Urinary Excretion of Thiol Compounds in Patients with Rheumatoid Arthritis

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The objective of the present study was to assess the excretion of urinary thiol compounds in patients with active and inactive rheumatoid arthritis (RA). Urinary thiol compounds were measured by the method of Kokonov (M. T. Kokonov, Lab. Delo 5:273–276, 1965) in 51 outpatients with active and inactive RA. Those with active disease had significantly higher levels of urinary thioamine excretion.

The reaction of Kokonov (6) measures the levels of thiol compounds excreted in the urine. Kokonov (6) published a description of this simple method for the detection of the thiol compounds in patients with neoplasms. This method was found to be suitable for the screening of patients with a high risk of malignancy (4, 7, 13), but patients with viral infection also displayed a positive test result (12). Patients suffering from bacterial infections, active autoimmune diseases, and acute pancreatitis or myocardial infarction also had positive test results. We assumed that the reaction is suitable for assessment of inflammatory processes in patients with infections and autoimmune diseases (3). If this assumption is correct, urinary thiol compound excretion depends on the level of autoimmune disease activity. Of the autoimmune diseases, rheumatoid arthritis (RA) was chosen since the activity of RA can be adequately assessed. The major thiol compounds found in plasma are cysteine, cysteinylglycine, homocysteine, and glutathione. Increased levels of thiol compounds were found in patients with RA (5, 8, 10). The elevated plasma and urinary thiol compound levels may be associated with the degree of inflammation and could explain the reason for the higher incidence of death due to cardiovascular disease in RA patients (5).

MATERIALS AND METHODS

Urinary thiol compound concentrations were measured by the method of Kokonov (6), with modifications: 100 ml of an aqueous solution of 2 M selenous acid (SeO2; Reanal, Budapest, Hungary) was added to 5 ml of fresh urine obtained in the morning, and the absorbance at 560 nm was measured after 10 min. Positive samples showed an orange, red, or dark red color. According to our previous work (6), the mean absorbance value for 36 healthy volunteers was 0.072 ± 0.067 (standard deviations [SDs]). The value 0.206, derived from the equation mean + 4 × SD, was used arbitrarily as the upper limit of normal values.

Fifty-one patients with RA, according to the American College of Rheumatology 1987 revised criteria (1), were examined randomly by a single clinician from the National Institute of Rheumatology. Patients with malignancy and viral infection were excluded. Fresh morning urine samples were obtained. It is of importance that dilution of the urine be avoided (6); i.e., the use of diuretics or the intake of excess fluids was not allowed.

Demographic data, disease duration, current treatment for RA, the need for a nonsteroidal anti-inflammatory drug, corticosteroid dose, and the use of a disease-modifying antirheumatic drug (DMARD) were recorded. Visual analog scale pain score, Ritchie index, the total number of 44 swollen joints (9), and the duration of morning stiffness were assessed. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) concentration were measured. The Health Assessment Questionnaire (HAQ) (2) was completed by the patients. Active disease was defined as at least the following: six or more tender joints, three or more swollen joints (but proximal interphalangeal joints, metacarpophalangeal joints, and metatarsophalangeal joints of each hand and foot were calculated as a single unit); ESR of more than 28 mm/h; or a CRP concentration of more than 20 mg/liter.

Statistics. The mean, standard error, and SD of all data were calculated. None of the variables fit a normal distribution; therefore, comparisons of active and inactive RA were made by Mann-Whitney's U test. The correlation between levels of thiol excretion and clinical and laboratory variables was calculated by the Spearman rank correlation method. The dependence of the presence of elevated levels of thioamine excretion was tested by the chi-square test on the basis of the appropriate two-by-two contingency table. Differences with P values of <0.5 were considered statistically significant.

RESULTS

Fifty-one patients with RA were studied. There were 10 males and 41 females, with an average age of 54 years (age range, 20 to 77 years); the mean disease duration was 6 years. With the exception of six patients, all had been treated with a DMARD. Twenty-three patients had active RA and 28 were in remission. Those with active disease had significantly more tender and swollen joints and higher ESRs and CRP levels, but the durations of morning stiffness and the levels of joint pain were not significantly different between the two groups. Disease duration, sex distribution, and age were also not significantly different (Table 1). There was a slight correlation (r = 0.30; P < 0.05) between thioamine excretion and the number of swollen joints. Patients with active disease displayed significantly higher levels of thiol compound excretion (Table 1), and the number of patients with a positive test result (Fig. 1) was also significantly higher for the group with active disease (9 versus 1). The odds ratios for the presence of elevated thioamine excretion were 3.37 times higher (95% confidence intervals, 1.06 to 10.69) for patients with a high level of disease activity than patients with a low level of disease activity.

DISCUSSION

The major thiol compounds found in plasma are cysteine, cysteinylglycine, homocysteine, and glutathione. Kokonov (6) suggested that the compounds in the urine of patients with malignant tumors that react with selenous acid are thiourea, thioethanolamine, and thioethylenoxide. In fact, L-homocysteine, L-homocysteine-thiolactone, and tri-(2-thioureido-S-ethyl)-amine in urine give a positive color reactions with selenous acid.

High levels of urinary thiol compounds measured by the method of Kokonov (6), are excreted from patients with stom-
ach and other neoplasms (6). The increased levels of excretion of thiol compounds in patients with malignant tumors can be explained by the activation of the immune system, probably by the inflammation around the tumor tissue, since patients suffering from bacterial infections, active autoimmune diseases, and acute pancreatitis or myocardial infarction also had positive test results (3). Inflammatory processes, i.e., activity and proliferation of cells of the immune system, might be responsible for the production of thiouamines.

 Increased levels of thiol compounds were found in patients with RA (5, 8, 10). The elevated thiol compound levels could explain the reason for the higher incidence of death due to cardiovascular disease in RA patients (5) by means of the toxic effect of homocysteine on the endothelium and the increased susceptibility of low-density lipoproteins to oxidation by increased amounts of thiol compounds such as cysteine in plasma.

The increase in the homocysteine levels in RA patients could be explained by an impaired metabolism of thiol complications.
Homocysteine may be either methylated to form methionine or condensed with serine to form the thioether cystathionine and then cysteine. Homocysteine metabolism is dependent on three vitamins, i.e., vitamin B₆, vitamin B₁₂, and folic acid. RA patients seem to have a reduced capacity to metabolize and detoxify thiol compounds by methylation (14), leading to a rise in the total thiol content of plasma. Elevated plasma homocysteine levels may result from low levels of vitamins (10). The levels of pyridoxal 5-phosphate, the biologically active form of vitamin B₆, in plasma, are lower in patients with RA and are inversely associated with tumor necrosis factor alpha production. The elevated plasma and urinary thiol compound levels may be associated with the degree of inflammation.

In conclusion, our results are in accordance with those found earlier (3), in that the Kokonov reaction is suitable for assessment of inflammatory activity, together with other laboratory parameters, in patients with certain autoimmune diseases. Patients with active disease displayed significantly higher levels of thiol compound excretion, but in five patients with active RA, it was outstandingly high (Fig. 1). These patients need further follow-up concerning the activity and progression of RA. While this test is simple and easy to perform, the assessment of its clinical value for patients with RA requires further extended and follow-up studies.

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