

Changes in Plasma Methionine and Total Homocysteine Levels in Patients Receiving Methotrexate Infusions¹

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ABSTRACT

Methotrexate reduces intracellular pools of 5-methyltetrahydrofolate and could result in reduced conversion of homocysteine to methionine by methionine synthetase. This study was designed to investigate the effects of moderate dose to very high dose methotrexate on methionine and total homocysteine as reflections of methotrexate induced intracellular events. Methionine and total homocysteine were measured prior to, during, and following twenty-six 24-h i.v. infusions of 33.6 g/m² methotrexate (very high dose methotrexate) in 16 children with acute lymphocytic leukemia and seven 4-h i.v. infusions of 8 g/m² methotrexate (high dose methotrexate) in 5 children with osteogenic sarcoma. Amino acids were measured by gas chromatography/mass spectrophotometry. Mean methionine levels decreased by 70.0 ± 3.1% (SE) with very high dose methotrexate and 72.6 ± 5.9% with high dose methotrexate at 24 and 4.5 h, respectively, after beginning methotrexate infusions. Mean total homocysteine levels increased by 61.7 ± 3.1% with very high dose methotrexate and 55.6 ± 17.5% with high dose methotrexate at 36 and 24 h, respectively, after beginning methotrexate infusions. No consistent or significant changes were noted in levels of total cysteine, leucine, isoleucine, or valine. Similar changes did not occur in patients receiving prednisone, vincristine, daunomycin, and intrathecal methotrexate as therapy for acute lymphocytic leukemia. These changes in homocysteine and methionine may reflect biological effects of methotrexate that may predict cytotoxicity of methotrexate.

INTRODUCTION

Methotrexate is a potent antifolate that binds to and inhibits dihydrofolate reductase. Recently, methotrexate, especially methotrexate polyglutamates, has been shown to inhibit other folate dependent enzymes and in this manner disturbs intracellular folate cycling (1).

Methionine synthetase (EC 2.1.1.13) is a key intracellular cobalamin dependent enzyme that catalyzes the transmethylation of homocysteine to methionine. The major methyl donor for this reaction is 5-methyltetrahydrofolate. Although nothing is known regarding the effect of methotrexate on the activity of this enzyme (1), methotrexate could interfere with methionine synthetase activity: by direct inhibition, which would tend to increase cellular 5-methyltetrahydrofolate levels; by competition for cellular uptake with 5-methyltetrahydrofolate present in serum; or by the known inhibition by methotrexate of methylene tetrahydrofolate reductase (EC 1.1.1.68), an enzyme responsible for intracellular regeneration of 5-methyltetrahydro-

folate (1), both of which would tend to decrease cellular 5-methyltetrahydrofolate levels.

Recent tissue culture studies reveal that incubation of human cells with methotrexate (10 μM) results in a dramatic and rapid (within hours) fall in intracellular 5-methyltetrahydrofolate (2, 3). This acute cellular folate deficiency could result in decreased activity of methionine synthetase and reduced activity of methionine synthetase could result in reduced cellular methionine (and increased homocysteine). Reductions of intracellular methionine would be predicted to have major consequences on methylation reactions involving DNA, RNA, and proteins (Fig. 1). Individuals who have dietary folate deficiency are known to have increased serum levels of homocysteine (4) and normal levels of serum methionine (4).

Refsum *et al.* (5) administered 1.0 to 13.6 g of methotrexate i.v. over 2-4 h to adults and demonstrated a modest increase in serum homocysteine that tended to decrease with subsequent treatments. No consistent changes in plasma methionine were observed. The present study was designed to measure the effects of 4-h infusions of 8 g/m² and a 24-h infusion of 33.6 g/m² of methotrexate on plasma methionine and total homocysteine. The results demonstrate a dramatic decline in the plasma methionine levels and a less striking increase in plasma total homocysteine levels. It is possible that the changes in metabolites that are the substrate and product of the methionine synthetase reaction in patients treated with methotrexate will prove to be important markers of cytotoxicity and therapeutic response to methotrexate.

MATERIALS AND METHODS

Patients. Five patients [age, 14.8 ± 1.4 year (SD)] with osteogenic sarcoma receiving high dose methotrexate, 8 g/m², prior to their definitive surgery were evaluated. Plasma methionine, total cysteine, and total homocysteine were measured during seven doses of high dose methotrexate.

Sixteen children [age, 6.6 ± 4.4 years (SD)] with acute lymphocytic leukemia receiving very high dose methotrexate, 33.6 g/m², were evaluated. Plasma methionine, total cysteine, and total homocysteine levels were measured during 26 doses of very high dose methotrexate. One adult, age 39 years, with Burkitt's lymphoma, treated with 3 g/m² of methotrexate over 32 h was also evaluated.

Protocols. Patients with osteogenic sarcoma were given 8 g/m² of methotrexate i.v. over 4 h followed by leucovorin beginning 24 h from starting high dose methotrexate infusions. According to Children's Cancer Study Group Protocol CCG-782, during high dose methotrexate infusions and until the methotrexate level was less than 0.1 μM, patients were alkalinized to keep the urine pH between 6.5 and 7.5 and fluid input was maintained at twice maintenance fluids. No chemotherapy was administered concomitantly with high dose methotrexate, but vincristine was given 24 h after the high dose methotrexate infusion. The four infusions of high dose methotrexate were given 1 week apart for two doses prior to and following a course of bleomycin, cyclophosphamide, and dactinomycin.

Patients with acute lymphocytic leukemia received 6 g/m² methotrexate i.v. over 1 h followed by 1.2 g/m²/h for the next 23 h, totaling 33.6 g/m² methotrexate during the 24-h infusion. These patients, treated according to Children's Cancer Study Group Protocol CCG-

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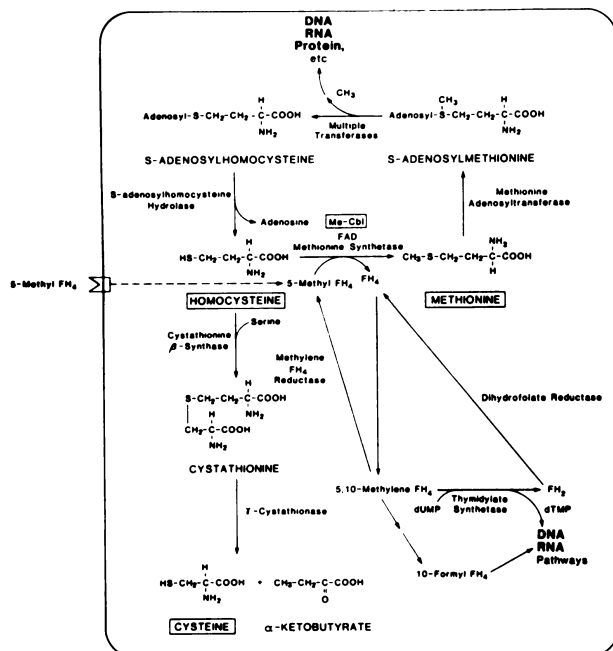


Fig. 1. Intracellular metabolism of homocysteine and methionine. The role of 5-methyltetrahydrofolate (5-methyl FH_4), tetrahydrofolate (FH_4), dihydrofolate (FH_2), 10-formyltetrahydrofolate (10-formyl FH_4) and 5,10-methylenetetrahydrofolate (5,10-methylene FH_4) are illustrated. FAD, flavin adenine dinucleotide; MeCbl, methylcobalamin.

144, received methotrexate infusion on the second day of a 1-month induction regimen; 1 methotrexate infusion on days 1, 15, and 29 of consolidation phase; and 1 methotrexate infusion on day 1 of six 6-month maintenance cycles. The first maintenance cycle was scheduled to begin 2-3 weeks following consolidation and patients received a total of 10 infusions of very high dose methotrexate during the 3 years of therapy. Alkalinization and hydration were similar to that given for patients receiving high dose methotrexate. Leucovorin is begun at hour 36, 12 h after completion of the very high dose methotrexate infusion. Chemotherapy administered concomitant with the first methotrexate dose includes prednisone; with the second through fourth dose, prednisone and vincristine; and with the fifth through tenth dose, prednisone, vincristine, and 6-mercaptopurine (prednisone, 40 mg/kg/day p.o.; vincristine, 1.5 mg/m² i.v.; and 6-mercaptopurine, 500 mg/m² i.v.).

All patients and/or their legal guardian gave written informed consent prior to participation in this study and in the chemotherapy protocols in accordance with the policies of the Medical Research Committee of The Children's Hospital and University Hospital in Denver.

Sample Collection and Analysis. One to 2 ml of blood were collected into EDTA sample tubes when methotrexate levels were drawn. The plasma was separated from the RBC after centrifugation and frozen at -20°C until amino acids were measured. Methotrexate levels were drawn at 0, 4.5, 24, and 48 h after beginning high dose methotrexate and at 0, 6, 24, 36, 48, and 72 h after beginning the very high dose methotrexate infusions. To be evaluable a baseline sample and all or all but one of the remaining samples had to be available. Plasma methionine, total cysteine, and total homocysteine were measured as described by Stabler *et al.* (6). Normal ranges (mean \pm 2 SD after log normalization) for these three amino acids were: methionine, 13.5-36.8 μ M; total homocysteine, 5.4-16.2 μ M; and cysteine, 186-335 μ M. Plasma levels of leucine, isoleucine, and valine were also measured (7) during seven methotrexate infusions. Serum methotrexate levels were measured using an enzyme immunoassay (Emit assay; Syva Co., Palo Alto, CA).

Pharmacokinetic Analysis. Systemic clearance of methotrexate (ml/min/m²) was calculated after each infusion from the zero-order i.v.

infusion rate constant for methotrexate (20.0 mg/min/m² for very high dose methotrexate and 33.3 mg/min/m² for high dose methotrexate) divided by the steady state serum methotrexate concentration as assessed at 24 or 4.5 h after very high dose and high dose methotrexate infusions began (8).

Statistical Calculations. Statistical analyses of the data were performed by Student's *t* test, linear regression, and Pearson correlation using Lotus 1-2-3 and Statistix II software programs. Results are reported as mean \pm SE unless otherwise indicated.

Methionine Synthetase Assay. Methionine synthetase was purified and assayed as described (9). For each assay where methotrexate was included, a duplicate containing no methionine synthetase served as a control. Methotrexate was obtained from Sigma Chemical Co., St. Louis, MO, and methotrexate heptaglutamate was obtained from Dr. Charles Baugh.

RESULTS

Effects of High Dose Methotrexate. A decrease in plasma methionine occurred in all patients with osteogenic sarcoma 4.5 h after starting high dose methotrexate. The mean decrease of 72.6 \pm 5.9% at 4.5 h represented a decline from 22.4 \pm 3.7 μ M at baseline to 5.6 \pm 1.0 μ M ($P < 0.004$). The mean plasma methionine level returned to near baseline at 24 h (Table 1; Fig. 2).

Plasma total homocysteine increased in all patients receiving high dose methotrexate and the peak occurred at 24 h after the

Table 1 Mean plasma methionine, total homocysteine, and cysteine levels; mean serum methotrexate levels; and mean homocysteine:methionine ratios during and following seven 4-h i.v. infusions of 8 g/m² of methotrexate in 5 patients with osteogenic sarcoma

Time (hr)	MTX	HCYS	METH	CYS	HCYS/METH ratio
0	0	9.9 \pm 1.1	22.4 \pm 3.7	244.7 \pm 15.4	0.49 \pm 0.1
4.5	722.9 \pm 52.8	11.9 \pm 1.3	5.6 \pm 1.0 ^a	215.7 \pm 12.7	2.50 \pm 0.4 ^b
24	3.9 \pm 1.1	15.2 \pm 2.1 ^c	26.4 \pm 7.6	220.0 \pm 15.2	0.82 \pm 0.2
48	0.5 \pm 0.2	12.3 \pm 2.0	17.1 \pm 2.0	248.0 \pm 13.9	0.79 \pm 0.2

^a $P < 0.004$.

^b $P < 0.002$.

^c $P < 0.05$.

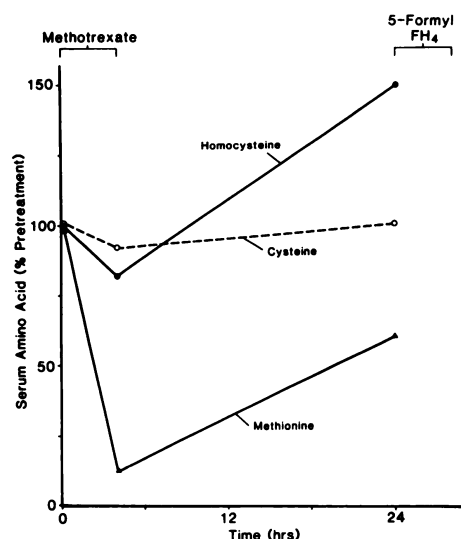


Fig. 2. Percentage of change of plasma methionine, total homocysteine, and cysteine from baseline levels during and following a 4-h i.v. infusion of 8 g/m² methotrexate in a patient with osteogenic sarcoma.

methotrexate infusions began in 5 of 7 doses administered. This elevation of mean plasma total homocysteine from $9.9 \pm 1.1 \mu\text{M}$ at baseline to $15.2 \pm 2.1 \mu\text{M}$ at 24 h ($P < 0.05$) represented a $55.6 \pm 17.5\%$ increase. This elevation was concomitant with the decline in methotrexate levels and the administration of leucovorin. The mean total homocysteine decreased to near mean baseline levels at 48 h after the methotrexate infusion began.

The mean total homocysteine:methionine ratio was significantly elevated above the mean baseline level at 4.5 h ($P < 0.002$) representing a $462.4 \pm 95.5\%$ increase. This ratio approached the baseline value at 48 h.

The mean plasma total cysteine level appeared to decrease during the infusions of high dose methotrexate, but the maximal decrease of $11.4 \pm 3.4\%$ at 4.5 h was not statistically different from the mean baseline value ($P = 0.17$).

Effects of Very High Dose Methotrexate. Plasma methionine decreased in all patients receiving very high dose methotrexate. The nadir for each patient occurred during the methotrexate infusions at either 6 or 24 h (14 at 6 h and 12 at 24 h) and then returned to baseline by 48 h. The mean methionine level decreased $70.03 \pm 0.1\%$ from a baseline of 22.1 ± 1.9 – $5.8 \pm 0.5 \mu\text{M}$ at 24 h ($P < 0.0001$) (Table 2; Fig. 3).

An increase in plasma total homocysteine levels occurred in 92% of the patients with the maximal change occurring between 24 and 72 h (41.8 ± 14.0 h) (SD). The mean plasma total homocysteine level increased significantly between 0 and 36 h ($P < 0.003$) and remained significantly elevated at 48 h ($P < 0.02$) before returning to near baseline values by 72 h. This change at 36 h represented a $61.7 \pm 12.8\%$ mean increase over baseline levels. Plasma total homocysteine levels tended to return to baseline values concurrent with the administration of leucovorin and the initial decline in serum methotrexate levels.

The mean total homocysteine:methionine ratio was significantly elevated above the mean baseline ratio at 6, 24, and 36 h ($P < 0.0001$, $P < 0.0004$, and $P < 0.0004$, respectively) with the largest increase of $550.4 \pm 133.0\%$ occurring at 24 h. This ratio tended to return to the baseline value by 48–72 h. A small (less than 15%) but statistically insignificant apparent decrease in the mean total cysteine level was observed at 6, 24, and 36 h ($P = 0.0575$, $P = 0.0919$, $P = 0.2152$, respectively).

Serum Methotrexate Levels. Comparable levels of serum methotrexate were attained in patients receiving high dose and very high dose methotrexate (Tables 1 and 2). As assessed by the Pearson correlation coefficient, the percentage of change from baseline levels in plasma methionine, total homocysteine, and the total homocysteine:methionine ratio did not appear to

Table 2 Mean plasma methionine, total homocysteine, and cysteine levels; mean serum methotrexate levels; and mean homocysteine:methionine ratios during and following twenty-six 24-h i.v. infusions of 33.6 g/m^2 of methotrexate in 16 children with acute lymphocytic leukemia

Samples were drawn at hours specified with methotrexate infusions beginning at hour 0. Unless otherwise indicated, all levels are expressed as $\mu\text{mol/liter}$. MTX, methotrexate; HCYS, homocysteine; METH, methionine; CYS, cysteine.

Time (hr)	MTX	HCYS	METH	CYS	HCYS/METH ratio
0	0	5.2 ± 0.4	22.1 ± 1.9	180.0 ± 9.1	0.27 ± 0.03
6	877.8 ± 56.1	5.3 ± 0.4	7.5 ± 1.0^a	158.0 ± 6.6	0.93 ± 0.14^a
24	790.0 ± 42.2	6.6 ± 0.7	5.8 ± 0.5^a	159.2 ± 7.8	1.33 ± 0.20^a
36	28.7 ± 5.4	7.8 ± 0.7^b	13.1 ± 1.0^c	164.9 ± 7.2	0.69 ± 0.10^d
48	4.7 ± 1.1	6.9 ± 0.4^e	24.1 ± 2.6	185.8 ± 6.2	0.38 ± 0.06
72	0.8 ± 0.1	5.3 ± 0.3	28.3 ± 2.5	175.0 ± 7.3	0.23 ± 0.03

^a $P < 0.0001$.

^b $P < 0.003$.

^c $P < 0.0002$.

^d $P < 0.0004$.

^e $P < 0.02$.

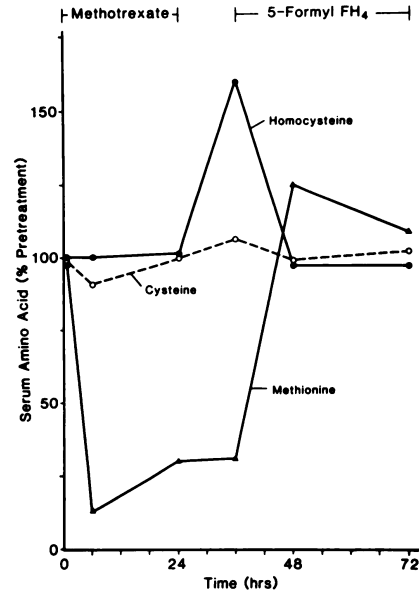


Fig. 3. Percentage of change of plasma methionine, total homocysteine, and cysteine from baseline levels during and following a 24-h i.v. infusion of 33.6 g/m^2 methotrexate in a child with acute lymphocytic leukemia.

correlate with serum methotrexate levels at each point of assessment for either high dose or very high dose methotrexate. Similarly, the peak serum methotrexate levels did not appear to correlate with the maximum changes in plasma methionine or total homocysteine for high dose and very high dose methotrexate.

Methotrexate Clearance. The peak homocysteine:methionine ratio did not appear to be related to the peak methotrexate level, but the peak homocysteine:methionine ratio was 2.50 for the high dose methotrexate patients whose clearance was $59.5 \pm 15.6 \text{ mg/min/m}^2$ while the peak homocysteine:methionine ratio was 1.33 for the very high dose methotrexate patients whose clearance was $105.0 \pm 25.6 \text{ mg/min/m}^2$. The systemic clearance of methotrexate may have prognostic significance in children with acute lymphocytic leukemia (10, 11) and in these studies a higher homocysteine:methionine ratio was obtained in patients with the slower clearance rates.

Effects in Patients not Receiving High Dose or Very High Dose Methotrexate. None of the changes in plasma methionine or total homocysteine noted with very high dose and high dose methotrexate were seen in 3 patients receiving intrathecal methotrexate, daunorubicin, prednisone, and vincristine during induction for acute lymphocytic leukemia. Mean plasma total homocysteine at baseline, $7.2 \pm 1.7 \mu\text{M}$, was similar to the mean at time of maximum change, $7.0 \pm 0.4 \mu\text{M}$. The mean plasma level of methionine increased from $16.6 \pm 3.9 \mu\text{M}$ at baseline to $29.0 \pm 2.7 \mu\text{M}$ at the points of maximum change. Mean plasma cysteine decreased from $176.3 \pm 23.7 \mu\text{M}$ at baseline to a mean of $153.7 \pm 9.6 \mu\text{M}$ at the points of maximum change.

Effect of Moderate Dose Methotrexate in an Adult. Fig. 4 shows the results of treatment of an adult patient with a 3-g/m^2 dose of methotrexate. As observed with children receiving higher doses of methotrexate, this adult patient with Burkitt's lymphoma also showed the characteristic decrease in methionine and rise in total homocysteine although the decrease in methionine was less pronounced at this lower dose of methotrexate.

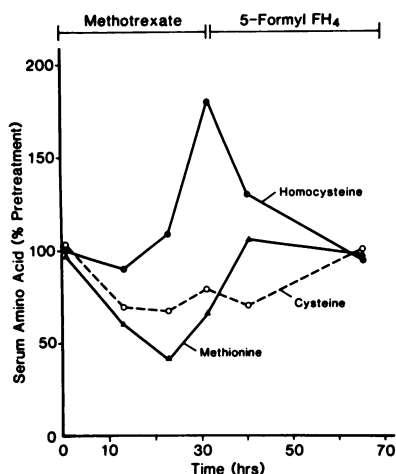


Fig. 4. Percentage of change of plasma methionine, total homocysteine, and cysteine following i.v. infusion of 3 g/m² methotrexate in an adult with Burkitt's lymphoma.

Table 3 Effect of methotrexate (10 μ M) and methotrexate heptaglutamate (10 μ M) on partially purified and homogeneous human placenta methionine synthetase activity

Addition	Crude	Homogeneous
None	10.1 \pm 0.3*	10.0 \pm 0.3
Methotrexate	9.9 \pm 0.3	9.8 \pm 0.1
Methotrexate heptaglutamate	9.9 \pm 0.4	9.9 \pm 0.3

* The *P* value for all values shown in this table is 0.20 or greater.

Determination of Direct Inhibition of Methionine Synthetase by Methotrexate. To determine if direct inhibition of human methionine synthetase occurs with methotrexate (monoglutamate) or methotrexate polyglutamate, both homogeneous and partially purified human placental methionine synthetases were incubated with 10 μ M methotrexate (monoglutamate) and 10 μ M methotrexate heptaglutamate (Table 3). Less than 10% inhibition of the activity of human methionine synthetase was observed in triplicate experiments. No difference in inhibition was noted between partially purified or homogeneous methionine synthetase. Thus, we conclude that methotrexate or methotrexate polyglutamates do not directly inhibit methionine synthetase *in vitro*. The concentration of methotrexate of 10 μ M results in complete inhibition of dihydrofolate reductase (12) and greater than 90% inhibition of DNA synthesis in fibroblasts (13).

DISCUSSION

The three doses of methotrexate evaluated in this study produced profound hypomethioninemia during infusion. The degree of hypomethioninemia decreased as the serum methotrexate level decreased and methionine rebounded above baseline levels at 48–72 h after methotrexate infusions began. Baram *et al.* (2, 3) have shown that the intracellular concentration of 5-methyltetrahydrofolate is reduced within hours in cells exposed to methotrexate. Thus decreased intracellular conversion of homocysteine to methionine (Fig. 1) would be expected to occur and cause an intracellular depletion of methionine as is seen in genetic defects of methionine synthetase (14). Metabolic functions of methionine include (a) utilization for protein synthesis and (b) conversion to *S*-adenosylmethionine which serves as the predominant methyl group donor, a precursor in polyamine synthesis, and as an intermediate in the transsulfuration pathway (15, 16). Borsi *et al.* (17) have suggested that hepato-

toxicity of methotrexate may result from reduced biosynthesis of methionine which leads to a deficiency of certain vital lipotropes formed via one-carbon metabolism. Many malignant cell lines require exogenous methionine for growth *in vitro* (15). By decreasing protein synthesis the profound protracted hypomethioninemia induced by methotrexate in this study may contribute to the cytotoxicity of methotrexate. However, until patients who are treated with low dose methotrexate where clinical toxicity can be severe in the absence of rescue with leucovorin are studied, the role of hypomethioninemia in the toxicity of methotrexate cannot be determined. Furthermore, whether or not such patients develop hypomethioninemia associated with low doses of methotrexate is presently unknown.

Total homocysteine levels increased during the infusions of methotrexate and continued to rise after the infusions were completed. The decline toward baseline occurred concomitantly with the administration of leucovorin and decreasing methotrexate levels. Reduced 5-methyltetrahydrofolate pools would be expected to cause a build up of intracellular homocysteine (Fig. 1). When normal and malignant mouse fibroblasts are exposed to methotrexate in culture there is a marked efflux of homocysteine with only a small increase in intracellular homocysteine (18). This efflux of homocysteine is much more pronounced in malignant than in normal mouse fibroblasts and is almost completely prevented by the addition of leucovorin to the culture media (18).

Previously, Refsum *et al.* (5) noted an increase in total homocysteine which peaked at 24 h after 2–4-h infusions of 1–13.6 g of methotrexate in 7 adults. Hypomethioninemia was not noted except in one patient receiving 13.6 g of methotrexate. This lack of hypomethioninemia may be due to several factors: (a) samples were not obtained during methotrexate infusions in all patients; (b) the duration of administration of methotrexate was shorter than that in our study; and (c) the dose for 5 of 7 patients was only 1 g of methotrexate.

The changes in total homocysteine and methionine noted in our study appear to be specific for methotrexate, since these changes were seen with high dose methotrexate when it was used without concomitant chemotherapy. In addition, the administration of intrathecal methotrexate and systemic vincristine, daunomycin, and prednisone to patients with acute lymphocytic leukemia did not produce similar alterations in plasma methionine and total homocysteine levels.

Hypomethioninemia (35% of normal) has been found to occur following i.v. cisplatin which was more pronounced when etoposide was given concomitantly with cisplatin intraperitoneally in humans (19). Cisplatin complexes with methionine (20) which may explain the hypomethioninemia observed. Such a complex of methionine with methotrexate has not been described.

In addition to the increase in plasma total homocysteine with methotrexate infusions, Refsum *et al.* (5) noted reduced plasma levels of total homocysteine and decreasing magnitude of the changes in total homocysteine with subsequent doses of methotrexate. Only one patient entered on the present study had four sequential doses of methotrexate evaluated from diagnosis of acute lymphocytic leukemia. Her baseline total homocysteine level increased by the second dose and remained stable for the next two doses. However, the maximum percentage of change in plasma total homocysteine did decrease from 165.4% to 26.9% over four doses. Her baseline plasma methionine decreased from 33.1 μ M to 13.9 μ M with a concurrent decrease in the percentage of change of methionine from –81.0% to –47.5%. This decrease in percentage of change of plasma

methionine and total homocysteine levels may reflect decreasing tumor burden since methotrexate appears to cause more pronounced efflux of homocysteine in malignant than in normal cells *in vitro* (18). However, this observation could also reflect the development of methotrexate resistance following repetitive treatment. Studies in patients who have developed methotrexate resistance would help distinguish whether the changes in homocysteine and methionine reflect a generalized metabolic effect of methotrexate or if these changes reflect changes in the tumor burden.

It is unknown whether the effect of methotrexate on methionine and homocysteine levels is related to organ specific effects such as on the liver or changes in peripheral utilization and metabolism of methionine and homocysteine. Lower extracellular methotrexate concentrations (0.1–10 μM) appeared to inhibit methionine uptake by L1210 mouse leukemia cells (21). Although the peak levels of methotrexate reached in the present studies were much higher, we would have expected serum levels of methionine to increase, not decrease, if inhibition of cellular uptake of methionine by cells in general was the sole effect of methotrexate on methionine metabolism. Others (22) have shown that the antimetabolic effect of methotrexate measured in human bone marrow cells and leukemic cells by the deoxyuridine suppression test was aggravated by methionine supplementation and improved by homocysteine supplementation (22), an effect opposite of the effect in hepatocytes suggesting that organ specificity is significant. Studies of isolated perfused liver and injection of radiolabeled forms of homocysteine and methionine in animals will be required to sort out the effects of methotrexate on intracellular folate levels and effects on specific organs as well as peripheral tissues. Furthermore, the levels of methotrexate required to produce depletion of intracellular 5-methyltetrahydrofolate were considerably less (1–10 μM) than the serum levels of methotrexate reached in the present study. The higher extracellular methotrexate levels of the present study could have additional or different mechanisms that result in the observed changes in methionine and homocysteine levels.

Regardless of the mechanisms of hypomethioninemia and homocystinemia, administration of moderate dose to very high dose methotrexate in this study was associated with decreases in plasma methionine levels and late increases in plasma total homocysteine levels. Further studies are indicated to determine if the effects on plasma methionine and total homocysteine occur with even lower doses of methotrexate and with various methods of administering methotrexate, as well as to determine if methionine and total homocysteine levels or changes in their levels can be used to predict clinical toxicity or therapeutic responses to methotrexate. These measurements might be used to reduce toxicity, to monitor for the development of resistance, and to enhance the tumor cytotoxicity of methotrexate.

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