REPEATED INVESTIGATIONS OF CYCLOPHOSPHAMIDE DISPOSITION IN MYELOMA PATIENTS RECEIVING INTERMITTENT CHEMOTHERAPY

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- 1 The disposition of cyclophosphamide was investigated in a group of myeloma patients.
- 2 Marked changes in clearance and volume of distribution of cyclophosphamide occurred between investigations.
- 3 The observed changes in disposition did not correlate with biochemical estimates of renal and hepatic function or plasma protein status.

Introduction

Current trends in the chemotherapy of certain forms of malignant disease favour the use of cytotoxic drugs, either alone or in association given as a repetitive course of treatment at fixed intervals. For example the COP schedule used in lymphosarcoma (Holoye & Samuels 1975) is given every 14 days for six courses. The dose of drug used in these treatments is usually adjusted for body weight but no other allowance for individual variation in drug disposition is made. It is assumed that the same dose will produce similar clinically effective blood levels in all patients.

It is now known that for some drugs, a group of individuals can show a wide range of blood levels for a given dose (Koch-Weser 1975; Wilkinson & Shand 1975). It would, therefore, seem reasonable to attempt to optimize these schedules by adjusting the dose to achieve some previously defined blood concentration. One of the causes of variability is the variation in physiological function within the group. It would be reasonable to expect changes in physiological function, and hence in drug handling to occur as a disease progresses. Disease states have been shown to have significant effects on drug disposition (Rowland, Blaschke, Meffin & Williams, 1976).

A number of studies have been carried out in patients with cancer of various types. For example,

Bagley, Bostick & de Vita (1973) suggested that cancer *per se* did not appear to contribute to the observed variation in drug disposition. However, multiple myeloma, for which cyclophosphamide is regularly prescribed is often associated with moderate to severe renal impairment, and changes in renal function are known to markedly alter the disposition of drugs or metabolites, largely eliminated by this route (Fabre & Balant, 1976).

We have investigated the disposition of one drug, cyclophosphamide, in six patients, when given as part of a schedule for the treatment of myeloma, at intervals throughout the period of treatment. Cyclophosphamide *per se* is inactive but is converted by the mixed function oxidases of the liver to alkylating metabolites (Cohen & Jao, 1970). These can be measured collectively as plasma alkylating activity which has been shown to correlate with cytotoxic effect in an animal model (Alberts & van Daalen Wetters 1976). The schedule was as follows: Day 1, cyclophosphamide 10 mg kg⁻¹, days 2, 3, 4 and 5, prednisolone 1.5 mg kg⁻¹, melphalan 0.15 mg kg⁻¹. This procedure was repeated at 6-weekly intervals. In all patients the disposition of cyclophosphamide was investigated after the initial dose and 12 weeks subsequently. In four patients it was reinvestigated after a further 24 weeks.

Figure 1 Plasma concentration/time profile of cyclophosphamide in Patient 2 after the first (○), second (△) and third (□) studies after the results were normalized to a dose of 10 mg/kg. The symbols represent data points and the lines the computer derived best fit to these points.

Methods

Cyclophosphamide was given by slow intravenous injection. Blood samples were collected just before the dose and at 0.15, 0.5, 1, 2, 3, 5, 8, 12 and 24 h subsequently. In patients 5 and 6 no 24 h sample was taken following the initial cyclophosphamide dose. The samples were centrifuged immediately, the plasma frozen and stored at -20°C until assayed. Urine was collected for 24 h after the dose at intervals of 0–3, 3–7, 7–12 and 12–24 h. No 12–24 h collection was made from patients 5 and 6 following the initial dose of cyclophosphamide. The volume of urine produced was measured, a sample taken, frozen and stored at -20°C until assayed. Cyclophosphamide and alkylating activity remained stable, both under these conditions of storage and to freezing and thawing. Standard tests of renal function—serum

Figure 2 Plasma concentration/time data of alkylating activity in Patient 2 after first (□), second (○) and third (△) studies after the results were normalized to a dose of cyclophosphamide of 10 mg/kg.

creatinine (Cr_s) and creatinine clearance (Cl_{Cr}) were made and blood cell counts carried out at each stage of treatment.

Cyclophosphamide concentrations were determined following extraction at pH 7.0 of plasma (0.5 ml) or urine, suitably diluted (0.5 ml) with dichloromethane (3 volumes) using an appropriate amount of isophosphamide (Asta-Werke, Bielfeld, West Germany) in sodium phosphate buffer (0.15M, pH 7.0) as an internal standard. The dichloromethane layer was evaporated to dryness and derivatized with a mixture of trifluoroacetic anhydride and ethyl acetate (1:2 v/v) according to the method of Pantarotto, Bossi, Belvedere, Martini, Donelli & Frigiero (1974). The derivatized material was analysed on a Perkin Elmer F33 gas chromatograph using an alkali flame ionization detector. The column was 3% OV-17 on 100–120 Mesh Gas-Chrom Q (Phase Separations Ltd). The alkylating activity of the samples was determined according to the method of Field, Gang, Kline, Venditti & Waravdekar (1973), modified from that of Friedman & Boger (1961) using a Pye Unicam SP 1750 double-beam spectrophotometer. The precision of the assay methods was determined by analysis of five plasma samples spiked with either cyclophosphamide (Koch-Light Laboratories Ltd) or nor-nitrogen mustard (Aldrich Chemical Co. Ltd).

The plasma concentration/time data for cyclophosphamide and alkylating activity, expressed in terms of cyclophosphamide equivalents, were normalized to a dose of 10 mg kg^{-1} . The best fit exponential expression for each data set was determined using the non-linear least squares regression programme NONLIN (Metzler, Elfring & McEwen, 1973). The expression which contained the least number of terms but adequately described the

Table 1 Pharmacokinetic parameters for cyclophosphamide and alkylating activity for the three investigations

Patient	Age (years)	Weight (kg)	Sex	Study	Cyclophosphamide					Alkylating activity				
					A	B	T _{1/2}	Cl _s	α	β	Cl _s	Cl _R	V _d	AUC
					(mg l ⁻¹)	(mg l ⁻¹)	(h)	(mm)	(h ⁻¹)	(h ⁻¹)	(ml min ⁻¹)	(ml min ⁻¹)	(l kg ⁻¹)	(mg l ⁻¹ h)
1	41	71	M	1	95	7.8	11.8	0.21	0.3	0.06	56	6.0	0.73	90
				2	76	12.7	11.2	0.16	1.7	0.09	83	5.1	0.74	116
				3	88	24.2	9.1	0.15	0.7	0.09	88	12.5	0.84	213
2	67	51	F	1	115	30.4	15.7	0.06	3.6	0.17	109	8.6	0.57	98
				2	53	25.2	7.0	0.07	3.6	0.11	160	8.7	1.16	178
				3	80	30.4	17.1	0.09	3.4	0.20	116	7.2	0.53	62
3	52	42	F	1	24	36.2	17.2	0.23	2.7	0.15	54	6.2	0.51	177
				2	31	50.1	5.4	0.24	3.0	0.10	103	2.9	1.47	114
				3	6	14.4	11.3	0.43	0.5	0.13	61	4.8	0.66	218
4	61	60	M	1	77	18.3	10.9	0.07	2.1	0.04	44	5.4	1.00	111
				2	96	—	12.3	0.08	—	*0.12	99	9.1	0.81	67
				3	119	6.3	97.3	0.08	6.3	0.15	86	3.5	0.59	N.D.
5	47	68	F	1	64	29.3	17.0	0.09	1.7	0.08	43	11.2	0.49	66
				2	100	25.1	7.5	0.10	1.8	0.06	82	16.5	1.20	173
				3	20	28.8	11.9	0.21	1.2	0.06	62	4.8	0.78	146
6	69	78	M	1	N.D.	26.8	11.9	0.12	5.4	0.05	52	6.0	0.82	399
				2			14.2							31
														6.2

*monoexponential decline
N.D. not determined

data according to the criteria of Boxenbaum, Riegelman & Elashoff (1974) was used for evaluation of pharmacokinetic parameters. In the case of alkylating activity it is necessary to refer to the pharmacokinetic parameters as 'apparent' since the assay used is not specific for any one metabolite of cyclophosphamide. Plasma protein binding of cyclophosphamide was determined *in vitro* over the concentration range 1 to 50 mg l⁻¹. Pooled human plasma containing 5-[¹⁴C]-cyclophosphamide (1 × 10⁴ d/min,—National Cancer Institute, Bethesda, Maryland, U.S.A.) was subjected to ultracentrifugation at 1.5 × 10⁵g for 4h at 37°C *in vacuo* using an MSE superspeed 65 ultracentrifuge.

Results

Investigation of the precision of the analytical methods gave a coefficient of variation of 6% for the analysis of cyclophosphamide and 9% for the analysis of alkylating activity.

The plasma concentration/time profiles obtained after studies 1, 2 and 3 in patient 2 are presented for cyclophosphamide in Figure 1 and for alkylating activity in Figure 2 showing the best fit line obtained using the method of least squares. A bi-exponential expression $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, adequately described the data in all cases with the exception of patient 4 (second study) where a mono-exponential expression was more appropriate.

Pharmacokinetic parameters were calculated from the above as follows and are shown in Table 1:

$$\text{Elimination } T_{1/2} = \frac{0.693}{\beta}$$

$$\text{Total plasma clearance } Cl_s = \frac{\text{Dose}}{\text{AUC}}$$

where

AUC = area under the plasma concentration/time curve

$$= \frac{A}{\alpha} + \frac{B}{\beta}$$

Apparent volume of distribution

$$V_d = Cl_s \times \beta / \text{body weight.}$$

The results for alkylating activity could be fitted only by using multi-exponential expressions. Therefore half-life and area under curve were determined by linear regression and trapezoidal rule respectively.

Renal clearance of cyclophosphamide or alkylating activity was calculated as follows:—

$$\text{Renal clearance} = \frac{\text{Urinary excretion rate}}{\text{AUC}}$$

Cyclophosphamide was found to exist predominantly in the free form (fraction bound = 20.3 ± 1.4% over the plasma concentration range of 1–20 mg l⁻¹). This value is in approximate agreement with Jardine, Fenselau, Appler, Kan, Brundrett & Colvin (1978) who obtained a value of 24% in a single study, but greater than the 12–14% of Bagley, *et al.* (1973).

A two-way analysis of variance (Chatfield, 1978) was used to assess the significance of differences in values of Cl_s , V_d and $T_{1/2}$ among treatments and patients. The effect of treatment on Cl_s was marked and significant at the 5% level. Changes in V_d were often marked and approached, but did not attain significance (5% level). The treatment had no significant effect (5% level) on cyclophosphamide $T_{1/2}$ or any of the parameters of alkylating metabolite disposition. The differences in value of Cl_s among patients were significant at the 1% level.

Discussion

The plasma concentration time profiles for cyclophosphamide in the second study differed significantly from those in the initial study in all six patients. These differences resulted from increased clearance in five of the six patients and increases in volume of distribution in four of the subjects. These changes generally resulted in lower plasma concentrations of alkylating activity at study two compared to study 1. These data showed little change in half-life. This would be expected since half life is related to clearance and volume, increased clearance resulting in a shorter half-life whereas increased V_d results in a longer half-life.

The changes in alkylating activity disposition were not as uniform as that of cyclophosphamide. The alkylating activity did show a surprising persistence in plasma for such a chemically reactive species.

When the disposition of cyclophosphamide was re-determined in four of this group of six patients after a further 24 weeks, the trends shown from studies 1 and 2 were not maintained the values of clearance and volume of distribution tending to return to those obtained at treatment one in two of the four patients.

It is apparent from these results that the disposition of cyclophosphamide changes during the course of repetitive treatment. The magnitude of the changes found is not sufficient to warrant plasma concentration monitoring since the difference

between individuals at study one are as great as the difference in the same individuals between studies 1 and 2.

Several reasons for these changes were explored. Initially changes in plasma protein binding were thought to be involved since increase of free drug would increase clearance and volume. However, cyclophosphamide is bound only to the extent of 20% over the concentration range applicable, and therefore decreases in binding, which would lead to increase in volume of distribution and clearance could not account for the changes found between studies 1 and 2. Neither was there any consistent relationship between haemoglobin, leucocyte and

platelet counts and changes in clearance. These values are not reported here but are available on request. Therefore no satisfactory explanation can be put forward at present for the changes in clearance observed between studies 1 and 2. In the case of cyclophosphamide these changes may not be clinically significant. This observation of changes in disposition during treatment could be important for other drugs used in repetitive treatments which have a lower therapeutic index or where the therapeutic plasma concentration range is known. In these cases it would be important to monitor plasma concentrations of these drugs to optimize treatment with them.

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