

Comparison of urinary glycosaminoglycan excretion in rheumatoid arthritis, osteoarthritis, myocardial infarction, and controls

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SUMMARY Urinary glycosaminoglycan (GAG) excretion was measured in 24 patients with active rheumatoid arthritis (RA) before and after treatment with conventional second-line agents. Urinary GAG excretion was also measured in normal controls, patients with osteoarthritis (OA), and patients with acute myocardial infarction (MI). Total GAG excretion was increased in the RA group and fell after second-line therapy ($p < 0.01$). More low than high molecular weight GAG was excreted in the active RA group, and this pattern was reversed after treatment. Excretion of total, high and low molecular weight GAG in the OA group did not differ significantly from controls. Total GAG excretion was increased in the MI group when compared with controls ($p < 0.02$) and consisted mainly of high molecular weight GAG. The serial measurement of urinary GAG provides a further index of disease activity and may help to monitor response to treatment.

Key words: serial measurements, alcian blue, disease activity.

Previous workers have shown that urinary excretion of sulphated glycosaminoglycans (GAG) is increased in connective tissue disorders such as progressive systemic sclerosis¹ and rheumatoid arthritis (RA).² The amount of high molecular weight GAG and low molecular weight GAG excreted varies in different disorders and with disease activity.^{3,4} To establish whether measurement of urinary GAG excretion and the relative amounts of high and low molecular weight GAG provide a useful additional index of inflammatory disease activity, patients with RA were studied before and after commencing second-line drug therapy with chloroquine, gold, or D-penicillamine. The results of GAG excretion in RA were compared with GAG excretion in patients with osteoarthritis (OA), in patients after acute myocardial infarction (MI), and in normal controls. The OA and MI groups were chosen to study the effects of non-inflammatory joint disease and acute non-articular tissue damage respectively on GAG excretion.

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Patients and methods

All patients were attending one of two rheumatology departments or were inpatients. Serial assessments (RA patients) were performed by the same clinician, and the MI group was obtained from admissions to two coronary care units. The control group comprised laboratory and medical personnel with no history of joint disease, and patients with soft tissue rheumatism. No subject with significant renal or hepatic impairment was included in the study. Patient numbers and ages are recorded in Table 1.

The RA patients all had definite or classical RA by American Rheumatism Association (ARA) criteria.⁵ At each assessment the following parameters of disease activity were recorded: global clinical assessment (0-3); duration of morning stiffness (EMS) (minutes); erythrocyte sedimentation rate (ESR) (mm/1st h). Disease duration (range two months to 20 years, mean 6.7 years) at entry into the study and drug treatment were noted. Six out of the 24 patients were taking no drugs or pure analgesics only, the remaining 18 were taking non-steroidal anti-inflammatory agents regularly, which were not

Table 1 Characteristics of the groups (patients and controls) studied: numbers in each group and age range (mean) in years

Group	No in group	Age range (mean)	Males	Age range (mean)	Females	Age range (mean)
Rheumatoid arthritis	24	22-80 (56.5)	8	31-74 (60.7)	16	22-80
Osteoarthritis	16	42-79 (66.6)	5	42-75 (62.4)	11	57-79 (68.8)
Myocardial infarction	21	37-73 (56.1)	16	41-73 (56.3)	5	37-65 (55.6)
Controls	30	26-68 (43.7)	17	27-68 (43.6)	13	29-65 (43.9)

altered during the study period. Samples from the RA group were obtained while the patients were on a gelatin-free diet, as a concurrent study requiring hydroxyproline excretion estimation was in progress. The other groups were on normal diets. The OA patients had predominantly primary generalised disease with no evidence of inflammatory joint disease. They were assessed clinically, with radiological assessment of the affected joints and measurement of the ESR. Patients who had sustained an MI in the previous 24 hours were entered into the study after excluding those with joint disease or other inflammatory condition. Serial 'cardiac enzymes' (aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)) and plasma viscosity or ESR were recorded. The height and weight of each subject were recorded, and the body surface area was calculated from a nomogram. Initially this was used to express hydroxyproline excretion, as it eliminated the effect of differing bone masses. It was retained in calculating GAG excretion, as we believe it also reflects the total connective tissue content of the body and hence turnover of GAG.

A single 24-hour urine sample was collected from each subject, except those in the RA group. From these, at least two specimens were obtained, one before commencing second-line treatment and a second after treatment had been established. Treatment periods ranged from two to 12 months, mean 18.6 weeks. In the MI group, to exclude the effects on renal function of transient hypotension post-infarction, the GAG:creatinine ratio was calculated on three successive days postinfarct. All samples were stored at -20°C without preservative until assayed. Sample storage time did not affect GAG content and this has been the experience of other workers.⁴

The urinary GAG content was measured before and after dialysis against distilled water. The method used was a modification of that developed

by Whiteman⁶ and involves complex formation with the cationic dye alcian blue 8 GX. In this procedure a standard GAG solution (200 μl) or centrifuged urine (200 μl) was mixed with 4 ml of freshly prepared 0.05% w/v alcian blue 8 GX and 50 mM MgCl_2 in a 50 mM sodium acetate buffer (pH 5.8). The mixture was equilibrated for two hours at room temperature and an alcian blue-GAG complex was separated by centrifugation at 2000 g for 20 minutes. The precipitate was washed twice with ethanol followed by centrifugation and then dissociated with a 5% solution of sodium dodecyl sulphate in distilled water (4 ml). The absorbance of the resulting solution was measured in 1 cm microcuvettes at 620 nm. The GAG content of the urine was determined by reference to a calibration curve constructed with chondroitin 4-sulphate as standard. The results are expressed as milligrams per square metre surface area per 24 hours. Total GAG represents results on undialysed urine, high molecular weight GAG the dialysed urine, and low molecular weight GAG the difference between these two values.

Differences between urinary GAG excretion in the RA and other groups were analysed by the Mann-Whitney U test; p values of 0.05 or less were considered statistically significant. Two-tailed tests were applied to the analysis of GAG excretion in all groups. A one-tailed Wilcoxon's rank sum test was used for the analysis of changes in clinical assessment in the RA group.

Results

The results of urinary GAG excretion in the pretreatment RA group, OA, MI, and control groups are recorded in Table 2 and show that the mean total urinary GAG excretion was higher in the pretreatment RA group than in the controls, though this did not reach statistical significance. The cross-sectional statistical analysis of total GAG was biased

Table 2 *Mean urinary GAG excretion and ranges (in brackets) in patient groups and controls: statistical analysis by the Mann-Whitney U test*

Group	Total GAG excretion (mg/m ² /24 h)	High mol. wt GAG excretion (mg/m ² /24 h)	Low mol. wt GAG excretion (mg/m ² /24 h)	Ratio high:low mol. wt GAG
Rheumatoid arthritis (pretreatment)	32.2 (4.6-155.3) NS	15.4 (1.5-48.2) NS	16.8 (0.4-137.2) NS	0.9
Rheumatoid arthritis (excluding two patients see text)	34.6 (10.1-155.3) p<0.04	16.5 (3.7-48.2) p<0.08	18.1 (0.6-137.2) p<0.075	0.9
Osteoarthritis	22.0 (9.0-75.2) NS	12.75 (2.8-52.1) NS	9.3 (1.4-41.7) NS	1.4
Myocardial infarction	40.8 (8.3-182.9) p<0.02	35.6 (6.3-117.4) p<0.001	5.24 (0.38-7) p<0.01	6.8
Controls	20.8 (2.6-58.0)	11.7 (1.8-35.3)	8.9 (0.1-36.6)	1.3

Table 3 *Mean urinary GAG excretion and ranges (in brackets) in RA patients pre- and post-treatment: statistical analysis by the Mann-Whitney U test*

Group	Total GAG excretion (mg/m ² /24 h)	High mol. wt GAG excretion (mg/m ² /24 h)	Low mol. wt GAG excretion (mg/m ² /24 h)	Ratio high:low mol. wt GAG
Pretreatment RA	32.2 (4.6-155.3)	15.4 (1.5-48.2)	16.8 (0.4-137.2)	0.9
Post-treatment RA	15.1 (2.5-52.1) p<0.01	9.0 (2.3-20.4) p<0.04	6.0 (0.2-25.2) p<0.25 (NS)	1.5

by the inclusion of two patients in the RA group whose values for total GAG of 4.6 and 6.6 mg/m²/24 h lay at the lower limit of the normal range. Articular disease activity in one was low, and the reasons for commencing second-line drug therapy had been the presence of systemic features, and in the other articular disease activity remained unchanged by second-line therapy. If these two values are excluded from the cross-sectional statistical analysis, the difference between the pretreatment RA group and controls becomes statistically significant at the 5% level. There was no difference in total GAG excretion in those RA patients in the pretreatment group on non-steroidal anti-inflammatory drugs compared with those on no drugs or pure analgesics only. The high and low molecular weight GAG fractions were excreted in approximately the same amounts in the pretreatment RA group (ratio 'high' to 'low' 0.9), whereas in the control group there was a predominance of high molecular weight GAG (ratio 'high' to 'low' 1.3) excreted. Urinary excretion of total, high and low molecular weight GAG fell in the post-treatment RA group; the total and high molecular weight GAG excreted reached statistical significance compared with the pretreat-

ment group, but the low molecular weight GAG did not. This was because of the wide range in this group. The proportion of high to low molecular weight GAG excreted in the post-treatment RA group increased to resemble the control group (ratio 'high' to 'low' 1.5) (Table 3).

There was an overall fall in the disease activity as measured by clinical assessment, duration of morning stiffness, and ESR (Table 4) in the post-treatment RA group when compared with the pretreatment values, and this reached statistical significance in the clinical assessment and ESR but

Table 4 *Clinical assessment pre- and post-treatment in RA group: statistical analysis by Wilcoxon's rank sum test (one-tailed)*

Group	Mean global clinical assessment	Duration morning stiffness (minutes)	ESR (mm/1st h)
Pretreatment RA	1.7	106.3	57.2
Post-treatment RA	1.36 p<0.05	72.4 NS	46.5 p<0.05

not in the duration of morning stiffness. Fig. 1 compares the change in ESR and total urinary GAG excretion in the RA groups pre- and post-treatment. In the seven patients in whom the ESR rose over the study period, the total urinary GAG excretion fell in all but one. In four of these six patients the remaining parameters (morning stiffness and global activity) suggested improvement. In only one patient did the urinary GAG rise while the other parameters fell, but this was one of the two patients with a low pretreatment GAG value.

There were no differences in GAG excretion between the OA and control groups. In the MI group total urinary GAG excretion was significantly higher than in controls ($p < 0.02$) and appeared to be due to an excess of high molecular weight GAG when compared with controls ($p < 0.001$). From the GAG:creatinine ratio measured on random samples on three successive days postinfarct it was concluded that the high levels were not artefactual and neither were peak levels of urinary GAG being missed by collecting the study sample in the 24 hours post-infarction. By obtaining 24-hour urine samples from all subjects in the main study creatinine estimation was considered unnecessary. There was no difference

in day to day variation between the GAG:creatinine ratio or the GAG excretion per 24 hours.

Discussion

This study shows that there is an increase in total urinary GAG excretion in patients with active RA and in patients after myocardial infarction but no increase in GAG excretion in patients with osteoarthritis. In RA, total urinary GAG excretion falls after response of the disease to second-line drug therapy but appears uninfluenced by treatment with non-steroidal anti-inflammatory drugs. Urinary GAG is thought to be derived from high molecular weight proteoglycans which have probably been released from the parent molecule by proteolytic digestion *in vivo*.⁷ The method used for the precipitation of GAG complexes has been designed specifically for the measurement of urinary GAG and measures both sulphated and non-sulphated uronic complexes. Thus hyaluronic acid as well as the chondroitin sulphates and heparan sulphate are measured.⁶ The low molecular weight GAG fraction probably indicates further enzymic digestion of high molecular weight GAG. Thus in the present study

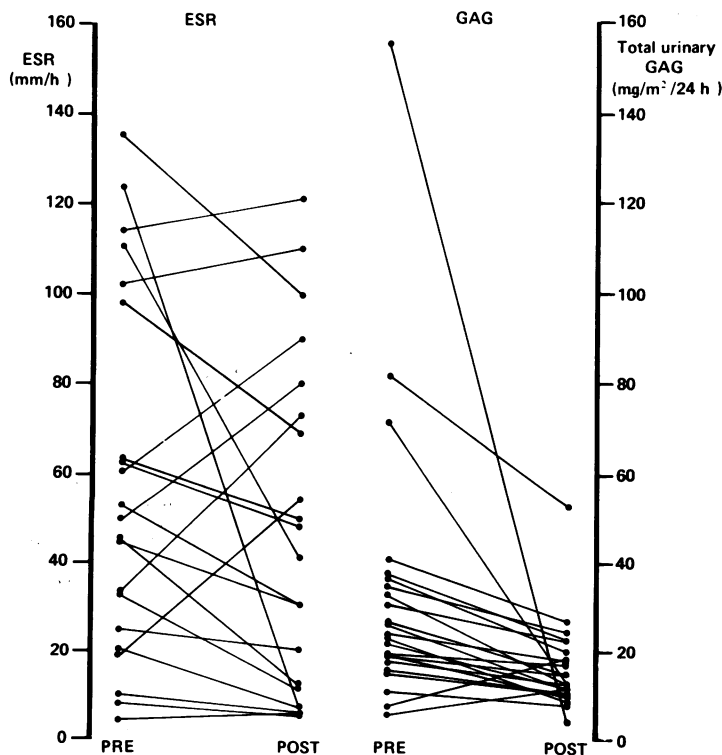


FIG. 1 Changes in ESR and total urinary GAG excretion in the RA group pre- and post-treatment.

the increase in total urinary GAG excretion in both RA and MI groups probably reflects tissue destruction in both disorders. In the RA group, however, there is a disproportionate increase in the low molecular weight fraction, which could be explained by further local enzymic digestion of GAG released from articular tissues. This interpretation is in keeping with the normalisation of the high molecular weight to low molecular weight GAG fractions with response of the articular disease to the second-line drug therapy. Whether the increase in urinary GAG excretion in RA reflects tissue destruction or not is unproved, and an alternative explanation is that it reflects an overall increase in connective tissue metabolism. This is a less likely explanation of the disproportionate increase in the low molecular weight GAG fraction.

The increased GAG excretion in the MI group is of interest in the light of previous studies which have shown a complex inter-relationship between tissue and plasma GAG, atherosclerosis, and myocardial infarction. The earliest pathological change in lipid deposition and atherosclerosis has been linked with change in GAG composition and concentration in the ground substance of the mesenchyme of the arterial intima and cardiovascular connective tissue.^{8,9} During the early oedematous reaction following experimentally induced MI in rats an alteration in GAG concentration in heart muscle has been reported.¹⁰ An increase in chondroitin sulphate in rat heart muscle was found to be associated with the earliest phase of the infarction process,¹¹ and similar findings were noted in the dog 10 days after ligation of the circumflex artery.¹² In view of these findings following MI it may be that the earliest sign of tissue damage in RA is also an alteration in GAG composition and concentration. However, because of the larger amounts of 'connective tissue' at risk of involvement in an inflammatory condition affecting joints it seems surprising that the urinary GAG levels are not even higher in the active RA group, though this might reflect a more insidious process than the single insult of a myocardial infarction.

Conventional monitoring of disease activity in RA uses several different measures each of which may reflect a different facet of the inflammatory disease process. As urinary GAG excretion is the presumed end point of tissue breakdown and is not an indirect measure (cf. ESR, a composite of various acute-phase reactants), it may be a useful addition to

conventional assessment techniques. The poor correlation noted between the change in ESR and change in urinary GAG excretion before and after second-line drug therapy in our minds invalidates neither assessment but rather supports the concept that the two measure different facets of the disease process. There is a wide variation in GAG excretion in both normal and disease groups, with considerable overlap, so that a single GAG measurement is of limited value. Serial measurements in individual patients may, however, be a useful indicator of either increasing activity or of response to second-line therapy.

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References

- 1 Murata K, Takeda M. Compositional changes in urinary acidic glycosaminoglycans in progressive systemic sclerosis. *Clin Chim Acta* 1980; **108**: 49-59.
- 2 Di Ferrante N. Urinary excretion of acid mucopolysaccharides by patients with rheumatoid arthritis. *J Clin Invest* 1957; **36**: 1516-20.
- 3 Firman C, Eronen I, Hämäläinen E. Increased urinary excretion of glycosaminoglycans degradation products in rheumatoid arthritis [Abstract]. *European Congress on Rheumatology*. Paris, 1981; 1077.
- 4 Mbuyi J, Dequeker J, Teblich M, Merlevede M. Relevance of urinary excretion of alcian-blue-glycosaminoglycans complexes and hydroxyproline to disease activity in rheumatoid arthritis. *J Rheumatol* 1982; **9**: 579-83.
- 5 Ropes M W, Bennett G A, Cobb S, Jacox R, *et al.* Revision of diagnostic criteria for rheumatoid arthritis. *Arthritis Rheum* 1958; **2**: 16-20.
- 6 Whiteman P. The quantitative determination of glycosaminoglycans in urine with alcian blue 8 Gx. *Biochem J* 1973; **131**: 351-7.
- 7 Salposky A K, Howell D S, Woessner J F. Neutral proteases and cathepsin D in human articular cartilage. *J Clin Invest* 1974; **53**: 1044.
- 8 Gerő S, Bihari-Varga M, Virág S, Vegh M. Investigations of the role of mucopolysaccharides in atherosclerosis. In: *Le rôle de la paroi artérielle dans l'athérogénèse*. Proc 169th Int Coll Cen Nat Rech Sci Paris, 1968: 789-819.
- 9 Smith E B. Acid glycosaminoglycans, collagen and elastin content of normal artery, fatty streaks and plaques. *Adv Exp Med Biol* 1974; **43**: 125-39.
- 10 Judd J T, Wexler B C. Myocardial connective tissue metabolism in response to injury. *Circ Res* 1970; **26**: 101-9.
- 11 Judd J T, Wexler B C. Sulphur-35 uptake in acid mucopolysaccharides of the rat heart during injury. *Am J Physiol* 1973; **224**: 312-7.
- 12 Shetlar M R, Davitt W F, Shetlar C L, *et al.* Glycosaminoglycan changes in healing myocardial infarction. *Proc Soc Exp Biol Med* 1978; **158**: 210-4.