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Renal Clearance of Methotrexate in Man During High-dose Oral and Intravenous Infusion Therapy

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Summary. *The renal excretion and clearance of methotrexate (MTX) following high-dose (800 mg) therapy followed by folinic acid rescue was studied in 12 patients (2 female, 10 male): the mean age was 49.3 ± 5.5 (SE), weight 68.6 ± 3.9 (SE) and body surface area 1.8 ± 0.1 m^2. Plasma and urine were collected over 154 h at intervals of 2–24 h, and the collection times, volume, and pH of urine samples recorded. Total MTX concentrations in urine and plasma were measured by the highly specific competitive protein-binding assay method. Plasma and urinary creatinine levels were measured on an SMA-12 autoanalyser. The renal clearance of MTX was calculated for each urine collection period. Following oral administration, clearance values during the first 6 h were high at 257 ± 8.3 (ml/min), followed by a trough in clearance of 27.9 ± 4.2 (ml/min) in the 20- to 30-h period. This was followed by a secondary rise of MTX renal clearance to 180.4 ± 14.6 ml/min during the 68- to 84-h period and again to 84.9 ± 17.1 ml/min between 84 and 112 h. In the last two periods it rose to 209 ± 57.9 ml/min. Similar fluctuations were seen following IV administration. The changes in clearance were statistically significant at the $P < 0.005$ level. It is suggested that high concentrations of MTX in the renal tubules result in inhibition of carrier protein synthesis, leading to a fall in active tubular secretion. When MTX concentrations fall the tubular cell recovers and a secondary rise in renal clearance occurs, leading to cyclical changes in MTX elimination.*

Introduction

It is well established that the major route of elimination of MTX is via the kidney and that reduced renal function predisposes to MTX toxicity [4]. Methotrexate is an organic acid which is cleared by the kidney by glomerular filtration and by active tubular secretion, probably involving a carrier protein in the proximal tubule [1, 6]. Although some studies have shown periodic changes in renal clearance following high-dose MTX therapy, detailed examination of this important aspect of therapy is lacking [3]. It is conceivable that a metabolic inhibitor such as MTX, which is concentrated in the kidney, may inhibit its own secretion. The mechanism of such inhibition may be mediated by an effect on the active transport process dependent on protein synthesis.

This study presents the results of measurements of the urinary excretion and renal clearance of MTX in 12 patients during and following high-dose MTX therapy. The renal clearance was found to fluctuate cyclically in spite of a lack of significant change in glomerular filtration rate assessed by creatinine clearance. A possible explanation for these cyclical changes based on inhibition of transport protein synthesis is presented.

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Materials and Methods

Twelve patients with solid tumours were studied. There were two females and ten males, with a mean age (± SEM) of 49.3 ± 5.5 years, weight 68.6 ± 3.9 kg, and body surface area 1.8 ± 0.1 m^2. Patient details are given in Table 1 and include baseline renal function tests. Each course of cytotoxic drug therapy consisted of 800 mg MTX in combination with cyclophosphamide (250 mg/m^2), adriamycin (1 mg/kg), and vincristine (1 mg/m^2), and was given at 4-weekly intervals. Methotrexate was usually given PO in 50-mg doses each hour over 16 h, starting at 7 a.m. on day 1 of each course. On one occasion, five patients (patients 4, 9, 10, 11, and 12) were given the drug by IV infusion over 16 h for comparison. Leucovorin rescue was given 24 h after the start of MTX therapy and was continued for 2 days. The rest of the combination was given IV between 2 and 4 p.m. on day 2 of each treatment course. Urine pH was measured daily and maintained above pH 6.5 by administration of citravescent PO when necessary.

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Table 1. Details of patients in studies of renal clearance of methotrexate

Patient	Serial no.	Sex	Age (years)	Weight (kg)	S.A. (m^2)	Se Cr.	CCR (ml/s)	Diagnosis
K. P.	1	M	33	65	1.8	0.09	1.8	Giant cell bone tumour
C. D.	2	M	68	70	1.8	0.10	0.9	Metastatic carcinoma? Primary site
G. O.	3	M	65	64	–	0.10	0.9	Carcinoma, ethmoid
F. N.	4	M	65	95	1.8	0.07	1.5	Osteogenic, sarcoma
J. O.	5	M	55	50	–	0.07	1.3	Carcinoma, tongue
R. R.	6	M	19	70	1.95	0.09	1.9	Ewing sarcoma
E. W.	7	F	49	47	1.5	0.07	1.2	Carcinoma, lung
R. W.	8	M	27	74	1.95	0.10	1.9	Rhabdomyosarcoma
B. K.	9	M	25	87	2.1	0.07	2.4	Fibrosarcoma
E. H.	10	M	75	61.7	–	0.11	1.4	Rhabdomyosarcoma
S. D.	11	M	50	74	1.8	0.08	1.9	Carcinoma, lung
E. B.	12	F	61	65	1.75	0.08	0.9	Carcinoma, breast
Mean			49.3	68.6	1.8	0.09	1.5	
SD			18.9	13.5	0.2	0.01	0.5	
SE			5.5	3.9	0.1	0.004	0.1	

Drugs likely to interfere with the renal excretion of MTX were carefully omitted during treatment. These included salicylates, indomethacin, sulphonamides, penicillin, and probenicid. Drugs likely to interact with MTX at the plasma protein-binding level were also carefully omitted during treatment.

Methotrexate Measurements. Plasma samples were collected via an indwelling IV line and stored at −20° C until assayed for MTX. Sampling times were 15 min pre-dose and then at 2-h intervals for the first 20 h and then at 4- to 12-h intervals for up to 154 h from the start of treatment.

Urine was collected over the whole of this period at intervals of 6–24 h. The collection times, volume and pH of urine samples were recorded. Aliquots were stored at −20° C until assayed for MTX.

Methotrexate was measured in plasma and urine by a competitive protein-binding assay [5]. Plasma and urinary creatinine levels were measured on an SMA-12 autoanalyser.

The renal clearance (C, ml/min) was calculated by dividing the urinary excretion rate (UV/T, in units of μg/min, where U = urinary MTX concentration [μg/ml], V = urine volume [ml] and T = the collection time [min]), by the plasma concentration (P in units of μg/ml) at the midpoint of each urine collection period. P was determined from a graph relating total plasma MTX concentration to time [2]. Estimation of the free MTX plasma concentration was not necessary since MTX is cleared by active tubular secretion.

The net renal clearance was measured from the slopes of curves relating the cumulative urinary excretion of MTX (μg) to the area under the plasma concentration–time curve (AUC, $\mu g \cdot h \cdot ml^{-1}$) [2].

Results

The total urinary recovery of MTX following oral administration ranged from 482–592 mg (mean ± SE = 513.8 ± 8.2), compared with 718–778 (mean ± SE = 721.2 ± 6.2) following IV administration. Thus almost all (90%) of the IV administered dose of MTX was recovered in the urine, confirming the renal excretion is the major route of elimination of MTX. The renal clearance of MTX varied with time. Following oral administration, renal clearance values were high during the first 6 h, at the level of 257 ± 8.3 (mean ± SE) ml/min, and subsequently fell, with a trough clearance value of 27.9 ± 4.2 (mean ± SE) ml/min during the 20- to 40-h time period. This was followed by a secondary rise to 180.4 ± 17.3 (mean ± SE) ml/min during the 68- to 84-h time period and again fell to 84.9 ± 17.3 (mean ± SE) ml/min between 84 and 112 h. In the last time period, the renal clearance rose again to 209 ± 57.9 (mean ± SE) ml/min, almost equal to that in the first 6-h collection period. Figure 1 shows these changes graphically.

Data from five patients who received the drug IV on one occasion and PO on another shows that following administration PO there was, initially, in the first collection period, a high MTX renal clearance of 295 ± 26.8 (mean ± SE) ml/min followed by a progressive fall in clearance with a trough value of 41.7 ± 7.3 (mean ± SE) ml/min in the 12- to 20-h collection period. This was followed by an increase in clearance, with a peak of 148.5 ± 37.9 ml/min (mean ± SE), again followed by a progressive fall with a trough value of 60.9 ± 24.0 (mean ± SE) ml/min.

Figure 2 shows the mean values (± SEM) in renal clearance of MTX in patients who received the same dose of high-dose oral MTX therapy IV and PO on different occasions for comparison. In each case there is evidence of a high initial rate of excretion, followed by a trough and then by repeated cyclical peak levels and minimal excretion rates in the corresponding period.

Figure 3 is a histogram of the peaks and troughs of the MTX renal clearance in patients receiving the drug PO, and emphasizes the marked fluctuations in

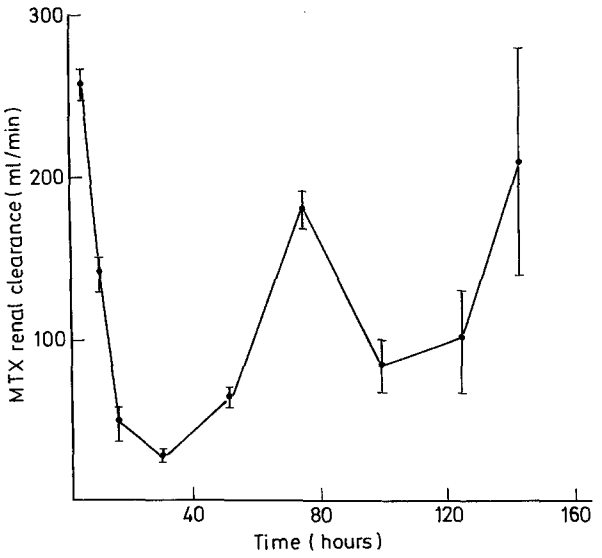

Fig. 1. Renal clearance of MTX (ml/min) in relation to time (h) in eight patients given 800 mg MTX (50 mg/h for 16 h) PO. Each *point* represents the mean ± SEM of the results from the eight patients

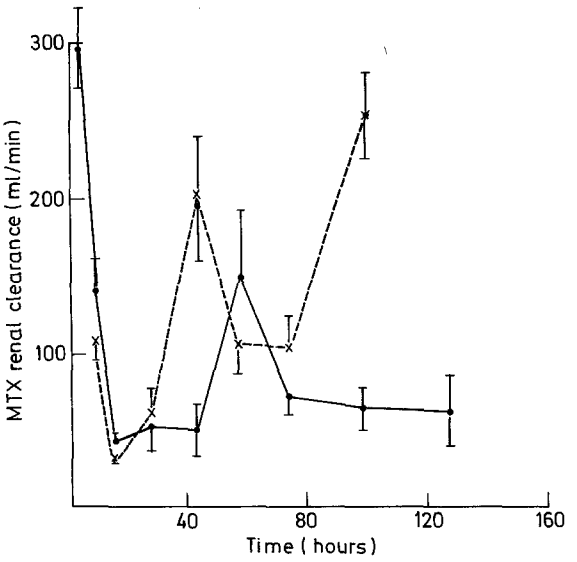

Fig. 2. Renal clearance of MTX (ml/min) in relation to time (h) in five patients following administration of 800 mg MTX IV by infusion over 16 h (. . . . .) and PO, 50 mg/h for 16 h (———). Each *point* represents the mean ± SEM of the results from the five patients

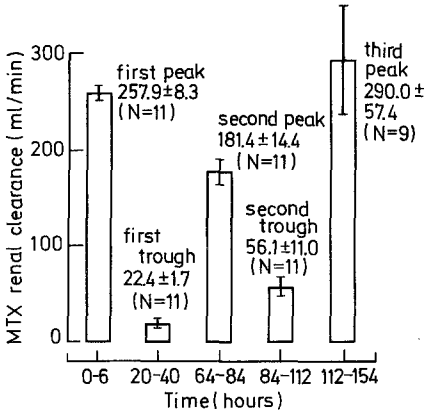

Fig. 3. Histogram of the peaks and troughs in renal MTX clearance following administration of 800 mg MTX PO. These changes in renal clearance of MTX were statistically significant according to the paired *t*-test ($P < 0.001$)

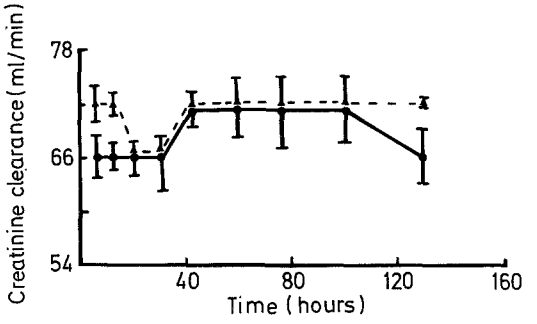

Fig. 4. Creatinine clearance of MTX in relation to time in five patients following 800 mg MTX administered IV (. . . . .) on one occasion and PO (———) on another. The changes in creatinine clearance were not statistically significant according to the paired *t*-test ($P > 0.05$)

renal clearance. Two-tailed Student's *t*-tests comparing successive peaks and troughs in MTX renal clearance were statistically significant at $P < 0.001$ level. The first peak in MTX renal clearance always occurred in the 0- to 6-h urine collection period and was significantly greater than the first trough ($t = 32.4$, d.f. = 10, $P < 0.001$). The first trough in renal clearance occurred during the 20- to 40-h period in eight of the studies, and between 12 and 20 h in the remaining three. The first trough was significantly lower than the second peak in MTX renal clearance ($t = -10.6$, d.f. = 10, $P < 0.001$). The second peak almost always occurred in the 64- to 84-h period and was significantly higher than the second trough ($t = 5.76$, d.f. = 10, $P < 0.001$). The second trough in MTX renal clearance occurred in the 84- to 112-h period in four studies, in the 112- to 136-h period in four studies, and in the 136- to 148-h period in three studies. The second trough was significantly lower than the third peak ($t = -4.14$, d.f. = 8, $P < 0.005$). The third peak usually occurred in the 136- to 148-h collection period.

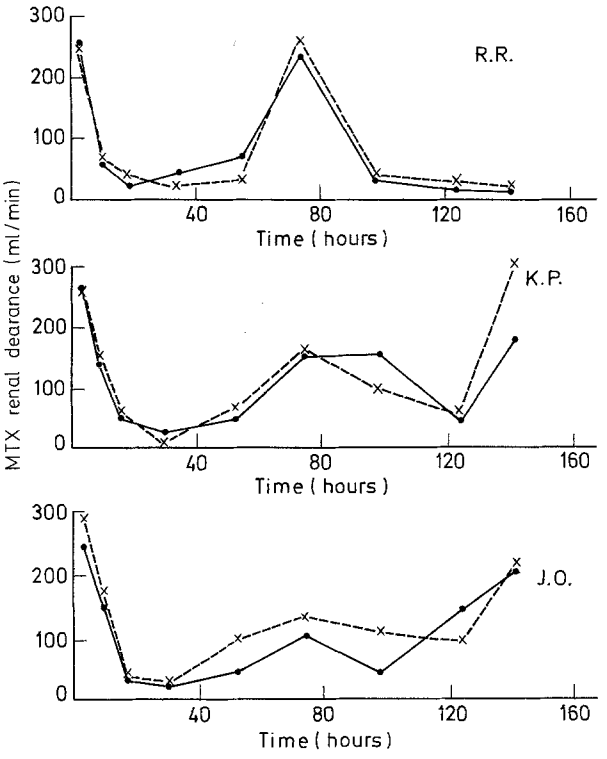

Fig. 5. Renal clearance of MTX (ml/min) in relation to time (h) in three patients studied on two separate occasions at least 1 month apart. MTX was given PO on both occasions. Analysis by the paired *t*-test showed no significant change in the fluctuations in renal MTX clearance with repeat administration of the drug

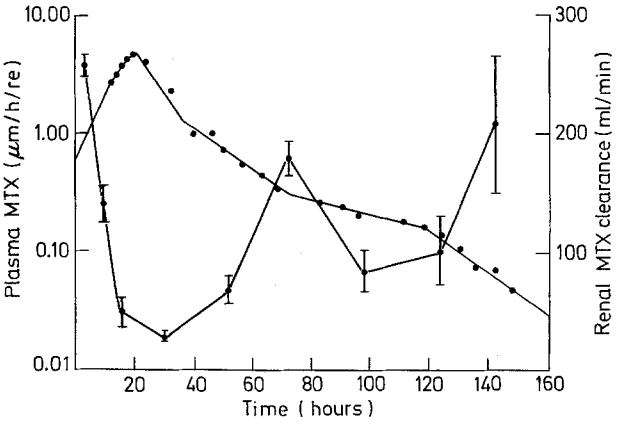

Fig. 6. Mean plasma MTX concentrations (μmol/l, *left vertical logarithmic scale*) and mean ± SEM (ml/min, *right vertical linear scale*) renal MTX clearance in relation to time (h, *horizontal scale*) in 12 patients. In the post-distribution phase of the plasma MTX concentration-time curve, there were three phases in the plasma MTX decay curve. The first and third phases were relatively rapid and were associated with increases in the renal clearance of MTX. The middle phase was slower and was associated with a decrease in renal clearance. The inverse relationship between renal clearance and plasma half-life of MTX is shown in Fig. 7

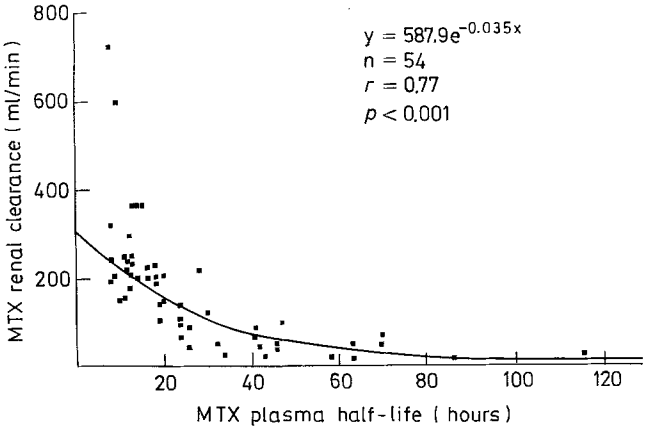

Fig. 7. Renal clearance of MTX (ml/min) in relation to plasma MTX half-life (h) in 12 patients. The relationship was an inverse one and was statistically significant ($P < 0.001$)

The results of creatinine clearance studies in the five patients who received MTX both PO and IV are shown in Fig. 4. There was no significant fluctuation in creatinine clearances to explain the changes with MTX renal clearance with time. Creatinine clearance values throughout the 142 h of collection ranged from 0.9–2.0 ml/s and did not change significantly with time ($P > 0.05$).

A comparison of the peaks and troughs in the renal clearances of MTX and the corresponding creatinine clearances shows that peaks in renal clearance of MTX were significantly higher than the corresponding creatinine clearances, whereas the troughs were lower. The fact that MTX clearances during the peak periods were higher than the creatinine clearances during these periods indicates that in the periods of peak MTX clearance there is tubular secretion of MTX in addition to glomerular filtration. Glomerular filtration is reflected by creatinine clearance.

The effect of repeated dosing on the MTX clearance pattern was studied in three patients (patients 1, 5, and 6) following 800 mg MTX given PO on two separate occasions 1 month apart. The results are shown in Fig. 5. There was no statistical difference ($t = 0.83$, 1.9, and 0.43; $P > 0.05$, respectively) in the clearance rates during the two separate study periods in each of the three patients.

In all 12 patients, the fluctuations in renal clearance were accompanied by changes in the rate of fall in plasma concentration of MTX in the post-distribution phase of the plasma concentration-time curve (Fig. 6). There was a significant ($r = 0.77$, d.f. = 53, $P < 0.001$) inverse relationship between each elimination phase plasma half-life of MTX and

the value of renal MTX clearance related to that time period (Fig. 7).

Discussion

Methotrexate has potential severe toxicity, which may be reduced by careful monitoring. Prevention of renal toxicity is an important component of this. To prevent renal toxicity, adequate hydration and alkalinization were maintained during and following high-dose MTX therapy. The renal clearance of MTX, as assessed by measurements of plasma concentration, urinary recovery, and well-defined periods of urine collection over a 150-h period, showed a statistically significant cyclical fluctuation with time following both PO and IV administration of the drug. The fluctuations in clearance could not be attributed to changes in filtration since creatinine clearances remained unchanged throughout. The possibility of competition with leucovorin for the active secretory process in the proximal tubule was excluded by the fact that the fall in renal clearance of MTX occurred within 12–20 h after MTX administration, preceding the administration of leucovorin. However, the possibility of competion between MTX and metabolites such as 7-hydroxy MTX for the active transport process in the proximal renal tubule could not be excluded. Other drugs which may interfere with this process, such as aspirin, indomethacin, and sulphonamides, were excluded in the protocol of this study. A possible mechanism to explain the cyclical fluctuation in the renal clearance of MTX is shown in Fig. 8. The initially high renal clearance rate is due to combined filtration and active tubular secretion. The active transport of MTX results in high intracellular levels, which results in inhibition of nucleic acid and protein synthesis so that synthesis of new carrier protein ceases. The existing amount of carrier becomes saturated with a resultant inhibition of the active tubular secretion, and hence renal clearance of MTX falls, as is reflected in slowing of the rate of fall of MTX in the plasma concentration–time curve. In our studies, during these periods of diminished tubular secretion of MTX the renal clearance of MTX fell significantly below the glomerular filtration rate (GFR), reflecting a possible back-diffusion of MTX out of the tubule cell and into the plasma. This results in a fall of MTX concentration inside the tubule cell, which then recovers, leading to a return of active secretion with a secondary rise in renal clearance. The cycle is then repeated.

The fluctuations in renal clearance and the related fluctuations in the plasma half-life of MTX following high doses may in part explain the complexity of the pharmacokinetic models previously proposed, and also the disparate literature results. Studies made over a short period of time have shown MTX clearances greater than GFR [4], but studies done over 0–96 h have shown them to be less [3]. The overall net renal clearance in our patients studied over 154 h was 59.1 ± 4.1 ml/min, in agreement with the latter. The possible role of leucovorin rescue in influencing the observed changes in MTX clearance with time is the subject of further studies.

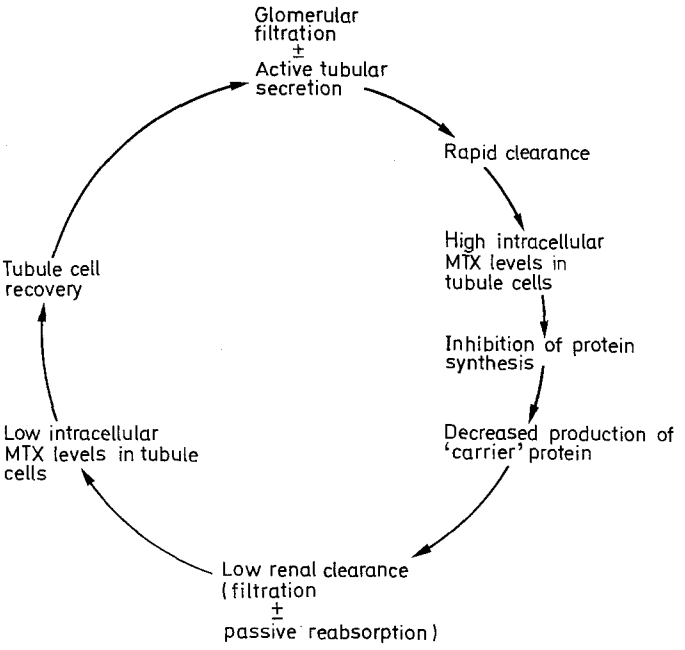

Fig. 8. Hypothesis for the observed cyclical fluctuations in renal MTX clearance. MTX is a weak organic acid that is cleared primarily by the kidney by glomerular filtration and active tubular secretion. This rapid clearance leads to high intracellular concentrations of MTX, resulting in inhibition of protein synthesis and decreased production of 'carrier' protein. This results in reduced tubular secretion of the drug; the concentration of MTX inside the tubular cell falls and the cell recovers, resulting in increased clearance

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