Fasting plasma homocysteine as a sensitive parameter of antifolate effect: A study of psoriasis patients receiving low-dose methotrexate treatment

We have investigated the effect of low-dose methotrexate (25 mg weekly) on plasma homocysteine in 13 patients who had psoriasis. Total, free, and protein-bound homocysteine were determined both during fasting and after methionine loading. Psoriasis patients had significantly higher basal plasma homocysteine levels than age-matched control subjects. In addition, the methionine loading test was abnormal in four of the patients, but this was not significant. Psoriasis patients, although not folate deficient, did have lower serum folate levels than control subjects. There was a significant and transient increase in fasting plasma homocysteine levels within 48 hours after administration of low-dose methotrexate. This response was repeated after each administration and was observed eight to 20 times in three patients whose progress was monitored for 2 to 6 months. Notably, methotrexate did not affect the plasma profile for homocysteine after methionine loading. This study showed the level of fasting plasma homocysteine to be a sensitive and responsive parameter of antifolate drug treatment. (CLIN PHARMACOL THER 1989;46:510-20.)

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There is growing interest in the sulfur amino acid homocysteine inasmuch as moderate homocysteinemia may produce atherosclerosis.[1-5] Increased levels of plasma homocysteine have been observed in several clinical conditions including cancer,[6,7] renal failure,[8,9] cobalamin deficiency,[10] and folate deficiency.[10,11]

Homocysteine is not supplied by food but is a product of transmethylation.[12] Intracellular homocysteine is either remethylated to methionine or converted to cysteine via the so-called transsulfuration pathway. The salvage to methionine is, in most tissues, catalyzed by the enzyme 5-methyltetrahydrofolate-homocysteine methyltransferase, requiring 5-methyltetrahydrofolate as a methyldonor and methylcobalamin as a cofactor. In this way, the metabolic fate of homocysteine is linked to the metabolism of reduced folates.[13] Notably, studies on isolated cells suggest that altered homocysteine production or utilization is correlated to cellular homocysteine egress.[14] Thus homocysteine in extracellular media, such as plasma and urine, may reflect the intracellular homocysteine metabolism. Homocysteine metabolism and its relation to folate and methionine metabolism are depicted in Fig. 1.

We have previously shown that the antifolate drug methotrexate, given to cancer patients in doses of 1 to 13.6 gm, induced a transient increase in plasma and urinary homocysteine levels. The homocysteinemia subsided within 1 week, and plasma homocysteine returned to levels that were usually below pretreatment values.[6] In vitro experiments gave analogous results in that methotrexate enhanced the release of homocysteine from cultured cells into the medium.[15] These clinical and experimental observations can be explained by known effects of methotrexate on intracellular pools of reduced folates. Among the different species, 5-methyltetrahydrofolate is most efficiently depleted after methotrexate exposure.[16,17] These findings point to the possibility that the level of plasma homocysteine may be a sensitive and responsive parameter of antifolate effect.

Low-dose methotrexate treatment is used in the long-

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Supported in part by grants from Norwegian Research Council for Science and the Humanities and the Norwegian Cancer Society.
Received for publication March 3, 1989; accepted June 22, 1989.

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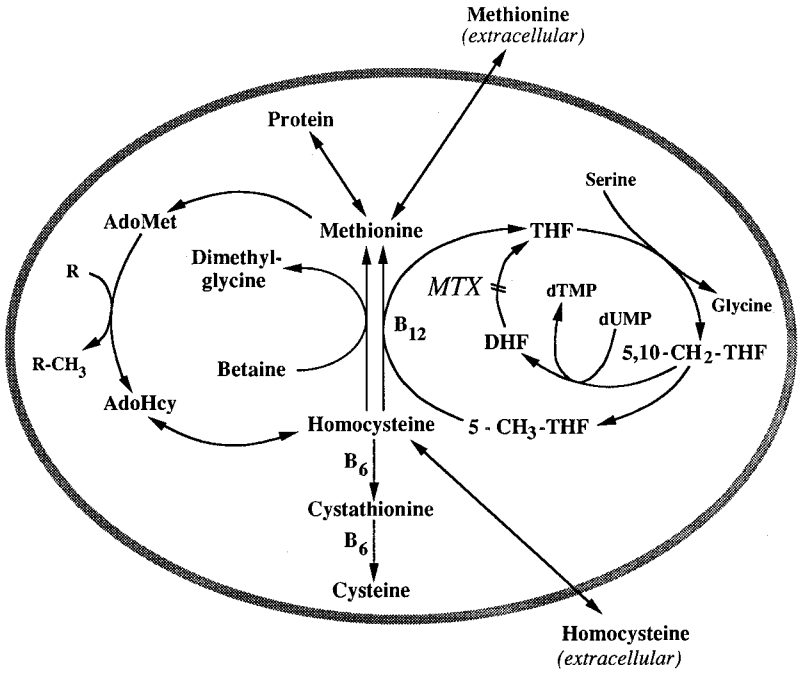

Fig. 1. Interrelationship between homocysteine, methionine, and reduced folates. AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; DHF, dihydrofolate; THF, tetrahydrofolate; 5-CH_3-THF, 5-methyltetrahydrofolate; 5,10-CH_2-THF, 5,10-methylenetetrahydrofolate.

term management of severe psoriasis[18] and of rheumatoid arthritis.[19] In the present study, we investigated the effect of such treatment on the plasma homocysteine levels of psoriasis patients. This study was performed to evaluate the hypothesis[14] that plasma homocysteine levels reflect cellular antifolate effects and to investigate whether methotrexate may affect basal or postload plasma homocysteine levels. Because therapy with methotrexate of psoriasis patients usually lasts for months or even years, the study was also motivated by the growing evidence that homocysteinemia is a risk factor for vascular disease.[1-5]

METHODS

Chemicals. Sources of reagents used for the homocysteine assay (DL-homocysteine, adenosine, [^{14}C]adenosine (59 mCi/mmol), *S*-adenosylhomocysteine, 2′-deoxycoformycin and dithioerythritol) have been given previously.[6,20] L-Methionine and *o*-phthaldialdehyde were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Patients and control subjects. A total of 13 seriously affected psoriasis patients were included in this study. Their characteristics are listed in Table I. Thirteen control subjects were selected from other patients in the dermatologic ward. The control subjects were matched for age and sex with the psoriasis patients (Table II). All women included in the study were postmenopausal.

Methotrexate treatment. All patients, except A. N. and S. W., were treated with 25 mg methotrexate, given as a weekly intramuscular injection. In his first treatment, patient A. N. received 35 mg intramuscularly, and 25 mg intramuscularly thereafter. S. W. received an oral dose of 10 mg.

Methionine loading test. Methionine, mixed in orange juice, was given orally at a dose of 100 mg/kg body weight. Blood samples were drawn immediately before and at 4, 8, 12, 24, 28, 48, and 72 hours after methionine ingestion.

The results from the test were described in two different terms, that is, either as the maximal increase in plasma homocysteine above fasting level (C_p) or as the area under the plasma profile above the fasting level in the interval 0 to 72 hours after loading [AUC_p(0-72)]. The latter term has been adopted from the concept of AUC (area under the plasma concentration curve), which is used in pharmacokinetic evaluation of drug action.[21]

Protocol. The levels of fasting basal homocysteine, plasma methionine, *S*-cobalamin, and *S*-folate were measured before methotrexate treatment and methionine loading in all patients and control subjects.

Table I. Characteristics of patients

Patient	*Sex*	*Age (yr)*	*Diagnosis*	*Protocol (condition)**	*S-folate† (nmol/L)*	*S-cobalamin‡ (pmol/L)*
S. W.	F	62	Plaque psoriasis	1,3	11	297
A. N.	M	46	Plaque psoriasis, arthropathy	1,2,3,4	10	267
K. H.	F	81	Pustulous psoriasis, kidney and liver disease	1,2,4	15	285
O. L.	F	80	Plaque psoriasis	1,2,3,4	17	437
F. H.	M	65	Plaque psoriasis, arthropathy	1,2,3,4	14	233
O. H.	M	55	Plaque psoriasis, alcohol abuse	1,2,3,4	10	419
O. J.	F	70	Plaque psoriasis	1,2,3,4	9	389
E. S.	M	66	Plaque psoriasis	1,3,4	15	208
T. S.	M	37	Plaque psoriasis	1,3,4	8	271
N. P.	M	78	Plaque psoriasis	1,2,3,4	11	314
M. B.	M	40	Plaque psoriasis	1,2,3,4	17	459
R. E.	F	45	Plaque psoriasis, arthropathy PPP§	1,2,3,4	18	224
Mean ± SD		60.8 ± 15.1			12.8 ± 3.4	308 ± 89

*1, Fasting before start of therapy; 2, methionine loading; 3, methotrexate administration; 4, methionine loading while receiving methotrexate.
†Normal range, 5 to 22 nmol/L.
‡Normal range, 130 to 800 pmol/L.
§Pustulosis palmaris et plantaris.

Table II. Characteristics of control subjects

Patient	*Sex*	*Age (yr)*	*Diagnosis*	*S-folate (nmol/L)*	*S-cobalamin (pmol/L)*
M. L.	F	68	Dermatitis artefacta	30	224
A. R.	M	70	Nodular prurigo	18	339
S. S.	F	75	Venous ulceration of the leg	35	387
K. S.	M	68	Allergic contact dermatitis	15	198
R. R.	M	60	Allergic contact dermatitis	12	129
T. R.	M	66	Atherosclerotic ulceration of the leg	17	261
L. G.	M	50	Dermatitis NOS	29	342
A. J.	F	74	Venous ulceration of the leg	9	342
N. S.	M	51	Post-thrombotic ulcer of the leg	11	401
G. T.	F	50	Nodular prurigo	15	350
A. C.	F	76	Fracture of the femoral neck	21	272
M. B.	F	68	Rheumatoid arthritis	30	224
A. N.	M	44	Allergic contact dermatitis	21	421
Mean ± SD		63.1 ± 10.9		19.8 ± 8.0	297 ± 90

NOS, Not otherwise specified.

The psoriasis patients were investigated under three different conditions: (1) methionine loading before the start of methotrexate therapy, (2) fasting after administration of methotrexate, and (3) methionine loading during methotrexate exposure. The three phases were separated by intervals of 7 days. The protocol of each patient is listed in Table I.

In the 13 control subjects, fasting homocysteine levels were determined. Ten control subjects were subjected to one methionine loading test.

Blood sample collection and processing. Blood samples were collected into EDTA vacutainers, immediately placed on ice, and prepared by centrifugation within 15 minutes. A portion of plasma was immediately deproteinized by addition of perchloric acid, which was neutralized and removed, as previously described.[6,20] Native and deproteinized plasma were stored at −80° C until analysis.

Determination of free, total, and protein-bound homocysteine in plasma. Homocysteine was deter-

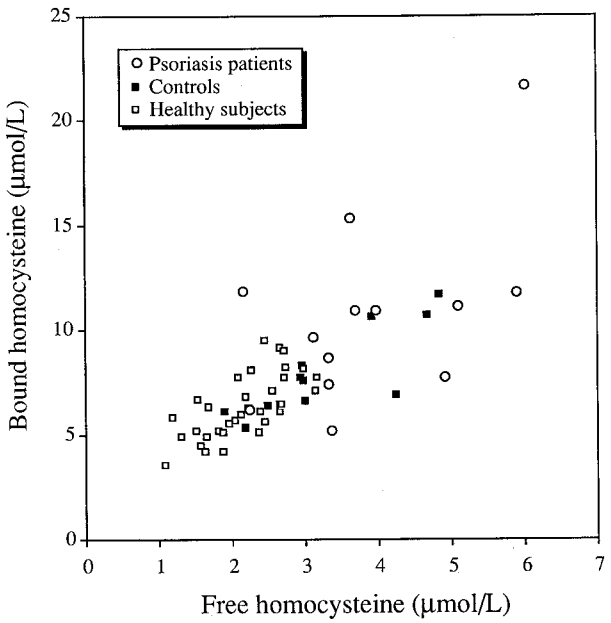

Fig. 2. Free and protein-bound homocysteine in plasma from psoriasis patients, control patients, and healthy subjects (20 to 40 years of age).

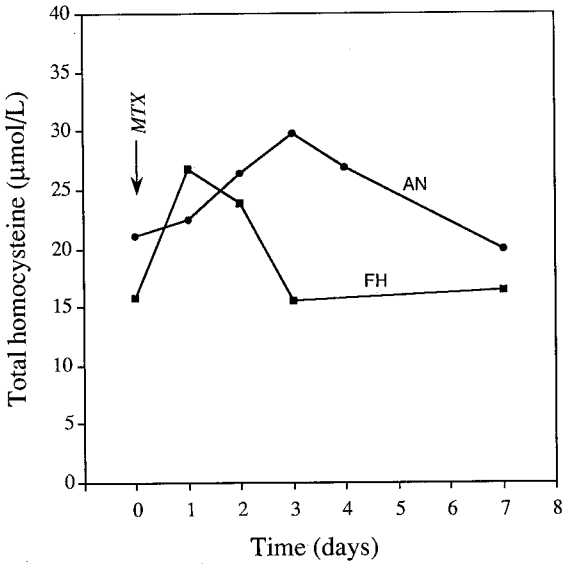

Fig. 3. Time course for the increase in total plasma homocysteine after administration of methotrexate (MTX) in two patients. Intramuscular dose of 25 mg MTX was given at time indicated by the *arrow*.

mined with a radioenzymic assay that is based on the conversion of homocysteine to *S*-[^{14}C]adenosylhomocysteine in the presence of [^{14}C]adenosine and the enzyme *S*-adenosylhomocysteine hydrolase.[20]

Free plasma homocysteine, which refers to acid-soluble homocysteine, was determined in deproteinized plasma. Total plasma homocysteine was determined in native plasma in the presence of the reducing agent dithioerythritol, which liberates homocysteine that is associated with the plasma proteins. Protein-bound homocysteine is total homocysteine minus free homocysteine. The assay procedure has been described in detail and verified elsewhere.[20]

Various assay procedures. Methionine was determined in deproteinized plasma with an assay based on derivatization with *o*-phthaldialdehyde and fluorescence detection.[22] Serum cobalamin and serum folate were determined with radioassay kits from Diagnostic Product Corporation (Los Angeles, Calif.).

Calculations and statistics. We determined the $AUC_p(0\text{-}72)$ by plotting the plasma homocysteine values between 0 and 72 hours after methionine loading on high-quality paper of uniform thickness. We calibrated the paper by weighing and calculated the $AUC_p(0\text{-}72)$ by cutting and weighing. The data were given in μmol · hr/L.

The effect of methotrexate treatment was evaluated by means of the Wilcoxon signed-rank test for paired samples. Unpaired samples were evaluated by means of the Mann-Whitney *U* test.[23] Simple and multiple regression analyses were performed to test for possible relation between different parameters. Significant levels were always expressed as two-tailed.

RESULTS

Basal plasma homocysteine in psoriasis patients and control subjects. Free, protein-bound, and total homocysteine in plasma were determined in 13 fasting patients who had severe psoriasis. The homocysteine values were compared with those of matching control subjects. Psoriasis patients had significantly higher fasting plasma homocysteine levels than those of control subjects ($p < 0.05$; Fig. 2, Table III).

The relation between free and bound homocysteine in fasting psoriasis patients, matching control subjects, and healthy young subjects is shown in Fig. 2. Linear regression analysis of these data confirmed our previous finding[20] of a positive correlation between free and bound homocysteine in plasma (psoriasis patients, $y = 1.77x \pm 3.41$, $r = 0.49$; control subjects, $y = 1.85x \pm 1.86$, $r = 0.86$; healthy subjects, $y = 1.92x \pm 2.20$, $r = 0.70$). The ratio between free and bound was the same in the control subjects and the patients (0.4), but the psoriasis patients showed a

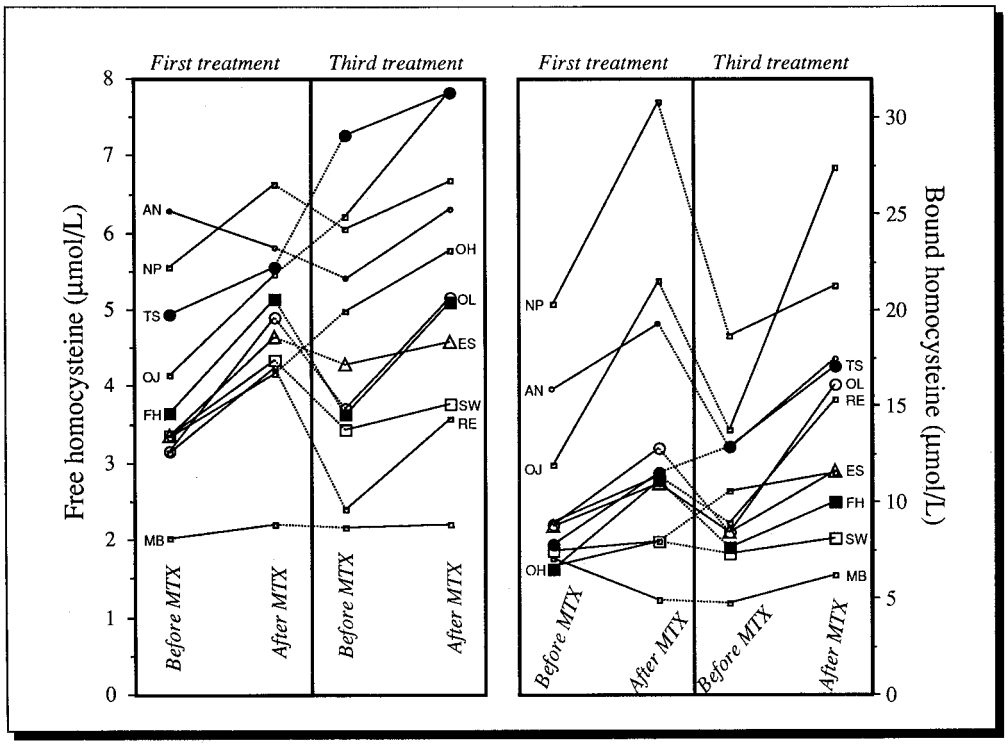

Fig. 4. The effect of first and third methotrexate (MTX) treatments on free and protein-bound homocysteine in 11 patients with psoriasis (patients K. H. and A. L. not included).

Table III. Basal plasma homocysteine levels in psoriasis patients and control subjects

	Plasma homocysteine			
Subjects	*Free (μmol/L)*	*Bound (μmol/L)*	*Total (μmol/L)*	*Free/bound ratio*
Control subjects	3.1 ± 1.0	7.7 ± 2.1	10.8 ± 2.9	0.41 ± 0.07
Psoriasis patients	4.0 ± 1.2*	10.4 ± 4.3*	14.4 ± 4.8*	0.41 ± 0.14

Fasting plasma homocysteine was determined before the patients were exposed to methotrexate or subjected to methionine loading test. The data are the same as those presented in Fig. 1 and are given as mean values ± SD.

*Significantly different from control subjects at a probability level of $p < 0.05$ (Mann-Whitney U test).

greater spread (Table III), which indicated heterogeneity.

Methionine, folate, and cobalamin. Psoriasis patients had significantly lower ($p < 0.01$) plasma methionine (13.2 ± 5.0 μmol/L) and serum folate (12.8 ± 3.4 nmol/L) before therapy than the control group (methionine, 20.3 ± 4.1 μmol/L, folate, 19.8 ± 8.0 nmol/L). Only one psoriasis patient (S. W.) had a higher methionine concentration than the average of the control subjects, and six had values that were more than two standard deviations below the mean of the control subjects. All patients had serum folate values within the normal range (5 to 22 nmol/L). Serum cobalamin levels were not significantly different between psoriasis patients (308 ± 89 pmol/L) and control subjects (297 ± 90 pmol/L). Serum folate and serum cobalamin levels for the separate patients and control subjects are listed in Tables I and II.

Relation between homocysteine, methionine, folate, and cobalamin. Multiple regression analysis showed a strong correlation between total fasting homocysteine levels and plasma methionine, serum folate, and serum

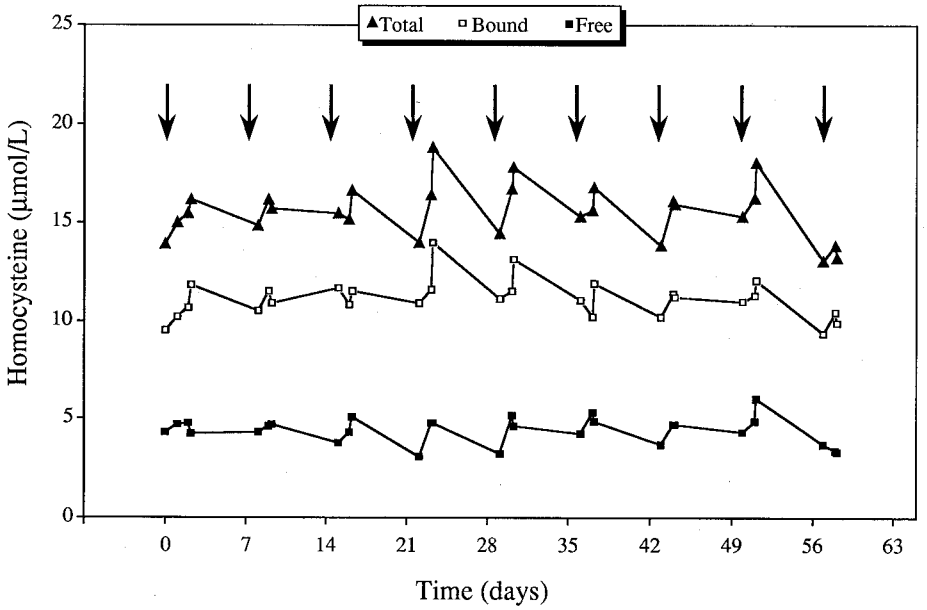

Fig. 5. Variation in plasma homocysteine in patient O. H., who received weekly doses of methotrexate (MTX) for a period of 2 months. Administration of MTX was done at times indicated by *arrows*. Dose administered was 25 mg intramuscularly except for the last two treatments (20 mg intramuscularly and 15 mg orally, respectively).

Table IV. The effect of methotrexate on basal plasma homocysteine

MTX treatment No.	*n*	*Plasma homocysteine*			
		Free (μmol/L)	*Bound (μmol/L)*	*Total (μmol/L)*	*Free/bound ratio*
1	11				
Before MTX		3.9 ± 1.2	9.5 ± 4.4	13.8 ± 5.4	0.41 ± 0.11
After MTX		4.8 ± 1.5*	13.6 ± 7.5*	18.4 ± 8.4*	0.40 ± 0.11
3	11				
Before MTX		4.5 ± 1.6†	10.3 ± 3.8†	14.8 ± 5.3†	0.44 ± 0.08
After MTX		5.3 ± 1.8*	14.7 ± 6.2*	20.0 ± 6.1*	0.38 ± 0.09
4	5				
Before MTX		5.1 ± 1.8	10.3 ± 3.9	15.4 ± 5.6	0.50 ± 0.08
After MTX		6.7 ± 2.0	15.1 ± 6.7	21.8 ± 8.5	0.47 ± 0.09

MTX, Methotrexate.
Fasting plasma homocysteine levels were determined immediately before and at various time period after methotrexate administration. The values listed as after methotrexate refer to those levels showing the greatest deviation (above or below) within a time period of 72 hours from the plasma homocysteine level determined immediately before treatment. Data are mean values ± SD.
*Significantly different from before treatment at a probability level of $p < 0.005$ (Wilcoxon signed-rank test).
†Not significantly different from before first methotrexate treatment.

cobalamin levels ($r = 0.94$, $p < 0.01$) in the control subjects. The homocysteine level was negatively correlated to these three parameters. In the psoriasis patients, a much weaker correlation between these parameters was found ($r = 0.45$, not significant).

Methotrexate and basal plasma homocysteine. Methotrexate in intramuscular doses of 25 mg was administered once a week to all patients except A. N. and S. W., who received 35 mg intramuscularly and 10 mg orally, respectively. The effect of methotrexate on fast-

ing plasma homocysteine levels was determined as a function of time. In some patients, methotrexate induced a relatively rapid increase in plasma homocysteine levels, which then returned to basal levels within 3 days. In other patients the response was slower, reaching a maximum in 2 to 3 days and returning to pretreatment levels within 6 to 7 days. Examples of such plasma profiles after methotrexate administration are shown in Fig. 3.

Because the maximum increase was reached at different times in different patients, the response was routinely recorded as the concentration of homocysteine in plasma, deviating (increase or reduction) most from the pretreatment value within a time period of 3 days after methotrexate administration. Effect of the first and third methotrexate treatment on plasma homocysteine levels is shown in Fig. 4. (The second treatment was combined with a methionine loading test.)

All patients, except patient M. B. in his first treatment, showed an increase in free, bound, (Fig. 4) and total (data not shown) plasma homocysteine after methotrexate administration, corresponding to a significance level of $p < 0.005$. This response was associated with only marginal changes (reduction) in the ratio between free and bound homocysteine, in contrast to the increased ratio during the marked homocysteinemia induced by methionine ingestion. The first methotrexate treatment induced an average increase in total plasma homocysteine levels of 4.6 μmol/L (33%), and the third treatment induced an average increase of 5.7 μmol/L (40%). This difference between treatments was not significant ($p > 0.05$). The data are summarized in Table IV.

Plasma methionine levels did not change after methotrexate administration (data not shown).

The progress of three patients (A. N., O. H., and F. H.) was monitored 2 to 6 months, corresponding to eight to 20 methotrexate administrations, and the data from O. H. is shown in Fig. 5. There were some fluctuations both in response and in basal homocysteine levels (Fig. 5), but only one patient (F. H.) showed a gradual increase in basal plasma homocysteine levels (from 8.6 to 16.6 μmol/L) during a period of 2 months (data not shown).

Methionine loading test in psoriasis patients and control subjects. The methionine loading test has been used for the detection of reduced metabolic capacity for homocysteine.[4,24] The plasma profiles for free, bound, and total homocysteine after methionine loading were recorded, as shown for the psoriasis patients in Fig. 6, and the response determined either as $AUC_p(0\text{-}72)$ or as maximal increase above fasting homocysteine level (C_p). Both $AUC_p(0\text{-}72)$ and C_p were higher in the patients than in the control subjects, but the difference was not significant (Table V). In four patients, C_p (A. N., A. L., O. J., and K. H.) and $AUC_p(0\text{-}72)$ (A. N., A. L., O. J., and R. E.) were more than two standard deviations above the mean of the control subjects, which indicated an abnormal methionine metabolism in these patients.

Administration of methotrexate to the patients 12 hours before the methionine loading did not affect the homocysteine response (Table V).

In contrast to the absence of a change in ratio between free and bound homocysteine when methotrexate was given alone, there was a significant increase in ratio when methionine was administered. The ratio was usually maximal about 4 hours after methionine administration, and it rapidly returned to normal in the next 4 to 8 hours (data not shown).

DISCUSSION

We have determined total, free, and protein-bound homocysteine in plasma from psoriasis patients before and after methotrexate administration, during fasting, and after methionine loading. Estimation of free and bound homocysteine is made in plasma that is rapidly deproteinized before redistribution between these forms takes place.[14,20] Our data show that free and protein-bound homocysteine in freshly prepared plasma are positively correlated both in different patients (Fig. 2) and during slow fluctuations in plasma homocysteine in the individual patient (Fig. 4). However, when there is a rapid increase in plasma homocysteine, as with methionine loading, free homocysteine is more responsive, indicating that binding to proteins is a time-dependent process in vivo.

The fasting level of plasma homocysteine in psoriasis patients was determined and compared with that of control patients who were matched with respect to age and sex. The psoriasis patients had significantly higher homocysteine level than the control subjects, who in turn had higher levels than young healthy subjects. The reason for the elevated levels in control patients was not evaluated but may be related to the fact that plasma homocysteine is dependent on several factors that include age, sex,[11] renal function,[8,9] serum folate, and serum cobalamin.[10] Elevated plasma homocysteine in the control patients, compared with young healthy subjects, emphasizes the importance of the use of proper control subjects when evaluating factors that modulate plasma homocysteine.

Psoriasis patients, although not folate deficient, had significantly lower serum folate levels than those

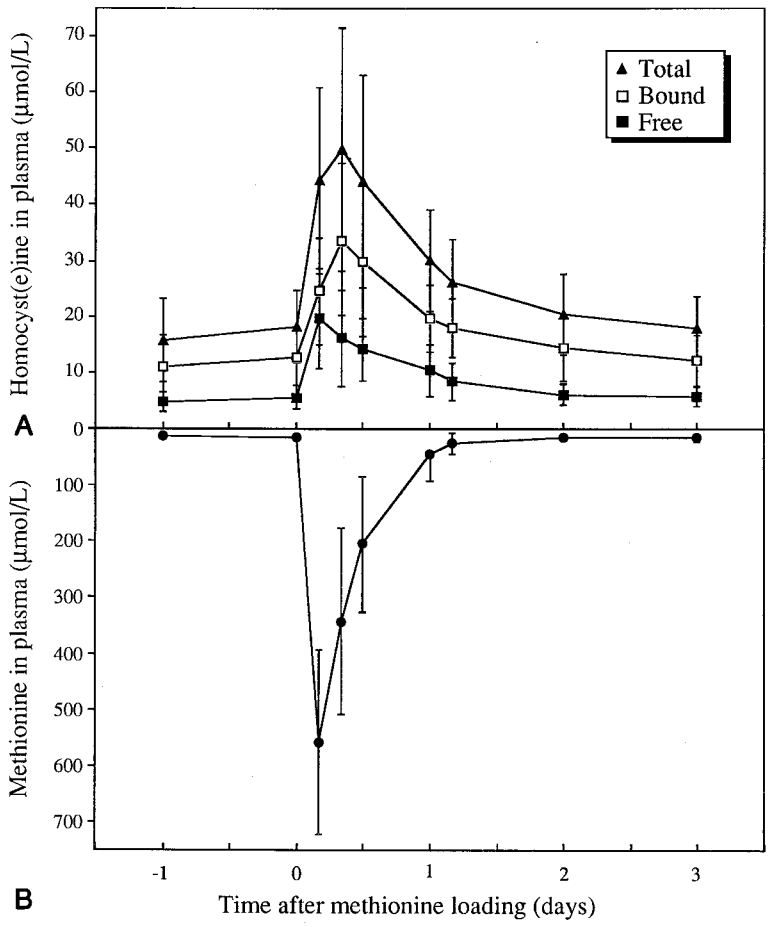

Fig. 6. Profiles for homocysteine and methionine in plasma after administration of methionine to psoriasis patients while they were receiving methotrexate (MTX). Eleven patients (S. W. and A. L. not included) were subjected to a standard methionine loading test. The results are given as mean values ± SD. **A,** Plasma profiles for free, bound, and total homocysteine. **B,** Profile for plasma methionine.

of control patients (Table I). This finding points to low tissue folate as a cause of high plasma homocysteine in psoriasis patients. However, we found a much weaker correlation between serum folate and plasma homocysteine levels in psoriasis patients ($r = -0.33$, $p > 0.05$) compared with control subjects ($r = -0.54$, $p < 0.05$), suggesting that additional factors are involved in the elevation of plasma homocysteine levels in psoriasis patients.

Some patients with cancer,[6,7] like patients with severe psoriasis, have elevated plasma homocysteine levels, and both patient categories have a large burden of rapidly proliferating cells. The germinative cell population in the skin of psoriasis patients may be increased up to ninefold, and the cell cycle is probably the fastest in human tissue in vivo.[25] Studies with cultured cells show that homocysteine egress is proportional to the specific growth rate.[26] Increased fasting plasma homocysteine levels in psoriasis patients may therefore be caused by homocysteine export from the rapidly proliferating germinative cell layer.

The methionine loading test has been used to stress homocysteine metabolism and thereby reveal defects in various pathways, including the reaction catalyzed by cystathionine β-synthase.[4,24] In most studies based on this test, the homocysteine response has been determined at a fixed time period after methionine ingestion, usually 4 hours. We determined the whole profile for plasma homocysteine after methionine loading, and the results were given as $AUC_p(0\text{-}72)$ (Fig. 6, Table V), a term adopted from pharmacokinetics.[21] This procedure is laborious but was preferred because the increase in

Table V. Homocysteine response after methionine administration

Subjects	*MTX*	*Parameter*	*Plasma homocysteine levels*		
			Free	*Bound*	*Total*
			(μmol · hr/L)	*(μmol · hr/L)*	*(μmol · hr/L)*
Control subjects	−	AUC_p(0-72)	239 ± 96	497 ± 154	712 ± 217
Patients	−	AUC_p(0-72)	370 ± 175*	592 ± 282†	928 ± 458†
Patients	+	AUC_p(0-72)	357 ± 199‡	553 ± 231‡	880 ± 387‡
			(μmol/L)	*(μmol/L)*	*(μmol/L)*
Control subjects	−	C_p	8.2 ± 2.2	15.3 ± 2.2	23.5 ± 4.4
Patients	−	C_p	11.8 ± 5.2†	17.6 ± 12.7†	29.4 ± 16.1†
Patients	+	C_p	12.2 ± 5.9‡	16.6 ± 9.0‡	28.8 ± 12.4‡

MTX, Methotrexate; AUC_p(0-72), Area under plasma concentration curve above fasting levele in the time interval 0 to 72 hours after administration; C_p, increase in plasma above fasting level.

The profiles for plasma homocysteine (free, protein-bound, and total) after a standard methionine loading test were determined in control subjects (n = 10), psoriasis patients before methotrexate exposure (n = 10), and psoriasis patients 12 hours after receiving methotrexate (n = 11). The methionine loading tests were separated by at least 14 days. Data are given as mean values ± SD.

*Significantly different from control subjects ($p < 0.05$, Mann-Whitney U test).

†Not significantly different from control subjects ($p < 0.05$, Mann-Whitney U test).

‡Not significantly different from before treatment ($p < 0.05$, Wilcoxon signed-rank text).

fasting homocysteine induced by methotrexate reached a maximum after a variable time period (Fig. 1), and it was conceivable that the effect of methotrexate on homocysteine metabolism under condition of excess methionine also showed variable kinetics. We therefore assumed that the AUC_p(0-72) was less sensitive to such variability than a single-point registration.

Four patients did have abnormal methionine loading test, that is, more than two standard deviations above the mean of the control group, indicating that homocysteine metabolism in these patients was impaired. This did not, however, result in a significant difference between the two groups (Table V). Obviously, excess methionine also greatly enhances the homocysteine egress from cells other than the germinative cells in the psoriatic skin lesions. For example, methionine loading to isolated liver cells stimulates homocysteine egress severalfold.[27] In this way, enhanced egress from the germinative cells may be totally masked.

In all patients except one (Fig. 4, Table IV), methotrexate administered weekly in doses of about 25 mg induced a transient increase in fasting plasma homocysteine levels, which then returned to normal. The patient (M. B.) who did not respond had the lowest basal plasma homocysteine level, a high methionine level, and the highest serum folate and serum cobalamin levels of all of the psoriasis patients. This is in accordance with the relation between these parameters and plasma homocysteine levels.[10,11]

The dose-dependency of the homocysteine response to methotrexate has not been dealt with in this article, but data suggest that such a relation does not exist. For instance, in a patient with rheumatoid arthritis treated with 7 mg methotrexate, we observed a rise in total plasma homocysteine from about 5 to 8 μmol/L (40%) on two occasions (Refsum H, Helland S, Ueland PM. Unpublished data, 1989), and in a cancer patient receiving 13.6 gm methotrexate, plasma homocysteine increased by 11% to 50%.[6] Absence of dose-dependency may be explained by different responses among target cells, duration of exposure to methotrexate before rescue therapy, excretion of homocysteine into urine, reduced homocysteine export after inhibition of cell division, and homocysteine metabolism through nonfolate-dependent pathways in the liver.

The response to methotrexate was repeated up to eight to 20 times in the three patients who were monitored for several months. Basal plasma homocysteine remained stable except in one patient (F. H.) in whom the fasting plasma homocysteine level showed a marked increase within 2 months of treatment. The effect from low-dose methotrexate was therefore different from cytotoxic doses of 1 to 13.6 gm that caused both a transient homocysteinemia and a progressive reduction in the basal level observed in the intervals between methotrexate infusions.[6] Because folic acid has been shown to be an efficient means to reduce plasma homocysteine,[28] it is conceivable that the reduced level in cancer patients after each treatment may be related to the 5-formyltetrahydrofolate (leucovorin) administration. Alternatively, the different responses to low and high doses of methotrexate may reflect the rapid loss of proliferating cells after high cytotoxic doses of methotrexate. This is supported by our preliminary findings,

which show that homocysteine levels in children with acute lymphoblastic leukemia are markedly reduced after cytotoxic drug regimens.

At present, it seems reasonable to postulate a similar mechanism for the transient homocysteinemia after high- and low-dose methotrexate. Methotrexate decreases the intracellular pools of reduced folates and, among these species, 5-methyltetrahydrofolate, the methyldonor in the methionine synthase reaction, is the most efficiently depleted.[16,17] Elevated intracellular homocysteine may, in turn, lead to enhanced cellular homocysteine egress. This explains the main point of the present article, that is, that homocysteine in plasma is a responsive parameter of the antifolate effect of methotrexate.

All patients receiving low-dose methotrexate (except R. E.) had stable basal plasma homocysteine levels between the first and third methotrexate treatments. Moreover, two of three patients monitored for at least 2 months also had stable fasting homocysteine levels (Fig. 5). Because plasma homocysteine has been shown to be negatively correlated to serum folate,[10] our findings indicate that low-dose methotrexate did not induce folate deficiency within the time period investigated. This is in accordance with the finding that methotrexate did not reduce serum folate below normal levels in our patients. Alternatively, the intracellular folate pools are reduced, but the loss of rapidly proliferating basal cells that export homocysteine may compensate for this deterioration of folate status.

The limited data on serum folate in patients receiving long-term low-dose methotrexate suggest that treatment for years,[29,30] but not for months,[31] may deplete the folate stores. Long-term monitoring of plasma homocysteine levels in such patients is warranted because increased levels may reflect low tissue folate. Inasmuch as intermediary homocysteinemia has been established as a risk factor for premature arteriosclerotic disease,[1-5] a rise in homocysteine levels as a result of therapy must be considered as an untoward effect of long-term methotrexate treatment.

Methotrexate did not increase the plasma homocysteine levels after methionine loading in psoriasis patients (Table V). This finding should be related to the recent observation made by Brattström et al.[32] that basal but not postload homocysteine is increased in folate- or cobalamin-deficient subjects. The possibility should be considered that fasting homocysteine levels are strongly influenced by the remethylation of homocysteine catalyzed by methionine synthase, whereas abnormal postload homocysteine levels reflect impaired homocysteine catabolism.

In conclusion, basal plasma homocysteine levels were increased above normal in patients with severe psoriasis. The patients were not deficient in cobalamin, and the low *S*-folate could only partly explain the homocysteinemia. The finding may be related to the large amount of rapidly proliferating germinative cells in the psoriatic lesion. Administration of low-dose methotrexate to these patients induced a transient increase in plasma homocysteine levels, probably by interfering with the folate-dependent remethylation of homocysteine. Plasma homocysteine levels returned to normal within 6 days, but the basal levels between treatments remained stable. This suggests that methotrexate treatment had no cumulative effect on tissue folates during the time of the investigation. Methotrexate did not affect the homocysteine response to methionine administration, a finding that further emphasizes that the fasting homocysteine level in plasma is a sensitive and potentially useful parameter of antifolate effect. The relationship between methotrexate-induced homocysteinemia and the therapeutic benefits and side effects of this drug should be investigated.

The authors thank Gry Kvalheim and Halvard Bergesen for their excellent technical assistance and the staff of the dermatologic ward, Haukeland Hospital, for making this work possible.

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