The in vivo Distribution of Methotrexate Between Plasma and Erythrocytes


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Summary. 1. The concentration of methotrexate in whole blood, plasma and erythrocytes was measured in three patients receiving 250 mg methotrexate by continuous intravenous infusion over 12 h for different malignant diseases.

2. Methotrexate was measured using a double-antibody radioimmunoassay which facilitated drug monitoring for 1–2 weeks.

3. The concentration of methotrexate in plasma was much higher than that in whole blood and erythrocytes during the period of infusion, but this profile was reversed during the elimination phase.

4. The concentration in erythrocytes fell rapidly immediately after the infusion ended, but thereafter, in contrast to plasma levels, methotrexate concentrations in erythrocytes did not appear to decay during the elimination phase. In one patient the concentration/time profiles differed between treatment days. On the first occasion, at the initiation of chemotherapy, erythrocytes progressively accumulated methotrexate in the elimination phase against an apparent concentration gradient. On the second occasion this progressive increase was not observed, but as in the other two patients, methotrexate levels in red cells remained many times higher than drug levels in plasma throughout the period of observation.

5. Folinic acid administration did not appear to influence the distribution of methotrexate between red cells and plasma.

6. It was concluded that while the distribution between plasma and erythrocytes was probably mediated by complex mechanisms, the results were consistent with the erythrocyte mass behaving as a slowly exchanging kinetic compartment. Accumulation and persistence of a drug such as methotrexate in red cells might be expected to promote resistance and perhaps influence the expression of toxicity.

Introduction

Methotrexate (MTX) has not only been used extensively in cancer chemotherapy, but has gained wide acceptance in the treatment of recalcitrant psoriasis [11, 14]. Since a protracted drug regimen is often necessary to control disease, drug toxicity is an important consideration. Reticuloendothelial system toxicity, hepatocellular damage, and nephrotoxicity [2, 3, 12] are common manifestations, which in some cases can limit drug treatment [4].

While knowledge of MTX distribution between plasma and erythrocytes would be of interest, apart from a few reports [15, 1]; little attention has been focussed on this aspect of the drug's kinetics. In particular, there have been no studies of MTX levels attained in erythrocytes in vivo.

A specific and sensitive radioimmunoassay [13] has enabled levels of this agent in serum, whole blood, and erythrocytes to be measured up to a time after administration when serum concentrations have been hitherto immeasurable. The changes in MTX concentrations with time in plasma, whole blood, and erythrocytes were monitored during and after high-dose IV infusion.

Patients and Methods

Three patients with various forms of malignant disease (Table 1) received 250 mg MTX by continuous IV infusion over 12 h as part of their regular chemotherapy. In patient JM therapy was monitored on two occasions, with an intervening period of 1 month. On the first occasion this patient had not previously been exposed to cytotoxic chemotherapy, in contrast to the other two patients, who had both been receiving treatment for a few months. Folinic acid ‘rescue’ was a routine part of the regimen and comprised 15 mg folic acid given IV 24 h after initiation of therapy followed by 5 mg folic acid q.i.d.

Blood samples were withdrawn at appropriate intervals from zero time until between 8 and 16 days after administration, and each sample was divided into three. Plasma was obtained from one, and another was haemolysed by freezing and thawing and diluting with water. From the third erythrocytes were prepared as follows. Immediately blood was withdrawn the packed cell volume (PCV) was found for each specimen of blood. A 1.0-ml aliquot of blood was then centrifuged and the plasma and buffy layer withdrawn and discarded. The packed cells were resuspended in normal saline to give a total volume of 2 ml and mixed by repeated inversion. After recentrifuging at 800 g for 10 min the supernatant was removed without disturbing the erythrocyte pellet and discarded. This procedure was repeated twice, and when the last supernatant was removed the volume was made up to 1 ml total volume with normal saline. The PCV was checked again, and the difference between pre- and post-washing values

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Table 1. Ratios of red cell: plasma methotrexate during infusion and elimination of methotrexate

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>Ratios RBC/plasma methotrexate$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–12 h</td>
</tr>
<tr>
<td>JM (1)</td>
<td>Oesophageal carcinoma</td>
<td>0.03</td>
</tr>
<tr>
<td>JM (2)</td>
<td>Oesophageal carcinoma</td>
<td>0.02</td>
</tr>
<tr>
<td>JC</td>
<td>Carcinoma of breast</td>
<td>0.01</td>
</tr>
<tr>
<td>MK</td>
<td>Carcinoma of lung</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$ 0–12 h values represent the ratio of areas under the relevant concentration-time curves, while ratios quoted for later times are mean values of estimates obtained between those time limits.

The half-life of MTX was determined from the slope of the log/linear regression line (least-squares fitting) drawn through the terminal portion of the serum concentration/time curve. To give an estimate of the increase in drug concentration in erythrocytes with time which was observed in patient JM's first exposure to the drug (Fig. 1a) log/linear regression analysis was also performed on the data from day 2 to day 16. The areas under the concentration/time curves were measured by the trapezoidal method.

Results

During the period of drug administration the concentration in the three inter-related 'compartments' increased with time, reaching a peak at the end of the infusion. The concentration in plasma and whole blood followed a similar pattern, with the plasma levels during infusion being consistently higher than in whole blood (Figs. 1 and 2). Erythrocyte MTX during infusion attained levels of only a few hundredths of the plasma levels throughout the 12-h period, as shown by the AUC ratios. While during the post-infusion period the MTX concentration in all three compartments fell sharply initially (Figs. 1 and 2), the mean plasma biologic half-life ($t_{1/2}$) measured over the terminal portion of the concentration time curve was 43.0 ± 6.22 (SD). The concentration in plasma fell below the limit of sensitivity of the assay between days 8 and 10, and by this time the concentration was very much greater in the erythrocytes and whole blood. The concentration of MTX in erythrocytes tended to plateau and therefore did not have an apparent elimination phase. Between days 3 and 4 the ratio of erythrocyte to plasma MTX ranged from 2.07 to 13.25, compared with a ratio ranging from 14.94 to 24.37 between days 6 and 8 after dosing. The individual data are summarised in Table 1.

In patient JM the course of drug levels was observed on two occasions, with an interval of 1 month. On the first occasion, when the patient had no previous history of chemotherapy, there was a general increase in erythrocyte MTX concentration from day 2 until day 16. The concentration increased from 46.78 nM at 46 h to 185.0 nM at 381 h (Fig. 1a). This increase in concentration occurred at a time when plasma levels were decaying, and was maintained even after the levels had become undetectable. When the study was repeated the concentration in erythrocytes levelled off at 48 h and no net increase in drug concentrations was observed at the end of the study after 14 days (Fig. 1b). The mean drug concentration between 24 h and 336 h was 33.58 nM ± 3.08 (SD).