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P. Winter · K. Ganter · U. Leppin
G. Schoeneich · A. Hesse

Glycosaminoglycans in urine and extracorporeal shock wave lithotripsy

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Abstract In 50 calcium oxalate stone-forming patients, the total excretion of glycosaminoglycans (GAGs) and of four subgroups [chondroitin-4-sulfate (CS-A), chondroitin-6-sulfate (CS-C), dermatan sulfate (DS) and hyaluronic acid (HY)] were investigated before extracorporeal shock wave lithotripsy (ESWL) and during the subsequent 5 days. The standard value was determined by reference to a group of healthy test subjects. The excretion of GAGs was significantly higher in healthy test persons than in stone-forming patients. Twenty-four hours after ESWL administration, GAG excretion was enhanced significantly but returned to normal values over the course of 3 days. ESWL had no influence on the proportional composition of GAG subgroups CS-A, CS-C, DS and HY. The increase in GAG excretion after ESWL indicates a transient injury of renal tissue or of the mucus layer lining the urothelium. This lesion, however, can be regarded as temporary with later restitutio ad integrum.

Key words Glycosaminoglycans in urine · Stone patients · Extracorporeal lithotripsy

Glycosaminoglycans (GAGs) are the major components of intercellular ground substance and are found in connective tissue, bones, cartilage, organs (skin, eyes, liver, kidney, brain) and body fluids [11, 27, 33]. They can be traced in varying forms depending on the nature of tissue. In the kidney they are part of the mucus layer lining the urothelium of the urinary tract and are also distributed along the membranes of glomeruli, Bowman's capsule, tubular and peritubular capillaries and arterioles [4]. The urinary bladder is covered with a layer of mucin and GAGs which serves as a shield against bacteria and noxious substances [20].

So far eight substances of natural origin are known to be glycosaminoglycans, viz., chondroitin, hyaluronic acid (HY), chondroitin-4-sulfate (CS-A), chondroitin-6-sulfate (CS-C), dermatan sulfate (DS), heparin, heparan sulfate and keratan sulfate.

Initial reports have shown that GAG excretion can be influenced by extracorporeal shock wave lithotripsy (ESWL) in both humans and dogs [1, 21, 22].

The aim of our investigations was to clarify the following questions:

1. How does ESWL influence the total amount of GAG excretion?
2. Is there any change in the GAG excretion pattern after ESWL?
3. Can these parameters serve as an indicator for possible renal/urothelial damage after ESWL treatment?

Material and methods

Patients

In 50 patients (21 women, 29 men, age range 24–84 years, mean age 49.8 years) with calcium oxalate stones in the renal pelvis ($n = 23$) and renal calices ($n = 27$), 24-h urine samples were taken 1 day before and on 5 consecutive days after ESWL treatment in order to measure total GAG excretion. Standard values of GAG excretion were taken from a group of healthy test subjects ($n = 23$, 13 women, 10 men).

At the same time endogenous creatinine clearance was determined in all patients before and after ESWL treatment (days 0, 3, 5). To this end, 24-h urine samples and serum creatinine values were measured. Care was taken to make sure that serum creatinine concentration did not exceed 3 mg/dl and urine volume was above 1500 ml/24 h. Creatinine clearance, taken as an indicator of glomerular filtration, was set in relation to body surface area (m^2).

P. Winter (✉) · K. Ganter · U. Leppin · G. Schoeneich · A. Hesse
Department of Urology, Division of Experimental Urology, University of Bonn, Sigmund-Freud-Strasse 25, D-53105 Bonn, Germany
Fax: (+49) 228 287 4285

Lithotripsy

Concrements, disintegrated by ESWL, had the following diameters: 0–5 mm ($n = 15$), 6–15 mm ($n = 23$) and 16–30 mm ($n = 12$). ESWL was given using a Siemens Lithostar II Plus. Each treatment comprised a maximum of 2000 shock waves per renal unit. All patients were treated under clinical conditions. Results were checked by conventional single-side X-rays (size 20/40). Disintegrated particles <0.2 cm were classified as stones passing spontaneously with no further need of treatment.

In 10 patients sufficient disintegration was achieved after the first session, but 40 patients had to undergo 2 or more sessions. The mean number of sessions was 3.4, with a total of 6200 shock waves applied. Before ESWL treatment, relevant renal parameters in serum were within the normal range. One day before (day-1) and 1–5 days after ESWL treatment GAG excretion was investigated in 24-h urine.

Analytical methods

For the determination of total GAG excretion a method was employed that had proved to deliver the best recovery rates in earlier investigations [6, 16]. Using the quaternary ammonium salt cetylpyrdinium chloride (CPC, Sigma) GAGs were precipitated in urine, forming sparingly soluble compounds. The precipitation was due to a chemical reaction between polyanions (GAGs) and cationic precipitation reagents forming insoluble compounds in urine. After centrifugation, these compounds were cleft by adding ethanol (Merck) and potassium salts. While quaternary ammonium compounds dissolve in this medium, GAGs do not. Having precipitated as potassium salts, they were then used for further analysis. For the determination of glucuronic acid the method originally applied by Blumenkrantz and Asboe-Hansen [2] was modified using *m*-hydroxy-diphenyl after acid hydrolysis. The GAG concentration refers to the recorded glucuronic acid content (micromoles).

Method of enzymatic cleavage and ionochromatographic separation of GAGs

When urine was prepared for analysis, ions such as chloride, nitrate, phosphate and sulfate proved to be inseparable from GAGs. For this reason urine samples were desalted before analysis by using porous resin on a polyacryl basis (Amberlite XAD-2). This resin was suspended in methanol and filled into a glass cylinder (diameter 2.5 cm). After conditioning the column with distilled water, the urine sample was added. Irrigation with 100 ml distilled water was followed by the extraction with methanol. The eluate was collected in three fractions and dried. The residue was diluted in distilled water and served as sample material for ionochromatography or GAG separation [18].

Enzymes used for cleavage were chondroitinase ABC (Sigma) and chondro-4-sulfatase (Sigma) from *Proteus vulgaris* as well as chondroitinase AC (Sigma) from *Flavobacterium heparinum*. Synthetic chondroitin sulfates, dissolved in distilled water and cleft by enzymes, served as reference substances.

Enzymes were dissolved in as much distilled water as was necessary to achieve a solution activity of 1 U/ml. Synthetic GAG solutions in distilled water, TRIS buffer (pH 8.0) or sodium phosphate buffer (pH 7.0 or pH 7.8) of a concentration of 1 mg/ml were diluted to between 1:10 and 1:100. Pooled test urines were taken in pure form or in dilutions up to 1:5. The dilution of CPC precipitations was between 1:3 and 1:5. For enzymatic cleavage, 500 µl of the solution was pipetted into 5-ml vials as required for the desired degree of dilution; then 25 µl enzymatic solution (1 U/ml) and 475 µl distilled water were added. One test specimen containing no enzymes was included for each solution as a blank value. The following enzymatic cleavage was continued over a period of 12 h at 37 °C and then stopped by 5 min of heating in boiling water. Prior to each recording unit, a calibration curve was set up, each based on four chondroitin sulfate and hyaluronic acid solutions with a concentration range of up to 200 µg/ml.

An ionochromatographic separation method (Dionex 2000 i) was used for the determination of the qualitative and quantitative composition of GAGs in the tested material. Chromatographic separation was performed by means of a CarboPac PA1 column (Dionex). The eluant consisted of sodium carbonate/sodium hydrogen carbonate (Merck) and detection was carried out by suppressed conductivity [18].

The following substances were identified, viz., chondroitin-4-sulfate (CS-A), chondroitin-6-sulfate (CS-C), dermatan sulfate (DS) and hyaluronic acid (HY). Heparan sulfates and keratan sulfates could not be identified using this method.

Applying the principle of linear regression and using the formula $c = h \times f = (a \times H + b) \times (4/V)$, the concentration of GAGs in urine was determined by the changes in conductivity. (C = concentration expressed in mg/l; h = concentration determined from linear regression in mg/l; f = concentration factor determined by specimen preparation; a = gradient of regression line; H = height of peak; b = axis intercept of regression line; V = urine volume.) The excretion of individual substances (mg/24 h) was determined by multiplying the various concentrations by the volume of 24-h urine.

Proportional distribution of the individual substances is obtained by dividing a given excreted substance by the sum of excretion of all substances.

Results

GAG excretion in the group of healthy subjects was 30.1 ± 3.9 µmol/day in men and 29.3 ± 3.3 µmol/d in women. GAG excretion in calcium oxalate stone patients 1 day before ESWL (day − 1) was 17.6 ± 1.6 µmol/day (16.9 ± 1.8 µmol/day in men and 18.7 ± 2.9 µmol/day in women). While on the day of ESWL treatment (day 0) there was no change in GAG excretion, a significant increase to 21.2 µmol/day was observed 1 day after ESWL. On the 2nd, 3rd and 4th days after ESWL treatment excretion dropped to 19.4 µmol/day, 18.2 µmol/day and 18.4 µmol/day, respectively. The significant increase to 26.0 µmol/day on day 5 is attributable to another ESWL treatment. There was no difference in the pattern of excretion between male and female patients (Table 1).

In all patients taking part in our investigations there was a significant increase in the excretion of all GAG subgroups one day after ESWL treatment (day 1), compared to excretions recorded 1 day before ESWL treatment (day-1). Performing two different analyses, one for male and one for female patients, there was no difference in the results of GAG subgroups. A comparison of excretion patterns of the 2 days in question is shown in Table 2. No evidence of any significant difference specific to sex could be found either in the total amount of excretion or in the proportional distribution of GAG subgroups.

There was, however, a tendency toward higher excretion of chondroitin-4-sulfate, dermatan sulfate, hyaluronic acid and chondroitin in men than in women.

Table 1 GAG excretion (means ± SD) of 50 calcium oxalate stone-forming patients before and after shock wave lithotripsy

Day	Glycosaminoglycan excretion (μmol/day ± SD)		
	Total ($n = 50$)	Men ($n = 29$)	Women ($n = 21$)
1	17.7 ± 2.1	16.9 ± 1.8	18.7 ± 2.9
0	17.6 ± 1.6	18.0 ± 1.3	17.0 ± 1.7
1	23.2 ± 1.8*	22.9 ± 2.5*	23.6 ± 2.8*
2	19.4 ± 1.3	18.2 ± 1.6	21.2 ± 2.2
3	18.2 ± 1.3	17.0 ± 1.2	19.8 ± 2.4
4	18.4 ± 1.6	18.1 ± 2.3	18.9 ± 1.9
5	26.0 ± 5.2*	32.1 ± 9.2*	21.1 ± 2.3*

* Significant in comparison to basic value (day-1)

Average endogenous creatinine clearance before ESWL treatment was 69.2 ± 7.7 ml/min m^2 (79.3 ± 9.8 ml/min m^2 in men, 59.1 ± 5.5 ml/min m^2 in women), thus being within the normal range. After ESWL there was a slight increase in endogenous creatinine clearance, which cannot be regarded as significant (Table 3).

Table 3 Endogenous creatinine clearance (mean value ± SD) before and after ESWL treatment.

	Endogenous creatinine clearance (ml/min m^2 ± SD)		
	Total ($n = 50$)	Men ($n = 31$)	Women ($n = 19$)
Day-1	69.2 ± 7.7	79.3 ± 9.8	59.1 ± 5.5
Day 0	71.5 ± 6.8	81.4 ± 9.3	61.6 ± 4.3
Day 3	73.1 ± 6.7	83.4 ± 8.7	62.8 ± 4.7
Day 5	77.1 ± 6.0	88.8 ± 7.9	65.3 ± 4.1

Discussion

GAGs have been traced in various organs such as liver, brain and kidney [7, 12, 24, 27]. According to the investigations by Hautmann et al. [14] and Hesse et al. [15–17], GAGs are found in renal tissue, mainly in renal papillae but also (though in decreasing concentration) in medulla and cortex. In addition the urothelial lining of the urinary tract has a protective coating containing GAGs (mucus coat) with anti-adherence properties against bacteria, proteins and urinary crystals [10, 13, 30–32]. Being proteoglycans, GAGs are components of all basement membranes and cell membranes in renal tissue [37]. Being the most important structural components in the glomerular basement membrane, they are responsible for its selective permeability properties [36].

If high molecular GAGs are found in urine it has to be considered whether this is attributable to tissue injuries or cell lesions of renal tubuli and papillae, thus enabling GAGs to penetrate into urine [15, 16]. The investigations by Kerby [23] show that usually GAG is excreted into the upper urinary tract.

Tubular reabsorption or secretion in GAG excretion has not been proved beyond reasonable doubt. GAGs are excreted into urine, the majority from the surface of the upper urinary tract, and not by way of glomerular filtration [19, 35]. Karlsen et al. [22] investigated urinary excretion of GAGs in dogs. After treatment with ESWL, urine was collected separately from both kidneys via a ureteric balloon catheter for subsequent investigation. Compared to the nonexposed side there was a significantly increased level of GAG excretion on the exposed side. Having carried out the same investigations on single-kidney patients there were no signs of any increase in GAG excretion levels in urine after ESWL administration (Dornier HM3).

In our investigation there was a significant increase in GAG excretion on the 1st day after ESWL, which

Table 2 GAG excretion pattern (mean value ± SD) of 50 stone-forming patients before and after lithotripsy (*CS-A* chondroitin-4-sulfate, *CS-C* chondroitin-6-sulfate, *DS* dermatan sulfate, *HY* hyaluronic acid and chondroitin)

Day-1	CS-A (mg)	CS-A (%)	CS-C (mg)	CS-C (%)	DS (mg)	DS (%)	HY (mg)	HY (%)
Xtot	1.92	31.6	2.21	35.0	0.39	5.6	1.73	27.8
SD	0.98	6.2	1.2	8.7	0.35	2.8	0.92	6.5
Men	2.05	33.6	2.06	32.1	0.42	5.8	1.73	28.5
SD	1.35	5.0	1.45	5.4	0.4	1.7	1.21	7.7
Women	1.80	29.9	2.33	37.5	0.36	5.5	1.72	27.1
SD	0.61	7.0	1.04	10.6	0.35	3.7	0.68	5.9
Day 1								
Xtot	5.23*	35.2	4.59*	32.3	0.84*	5.6	4.02*	26.9
SD	2.73	5.3	1.88	4.7	0.59	2.5	2.21	4.6
Men	5.55*	35.8	4.74*	30.9	0.82*	5.4	4.36*	27.9
SD	3.2	4.7	2.5	2.9	0.52	1.5	2.47	3.8
Women	4.96*	34.6	4.47*	33.4	0.86*	5.8	3.73*	26.2
SD	2.44	6.2	1.27	5.8	0.68	3.3	2.1	5.8

* Significant in comparison to basic value (day-1)

slowly decreased on the subsequent days to finally reach normal values on the 4th day after ESWL. With an almost constant endogenous creatinine clearance this increase in GAG excretion was observed to be the same in men and women. The proportional composition of GAG subgroups was the same before and after administration of ESWL. The increase in GAG excretion 24 h after ESWL gives rise to the assumption that ESWL therapy leads to damage in the urothelial mucus layer and renal parenchyma or to increased glomerular filtration.

Our investigations as well as those of other authors show, however, that the endogenous creatinine clearance and the glomerular filtration rate do not change upon ESWL administration, thus indicating a merely transient lesion of the urothelium and renal parenchyma, respectively [9, 21, 22].

GAGs are excreted into urine, the majority from the surface of the upper urinary tract, and not by way of glomerular filtration [20, 34]. If an increase in glomerular filtration as a result of a damaged glomerulus was the cause of an elevated GAG excretion, the excretion pattern of GAG composition would be different. We could not confirm the results of Karlsen and Berg [21], who found no changes in GAG excretion in patients with functioning single kidneys. Like Alkibay et al. [1], we observed a significant increase in GAG excretion in stone-forming patients 24 h after ESWL treatment.

There was also no significant difference in the total amount or in the proportional composition of GAG excretion in men and women. There is, however, a general tendency toward higher excretion of chondroitin-4-sulfate, dermatan sulfate, hyaluronic acid and chondroitin as well as a higher total amount of GAGs in men than in women. As far as the total GAG excretion is concerned, our findings confirm earlier investigations, even though these had shown a significant difference between males and females [16].

A comparison between excretion values of healthy test subjects and those of calcium oxalate stone formers shows that healthy persons excreted twice as much GAGs as calcium oxalate stone-forming patients.

This result differs from earlier investigations, which found no differences between stone-forming patients and healthy persons [16]. Our latest results thus confirm the statement by Robertson et al. [34] that urolithiasis patients excrete significantly less GAGs than healthy people. This has also been stated previously by many other authors [3–5, 8, 25, 26, 28, 29, 36]. The noncomparability of results in GAG excretion of healthy test subjects and urolithiasis patients indicates differences in analytical methods.

Future investigations should, however, put more stress on certain criteria when choosing test persons and patients, such as comparability of age, first-time stone formers or recurrent stone formers.

References

1. Alkibay T, Karabas Ö, Hizel N, Bozkirli I (1993) Urinary glycosaminoglycans excretion following extracorporeal shock wave lithotripsy. Int Urol Nephrol 25:321
2. Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54:484
3. Boevé ER, Cao LC, Verkoelen CF, Romijn JC, de Bruijn WC, Schröder FH (1994) Glycosaminoglycans and other sulphated polysaccharides in calculogenesis of urinary stones. World J Urol 12:43
4. Caudarella R, Rizzoli E, Malavolta N (1987) Clinical and metabolic aspects of urinary glycosaminoglycans excretion in calcium stone formers. In: Martelli A, Buli P, Marchesini B (eds) Inhibitors of crystallization in renal lithiasis and their clinical application. Proceedings of International Meeting, Bologna. Acta Med Rome, p 187
5. Conte A, Roca P, Genestar C, Grases F (1989) Uric acid and its relationship with glycosaminoglycans in normal and stone-former subjects. Nephron 52:162
6. Diferante N, Robbins WC, Rich C (1957) Urinary excretion of acid mucopolysaccharides by patients with lupus erythematosus. J Lab Clin Med 50:897
7. Farber SJ, Cohen GL, Castor JA (1962) The chemical and metabolic properties of acid mucopolysaccharides of renal papillae. Trans Assoc Am Physicians 75:154
8. Gianotti M, Genestar C, Palou A, Pons A, Conte A, Grases F (1989) Investigation of GAGs on 24-hour and 2-hour urines from calcium oxalate stone formers and healthy subjects. Int Urol Nephrol 21:281
9. Gilbert BR, Riehle RA, Vaughan ED Jr (1988) Extracorporeal shock wave lithotripsy and its effect on renal function. J Urol 139:482
10. Gill WB, Jones KW, Ruggiero KJ (1982) Protective effects of heparin and other sulfated glycosaminoglycans on crystal adhesion to injury urothelium. J Urol 127:152
11. Greiling H, Stuhlsatz HW (1969) Glycosaminoglycan-Peptide aus dem humanen Kniegelenkknorpel. Hoppe-Seyler's Z Physiol Chem 350:449
12. Gressner AM, Scherer R, Stuhlsatz HW (1983) Laser nephelometric determination of glycosaminoglycans – method of application. J Clin Chem Biochem 21:407
13. Grenabo L, Hedlin H, Pettersson S (1988) Adherence of urease-induced crystals to rat bladder epithelium. Urol Res 16:49
14. Hautmann RE, Hoffmann-Flölkersamb D, Stein T, Stuhlsatz HW, Greiling H (1985) On the possible role of intrarenal (tissue) glycosaminoglycans in man – preliminary data. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and related clinical research. Plenum, New York, p 345
15. Hesse A, Hartmann U, Schneider HJ, Horn G (1975) Zur Bedeutung der Mucopolysaccharidausscheidung beim Harnsteinleiden. Z Urol 68:401
16. Hesse A, Wuzel W, Vahlensieck W (1986) The excretion of glycosaminoglycans in the urine of calcium oxalate-stone patients and healthy persons. Urol Int 41:81
17. Hesse A, Wuzel W, Vahlensieck W (1991) Significance of glycosaminoglycans for the formation of calcium oxalate stones. Am J Kid Dis 17:414
18. Hesse A, Leppin U, Bongartz D, Winter P (1995) Separation of GAG fractions by means of ionochromatography. in preparation
19. Hurst RE (1994) Structure, function and pathology of proteoglycans and glycosaminoglycans in the urinary tract. World J Urol 12:3
20. Hurst RE, Parsons CL, Roy JB, Young JL (1993) Urinary glycosaminoglycan excretion as a laboratory marker in the diagnosis of interstitial cystitis. J Urol 149:31

21. Karlsen SJ, Berg KJ (1992) Changes in renal function after extracorporeal shock wave lithotripsy in patients with solitary functioning kidney: long-term follow-up. J Endourol 6:205
22. Karlsen SJ, Smevik B, Klingenberg Lund B, Berg KJ (1991) Do extracorporeal shock waves affect urinary excretion of glycosaminoglycans? Br J Urol 67:24
23. Kerby GP (1954) The excretion of glucuronic acid and of acid mucopolysaccharides in normal human urine. J Clin Invest 33:308
24. Margolis RU (1967) Acid mucopolysaccharides and proteins of bovine whole brain, white matter and myelin. Biochem Biophys Acta 141:91
25. Martelli A, Marchesini B, Buli B (1985) Urinary excretion pattern of main glycosaminoglycans in stone formers and controls. In: Schwille PO, Smith LH, Roberston WG (eds) Urolithiasis and related clinical research. Plenum, New York, p 355
26. Michelacci YM, Glashan RQ, Schor N (1989) Urinary excretion of glycosaminoglycans in normal and stone forming subjects. Kidney Int 36:1022
27. Murata K (1975) Acid mucopolysaccharides in human kidney tissue. Clin Chim Acta 63:157
28. Nesse A, Garbossa G, Romero MC, Bogado CE, Zanchetta JR (1992) Glycosaminoglycans in urolithiasis. Nephron 62:36
29. Nikkilä MT (1989) Urinary glycosaminoglycan excretion in normal and stone-forming subjects: significant disturbance in recurrent stone formers. Urol Int 44:157
30. Parsons CL, Greenspan C, Moore SW (1977) Role of surface mucin in primary antibacterial defence of bladder. Urology 9:48
31. Parsons CL, Stauffer C, Feldström B (1980) Bladder surface glycosaminoglycans: an efficient mechanism of environmental adaption. Science 208:605
32. Parsons CL, Danielson B, Feldström B (1985) Inhibition of sodium urate crystal adherence to bladder surface polysaccharide. J Urol 134:614
33. Poulsen JH (1986) Urine and tissue glycosaminoglycans and their interrelations. Dan Med Bull 33:75
34. Robertson WG, Peacock M, Heyburn PJ, Marshall DH, Clark PB (1978) Risk factors in calcium stone disease of the urinary tract. Br J Urol 50:449
35. Shum D, Bayliss C, Scott JE (1984) A micropuncture and renal clearance study in the rat of the urinary excretion of heparin, chondroitin sulfate and metabolic breakdown products of connective tissue proteoglycans. Clin Sci 67:205
36. Sidhu H, Hemal AK, Thind SK, Nath R, Vaidyanathan S (1989) Comparative study of 24-hour urinary excretion of glycosaminoglycans by renal stone formers and healthy adults. Eur Urol 16:45
37. Stow JL, Sawada H, Farquhar MG (1985) Basement membrane heparan sulphate proteoglycans are concentrated in the lamina rarae and in podcytes of the renal glomerulus. Proc Natl Acad Sci USA 82:3296