration is localized in the bulbar urethra.¹⁰

We exclusively analyzed strictures of the bulbar urethra, which is the most commonly injured segment, because the concentration and composition of GAGs varies in the several segments of the spongy urethra.¹⁰ Obtaining accurate controls for precise comparisons is difficult,¹⁷ although we were able to examine an adequate group of normal bulbar urethras with a significant number of age matched samples. In regard to the strategy for investigating ECM component changes an accurate control group avoids important bias. For example, Baskin et al evaluated changes in the collagen metabolism of human urethral strictures.¹⁷ They observed no significant differences in the amount of total collagen on hydroxyproline analysis. However, they did not use an adequate control group. Although we agree that the main change in collagen metabolism in urethral stricture disease is probably a decreased ratio of collagen III:I, we also noted a significant increase in total

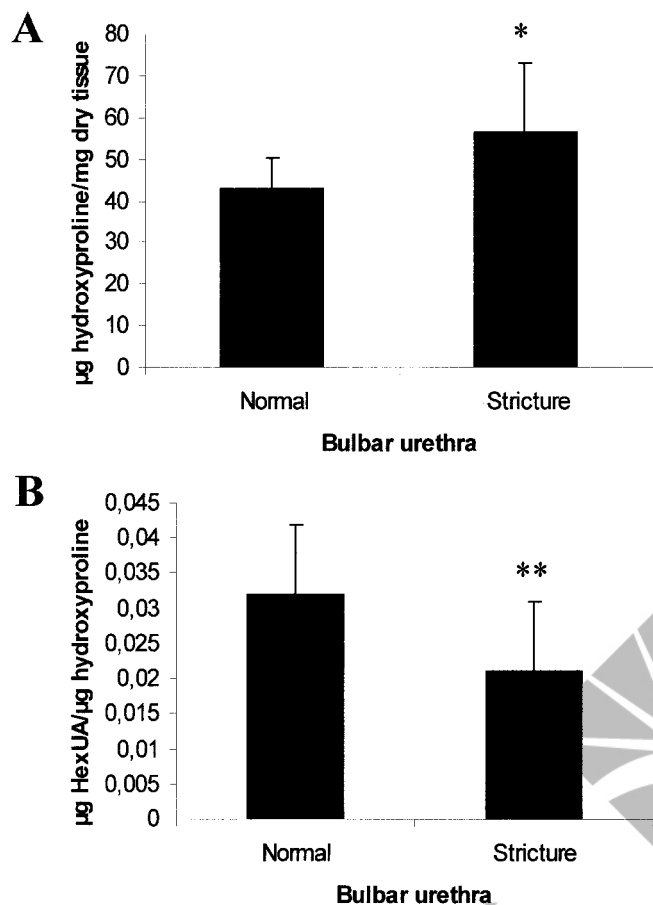


FIG. 3. Quantification of mean total collagen content in 10 normal and 10 strictured segments of bulbar urethra on hydroxyproline assay. *A*, total collagen was significantly increased in stricture group. *B*, GAG-to-collagen ratio was significantly decreased in stricture group. *HexUA*, hexuronic acid. Error bars represent 1 SD. Single asterisk indicates $p < 0.05$. Double asterisks indicate $p < 0.01$.

collagen content. This difference was more evident when the GAG-to-collagen ratio was considered (fig. 3, *B*).

GAGs interact with collagen and other components of the ECM.⁸ Therefore, they may have a key role in urethral compliance, although to our knowledge this fact has not yet been studied. Compliance resulting from wound healing may determine the caliber of the urethral lumen, severity of obstruction, patient complaints and urodynamic features. Dermatan sulfate proteoglycans interact with collagen and cause increased stability of collagen fibrils and orient fibrillogenesis.¹⁸ Dermatan sulfate, which has a predominant interstitial distribution, was the predominant GAG in strictures of the bulbar urethra. Furthermore, an important decrease in the concentration of hyaluronic acid and total GAGs in the urethral stricture as well as the high total collagen content may account for the undesirable biomechanical properties of urethral scar tissue. Matrix components other than GAGs and collagen can also be implicated in the final characteristics of urethral stricture.¹⁹

Tissues have a high hyaluronic acid content in the early stages of wound healing and as healing develops, sulfated GAGs progressively increase.^{9,20} Although it is almost impossible to determine the time of stricture onset, the high concentration of sulfated GAGs and low concentration of hyaluronic acid suggest that the urethral strictures analyzed in the current study were at a late stage of wound healing.

CONCLUSIONS

The development of numerous urethroplasty techniques for urethral stricture disease during the last decades should not

blind us to the fact that we are intervening in a disease with a poorly known pathogenesis. Understanding the biological behavior of cells in the tissues implies an understanding of the normal and pathological composition of individual tissues and the structure of ECM macromolecules with their associated functions. The results of our study of matrix component alterations may partly explain the clinically observed impairment of urethral biomechanical properties and may be useful for defining new targets for therapy for urethral stricture disease.

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