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The effect of abolition of the endogenous corticosteroid rhythm on the circadian variation in methotrexate toxicity in the rat

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Summary. Monitoring of indices of haematological, renal and hepatic toxicity in rats after a single i.v. bolus of methotrexate has shown that they vary with the time of day at which the drug is administered. Maximum toxicity occurs after administration at 0600 h. Further experimentation has shown that the amount of corticosteroid present in the blood has a profound effect on the toxicity of methotrexate in the rat. If the endogenous production of corticosterone is suppressed by treatment with dexamethasone the toxicity of methotrexate is markedly increased at whatever clock time it is administered. However, if constantly high plasma levels are achieved by giving supplementary corticosterone methotrexate toxicity is diminished regardless of what time it is given.

Since the timing of maximum methotrexate toxicity corresponds to the circadian nadir of endogenous plasma corticosterone concentration in the rat the possibility that it might be related to corticosterone production must be considered. Whether this phenomenon occurs in man and has any clinical relevance has yet to be investigated.

Introduction

Substantial evidence exists of a circadian rhythm in the toxicity of a number of cytotoxic agents. The overall death rate in mice has been shown to vary with the time of administration of single drugs, such as cytosine arabinoside [9], adriamycin [11] and 5-fluorouracil [2], or of drug combinations, such as those of cytosine arabinoside, cyclophosphamide, vincristine and methylprednisolone [3]. More specific parameters of toxicity also vary in rats; for example, the time of administration affects the gastrointestinal toxicity of 5-fluorouracil [7] and the nephrotoxicity of *cis*-diamminedichloroplatinum [10]. In both these cases it was suggested that variations in toxicity were dependent upon circadian changes in target organ susceptibility.

Several cellular mechanisms are influenced by circadian changes in adrenal steroid production: glomerular filtration of potassium in the squirrel monkey, for instance [12], and epidermal cell proliferation in man [17]. Disease activity in rheumatoid arthritis also exhibits a circadian rhythm corresponding to that of cortisol secretion, the high concentration of plasma cortisol in the morning being postulated to produce a decrease in the immune processes responsible for the symptoms of the disease [8]. The activities of several hepatic drug-metabolising enzymes are influenced by corticosterone in the male rat; the normal circadian differences in metabolism of ethylmorphine and aniline, for example, are abolished by adrenalectomy, and those in the oxidative metabolism of aminopyrine, *p*-nitroanisole, and hexobarbital by either adrenalectomy or corticosterone administration [14].

We have previously reported a circadian rhythm in the toxicity of methotrexate in the rat, with maximum toxicity coinciding with the nadir for plasma corticosterone concentration [5]. In view of these findings we decided to investigate the possibility of altering methotrexate toxicity by abolishing the normal diurnal corticosterone rhythm.

Materials and methods

All experiments were performed on male Norwegian hooded rats weighing 200–250 g, maintained on a light:dark regimen of 0630 h–1830 h light:1830 h–0630 h dark, and fed powdered diet ad libitum (Spratts Laboratory Animal Diet No. 1). Three groups of animals were used. The first were untreated and served as controls. In the second, adrenocortical rhythm was suppressed by adding dexamethasone to the diet so that each rat received approximately 25 μg/kg body weight per day for 10 days prior to the administration of methotrexate. In the third group, constantly high plasma corticosterone concentrations were maintained by adding corticosterone to the diet at a dose level of 250 μg/kg body weight per day for a similar period.

Experiments were performed on groups of six rats. The methotrexate was injected at 0600 h, 1200 h, 1800 h or 2400 h. Each animal was used in one experiment only.

After induction of anaesthesia with Sagatal (0.1 mg/100 g body weight, i.p.), methotrexate (Lederle) dissolved in 0.9% saline made slightly alkaline (pH 7.5–8.0) with $NaHCO_3$ was injected into a surgically exposed femoral vein at a dose of 100 mg/kg body weight. Blood samples (0.2 ml) were collected from the tail vein before and at daily intervals for 10 days after the methotrexate injection. The blood samples were separated and the serum was stored at −20 °C until assayed.

Total white cell counts were made by cytometry. Serum urea was measured by Berthelot's reaction after cleavage with urease [6], serum asparate transferase (Asp T) by a co-

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lorimetric method [15] and serum corticosterone concentration by radioimmunoassay.

The statistical significance of all differences found was tested using Student's *t*-test for paired observations, comparing each time with the zero time value for that parameter.

Results

Effects of the various steroid treatments on plasma corticosterone are shown in Fig. 1. Dexamethasone suppressed corticosterone production by the adrenals, and the resulting low plasma levels showed no circadian variation. In contrast, administration of exogenous corticosterone resulted in high plasma levels throughout the day.

Parameters for haematological, renal and hepatic damage followed the same pattern in all three experimental groups. The effects on myelosuppression caused by methotrexate given at different clock times are shown in Fig. 2. Those on plasma urea and Asp T levels are given in Table 1.

In untreated control rats the pattern followed that reported previously, with maximum toxicity occurring after methotrexate dosage at 0600 h. In rats in which corticosteroid production was suppressed by dexamethasone, methotrexate toxicity was so great that all of them died within 5 days irrespective of what time the methotrexate was given. In rats given corticosterone, whose plasma concentration was consequently high throughout the day, on the other hand, methotrexate was comparatively innocuous.

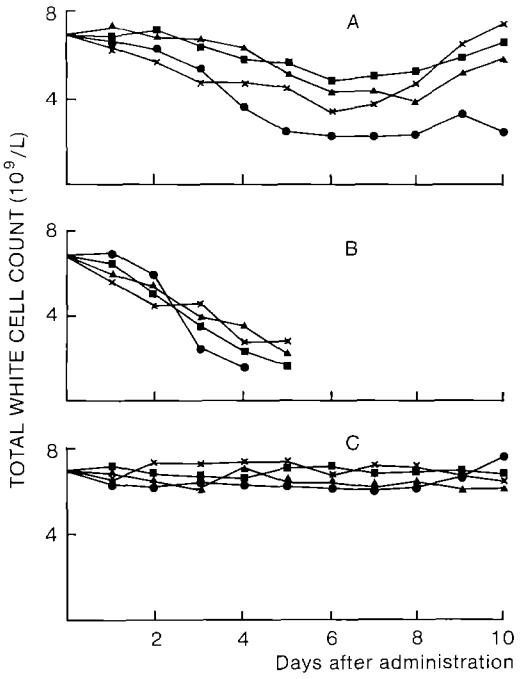

Fig. 2 A–C. Total white cell counts after methotrexate injection (100 mg/kg body weight i.v.) in normal rats **(A)** and rats treated with dexamethasone **(B)** or corticosterone **(C)**. The methotrexate was given at 0600 h (●———●), 1200 h (■———■), 1800 h (▲———▲) or 2400 h (×———×). Each *point* represents the mean of six animals

Discussion

Our results indicate that in the rat the toxicity of methotrexate is affected, directly or indirectly, by the circulating level of corticosterone. The decrease in toxicity observed when corticosterone levels were maintained at a high plasma concentration by oral administration reinforce the possibility of a link between the low toxicity of methotrexate administered at 1200 h, 1800 h and 2400 h and the high endogenous corticosterone production at these times. It is tempting to link the increased methotrexate toxicity when administered to rats which had been pretreated with dexamethasone, and were therefore low in endogenous corticosterone, with the high toxicity observed when methotrexate was administered at 0600 h, the circadian nadir in plasma corticosterone levels. However, dexamethasone is itself a corticosteroid, which would have three to four times greater glucocorticoid activity than that associated with corticosterone at the dose level used, although the mineralocorticoid activity would be lower. Dexamethasone, at a higher dose level, causes hepatotoxicity characterised by elevated plasma Asp T and Ala T levels in rats [13], and it is possible that some of the toxic symptoms seen in our rats were caused by co-toxicity between methotrexate and dexamethasone.

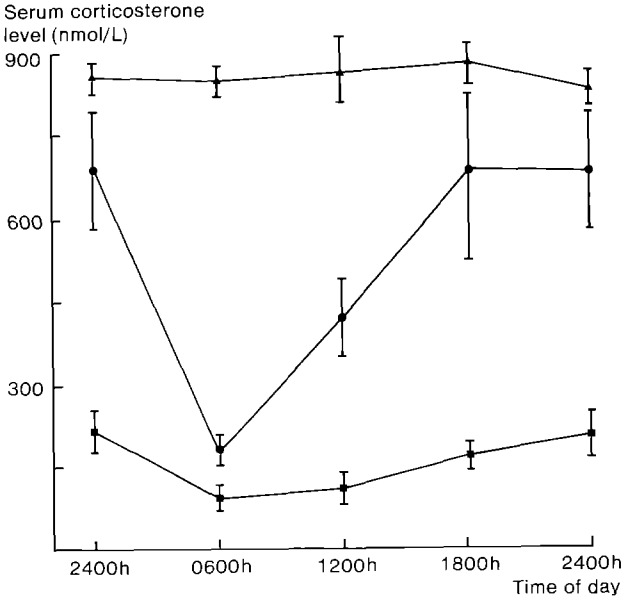

Fig. 1. Serum corticosterone levels in normal rats (●———●) and in rats treated with either dexamethasone (25 μg/kg body weight per day) (■———■) or corticosterone (250 μkg/kg body weight per day) (▲———▲), added to the diet for 10 days prior to measurement. Each *point* represents the mean ± SEM of six animals

Previous work from this laboratory [5] has indicated that variation in methotrexate toxicity is secondary to circadian changes in its rate of removal from the circulation, but whether or not corticosteroids influence methotrexate pharmacokinetics remains to be investigated.

Methotrexate is excreted largely unchanged in the bile and urine. There is a circadian variation in glomerular filtration rate [4], which might influence methotrexate excretion by this route, but the control mechanism, and any link it may have with circulating corticosteroid concentration, have yet to be clarified.

Table 1. Effects of timing of methotrexate administration and steroid pretreatment upon maximum and 10-day post-treatment levels of plasma urea and serum aspartate transferase activity in rats

Steroid treatment group	Time of administration of methotrexate			
	0600	1200	1800	2400
Plasma urea (mmol/l)				
No treatment				
Pre-experimental	7.0±0.7	6.7±0.1	7.1±0.3	7.1±0.4
Highest	12.2±0.2**	9.7±0.3**	9.1±0.6*	8.9±0.2*
10 days post dose	12.2±0.2**	6.5±0.2	9.1±0.6*	7.8±0.4
Dexamethasone suppressed				
Pre-experimental	7.3±0.2	7.3±0.3	7.5±0.2	7.5±0.3
Highest	18.6±3.0**	16.1±2.4**	16.0±0.7**	15.9±0.6**
10 days post dose	N-A	N-A	N-A	N-A
Corticosterone treated				
Pre-experimental	7.1±0.1	6.8±0.2	7.1±0.1	7.3±0.2
Highest	7.2±0.2	7.4±0.2	7.6±0.2	7.5±0.1
10 days post dose	7.0±0.1	6.7±0.1	8.2±0.2*	8.0±0.1
Serum Asp T (SF units)				
No treatment				
Pre-experimental	56±2	52± 3	50± 5	45± 3
Highest	420±7**	170±20**	126±50**	108±18**
10 days post dose	251±4**	81±18*	61± 9	68± 8**
Dexamethasone suppressed				
Pre-experimental	52±1	55±2	60±5	52±3
Highest	294±37**	426±190**	573±39**	310±66**
10 days post dose	N-A	N-A	N-A	N-A
Corticosterone treated				
Pre-experimental	58±3	54±3	52±3	46±2
Highest	77±3*	82±5*	80±5*	72±5*
10 days post dose	48±3	56±2	56±3	53±3

Values given are means ± SEM for groups of six animals
* = $P < 0.01$; ** = $P < 0.001$; N-A = not available (all animals dead)

In vitro work with L1210 murine leukaemia cells has indicated that both cortisol and methylprednisolone can inhibit uptake of methotrexate by cells [19]. If this is a general effect of corticosteroids on membrane transport and also occurs in vivo, it could explain the apparent protection afforded by corticosterone against methotrexate toxicity. An indirect effect on plasma clearance rates might also result since reduction in cellular uptake would leave a greater proportion of the injected methotrexate available for renal or biliary excretion.

Some corticosteroids have been reported to inhibit the (killing) efficacy of methotrexate in sensitive cell lines [1] whilst others do not. The results are particularly difficult to interpret, however, since prednisolone had no effect, whilst prednisone, which is generally considered to be biologically inactive until converted into prednisolone in the liver, was just as inhibitory as cortisol.

Prednisolone reputedly improves the therapeutic index of several alkylating agents in non-steroid-responsive cancerous cell lines [18] but the mechanism has not been elucidated. It seems possible, therefore, that the toxicity of methotrexate, when used therapeutically, could be reduced either by appropriate timing of its administration or by concomitant corticosteroid therapy. A recent report [16] suggests that children with acute lymphoblastic leukaemia had better survival rates when maintenance chemotherapy consisting of daily 6-mercaptopurine and weekly methotrexate was given in the evening rather than in the morning. The drugs were therefore more efficient when administered at a time when plasma cortisol levels were low; by analogy with our rat work this should result in high toxicity. The result of concomitant corticosteroid administration with evening timing of the cytotoxic drugs would therefore be of interest. It would, however, be important to assess the effect of pretreatment with corticosterone on the (killing) efficiency of methotrexate in a methotrexate-sensitive tumour cell line.

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