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Theoretically required urinary flow during high-dose methotrexate infusion

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Summary. *The renal excretion of methotrexate (MTX) and its major metabolite 7-hydroxymethotrexate (7-OH-MTX) was analysed in 12 children with malignancies during 52 courses of high-dose methotrexate (H-D-MTX) infusion at dosages ranging from 0.7 to 8.4 g/m².*

The peak concentrations of both MTX and 7-OH-MTX exceeded the aqueous solubilities of these compounds at low pH (≦ 6.0). The cumulative MTX excretion in urine was 75%–98% of the administered amount of MTX, and the cumulative 7-OH-MTX excretion in the urine was 3%–15%. The theoretically required urinary flow (TRUF) was estimated as the minimum urine volume needed for complete resolution of MTX and its metabolites in urine. TRUF during MTX infusion from 0 to 6 h and from 6 to 12 h was correlated with the dosage of MTX, and these values were 0.1–1.8 ml/min/m² at pH 7.0, 0.5–11.1 ml/min/m² at pH 6.0, and 1.9–42.2 ml/min/m² at pH 5.0 with dosages of 0.7 to 8.4 g/m². The value of the theoretically required urinary flow is important to ensure adequate hydration and the optimum alkalinization schedule for massive MTX infusion.

Introduction

High-dose methotrexate (H-D-MTX) therapy followed by citrovorum factor rescue offers a new and potentially more efficacious treatment for childhood malignancies, but is inevitably accompanied by an increased risk of severe host toxicity [6]. Nephrotoxicity is a special problem with H-D-MTX therapy, because the drug is retained as a result of renal dysfunction and further toxicity may ensue [9]. Methotrexate (MTX) has two carboxylic acid groups and acts as a weak acid with PKa values in the range of 4.8–5.5 [7]. Thus, at low pH, one carboxyl group is not ionized and MTX becomes insoluble [2]. 7-Hydroxymethotrexate (7-OH-MTX) was the major metabolite detected in urine after high-dose infusion [4], and was also considered to play an important role in the renal toxicity, because it is less soluble in urine than the parent drug [5].

Vigorous hydration and alkalinization of the urine have reduced the incidence of clinical nephrotoxicity to 15% of the treatment courses in 39% of patients [8, 10] but have not eliminated the problem altogether. To facilitate the use of H-D-MTX in clinical trials, we analysed MTX and 7-OH-MTX excretion in urine, and estimated the theoretically required urinary flow during and after H-D-MTX infusion.

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Material and methods

Twelve patients, seven males and five females, (8 with acute leukemia, 2 with osteosarcoma, 1 with rhabdomyosarcoma, and 1 with lymphoma) with ages ranging from 2 to 8 years (mean age 7.1 years) were studied.

Methotrexate was administered as a 6-h IV infusion in 500 ml of balanced salt solution (Na: 35 mEq/l, K: 20 mEq/l, Cl: 35 mEq/l, lactose: 20 mEq/l) at dosages ranging from 0.7 to 8.4 g/m² body surface area. Citrovorum factor was started 3 h after the completion of the infusion at a dosage of 15 mg/m² IV every 3 h for a total of nine doses, after which the same dosage was given IV every 6 h for the next 48 h. Balanced salt solution as above was administered IV alone at the rate of 100 ml/h/m², beginning 12 h prior to MTX infusion. Intravenous sodium bicarbonate (33 mEq/l) in the same balanced salt solution was started immediately after the MTX infusion and continued for 72 h. Acetazolamide (Diamox) (250 mg for patients older than 5 years and 125 mg for patients under 5 years) was given PO 12 h before the MTX infusion and continued every 12 h for 3 days.

Fractional urines for 0–6, 6–12, 12–24, 24–48, and 48–72 h were collected following 52 nontoxic H-D-MTX infusions. Urine volumes were carefully measured.

The urinary concentrations of MTX and 7-OH-MTX were analysed by high-pressure liquid chromatography [3], using the Hitachi HPLC system (Hitachi 633-A, Japan) with a Spherisorb C18 5μODS (4.6 mm × 200 mm) column (Custom LC Inc., Texas, USA), and a changeable ultraviolet detector (Hitachi 6335-0900, Japan) was also connected to the system. We used a digital integrator (Hewlett Packard 3390A, USA) to estimate the area under the peaks. A 50-μl aliquot of diluted urine (1 : 1 to 1 : 10,000 v/v with distilled water) was injected into the liquid chromatograph; the mobile phase consisted of a 30/70 (by vol) mixture of methanol and 5 mmol/l 1-hexane-sulfonic acid pH 3.75; the flow rate was 1 ml/min. Identification and measurement of MTX and 7-OH-MTX were based on retention times and the area under the peak, and were compared with authentic MTX and 7-OH-MTX standard solutions.

The cumulative urinary excretion of MTX or 7-OH-MTX ($CUE_{MTX/or\ 7\text{-}OH\text{-}MTX}$) was expressed as the percentage of administered MTX and calculated by the standard formula. The urinary excretion rate of MTX/or 7-OH-MTX ($UER_{MTX/or}$

$_{7\text{-OH-MTX}}$) was expressed in mol/min/m^2 and determined by the formula:

$$\mathrm{UER}_{\mathrm{MTX/or\ 7\text{-}OH\text{-}MTX}} = \frac{UV \cdot C_{\mathrm{MTX/or\ 7\text{-}OH\text{-}MTX}}}{t \cdot BSA} \quad (1)$$

where UV is the volume of urine collected over the time interval, $C_{\mathrm{MTX/or\ 7\text{-}OH\text{-}MTX}}$ is the concentration of MTX/or 7-OH-MTX, t is the duration of the collection period, and BSA is the body surface area (m^2) of the patient. The theoretically required urinary flow ($\mathrm{TRUF}_{\mathrm{pH\ 5.0/or\ pH\ 6.0/or\ pH\ 7.0}}$) is the

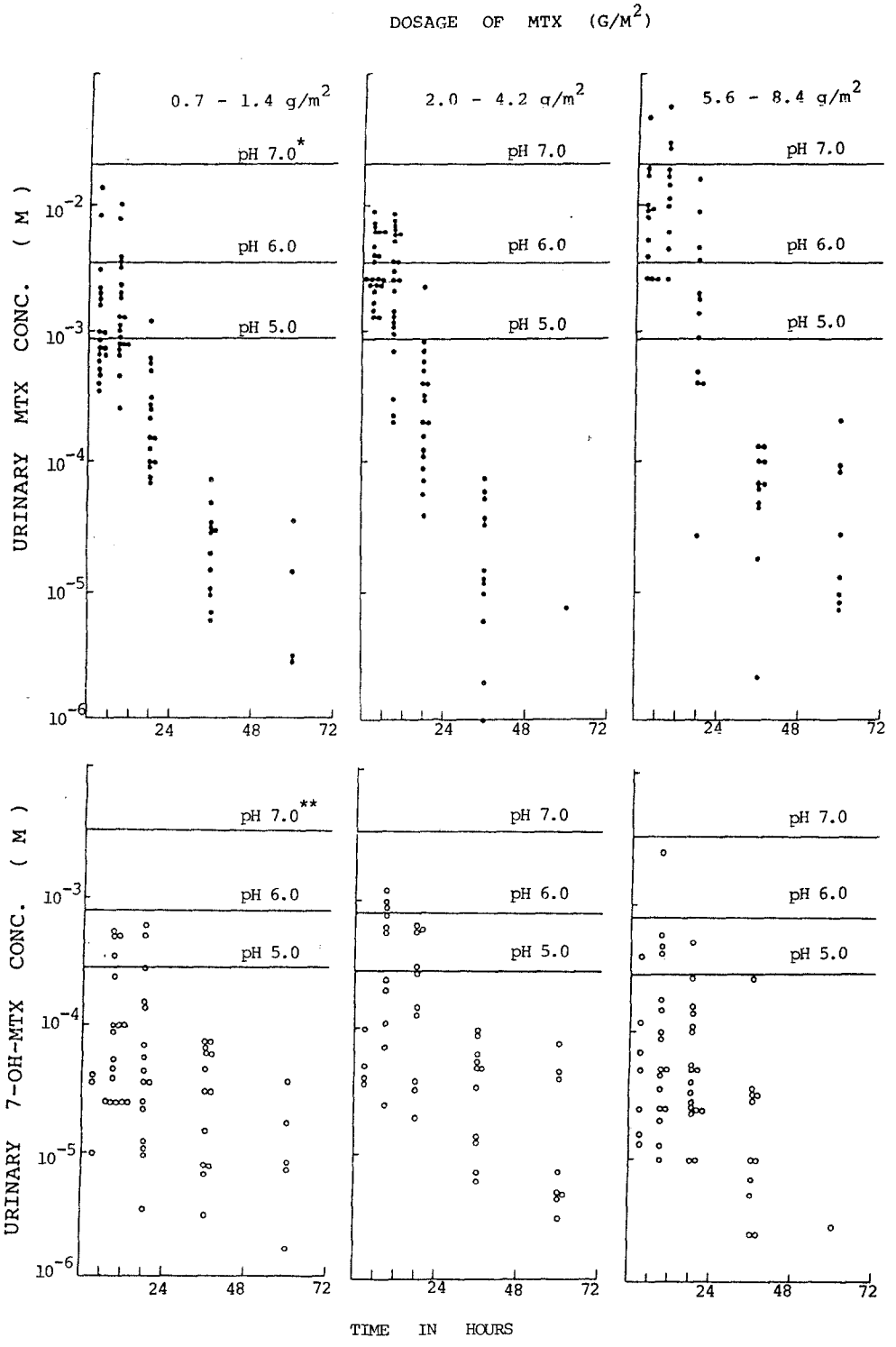

Fig. 1. Urinary MTX (●) and 7-OH-MTX (○) concentrations with high-dose MTX infusion. ★ Shows the aqueous solubilities of MTX at pH 7.0, pH 6.0, and pH 5.0 (range $1.99 \times 10^{-2} M$ at pH 7.0, 3.41×10^{-3} M at pH 6.0, and 8.59×10^{-4} M at pH 5.0). ★★ Shows the aqueous solubilities of 7-OH-MTX at pH 7.0, pH 6.0, and pH 5.0 (range 3.30×10^{-3} M at pH 7.0, 7.88×10^{-4} M at pH 6.0, and 2.77×10^{-5} M at pH 5.0). The time is measured from the start of MTX infusion

minimum urine volume required to saturate both MTX and 7-OH-MTX and is calculated by the formula:

$$TRUF_{pH\ 5.0/or\ pH\ 6.0/or\ pH\ 7.0} = \frac{UER_{MTX}}{S_{MTX\ pH\ 5.0/or\ pH\ 6.0/or\ pH\ 7.0}} + \frac{UER_{7\text{-}OH\text{-}MTX}}{S_{7\text{-}OH\text{-}MTX\ pH\ 5.0/or\ pH\ 6.0/or\ pH\ 7.0}} \quad (2)$$

where $UER_{MTX/or\ 7\text{-}OH\text{-}MTX}$ is the urinary excretion rate of MTX/or 7-OH-MTX determined before [formula (1)]; $S_{MTX\ pH\ 5.0/or\ pH\ 6.0/or\ pH\ 7.0}$ is the aqueous solubility of MTX at pH 5.0, pH 6.0, or pH 7.0; and $S_{7\text{-}OH\text{-}MTX,\ pH\ 5.0/or\ pH\ 6.0/or\ pH\ 7.0}$ is the aqueous solubility of 7-OH-MTX at pH 5.0, pH 6.0, or pH 7.0, respectively. The aqueous solubility values used for MTX and 7-OH-MTX in this formula were MTX: 8.59×10^{-7} mol/ml (pH 5.0), 3.41×10^{-6} mol/ml (pH 6.0), 1.99×10^{-5} mol/ml (pH 7.0); and 7-OH-MTX: 2.77×10^{-8} mol/ml (pH 5.0), 7.88×10^{-7} mol/ml (pH 6.0), 3.30×10^{-6} mol/ml (pH 7.0) [1].

Results

The common logarithms of the concentrations of MTX and 7-OH-MTX in urine were plotted as a function of the actual time of fractional urine collection for three dosage groups (0.7–1.4 g/m^2, 2.0–4.2 g/m^2, and 5.6–8.4 g/m^2) (Fig. 1). The peak concentration of MTX in urine was observed in the 0–6 h or 6–12 h urine, and the mean values were 1.52×10^{-3} *M* for 0.7–1.4 g/m^2, 3.03×10^{-3} *M* for 2.0–4.2 g/m^2, and 1.08×10^{-2} *M* for 5.6–8.4 g/m^2. The peak concentration of 7-OH-MTX was observed in the urine collected in the first 6 h or the next 12 h after infusion, and these mean values were 1.37×10^{-4} *M* for 0.7–1.4 g/m^2, 1.83×10^{-4} *M* for 2.0–4.2 g/m^2, and 2.83×10^{-4} *M* for 5.6–8.4 g/m^2.

Figure 2 shows the cumulative MTX and 7-OH-MTX excretions in urine from the start of infusion, indicating 75%–98% as MTX and 3%–15% as 7-OH-MTX by 72 h.

Figure 3 shows the renal excretion rates of MTX and 7-OH-MTX. The maximum renal excretion rate of MTX was observed during MTX infusion of the dose from 0.7 to 4.2 g/m^2 and its value was proportional to the dosage (1.6×10^{-6} mol/min/m^2 to 6.5×10^{-6} mol/min/m^2). However, for dosages between 5.6 and 8.4 g/m^2, the maximum renal excretion rate fell in the first 6 h after infusion and its value ranged from 1.9×10^{-5} mol/min/m^2 to 3.4×10^{-5} mol/min/m^2. The maximum excretion rate of 7-OH-MTX was observed in the first 6 h after infusion and ranged from 5.6×10^{-7} mol/min/m^2 to 1.2×10^{-6} mol/min/m^2.

Figure 4 shows the TRUF for variable pH at 5.0, 6.0, and 7.0. The graphs were drawn from the linear regression analyses. During MTX infusion, the values of TRUF correlated well with the logarithmic value of administered MTX and matched the equation;

$$Y = a + b \ln X \quad (3)$$

where Y is TRUF and X is the dosage of MTX (g/m^2). The value of a is 2.26 and that of b is 9.05 at pH 5.0 ($r^2 = 0.65$); $a = 0.58$ and $b = 2.30$ at pH 6.0 ($r^2 = 0.66$); and $a = 0.1$ and $b = 0.39$ at pH 7.0 ($r^2 = 0.65$) During the first 6 h and the next 12 h after infusion TRUF correlated linearly with the dosage of MTX, and matched the equation;

$$Y = a + b X \quad (4)$$

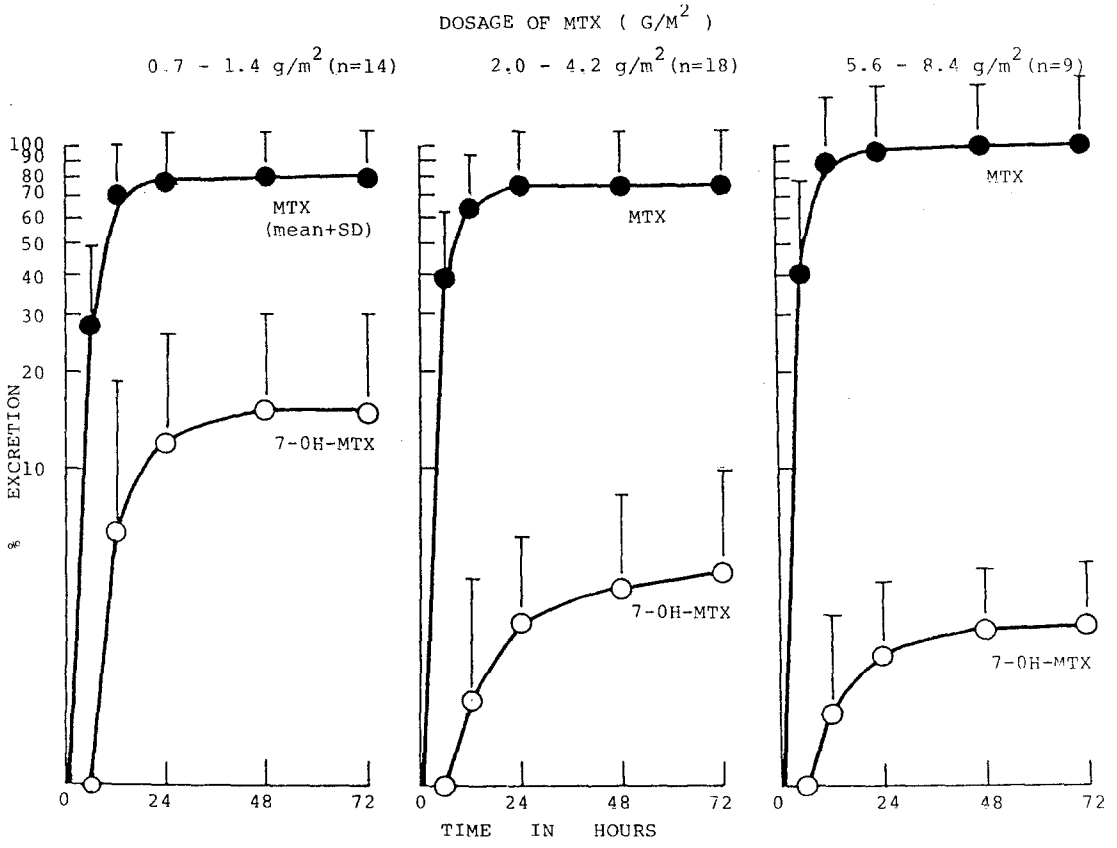

Fig. 2. Cumulative urinary excretion of MTX (●) and 7-OH-MTX (○) (% of administered MTX) with high-dose MTX infusion. The data are shown as mean + SD. Time is counted from the start of MTX infusion

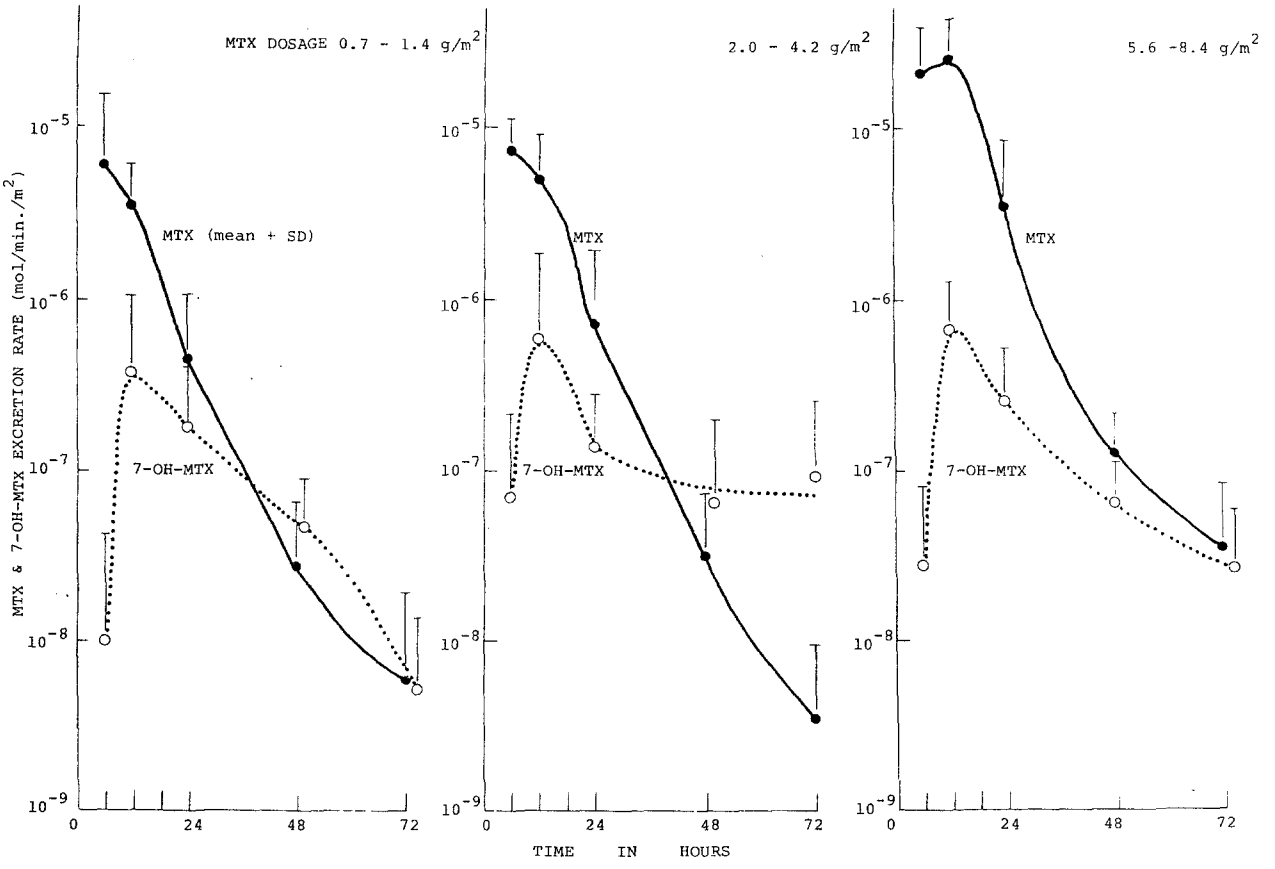

Fig. 3. Urinary MTX (●) and 7-OH-MTX (○) excretion rate (mol/min/m^2) with high-dose MTX infusion. The data are shown as means ± SD. The time is counted from the start of MTX infusion

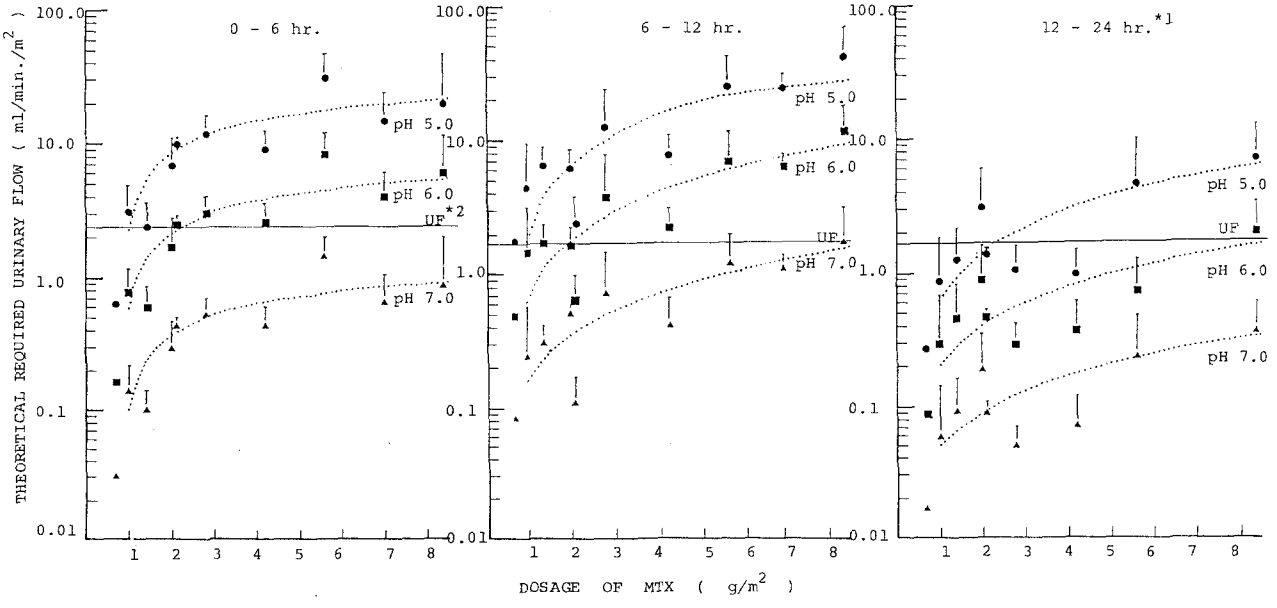

Fig. 4. Theoretically required urinary flow (ml/min/m^2) during and after high-dose MTX infusion. Hour^{*1} is the period of urine collection after the start of MTX infusion. Theoretically required urinary flows at pH 5.0 (●), pH 6.0 (■), and pH 7.0 (▲) are shown as the mean values ± 1 SD. The graph was drawn from the linear regression: $Y = a + b \ln X$ at 0–6 h, and $Y = a + b X$ at 6–12 h and 12–24 h. The values of 'a' and 'b' and 'r^2' are reported in the *Results*. UF^{*2} is the actual mean urinary flow (ml/min/m^2) observed with hydration (100 ml/h/m^2) and alkalinization (see text)

where X and Y are the same as in Eq. (3). For the first 6 h after infusion, the values of a and b are -2.81, 4.60, respectively, at pH 5.0; -0.56 and 1.19 at pH 6.0; and -0.03 and 0.19 at pH 7.0. From 12 to 24 h after the start of infusion, the values of a and b are -0.18 and 0.8 at pH 5.0; 0.005 and 0.20 at pH 6.0; and 0.01 and 0.04 at pH 7.0 ($r^2 = 0.69-0.88$). After 24 h from the start of MTX infusion TRUF was not correlated with MTX dose and its value was less than 0.5 ml/min/m^2 at any pH value.

The mean urinary flows with our hydration and alkalinization schedule were 2.31 ml/min/m^2 during the infusion, 1.78 ml/min/m^2 at 6–12 h, and 1.65 ml/min/m^2 at 12–24 h.

Discussion

MTX was mainly excreted during the infusion hour with MTX dosages up to 4.2 g/m^2, and the dose-dependent peak concentration of MTX in urine and the maximum excretion rate were observed in this period for these dosages. However, the peak concentration of MTX and the maximum excretion rate of MTX fell in the first 6 h after MTX infusion with dosages over 5.6 g/m^2. In children, the limit of urinary MTX excretion rate was considered to be 2.0×10^{-5} mol/min/m^2, and MTX not filtered during the infusion was excreted in the urine during the next 6 h. The peak urinary concentration and maximum excretion rate of 7-OH-MTX were observed in the first 6 h after infusion.

We theoretically estimated the minimum urinary flow for the complete resolution of MTX and 7-OH-MTX in urine at variable pH conditions as the theoretically required urinary flow (TRUF). The values of TRUF were dependent mainly on the pH of the urine, and decreased to 1/25 with alkalinization of urine from pH 5.0 to pH 7.0. Accurate alkalinization of urine ($\geqq$ pH 7.0) is considered to be essential for H-D-MTX at dosages over 2.0 g/m^2. With alkalinization ($\geqq$ pH 7.0), the values of TRUF (pH 7.0) during the 0–6 h, 6–12 h, and 12–24 h periods were well correlated with the dosage of MTX. The maximum TRUF (pH 7.0) was required during the first 6 h after MTX infusion. In this period, TRUF (pH 7.0) with massive MTX infusion ($\geqq$ 8.4 g/m^2) was larger than the urinary flow actually observed (1.78 ml/min/m^2) with conventional schedules of hydration (100 ml/h/m^2). More vigorous hydration is necessary for massive MTX infusion in this period. The values of TRUF (pH 7.0) during the 0–6 h and 12–24 h periods were less than 1 ml/min/m^2, and the conventional schedules of hydration and urinary alkalinization are considered to be sufficient for these periods, even with massive dosages of MTX.

The values of TRUF are very important to enable adequate hydration and alkalinization to be provided with H-D-MTX infusions. Further, the comparison of actual urine flow with these TRUFs will give early warning of delayed MTX clearance from the kidney. With these precautions, the risks of nephrotoxicity will be minimized.

References

1. Donehower RC (1980) Metabolic conversion of methotrexate in man. Recent Results Cancer Res 74: 37
2. Fox RM (1979) Methotrexate nephrotoxicity. Clin Exp Pharmacol Physiol 5: 43
3. Howell SK, Wang YM, Hosoya R, Sutow WW (1980) Plasma methotrexate as determined by liquid chromatography, enzyme inhibition assay after high-dose infusion. Clin Chem 26: 734
4. Jacobs SA, Stoller RG, Chabner BA, Johns DG (1976) 7-Hydroxymethotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high dose methotrexate. J Clin Invest 57: 534
5. Jacobs SA, Stoller RG, Chabner BA, Johns DG (1977) Dose-dependent metabolism of methotrexate in man and rhesus monkeys. Cancer Treat Rep 61: 651
6. Jaffe N, Traggis D (1975) Toxicity of high-dose methotrexate (NSC-740) and citrovorum factor (NSC-3590) in osteogenic sarcoma. Cancer Chemother Rep 6: 31
7. Liegler DG, Henderson ES, Hahn MA, Oliverio VT (1969) The effect of organic acids on renal clearance of methotrexate in man. Clin Pharmacol Therap 10: 849
8. Pitman SW, Frei III E (1977) Weekly methotrexate-calcium leucovorin rescue: effect of alkalinization on nephrotoxicity; pharmacokinetics in the CNS; and use in CNS non-Hodgkin's lymphoma. Cancer Treat Rep 61: 695
9. Pitman SW, Parker LM, Tattersall MHN, Jaffe N, Frei E III (1975) Clinical trial of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590) – toxicologic and therapeutic observations. Cancer Chemother Rep 6: 43
10. Stoller RG, Jacobs SA, Drake JC, Lutz RJ, Chabner BA (1975) Pharmacokinetics of high-dose methotrexate (NSC-740). Cancer Chemother Rep 6: 19

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