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Pharmacokinetics and metabolism of cyclophosphamide in paediatric patients*

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Summary. The pharmacokinetics and metabolism of cyclophosphamide were studied in nine paediatric patients. Plasma samples were obtained from eight subjects and urine was collected from six children during a 24-h period after drug administration. Cyclophosphamide and its major metabolites phosphoramide mustard (PM), carboxyphosphamide (CX), dechloroethylcyclophosphamide (DCCP) and 4-ketocyclophosphamide (KETO) were determined in plasma and urine using high-performance thin-layer chromatography-photographic densitometry (HPTLC-PD). Cyclophosphamide (CP) was nearly, if not completely, cleared from plasma by 24 h after its administration. The plasma half-life of CP ranged from 2.15 to 8.15 h; it decreased following higher doses and was shorter than that previously reported for adult patients. Both the apparent volume of distribution (0.49 ± 1.4 l/kg) and the total body clearance (2.14 ± 1.4 l m^{-2} h^{-1}) increased with increasing dose. Renal clearance ranged between 0.12 and 0.58 l/h (mean, 0.43 ± 0.19 l/h). Between 5.4% and 86.1% of the total delivered dose was recovered as unchanged drug in the urine. The major metabolites identified in plasma and urine were PM and CX. One patient appeared to be deficient in CX formation. This study suggests that there is interpatient variability in the pharmacokinetics and metabolism of CP in paediatric patients. The shorter half-life and higher clearance as compared with adult values indicate faster CP metabolism in children.

Introduction

The oxazaphosphorine cyclophosphamide (CP), first synthesized in 1958, is an alkylating agent that is widely used in both adults and children with malignancies [1, 6]. CP is a prodrug that is activated primarily in the liver (Fig. 1). It is first converted to 4-hydroxycyclophosphamide by a cytochrome P-450 monooxygenase enzyme [2]. Tautomerisation of this metabolite yields the ring-opened aldophosphamide, which acts as the branching point for either detoxication to carboxyphosphamide (CX) or further activation to the cytotoxic metabolite phosphoramide mustard (PM). The former reaction is catalysed by a cytosolic aldehyde dehydrogenase (ALDH1) [14]. The latter is a spontaneous degradation reaction that also releases an equimolar quantity of acrolein, which is thought to be responsible for the urotoxicity of CP [16].

Few detailed investigations of the metabolism of CP have been carried out in the past because of the lack of a sensitive assay for the various metabolites. A preliminary study in adults demonstrated that there are interindividual differences in the balance of production of PM, which is the active alkylating agent, and CX, which is the product of detoxication; some individuals, termed "low carboxylators", were reported to excrete 0.3% or less of the total delivered dose as CX [9], suggesting a phenotypic deficiency in CX formation that may indicate a genetic polymorphism of the ALDH1 enzyme responsible for the conversion of aldophosphamide to CX. This would be of considerable clinical importance since variations in CP metabolism may influence both its toxicity and its efficacy.

There are few reports in the literature regarding the pharmacokinetics and metabolism of CP in children. Sladek et al. [16] described the plasma half-life ($t_{1/2}$) and urinary excretion of CP after its intravenous infusion in 13 children with malignancies, and Juma et al. [13] reported on a pharmacokinetic study of CP following its bolus administration to 8 Kenyan children with lymphoma; however, the metabolites of CP were not measured in either of these studies.

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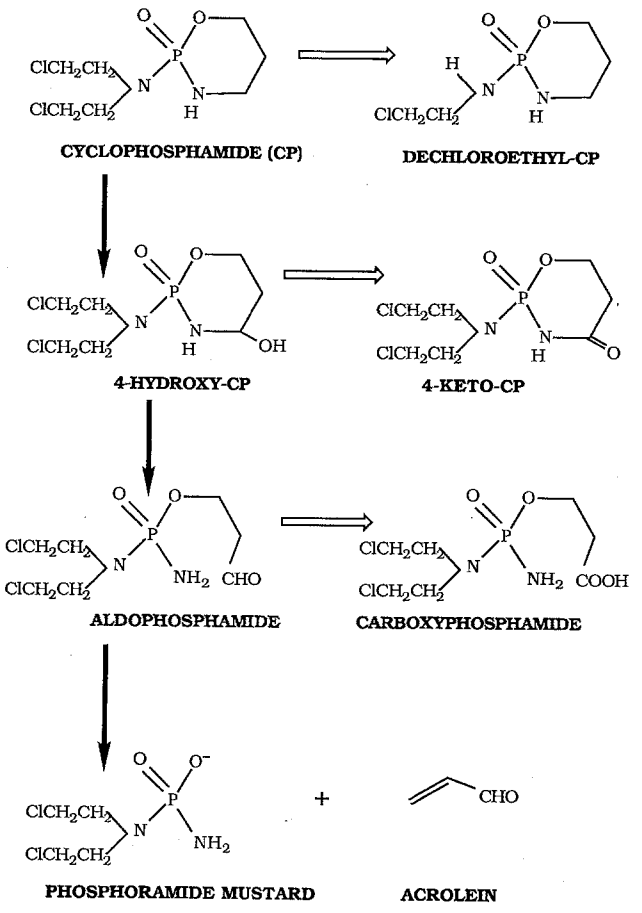

Fig. 1. Metabolism of CP

We studied the pharmacokinetics and metabolism of CP in children using high-performance thin-layer chromatography-photographic densitometry (HPTLC-PD). This method, which enables the identification and quantification of the metabolites in urine [10], was modified for the analysis of plasma samples.

Patients and methods

Nine children aged from 0.7 to 16.8 years (median, 4.3 years), including four boys, were given CP as therapy for their underlying diseases (Table 1). At the time of treatment, renal and hepatic functions were normal as shown by standard clinical tests.

CP was given intravenously as a 1-h infusion at a dose ranging from 125 to 1500 mg/m^2 (median dose, 1057 mg/m^2), except for one child (patient 3), who received 500 mg/m^2 in a short infusion given every 12 h daily. The latter patient was studied during the last two doses of a course of six. Two other subjects (patients 4 and 9) had received CP at the doses shown in Table 1 on the day immediately preceding the study. Prior to the infusion and at approximately 0.5, 1, 2, 4, 6, 12, 18 and 24 h after drug administration, blood samples were collected in tubes coated with ethylenediaminetetraacetic acid (EDTA) and immediately centrifuged to obtain plasma, which was stored at –20° C prior to analysis for CP and its metabolites. Urine was collected over 6-h periods for 24 h after the start of the infusion. The urine volume was measured and an aliquot of each urine sample was stored at –20° C prior to analysis. Blood samples were obtained from eight of the children through a central venous line separate from that through which the drug was infused. Urine was obtained only from the six children aged more than 3 years because of the difficulty of collecting urine from the younger patients.

CP was obtained from Sigma (Poole, UK) and the metabolites were kindly supplied by Asta Werke (Bielefeld, FRG). All other reagents were of appropriate analytical grade. Determinations of CP, PM, CX, dechloroethylcyclophosphamide (DCCP) and 4-ketocyclophosphamide (KETO) were carried out using HPTLC-PD.

Urine samples were loaded onto XAD-2 Spe-Ed solid-phase extraction cartridges (500 mg/3 ml; Laboratory Impex Ltd., Teddington, UK). The cartridges were washed with water and eluted with methanol. Plasma samples were added to an equivalent amount of cold acetonitrile for the removal of proteins. Following centrifugation, the supernatants were transferred into Eppendorfs. The eluates and supernatants were then evaporated to dryness and reconstituted in a small volume of methanol. Reconstituted samples were applied to a 100- × 200-mm HPTLC silica plate (Merck, Darmstadt, Germany) using a Linomat (Camag, Muttenz, Switzerland). The plates were run in a mixture of either butanol and water (20 : 3 v/v) or chloroform, ethanol and glacial acetic acid (20 : 5 : 0.1, by vol.) to a height of 90–95 mm from the lower edge. When the latter mobile phase was applied, a second chromatographic step was performed to elute PM from the point of application, running the plates in a mixture of dichloromethane, methanol and glacial acetic acid (18 : 12 : 0.1, by vol.) to a height of 20 mm from the lower edge. The plates were then sprayed twice with a solution of 15% *p*-nitrobenzylpyridine in acetone and acetate buffer (pH4; 80 : 20, v/v) and then heated in an oven at 150° C for 10 min. After cooling, the plates were dipped in a solution of potassium hydroxide and immediately photographed using a Polaroid MP4 Land camera. The negative was used to produce a print of the same size as the original plate.

The print was scanned with a Camag photodensitometer at 500 nm using a tungsten lamp. The scans were then analysed using the CATS3 software package (Camag, Muttenz, Switzerland), each plate containing tracks from samples and tracks from standards for the construction of a calibration line. The recovery of CP and metabolites from plasma varied

Table 1. Patients' characteristics

Patient number	Sex	Age (years)	Diagnosis	CP dose (mg m^{-2} day^{-1})	Other drugs
1	M	16.8	Relapsed MB	1500	VP16, Vinc, M
2	M	15.4	OS	600	Bleo, ActD, M
3	F	11.0	T-cell lymphoma	1000	Vinc, Doxo, Mtx, M
4	F	10.0	BMT[a]	125	Cot, Acy, Ran M
5	F	4.3	NB	1057	Vinc, VP16, M
6	M	3.7	NB	1050	Vinc, VP16, M
7	F	1.4	NB	638	Vinc, VP16, M
8	F	0.8	STS	850	VP16, Imi, M
9	M	0.7	BMT[b]	882	Cef, Gent, Fluc, Rib, Frus, M

MB, Medulloblastoma; OS, osteosarcoma; BMT, bone marrow transplantation; NB, neuroblastoma; STS, soft-tissue sarcoma; Vinc, vincristine; VP16, etoposide; M, mesna; Bleo, bleomycin; Doxo, doxorubicin; ActD, actinomycin D; Mtx, methotrexate; Acy, acyclovir; Cot, cotrimoxazole; Ran, ranitidine; Imi, imipenem; Cef, cefotaxime; Gent, gentamicin; Fluc, fluconazole; Rib, ribavarin; Frus, frusemide

[a] Fanconi's anaemia

[b] Severe combined immunodeficiency

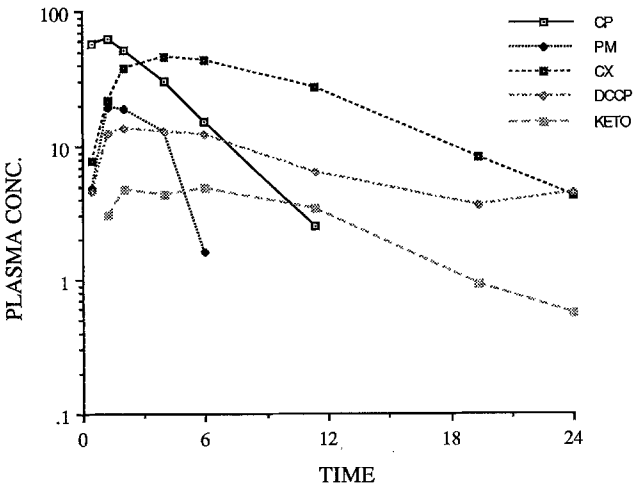

Fig. 2. Plasma-concentration (*conc.*, μg/ml) profiles obtained for CP and its metabolites during a 24-h period after the administration of CP to one subject (patient 1)

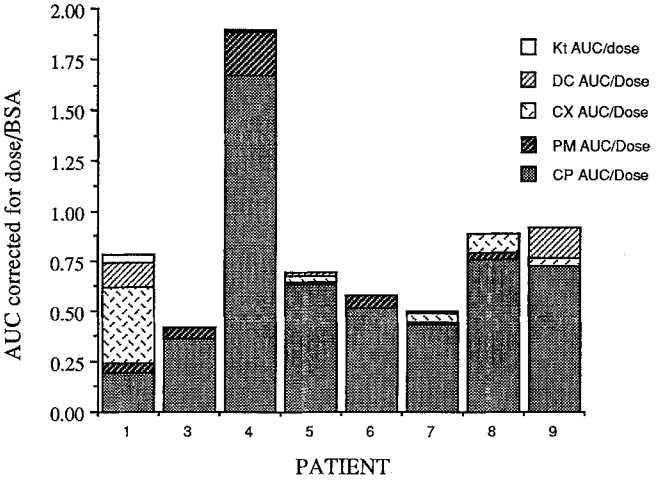

Fig. 3. Histogram of plasma AUC values [corrected for dose, μg/ml h/(mg/m^2)] obtained for CP and its metabolites in 8 paediatric subjects

from 50% (PM) to 90% (CX). The coefficient of variation was less than 10% for single plates and less than 20% between plates. The lower limit of detection in plasma varied from 0.5 μg/ml for the keto metabolite to 2 μg/ml for the parent drug. Details of recovery as well as the precision and sensitivity of the assay in urine have been described elsewhere [10].

Urinary concentrations were used to calculate the total recovery of metabolites expressed as a percentage of the total dose given (corrected for molecular weight). Plasma concentrations were used to calculate areas under the curve (AUCs) and half-lives when possible. For the parent drug, the clearance (C) and the volume of distribution at steady state (V_{ss}) were calculated using conventional methods [7]. Renal clearance (C_R) was calculated for four patients as the product of C and the total fraction of CP excreted unchanged in the urine. Correlations were analysed using simple least-squares regression or Spearman rank correlation.

Table 2. Pharmacokinetic parameters for CP

Patient number	$t_{1/2}$ (h)	V_{ss} (l/kg)	C (l h^{-1} m^{-2})	C_R (l/h)
1	2.15	0.50	5.24	0.48
2	5.16[a]	-	-	-
3	2.41	0.79	2.75	0.57
4	8.15	0.19	0.60	0.41
5	3.09	0.51	1.58	-
6	4.25	0.59	1.93	0.58
7	2.57	0.41	2.32	-
8	4.95	0.40	1.33	-
9	2.91	0.53	1.38	-

[a] Half-life calculated from excretion-rate data

Results and discussion

CP was nearly, if not completely, cleared from plasma by 24 h after its administration. The two children (patients 4 and 9) who had received CP on the day preceding the study exhibited detectable plasma concentrations of the parent drug but not its metabolites prior to drug administration. Determination of the metabolites in plasma was sometimes difficult due to the limitations of the assay in detecting low concentrations (<1 μg/ml). PM and DCCP were detected in eight patients; CX, in 7; and KETO, in only 3. Figure 2 shows the plasma-concentration profiles obtained for CP and its metabolites in patient 1. The AUC values [corrected for dose/body surface area (BSA)] calculated for CP and its metabolites in each patient are shown in Fig. 3.

The mean plasma half-life for CP was 3.96 ± 1.92 h (Table 2). These results agree with those previously reported by other authors. Sladek et al. [16] found a half-life of 2.4–6.5 h in a series of 13 paediatric patients receiving CP at a dose of 24–60 mg/kg, and Juma et al. [13] reported a half-life of 4.1 ± 0.50 h in a series of 8 children with lymphoma. The plasma half-life in children appears to be shorter than that previously reported for adults (5–8.2 h) [4, 11, 12, 15, 18]. In patient 3, who received two infusions of CP 12 h apart, the half-life was calculated for the first and the second infusion, being 2.41 and 2.11 h, respectively. Patients who had undergone treatment with CP immediately prior to the study did not appear to exhibit marked differences in CP pharmacokinetics or metabolism, although the influence of pretreatment on these parameters cannot be ruled out. The plasma half-life could be estimated for PM in three children (patient 1, 1.12 h; patient 3, 3.07 h; and patient 6, 1.63 h), for CX in five (patient 1, 5.21 h; patient 5, 11.18 h; patient 7, 2.39 h; patient 8, 14.44 h; and patient 9, 2.78 h), for DCCP in two (patient 1, 11.18 h; patient 9, 9.24 h) and for KETO in one child (patient 1, 4.8 h).

The apparent volume of distribution (V_{ss}) for CP was 0.49 ± 0.17 l/kg, slightly lower than the values reported by Juma et al. [13] (0.61 ± 0.17 l/kg) and Sladek et al. [17] (0.67 ± 0.09 l/kg) and lower than that reported for adult patients (0.57–0.70 l/kg) [4, 11, 12, 15]. The V_{ss} value was found to increase significantly with increasing dose/BSA (P = 0.025) and was lowest in the only child (patient 4) who received 125 mg/m^2 (V_{ss}, 0.19 l/kg). This relationship was not significant when patient 4 was omitted from the

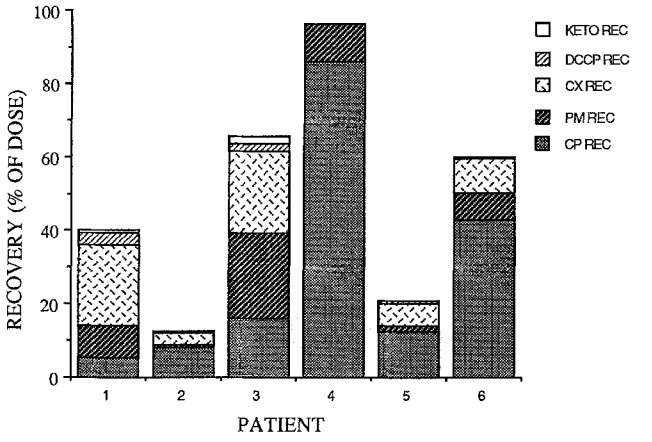

Fig. 4. Histogram of the urinary recovery *(REC)* (as a percentage of the delivered dose) of CP and its metabolites in 6 paediatric subjects (incomplete urine collection in patient 5)

Table 3. Plasma AUC values and urinary recovery expressed as a percentage of the total delivered dose as determined for CP and its metabolites

Patient number		CP	PM	CX	DCCP	KETO	Total % recovery
1	AUC[a]	0.19	0.05	0.38	0.12	0.04	
	% rec.	5.4	8.5	22.3	3.2	0.5	40.0
2[b]	% rec.	8.1	0.7	3.4	0.3	–	12.5
3	AUC	0.36	0.05	–	–	–	
	% rec.	16.1	23.4	21.8	2.1	1.9	65.3
4	AUC	1.67	0.22	–	0.01	–	
	% rec.	86.1	10.5	–	–	–	96.6
5[c]	AUC	0.63	0.01	0.04	0.01	–	
	% rec.	12.5	1.6	6.0	0.7	–	20.8
6	AUC	0.52	0.06	–	–	–	
	% rec.	43.2	7.2	8.9	0.3	–	59.7
7[d]	AUC	0.43	0.01	0.05	0.01	–	
8[d]	AUC	0.76	0.03	0.10	–	–	
9[d]	AUC	0.72	–	0.05	0.15	–	

[a] AUC corrected for dose: $\frac{(\mu g\ ml^{-1}\ h)}{mg/m^2}$. For patients 3, 4 and 9, AUC values were calculated from time zero to 24 h

[b] No plasma

[c] Urine collection incomplete

[d] No urine

% rec., Percentage of drug or metabolite recovered relative to the total CP dose delivered

analysis, but there was nevertheless an apparent trend for this parameter to rise with increasing dose.

The mean total body clearance (C) of CP was $2.14 \pm 1.4\ l\ h^{-1}\ m^{-2}$ and was positively correlated with the dose/BSA ($P = 0.025$). This finding is slightly lower than that reported by Juma et al. [12], but the variability in clearance values was higher in our patients. Total body clearance appears to be greater in children than in adults [12]. Although both V_{ss} and C increased with increasing dose, the net effect was a decrease in half-life as the dose was increased ($P = 0.012$). The changes in pharmacokinetics observed over the dose range studied are consistent with saturation of plasma protein binding at higher doses. This would result in an increase in both the extent of distribution and the unbound fraction of drug in plasma that is available for elimination. However, the protein binding of CP in adults has been reported to be low (13%–20%) and independent of concentration [3, 8, 15].

The recovery of CP and its metabolites in urine was expressed as a percentage of the total dose given (Fig. 4, Table 3). Total recovery of CP and its metabolites in the urine ranged between 12.47% and 96.58% (median value, 49.8%). Low total recovery may reflect incomplete urine collection in children, but recovery rates were less than 50% in the two oldest patients studied, and similarly low and variable rates of recovery have been reported in adults [5, 11]. Between 5.4% and 86.1% of the delivered dose was excreted as unchanged drug in the urine (median value, 14.3%). The rate of excretion was maximal during the first 6 h (>45% of the total amount recovered), except for one child (patient 5), in whom the collection of urine was disrupted by concurrent diarrhoea. The recovery of unchanged CP increased with the half-life ($P = 0.003$), probably because a shorter half-life indicates a higher rate of metabolism and, hence, less of the parent drug is recovered in the urine. We could not find any correlation between recovery of the parent drug and that of the measured metabolites as would be expected as a consequence of an increase in these metabolic pathways. The recovery of unchanged CP tended to decrease with increasing dose, but the correlation was not significant.

PM could be measured in all six patients from whom urine was collected (median recovery, 7.85%; range, 0.7%–23.4%), and CX could be detected in urine samples from five of six children (median recovery, 8.9%; range, 3.4%–22.3%). Patient 4, who showed no CX in plasma or urine, may be considered phenotypically as a "low carboxylator" [9]. DCCP was recovered from five subjects (median recovery, 0.7%; range, 0.3%–3.2%) and KETO, from only two children (0.47% and 1.92%, respectively). CX and DCCP were found in urine samples from three individuals (patients 3, 5 and 6) who showed no detectable amounts of these metabolites in plasma.

Renal clearance (C_R) was calculated in four patients in whom plasma and urine samples were available and urine collection was reliable. This parameter did not vary greatly amongst the four patients in whom it could be reliably determined (mean, 0.5 ± 0.08 l/h) and was greater than that previously reported for adults [11].

The present study demonstrates variability in CP pharmacokinetics and metabolism amongst paediatric patients. A positive correlation was found between the plasma half-life and the recovery of unchanged drug in the urine, and these two parameters correlated negatively with the dose/BSA. These relationships suggest an enhancement of CP metabolism at higher doses, possibly due to saturable plasma protein binding. In our paediatric patients, the half-life was shorter and the total body clearance and renal clearance were greater than the corresponding adult values, suggesting faster CP metabolism in children.

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