Lack of Relationship between Urinary Glycosaminoglycans and Indices of Tubular or Glomerular Renal Damage

Urinary GAG Are an Unreliable Nephrotoxicity Index

A.M.		Bernard ^a
A.		Ouled ^a
H.		Roels ^a
R.		Lauwerys ^a
B.		Vandeleene ^b
A.		Lambert ^b

Units of ^aIndustrial Toxicology and of ^bDiabetology and Nutrition, School of Medicine, Catholic University of Louvain, Brussels, Belgium

Alfred Bernard, PhD, Unit of Industrial Toxicology and Occupational Medicine, Clos Chapelle-aux-Champs 30.54, B-1200 Brussels (Belgium)

Dear Sir,

Glycosaminoglycans (GAG) are the major constituents of the anion layer of the glomerular basement membrane which provides an electrostatic barrier to the filtration of anionic macromolecules like albumin [1]. It has been recently suggested that the increased urinary excretion of GAG observed in diabetics [2, 3] might reflect a derangement of GAG in the glomerulus, which would represent the first step toward the development of microalbuminuria [4]. In support for this hypothesis, a correlation between urinary GAG and albuminuria has been reported in 25 insulin-dependent diabetics with daily excretion levels of albumin between 25 and 250 mg [5].

To assess the validity of urinary GAG as index of the charge selectivity of the glomerular basement membrane, we have compared the urinary excretion of GAG with that of albumin, retinol-binding protein (RBP; a marker of proximal tubular function), Tamm-Horsfall glycoprotein and β -N-acetylglucosaminidase (NAG) in 69 diabetics (duration of diabetes 1–32 years, mean 13 years), in 34 workers chronically exposed to cadmium and in 15 patients with acute tubular damage due to multiple injuries, rhabdomyolysis or acute poisoning. The control group consisted of 31 age-matched subjects (age 20–65 years).

GAG were assayed by the method of Whiteman [6] as in the studies by Baggio et al. [4, 5]. Albumin, RBP and Tamm-Horsfall glycoprotein were measured by latex immunoassay [7] and NAG by fluorimetry [8]. In comparison with the control group, cadmium workers, patients with acute tubular injury and diabetics exhibited an increased prevalence of elevated GAG values in urine (fig. 1: χ^2 test significant for diabetics and patients with tubular injury). This prevalence parallels the mean urinary excretion of albumin, NAG and RBP, which suggests that urinary GAG might have some link with renal da-

600

(0 1,000)

500

1/800-
< B 300
100

Healthy subjects Cadmium Patients with Diabetics
 workers acute tubular
 injury

Fig. 1. Effect of cadmium exposure, acute tubular injury and diabetes on the urinary excretion of GAG. The horizontal dashed line represents the mean +2 SD of control values. The symbols indicate the subjects with different patterns of proteinuria, using as upper limit of normal 20 mg/g creatinine for albumin, 300 µg/g creatinine for RBP and 5 U/g creatinine for NAG. O = Normal; Δ = increased RBP; · = increased albumin; □ = increased NAG; V = increased RBP+NAG; A = increased albumin + RBP; ▼ = increased albumin + NAG; ■ = increased albumin + RBP + NAG.

mage. But, it can be readily seen from figure 1 that quantitatively the urinary excretion of GAG shows no relationship with that of albumin, RBP or NAG. The latter parameters were normal in the two subjects excreting the highest amounts of GAG and in 6 of the 12 subjects with GAG values above 200 mg/g creatinine. No

Urinary Glycosaminoglycans as Nephrotoxicity Index
83

Healthy subjects Cadmium workers Patients with acute Diabetics
tubular injury

Subjects, n	31	65	15	69
Percentage of increased GAG values	32	15.4	87	26
Albuminuria, mg/g creatinine	4.2	14	390	32
	(0.9–17)	(1.6–190)	(120–2,400)	(3.4–2,000)
NAG in urine, U/g creatinine	0.58	1.1	21	2.6
	(0.29–24)	(0.1–6.6)	(6.5–170)	(0.6–15)
RBP in urine, µg/g creatinine	79	350	9,870	202
	(56–190)	(37–106,000)	(400–160,000)	(44–7,400)

Measured data are given as geometric mean values and, below, ranges in parentheses. correlation could be demonstrated either, within each group or in the total population between the urinary excretion of GAG and that of albumin, RBP, NAG and Tamm-Horsfall glycoprotein. In the case of diabetics, it could be argued that the relationship between GAG and albumin is not linear over the whole range of albuminuria. To test this hypothesis, we have distributed the diabetics in three groups according to their albuminuria (mg/g creatinine): below 20 (n = 32), between 20 and 100 (n = 21) and higher than 100 (n = 16). But no significant correlation between GAG and albumin emerged in these three groups (r = 0.27, 0.17 and 0.067, respectively), and no effect of albuminuria on GAG excretion could be demonstrated by one-way analysis of variance (mean: 79, 73 and 84 mg/g creatinine, respectively).

In conclusion, the present data lead us to question the validity of urinary GAG as an index of the charge-selectivity of the glomerulus or even as a nonspecific marker of renal damage. The fact that GAG are constituents of the extracellular matrix and basement membranes present all along the nephron, and that an enhanced urinary excretion of these compounds may reflect tissue injury or an increased cellular turnover associated with tissue regeneration or else a derangement of GAG metabolism, probably accounts for the lack of reliability of this parameter as a nephrotoxicity index.

Acknowledgements

We gratefully acknowledge Dr. J. Daniel (Rhône-Poulenc Industries, France) for providing batches of polystyrene latex particles. A.M.B. is Chercheur Qualifié du Fonds National Beige de la Recherche Scientifique.

References

- Rosenzweig, L.J.; Kanwar, Y.S.: Removal of sulfated (heparan sulfate) or nonsulfated (hyaluronic acid) glycosaminoglycans results in increased permeability of the glomerular basement membrane to 125-bovine serum albumin. *Lab. Invest.* 47:177–184 (1982).
- Clamp, J.R.; Hartog, M.; Shelly, J.H.: Carbohydrate-containing materials in urine from normal and diabetic subjects. *Clin. Sci.* 56:193–196 (1979).
- Lubec, G.; Legenstein, E.; Pollack, A.; Meznick, E.: Glomerular basement membrane changes, $HbA_{1c} < .$ and urinary excretion of acid glycosaminoglycans in children with diabetic mellitus. *Clin. chim. Acta* 103:45–49 (1980).
- Baggio, B.; Briani, G.; Cicerello, E.; Gambaro, G.; Brutto-messo, D.; Tiengo, A.; Borsatti, A.; Crepaldi, G.: Urinary glycosaminoglycans, sialic acid and lysosomal enzyme increase in nonalbuminuric diabetic patients. *Nephron* 43: 187–190 (1986).
- Baggio, B.; Briani, G.; Cicerello, E.; Gambaro, G.; Borsatti, A.; Crepaldi, G.: Urinary glycosaminoglycans excretion and micro-albuminuria in diabetics. *J. Am. Med. Ass.* 255: 3250–3251 (1986).
- Whiteman, P.: The quantitative measurement of alcian blue-glycosaminoglycan complexes. *Biochem. J.* 757:343–350 (1973).
- Bernard, A.; Lauwerys, R.: Continuous flow system for the automation of latex immunoassay. *Clin. Chem.* 29: 1007–1011 (1983).
- Tucker, S.M.; Boyd, P.J.R.; Thompson, A.E.; Price, R.G.: Automated assay of N-acetyl- β -glucosaminidase in normal and pathological urine. *Clin. chim. Acta* 62:333–339 (1975).