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Pharmacokinetics of high-dose cyclophosphamide in patients with metastatic bronchogenic carcinoma

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Summary. *Cyclophosphamide (CP) was administered to eight patients with metastatic bronchial carcinoma in escalating doses of 1.5, 2.5, and 3.5 g/m² at intervals of 3 weeks. The proportion of the administered dose converted into alkylating metabolites was similar for each dose and there was no evidence to suggest that the enzyme system responsible for activating CP was saturated even with the highest dose. Considerable between-patient variation in drug metabolism was observed, but within each patient the fraction metabolised remained constant.*

Introduction

Cyclophosphamide is now used widely in the treatment of disseminated malignancy, particularly oat cell carcinoma of the bronchus [9], and there has been a recent trend towards the use of large doses IV in attemps to improve therapeutic efficacy. In view of this we felt it important to re-examine the pharmacokinetics and to determine the between-patient variation in biotransformation with incremental doses.

Patients, materials and methods

All patients admitted to the study had evidence of metastatic disease. Renal function was judged to be normal if the urea, electrolytes, and creatinine were in the normal range; more detailed assessment was not considered appropriate. Liver function was assessed by routine liver function tests and isotopic scanning. Patients were accepted for the study if the bilirubin was within the normal range; abnormalities of liver enzymes were noted, but more subtle tests were not performed as there is no general agreement on the best available test of liver function which relates the degree of hepatic impairment to impairment of hepatic drug clearance [19, 20], and because there appears to be no consistent pattern between which cytotoxic drugs show a change in elimination and the type of liver disease [10].

All treatment was carried out as in-patients procedures. Cyclophosphamide (CP) was administered in incremental doses of 1.5, 2.5, and 3.5 g/m² by rapid IV infusion over 30 min followed by IV hydration with 3 l/m² of normal saline over 24 h. The doses were repeated at intervals of 3 weeks, blood count permitting.

Serial samples of blood were collected over 0–24 h in sterile tubes and allowed to clot; the serum was then obtained by centrifugation and stored at −20° C prior to assay. Urine was collected in three aliquots 0–6, 6–12, and 12–24 h after administration, the volume recorded, and an aliquot stored for assay.

Cyclophosphamide. CP was determined by a modification of the method of Boughton et al. [1]. To 1 ml serum (or urine diluted × 10) was added an equal volume of standard isophosphamide solution, and the volume was adjusted to 3 ml with phosphate buffer, pH 7. To this was added 10 ml dichloromethane, after which the mixture was shaken for 10 min and centrifuged, and the resultant supernatant decanted and discarded. To the organic phase was added 0.5 g anhydrous $MgSO_4$, and then the mixture was shaken and centrifuged and the supernatant decanted into a second tube. After evaporation to dryness under N_2 at 40° C the residue was dissolved in ethylacetate (0.1 ml) to which trifluoroacetic anhydride (0.05 ml) was then added; the mixture was subsequently heated at 70° C for 20 min. Following further evaporation to dryness under N_2 at 40° C the residue was dissolved in 0.1-ml ethyl acetate and 0.5–1 µl was injected into a Perkin-Elmer chromatograph. The amount of CP was then determined by reference to a calibration curve of peak height ratios vs weight ratios of CP/isophosphamide.

Alkylating metabolites. These were determined using a modification of the methods described by Pantarotto et al. [17] and Field et al. [6]. To 1 ml serum was added an equal volume of 0.6 *M* perchloric acid, and the mixture was then stirred and centrifuged at 2,000 rpm for 10 min. The supernatant was decanted and the precipitate washed twice with 2 ml sodium acetate buffer (0.2 *M*, pH 4.6), centrifuged, and added to the first aliquot. The pH was adjusted to 4.6 with 0.5 *M* NaOH and 0.5 ml 5% solution of 4 (4-nitrobenzyl) pyridine (NBP) in acetone was added. After thorough mixing the tubes were heated at 100° C for 20 min and then cooled in ice; this was followed by sequential addition of 1-2-dichloroethane (5 ml), acetone (1 ml) and 5 *M* NaOH (0.5 ml). The mixture was stirred, centrifuged at 2,000 rpm for 5 min, and the supernatant collected. The absorbance of the lower organic phase was read using a Unicam SP 1750 spectrophotometer at 540 nm. The assay was calibrated by adding to blank serum known concentrations of bis(2-chloroethyl) amine. Alkylating activities of unknown samples was determined with reference to a calibration curve by multiplying the appropriate value by the weight ratio of the two compounds (1.46).

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Table 1. Details of the eight patients who received incremental doses of cyclophosphamide

Pt no.	Age	Sex	Wt (kg)	SA/m^2	Pathology	Sites of disease
1	39	M	68.5	1.8	Small cell	Hemithorax
2	63	M	70	1.7	Adenocarcinoma	Hemithorax with effusion
3	39	F	66	1.7	Not known	Hemithorax, bilateral neck nodes
4	49	M	64	1.8	Small cell	Hemithorax, bone marrow
5	53	M	70	2.0	Not known	Hemithorax, neck nodes, liver
6	56	M	52	1.5	Small cell	Hemithorax, neck nodes
7	62	M	66	1.8	Adenocarcinoma	Hemithorax
8	57	M	56	1.7	Squamos carcinoma	Hemithorax

Kinetic analysis. Individual serum concentration/time curves were fitted to the appropriate one- or two-open-compartment model according to the type of decay curve identified. A non-linear iterative computer program was used to fit the decay curves, and the dispositional rate constants, clearances, half-times, and distribution volumes were calculated in the normal manner.

An estimate of the rate of metabolism (*Km*) of CP was determined as follows. The renal clearance (*Clc*) was calculated using the log mean serum concentration for each of the urinary excretion intervals and deriving the total mass of CP excreted in the urine:

$$Clc = UV/P. \quad (1)$$

The rate constant for renal excretion (*Ke*) could then be determined using the equation

$$Ke = Clc/Vc \quad (2)$$

where Vc = central compartment volume.

Km (the rate constant for metabolism) was then calculated using the equation

$$Km = Kel - Ke \quad (3)$$

where *Kel* is the rate constant for total clearance.

The fraction metabolised was then obtained from the ratio of *Km* to *Kel*.

Results

Patient details are given in Table 1. In six patients there was histological proof of disease, while in the remaining two disease was accepted on clinical and radiological grounds. All patients completed the prescribed therapy with the exception of patient 3, who died of a pulmonary embolism prior to the third treatment.

The mean serum concentration/time curve of CP is shown in Fig. 1. The mean curve could be fitted to a two-compartment open model but some of the individual curves could only be fitted to a single exponential function. The mean terminal half-life of successive doses was 10.7, 11, and 11 h, respectively. The mean area under the concentration/time curve (AUC) increased from 2.241 ± 0.4 to 3 ± 3.5, to 3.6 ± 0.5 μg/l/h^{-1}.

The mean decay curve of alkylating activity is shown in Fig. 2. There was a very rapid accumulation of alkylating metabolites (AM) within 30 min of drug administration. It was

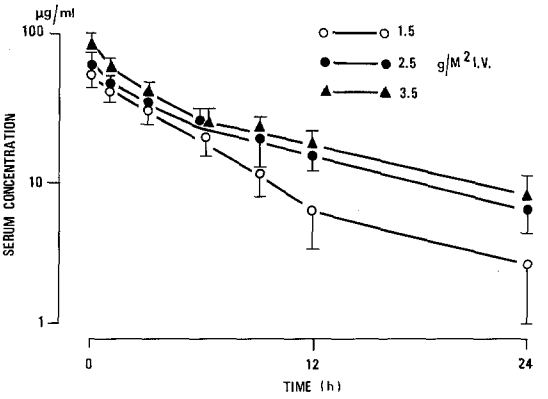

Fig. 1. Concentration/time curve (mean ± SEM) in eight patients with metastatic bronchogenic carcinoma who received incremental doses of cyclophosphamide IV

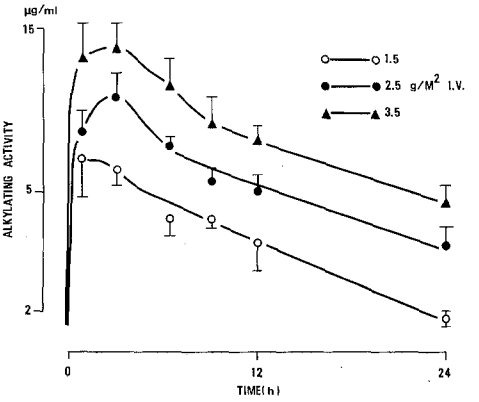

Fig. 2. Concentration/time curve (mean ± SEM) of total alkylating activity in eight patients with bronchogenic carcinoma who received incremental doses of cyclophosphamide IV

Table 2. Ratio of AUC alkylating metabolites to AUC of cyclophosphamide in eight patients who received incremental doses of 1.5, 2.5, and 3.5 g/m^2 IV

	Dose (g/m^2)		
	1.5	2.5	3.5
Mean dose (g)	2.64	4.4	6.2
Ratio (mean)	0.28	0.29	0.27
SE	0.06	0.06	0.03
Range	0.12–0.71	0.15–0.70	0.19–0.40

Table 3. Renal clearance Clc and % of (CP) metabolised in eight patients with bronchial carcinoma who received incremental doses of cyclophosphamide IV

Parameter	Dose (g/m^2)								
	1.5			2.5			3.5		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Clc (ml/min)	16	5.4	8–49	22	8.2	10–67	17	3.9	9–36
% Metabolised	86	3.78	71–94	85	4.7	59–95	90	2.75	75–97

not possible to fit this assumed metabolism by Michaelis-Menton kinetics. In all instances a single exponential function would fit the decay curve and the respective half-lives were 14, 14.7, and 12.6 h. The mean AUC increased from 0.53 ± 0.64 to 0.89 ± 0.11 to 1.291 ± 0.264 $\mu g/l/h^{-1}$.

The ratio of AUC (AM) to AUC (CP) is given in Table 2. The ratio was similar with incremental dose within any one patient, but there was considerable between-patient variation, particularly with the 3.5 g/m^2 doses. The renal clearance of CP and the fraction metabolised are given in Table 3. The proportion of drug metabolised was similar for each dose, but there was moderate between-patient variation in the proportion metabolised.

Toxicity

Nausea, vomiting, and complete epilation were universal in all patients. There was no evidence of cystitis in any patient treated, and there were no cardiac deaths. Five patients developed septicaemia requiring hospital admission and appropriate supportive care, and recovery was complete in all instances. The relationship between alkylating activity and percent fall in neutrophil count is shown in Fig. 3. Absolute neutropoenia was the rule with the second and third doses; the mean times to recovery were 18.5 ± 6.8, 18 ± 3.5, and 22 ± 9.1 days, respectively. A significant correlation was observed with the AUC of AM from 6 to 24 h and percent change in neutrophil count ($P < 0.015$), but not for the areas from 0 to 6 h.

Response was assessed in the usual manner. A complete response was obtained in patient 5, and > 50% regression of measurable disease was maintained for 1 month in patients 4, 6, and 8. Patients were ranked according to the degree of response and a significant difference was observed between the ratio of AUC (AM) to AUC (CP) ($P < 0.05$, 2-way Kriskal Wallace test): the higher the ratio the more likely the patient was to respond.

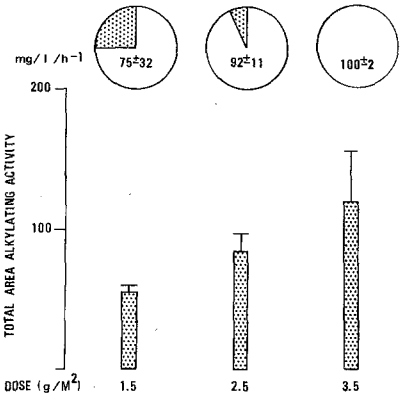

Fig. 3. Relationship between total alkylating activity and percent fall in neutrophil count

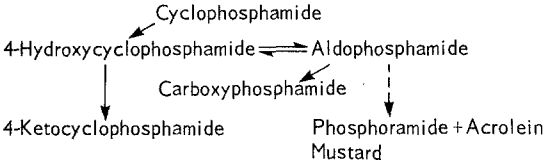

Fig. 4. Metabolism of cyclophosphamide (after Connors et al. [4])

Discussion

CP is a prodrug requiring activation by the mixed function oxidase system [7], after which further biotransformation is part enzymatic, part chemical, the two alternative pathways leading either to the formation of the active principle and acrolein or to the formation of non-toxic metabolites (Fig. 4). Aldophosphamide is a key intermediate metabolite [11] and phosphoramide mustard is probably the active principle [4]. Although detailed methodology is available for determining individual members, including phosphoramide mustard [12], it is relatively complex and beyond the scope of most clinical laboratories. It was felt that the nitrobenzyl pyridine assay selected here to determine total activitiy would accurately provide the *proportion* of drug activated in vivo and thus allow estimation of the between- and within-patient variation in metabolism with incremental doses.

Although theoretically it is possible to saturate the mixed-function oxidase system with very large doses, it is clear that a dose increase from 1.5 to 3.5 g/m^2 does not, even in patients with biochemical evidence of liver metastases and in the one patient with isotopically proven metastases.

The *km* for the hepatic metabolism of CP is 1 m*M* [2, 18], which is considerably in excess of serum concentrations even at high doses [12], and this accounts for the fact that CP does not show dose-dependent kinetics [8].

Within patients there was no significant variation in drug metabolism with incremental doses. Treatment was administered over a 6-week period and it is unlikely that enzyme induction would have occurred during this time, as Mouridsen et al. observed no change in metabolism over a period of 22 days [15]. However, more repetitive treatment is likely to result in increased metabolism by means of enzyme induction as a change in serum half-life has been noted in patients receiving oral CP for 6 months [5, 16].

The situation is made more complex by the fact that the metabolites 4-hydroxy-CP and acrolein cause denaturation of cytochrome P 450 in the rat, thereby depressing microsomal

function oxidase activities; however CP and phosphoramide mustard have no effect [13]. The inference from this study, however, suggests that activity is not inhibited, in view of the fixed proportion of the administered dose metabolised with each dose increment. Evidence from other studies suggests that enzyme induction is the more crucial factor, with prolonged administration resulting in increased metabolism [5, 16].

Between patients there was considerable variation in metabolism when the ratio of AUC (AM) to AUC (CP) was compared. A similar but less dramatic change was observed in the fraction metabolised, and these observations have been noted by other workers [14]. Even with smaller doses, i.e., 200 mg to 1 g, although there is no dose-dependency there is again considerable variation between subjects [3]. In this series the variation in metabolism did not correlate significantly with haematological toxicity, but this is probably because the initial dose was already at the maximum point of the dose-response curve for the bone marrow. There is, however, a suggestion that the response was directly related to the proportion of drug activated. The numbers are too small to draw any valid conclusion and could only be tested by titrating individual doses. If this is not possible the only means by which this can be overcome is by the administration of a large dose at 3- to 4-week intervals rather than chronic oral administration or fractionation of a larger oral dose.

Chemotherapy for small cell bronchial carcinoma of limited extent is now capable of producing useful remissions in up to 60% of patients treated, and cyclophosphamide is clearly going to be an important drug whether used alone or in combination. Whatever dose is selected the kinetics remain constant within patients, but in a large series there must inevitably be considerable variation between patients, which may be reflected in the response rate.

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