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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 folinic acid given IV 24 h after initiation of therapy followed by 5 mg folinic 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

Patient	Disease	Ratios RBC/plasma methotrexate[a]			
		0–12 h	3–4 days	6–8 days	Plasma $t_{1/2}$ (h)
JM (1)	Oesophageal carcinoma	0.03	2.07	24.37	36.0
JM (2)	Oesophageal carcinoma	0.02	3.10	17.40	40.0
JC	Carcinoma of breast	0.01	2.48	14.94	46.0
MK	Carcinoma of lung	0.01	13.25	18.75	50.0

[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

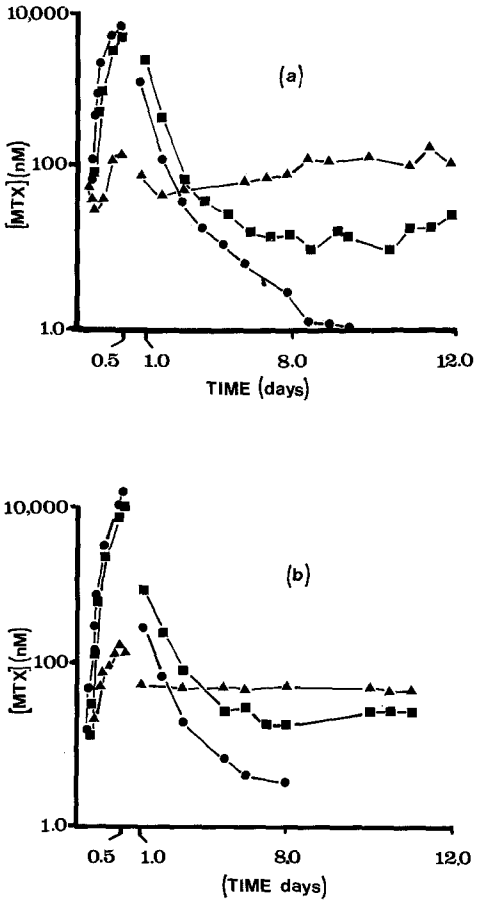

Fig. 1 a and b. The concentration/time curves for MTX in plasma (●), whole blood (■), and erythrocytes (▲) for patient JM at the beginning of MTX medication (**a**) and after 1 month (**b**)

allowed a correction factor for loss of cells to be calculated. The saline was removed after centrifuging and the volume made up to 1 ml with distilled water to promote haemolysis. The washing procedure and centrifugation were performed at between 0° C and 4° C.

All specimens were kept at −20° C until assayed.

Methotrexate concentrations were measured by a specific and sensitive radioimmunoassay [13] employing ^{75}Se-labelled MTX. The assay had a limit of sensitivity of 250 p*M* and the inter-assay precision was within 10% (c.v.).

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 n*M* at 46 h to 185.0 n*M* 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 n*M* ± 3.08 (SD).

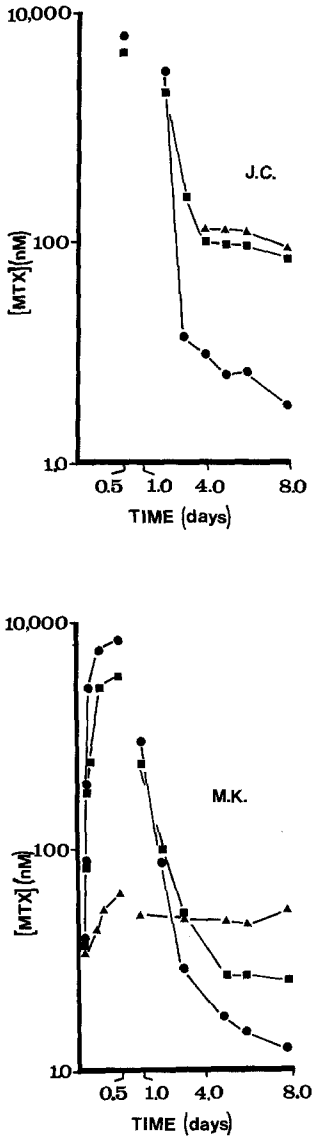

Fig. 2. The concentration/time curves for MTX in plasma (●), whole blood (■), and erythrocytes (▲) for patients JC and MK

Discussion

The persistence of drug in erythrocytes suggests that MTX is bound in some way either within the cell or on the cell membrane. Indeed, it is possible that two separate mechanisms may be involved. The initial rise in concentration during the infusion followed by a sharp drop in erythrocyte drug concentration immediately following the termination of the infusion suggests that some MTX is partly associated with erythrocytes in a rapidly reversible manner. This initial association might perhaps be influenced by the concentration gradient, so that uptake was mainly by diffusion. In addition, since MTX concentration in erythrocytes increased after day 2 in the first treatment of JM, apparently against a concentration gradient, an energy-dependent process is possible. The time course of this reaction is longer than one would normally expect from a simple active uptake process; however, there seems little doubt that this profile is consistent with erythrocytes being a slowly exchanging kinetic compartment.

Other explanations for the persistense of MTX in erythrocytes could be postulated. Dihydrofolate reductase activity would be expected to be high in blast cells, and may bind considerable quantities of MTX. The increase in erythrocyte MTX levels from day 2 after the first treatment in JM, and the maintenance of a plateau in the face of falling plasma drug levels in other cases, may reflect de novo erythrocyte synthesis and erythrocyte turnover. This assumes that MTX levels in the reticuloendothelial system may be considerably higher than serum levels. It is also possible that MTX polyglutamate synthesis within erythrocytes contributes to the observed persistence in these cells. The relative quantities of MTX and MTX polyglutamates could not be determined in this study, however.

That peak and plateau ratios found in one patient on the first occasion were also considerably different, not only from that obtained when the treatment was repeated but from those recorded in the other two patients, who had received regular chemotherapy, suggests that other factors may influence erythrocyte MTX levels. It certainly appears possible that distribution of MTX between plasma and erythrocytes may change with repeated chemotherapy. Prolonged exposure to low concentrations of MTX could promote biochemical resistance such as that associated with decreased binding or altered transport properties [6, 10]. This might explain why the second uptake phase was not observed in the other two patients or when the treatment was repeated in this patient.

Changes in nutritional status could also affect the phenomenon, since there is considerable evidence that other folic acid analogues can displace MTX from intracellular sites [7–9, 16]. However, it appears that folinic acid did not affect the release of MTX from erythrocytes, since the slope of the concentration/time curve did not alter in response to initiation of rescue. It is possible that the relatively small concentration of folinic acid used in rescue compared with the dose of MTX may not cause detectable displacement of MTX from erythrocytes. Another possibility is that folinic acid is taken up by erythrocytes by the same process and over a similar time course to MTX. It may be progressively altering the uptake of MTX, the major effect of which is not observed until later times than covered by this study. Indeed, folinic acid rescue may be at least partially responsible for the change in distribution when patient JM was studied a second time.

The persistence of MTX in erythrocytes could contribute to toxicity, since MTX toxicity is dependent not only on the concentration but also on the length of exposure to the drug [5, 17]. Even although the concentration in erythrocytes is relatively low, MTX nevertheless persists for long periods of time after high doses, and may exceed the time threshold for toxicity.

In conclusion, the distribution of MTX between plasma and erythrocytes appears to be mediated by complex mechanisms. Methotrexate persists for a long time in erythrocytes compared with plasma, and could contribute to the expression of toxicity by exceeding the time threshold for toxicity. Exposure to low concentrations of MTX for such long periods of time may also promote tolerance, as reflected initially in altered association/uptake properties observed when treatment is repeated.

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