N-ACETYL-β-D-GLUCOSAMINIDASE URINARY EXCRETION AS AN EARLY INDICATOR OF KIDNEY DYSFUNCTION IN RHEUMATOID ARTHRITIS PATIENTS ON LOW-DOSE METHOTREXATE TREATMENT

P. WILAND, J. ŚWIERNOT and J. SZECHIŃSKI
Institute of Rheumatology, Medical Academy and PKP Hospital, Wrocław, Poland

SUMMARY
The N-acetyl-β-d-glucosaminidase (NAG) activities and albumin levels in the urine of 32 patients with active rheumatoid arthritis treated with low-dose pulse methotrexate (MTX) have been investigated. An increase in NAG urinary excretion was more frequent than the incidence of micro- or macroalbuminuria on entry, and during treatment with MTX. There was also a significant decrease in NAG levels observed at week 24. Parameters such as patient's age, time from onset, previous and current treatment did not allow us to predict the degree of NAG enzymuria. We conclude that MTX does not cause marked damage to renal proximal tubules; on the contrary, the observed significant decrease of urinary NAG on week 24 could be interpreted as a beneficial effect of MTX on kidney function. Early detection of high NAG enzymuria and elevated albumin levels in urine before the initiation of MTX therapy could be helpful in predicting possible MTX toxicity probably related to impaired renal clearance of MTX. Patients withdrawn from the study for non-renal-related adverse events also had an unusually large increase in urine NAG activity and urine albumin levels.

KEY WORDS: Methotrexate, N-Acetyl-β-d-glucosaminidase (NAG), Urine albumin, Rheumatoid arthritis.

Low-dose pulse methotrexate (MTX) therapy is regarded as being an effective drug treatment in rheumatoid arthritis (RA). MTX toxicity is increased in patients with renal insufficiency due to delayed excretion of the drug, since it is predominantly excreted by the kidneys through glomerular filtration and tubular secretion.

The co-administration of sodium salicylate with MTX results in decreased renal clearance of MTX and increased toxicity as leucopenia [1]. There are conflicting results between putative interactions of low-dose pulse MTX with other non-steroidal anti-inflammatory drugs (NSAIDs). Some papers suggest no significant interactions in patients on low-dose MTX treated with piroxicam, sulindac and naproxen [2, 3], other work reports a possible interaction in patients treated with naproxen and ibuprofen [4]. Impairment of renal function has been observed in cancer patients on high-dose MTX treatment and it remains unclear whether the administration of low doses of MTX may have similar effects [5].

The elevation of N-acetyl-β-d-glucosaminidase (NAG) in urine has been shown to be associated with reversible tubular damage [6, 7]; therefore, any elevation in urinary NAG levels may herald renal impairment [8, 9].

The purpose of the present study was to determine the effect of low-dose MTX therapy on glomerular and tubular function. Urine albumin was used as a marker of glomerular function. NAG served as an indicator of proximal tubular damage.

PATIENTS AND METHODS

Patients
All the patients met at least four of the ARA 1987 revised criteria for RA [10]. Thirty-two patients (27 women, five men) were included in the study. Their mean age was 59 yr (range 24–74 yr). The mean time from onset was 94.5 months (range 4–468 months). All the patients had active disease with clinical and biochemical indices of active RA. No patient had a history of, or current, renal disease (Table I). The majority of patients were taking NSAIDs. MTX was given once a week as a single morning dose; starting with 7.5 mg. The dose adjustments were not made before the sixth week of treatment and the final total dose did not exceed 10 mg/week.

Clinical assessments
Clinical evaluations were performed by the same physician–investigator every 4 weeks for the first 3 months and finally at 6 months. They included pain scale, modified Ritchie articular index (maximum score = 26) and patient’s global impression assessment with overall rating of change on a scale of 0–4, from the start of treatment.

Laboratory assessments
Full blood cell count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum creatinine, serum aspartate aminotransferase, serum alanine aminotransferase and alkaline phosphatase measurements were taken at every clinical assessment. Urine samples were obtained from patients for routine analysis as well as for the determination of NAG, creatinine and albumin levels.

© 1997 British Society for Rheumatology
Albumin was measured in fresh and centrifuged urine samples. A 500 μl volume of reagent was added to 50 μl of undiluted urine (Turbiquant Albumin/Behring). The turbidity generated in the reaction was measured photometrically. Reference values for albumin in urine were <20 mg/l. NAG was assayed in 162 urine samples from 32 patients in the course of MTX treatment. An average of five (3–8) specimens were performed for each patient at baseline, and sequentially on week 1, 2, 4, 8, 12 and 24. The estimations were performed on early morning mid-stream urine [11].

Urinary NAG activity was assessed by the method of Merle et al. [12] with some elements of Price et al.’s [9] method added. A 0.05 ml volume of 20-fold diluted urine was diluted again with 0.95 ml of a solution of 4-methylumbelliferyl-N-acetyl-b-D-glucosaminide (MUNAG; Sigma Chemical Co., St Louis, MO, USA) in 0.2 mm citrate/phosphate buffer (pH 4.74). It was incubated at 37°C for 30 min. Hydrolysis was stopped by the addition of 3.0 ml of 0.25 M glycine–sodium hydroxide buffer (pH 10.4). Fluorescence released by the 4-methylumbelliferone (4-MU) was measured with a spectrofluorometer (Perkin-Elmer Corp.). The final result was expressed as nanomoles of 4-MU released from the substrate per hour per milligram of urinary creatinine which provides a measure independent of urine concentration and avoids the necessity for 24 h urine collections [13].

The creatinine concentration was measured by the Jaffe reaction using alkaline picric acid reagent.

Statistical analysis

The χ² test with Yate’s correction was used for comparison of two groups of patients with decreased and increased NAG activity for the parameters characterizing activity of disease, and for the incidence of microalbuminuria and high NAG values in the course of MTX treatment. The paired values for individual patients were compared between periods of treatment by the Wilcoxon matched-pairs signed-ranks test. P values below 0.05 were considered statistically significant. When discussing the results, a P value between 0.05 and 0.1 was considered as almost significant.

RESULTS

Values for urinary NAG/creatinine in the control group

The NAG activity in the urine of 30 control subjects was 25.4 ± 17.2 (3.4–71.3 nmol/h/mg of creatinine) and the 95% one-sided upper prediction value was 56.8 nmol/h/mg of creatinine. The control subjects were 30 patients (24 women and six men, mean age 56 yr, range 30–71 yr). They comprised healthy subjects and patients with non-inflammatory musculoskeletal pains and neurosis. They were matched by age and gender. None of the control subjects were suspected of having renal diseases and they were not receiving potentially nephrotoxic drugs.

Patient results

The clinical characteristics of patients with RA on entry to the treatment with MTX are summarized in Table I. In three patients, MTX had to be stopped due to adverse reactions for the following reasons: one patient for mucocutaneous lesions (at week 2) and two patients for leucopenia (at week 4 and 12). All three patients withdrawn from the study had a large increase in the NAG value at the time of withdrawal. Two of them had both abnormal NAG and albumin excretion values at baseline. One patient was lost to follow-up after 8 weeks. In four patients, we could not obtain urine samples for NAG activities at week 24, so we submitted statistical analysis for only 24 patients, who finished the treatment. In 21 patients, albumin and NAG values in urine were performed simultaneously at baseline, week 12 and week 24.

High values of urinary NAG/creatinine were more common than the incidence of micro- or macroalbuminuria on entry (71.4% vs 14.3%, P < 0.001) as well as during MTX treatment at week 12 (38% vs 0%, P < 0.001) and at week 24 (47.6% vs 19%) (Table II).

Five patients had both abnormal NAG and albumin excretion values at baseline. Two of the five patients were withdrawn from the study; in the third patient, who continued the study, we observed transient proteinuria and macroalbuminuria (7- to 20-fold increase) at week 16. This was accompanied by a 4.6- to 10-fold increase in urine NAG activity.

The median NAG value for the 24 patients, who finished the study, was 125.95 (interquartile range 79.2–180.1) nmol/mg of creatinine at baseline. High NAG values were seen in 18/24 (75%) patients. We observed a gradual decrease in pathological NAG values and the percentage of high NAG excretion at weeks 12 and 24 (Fig. 1). The significant fall in NAG after 24 weeks (P < 0.05) of MTX therapy, but not after 12 weeks (P < 0.25), was demonstrated using Wilcoxon matched-pairs signed-ranks test (Table III).

We compared the median of NAG estimations (it was measured in each patient 3–8 times during the whole treatment with MTX) to NAG excretion in urine

### Table I

| Clinical characteristics of patients with RA on entry to the treatment with MTX |
|---------------------------------|-----------------|-----------------|-----------------|
| Female: male ratio              | 27:5            | 25:7            | 27:5            |
| Median age, yr (range)          | 59 (24–74)      | 59 (24–74)      | 59 (24–74)      |
| Median duration of disease, months (range) | 94.5 (4–468)   | 94.5 (4–468)   | 94.5 (4–468)   |
| Previous treatment with gold salts (no. of patients) | 19              | 19              | 19              |
| Positive rheumatoid factor (no. of patients) | 27              | 27              | 27              |
| Oral corticosteroid therapy ever | 17              | 17              | 17              |
before the onset of treatment. This allowed us to distinguish all patients into two groups: 11 patients with a rise and 20 patients with a decrease in median NAG activities (for one patient, the NAG value before treatment was not available). Patients’ age, time to onset, current and previous consumption of NSAIDs, previous history of gold treatment and rheumatoid factor did not differ between both groups. Thus, these parameters could not influence the occurrence of eventual damage to renal proximal tubules during MTX treatment (Table IV). We compared the two groups, those with increased and those with decreased urinary NAG, for the following variables characterizing disease activity: ESR, modified Ritchie articular index, pain scale and patient’s global impression at weeks 12 and 24. There were no statistically significant differences between the occurrence of these parameters in both groups with one exception of the pain scale at week 24 using the $\chi^2$ test with Yate’s correction (Table V).

We noticed elevated urine albumin values in 11 patients (16 of 118 urine samples) during the whole period of treatment. In eight out of 11 of these patients, pathological NAG activity was noted. Greater than 2-fold rises in urine albumin were seen in three patients and all of them showed very large rises in NAG enzymuria (Fig. 2).

Only 3/32 patients did not require NSAID before initiation of MTX therapy, but 8/23 (35%) were NSAID free at week 24. High NAG values greater than twice the normal range (113 nmol/mg creatinine) observed at week 24 were less frequent in patients who did not take NSAID (12.5%) than in those who were treated with NSAID (36%, predominantly diclofenac—5/6). However, due to the small number of patients involved, these figures are not statistically significant.

### DISCUSSION

The major finding of the present study is that despite a high frequency of abnormal urinary excretion of NAG on entry, we observed a decrease in the frequency of high NAG values in the course of MTX treatment. NAG is a hydrolytic enzyme present in high concentration in the lysosomes of proximal renal tubular cells. It has a mol. wt of $\sim$140 000 Da and finds its way into urine when tubule cells undergo alterations. Although NAG is not organ specific, the appearance of increased quantities of this macromolecule in urine could, after exclusion of damage to the glomerular basement membrane, be interpreted as evidence of tubular damage. An increase in the urinary NAG excretion was observed in patients with progressive or active renal parenchymal primary diseases, diabetes and RA [12, 14–16]. The detection of elevated levels of NAG allows us to predict the potentially nephrotoxic action of many drugs, e.g. NSAIDs, aminoglycosides, cyclosporin and gold [8, 12, 18–20]. Most of them are often used in the treatment of RA.

Although proteinuria has been reported in 1–11% of gold-treated patients, MTX is mentioned as a nephrotoxic drug only during the treatment of cancer with high doses [5]. The most significant route of MTX excretion is through the kidney. Seventy to ninety per cent of an i.v. dose of 10–20 mg MTX appears in the urine within 24 h [22]. Since MTX is secreted by the renal tubules, its elimination in urine may be reduced by the concomitant administration of such drugs as salicylates, which are also excreted by the organic transport system [23]. MTX toxicity is largely dependent on the duration of exposure to the drug (dependent on the duration of the drug elimination phase). Thus, increased MTX toxicity occurs more

![Fig. 1.—NAG excretion in urine (expressed in nmol/h/mg of creatinine) compared with the ratio of NAG activity at weeks 1, 2, 4, 6, 8, 12 and 24 to the baseline result (expressed as a percentage) during MTX treatment course. The results of 32 patients are given as median values.](image-url)
TABLE V

<table>
<thead>
<tr>
<th></th>
<th>Week 12</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decrease</td>
<td>Increase</td>
</tr>
<tr>
<td>Fall/rise of ESR</td>
<td>14/5</td>
<td>3/4</td>
</tr>
<tr>
<td>Fall/rise of Ritchie index</td>
<td>16/3</td>
<td>4/4</td>
</tr>
<tr>
<td>Fall/rise of pain scale</td>
<td>16/2</td>
<td>4/4</td>
</tr>
<tr>
<td>Better/worse in patient’s GPI‡</td>
<td>18/1</td>
<td>5/3</td>
</tr>
</tbody>
</table>

*No. of patients.
†$P$ value using $\chi^2$ test with Yate’s correction.
‡GPI, global patient’s impression.

often in patients with reduced creatinine clearance and receiving drugs such as salicylates [11]. Although the results of our study do not show a definite negative influence of MTX on renal function, we should not ignore such an effect even with low-dose MTX therapy.

There were two of three patients withdrawn in our study for leucopenia and mucocutaneous lesions who had high values of urinary NAG and albumin on entry as well as during MTX therapy. In the third patient who was withdrawn, we observed a gradual increase in NAG value during treatment with peak NAG excretion at the time of withdrawal.

None of the withdrawn patients was found to have elevated levels of creatinine in serum or proteinuria. Thus, estimation of urinary NAG activity and albumin could be helpful in predicting possible adverse events of MTX treatment. Subclinical, renal glomerular and tubular dysfunction may be a possible reason for impairment of MTX elimination and the development of non-renal-related adverse events.

Previous workers have suggested the usefulness of serial measurements of microalbuminuria in patients with RA, especially those receiving gold sodium thiomalate [24]. In their serial estimations in a patient on gold therapy, urine albumin was elevated first and followed by increased NAG excretion. Our study shows more frequent pathological values of urinary NAG excretion than albumin both on entry and during MTX treatment.

There have been only a few studies specifically analysing serial measurements of early indicators of kidney dysfunction in RA patients treated with disease-modifying anti-rheumatic drugs [12, 21, 24]. We are not aware of any previous studies using NAG or albumin levels in urine in assessing renal function in RA patients during low-dose MTX treatment. In our rheumatoid MTX-treated patients, urinary NAG levels were found to be significantly lower at week 24 and this could be due to a beneficial effect of MTX on inflammation in RA patients. However, a positive correlation between urinary NAG levels and pain scale was found at week 24. The results for ESR were almost significant. On the other hand, lower NAG values at week 24 were found in patients who had not received NSAIDs. This may suggest that NAG excretion was influenced by NSAID consumption. In previous reports, there have been conflicting conclusions as to whether abnormal urinary NAG excretion is inherent to RA or is caused by drugs [1, 2, 18, 25]. We believe that these findings showed renal proximal dysfunction probably being intrinsic to RA in a significant number of cases.

Low-dose MTX treatment does not cause marked injury of the renal proximal tubule in the majority of observed patients and the observed significant decrease of urinary NAG excretion at week 24 could have resulted from a reduction in the activity of RA itself.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Jan Charamsa and Bernadette Charamsa for their help and advice.

REFERENCES


