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Repeated high-dose cyclophosphamide administration in bone marrow transplantation: exposure to activated metabolites

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Summary. Blood levels of cyclophosphamide (CP) and activated metabolites were measured in 11 patients undergoing a 2- to 4-day conditioning chemotherapy for bone marrow transplantation. Urinary excretion of CP was determined in five patients. CP half-life decreased after pretreatment from an average of 7.1 h on the 1st day to 5.5 h on the 2nd day ($P < 0.005$) and to 4.3 h on the 4th day ($P < 0.005$). No characteristic changes in urinary excretion could be observed. At the same time the exposure to non-protein-bound activated metabolites increased from 10.5 to 19.5 and 26.0 nmol × h/ml respectively ($P < 0.005$ and $P < 0.04$). Thus, in contrast to in vitro and animal studies, no evidence for an inhibition of activating enzymes could be found. On the contrary, pretreatment seems to enhance the production of the cytotoxic metabolites. The possible explanation of these changes by enzyme induction and by the role of saturated protein binding sites is discussed. Exposure to active metabolites might be altered by dose splitting or even by a change in the duration of the infusion.

Introduction

Cyclophosphamide (CP) is one of the most widely used antineoplastic agents. Its antineoplastic activities are mediated by metabolites produced by activation in hepatic microsomes by mixed function oxidases [5]. The accepted metabolic schema is shown in Fig. 1. The primary metabolite, 4-hydroxycyclophosphamide (4-HOCP), and the tautomeric aldophosphamide, which have also been called "activated metabolites" [12, 21, 25], degrade spontaneously to alkylating and cytotoxic products. Tardiff and Dubois [19] observed an inhibition of hepatic microsomal enzymes after CP. Gurtoo et al. [9] also measured a decrease to 50% in the specific activities of several microsomal mixed function oxygenases and in the contents of cytochromes P-450 and b_5. This inhibition may be caused by interaction between the metabolite acrolein and critical sulfhydryl groups in enzymes [13, 14].

Several authors observed a significant decrease in CP half-life after repeated doses [2, 6, 8, 16]. It has been suggested that these changes are due to induction of CP metabolism [16], impairment of renal reabsorption of CP due to damage by prior dose or reduction of binding sites to plasma proteins following each dose [8].

In bone marrow transplantation high-dose CP (followed by total body irradiation) is used as a cytotoxic drug to eradicate leukemic and host cells and as an immunosuppressive agent to facilitate engraftment of donor cells. CP is usually administered at a dose of 60 mg/kg body weight (BW) on 2 consecutive days i.v. in leukemic patients or 50 mg/kg BW on each of 4 consecutive days in transplantation for severe aplastic anemia [18, 20].

In this study we measured blood levels of CP and "activated metabolites" and urinary CP excretion in patients undergoing bone marrow transplantation to elucidate the pharmacokinetics of high-dose CP in repeated doses.

Methods

Eleven patients with severe aplastic anemia or leukemia were transplanted according to a modified Seattle protocol. All patients had normal renal and hepatic function measured by creatinine, creatinine clearance, blood urea nitrogen, bilirubin, cholinesterase, coagulation profile and albumin. In patients with aplastic anemia, CP was given in a dose of 50 mg/kg BW i.v. over 60 min on days −5, −4, −3 and −2 prior to bone marrow infusion. In patients with leukemia, CP was given in a dose of 60 mg/kg at day −7 and −6 followed by fractionated total body irradiation from day −4 to day −1 in a daily dose of 400 rad.

Mesna (12 mg/kg) was administered prior to CP and 0, 3, 6, 9, 12, 15 and 18 h after CP. Hydration with 3000 ml/m^2/day 5% dextrose in 0.5 *N* saline was used.

CP levels were measured by N/P flame ionization gas chromatography after extraction with dichloromethane and derivatization with heptafluorobutyric acid [7].

Activated CP metabolites were determined by liberation of acrolein and its fluorometric determination according to Alarcon et al. [1]. Acrolein has been shown to originate from 4-HOCP and aldophosphamide only; inactive keto and carboxy derivatives are not measured with this method [23, 26, 27].

A quantity of 1.0 ml blood was directly added to 1.0 ml ice-cold 10% TCA (w/v) and centrifuged (2000 *g*) for 5 min. Then 0.6 ml of a solution containing 2.5 mg/ml 3-aminophenol and 3 mg/ml hydroxy-ammonium-chloride dissolved in 1.0 *N* HCl was added to 0.7 ml of the supernatant and the resulting mixture heated for 20 min at 95° C in a thermoblock. The solution was cleared through

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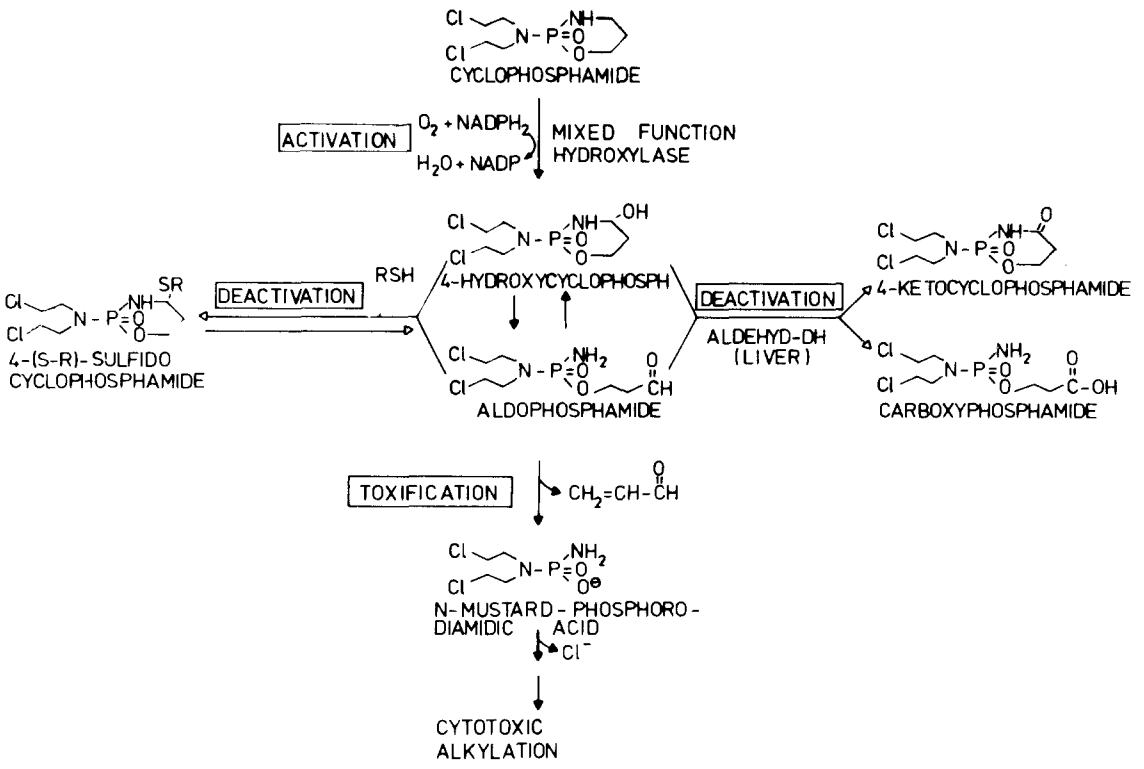

Fig. 1. Metabolic schema for cyclophosphamide

a Millipore 0.22-μm filter and measured at room temperature in an Aminco-Bowman ratio spectrophotofluorometer at an excitation wavelength of 350 nm and an emission wavelength of 515 nm. A standard solution of 7-hydroxyquinoline was derived from 10.01 nmol hydroxyperoxycyclophosphamide as described above and added to supernatant of a blank. The concentration (c) was calculated according to the following equation:

$$c = (S - B)/(100 - RB) \times 28.6 \text{ nmol}$$

where S represents relative intensity of sample, B that of blank and RB that of reagent blank.

Blood samples were taken before and 20, 40, 60, 80 and 100 min and 2, 3, 4, 6, 8, 10 and 24 h after beginning the CP infusion on each of the treatment days. In five patients an aliquot of each voided urine was sampled and frozen until CP determination.

All available data were fitted simultaneously using TOPFIT [10, 11].

Results

Patients' demographic data, diagnoses and interval since prior treatment are listed in Table 1. Blood concentrations of CP and activated metabolites representative of patient 11 are shown in Fig. 2 and 3. The increase in exposure to activated metabolites is most obvious at the end of the infusion and levels of after about 8 h. A decline in half-life of CP and higher peaks of activated CP on consecutive days could be observed in all other patients except one. Peak concentrations and the calculated pharmacokinetic parameters are listed in Table 2. No characteristic change in urinary excretion of CP in subsequent cycles could be observed (Table 3).

Table 1. Demographic data, diagnosis and interval since prior treatment

No.	Age	Sex	Diagnosis	Interval (months)
1	17	M	AML	2
2	15	F	CML, chronic phase	6-TG until BMT
3	22	F	SAA	–
4	16	M	SAA	–
5	5	M	CML, second chronic phase	2
6	16	M	CML, chronic phase	2
7	7	M	ALL, second CR	1
8	21	M	ALL, third CR	1
9	8	F	AMoL, second CR	1
10	23	F	SAA	–
11	19	M	SAA	–

AML, acute myelocytic leukemia; AMoL, acute monocytic leukemia; CML, chronic myelocytic leukemia; SAA, severe aplastic anemia; BMT, bone marrow transplantation; 6-TG, 6-thioguanine; CR, complete remission; –, no prior treatment

The parameters are based on an average of 10.8 data points for CP (10.5 for metabolites) per day and patient. The differences in CP half-life between the 1st and the 2nd day were highly significant (*t*-test for paired samples; $t = 5.07$, $P < 0.0005$). The corresponding values for the 1st and 4th days were $t = 4.87$, $P < 0.005$. The differences of peak concentrations of CP were not significant.

The area under curve (AUC) values for the active metabolites were calculated for the data points from 0 to 8 h.

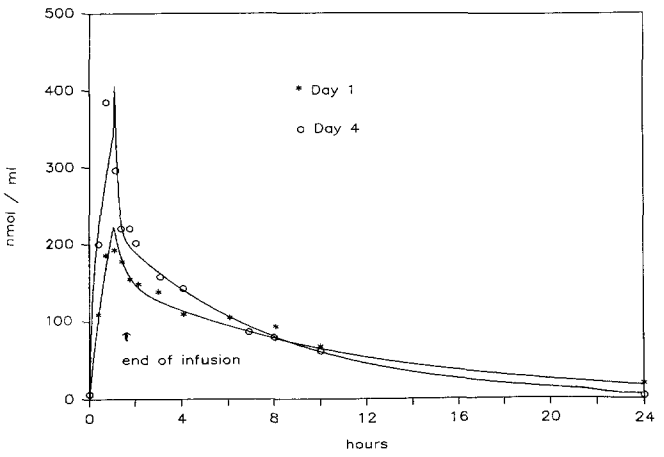

Fig. 2. Bloodlevels of cyclophosphamide after repeated high doses in patient no. 11 (* day 1; o day 4)

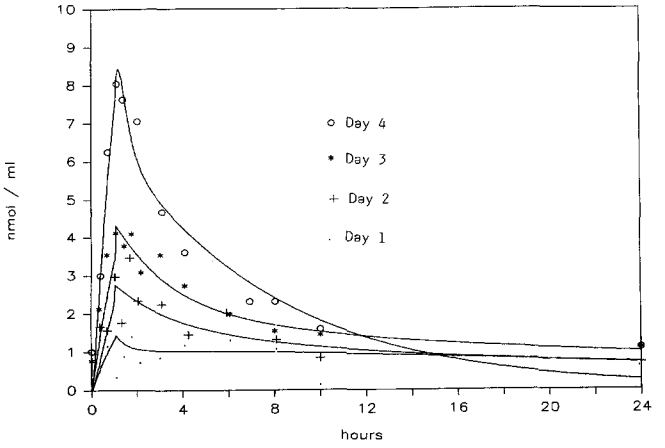

Fig. 3. Bloodlevels of activated cyclophosphamide after repeated high doses in patient no. 11 (* day 1; + day 2; * day 3; o day 4)

Table 2. Pharmacokinetic parameters

Patient no.	Cyclophosphamide						Activated metabolites					
	Peak concentration (nmol/ml)			Half-life (h)			Peak concentration (nmol/ml)			AUC (nmol · h/ml)		
	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
1	370.0	360.1	–	5.7	4.0	–	3.0	4.4	–	22.7	21.5	–
2	247.1	207.5	–	7.2	10.3	–	2.9	4.9	–	15.0	16.2	–
5	323.1	250.7	–	6.9	3.4	–	2.1	3.8	–	6.5	12.5	–
6	246.4	154.0	–	5.2	4.0	–	1.1	2.7	–	11.4	19.5	–
7	272.2	333.9	–	5.4	4.7	–	0.8	7.5	–	4.8	28.5	–
8	442.4	201.5	–	6.5	4.0	–	1.0	2.5	–	4.5	9.1	–
9	596.0	845.1	–	5.7	2.4	–	2.8	7.0	–	17.6	34.9	–
3	230.3	151.0	196.7	10.9	11.5	4.3	1.5	2.9	3.4	8.2	13.3	18.0
4	186.6	236.0	215.9	8.6	4.9	3.1	1.1	2.5	9.6	5.8	11.5	26.9
10	255.8	509.2	203.3	9.1	5.0	5.0	–	–	–	–	–	–
11	190.0	438.4	385.0	7.3	6.6	4.9	1.4	3.5	8.1	8.9	15.4	33.3
Mean	305.5	335.2	250.2	7.1	5.5	4.3	1.7	4.2	7.0	10.5	18.2	26.1

Table 3. Urinary excretion of cyclophosphamide (% of administered dose)

Patient no.	Day 1	Day 2	Day 3	Day 4
7	17.5	6.7	–	–
8	16.2	9.0	–	–
9	23.0	18.6	–	–
10	6.7	9.2	11.9	18.5
11	5.6	5.2	6.8	3.0
Mean	13.8	9.7	9.4	10.8

The increases from day 1 to day 2 ($t = 3.31$, $P < 0.005$) and from day 1 to day 4 respectively ($t = 4.19$, $P < 0.04$) were significant.

Discussion

Our results regarding the decrease of CP half-life in blood after repeated high doses are in accordance with several other publications. This phenomenon was first reported in 1973 by Bagley et al. [2]. Sladek et al. [16] demonstrated a decreased half-life after daily administration of 50–60 mg/kg BW for 2 (4) days from 3.4 h to 2.1 h (4.4 h to 2.11 h). Graham et al. [8] observed a decrease from 5.1 h to 2.6 h after a daily dose of 50 mg/kg BW over 4 days. Their suggested explanation, that these changes might be accounted for by an impaired renal reabsorption, is, however, unlikely to be true because of the observation of a decrease of urinary excretion of CP in four of the five patients presented in our study. Similar results have been obtained by others [2, 16]. At a lower dose level of 2 mg/kg BW the duration of treatment seems to play an important role. While Mouridsen et al. [15] measured no change over a period of 22 days, D'Incalci et al. [6] showed a decrease from 7.3 h to 4.9 h after a treatment period of 6 months. It has been suggested that CP rapidly induces enzymes and therefore enhances its own metabolism. However, several animal studies have shown an inhibition of microsomal enzymes [9, 13, 14, 19]. Berrigan et al. [3] attributed this to the interaction between acrolein and sulfhydryl groups in enzymes, an interaction which could be prevented by sulfhydryl-containing drugs like *n*-acetylcysteine and mesna. The relevance of these observations for clinical treatment regimens has never been determined.

In our study the activation of CP was obviously not inhibited by prior administration of the drug, as assessed by an increase of the AUC of activated metabolites. A study taking a similar appproach has been published recently by Sladek et al. [17]. They did not observe any significant change of AUC values of plasma 4-HOCP in eight patients without mesna, although in every single patient higher peak concentrations were found after the second infusion. Because of the short half-life of 4-HOCP, blood levels as presented here cannot be compared with plasma levels measured after a time-consuming centrifugation step.

The influence of mesna in regard to enzyme protection could not be investigated for ethical reasons, since a control group without mesna would run an increased risk of hemorrhagic cystitis. Berrigan et al. [3] showed that both aryl hydrocarbon hydroxylase and demethylase activity could be protected by mesna from depression caused by CP in the rat. This is therefore probably also the case in man.

Enzyme induction by CP could explain the decreasing half-life of the parent compound. In that case one would expect higher blood levels of activated metabolites and a decrease in urinary excretion. Patient 7 seems to show this pattern (Table 3): shorter half-life of CP on the 2nd day combined with a increasing AUC of 4-HOCP (and reduced renal excretion). One would expect some sort of negative correlation between the degrees of changes of the two variables in individual patients; however, we saw only a weak correlation between the following quotients:

$$\frac{T\,1/2\ (\text{day } n)}{T\,1/2\ (\text{day } n+1)} \quad \text{compared with} \quad \frac{AUC\ (\text{day } n)}{AUC\ (\text{day } n+1)} \qquad r = -0.23$$

In our opinion two additional mechanisms may play an important role: first an accumulation of 4-HOCP and aldophosphamide due to an inhibition of their degradation enzymes [4] and secondly a saturation of protein binding sites. Voelcker et al. [22] could still demonstrate protein-bound fraction of 4-HOCP up to 48 h after CP administration, at a time when the free fraction was below the detection limit. Thus the number of reacting protein thiols might well be reduced when the second dose is given within this time.

Voelcker et al. [24] recently demonstrated in an animal model that 4-HOCP showed more therapeutic efficacy when present in blood at relatively low levels for longer times compared to a sharp peak level with the same AUC. Therefore different protocols with the same total CP dose in different schedules, e.g., 120 mg/kg BW on day 1 or 60 mg/kg BW on days 1 and 2, or even a change in the duration of the infusion, might cause different exposition to cytotoxic metabolites and in clinical terms might influence the response rate.

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