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

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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 spectrometry. 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-methyltetrahydrofolate (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 daunomycin.

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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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 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. Alkalization and hydration were similar to that given for patients receiving high dose methotrexate. Leucovorin is begun at hour 0. Unless otherwise indicated, all levels are expressed as (imol/liter.

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 ± 5.9% at 4.5 h represented a decline from 22.4 ± 3.7 µM at baseline to 5.6 ± 1.0 µM (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 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).

Fig. 1. Intracellular metabolism of homocysteine and methionine. The role of 5-methyltetrahydrofolate (5-methyl FH₄), tetrahydrofolate (FH₄), dihydrofolate (FH₂), 10-formyltetrahydrofolate (10-formyl FH₄) and 5,10-methylene tetrahydrofolate (5,10-methylene FH₄) are illustrated. FAD, flavin adenine dinucleotide; MeCbl, methylcobalamin.

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

<table>
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<tr>
<th>Time (hr)</th>
<th>MTX</th>
<th>HCYS</th>
<th>METH</th>
<th>CYS</th>
<th>HCYS/METH ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>9.9</td>
<td>22.4</td>
<td>15.4</td>
<td>0.49 ± 0.1</td>
</tr>
<tr>
<td>4.5</td>
<td>722.9 ± 52.8</td>
<td>11.9 ± 1.3</td>
<td>5.6 ± 1.0*</td>
<td>215.7 ± 12.7</td>
<td>2.50 ± 0.4*</td>
</tr>
<tr>
<td>24</td>
<td>3.9 ± 1.1</td>
<td>15.2 ± 2.1*</td>
<td>26.4 ± 7.6</td>
<td>220.0 ± 15.2</td>
<td>0.82 ± 0.2*</td>
</tr>
<tr>
<td>48</td>
<td>0.5 ± 0.2</td>
<td>12.3 ± 2.0</td>
<td>17.1 ± 2.0</td>
<td>248.0 ± 13.9</td>
<td>0.79 ± 0.2*</td>
</tr>
</tbody>
</table>

* P < 0.004.
* P < 0.002.
* P < 0.05.
methotrexate infusions began in 5 of 7 doses administered. This elevation of mean plasma total homocysteine from 9.9 ± 1.1 μM at baseline to 15.2 ± 2.1 μM at 24 h (P < 0.05) represented a 55.6 ± 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 at 4.5 h (P < 0.002) representing a 462.4 ± 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 ± 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 ± 0.1% from a baseline of 22.1 ± 1.9–5.8 ± 0.5 μM 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 ± 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 ± 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 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 ± 15.6 mg/min/m² while the peak homocysteine:methionine ratio was 1.33 for the very high dose methotrexate patients whose clearance was 105.0 ± 25.6 mg/min/m². 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 ± 1.7 μM was similar to the mean at time of maximum change, 7.0 ± 0.4 μM. The mean plasma level of methionine increased from 16.6 ± 3.9 μM at baseline to 29.0 ± 2.7 μM at the points of maximum change. Mean plasma cysteine decreased from 176.3 ± 23.7 μM at baseline to a mean of 153.7 ± 9.6 μM 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/m² 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.
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thesis and (b) conversion to S-adenosylmethionine which serves
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lne synthesis, and as an intermediate in the transsulfuration
pathway (15, 16). Borsi et al. (17) have suggested that hepato-

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toxicity of methotrexate may result from reduced biosynthesis
of methionine which leads to a deficiency of certain vital lipo-
tropes formed via one-carbon metabolism. Many malignant cell
lines require exogenous methionine for growth in vitro (15). By
decreasing protein synthesis the profound protracted hypome-
thioninemia induced by methotrexate in this study may contrib-
ute 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 metho-
trexate 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 hom-
cysteine (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 methotrex-
ate. 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 ad-
ministration of intrathecal methotrexate and systemic vincris-
tine, daunomycin, and prednison to patients with acute lympho-
cytic 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 intraperito-
neally in humans (19). Cisplatin complexes with methionine
(20) which may explain the hypomethioninemia observed. Such
a complex of methionine with methotrexate has not been de-
scribed.

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 metho-
trexate. 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 de-
creased from 33.1 μM to 13.9 μM 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

![Figure 4](image-url)
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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