β_2 -microglobulin levels in serum and urine of rheumatoid arthritis patients on gold therapy

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SUMMARY The levels of β_2 -microglobulin (β_2 -m) in serum and urine of 24 seropositive patients with rheumatoid arthritis (RA) treated with regular gold (sodium aurothiomalate) injections have been investigated. The values obtained were compared with levels from 20 seropositive patients with RA treated only with nonsteroidal anti-inflammatory drugs and 20 age and sex matched normal controls who had received no medication. A significant increase of urinary β_2 -m levels was found in the gold-treated RA group. No correlation between dose of gold received and the levels of β_2 -m in the urine could be established. There was also no correlation between the erythrocyte sedimentation rate (ESR) or total lymphocyte count and β_2 -m levels in serum or urine. We conclude that serum and urinary β_2 -m levels appear to be poor indices of joint inflammation, but sequential urinary β_2 -m levels may prove valuable in monitoring the development of renal tubular lesions due to gold therapy.

β_2 -microglobulin (β_2 m), a low molecular weight protein (11 800), was first isolated from urine of patients with renal tubular disorders.¹ Since then urinary β_2 m estimation has been regarded as an index of tubular reabsorptive capacity.² Quantitative analysis of β_2 -m by radioimmunoassay is a sensitive method³ and has been widely used in screening for tubular dysfunction in general epidemiological studies⁴ and in studies on 'cadmium exposed' workers⁵, proteinuria being the first sign of the renal abnormality due to prolonged exposure to the heavy metal cadmium.⁶ The proteinuria of cadmium toxicity has been considered to be tubular in origin, on the basis of a very high β_2 -m excretion despite only moderate increases in the total protein excretion, a characteristic of tubular proteinuria.²

This study was carried out to investigate whether gold, also a heavy metal, may cause tubular dysfunction similar to that observed in cadmium toxicity and whether measurement of serum and urinary β_2 -m could be used to predict early renal dysfunction in the gold treated patients. Further, the value of determining levels of β_2 -m in serum and urine as an objective measurement of inflammation in rheumatoid arthritis, as previously suggested,⁷ was also assessed.

Subjects and methods

All patients selected had normal renal function, defined as a creatinine clearance of at least 80 ml/minute, and none had proteinuria at the commencement of the study. Patients with amyloidosis, Sjögren's syndrome, or renal insufficiency from any other causes were excluded. Three groups of patients were studied.

Group 1. Twenty age and sex matched normal controls who were not receiving any form of medication (mean age 47 years, 10 females and 10 males).

Group 2. Twenty patients with classical seropositive rheumatoid arthritis (RA) aged between 21 and 68 years (mean age 48 years, 12 females and 8 males) and who were not on gold or penicillamine but were receiving treatment with nonsteroidal anti-inflammatory drugs such as naprosyn, ibuprofen, and indomethacin. Duration of disease ranged from 9 months to 20 years.

Group 3. Twenty-four patients with classical seropositive rheumatoid arthritis aged between 21 and 67 years (mean age 47 years, 14 females and 10 males). Duration of disease ranged from 9 months to 15 years. Patients in this group were already receiving regular gold (sodium aurothiomalate) injections in addition to nonsteroidal anti-inflammatory drugs. The average total dose of gold

received was 1100 mg, the duration of gold therapy ranging from 2 months to 3 years.

A further group comprising 8 patients with rheumatoid arthritis (mean age 46 years range 26–64 years, (6 females and 2 males) was also studied. In these patients, who had disease of recent onset (6–18 months' duration), serial measurements of serum and urinary β_2 -m levels were performed, starting before the administration of gold therapy and subsequently at fortnightly intervals for a period of 6 months.

Blood for serum creatinine, serum β_2 -m, full blood count, and erythrocyte sedimentation rate (ESR) was collected from patients on the same day as the 24 hour urine specimen. In order to avoid degradation of β_2 -m, which is known to occur in acid conditions,⁸ the 24 hour urine samples were rendered alkaline by the addition of 5 g of sodium bicarbonate to each container before collection. This ensured a urinary pH of approximately 6. Addition of sodium bicarbonate in concentrations of 2–4 g/l had no effect on concentration of β_2 -m determined by the radioimmunoassay technique. Samples were stored at -20°C prior to assay. β_2 -m was measured by the Phadebas radioimmunoassay kit (Pharmacia).

The values of serum and urinary β_2 -m showed a skewed distribution within groups, and for this reason nonparametric tests, the Kruskal-Wallis 1-way analysis of variance by ranks and the Mann-Whitney U test,⁹ were used and results expressed as median values and range.

Results

There was a significant difference ($P < 0.05$) in the urinary β_2 -m levels between the RA group on gold therapy and the RA group on nonsteroidal anti-

inflammatory drugs alone (Table 1). Significant differences ($P < 0.05$) were also observed in the urine β_2 -m levels between controls and the RA group on gold therapy. There was no difference in the urine β_2 -m levels between controls and the RA group treated only with nonsteroidal anti-inflammatory drugs.

For the serum β_2 -m levels there was no significant difference ($P > 0.1$) between the RA group on gold therapy and the RA group on nonsteroidal anti-inflammatory drugs, but there was a significant difference between RA groups and controls ($P = < 0.01$ for RA group on gold and $P = < 0.05$ for RA group on nonsteroidal anti-inflammatory drugs).

There was a highly significant difference ($P = 0.001$) in ESR levels between the groups, but there was no correlation between the ESR and either serum or urinary β_2 -m levels. There were no significant differences between the total lymphocyte counts for each group, nor did the total lymphocyte count correlate with either serum or urine β_2 -m levels.

In the group of 8 rheumatoid patients on whom sequential measurements of serum and urinary β_2 -m levels were performed no significant change in the levels of β_2 -m in serum was seen after 6 months of regular gold injections (total dose 1200 mg) from that seen prior to gold therapy. The urinary and serum β_2 -m levels prior to gold therapy were not significantly different from those of other rheumatoid patients treated with nonsteroidal anti-inflammatory drugs alone. The mean value for urinary β_2 -m levels rose from 104 $\mu\text{g/l}$ to 183 $\mu\text{g/l}$ during the 6-month period. Although because of the small number of patients involved these figures are not statistically significant, the urinary β_2 -m levels in these patients after 6 months of gold therapy was similar to the urinary β_2 -m levels in the 'gold-treated' patients (group 3).

Table 1 Creatinine clearance, serum β_2 -m, urine β_2 -m, ESR, and total lymphocyte count in RA patients and controls. The results are expressed as median values and range

	Creatinine clearance ml/minute	Serum β_2 -m mg/l	Urine β_2 -m $\mu\text{g/l}$	ESR mm/1st hour	Total lymphocyte count $\times 10^9/\text{l}$
Group 1, normal controls n=20	91 (82–118)	2.0 (0.6–2.8)	81 (3.6–320)	5 (3–24)	1.305 (1.1–2.258)
Group 2, RA on NSAID n=20	92 (78–125)	3.1 (1–7)	90 (3.6–533)	60 (13–131)	1.602 (0.9–3.21)
Group 3, RA on gold n=24	90 (78–126)	3.71 (1.3–8)	183 (18–980)	28 (8–92)	1.582 (0.95–3.1)
Kruskal-Wallis test (3 groups)	$\chi^2 = 2.05$	$\chi^2 = 6.62$	$\chi^2 = 7.17$	$\chi^2 = 39.88$	$\chi^2 = 0.48$
DF = 1 for all χ^2 tests	NS	$P < 0.05$	$P = < 0.05$	$P = < 0.001$	NS
Mann-Whitney U test					
Group 1 vs. 2	NS	$P < 0.05$	NS	$P < 0.001$	NS
Group 1 vs. 3	NS	$P < 0.01$	$P < 0.05$	$P < 0.001$	NS
Group 2 vs. 3	NS	NS	$P < 0.05$	$P < 0.001$	NS

NSAID = nonsteroidal anti-inflammatory drugs.
NS = not significant.

Discussion

Proteinuria is a well recognised complication of gold therapy in rheumatoid arthritis and has been reported to occur in 1–11% of gold treated patients.^{10 11} Both glomerular and tubular mechanisms may be involved. An immune complex nephritis due to gold salts has been described.^{12 13} Tubular involvement is known from the development of acute tubular necrosis during gold therapy,¹⁴ and the development of tubular lesions in gold treated animals.^{15–17} Further, it has been shown that gold inclusions appear in the proximal tubules within a few hours of administration of the drug,¹⁸ and that the proximal tubule is the major site of gold deposition in the kidney.^{19–21} Immunoglobulins and complement have also been demonstrated in the endothelium and basement membranes of renal tubules in immunofluorescence studies.¹²

Urinary β_2 -m estimation is said to be a sensitive index of tubular dysfunction in several diseases, including heavy metal toxicity.^{2 3} In our rheumatoid patients treated with gold urinary β_2 -m levels were found to be significantly raised when compared to the rheumatoid patients treated with nonsteroidal anti-inflammatory drugs alone. In fact the latter group had similar urinary β_2 -m levels to the normal controls, which implies that the high β_2 -m levels in the gold treated patients could well be related to the gold therapy.

Previous workers have suggested a correlation between serum β_2 -m levels and glomerular function,²² but not between serum and urinary β_2 -m levels. Glomerular function as measured by creatinine clearance was normal in all groups of patients in this study. However, the serum levels of β_2 -m were significantly higher in both groups of rheumatoid patients than in the control group, though there was no significant difference in the serum β_2 -m levels between the 2 rheumatoid groups. This would tend to support the view that the higher urinary β_2 -m levels in the gold treated rheumatoid patients were neither due to differences in glomerular function in the gold treated rheumatoid patients, nor due to raised serum levels of β_2 -m per se, but rather to the gold therapy.

However, the reason for the increase in urinary β_2 -m levels in gold treated patients may not be straightforward. Although the urinary β_2 -m levels in these patients were raised relatively to the controls and to the group treated with nonsteroidal anti-inflammatory drugs, there was no apparent correlation between the urinary β_2 -m levels and either the duration of gold therapy or the total dose of gold administered. In the patients followed up sequentially serum and urinary β_2 -m levels before

gold therapy were similar to those in the group not treated with gold. The mean dose of gold given to the 2 groups was also similar. The urinary β_2 -m levels after 6 months of gold therapy reached the levels seen in the 'gold treated' group (group 3), suggesting that gold was responsible for the rise in the urinary β_2 -m levels. It is probable that a six-month sequential study is too short, and it is hoped to continue this study for a further 3-year period.

The serum β_2 -m levels were significantly higher in both rheumatoid arthritis groups than in the controls, and this confirms the reports of other workers.⁷ A correlation between serum β_2 -m levels and total lymphocyte count has previously been reported in a group of 21 patients with RA.⁷ We have been unable to confirm this finding. In fact we could establish no correlation between ESR values and levels of β_2 -m in either serum or urine or between the total lymphocyte count and β_2 -m levels in serum and urine. This would indicate that β_2 -m levels do not reflect inflammation per se and are not therefore useful as an objective measurement of inflammation.

However, the finding of raised levels of β_2 -m in urine of patients on gold therapy may suggest slow or latent development of renal tubular dysfunction in some patients. It would seem to be important when studying β_2 -m levels in rheumatoid disease that adequate importance is given to the patient's drug therapy, as it is possible that other drugs may potentiate the tubular effect of gold.

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