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Renal Excretion and Pharmacokinetics of Methotrexate and 7-Hydroxy-Methotrexate Following a 24-h High Dose Infusion of Methotrexate in Children

B. Winograd[1], R. J. J. Lippens[2], M. J. M. Oosterbaan[1], M. J. M. Dirks[1], T. B. Vree[1], and E. van der Kleijn[1]

[1] Departments of Clinical Pharmacy and [2] Pediatrics St. Radboudziekenhuis, University of Nijmegen, Nijmegen, The Netherlands

Summary. In children with lymphoid malignancies 18 courses of methotrexate (18-200 mg/kg) administered as a 24-h infusion were monitored. Plasma concentrations and renal excretion rates of methotrexate (MTX) and 7-hydroxymethotrexate (7-OHMTX) were determined. A low correlation was found between the administered dose of MTX and the body exposure to MTX or 7-OHMTX. Although 84% of the MTX eventually recovered from the urine was excreted during the 24 h of the infusion, the renal clearance of MTX was markedly lower during the time of the infusion than after it. There were courses with a low and others with a high renal clearance of MTX during the infusion, despite the same urine flow. A low MTX renal clearance was correlated with a high body exposure to MTX. As the same variations were also seen in the same patient during successive courses, pharmacokinetical characterization of patients appears questionable. The renal clearance of 7-OHMTX was significantly lower than the renal clearance of MTX, and the body exposure to 7-OHMTX ranged from 2-40% of the MTX body exposure. Treatment courses with a low or a high body exposure to 7-OHMTX were not associated with different urinary recoveries of the metabolite.

Differences in MTX hydroxylation could not be substantiated. Because the concentration of 7-OHMTX is high soon after the end of an infusion, a specific method of MTX determination should be chosen for controlling treatment.

Key words: methotrexate, hydroxymethotrexate; lymphoid malignancy, renal excretion, metabolism, pharmacokinetics

In contrast to the folic acid antagonist methotrexate (MTX), the pharmacokinetics and especially the renal excretion of its main metabolite 7-hydroxymethotrexate (7-OHMTX) have been little studied [1, 16, 18, 19]. In 1964 7-OHMTX was described as a metabolite of MTX in vitro [11], and in 1976 it was identified as a metabolite of MTX in man and monkey [10]. It is a much less effective inhibitor of mammalian dihydrofolate reductase than MTX [12, 13].

The concentration of 7-OHMTX cannot be determined by commonly used methods of determination of MTX, such as radioimmunoassay (RIA), the competitive protein binding assay (CPB), Emit and the competitive dihydrofolate reductase immunoassay. There is good evidence, that 7-OHMTX interferes with the determination of MTX in the RIA, CPB and Emit assay [2, 3, 5, 6, 8].

7-OHMTX is about five times less soluble than MTX at pH 7 and it has been found as crystalline material in the renal tubules of monkeys after high dose MTX administration [10]. The concentrations of 7-OHMTX are much higher than those of MTX in plasma and urine from patients soon after termination of a 6-h or 24-h MTX infusion [1, 5, 16-19].

High concentrations of 7-OHMTX have been shown to influence the intracellular concentration of MTX in vitro [16].

The aim of the present investigation was to study the pharmacokinetics and renal excretion of 7-OHMTX and MTX in a homogeneous group of children during and following a 24-h high dose infusion of MTX. 7-OHMTX and MTX in plasma and urine were determined by a sensitive HPLC method [15].

Patients and Methods

Eight children, aged 9-12 years, were treated with a total of 18 courses of MTX (18-200 mg/kg), administered as 24-h continuous infusions. The MTX treatment was part of the consolidation therapy of their lymphoid malignancies (5 non-Hodgkin lymphomas, 3 ALL relapses). There is a summary of the clin-

Table 1. Clinical data of the patients in the study

Patient	Age (year)	Sex	Diagnosis	Weight (kg)	Previous[a] Treatment	Side-effects Course 1 (WHO)
I.M.	8	f	ALL relapse bonemarrow	24.0	VCR, Pred Cycl	stomatitis 2 myelosup. 3
F.B.	10	m	Non Hodgkin stomach	38.0	VCR, Pred DXR, Cycl	naus/vom. 2 myelosup. 4
B.H.	11	f	B-cell lymphoma	42.3	VCR, Pred DXR, Cycl	stomatitis 3 myelosup. 2
J.E.	9	f	B-cell lymphoma	24.3	VCR, Pred DXR, Cycl	stomatitis 1 myelosup. 2
M.P.	9	m	B-cell lymphoma	26.0	VCR, Pred DXR, VM-26 ARA-C	naus/vom. 2 myelosup. 1
G.B.	12	m	B-cell lymphoma	34.0	VCR, Pred DXR, Cycl	stomatitis 3
J.B.	7	m	ALL relapse testicular bonemarrow	21.2	VCR, Pred Cycl	none
K.W.	9	m	Pre B-cell ALL	28.0	VCR, Pred DXR, Cycl	stomatitis 2

[a] ARA-C = cytosine arabinoside; Cycl = Cyclophosphamide; DXR = doxorubicine; Pred = Prednison; VCR = vincristine; VM-26 = teniposide

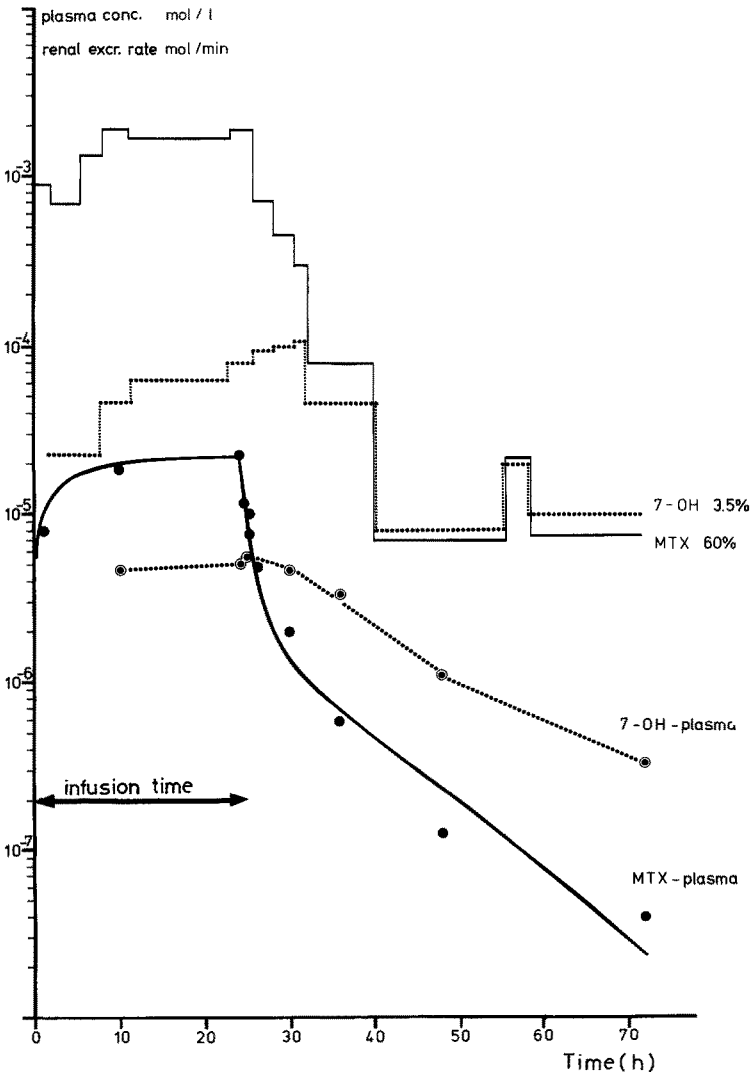

Fig. 1. Findings during treatment course 1 of patient G.B. given 49 mg/kg MTX in a 24-h infusion: plasma-concentration-time curve of MTX (● $t_{1/2}$ = 9.6 h) and of 7-OHMTX (⊙ $t_{1/2}$ = 14.0 h); renal excretion rates of MTX and 7-OHMTX per urine sample; of the administered dose 60% was recovered as MTX and 3.5% as 7-OHMTX in the urine after 71 h

ical data in Table 1. MTX was given at intervals of 1–2 weeks. Treatment courses with the index "1" refer to the first treatment with MTX, etc.

The treatment plan called for oral alkalization with 1 g $NaHCO_3$ every 4 h starting 24 h prior to MTX. Intravenous hydration with 100 ml/m^2/per h saline/dextrose was started 3–6 h prior to MTX and was continued until the end of the MTX infusion. MTX was dissolved in 5% dextrose, less than 3 g in 480 ml and more than 3 g in 960 ml. Six hours after discontinuation of the MTX infusion, rescue with leucovorin started: 15 mg leucovorin was given p.o. or i.v. every 6 h for 3 days. Alkalinization was stopped at the same time as the rescue. The pharmacokinetic studies on MTX in children were approved by the local Ethical Committee.

An example of the monitoring during the 18 courses of MTX treatment is given in Fig. 1. Blood was collected at regular time intervals into heparinized tubes at least until 60 h after the start of the MTX infusion. Plasma was kept frozen at −20 °C until HPLC analysis.

Urine was collected as spontaneously voided portions during 42–90 h after the start of the MTX infusion. In order to prevent MTX renal toxicity, each urine portion was checked for ph ⩾ 7 to ensure sufficient alkalization; an aliquot was kept frozen at −20 °C until analysed.

MTX and its metabolite 7-OHMTX were measured by HPLC [15]. Because of better peak resolution, a reversed phase column with spherical 7 µm particles (25 cm × 3 mm ID, CPtm spher C 8, Chrompack, The Netherlands) was used.

The analytical procedure clearly discriminated between 7-OH-MTX and 2,4-diamino-N^{10}-methylpteroic acid (APA), as shown in plasma samples spiked with APA.

The areas under the plasma concentration-time curves for MTX (AUC-MTX) and for 7-OHMTX (AUC-7-OHMTX) were calculated by the trapezoidal rule, using the measured plasma concentrations and the terminal half-life. The pharmacokinetic parameters were calculated by means of NONLIN according to a two compartment infusion model.

The renal excretion rates of MTX and 7-OHMTX were determined for each collected urine portion as shown in Fig. 1. By adding up the cumulative amounts excreted, the total recovery in urine of MTX and 7-OHMTX was calculated as percentage of the administered dose of MTX.

Renal clearances of MTX and 7-OHMTX were calculated by dividing the renal excretion rate by the plasma concentration. This was done at the midpoint of each urine collection period and at the end of each period, taking the mean of the two excretion rates.

Results

The body exposure to MTX (AUC-MTX) during the 18 treatment courses is shown in relation to the administered dose in Fig. 2. After omitting the courses with an extreme MTX dose (I.M.) and extreme AUC-MTX values (B.H. 1, J.E. 1, K.W. 2), a correlation of $r=0.63$ was found ($n=14$). The overall correlation of the AUC-MTX with the dose of MTX for all 18 treatment courses was as low as $r=0.18$.

The relation of AUC-7-OHMTX to the administered dose of MTX is shown in Fig. 2 (bottom). Only treatment course I.M. has been omitted. The correlation coefficient r was 0.31 ($n=17$). Adding in the course of I.M. r increased to 0.52 ($n=18$). Courses with a high AUC-MTX (B.H. 1, J.E. 1, K.W. 2) were not consistently associated with a high AUC-7-OHMTX (Fig. 2 bottom).

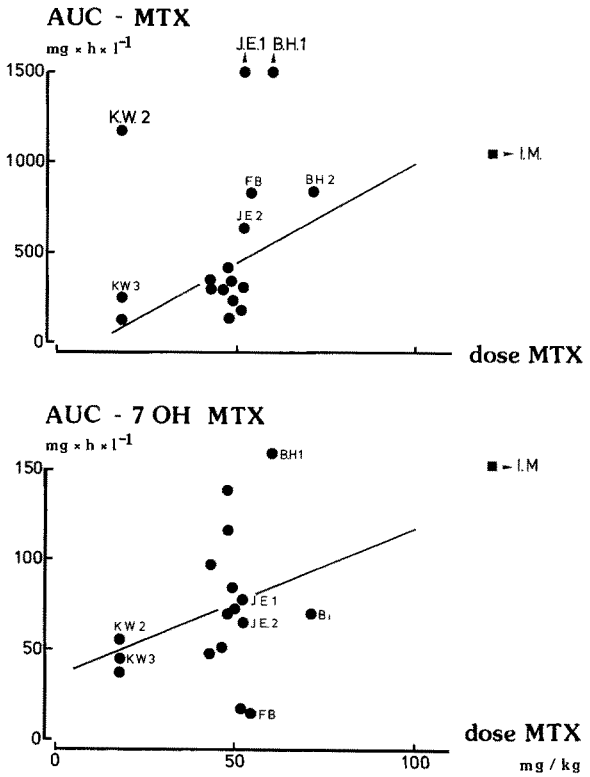

Fig. 2. *Top* Dose of MTX versus AUC-MTX following 18 courses of MTX. Correlation calculated on 14 courses, $r=0.63$, $y=11x-109$ (except I.M., B.H. 1, J.E. 1, K.W. 2). *Bottom* Dose of MTX versus AUC-7-OHMTX following 18 courses of MTX. Correlation calculated on 17 courses, $r=0.31$, $y=x+35$ (except I.M.)

As a further step the attempt was made to relate the AUC-MTX to the AUC-7-OHMTX following treatment with MTX (Fig. 3). In 17 courses the correlation r was 0.50 (solid line). Adding I.M. the correlation of AUC-MTX and AUC 7-OHMTX was 0.48.

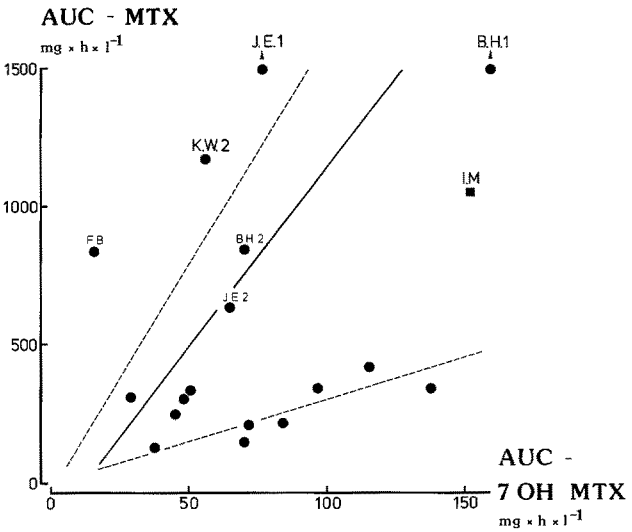

Fig. 3. AUC-7-OHMTX versus AUC-MTX following 18 courses of MTX. *Solid* regression line calculated on 17 courses, $r=0.50$, $y=13x-164$ (except I.M.). *Dotted* regression lines are calculated on courses with a percentage of the AUC-7-OHMTX below and above 20%. *Below* 20%: $n=11$, $r=0.71$, $y=17x-90$; *above* 20%: $n=7$, $r=0.88$, $y=3x+1$

The AUC-MTX, AUC-7-OHMTX, percentage AUC-7-OHMTX/AUC-MTX and the recovery of MTX and 7-OHMTX in urine as a percentage of the administered dose of MTX are summarized in Table 2. The total time of urine collection following each treatment course is given. The total recovery of MTX in the urine amounted to 71% ± 11% of the administered dose, of which 84 ± 9% was excreted during the 24 h of the infusion.

In Tables 2–4 mean values for each of the given parameters for all treatment courses are listed, as well as those for treatment courses with a percentage AUC-7-OHMTX below and above 20%.

The calculated clearances per urine sample of MTX and 7-OHMTX are summarized in Tables 3 and 4, as well as their flow dependency. Patient G. B. 1 in Fig. 1 may be taken as an example: during the 24 h of the MTX infusion, 6 urine samples were collected, and after the end of the infusion 7 more were obtained. The renal clearance of MTX could then be related 6 + 7 times to the urine flow. As outlined in Methods the renal clearance was calculated at 6 + 5 = 11 and 7 + 7 = 14 time points (Table 3). For 7-OHMTX the renal clearance in 9 urine samples could be related to the urine flow, and the renal clearance could be calculated at 18 time points (Table 4).

Table 2. Body exposure and urinary recovery of MTX and 7-OHMTX

Patient	MTX dose (mg/kg)	AUC-MTX (mg × h/l)	AUC-7 OH-MTX (mg × h/l)	AUC-7OH-MTX / AUC-MTX (%)	Recovery MTX (%)	Recovery 7 OH-MTX (%)	Time (h)
I.M.	200	1050	152	14	68	2.2	42
F.B.	54	830	16	2	79	0.4	44
B.H. 1	60	4028	159	4	77	6.5	92
B.H. 2	71	840	70	8	60	2.2	72
J.E. 1	52	2070	77	4	74	3.8	72
J.E. 2	52	632	65	10	72	1.7	72
M.P. 1	52	309	17	6	64	1.6	72
M.P. 2	46	325	51	16	80	2.5	72
M.P. 3	48	145	70	48	72	2.9	72
G.B. 1	49	219	84	38	60	3.5	71
G.B. 2	48	345	138	40	62	3.9	66
G.B. 3	50	207	72	35	54	2.2	47
J.B. 1	43	312	49	16	55	1.5	42
J.B. 2	48	413	116	28	66	2.6	42
J.B. 3	43	336	97	29	68	5.1	42
K.W. 1	18	130	38	29	94	7.0	72
K.W. 2	18	1169	56	5	86	6.0	48
K.W. 3	18	253	45	18	84	5.1	53
				19% ± 14%[a]	71% ± 11%[a]	3.4% ± 1.9%[a]	
				9.4% ± 5.7%[a]	73% ± 10%[a]	3.1% ± 2.0%[a]	
				35.3% ± 7.3%[a]	68% ± 13%[a]	4.1% ± 1.8%[a]	
Student's *t*-test				$p<0.001$	n.s.	n.s.	

[a] Mean ± SD

Table 3. Renal clearance of MTX and its dependence on urine flow

Patient	MTX renal clearance 0–24 h		MTX renal clearance/flow		MTX renal clearance 24+h		MTX renal clearance/flow	
	ml/min ± SD (range)	*n*	*r*	*n*	ml/min ± SD (range)	*n*	*r*	*n*
I.M.	65 ± 28 (13-110)	11	0.93	5	224 ± 154 (75-477)	7	1.0	4
F.B.	16 ± 8 (4- 32)	8	0.47	4	79 ± 30 (51-110)	3	*	2
B.H. 1	5 ± 4 (0.6- 11)	10	0.93	5	230 ± 224 (7-708)	9	0.81	4
B.H. 2	31 ± 23 (9- 75)	12	0.87	6	123 ± 57 (41-225)	23	0.94	12
J.E. 1	10 ± 7 (3- 25)	10	0.87	5	169 ± 131 (76-613)	20	0.55	11
J.E. 2	23 ± 7 (12- 38)	11	0.02	5	88 ± 62 (21-223)	23	0.30	12
M.P. 1	42 ± 22 (3- 78)	11	0.96	5	106 ± 80 (26-276)	23	0.79	12
M.P. 2	62 ± 24 (39-116)	11	0.67	5	58 ± 41 (8-164)	19	0.37	10
M.P. 3	96 ± 16 (71-120)	8	0.16	4	284 ± 70 (198-450)	21	0.50	11
G.B. 1	89 ± 15 (67-117)	11	0.70	6	172 ± 54 (56-294)	14	0.32	7
G.B. 2	42 ± 3 (38- 45)	5	*	2	115 ± 35 (62-161)	9	0.63	5
G.B. 3	100 ± 43 (39-169)	7	0.93	4	352 ± 116 (194-645)	10	0.00	5
J.B. 1	57 ± 17 (36- 82)	4	*	2	176 ± 67 (99-226)	3	*	2
J.B. 2	67 ± 25 (32- 97)	4	*	2	126 ± 19 (101-142)	5	0.39	3
J.B. 3	90 ± 18 (63-112)	4	*	2	400 ± 224 (176-671)	5	0.71	3
K.W. 1	106 ± 60 (15-214)	8	0.73	4	121 ± 58 (36-205)	9	0.76	4
K.W. 2	34 ± 39 (3-106)	11	0.74	5	187 ± 133 (75-415)	5	0.72	3
K.W. 3	44 ± 27 (13- 81)	8	0.97	4	171 ± 106 (53-339)	7	0.85	4
	54 ± 32[a]		0.71 ± 0.30[a]		177 ± 93[a]		0.60 ± 0.27[a]	
< 20%[a]	35 ± 21[a]		0.74 ± 0.30[a]		146 ± 59[a]		0.70 ± 0.24[a]	
> 20%[a]	84 ± 22[a]		0.63 ± 0.32[a]		224 ± 120[a]		0.47 ± 0.26[a]	
	$p < 0.001$		n.s.		$p < 0.1$		$p < 0.1$	

[a] Mean ± SD

Table 4. Renal clearance of 7-OHMTX and its dependence on urine flow

Patient	7-OHMTX renal clearance		Renal clearance/flow	
	ml/min ± SD (range)	*n*	*r*	*n*
I.M.	19 ± 13 (5-56)	18	0.90	8
F.B.	14 ± 1 (13-15)	3	*	2
B.H. 1	17 ± 13 (2-45)	21	0.30	11
B.H. 2	13 ± 5 (4-21)	28	0.89	14
J.E. 1	25 ± 19 (8-58)	21	0.81	11
J.E. 2	18 ± 18 (1-53)	28	0.00 (0.62)[a]	14 (7)[a]
M.P. 1	26 ± 15 (2-68)	24	0.47 (0.75)	12 (5)
M.P. 2	11 ± 6 (2-24)	27	0.86	13
M.P. 3	20 ± 9 (7-42)	27	0.55	13
G.B. 1	19 ± 6 (6-30)	18	0.60	9
G.B. 2	11 ± 3 (6-16)	13	0.69	7
G.B. 3	42 ± 17 (6-66)	13	0.69	6
J.B. 1	16 ± 3 (13-19)	5	*	2
J.B. 2	12 ± 3 (8-17)	6	*	2
J.B. 3	32 ± 18 (13-65)	7	0.46	3
K.W. 1	15 ± 7 (6-29)	17	0.07	8
K.W. 2	10 ± 2 (7-14)	14	0.86	6
K.W. 3	12 ± 5 (5-21)	13	0.45	7
	18 ± 8[a]		0.57 ± 0.29[a] (0.63 ± 0.24)[a]	
< 20%	16 ± 5[a]		0.59 ± 0.34[a]	
> 20%[a]	22 ± 11[a]		0.51 ± 0.23[a]	
	$p < 0.2$		n.s.	

[a] Mean ± SD

At 16 of the 18 treatment courses the renal clearance of MTX during the infusion was markedly lower than after its end (except M.P. 2 and K.W. 1). Therefore, the renal clearances of MTX have been separately listed in Table 3 under these two different time periods. A high correlation between MTX renal clearance and urine flow was more often found during than after the infusion period.

The pattern of lower clearance during the infusion and higher clearance thereafter, as found for MTX, was not observed for the renal clearance of 7-OHMTX. Accordingly, the renal clearances of 7-OHMTX in Table 4 have not been listed under these two different time periods. The mean renal clearance of 7-OHMTX (18 ml/min) was significantly lower than that of MTX in both periods (54 ml/min and 177 ml/min, respectively). The renal clearance of 7-OHMTX during many courses was dependent on urine flow. Late urine samples from courses J.E. 2 and M.P. 1 did not show this flow dependency; the 7-OHMTX renal clearance in them was higher than 40 ml/min. Omitting these late samples, the mean correlation for the flow dependent clearance of 7-OHMTX was $r = 0.63$ ([a] and [b] in Table 4).

The dose-corrected body exposure to MTX has been related in Fig. 4 to the mean 0-24 h renal clearance of MTX for the 18 treatment courses. For $n=15$ the correlation r was -0.75 (B. H. 1, J. E. 1 and K. W. 2 with extreme values of AUC-MTX omitted).

All the children had normal kidney function, as determined by serum creatinine and endogenous creatinine clearance. No decrease in kidney function during treatment with MTX was observed.

Discussion

As shown in Fig. 2 (top), treatment courses B. H. 1, J. E. 1 and K. W. 2 were well above the regression line for AUC-MTX on the dose of MTX. This means that following those three courses of MTX the patients were exposed to much more MTX than in the other 15 courses. Those three courses were followed by toxic reactions in the children (leukopenia $< 2.5 \times 10^9/l$ and stomatitis).

The renal clearance of MTX during the period of the infusion in the above mentioned three toxic treatment courses was far below the mean of 54 ml/min; namely, 5, 10 and 34 ml/min (Table 3). As during each of those courses a correlation was found between the renal clearance of MTX and the urine flow, it might be concluded that low urine flow was the reason for the low MTX renal clearance and the relative high body exposure to MTX (Fig. 4).

Another four of the treatment courses resulted in a high body exposure of MTX. In them, too, the data lay above the regression line in Fig. 2 (top) and in the right hand part of Fig. 4 (dose corrected AUC-MTX versus 0-24 h MTX renal clearance), namely F. B., B. H. 2, J. E. 2 and K. W. 3. Again mean renal clearances of MTX below the average were calculated during the first 24 h: namely 16, 31, 23 and 44 ml/min (Table 3).

Is the low urine flow during the 24 h of the infusion in the above seven courses of MTX the reason for the rather low mean renal clearance of MTX? The flow-dependent renal clearances per urine sample in those seven courses are shown in Fig. 5 (open symbols, $n=34$) in comparison with three patients with renal clearances of MTX above the mean (I. M., M. P. 1-3, G. B. 1-3; solid dots, $n=31$). In the two groups the most significant difference was in the renal clearance of MTX (21 ± 23 ml/min versus 74 ± 33 ml/min. No significant difference in urine flow was found (1.3 ± 1.2 ml/min versus 1.6 ± 1.1 ml/min). Although the MTX renal clearance was flow dependent to a certain extent (Table 3, Fig. 5), differences in urine flow could not account for the large variations in renal clearance observed in different patients as well as in consecutive courses (K. W. 2 and 3 versus K. W. 1). A drop in urinary pH could be ruled out as another possible reason for the low renal clearance of MTX.

The renal clearance of MTX depends upon the passive process of glomerular filtration minus tubular reabsorption (flow and pH dependent), and active tubular secretion (not flow dependent). The renal clearance of MTX can far exceed the creatinine clearance (Table 3) and can be inhibited by probenecid. These two facts indicate that active tubular transport processes play a role in its clearance. The low clearance of MTX during the period of the infusion as compared to the clearance after infusion, and the weak flow dependency of its renal clearance both indicate that active transport processes would have played a role in excretion during this period. A high concentration of MTX and/or 7-OHMTX in urine or plasma may inhibit active secretion. This is supported by the present finding that $84\% \pm 9\%$ of the MTX ultimately recovered was excreted during the 24 h of the infusion. Large variations in the clearance

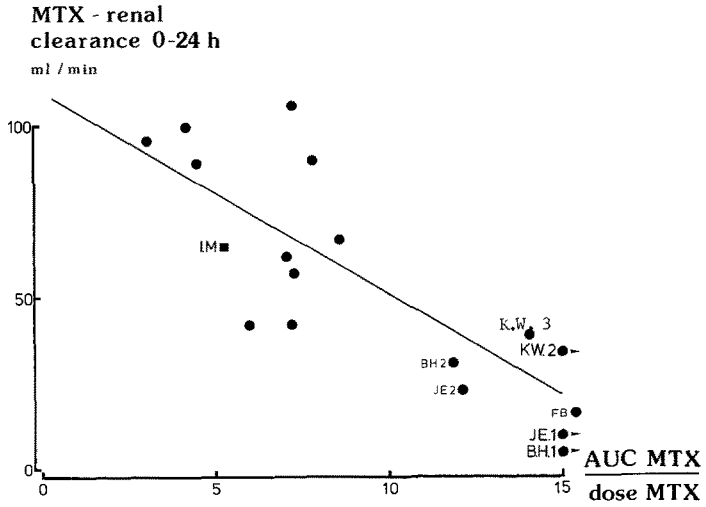

Fig. 4. Dose corrected AUC-MTX versus mean MTX renal clearance 0-24 h following 18 courses of MTX. Correlation calculated on 15 courses, $r=-0.75$, $y=-6x+110$ (except B. H. 1, J. E. 1, K. W. 2)

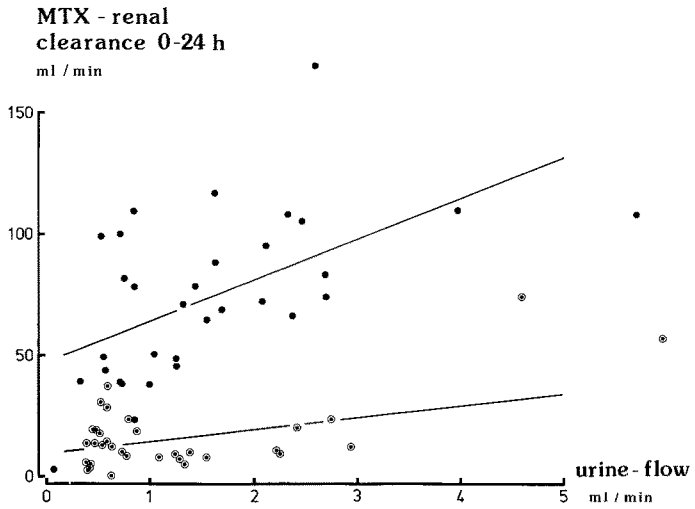

Fig. 5. Urine flow versus MTX renal clearance 0-24 h per urine sample in patients with low (⊙) and high (●) MTX renal clearance. *Open symbols* represent courses F. B., B. H. 1, B. H. 2, J. E. 1, J. E. 2, K. W. 2 and K. W. 3: mean clearance 21 ± 23 ml/min, mean flow 1.3 ± 1.2 ml/min, $n = 34$, $r = 0.40$, $y = 7.6x + 12$. *Closed symbols* represent courses I. M., M. P. 1-3, and G. B. 1-3: mean clearance 74 ± 33 ml/min, mean flow 1.6 ± 1.1 ml/min, $n = 31$, $r = 0.58$, $y = 17x + 46$

of MTX [3, 14], and in its renal clearance [4, 9], have been described by others. Christophidis [4] showed time-dependent fluctuations in its renal clearance and postulated self-inhibition by MTX of its own active tubular secretion.

Few pharmacokinetic data on the main metabolite 7-OHMTX have so far been published [1, 16, 18, 19]. Under physiological conditions it is less soluble than MTX, and so is potentially more nephrotoxic than MTX [10]. Plasma levels of 7-OHMTX in excess of those of MTX were found very soon after the end of the 24 h of the MTX infusion (Fig. 1; [1, 14, 15, 16, 18]). The same was also true for 7-OHMTX concentration in the urine. The renal clearance of 7-OHMTX has here been shown to be much smaller than that of MTX and also to be dependent on urine flow (Table 4); the clearance of 7-OHMTX was low, when the renal clearance of MTX was low (Patients F. B., B. H. 2 and G. B. 2), but this was not a consistent finding.

During the 18 treatment courses, body exposure to 7-OHMTX ranged from 2-40% of that to MTX. This and findings in previous studies suggesting differences in MTX metabolism [16, 18] led to an analysis of these data with regard to slow and fast MTX hydroxylation. The results in Tables 2-4 and in Fig. 3 have been grouped into the categories of body exposure to 7-OHMTX above or below 20% of that to MTX. Between these two subgroups no significant difference in urinary recovery of 7-OHMTX was found, so differences in MTX hydroxylation in these patients can be ruled out. The only difference found between the two subgroups was in the renal clearance of MTX during the infusion period ($p < 0.001$) and subsequently ($p < 0.1$). The data points in Fig. 3 were also divided into the same two subgroups; the upper dotted regression line shows those courses with an AUC-7-OHMTX of less than 20%, low MTX renal clearance and high AUC-MTX ($r = 0.71$, $y = 17x - 90$, $n = 11$), and the lower dotted regression line shows the courses with an AUC-7-OHMTX of more than 20%, high MTX renal clearance and low AUC-MTX ($r = 0.88$, $y = 3x + 1$, $n = 7$).

In conclusion, the renal clearance of MTX during the period of an infusion determines the overall body exposure to MTX, and in consequence the bone marrow and gastrointestinal toxicity. The present findings give only very weak support to the concept that patients should be pharmacokinetically characterized before administering high dose MTX regimens [7, 14], because large variations in renal clearance of MTX and body exposure to MTX were found in the same patient during consecutive courses of treatment. Althouth no indication of interindividual differences in MTX hydroxylation could be found, the possible nephrotoxicity and the pharmacokinetics of 7-OHMTX should be considered, because high concentrations of this metabolite in serum and urine were found soon after the end of the infusions. This may also be of importance in choosing a specific analytical method for controlling MTX treatment.

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Dr. B. Winograd
Department of Oncology
Free University Hospital
De Boelelaan 1117
Amsterdam, The Netherlands