Hyperhomocysteinemia in Rheumatoid Arthritis: Influence of Methotrexate Treatment and Folic Acid Supplementation

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ABSTRACT. ***Objective.*** To examine the effect of methotrexate (MTX) and folic acid supplementation on the homocysteine level in patients with rheumatoid arthritis (RA).

Methods. A cross sectional study was performed in 81 patients with RA, comprising a standardized clinical interview, an examination, and a blood specimen test.

Results. P-homocysteine tended to be lower in 41 patients receiving MTX, compared with 40 patients not receiving MTX. Of the MTX treated patients, 76% received folic acid supplementation. Multivariate analysis revealed a statistically significant association between P-homocysteine and P-creatinine ($p < 0.001$), and disease activity/progression measured by the Health Assessment Questionnaire score ($p < 0.001$). There was a tendency to negative association between P-homocysteine and folic acid supplementation.

Conclusion. P-homocysteine in patients with RA receiving MTX and folic acid supplementation did not differ significantly from P-homocysteine in RA patients receiving other types of treatment. (J Rheumatol 2002;29:1615–8)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
HYPERHOMOCYSTEINEMIA
METHOTREXATE
FOLIC ACID SUPPLEMENTATION

Hyperhomocysteinemia is an independent risk factor of arterial as well as venous thrombosis[1-3]. P-homocysteine is easily corrected by folic acid supplementation, although occasionally B6 and B12 vitamin supplementation is also required[4]. However, it remains unknown whether normalization of P-homocysteine reduces the risk of thrombosis[5].

Low dose methotrexate (MTX) is the choice of treatment in psoriatic and rheumatoid arthritis (RA)[6]. Treatment with MTX causes an increase of P-homocysteine[7], whereas folic acid supplementation causes a decrease[8]. The overall effect on P-homocysteine of treatment with both drugs is unknown, as this treatment has not been compared with other regimens in RA. The question was emphasized in a recent report on the mortality rate in RA[9]. MTX treated patients with arteriosclerotic risk factors had a higher mortality rate than corresponding patients treated by other disease modifying antirheumatic drugs.

We examined the overall effect of MTX and folic acid treatment on P-homocysteine in patients with RA.

MATERIALS AND METHODS

At the Department of Rheumatology, University Hospital of Aalborg, and the General Hospital of Hjørring, outpatients and inpatients fulfilling the 1987 American College of Rheumatology criteria for RA[10] were asked to join the study, if they had received MTX for at least 6 months or had not been treated with MTX during the last 6 months. Equal group sizes were intended. Pregnant women and patients undergoing estrogen replacement therapy were excluded.

Patients were enrolled from November 1998 through May 1999. On the same day we performed a standardized clinical interview and an examination. The interview included a Health Assessment Questionnaire (HAQ) score[11], a standardized measurement of physical limitations in daily life activities used in RA patients (minimum disability 0, maximum disability 3). A blood specimen was drawn from the cubital vein of the nonfasting patient in sitting position[12]. C-reactive protein (CRP) and P-creatinine were determined by standard analysis (CRP reference interval < 10 mg/l; P-creatinine reference interval: men 60–125 μmol/l, women 55–115 μmol/l). Part of the blood sample was centrifuged and frozen immediately for later analysis. P-homocysteine was determined by standard analysis using high performance liquid chromatography[12], reference interval: men 1.0–14.6 μmol/l, women 0.8–12.0 μmol/l.

The study was approved by the regional ethical committee.

Statistics. Data were analyzed using standard procedures in SPSS. The bivariate associations between P-homocysteine (continuous variable) and sex, MTX treatment, and folic acid supplementation (categorized variables), age, P-creatinine, HAQ score, CRP, and disease duration (continuous variables) were examined by linear regression. To control for potential

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confounding, the associations were subsequently examined by multivariate linear regression with P-homocysteine as the dependent variable. A significance level of 0.05 was chosen. In the subanalysis, we stratified the cohort according to MTX treatment.

RESULTS

We studied 81 patients with RA; rheumatoid factor was detected in 78 of these.

The mean disease duration of the patients was 8.3 years (range 2 mo to 44 yrs). Other characteristics of the patients are shown in Table 1. Thirty-one of the patients treated with MTX received folic acid supplementation. Twenty-six of these patients received 5 mg weekly, 2 patients 5 mg twice weekly, one patient 5 mg 3 times weekly, and 2 patients 5 mg daily. Low dose prednisone treatment was given to 14 of the MTX treated patients and 16 of the patients not receiving MTX. Two patients were in combination therapy with MTX-sulfasalazine and MTX-hydroxychloroquine. Of the patients not receiving MTX, 16 were treated with sulfasalazine, 7 with intramuscular gold, and 8 with azathioprine, penicillamine or hydroxychloroquine.

P-homocysteine was 12.2 μmol/l (SD 4.2) in patients treated with MTX, with no difference between patients receiving and those not receiving folic acid supplementation. P-homocysteine was 13.6 μmol/l (SD 10.4) in the patients not receiving MTX.

In the bivariate analysis, P-homocysteine was associated with P-creatinine, regression coefficient 0.197 ($p < 0.0001$), as well as HAQ score, regression coefficient 2.667 ($p < 0.025$). There was no statistically significant association with sex, age, MTX treatment, folic acid supplementation, disease duration, or CRP. The difference in P-homocysteine between the lowest and highest P-creatinine and HAQ score was 27 μmol/l and 7 μmol/l, respectively.

In the multivariate model, these 2 associations were consistent (Table 2), and remained so when the MTX group and the non-MTX group were analyzed separately (Table 3). MTX had very little influence on P-homocysteine. P-homocysteine tended to be negatively associated with folic acid supplementation, but this was not statistically significant. In the full model adjusted R^2 was 0.32. Accordingly, 32% of the variance was explained by the model.

Table 1. Patient characteristics.

			Taking MTX, n = 41		Not Taking MTX, n = 40	
Sex, n, male/female			15/26		13/27	
No. taking folic acid supplementation			31		1	
	Minimum*	Maximum	Mean	SD	Mean	SD
Age, yrs	21.0	80.0	60.4	11.1	55.6	13.3
P-creatinine, μmol/l	45.0	158.0	85.3	12.4	76.4	19.9
CRP, mg/l	10.0	144.0	27.1	32.4	26.9	26.3
HAQ score, 0-3**	0.0	2.63	0.87	0.66	1.20	0.79
MTX, mg/week	0	20	11.2	3.3	—	—

* Among the patients receiving MTX the minimum dose was 7.5 mg. HAQ: Health Assessment Questionnaire

Table 2. Multilinear linear regression coefficients and standard errors for the association between P-homocysteine and biomarkers among 81 patients with RA.

	Coefficient	SE	Student t	p
Predictor variable				
Constant	–12.83	4.79	–2.68	0.009
Age	0.005	0.06	0.08	0.94
Sex	–1.73	1.67	–1.04	0.3
Creatinine	0.299	0.05	5.81	0.0000
Folic acid	–3.3	2.28	–1.45	0.15
HAQ Score	3.86	1.08	3.58	0.0006
MTX	–0.19	2.25	–0.09	0.93

R^2	0.37	Residual mean square	42.1
Adjusted R^2	0.32	SD	6.5

Source	DF	SS	MS	F	p
Regression	6	1858.25	309.71	7.35	0.0000
Residual	74	3117.18	42.12		
Total	80	4975.43			

DF: degrees of freedom, SS: sum of squares, MS: mean of squares.

Table 3. Multilinear linear regression coefficients and standard errors for the association between P-homocysteine and biomarkers, stratified according to MTX treatment.

Predictor Variables	Coefficient	SE	Student t	p
Taking MTX, n = 41				
Age	–0.01	0.06	–0.14	0.89
Sex	–1.53	1.44	–1.07	0.3
Creatinine	0.17	0.06	2.73	0.0099
Folic acid	–1.4	1.46	–0.98	0.34
HAQ score	3.06	0.92	3.31	0.0022
Not taking MTX, n = 40				
Age	0.016	0.11	0.15	0.88
Sex	–1.03	3.19	–0.32	0.74
Creatinine	0.32	0.07	4.36	0.0001
Folic acid	–9.13	8.92	–1.02	0.31
HAQ score	4.61	2.01	2.29	0.028

DISCUSSION

According to our findings, P-homocysteine was not significantly different in patients treated with MTX and folic acid supplementation, compared with other patients with RA. Indeed, there was a tendency to a lower level. We did not see an elevated P-homocysteine in the 10 patients receiving MTX and no folic acid, probably because the number of patients was small. Further, these patients had lower P-creatinine than the rest, and 8 were women.

The tendency to a lower P-homocysteine level among the patients receiving MTX was still present when adjustment was made for age, sex, folic acid supplementation, and HAQ score, as shown by the negative coefficient of MTX in the regression analysis. Further, the well known association between P-homocysteine and P-creatinine was confirmed, whereas the associations with age and sex described in the Hordaland Homocysteine Study[14] of 16,176 healthy persons were too weak to be detected.

The explanation might be that the expected average sex and age difference would be considerably smaller than the maximal difference accounted for by P-creatinine (27 μmol/l) or HAQ score (7 μmol/l). In the Hordaland Study, the difference in both sex and age (40 to 65 years) was 1.5–2.0 μmol/l. Physical inactivity was also associated with an elevated P-homocysteine, but with a modest difference between maximal and minimal physical activity at 0.8–0.9 μmol/l. Therefore, physical inactivity as such would not explain the association between the HAQ score and P-homocysteine in this study. The lack of association with CRP might be explained by the fact that CRP reflects disease activity at the time of measurement, whereas the HAQ score can be interpreted as a measure of disease severity.

If hyperhomocysteinemia was the mechanism of the increased risk of thrombosis in the previous study[9], the explanation might be the lack of folic acid supplementation. This vitamin supplementation was not used regularly at that time (1984-95), because it was thought to reduce the effect of MTX. At present, however, folic acid supplementation is the rule rather than the exception. According to a Cochrane review, folic acid supplementation, 5–27.5 mg weekly, reduces the side effects of MTX but does not lessen the effect of MTX[15].

One study showed that the level of P-homocysteine was higher in patients with RA compared with controls[16]. Apparently, we found a "dose response effect": the higher the disease progression/activity measured by HAQ score, the higher the P-homocysteine. There is a need to reinvestigate this unexpected finding in a larger study. First, hyperhomocysteinemia might explain some of the well known increased cardiovascular mortality in patients with RA. Second, folic acid supplementation might be indicated, not only in patients treated with MTX, but also in other RA patients.

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