

CONCISE REPORT

Intermittent rises in plasma homocysteine in patients with rheumatoid arthritis treated with higher dose methotrexate

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Objectives: To investigate the effect of higher weekly maintenance dose methotrexate (MTX) (≥ 25 mg/week) on plasma homocysteine concentrations in adults with RA.

Methods: Patients with RA were treated with high doses of MTX with adjuvant folic acid. Plasma homocysteine was determined at baseline and 1, 2, 4, 8, 12, and 48 hours after subcutaneous MTX administration. Maximum homocysteine concentrations after MTX administration were compared with baseline concentrations.

Results: Fifteen patients with RA (11 women) were included, with a median age of 61 years (range 31–72) and median disease duration 7 years (range 2–32). Median MTX dose was 30 mg (range 25–40). All patients received folic acid supplementation (5–30 mg/week). Median plasma homocysteine concentration at baseline was 10.1 $\mu\text{mol/l}$ (range 6.6–12.7; normal 6–15). Homocysteine concentrations increased after MTX administration by a median of 2.5 $\mu\text{mol/l}$ (range 0.7–5.1). Median maximum plasma homocysteine was significantly higher than at baseline. Peak homocysteine was reached after 12 hours. No relation between serum folate concentrations and plasma homocysteine concentrations was found.

Conclusions: In patients with RA higher MTX doses with adjuvant folic acid do not increase baseline concentrations of homocysteine. An intermittent significant rise in plasma homocysteine occurs in the 48 hours after MTX administration.

Rheumatoid arthritis (RA) is associated with increased cardiovascular morbidity and mortality.^{1,2} Traditional risk factors (hypertension, smoking, dyslipidaemia), as well as mediators of inflammation and homocysteine are considered to play a part. Hyperhomocysteinaemia is associated with cardiovascular disease, in a concentration related manner.^{3,4} Different pathogenic mechanisms are involved, such as the effect of homocysteine on endothelium function, intima and media proliferation, and a procoagulant effect.³ In patients with RA higher homocysteine serum concentrations are found than in normal controls.⁵ Homocysteine plasma concentrations are influenced by methotrexate (MTX), which may result in increased cardiovascular morbidity and mortality. However, studies on cardiovascular mortality in patients with RA treated with MTX are not conclusive.^{6,7}

Most studies on the effect of MTX on homocysteine concentrations in patients with RA showed a substantial rise in homocysteine concentrations during MTX treatment,^{8–11} an effect that was diminished by folate supplementation.^{8,10,11} These studies were performed in patients treated with low dose MTX (maximum dose 25 mg/week). The homocysteine concentrations were measured at one time, some in a fasting

state. Higher doses than 25 mg/week are being used nowadays to improve efficacy. The effect of high dose maintenance MTX treatment on homocysteine concentrations in RA is not known. In this descriptive study we studied homocysteine concentrations in patients with RA who were included in a pharmacokinetic study of higher dose MTX (≥ 25 mg/week).

METHODS

Patients with RA who were treated with MTX in a stable dose of ≥ 25 mg/week were included in a pharmacokinetic study comparing the bioavailability of the oral and subcutaneous route of MTX administration. MTX concentrations were determined from intake of MTX until 48 hours later. This study has been described in detail elsewhere.¹² Folate supplementation was continued at the discretion of the attending physician. At the moment of sampling the patients had had breakfast at home, and during the day they had lunch and dinner in the hospital.

At baseline, blood was drawn for determination of serum creatinine, folate, and cobalamin. Creatinine was determined according to the Jaffe method on a modular P800 auto-analyser (Roche Diagnostic Systems, Penzberg, Germany); folate and cobalamin concentrations were determined by an immunoassay on an AxSYM analyser (Abbott Laboratories, Abbott Park, IL, USA).

Subcutaneous MTX was administered in the upper leg. At baseline and 1, 2, 4, 8, 12, and 48 hours after MTX subcutaneous administration, blood was drawn for homocysteine determination. Blood was collected in an EDTA tube and put on ice immediately. The blood samples were centrifuged and the plasma samples frozen at -20°C until analysis. Homocysteine concentrations were determined as previously described by Araki and Sato¹³ with the modification of Vester and Rasmussen.¹⁴ Proficiency testing shows a homocysteine coefficient of variation of 3.0%.

Analysis

Wilcoxon signed rank tests (paired samples) were performed to compare maximum homocysteine concentrations after MTX administration with baseline homocysteine concentrations. A p value < 0.05 was considered significant. The tests were performed two sided.

The relation between serum folate, serum cobalamin, creatinine clearance, age, pharmacokinetic parameters of MTX (maximum concentration (C_{max}), time till maximum concentration (T_{max}), area under the curve (AUC) of time versus MTX concentration, bioavailability(F)), and homocysteine was studied by linear regression analysis.

RESULTS

Fifteen patients with RA (11 women) were included. Median age was 61 (range 31–72), median disease duration 7 years (range 2–32), median MTX dose 30 mg (range 25–40). All

Abbreviations: MTX, methotrexate; RA, rheumatoid arthritis

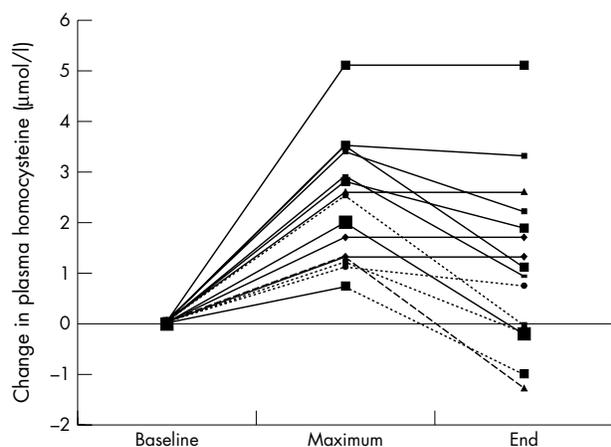


Figure 1 Change in plasma homocysteine concentrations after subcutaneous MTX (25–40 mg), from baseline to maximum and to the end (48 hours) of the study in 15 patients with RA (every line represents one patient). (Solid line: folic acid 5 mg once a week; dashed line: 3 days a week; dotted line: 6 days a week).

patients had folate supplementation, in doses varying from 5 to 30 mg weekly. Four patients received folate supplementation on 6 days of the week, one patient on 3 days of the week, the other patients once weekly 3–4 days after MTX administration. Foliates were not used on the day of MTX administration. Creatinine clearance, serum folate, and serum cobalamin concentrations were normal in all patients. The median plasma homocysteine concentration at baseline was 10.1 $\mu\text{mol/l}$ (range 6.6–12.7). The median maximum rise in homocysteine concentration after MTX administration was 2.5 $\mu\text{mol/l}$ (range 0.7–5.1).

Figure 1 shows the change in homocysteine concentrations from baseline ($t=0$) to maximum concentrations, and to 48 hours after MTX administration for the individual patients. The time until maximum homocysteine concentration varied among patients from 8 to 48 hours. In seven patients the time of maximum homocysteine concentration was 8 hours, in five patients 12 hours, and in three patients 48 hours.

No correlation between serum folic acid and homocysteine concentrations was found, and there was no significant correlation between homocysteine concentrations, change in homocysteine, time till maximum concentration, and pharmacokinetic parameters of MTX. Age and creatinine clearance were not related to homocysteine concentrations (data not shown).

DISCUSSION

Our data show an intermittent significant rise in plasma homocysteine concentrations during higher dose MTX maintenance treatment. This rise occurred in every patient and in most patients within 48 hours after MTX administration. Because these patients are receiving maintenance treatment with MTX, the homocysteine concentration at baseline indicates a steady state concentration. The homocysteine concentrations after 48 hours are considered to return to “baseline” concentrations before the next administration.

The effect of low dose MTX on homocysteine was evaluated in various studies in patients with RA starting MTX treatment. Homocysteine concentrations at baseline, before the start of MTX treatment, varied from 12.3 to 15.4 $\mu\text{mol/l}$, and during MTX treatment a significant rise was seen. In general, this effect could be reversed by folate administration.^{9–11} The course of homocysteine concentrations after MTX

administration was studied only once. That study comprised 13 patients with psoriasis and controls, treated with a weekly dose of 25 mg MTX. The patients had normal serum folate and serum cobalamin concentrations, and did not receive folate supplementation. Plasma homocysteine concentrations in the patients with psoriasis were higher than in control subjects (14.4 (4.8) v 10.8 (2.9) $\mu\text{mol/l}$). A variable increase in homocysteine concentration was found, reached within 3 days after MTX administration. A mean rise in homocysteine concentration of 37% occurred. A weak correlation between folate and homocysteine concentrations was found.¹⁵ In our study a lower baseline concentration and a smaller rise in homocysteine concentrations was found, possibly explained by the fact that all our patients received folates.

We chose to continue all comedication, including folic acid, to mimic daily practice. The rise in homocysteine seems smaller in the patients using folic acid every day (fig 1). The difference is not statistically significant. However, the numbers are too small to draw conclusions.

What do our results mean for clinical practice? Up to the present the relation between a periodic rise in homocysteine after MTX administration and clinical effects has not been demonstrated. In general, the relation between homocysteine and cardiovascular morbidity and mortality has not been fully elucidated. In patients with RA who are generally treated with MTX for a long time, and who have an increased risk of cardiovascular morbidity, a rise of homocysteine each week, might possibly lead to a substantial exposure to homocysteine, resulting in cardiovascular morbidity. Folate supplementation, as we used in our study, evidently cannot prevent the rise in homocysteine. The question arises whether a different dosing regimen of folates might diminish the effect on homocysteine, without reducing the effectiveness of MTX. A daily folic acid dose, or a weekly dose within 12 hours after MTX intake, may be preferable. This warrants further study.

In conclusion, we can say that patients with RA who are treated with higher dose MTX (25–40 mg), and who receive folate supplementation, do not have raised baseline homocysteine concentrations. However, in most patients, a significant rise in homocysteine concentration occurs in the 48 hours after MTX administration.

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