Eur. J. Clin. Pharmacol. 19, 453–456 (1981)

European Journal of Clinical Pharmacology

Effect of Urine pH and Flow on Renal Clearance of Methotrexate

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Summary. Hydration and urinary alkalinization are used with high doses of methotrexate (MTX) to prevent precipitation of the drug in the renal tubules and consequential nephrotoxicity. The quantitative effect of these measures on the renal clearance of MTX was studied in 8 patients with normal renal function, and in 3 patients with reduced renal function. Multiple regression analysis indicated an influence of both factors on the ratio of the renal clearances of MTX and creatinine. In the eleven patients there was a linear correlation between this ratio and urine pH ($p < 0.001$); the ratio increased from 0.88 at pH 5.5 to 2.62 at pH 8.4. The pH effect on this ratio was similar in the patients with normal and reduced kidney function. An increase in urine flow did not significantly increase the ratio between renal clearance of MTX and creatinine. The effect of urinary alkalinization on renal MTX clearance could be clinically exploited in patients with delayed elimination of MTX. The probable modifying effect of alkalinization of urine on the intentionally high plasma concentration after high dose MTX infusions should be further evaluated, particularly in patients with normal renal function.

Key words: methotrexate, renal clearance; urine pH, diuresis, poor renal function, alkalinization

Infusion of large doses of methotrexate (MTX) has been reported efficacious in the treatment of various malignant neoplasms [1, 2]. Such doses of MTX are permissible only when leucovorin (citrovorum factor) is administered after the MTX [1–3], and precipitation of MTX in urine is avoided. Even then, serious side effects from damage to bone marrow, gastrointestinal mucosa and kidneys [4] may occur, more frequently when elimination of MTX, which is mainly by the kidneys, is delayed [5]. Consequently, renal function should be evaluated before, and elimination rate and plasma concentrations of MTX should be determined after drug administration.

MTX is poorly soluble in water at low pH and may precipitate in kidney tubules during and after high-dose infusion of MTX, with subsequent nephrotoxicity [5] and decreased elimination of MTX. For this reason, intensive hydration and alkalinization of urine are routinely prescribed in patients receiving large doses of MTX, to avoid crystalluria [6].

Lower plasma concentrations of MTX have been observed with such a regimen [7, 8], possibly due to an increase in the elimination rate of the weak acid MTX, analogous to the effect of a forced alkaline diuresis on the renal elimination of barbiturates and salicylates [9, 10]. Thus, urinary alkalinization and hydration may have two implications in patients receiving high doses of MTX, first by increasing MTX solubility and reducing the risk of crystalluria, and second by increasing urinary excretion of MTX. The latter may affect treatment adversely by lowering the intended high plasma concentration after the high dose infusion, but desirably so by increasing elimination of MTX in patients with reduced kidney function. No studies have assessed the quantitative consequence of an alkaline diuresis on the urinary excretion of MTX.

The effect of urinary alkalinization and increased diuresis on renal clearance of MTX has been determined in patients with normal and reduced renal function.

Patients and Methods

After obtaining their informed consent, the renal clearance of MTX was determined in 11 patients (age 14–32 years; 7 males and 4 females) with various malignant diseases. Methotrexate (Lederle)

0031-6970/81/0019/0453/$01.00

Table 1. Data from urine

Patients	Creatinine clearance mean [ml/min]	Number of urine collection periods	Urine volume (diuresis) of different collection periods[a] [ml/h]	pH in urine[a]	Renal clearance[a] of MTX [ml/min]
1–8	98–121	42	30–324 (131± 97)	5.5–8.3 (6.7±0.6)	48–300 (158±66)
9	47	24	15–330 (85± 75)	5.5–8.5 (7.6±0.9)	40–210 (98±40)
10	55	5	30–432 (148±163)	7.1–8.3 (7.7±0.5)	95–224 (156±53)
11	67	9	132–372 (234± 81)	6.9–7.7 (7.4±0.25)	87–230 (161±49)

[a] range and mean ± SD

1000–2000 mg/m^2 in 1000 ml saline was administered as a four hour infusion, at minimum intervals of two weeks.

Leucovorin (Lederle; tetrahydropteroylglutamic acid) 15 mg i. v. and 9 mg p. o. was given four hours after the infusion and then (9 mg i. m. or p. o.) every 6 h for 72 h. Patients with slow elimination ($t_{1/2}$ of more than 18 h, or MTX concentration exceeding $5 \cdot 10^{-8}$ M 72 h after the infusion) received leucovorin up to 30 mg × 4 daily parenterally or orally until the plasma MTX concentration had fallen to approximately 2×10^{-8} mol/l. Voluntarily voided urine was collected for 2–6-h periods and blood samples were collected at the midpoint or at the beginning and end of the periods. Urine was collected for the last two hours of the infusion and immediately afterwards, in the morning and in the afternoon on the second and third days, and in the morning on the fourth day after the infusion. The volume and pH of urine were measured immediately (pH meter 27, Radiometer, Copenhagen), and aliquots of urine and plasma were kept at −20 °C until analyzed. To increase the spontaneous variation in urine pH and flow, patients with normal renal function were given up to 400 ml/h of fluid orally for seven hours on the second and third days, starting three hours before the collection period in the afternoon, and sodium bicarbonate 10 g was given orally over three hours before the afternoon collection period on the third day. Patients with impaired renal function were incouraged to drink as much as possible and were given orally up to 20 g/day sodium bicarbonate to establish a high urine pH until MTX plasma concentrations had fallen to approx. 2×10^{-8} mol/ l. MTX in plasma and urine was determined by a competitive binding assay [11], with minor modifications (potassium phosphate buffer strength was increased to 0.2 M, KCL reduced to 0.05 M and 10% V/V human plasma was added whenever plasma samples were analyzed). Renal clearance (C) of MTX was calculated as $C = \frac{U \cdot V}{P \cdot T}$, U and P are urine and plasma concentrations of MTX, V volume of urine and T is the duration of the collection period. The plasma concentration at the midpoint of the urine collection period was used in the calculation. When plasma concentrations were obtained from the start and end-points of a collection period, the clearance was calculated by using the area under the plasma concentration-time curve [12] and the amount of drug eliminated in the urine during the collection period. When the duration of the urine collection period exceeded the observed plasma half-life, clearances were corrected for the inaccuracy inherent in using the mean urinary excretion rate of the collection period [12]. When the creatinine clearance for a collection period was more then 50% higher or lower than the average creatinine clearance for the same patient, the urine collection period was excluded from the study.

Binding of MTX to serum was determined by equilibrium dialysis [13]. Serum 0.3 ml, obtained before MTX administration, with added MTX and ^{3}H-MTX $1 \cdot 10^{-11}$ M, 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 concentration [14] were determined before and after dialysis.

Multivariate and linear least-squares regression analyses were performed with equal weighting of all points. Graphs were drawn from the linear regression analysis.

Results

Increased fluid intake or sodium bicarbonate were administered at intervals subsequent to the MTX infusion to patients with normal renal function, and also simultaneously to patients with impaired renal function, to vary urine pH and flow. Spontaneous and induced individual variation in urine pH and flow were large. Observed values of creatinine clearance, diuresis and urine pH are given in Table 1. An average of 4.2 and 3.8 collection periods were obtained

after each infusion in the patients with normal and impaired renal function. This was mostly due to patient-dependent interruption of urine collection. There was no significant linear correlation between urinary pH and urine flow in the different collection periods.

To compare the renal clearance of MTX in patients with different renal function, the ratio between the clearance of MTX (C_{MTX}) and that of creatinine (C_{creat}) has been used. A multivariate analysis was performed on the data from these patient groups to assess the effect of varying urine pH and flow on this ratio. The relationship in the patients with normal kidney function is described by $C_{MTX}/C_{creat} = -1.1732 + 0.3739$ pH $+ 0.0012$ urine flow. For the patients with reduced kidney function the relationship is $C_{MTX}/C_{creat} = -2.5084 + 0.6067$ pH $+ 0.0008$ urine flow, and including the data from all the patients $C_{MTX}/C_{creat} = -2.5287 + 0.5963$ pH $+ 0.0009$ urine flow. The multivariate analysis indicated that in both patient groups urine pH affected renal clearance of MTX more than urine flow. When observed values of urine pH are plotted against the corresponding values of the ratio between renal MTX and creatinine clearance in the patients with normal renal function, there is a significant linear correlation ($r = 0.446$). The ratio increased from 0.96 at pH 5.5 to 2.15 at pH 8.3 ($p < 0.01$). MTX clearance increased with higher urine pH in three patients with reduced kidney function; the ratio between the corresponding values of MTX and creatinine clearance increased from 1.1 at urine pH 5.5 to 2.6 at urine pH 8.5 ($r = 0.450$; $p < 0.01$). When plotting all data from both patient groups (Fig. 1) the ratio between the clearance of MTX and creatinine increased from 0.88 at urine pH to 5.5 to 2.62 at pH 8.4 ($r = 0.596$; $p < 0.001$).

The ratio between methotrexate and creatinine clearance increased (data not shown) with increasing urine flow in the patients with normal kidney function; this relationship approached significance ($r = 0.299$, $p = 0.05$). There was no linear correlation between this ratio and urine flow in the patients with reduced kidney function.

Protein binding (Table 2) ranged from 44 to 57%. It remained stable in individual patients over a wide range of MTX concentrations ($2 \cdot 10^{-8}$ to $2 \cdot 10^{-4}$ M).

Table 2. Protein binding of methotrexate in serum (%)*

	MTX concentration [M]				
Patient	$2 \cdot 10^{-8}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-6}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-4}$
1	50	54	52	53	49
2	54	57	56	56	53
3	44	47	49	46	48
4	55	55	54	53	48

* Values are mean of duplicates

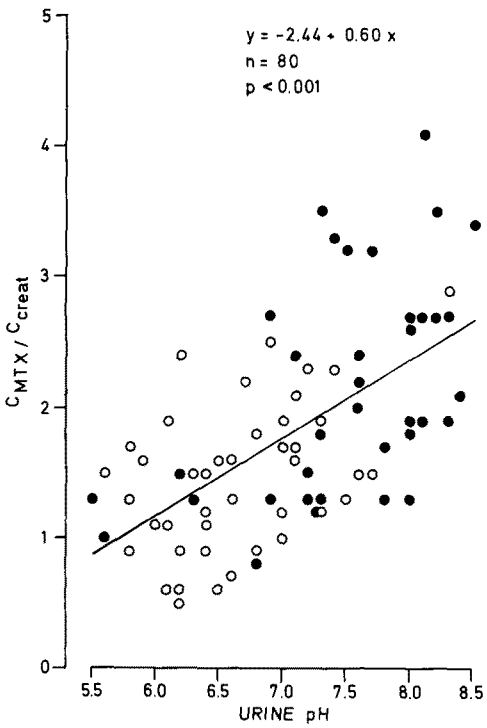

Fig. 1. Ratios between renal clearance of methotrexate and creatinine in 80 separate urine collection periods, at different urine pH, in 11 patients with normal (○) or impaired (●) renal function. $Y = -2.44 + 0.60x$, $p < 0.001$

Discussion

Endogenous creatinine clearance is correlated with the elimination rate of drugs that are excreted entirely or partly unchanged by the kidneys [15]. This permitted comparison of the urinary clearance of MTX in patients with large differences in kidney function by using the ratio between the urinary clearance of MTX and of creatinine.

The data show a considerable increase in renal MTX clearance when urine pH is increased, both in patients with normal and with impaired renal function. The multivariate analysis of the data suggested a small influence of high urine flow, which was not significant in this study. There were small interindividual differences in protein binding, and the protein binding did not change with changing MTX concentration. Total MTX plasma concentration could be used, therefore, when calculating renal clearance.

The scatter of data in Fig. 1 is partly explained by unavoidable, simultaneous changes in urine pH and flow and partly by the possible influence of other factors, such as the tissue distribution of MTX. Difficulties involved in the collection of voluntarily voided urine in severely ill patients may also have influenced the results.

There are two implications of our findings. MTX is poorly soluble in urine at low pH, and at the high concentrations encountered in urine during and shortly after high dose infusions, MTX may precipitate in renal tubules with consequential kidney damage and reduced clearance. A small increase in urine pH increases MTX solubility sharply – from 2.2 mM at urine pH 5.7 to 22 mM at pH 6.9 [5]. Peak urine concentrations of 11 mM have been measured after doses of MTX up to 200 mg/kg [5]. We suggest that a small increase in urine pH and a high urine flow during and shortly after infusion could prevent MTX crystalluria and would have little effect on elimination rate.

Intensive hydration and alkalinization of urine during and after the administration of large doses of MTX are recommended procedures to prevent crystalluria [5]. However, this is accompanied by lower plasma concentrations of MTX [7, 8], which, from the present results, can be explained by an increase in the elimination rate of MTX. The purpose of the regimen with large doses of MTX given as an infusion is to establish a high plasma/tissue gradient and so to enhance the distribution rate of the drug [16]. An increase in the elimination rate during and shortly after the infusion will reduce the concentration gradient and distribution of MTX, and will impair the objective of high dose infusions.

Patients with reduced renal function regularly have delayed elimination of MTX and prolonged administration of leucovorin is necessary. Our data suggest that alkalinization of urine could be tried clinically to increase MTX elimination in these patients, to avoid serious side effects and to abolish the need for prolonged administration of leucovorin. Preliminary data indicate that the increase in elimination provoked by urine alkalinization in these patients is also reflected by a shorter biological half-life of the drug.

Acknowledgements. We wish to thank Mrs. R. Patel and A. Killi for technical assistance, Mrs. E. Nandrup for aid in collecting the urines, and Mr. E. Fønstelien for running the multiple regression analysis. The study was supported by the Norwegian Council for Science and Humanities, and by the Norwegian Cancer Society and the Norwegian Drug Monopoly.

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Received: February 4, 1980
accepted in revised form: November 25, 1980

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