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Short communication

Circadian pharmacokinetics of methotrexate

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Summary. Six patients with intermediate- and high-grade non-Hodgkin's lymphoma were treated with 400 mg/m^2 i.v. methotrexate (MTX) at 0600 and 1800 hours. Despite evidence of circadian rhythms in renal function, the pharmacokinetics of total and free serum MTX showed no significant difference between these two times. The marked two-fold circadian variation in MTX pharmacokinetics previously reported in rats was not observed in these patients.

Introduction

Circadian rhythms in pharmacokinetics and in tissue susceptibility of anticancer agents are currently under study to identify times of administration that might result in optimal efficacy and minimal toxicity. Methotrexate (MTX) is a widely used anticancer agent, which after initial distribution disappears from the plasma in two phases. The first phase has a half-life of 2–3 h and mainly reflects renal clearance whereas the second has a half-life of approximately 10 h [1, 14].

In rats, different times of administration have resulted in a nearly two-fold variation in MTX clearance and variations in toxicity, all abolished by the administration of exogenous corticosteroids [2, 11]. Circadian variations in the toxicity of MTX have also been demonstrated in mice [10].

In humans, circadian rhythms in urine volume and pH, creatinine (CRN) clearance and plasma protein binding have been demonstrated [5, 8, 12, 13], each of which could affect MTX pharmacokinetics. Clinically significant circadian variations in the pharmacokinetics and toxicity of cisplatin have previously been demonstrated in association with changes in urinary excretion [7, 8]. The aim of this study was to establish whether or not patients demonstrate a diurnal variation in MTX pharmacokinetics.

Patients and methods

Six patients with intermediate- or high-grade non-Hodgkin's lymphoma who were responding to treatment were studied during two consecutive courses of chemotherapy. Cyclophosphamide (750 mg/m^2), doxorubicin (50 mg/m^2) and vincristine (1.4 mg/m^2) were given orally on day 1 of the 21-day treatment cycle, with prednisone (40 mg/m^2) given orally on days 1–5 (CHOP). On day 15, 400 mg/m^2 MTX (Lederle) was given by constant-rate i.v. infusion over 30 min, commencing at 0600 hours in one course and at 1800 hours in the other, in random order in each patient. I.v. hydration was standardised and folinic acid rescue was begun 24 h after MTX. Patients had to have a performance status of 0–1 (WHO scale) and were ineligible if they had an underlying endocrine disorder, were taking corticosteroids or other medication, had renal impairment (CRN clearance of <0.6 ml/s per m^2) or a "third space" such as ascites. However, one patient had mild hypertension that was treated with metoprolol, the dose of which was constant during the study.

Determinations of plasma CRN and albumin concentrations and urinary CRN clearance were carried out in the 24 h immediately preceding the administration of MTX and repeated every 6 h for 24 h. The isotopic ^{99m}Tc-DTPA glomerular filtration rate (GFR) was determined before MTX administration. Serum MTX samples were taken immediately before MTX, then at 0, 5, 10, 15, 20, 30, 45 and 60 min and at 1.25, 1.5, 2, 3, 4.5, 6, 9, 12 and 24 h after the end of the infusion. Total and free serum (unbound) MTX concentrations were measured by fluorescence polarisation immunoassay (Abbott TDx), each sample being assayed in duplicate (SD = 0.73 at 20 μmol/l; CV, 3.59%). Protein-free ultrafiltrates were prepared by centrifugation (2,000 *g* for 20 min at 37° C) using Amicon YMT membranes (Amicon Corporation; Danvers, Mass). Clearances of total and free MTX over the first 12 h were calculated from the AUCs using the log-trapezoidal rule.

Results

The characteristics of the patients are shown in Table 1, together with CRN clearance and MTX pharmacokinetic data. Renal function immediately before MTX administration was the same for the two courses in each patient. When morning and evening administration times were compared, no significant difference was found in the clearance of either free or total MTX. There was also no significant difference in MTX protein binding determined over the first 6 h following MTX. The protein binding of MTX did not correlate with plasma albumin concentration

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Table 1. Patient characteristics, renal function and MTX pharmacokinetics

Patient	Sex	Age (years)	Dose (mg)	Time MTX	DTPA[a] GFR pre-MTX	CRN clearance[a]		MTX clearance[a] (0–12 h)		Protein binding (%)
						pre-MTX	post-MTX	total	free	
1	M	56	680	a.m.	0.86	0.94	0.89	94	170	44.5
				p.m.t	0.63	0.91	0.66	78	150	50.2
2	M	56	650	a.m.t	0.71	0.63	0.95	90	162	42.5
				p.m.	0.74	0.67	0.74	94	156	41.6
3	M	59	750	a.m.t	0.61	0.64	0.70	134	270	49.5
				p.m.	0.68	0.61	0.67	179	317	46.8
4	M	38	750	a.m.	1.14	1.19	1.14	156	255	45.2
				p.m.t	0.81	1.25	1.40	167	295	44.3
5	M	58	740	a.m.t	0.91	0.82	0.78	93	165	43.0
				p.m.	0.65	0.74	0.74	96	175	45.1
6	F	65	640	a.m.	0.71	0.94	0.89	97	172	44.7
				p.m.t	0.85	0.80	0.84	102	261	49.3
Mean ± SEM				a.m.				111 ± 11	199 ± 20	44.9 ± 1.0
				p.m.				119 ± 17	226 ± 30	46.2 ± 1.3
P value for paired difference (Student's *t*-test):								0.33	0.23	0.38

[a] ml/min per m^2
t, treatment course given first

($r = 0.1236$). No toxicity attributable to MTX was seen after its administration at either time. Circadian variations were demonstrated in urine volume, CRN clearance and plasma albumin concentration in the four consecutive 6-h periods following MTX (Fig. 1). Values for all three parameters were greatest in the evening.

Discussion

Despite evidence of circadian rhythms in renal function, there were no clinically important variations in the pharmacokinetics of MTX. The values for total MTX clearance and protein binding fell within the ranges reported by oth-

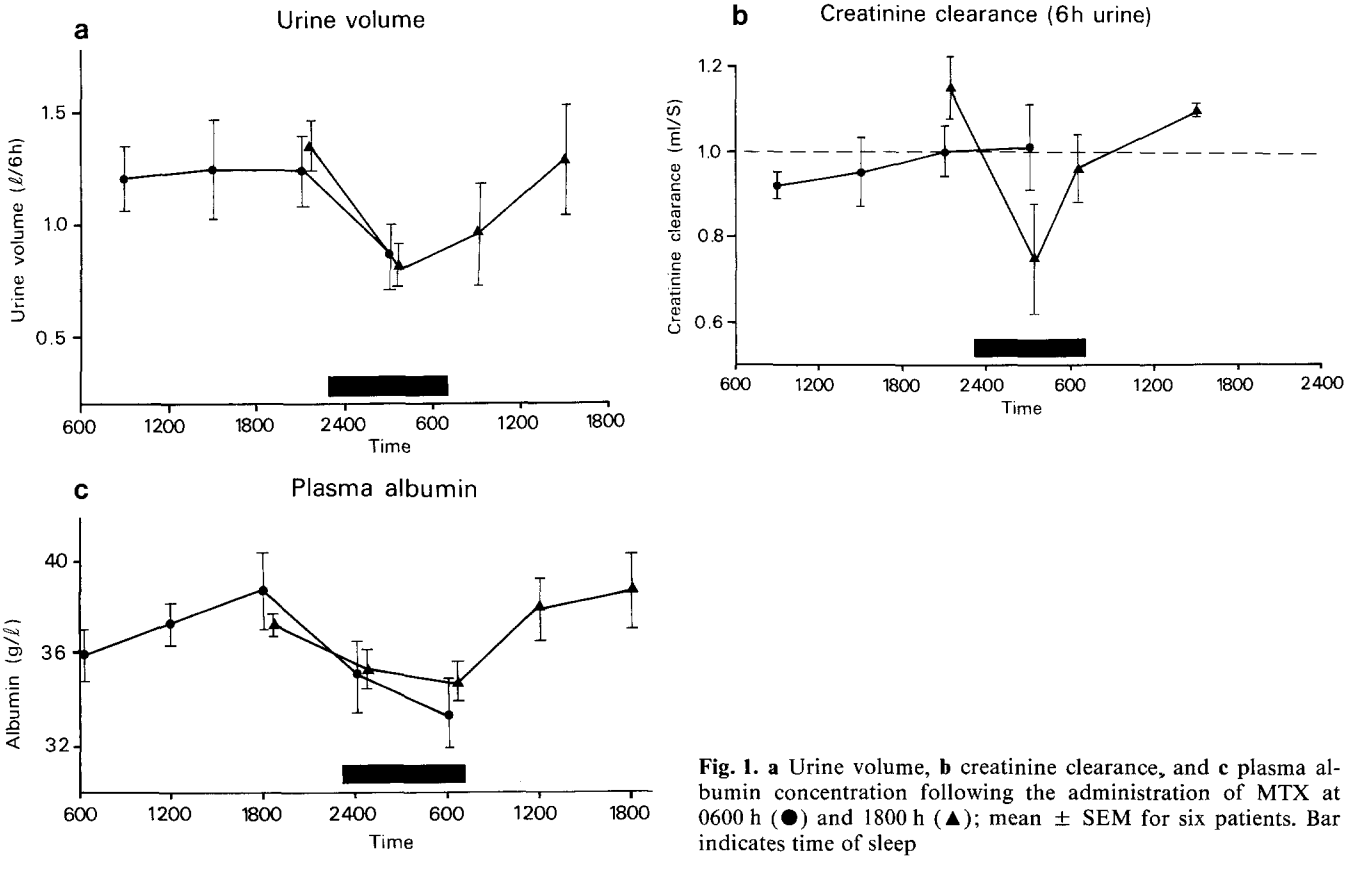

Fig. 1. a Urine volume, **b** creatinine clearance, and **c** plasma albumin concentration following the administration of MTX at 0600 h (●) and 1800 h (▲); mean ± SEM for six patients. Bar indicates time of sleep

ers [4, 9, 14, 16]. With an alpha level of 0.05, a power of 80%, and with each patient being their own control, this study could have demonstrated a 20% difference in total MTX Cl_{0-12}, a 37% difference in free MTX Cl_{0-12} and a 7% difference in MTX protein binding. Any true differences in the population values for these indices are likely to be smaller than these values and, therefore, unlikely to be great enough to alter decisions as to the time of administration of MTX.

Although various body processes peak at a similar stage in the day-night cycle, the exact timing varies by several hours in individuals. This might obscure a circadian time variation when externally fixed time points (e.g. 0600 hours) are used [5, 6, 13]. It may not be appropriate to extrapolate times of administration from rodent studies [2, 3, 11], in which MTX clearance was lowest after administration at sleep onset. In humans this might be nearer 2200 hours than 1800 hours. However, this time would not be clinically acceptable and the frequent blood tests in the first few hours after MTX administration might upset the circadian rhythms under study. Ideally, a larger number of administration times should be studied in each patient, but this was not acceptable to our patients.

By causing high endogenous corticosteroid concentrations, stress might have abolished circadian variations in MTX pharmacokinetics, as has been reported in rats [3]. However, circadian differences in cisplatin pharmacokinetics were detected in patients with ovarian cancer, who would be similarly stressed [7, 8]. In most cancer patients a 5-day course of 40 mg/m^2 prednisone suppresses adrenal function; however, in only a minority does it remain suppressed after 7 days [15], and after 14 days no suppression of adrenal function is evident [17], making it unlikely that prednisone in CHOP would have abolished the circadian rhythms of MTX.

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References

1. Chabner BA, Donehower RC, Schilsky RL (1981) Clinical pharmacology of methotrexate. Cancer Treat Rep 65 [Suppl 1]: 51–54
2. English J, Aherne W, Marks V (1982) The effect of timing of a single injection on the toxicity of methotrexate in the rat. Cancer Chemother Pharmacol 9: 114–117
3. English J, Aherne GW, Arendt J, Marks V (1987) The effect of abolition of the endogenous corticosteroid rhythm on the circadian variation in methotrexate toxicity in the rat. Cancer Chemother Pharmacol 19: 287–290
4. Evans WE, Hutson PR, Stewart CF, Cairnes DA, Bowman WP, Rivera G, Crom WR (1983) Methotrexate cerebrospinal fluid and serum concentrations after intermediate-dose methotrexate infusion. Clin Pharmacol Ther 33: 301–307
5. Halberg F (1983) Quo vadis basic and clinical chronobiology: promise for health maintenance. Am J Anat 168: 543–594
6. Hrushesky WJM (1983) The clinical application of chronobiology to oncology. Am J Anat 168: 519–542
7. Hrushesky WJM (1985) Circadian timing of cancer chemotherapy. Science 228: 73–75
8. Hrushesky WJM, Borch R, Levi F (1982) Circadian time dependence of cisplatin urinary kinetics. Clin Pharmacol Ther 32: 330–339
9. Jones RB, Collins JM, Myers CE, Brooks AE, Hubbard SM, Balow JE, Brennan MF, Dedrick RL, DeVita VT (1981) High-volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res 41: 55–59
10. Labat C, Mansour K, Malmary M-F, Terrissol M, Oustrin J (1987) Chronotoxicity of methotrexate in mice after intraperitoneal administration. Chronobiologia 14: 267–275
11. Marks V, English J, Aherne W, Arendt J (1985) Chronopharmacology. Clin Biochem 18: 154–157
12. Pasternack A, Kuhlback B (1971) Diurnal variations of serum and urine creatine and creatinine. Scand J Clin Lab Invest 27: 1–7
13. Reinberg A, Smolensky MH (1982) Circadian changes of drug disposition in man. Clin Pharmacokinet 7: 401–420
14. Shen DD, Azarnoff DL (1978) Clinical pharmacokinetics of methotrexate. Clin Pharmacokinet 3: 1–13
15. Spiegel RJ, Vigersky RA, Oliff AI, Echelberger CK, Bruton J, Poplack DG (1979) Adrenal suppression after short term corticosteroid therapy. Lancet I: 630–633
16. Wan SH, Huffman DH, Azarnoff DL, Stephens R, Hoogstraten B (1974) Effect of route of administration and effusions on methotrexate pharmacokinetics. Cancer Res 34: 3587–3591
17. Zamkoff K, Kirshner J, Cass D, Miller M (1981) Adrenal response to serial cosyntropin stimulation after repeated high-dose prednisone administration in patients with lymphoma. Cancer Treat Rep 65: 563–566

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