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The Disposition of Cyclophosphamide in a Group of Myeloma Patients

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Summary. *The disposition of cyclophosphamide and its alkylating metabolites was investigated in a group of myeloma patients with varying degrees of renal function impairment. No correlation between renal function and clearance of cyclophosphamide or its alkylating metabolites was found. No evidence of accumulation of cyclophosphamide or alkylating activity was found in four patients receiving radiolabelled cyclophosphamide. Renal function was found to be related to the reciprocal of the area under curve of alkylating activity, indicating that this area increased as renal function decreased. In view of the large nonrenal component of alkylating activity elimination and the large inter-subject variability, it is recommended that dose of cyclophosphamide is not altered in moderate impairment of renal function.*

Introduction

At present there are few guidelines as to the optimum use of cyclophosphamide (CP) in man. There is general agreement that it is best to give large doses of CP intermittently to minimize toxicity and maximize the antitumour effect, but the optimum dose size and frequency of dosing for any one individual are subjects of debate. Problems of this nature have been resolved for other drugs on the basis of knowledge of the optimum plasma concentration of a drug and its disposition parameters in the individual or population [3, 12]. In the case of CP the optimum plasma concentrations, which would lead to effective therapy but minimize toxicity, are not known; these concentrations would in any case be an indirect measure of effect, since it is the metabolites of CP that are the active species [7]. The disposition of CP itself has been well characterized by several groups [2, 4, 5]. However, little information is available about the disposition of CP metabolites in man. A major problem in any study of the metabolites of CP is the lack of suitable analytical methodology to characterize the plasma concentration/time profile of individual metabolites. The principal metabolites of CP have been identified and the proposed metabolic pathway for CP is given in Fig. 1 [7, 17, 18]. Recently the plasma concentration/time profile of two of these metabolites (IV and VI) has been determined in one patient after a 75 mg/kg dose of CP, by means of gas chromatography selective ion monitoring [6, 11]. This sophisticated technique is not widely available.

It is possible to quantitate the total alkylating metabolites present in plasma by means of a nonspecific colorimetric method of analysis [9, 15]. The results of this method have been shown to correlate with the pharmacological activity of CP in animals [1]. Therefore, although it is not a specific method, it does enable the pharmacologically active metabolites to be monitored in individual patients. These metabolites are polar compounds and would generally be expected to be excreted unchanged. In view of this, the dose of CP is frequently reduced in patients with renal impairment, since it is thought that a 'normal' dose would cause greater toxicity in these patients than in patients with normal renal function. Support for dose adjustment in these patients comes from the investigation of Mouridsen and Jacobsen [14], who, using 5-^{14}C-CP, found that non-CP radioactivity in the plasma of patients with severe renal failure had a much longer life than that in patients with normal renal function.

It has been demonstrated that this nonextractable radioactivity is equivalent to alkylating activity in patients with normal renal function [14]. This equivalence has been assumed to hold for patients with impaired renal function. It can be seen from Fig. 1 that not all alkylating metabolites retain the C-5 atom of CP; this is split off as part of acrolein on formation of phosphoram-

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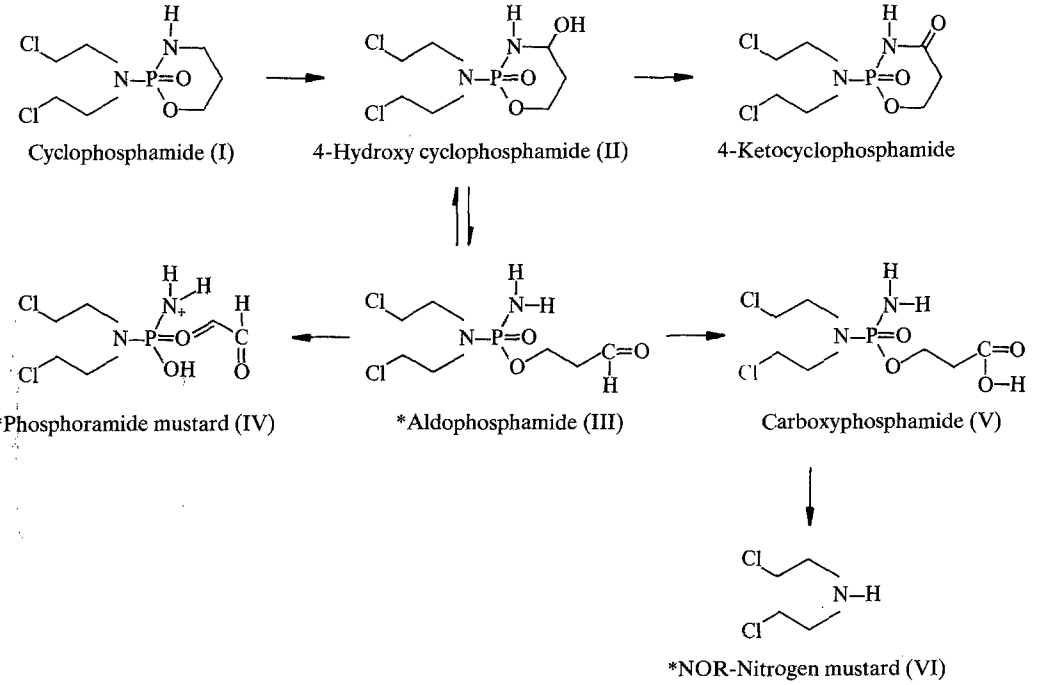

Fig. 1. Scheme for the metabolism of CP. *, active alkylating agent

ide mustard (IV). If this is the radiolabelled atom, then nonextractable, i.e., water-soluble, radioactivity will reflect acrolein and acrolein derivative concentrations. If acrolein derivatives and phosphoramide mustard are excreted by routes that are influenced differently by impairment of renal function, then monitoring nonextractable radioactivity concentrations alone in these patients could be misleading.

Therefore the kinetics of CP tritriated in the chloroethyl side chain, an essential part of all alkylating metabolites, was investigated in two patients with moderately impaired renal function. The disposition of 5^{-14}C-CP was also investigated in two patients.

Impairment of renal function is a frequent consequence of myeloma. It is desirable to give such of these patients as are receiving treatment for myeloma the largest dose of CP that is compatible with expected toxicity. Since toxicity is thought to be related to the amount of alkylating activity formed it is important to know the disposition of alkylating metabolites and whether their elimination is retarded in patients with moderate to severe renal impairment. Therefore the disposition of CP and its alkylating metabolites was investigated in a group of patients receiving treatment for myeloma. Other parameters that could affect drug disposition, such as weight, liver function, and plasma volume, were also monitored, to see whether a rational approach to choice of dose in any one patient could be deduced.

Methods and Materials

Cyclophosphamide was administered by slow IV injection to nine patients. Blood samples were collected just before the injection and at timed intervals after the dose for up to 24 h. The plasma was separated and stored at −22° C until assayed. Urine was collected for either 12 or 24 h after the dose in intervals of 0–3, 3–7, 7–12, and 12–24 h. The urine was stored at −22° C until assayed.

The CP content of the samples was determined by the method of Pantorotto et al. [16] with an alkali flame detector. The alkylating activity in plasma and urine samples was determined by the method of Friedman and Boger [9]. Renal function of the patients was assessed by estimation of serum creatinine and creatinine clearance.

All patients received melphalan (10 mg) and prednisolone (100 mg) for 4 days starting the day after CP administration. Three patients received melphalan on the day of CP administration. These were patients FC, DH, and FM. The effect of the combined treatment on serum protein, leucocyte count, and erythrocyte count was assessed by monitoring the decrease in these parameters after treatment in some patients. Aqueous solutions of melphalan over the concentration range 1.0–20 μg/ml were assayed for alkylating activity.

Radiolabelling Studies

Two patients (HH, PV) received 5^{-14}C-CP (HH 71.9 μCi 600 mg, PV 70.0 μCi 600 mg). Blood samples (10 ml) were collected at 0.16, 0.55, 1, 2, 3, 5, 8, 12, and 24 h, and urine was collected for 72 h and pooled in 0–3, 3–6, 6–12, 12–24, 24–48, and 48–72 batches. Plasma samples were assayed for CP and alkylating activity by the methods outlined above. Extractable radioactivity, equivalent to CP,

and nonextractable radioactivity, equivalent to alkylating activity, were determined by the method of Mouridsen et al. [14].

Two patients (DS, MH) received CP generally tritiated in the chloroethyl side chain (DS 61.9 μCi, 540 mg, MH 59.4 μCi, 540 mg). Samples were collected and assayed as above.

Analysis of Results

The plasma concentration/time data for CP and alkylating metabolites, expressed in terms of CP equivalents, were fitted with a suitable exponential expression by means of the nonlinear least-square-fitting programme NONLIN [13]. The expression containing the least number of terms that gave the best fit to the data, as judged by an F test in the residual sum of squares, was used for evaluation of the disposition parameters.

The disposition parameters for CP of half-life, total body clearance (Cl_T), renal clearance (Cl_R), and volume of distribution (V) were calculated by the methods of Gibaldi and Perrier [10]. The disposition parameters for alkylating activity were calculated in a similar manner. The accuracy of the parameters determined in this way is compromised by several factors. The assay used is not specific for any one metabolite but is responsive to several metabolites of CP, the relative concentrations of which may vary in urine compared with plasma; the metabolites may undergo hydrolysis in the urine whilst in the bladder [18]; certain metabolites such as carboxycyclophosphamide may degrade to alkylating metabolites during extraction from urine [6]; and finally, it is not possible to calculate the fraction of the dose of CP that is converted to alkylating metabolites. Therefore the parameters of clearance (Cl_TAA) and renal clearance (Cl_RAA) are referred to as apparent parameters. Other parameters of half-life and area under alkylating activity concentration/time profile (AUC_{AA}) are calculated by the methods of Gibaldi and Perrier [10].

Renal function is related to removal of drugs from the body by Equation 1 [10]

$$Cl_T = Cl_{NR} + \frac{Cl^F_{CR}}{Cl^N_{CR}} \times Cl_R \qquad (1)$$

where Cl^F_{CR} and Cl^N_{CR} refer to the patients' renal function and to normal renal function respectively, Cl_{NR} is the normal clearance and Cl_R is the renal clearance of the drug in question.

Therefore $Cl_T = Cl_{NR} + Cl_{CR} \times C$, where C is a constant, since Cl_R and Cl^N_{CR} are constant for normal renal function and total clearance will be linearly related to creatinine clearance. For small numbers of patients this will only hold true if Cl_{NR} is small compared with Cl_R, i.e., a large proportion of the dose is excreted unchanged. If renal function is an important determinant of drug clearance then Cl_T should be linearly related to Cl_{CR}. If volume of distribution remains constant with changes in renal function K, the elimination rate constant should be linearly related to Cl_{CR}, since $Cl_T = V \times K$. For alkylating activity, because of the assumptions made in calculating Cl_T, the relationship of $1/AUC_{AA}$ to Cl_R was investigated since $Cl^{AA}_T = DOSE/AUC_{AA}$ although this latter relationship does assume that a similar fraction of the dose of CP is metabolized to alkylating activity in each patient.

Results

The disposition parameters for CP and its alkylating metabolites are given in Tables 1 and 2. Typical plasma concentration/time profiles for CP and alkylating metabolites are shown in Fig. 2.

The results of linear regression of disposition parameters against parameters of renal function are given in Table 3. The results of those patients who received melphalan 6 h after the IV CP are included, since it was found that melphalan was unreactive in the alkylating assay over the concentration range 1–20 μg/ml and did not interfere with the CP assay with the methods used in this study.

The results obtained after administration of ^{3}H-CP to two patients (DS Cl_{CR} = 15 ml · min^{-1} and MH Cl_{CR} = 24 ml · min^{-1}) are given in Fig. 3. Alkylating activity and nonextractable radioactivity are expressed as percentages of the initial dose per litre. The rate of appearance of radioactivity and alkylating activity in the urine expressed as the percentage of the dose remaining to be

Table 1. Disposition parameters for CP in 13 patients

Patient	Weight (kg)	Dose (mg)	$T_{1/2}$ (h^{-1})	Cl_T (ml · min^{-1})	Cl_R (ml · min^{-1})	V (l/kg)	fm^{cp}	Cl_{CR} (ml · min^{-1})
HH	71	900	10.8	56	6.6	0.73	0.92	95
MH	42	500	4.6	53	6.2	0.51	0.88	24
DH	67	850	4.1	104	8.6	0.58	0.92	115
AV	50	500	4.3	78	4	0.58	0.97	28
GW	78	500	10.8	62	4.8	0.78	0.91	20
FC	68	900	9.0	43	11.2	0.49	0.74	64
FM	60	900	16.0	43	5.4	1.00	0.87	77
JH	84	950	3.9	78	1.8	0.31	0.98	31
MK	70	500	5.6	71	1.2	0.41	0.98	
JP	50	750	3.7	86	7.8	0.55	0.91	34
HC	82	1000	11.8	200	11.2	2.49	0.94	69
DS	60	540	4.2	82	2.6	0.50	0.97	15
PV	51	600	8.3	90	7.8	1.27	0.90	32
Mean			7.47	80		0.78	0.91	
SD			3.95	40		0.57	0.064	

Table 2. Disposition parameters for alkylating activity in 13 patients

Patient	Terminal $T_{1/2}$ (h)	AUC_{AA} (mg · ml^{-1} · h)	Cl_T^{AA} (ml · min^{-1})	Cl_R^{AA} (ml · min^{-1})	Cr_S (mM)
HH	23	90	120	22	0.21
MH	15	181	34	15	0.23
DH	33	98	105	50	0.06
AV	11	100	81	9	0.15
GW	13	137	86	14	0.21
FC	12	66	128	53	0.09
FM	29	111	78	24	0.07
JH	32	152	89	12	0.25
MK	36	476	24	5	0.87
JP	ND	ND	ND	54	0.34
HC	12	63	205	37	0.12
DS	40	461	21	5	0.28
PV	ND	197	39	23	0.12
Mean	21.6				
SD	10.1				

Table 3. Correlation of disposition factors for CP with parameters of renal function

Cl_T	= 0.188 Cl_{CR} + 71.7	$n = 12$	$r =$ 0.146	$P > 0.05$
K_{CP}	= 0.14–0.00053 Cl_{CR}	$n = 12$	$r =$ 0.31	$P > 0.05$
Cl_T^{AA}	= 0.85 Cl_{CR} + 45.1	$n = 11$	$r =$ 0.56	$P > 0.05$
$\frac{1}{AUC_{AA}}$	= 0.0047 + 0.000075 Cl_{CR}	$n = 11$	$r = -0.61$	$P < 0.05$
K_{AA}	= 0.045–0.00012 Cl_{CR}	$n = 10$	$r = -0.25$	$P > 0.05$

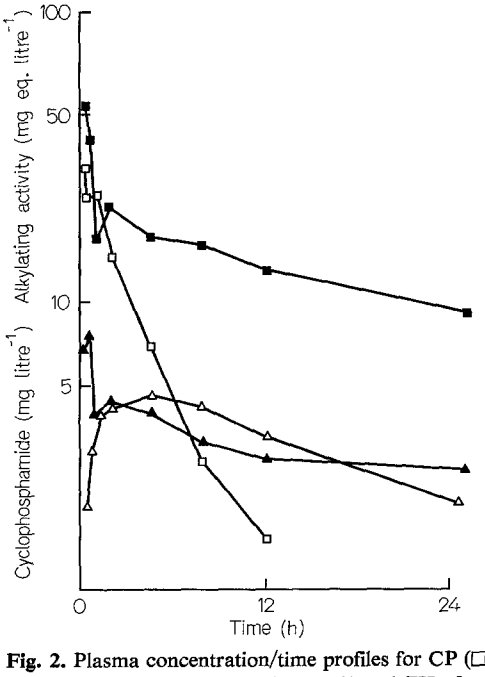

Fig. 2. Plasma concentration/time profiles for CP (□–□) and alkylating activities (△–△) in patients AV and FH after a 10 mg · kg^{-1} IV dose of CP (data for AV shown by *open symbols*)

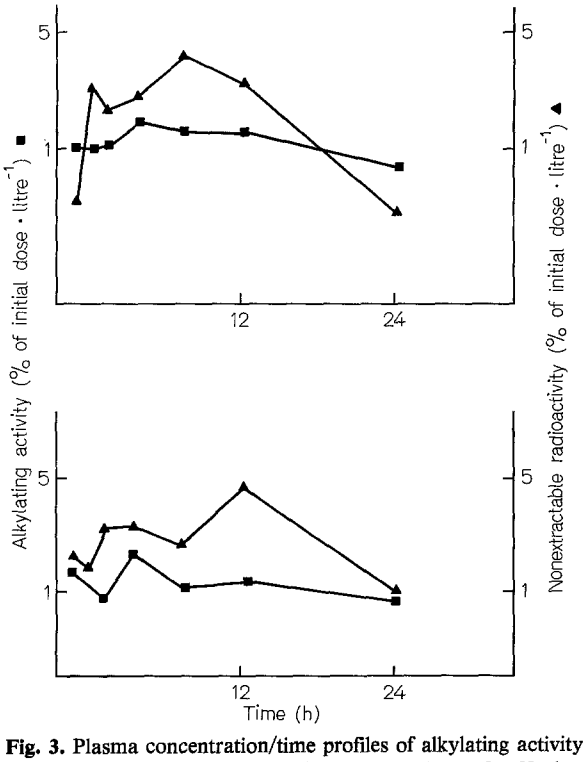

Fig. 3. Plasma concentration/time profiles of alkylating activity (■) and nonextractable radioactivity (▲) in two patients after IV doses of ^{3}H-CP. *Upper graph*, DS; *lower graph*, MH

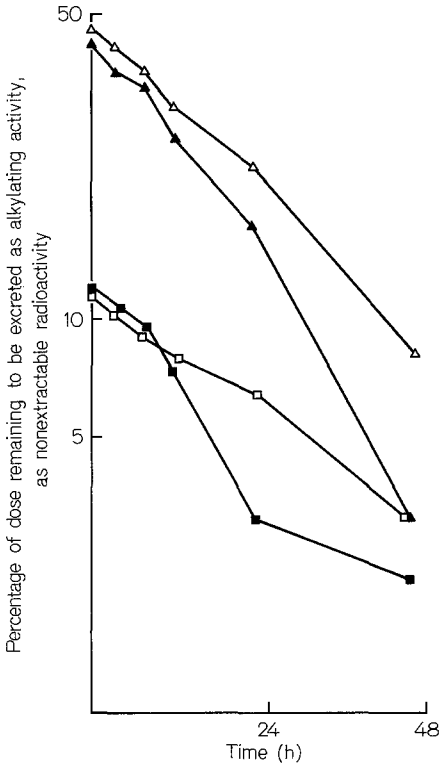

Fig. 4. Nonextractable radioactivity (△, ▲) and alkylating activity (□, ■) remaining to be excreted, expressed as percentages of dose at various times after IV doses of ^{3}H-CP to two patients (MH, *solid symbols*, and DS, *open symbols*)

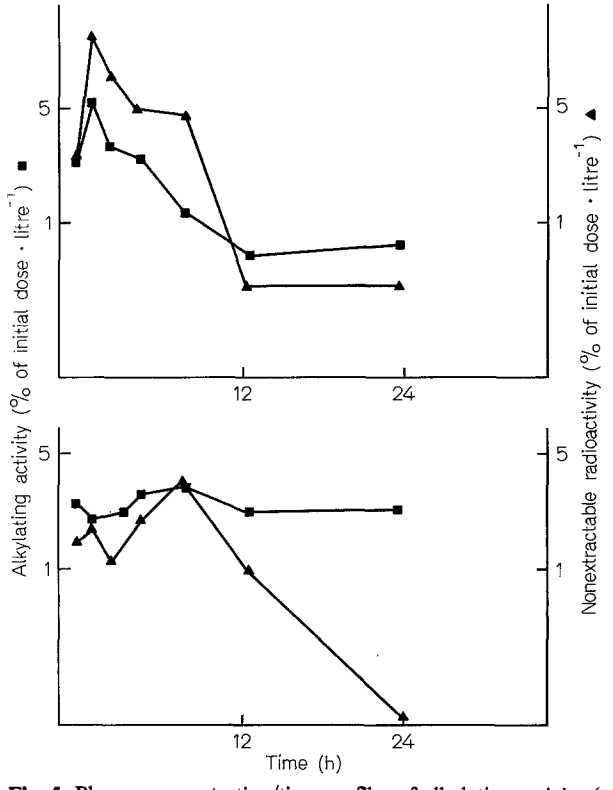

Fig. 5. Plasma concentration/time profiles of alkylating activity (■) and nonextractable radioactivity (▲) in two patients after IV doses of 5-^{14}C CP. *Upper graph*, PV; *lower graph*, HH

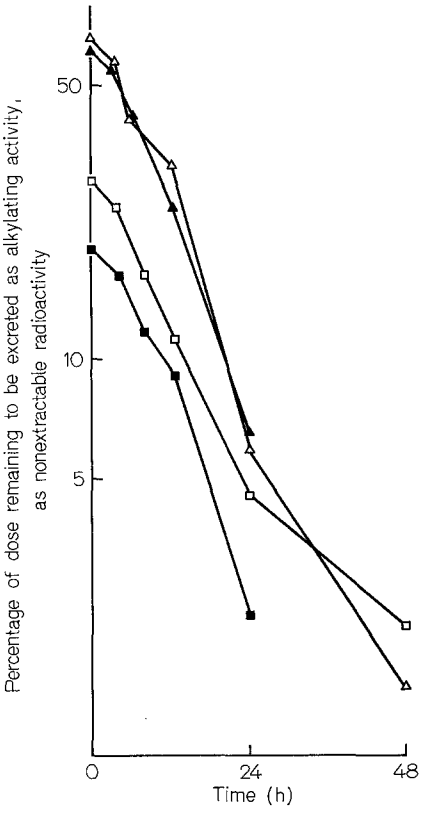

Fig. 6. Nonextractable radioactivity (△, ▲) and alkylating activity (□, ■) remaining to be excreted, expressed as percentages of dose at various times after IV doses of 5-^{14}C-CP to two patients (HH, *solid symbols*, and PV, *open symbols*)

excreted (ARE) against time plot in these patients is given in Fig. 4.

Results expressed in the same way, obtained from two other patients PV (Cl_{CR} 30 ml min^{-1}) and HH (Cl_{CR} 85 ml min^{-1}), who received 5-^{14}C CP are given in Figs. 5 and 6.

Discussion

The disposition parameters calculated for CP for the patients investigated in this study are similar to those previously reported [2, 5] and emphasize the wide variation found in the disposition of CP between individuals. The group of patients covered a wide range of renal function, creatine clearance varying from 15 to 115 ml · min^{-1}. No significant correlation was found between CP clearance or rate of elimination and renal function, as seen in Table 3. This was the expected result, in view of the extensive metabolism of CP found in this group. Although CP is extensively metabolized, no significant correlation was found between drug clearance and liver function as assessed by the concentrations of alkaline phosphatase and glutamyl transferase

enzymes in plasma; however, the results of liver function tests were in the normal range for all patients investigated. The three patients with appreciably longer half-livers of CP (HH, GW, and FM) all had expanded plasma volumes and larger volumes of distribution of CP than the others in the group, but there was no correlation for the group between those variables. In general there was no relationship between CP disposition and other variables, such as weight, sex, and liver function.

The disposition of alkylating activity again showed a wide variation between individuals and a surprising persistence in some patients. There was no significant positive correlation between the rate of elimination or clearance of alkylating activity and renal function. A second estimate of clearance $1/AUC_{AA}$ was significantly correlated to creatine clearance, indicating that the AUC_{AA} would increase as renal function decreased, and might indicate that the dose of CP and hence alkylating activity should be decreased in renal impairment. However the relationship is such that the area would only double when Cl_{CR} falls from $100\ ml \cdot min^{-1}$ to $20\ ml \cdot min^{-1}$, and individuals with similar renal function show greater differences in AUC_{AA}. Although there was no significant correlation between Cl_T^{AA} and Cl_{CR}, the nonrenal clearance, derived from linear regression of these values, of $43\ ml \cdot min^{-1}$ suggests that further metabolism before elimination is an important route of removal of alkylating activity. Therefore on the basis of these results it is recommended that there is no need to reduce the CP dose in patients with moderately impaired renal function. In cases of severe impairment dose reduction may be required.

The rate of elimination of nonextractable radioactivity, obtained from the slope of the graphs given in Figs. 4 and 6, was slower in the patients receiving tritiated CP than in those receiving ^{14}C-labelled CP. Of the two patients receiving tritiated CP, elimination was slower in MH, who had superior renal function to DS; similarly, both these patients showed slower elimination of radioactivity and alkylating activity than patient PV, who had a similar renal function to MH. In all patients the rate of elimination of radioactivity was similar to that of alkylating activity. However, a larger proportion of the dose was excreted as nonextractable radioactivity by the two patients who received ^{14}C-labelled CP than by the two receiving tritiated CP, as shown by the intercept value in Figs. 4 and 6. This may be due to elimination of acrolein derivatives in the urine with 5-^{14}C-labelled CP whilst some of the tritiated metabolites are retained in the body. The plasma concentration/time profiles of alkylating activity and nonextractable radioactivity were similar in all four patients. However, alkylating activity concentrations were generally lower. The results with the different ratio-labels were inconclusive in that no evidence of extensive accumulation of 5-^{14}C-CP was found in the one patient with impaired renal function receiving this compound. The kinetics of tritiated and 5-^{14}C-CP were similar to that of alkylating activity in all these patients apart from HH, who had normal renal function and in whom nonextractable radioactivity declined more rapidly in plasma than alkylating activity. These results reflect the larger inter-subject variation of CP disposition. They support the contention that renal function impairment cannot be used to predict the rate of elimination of alkylating activity and that the dose of CP should not be reduced in patients with moderately impaired renal function.

In conclusion, the present study has demonstrated that there is no basis for decreasing the dose of CP in myeloma patients with moderately impaired renal function. A wide range of disposition parameters for CP and its alkylating metabolites were found amongst this group of 13 patients; the variability amongst this group could not be explained on the basis of age, weight, or sex and therefore these parameters are of little value as a guide to the choice of dose of CP.

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