Lyphology 11 (1978) 181–185

Distribution of Methotrexate between Plasma and Peripheral Lymph in Man

T.E. Sand*, A. Engeset, Ø.P. Solheim**, S. Jacobsen***

*Institute of Pharmacology, University of Oslo, Oslo, Norway
**Laboratory of Hematology and Lymphology, Norwegian Radium Hospital and Norsk Hydro's Institute for Cancer Research, Oslo, Norway

Summary

The distribution of methotrexate (MTX) to interstitial fluid has been studied by determination of MTX in peripheral lymph from the leg and in plasma after parenteral administration to 6 patients. Peak concentrations of MTX in lymph appear 0.5 to 3.25 h after peak concentrations in plasma. Maximal concentrations in lymph are 16 to 48% lower than in plasma. MTX concentration ratios lymph/plasma were higher than 1.0 (1.0–2.8) in 4 patients, and lower than 1.0 (0.2–0.8) in 2 patients. 12 to 34% of MTX was protein bound in lymph, but the variation in the ratio of bound to free MTX per g lymph protein was only 0.13 to 0.18. The patients with a lymph/plasma MTX ratio above 1.0 will have high and favourable MTX concentrations for distribution to tumours in peripheral tissue, while in the patients with a low ratio conditions for distribution are less favourable. The results indicate that pharmacokinetics of MTX are in accordance with a two or multicompartment model with varying distribution characteristics. The present observations indicate that distribution of MTX is blood flow limited, but the influence of permeability cannot be excluded.

Methotrexate (MTX) is widely used in the treatment of various malignant neoplasms (1). The last decade the interest in MTX has been renewed because of therapeutic success with large doses followed by administration of leucovorin (citrovorum factor) (2). However, the therapeutic efficacy and the pharmacokinetics of MTX vary (2, 3). The recommended dosage schedules are partly based on our knowledge about the pharmacokinetics of MTX (4).

Pharmacokinetics of MTX is characterized by a low rate of distribution (5). By using large doses of MTX as infusions, high plasma concentrations of MTX are achieved in the infusion period. This should be favourable for distribution to tumours located in peripheral tissues (6).

The peripheral tissues are exposed to the effective MTX concentrations in interstitial fluid. The distribution of MTX between peripheral lymph reflecting interstitial fluid, and plasma has never been investigated. The purpose of this study has been to evaluate the relationship between MTX plasma levels and the levels of MTX in peripheral tissues at different times after administration. MTX concentration and protein binding in peripheral lymph and MTX concentrations in plasma have been determined after parenteral administration of MTX.

Patients and Methods

Six male patients (age 33–60 years) treated for various malignancies gave their informed consent to participate in this study. Five patients received 25–100 mg MTX i.m. and 1 patient received 3,800 mg MTX as a 4 h infusion with subsequent administration of leucovorin (citrovorum factor) 15 mg i.v. and 9 mg i.m. 4 h after end of infusion and 9 mg orally/6 h for 72 h.

A lymph vessel on the leg was cannulated before administration of MTX and lymph was collected according to a method described previously (7). Lymph was collected in a practically closed system. The patients were allowed to move freely after cannulation ensuring an ample lymph flow. In the 5 patients receiving low doses of MTX as intramuscular injections in the morning, lymph was collected for 2 h periods that day and thereafter for 4 h periods. Blood was obtained at the end of each collection period. Concentrations of MTX in lymph samples were plotted according to the midpoint of collection period, and in plasma at the time of blood sampling. In the patient

0024–7766/78 1600–0181 $ 04.00

Tab. 1 Peak MTX concentrations in plasma and lymph

Patient	Dosage	Time of peak plasma concentration h[a)]	Time of peak lymph concentration h[a)]	Peak concentration ratio lymph/plasma
a	25 mg i.m.	2	3	0.6
b	25 mg i.m.	2.25	3.25	0.8
c	50 mg i.m.	1	1.5	0.5
d	100 mg i.m.	2	3	0.8
e	100 mg i.m.	3.75	7	0.7
f	3,800 mg/4 h inf.	–	–	–

a)Time after administration

who received high dosage MTX as an i.v. infusion in the afternoon, lymph collection was started the next morning at the same intervals as in the other patients. During the night lymph was collected in batch to avoid disturbing the patients.

Plasma was separated immediately and samples of plasma and lymph kept at –20 °C until analysis.

MTX in plasma and lymph was determined by the competitive variant of a binding assay (8) with minor modifications. Recovery of MTX from lymph and plasma was identical. Heparin was added to some lymph samples to avoid clotting. Lymph and plasma samples were assayed in duplicate.

Binding of MTX to lymph was determined by equilibrium dialysis (9). 0.3 ml of lymph obtained at different times after MTX administration with $1 \cdot 10^{-11}$ mole ^{3}H-MTX added was dialyzed against Krebs-Ringer bicarbonate buffer in an atmosphere of air with 5% CO_2 for 18 h at 20–22 °C with standard shaking. pH and protein content (10) were determined before and after dialysis.

Results

The log plasma and lymph MTX concentration – time curves for the individual patients (Fig. 1a-f) show that course of the curves appear quite similar. However, the peak plasma concentrations after low doses of MTX are reached 0.5 to 3.25 h before peak concentrations in peripheral lymph. The peak lymph concentrations of MTX were 50 to 80% of the respective peak plasma concentrations (Table 1).

The rate of decline in MTX concentrations in plasma and peripheral lymph varies in the individual patients and also among the patients. The results in Table 2 show that the MTX concentration ratios lymph/plasma for each patient vary with time without any distinct pattern. These concentration ratios are higher than 1.0 in 4 patients (Fig. 1, b, d, e and f) and less than 1.0 in 2 patients (Fig. 1, a and c) after peak concentrations were achieved. MTX binding in lymph ranged from 12 to 34%. The average ratios of

$$B/F \cdot \frac{1}{p} \left(\frac{\text{Bound MTX}}{\text{Free MTX}} \cdot \frac{1}{\text{lymph protein concentration}} \right)$$

from the individual patients were 0.13–0.18 (Table 3). Binding varied between patients and between the different lymph samples obtained from each patient. Binding was similar at different concentrations of MTX and at different times after administration.

Discussion

A later appearance of peak MTX concentration in lymph than in plasma after intramuscular injection confirms the observation and theoretical prediction of late appearance of peak concentrations in many peripheral tissues (11). The interstitial fluid concentration of MTX will be critical for drug concentration in tissues. Therefore, the infusion period and the subsequent interval before administration of leucovorin should be sufficiently long to achieve adequate tissue concentrations of MTX.

The results show that in some patients the lymph MTX concentrations exceed the plasma levels after peak concentrations were reached.

Tab. 2 Methotrexate concentration ratios lymph/plasma

Patient	Time after MTX-administration, h 5	10	20	30	40	50	60
a	0.8	0.7	0.2				
b	1.7	2.2	1.8	2.4			
c	0.7	0.4	0.6				
d	1.1	1.4	1.0	1.7	1.1	0.8 ~ 2.0	
e	0.9	1.8	2.8	2.0	1.4		
f			1.0	1.8	1.5	1.4	

ratio lymph/plasma

Tab. 3 Binding of methotrexate in lymph

Patient	Per cent bound MTX[a]	$\frac{B}{F} \cdot \frac{1^b}{P}$ $(g^{-1})^c$
a	22 (19–26)	0.16 ± 0.03
b	34 (24–44)	0.14 ± 0.02
c	20 (16–23)	0.13 ± 0.04
d	12 (10–16)	0.15 ± 0.03
e	25 (10–33)	0.13 ± 0.02
f	16 (9–20)	0.18 ± 0.05

[a]Mean (range) [b]B, F and P represent concentration of bound MTX, unbound MTX and total protein in the lymph [c]Mean ± SD

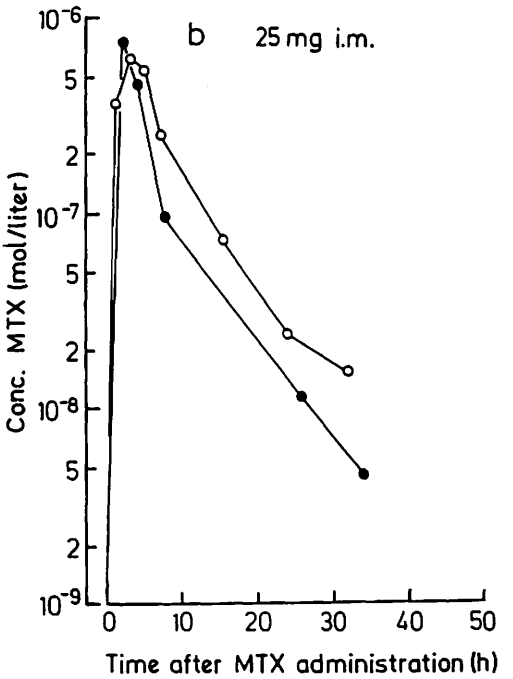

Fig. 1b

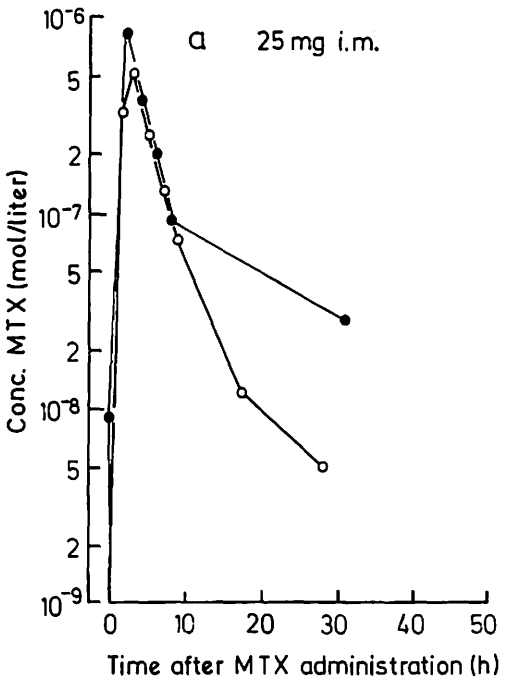

Fig. 1a Plasma (●) and lymph (○) concentrations of methotrexate after parenteral administration.

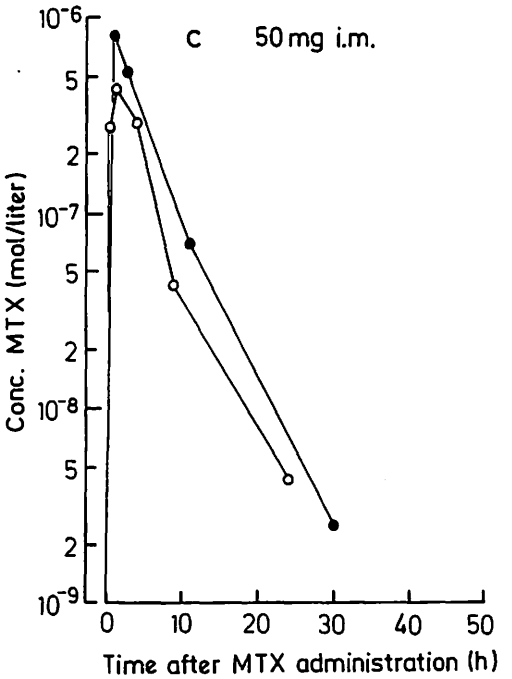

Fig. 1c

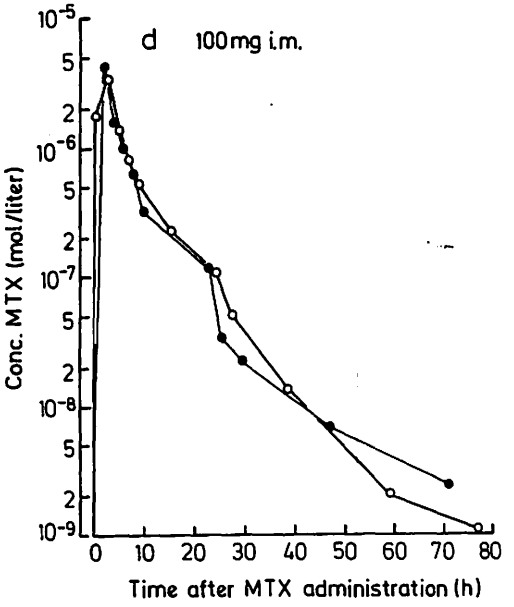

Fig. 1d

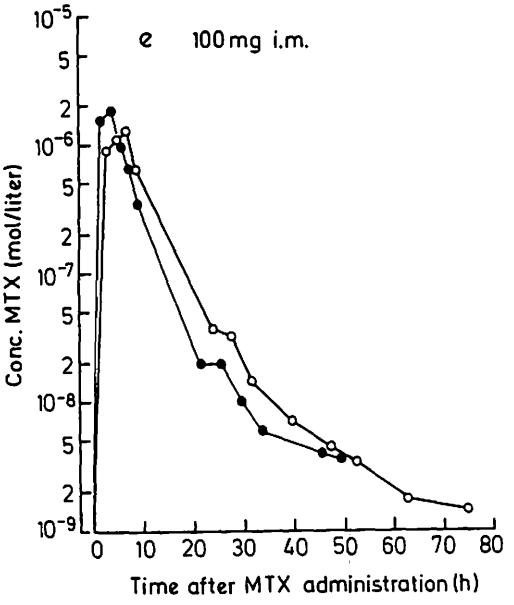

Fig. 1e

This could be explained by a lag in the collection of lymph. However, in some of our patients this lag would have to be around 5 hours to explain the observed concentration differences, also, MTX in high concentrations was measured in the lymph shortly after administration. When visualizing the lymph vessel with dye injected in the foot before cannulation the dye arrives on the leg within minutes. Late appearance of peak concentration and the high lymph/plasma concentration ratios indicate that the interstitial tissue fluid, reflected by the peripheral lymph, is a compartment separated from the central plasma compartment, and characterized by restricted distribution and redistribution rates (12). The observed distribution pattern may be explained by tissue binding, low permeability between tissue fluid and plasma and/or low blood flow (12).

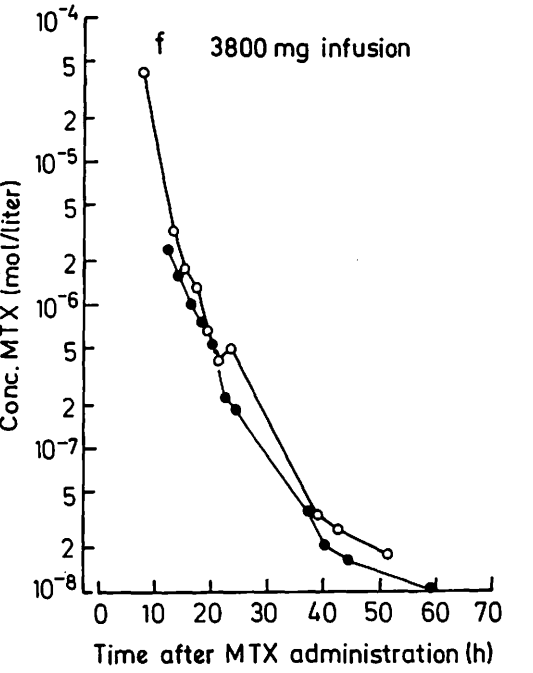

Fig. 1f

The collected lymph in this study was drained from skin, subcutaneous tissue and muscular fasciae (7) characterized by a low blood flow at rest dependent on the muscular activity (13).

Lymph/plasma concentration ratios vary with time without any distinct pattern in these patients. This may be explained by varying activity and blood flow in these patients who moved freely with intervals of resting after cannulation. The observed distribution characteristics of MTX and MTX concentration ratios

are indicative of an anticipated blood flow-limited multicompartment behaviour of MTX (11).

MTX is appr. 50% bound in serum (unpublished results) and the lower degree of binding observed in lymph can be explained by the lower protein concentration in lymph. The ratio between bound and free MTX in lymph was similar in all patients when corrected for differences in protein concentration of lymph, and could not explain the concentration differences between plasma and lymph.

In two patients the distribution pattern of MTX was different with MTX concentration ratios lymph/plasma lower than 1.0 after peak concentrations were achieved. This may be explained by different and lower binding characteristics in interstitial tissue, increased blood flow or increased permeability. This distribution pattern produces lower total MTX concentration in interstital fluid and may decrease the resultant drug concentrations in peripheral tissues compared with the patients with higher concentration ratios lymph/plasma.

The present study indicates that the distribution pattern of MTX to peripheral tissues is variable. In some patients the MTX concentrations in interstitial fluid are higher than in plasma, and consequently favourable for distribution of MTX to tumours in peripheral tissues. In other patients the MTX concentrations in interstitial tissue are lower than plasma levels, and thereby unfavourable for peripheral tissue distribution. This variation in distribution of MTX may be important when the dosage of MTX is determined.

Acknowledgments

Financial support from The Norwegian Research Council for Science and the Humanities, Norwegian Drug Monopoly and The Norwegian Cancer Society is gratefully acknowledged. We wish to thank Mrs. R. Patel and Mrs. A Killi for skilled technical assistance.

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T.E. Sand, M.D., Institute of Pharmacology, University of Oslo, P.O. Box 1057, Blindern Oslo 3, Norway