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Pharmacokinetics of Oral and Intravenous Melphalan during Routine Treatment of Multiple Myeloma*

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Abstract—*Plasma melphalan levels have been measured in nine (mostly stage IIIA) multiple myeloma patients after therapeutic doses of drug had been given p.o. and i.v. A new isocratic high-pressure liquid chromatographic (HPLC) method with a sensitivity limit of* 5 ng/ml *was used to quantify the melphalan. Patients receiving* 8–28.5 mg *melphalan i.v. showed* α *and* β *plasma decays with half-lives of* 7.7 ± 3.3 *(mean ± S.D.) and* 83 ± 14 min *respectively. The apparent volume of the central compartment was* 12.8 ± 4.3 l, *and the total volume of distribution was* 0.62 ± 0.21 l/kg. *Very variable absorption was seen in the same patients after receiving* 5–12 mg *melphalan p.o. The half-life of the absorption phase varied from* 2.1 *to* 62.1 min (22.8 ± 18.1 min) *with delays (before absorption started) of* 0–113 min. *The fraction of dose absorbed varied from* 0.32 *to* 1.03 (0.72 ± 0.23), *and the half-life of the* β *phase was* 92 ± 27 min. *The type of breakfast eaten before p.o. melphalan was found to correlate with the fraction of drug absorbed.*

INTRODUCTION

THE CYTOTOXIC drug melphalan has shown most activity against multiple myeloma [1] and ovarian carcinoma [2]. However, since its synthesis as L-phenylalanine mustard (4-bis(2-chloroethyl)amino-L-phenylalanine) in 1954 [3] or as the DL-racaemic mixture [4], few pharmacological studies have been undertaken due to the lack of sufficiently sensitive methods to quantitate levels of the drug in blood. Furner *et al.* [5] and Chang *et al.* [6] have published HPLC methods with a sensitivity of about 50 ng melphalan per ml of plasma, and more recently this has been extended to approximately 5 ng/ml with a GLC–Mass Spectrometry assay [7]. However, this latter technique "is expensive and has a low throughput" [7], and so we have developed a similarly sensitive assay using a simpler ion-pair HPLC method [8].

Alberts and co-workers [9] have undertaken pharmacological assessment of i.v. melphalan in patients with a variety of different neoplasms. They have also shown [10] very variable absorption of p.o. melphalan with one patient apparently not absorbing the drug at all. However, these latter studies were using melphalan doses 3–5 times the normal therapeutic range so that plasma levels of the drug could subsequently be measured with their HPLC assay. We therefore decided to investigate the pharmacokinetics of melphalan in multiple myeloma and, in particular, study the absorption of the drug using therapeutic p.o. doses. As variable absorption could be a factor in the better response seen in multiple myeloma patients receiving i.v. melphalan compared to p.o. melphalan [11], we have also looked to see whether absorption can be correlated in any way with patient response.

MATERIALS AND METHODS

Drugs and reagents

Melphalan for both i.v. and oral administration is marketed as Alkeran, (Burroughs Wellcome. [G-^{3}H] Melphalan (8 Ci/mmol in ethanol solution) was obtained roughs Wellcome. [G-^{3}H] Melphalan (8 Ci/mmol in ethanol solution) was obtained from the Radiochemical Centre, Amersham. For i.v. administration radioactive melphalan

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solution (60 μCi) was evaporated to near dryness before the non-radioactive dose, dissolved in the Alkeran diluent, was added to it. The solution was diluted to 20 ml with saline before injection as a bolus over approximately one minute. For oral administration the radioactive melphalan (60 μCi) was evaporated to dryness on the tablets.

HPLC grade methanol was obtained from Fisons Scientific, Loughborough, and dansyl arginine from Sigma Chemical Co., Poole. XAD-2 was obtained from B.D.H., Poole.

Patients

All patients gave informed consent before undergoing the studies. Most patients attended for the investigation on an out-patient basis, usually for two consecutive treatments 4–6 weeks apart. At one attendance they were given an oral dose, and on the other occasion the dose was administered i.v. Patients received melphalan 7 mg/m^2 orally or 15 mg/m^2 i.v. as part of their normal therapy, prednisolone being given concurrently. Reduction in the melphalan doses were made in the presence of leukopenia, and patients actually received 8 ± 3 and 17 ± 7 mg respectively.

Investigation design

Patients were allowed breakfast and drinks before coming to the hospital for a 9 a.m. start. A baseline blood sample was taken before administration of the drug(s). Blood samples (10 ml) were taken during i.v. investigations at 5, 15, 30, 45 and 75 min, and 2, 3, 4, 6, 8 and 10 hr, and during oral investigations at $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, 4, 5, 6, 8 and 10 hr. They were collected into heparinised tubes and placed on ice before centrifugation at 1800 *g* at 4°C to recover plasma. Urine was collected into 10% HCl in fractions of 0–6, 6–10 and 10–24 hr. Urine and plasma samples were processed immediately for radioactive counting, and the remainder of the samples frozen at −40°C until melphalan concentrations could be determined by HPLC.

Plasma analysis

Melphalan levels in plasma were measured by an HPLC method described in detail elsewhere [8]. Briefly, melphalan and added internal standard (500 ng dansyl arginine) were adsorbed from 1 ml plasma onto a 5 × 25 mm column of well-washed XAD-2 resin. The column was washed with 10 ml of water, the drug eluted with 1.5 ml methanol and the eluates kept at −40°C until HPLC analysis could be performed. Injections of 200 μl of eluate were used in an HPLC system made up of a Constametric I pump and UV III monitor (both from Laboratory Data Control, Stone), and a 4.6 × 250 mm 5 μm ODS-Spherisorb column. The mobile phase used was methanol : 1.5 mM sodium lauryl sulphate (2 : 1; v/v) adjusted to pH 3.2 with sulphuric acid. Melphalan concentrations in urine samples were measured by injecting 20 μl of filtered urine directly into the HPLC system.

Radioactive levels were determined in plasma and deproteinised plasma (giving a measure of melphalan plus its two degradation products monohydroxy- and dihydroxy-melphalan (M + MOH)) using liquid scintillation cocktail (NE 260) and counter (NE 8312) from Nuclear Enterprises, Edinburgh. Cpm were converted to dpm using a previously constructed quench curve. The same deproteinising method was used to prepare samples for both radioactive counting and amino acid analysis: sulphosalicylic acid (0.5 ml of a 5% w/v solution) was added to 0.5 ml plasma, the resultant precipitate removed by centrifugation and the supernatant used for analysis. For amino acid determinations a Rank Hilger Chromaspek amino acid analyser containing a 350 mm ion exchange column was used with a lithium citrate and borate buffer system [12].

Serum creatinine and paraprotein and urine Bence–Jones protein concentrations were measured by routine procedures in the Area Central Laboratories at the Royal United Hospital. Glomerular filtration rates (GFR) were calculated using the formula adapted from Cockcroft and Gault [13]:

$$\mathrm{GFR} = \frac{k[140 - \mathrm{age(yr)}] \cdot \mathrm{wt\ (kg)}}{\mathrm{plasma\ creatinine\ (\mu M)}},$$

where $k = 1.23$ for males or 1.04 for females.

Data analysis

Data analysis was performed with reference to Greenblatt and Koch-Weser [14] and Curry [15]. Biexponential curves of the form

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad (1)$$

(where C is the concentration of melphalan in plasma (ng/ml) at time t (min), A and B are parameters measured in ng/ml, and α and β are apparent first-order distribution rate constants measured in min^{-1}) were fitted to the measured i.v. melphalan concentration (by HPLC) vs time data with the aid of the non-linear regression computer program NONLIN [16]. In calculating the best fit each value of y was given a weighting proportional to $1/y^2$. Oral

data were fitted to a similar equation (but with equal weighting of all y values):

$$C = -B(e^{-k_a(t-t_0)} - e^{-\beta(t-t_0)}), \quad (2)$$

where k_a is the apparent absorption rate constant (min^{-1}) and t_0 is the delay (min) before absorption starts. No α term was used as k_a was generally smaller than the α determined from the i.v. data. If t_0 was found to be less than 5 min, then the data was re-run through NONLIN with $t_0 = 0$.

Plasma half-lives ($t_{\frac{1}{2}}$; min) were calculated using the general formula

$$t_{\frac{1}{2}}x = \frac{0.693}{x}, \quad (3)$$

where $x = \alpha$, β, or k_a. Areas under the curve (AUCs) ($min \cdot \mu g/ml \cdot (mg\ dose)^{-1}$) were calculated per mg dose of melphalan (as Brox *et al.* [17] had found a good correlation between dose and AUC) with the formula

$$AUC = \frac{10^{-3}}{dose} \cdot \left(\frac{A}{\alpha} + \frac{B}{\beta}\right), \quad (4)$$

where the term A/α is substituted for $-B/k_a$ for oral data, and the dose is measured in mg.

The apparent volume of the central compartment V_c (litres) and the apparent volume of distribution V_d (litres) were calculated for i.v. data only using equations 5 and 6 respectively:

$$V_c = \frac{10^3 \cdot dose}{A + B} \quad (5)$$

$$V_d = \frac{1}{AUC \cdot \beta}. \quad (6)$$

Total body clearance C_b (ml/min) and renal clearance C_r (ml/min) were calculated for i.v. data from the following relationships:

$$C_b = \frac{10^3}{AUC} \quad (7)$$

$$C_r = \frac{Exc}{AUC}, \quad (8)$$

where Exc = 0–24 hr renal excretion of melphalan measured in μg/(mg dose). The fraction of oral melphalan absorbed (F) was calculated as

$$F = \frac{AUC\ p.o.}{AUC\ i.v.}. \quad (9)$$

AUCs were also integrated over 0–10 hr for plasma total radioactivity (dpm/ml) and M+ MOH radioactivity by trapezoidal estimation (units calculated as $min \cdot 10^3$ dpm/ml $\cdot$ (10^6 dpm in dose)$^{-1}$ which give the same values as $min \cdot \mu g/ml \cdot (mg\ dose)^{-1}$ when converted to actual mass of melphalan). Equation 9 was then used on this data to give figures for the absorption of radioactivity. Correlation and regression analysis was performed using the computer statistical package MINITAB [18].

RESULTS

Table 1 shows the details of the six male and three female patients studied in this investigation. The average age was 68 yr. According to Salmon and Durie's system, most patients presented with stage IIIA myeloma [19]. Seven patients had IgG myeloma, JW had IgA and CL had light chain disease. The decrease in serum paraprotein level six months after first treatment, and how long this was sustained for, is given as an indication of patient response. Glomerular filtration rates calculated from plasma creatinine concentrations were very similar to the creatinine clearance values where the latter had been investigated.

As breakfast was allowed before oral (and i.v.) investigations and was found to possibly effect absorption, Table 1 also gives an indication of the food consumed. Plasma leucine and glutamine concentrations were measured as these amino acids reduce melphalan uptake into cells *in vitro* and therefore could effect melphalan disposition [20–22]. They averaged 127 ± 37 and 432 ± 135 μM respectively for the p.o. investigations and 119 ± 31 and 454 ± 105 μM before i.v. administration.

Figure 1 shows a typical plasma decay curve for melphalan in one patient after doses both p.o. (8 mg) and i.v.(28.5 mg). Figure 2 shows the same patient's radioactive decay data. This gives an indication of the very long half-life of radioactivity in plasma (in the order of 7–10 days), which is presumably due to alkylated plasma proteins. This long half-life was not followed for an adequate period to describe the curve accurately and thus the AUCs for the radioactive data were calculated for 0–10 hr (see Tables 3 and 4).

The result of fitting the melphalan concentration vs time data to biexponential equations is shown in Table 2. The β phase of decay is seen to have similar parameters after both i.v. and oral administration, whereas both k_a and α are seen to be very variable.

Table 1. Patient details

Patient	Age (yr)	Weight (kg)	Response PPL* decrease (%)	Response Sustained for (months)	Glomerular filtration rate (ml/min)	Breakfast eaten before p.o. investigation†	Melphalan dose (mg) p.o.	Melphalan dose (mg) i.v.
CL	76	60	−‡	−‡	35	4	5	8
NC	70	70	65	>30	38	1	5	12.5
JW	72	64	40	>27	62	1	7	16
PP	61	65	50	12	109	3	9	12
ED	77	80	50	>20	54	3	8	28.5
IB	65	51	75	>6	52	4	10	11
VT	71	71	?	>40	72	3	6	12.5
AH	66	67	70	>6	64	3	12	25
MW	57	69	35	20	65	2	12	25

*Paraprotein level decrease six months after first treatment.
†0, Drink only; 1, cereal; 2, cereal + toast; 3, egg; 4, fried breakfast.
‡CL had Bence-Jones proteinuria. He died 7 months after first treatment.

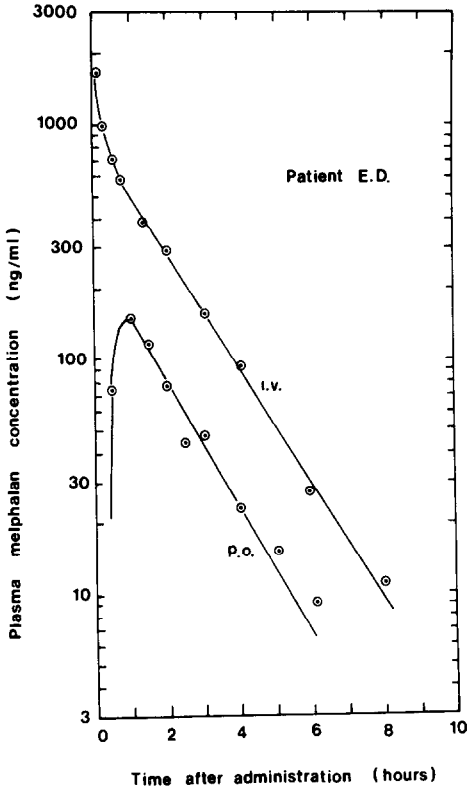

Fig. 1. Plasma levels of melphalan in patient ED after 28 mg *i.v. and* 8 mg *p.o. The computer-fitted curves are drawn through the data.*

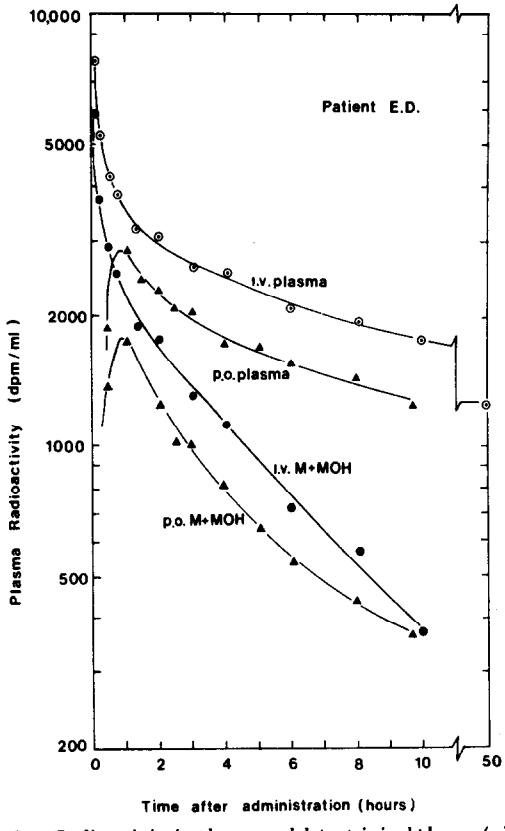

Fig. 2. Radioactivity in plasma and deproteinised plasma (giving a measure of melphalan plus its degradation products: M + MOH) from patient ED after 60 μCi [^{3}H]-*melphalan i.v. and p.o. Non-radioactive doses of melphalan were* 28.5 mg *i.v. and* 8 mg *p.o.*

Table 2. Parameters (calculated by computer) of the equations used to obtain the best fit of concentration versus time data

Parameter	p.o.	i.v.
A (ng/ml (mg dose)$^{-1}$)	—	65.1 ± 26.5
α(mins^{-1})	0.0737* ± 0.0997	0.147 ± 0.181
B (ng/ml (mg dose)$^{-1}$)	28.3 ± 15.9	21.0 ± 6.4
β (mins^{-1})	0.00818 ± 0.00275	0.00864 ± 0.00199

*Value of k_a.

Tables 3 and 4 give the kinetics of oral and intravenous administration respectively. The data show that from 0–10 hr (the time scale of the investigation) the AUC of M + MOH is approximately half of the AUC of total plasma radioactivity and approximately 60% of the M + MOH AUC is made up of melphalan. Half-lives of the absorption phase of oral melphalan ($t_{\frac{1}{2}}k_a$) read with delays that needed to be incorporated in some cases (Table 3) suggest a very variable absorption process. The extent of absorption (calculated as AUC p.o./AUC i.v.) is also seen to be very variable (Table 5).

Total body clearance and renal clearance of i.v. melphalan were calculated per kg body weight to be 5.26 ± 1.73 and 0.64 ± 0.26 ml/min/kg respectively. Although it is possible that there are actually three separable compartments (see Discussion), and therefore considerable caution should be exercised in interpreting the results, the pharmacokinetic data (Table 2) were fitted to a two-compartment open system model [15]. Values for k_e (rate of elimination), k_{cp} (rate constant from the central to the peripheral compartment) and k_{pc} were calculated to be 0.029 ± 0.013, 0.037 ± 0.020 and 0.089 ± 0.152 respectively. The high S.D. for k_{pc} was caused by eight of nine values

*Table 3. Kinetics of oral melphalan**

Patient	Delay† (min)	$t_{\frac{1}{2}}k_a$ (min)	$t_{\frac{1}{2}}\beta$ (min)	AUC(min · μg/ml · (mg dose)$^{-1}$) M (0–∞ hr)	Plasma (0–10 hr)	M + MOH (0–10 hr)
CL	113	18.2	145	2.29	7.0	3.4
NC	50	19.7	106	1.93	9.0	4.3
JW	0	26.5	78	1.41	3.8	1.4
PP	45	36.4	49	0.98	5.8	2.7
ED	23	6.3	68	2.39	7.9	3.4
IB	0	10.5	110	3.17	6.5	3.4
VT	0	23.4	95	4.37	9.2	5.5
AH	32	62.1	87	1.69	6.0	3.7
MW	27	2.1	94	1.86	6.8	4.0
Mean	32	22.8	92	2.23	6.8	3.5
S.D.	36	18.1	27	1.02	1.7	1.1

*Plasma melphalan concentration vs time data was fitted to an equation of the form $C = -B(\exp[-k_a(t-t_0)] - \exp[-\beta(t-t_0)])$ from which $t_{\frac{1}{2}}k_a$, $t_{\frac{1}{2}}\beta$ and the melphalan AUC were calculated. The AUCs for plasma and M + MOH were calculated from radioactive determinations of plasma and deproteinised plasma respectively.
†Delay before absorption of melphalan starts needed for the best fit of the data (t_0).

Table 4. Kinetics of i.v. melphalan

Patient	$t_{\frac{1}{2}}\alpha$ (min)	$t_{\frac{1}{2}}\beta$ (min)	AUC(min · μg/ml · (mg dose)$^{-1}$) M (0–∞ hr)	Plasma (0–10 hr)	M + MOH (0–10 hr)	V_c (litres)	V_d (litres/kg)
CL	1.1	94	2.22	7.6	3.3	7.7	1.02
NC	7.3	93	3.22	14.4	7.6	19.7	0.59
JW	10.5	77	4.40	14.7	7.0	9.1	0.40
PP	5.3	51	2.08	9.9	4.5	12.2	0.55
ED	6.0	75	3.71	11.2	5.6	12.0	0.36
IB	9.1	95	3.81	10.9	4.9	9.3	0.71
VT	8.2	96	4.39	12.0	5.6	10.8	0.44
AH	1.4	85	2.34	13.2	7.2	15.4	0.80
MW	10.7	83	2.32	9.5	5.6	18.9	0.75
Mean	7.7	83	3.17	11.5	5.7	12.8	0.62
S.D.	3.3	14	0.95	2.4	1.4	4.3	0.21

Plasma melphalan concentration vs time data were fitted to an equation of the form $C = A\exp(-\alpha t) + B\exp(-\beta t)$ from which $t_{\frac{1}{2}}\alpha$, $t_{\frac{1}{2}}\beta$, melphalan AUC, V_c and V_d were calculated. The AUCs for plasma and M + MOH were calculated from radioactive determinations of plasma and deproteinised plasma respectively.

Table 5. Fraction of oral melphalan absorbed (F) calculated as AUC p.o./AUC i.v.

Patient	F by HPLC	F by radioactivity
CL	1.03	0.92
NC	0.60	0.62
JW	0.32	0.26
PP	0.47	0.58
ED	0.64	0.70
IB	0.83	0.60
VT	1.00	0.98
AH	0.72	0.45
MW	0.80	0.72
Mean	0.71	0.65
S.D.	0.23	0.22

averaging 0.039 ± 0.013 and a value of 0.495 for patient CL.

Renal excretion of melphalan (as measured by HPLC and radioactivity) is given in Table 6. In agreement with Tattersall *et al.* [23], little excretion of radioactivity was seen after the first 24 hr.

DISCUSSION

Despite melphalan being in clinical use for over 25 yr, its pharmacokinetics are inadequately defined. Tattersall *et al.* [23] using [^{14}C]-melphalan described the kinetics of label after oral and i.v. administration, but little analysis of plasma-free melphalan was undertaken. Alberts and colleagues, using patients with a variety of different neoplasms and their HPLC method [6], have published the pharmacokinetics of i.v. melphalan [9] and high dose oral melphalan [10]. Three other groups have published a little data on melphalan pharmacokinetics [7, 17, 24].

In this paper we have described the pharmacokinetics of oral and i.v. melphalan as seen in multiple myeloma patients taking normal therapeutic doses of the drug. With our local regime, oral doses vary from 5 to 15 mg/day and i.v. doses from 10 to 30 mg. Plasma melphalan concentrations after the lower doses are only easily measured by HPLC using the sensitive method that we have developed [8], allowing detection of about 5 ng/ml. Peak plasma levels of melphalan after oral administration averaged 11.5 ± 5.5 ng/ml per mg dose (range 6.4–20.7), with four out of the nine patients having peak levels between 40 and 60 ng/ml—the limit of sensitivity of the method of Chang *et al.* [6].

Our results from multiple myeloma patients presented here are similar to the work of Alberts *et al.* [9, 10] and Tattersall *et al.* [23] using patients with different neoplasms. We have found a very long terminal half-life for plasma [^{3}H], of which only a small proportion was soluble by 10 hr. This suggests that melphalan alkylates plasma proteins extensively, the half-life of the label being approximately the same as that of plasma proteins (Fig. 2). Melphalan itself, on the other hand, decays biphasically after i.v. administration, with half-lives of 7.7 and 83 min (Table 4), values similar to those of Alberts *et al.* [9] but with less scatter. However, there must be an earlier phase of distribution from the plasma to the rest of the 'central' compartment (average apparent volume = 12.81, Table 4) which is extremely rapid and is almost over by the time the first sample is taken at 5 min. The $t_{\frac{1}{2}}$ for patient CL of 1.1 min might be an indication of this very rapid phase, the data seeming to lack the α phase. This very rapid and quite extensive removal of material from the plasma compartment is likely to be the influx of melphalan into cells well perfused with plasma. We have recently confirmed the findings of Vistica [25] and Begleiter *et al.* [26] that melphalan is taken up into L1210 cells *in vitro* with a half-life of less than 5 min [27], and a very similar observation was made using peripheral blood lym-

Table 6. Urinary excretion of melphalan

	0–6 hr	6–24 hr	24–48 hr	0–24 hr
Melphalan i.v.	12.3	1.9	—	14.2
Melphalan p.o.	9.8	5.4	—	14.2
Radioactivity i.v.	31.3	13.9	2.7	45.2
Radioactivity p.o.	19.1	11.3	3.9	30.5

Values are mean percentages of dose excreted with $n = 9$ except for the 24–48 hr figures, where $n = 2$.

phocytes from patient JW *in vitro* (A. D. Martin, A. G. Bosanquet and E. D. Gilby, unpublished observation). Thus three compartments would most likely provide the best fit of the data if earlier time points were possible.

This same work [25–27] had led us to suspect that amino acid levels might show some correlation with the pharmacokinetics of melphalan due to the effect of leucine and glutamine on the uptake of melphalan by cells *in vitro*. However, although a number of possible relationships were seen, none were statistically significant.

The figures for excretion of melphalan (Table 6) are similar to those of Alberts *et al.* [9, 10] and Tattersall *et al.* [23]. Although a correlation was found to exist between the glomerular filtration rate (Table 1) and the i.v. $t_{\frac{1}{2}}\beta$ (Table 4) ($r = 0.782$, $P < 0.02$), it was surprising to find no significant relationship between glomerular filtration rate and excretion or renal clearance of melphalan.

No correlation was found between response (Table 1) and fraction of oral melphalan absorbed (Table 5) when all the patients were studied. However, if the one patient with light chain disease (CL) is not included (as the prognosis is so much poorer) a trend ($P \simeq 0.1$) does suggest that greater absorption leads to a better response. If this is confirmed, it could be the reason for the observation of McIntyre and colleagues [11] that i.v. melphalan elicits a better response than the more usual oral administration.

So that the pharmacokinetics of melphalan could be determined in patients in a situation as similar to the way in which they would normally take the drug, we have allowed breakfast to be eaten before the investigation started. Concurrent steroids were also given with the melphalan as the two drugs are almost invariably given together and prednisolone had been found not to effect the uptake of melphalan into L1210 cells *in vitro* [27]. That the fraction of melphalan absorbed in our patients (0.71 ± 0.23; Table 5) was larger than the value of Alberts' fasting patients (0.56) [10] suggests that taking melphalan after a meal increased the absorption of the drug. A Spearman's rank correlation coefficient calculated between the fraction of drug absorbed (by HPLC) and the breakfast eaten (Table 1) confirmed this ($r = 0.708$; $P < 0.05$). Thus the data could suggest that melphalan should be taken after a good meal to obtain greatest absorption. This possibility is currently being investigated.

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