Cancer Chemother Pharmacol (1983) 10:212–216

Cancer Chemotherapy and Pharmacology

Pharmacokinetics of Melphalan in Children Following High-Dose Intravenous Injection

I. A.-K. Taha[1], R. A. Ahmad[1], D. W. Rogers[2], J. Pritchard[2], and H. J. Rogers[1]

[1] Department of Clinical Pharmacology, Guy's Hospital Medical School, London SE1 9RT
[2] Department of Haematology and Oncology, The Hospital for Sick Children, Great Ormond Street, London WC1N 3JH, England

Summary. *The pharmacokinetics of high-dose IV melphalan (140 or 220 mg m^{-2}) were studied after 12 administrations in 10 children (aged 2.5–16 years) undergoing chemotherapy for either neuroblastoma or Ewing's tumour. To assess whether a simpler and less expensive nitrobenzylpyridine (NBP) spectrophotometric assay for alkylating activity was a satisfactory alternative to high-pressure liquid chromatography (HPLC), the plasma melphalan concentration was estimated by both methods in five cases.*

Analysis of the disposition of melphalan gave a mean half-life of 1.3 ± 1.0 (SD) h, clearance 18.4 ± 9.4 $l \cdot h^{-1} \cdot m^{-2}$, and apparent volume of distribution 26.3 ± 18.0 $l \cdot m^{-2}$. These pharmacokinetic parameters were similar to those found in adults: no correlation was found between any parameter and age or glomerular filtration rate.

NBP alkylating activity determinations yielded consistent results and good correlation with plasma melphalan concentration. However, concordance analysis indicated a consistent bias, the NBP assay always giving lower estimates of plasma melphalan concentration: HPLC assay therefore remains the method of choice for determining plasma melphalan pharmacokinetics.

Introduction

High-dose chemotherapy followed by autologous marrow transplantation is currently under trial in the treatment of advanced malignancy [12]. Melphalan is particularly suitable for such treatment, because in the doses employed toxicity is largely confined to myelosuppression and because its relatively short half-life means that the marrow can be reinfused soon (up to 24 h) after marrow harvest without the need for cryopreservation. This procedure has now been introduced in paediatric oncology [13]. It is a paediatric maxim that children cannot be treated as miniature adults, and many drugs show pharmacokinetic characteristics in children which differ from those observed in adults [14]. Little information is available relating to the pharmacokinetics of alkylating agents in children, although recently it has been shown that the half-life of cyclophosphamide is shorter in children than in adults [15].

Children undergoing treatment for disseminated neuroblastoma may have sustained a variable degree of renal impairment as a result of prior treatment with cisplatin. Experience in adult patients has suggested, in addition, a direct toxic effect of mephalan on the kidneys, and for this reason children receiving melphalan have been subjected to a forced diuresis at the time of melphalan infusion. Melphalan pharmacokinetics might thus be influenced both by pre-existing renal impairment and by forced diuresis after administration.

Information on melphalan pharmacokinetics in children is therefore needed for four reasons:
1) To determine whether melphalan dosage can be directly extrapolated from adults to children;
2) To decide whether melphalan dosage needs adjustment in children with reduced renal function;
3) To evaluate the need for forced diuresis; and
4) To establish the earliest time at which marrow can be reinfused without risk of persisting melphalan activity.

It is probable that unchanged melphalan, rather than its hydroxylated products monohydroxy- and dihydroxymelphalan, is the major or perhaps even the sole source of the cytotoxic, alkylating activity of the drug. Careful studies of the pharmacokinetics of unchanged melphalan are therefore basic for an understanding of its clinical efficacy.

Materials and Methods

Patients. Twelve studies were carried out in children under treatment for either neuroblastoma (8 patients, 9 studies) or Ewings's sarcoma (2 patients, 3 studies). All received either 140 mg · m^{-2} or 220 mg · m^{-2} melphalan IV prior to marrow autografting. All except SD were patients at the Hospital for Sick Children, Great Ormond Street. Clinical details of the patients are given in Table 1. Glomerular filtration rates were measured in six patients by the ^{51}Cr EDTA method. A forced diuresis regimen (details shown in Table 1) was used on six occasions.

This procedure was thought to be unneccessary for patients studied in the later part of our series and the practice was discontinued. All patients had received other chemotherapy before high-dose melphalan, those with neuroblastoma receiving 3-weekly pulses of the OPEC regimen, and those with Ewing's sarcoma receiving 3-weekly pulses of CVA and, in one of the two patients, VAC. Details of these regimens are given as a footnote to Table 1. Blood samples (3–5 ml) were taken at times as close as were compatible with patient care to 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h. Both peripheral and central venous lines were placed under the anaesthetic for the

Reprint requests should be addressed to H. J. Rogers

Table 1. Details of patients studied

	Patient									
	SH	LC	SD	MG	HC	NM	JP	SJ	NH	MW
Sex	M	M	F	F	M	M	M	M	F	M
Age (years)	16	5	5	6	6	6	2½	10	3	4
Weight (kg)	52	16.5	[b]	17.9	20	16	13.7	16.9[c]	11.5	19.6
Melphalan dose (mg/m²)	140	140	140	220	140	140	140[c] 140[d]	140[c] 220[d]	220	140
Serum creatinine (μmol/l[1])										
Pre-treatment	70	54	[b]	30	130	82	120[c]	31[c]	83	93
Post-treatment	98	62	[b]	45	92	78	125[c] [b, d]	25[c] [b, d]	43	70
Glomerular filtration rate (ml/min[1]/1.73 m²)	[b]	60	[b]	[b]	40	33	30.5[c]	[b]	46	47
Diuresis	Yes	Yes	[b]	Yes	Yes	Yes	Yes[c] No[d]	No[c] No[d]	No	No
Diagnosis	Neuro-blastoma	Neuro-blastoma	Neuro-blastoma	Ewing's sarcoma	Neuro-blastoma	Neuro-blastoma	Neuro-blastoma	Ewing's sarcoma	Neuro-blastoma	Neuro-blastoma
Previous treatment[a]	OPEC × 6	OPEC × 6	[b]	CVA × 6	OPEC × 6	OPEC × 11	OPEC × 7	VAC × 9 CVA × 8	OPEC × 8	OPEC × 9

[a] OPEC: Day 0, vincristine (Oncovin) 1.5 mg/m², cyclophosphamide 600 mg/m²;
Day 1, cisplatinum 100 mg/m² preceded by hydration and followed by mannitol diuresis;
Day 4, VM 26 (Epipodophyllotoxin) 150 mg/m²
CVA: cyclophosphamide 600 mg/m², vincristine 1.5 mg/m², actinomycin 40 mg/m² together on day 0
VAC: vincristine 1.5 mg/m², actinomycin D 1.0 mg/m², cyclophosphamide 600 mg/m²

Forced diuresis:
h 1: 0.9% NaCl with added KCl 20 ml/kg/h;
h 2–3: 0.45% NaCl with added KCl 15 ml/kg/h;
h 3–6: 0.45% NaCl with added KCl 10 ml/kg/h
[b] Information unavailable
[c] First administration
[d] Second administration

bone marrow harvest, the central line being a Hickman catheter for blood product, antibiotic, and nutritional support. Melphalan and, in one patient, VAC (vincristine 1.5 mg/m², actinomycin D 1.0 mg/m², cyclophosphamide 600 mg/m², all given on day 0) were administered through the central line, and blood samples were taken through the peripheral line without any distress or disturbance to the children. Samples were immediately centrifuged and the plasma removed and stored at −20° C until analysed.

Chemicals. Melphalan was donated by Wellcome Research Laboratories, Beckenham, Kent, England. Standard stock solutions of 10 mg in a mixture of 98 ml methanol and 2 ml acetic acid were stored at −20° C for up to 1 week. 4-4-Nitrobenzylpyridine and nor-nitrogen mustard were purchased from Aldrich Chemical Co. Ltd (Gillingham, Dorset, England). All other reagents were of Analar grade and purchased from BDH Ltd (Poole, Dorset).

Estimation of Plasma Melphalan Concentration. This was by a high-pressure liquid chromatographic technique based upon the methods of Chang et al. [5] and Flora et al. [10]. One millilitre of plasma was vortex-mixed for 30 s with 6 ml ethyl acetate containing 5% ethanol after addition of an appropriate concentration of benzophenone as internal standard. Of the organic phase, 5 ml was quantitatively transferred to a dry conical glass tube and carefully evaporated to dryness under a stream of cold air at room temperature. The residue was redissolved in 30 μl methanol and introduced onto the column via a Rheodyne 7125 valve fitted with a 20-μl loop. The column was a stainless steel tube (150 × 4.6 mm) packed with C_{18} Magnusphere (Magnus Scientific, Sandbach, Cheshire, England). The mobile phase consisted of a 60 : 40 mixture of water and methanol delivered at a rate of 1.5 ml/min at a pressure of 2,000 p.s.i. by an Altex 110 A pump. Separations were effected isocratically at ambient temperature and detection was by a fixed-wavelength (254 nm) ultraviolet detector (Applied Chromatography Systems Limited, Luton, Bedfordshire, England). Quantitation was made by construction of a standard curve of the ratio of peak heights of mephalan to internal standard versus the known concentrations of standard plasma samples run simultaneously with the unknown samples.

The mean recovery of melphalan by this method over the range 0.1–15 $\mu g \cdot ml^{-1}$ was 85.3% ± 4.3% (SD). The calibration curve was linear over the range

0.025–15 μg · ml^{-1} and quadruplicate standards were not more than 9% different at 0.2 μg · ml^{-1} and 8% at 15 μg · ml^{-1}. The lower limit of detection (peak height greater than twice background recorder deflection) was 25 ng · ml^{-1}. No endogenous plasma constituents produced interfering chromatographic peaks with the same retention time as melphalan, but in some cases substances were present which made estimation of monohydroxy- and dihydroxymelphalan impossible. No interference by the other drugs taken by this group of patients was noted.

Determination of Plasma NBP-Alkylating Activity. One millilitre of plasma was deproteinised with 1 ml acetone-ethanol (1 : 1) by vortex-mixing for 20 s, after which it was centrifuged for 10 min at 2,000 *g*. Of the protein-free supernatant, 1 ml was transferred to a 15-ml tube with a PTFE-lined screw top and 0.5 ml 5% 4-4′-nitrobenzylpyridine in acetone and 0.1 ml acetic acid were added. The pH was adjusted to pH 4.6 with 0.2 *M* acetate buffer (pH 4.6) and the tubes put into a water bath at 85° C for 15 min. They were then cooled in ice for 15 min and centrifuged. Then 1 ml supernatant was transferred to a glass cuvette, to which 1 ml triethylamine : acetone (1 : 1) was added. Absorbance was then measured immediately at a wavelength of 565 nm. This assay had a coefficient of variation between assays of 12.8% at 1 μg · ml^{-1} and 6.5% at 10 μg · ml^{-1}, with a lower limit of detection of 0.25 μg · ml^{-1}. The mean ratio of the absorbance of melphalan to that of the standard alkylating reference compound nor-nitrogen mustard (Nor-HN2), which can also be assayed by this method, was 0.445 over a concentration range of 1–6 μg · ml^{-1} and results from patient studies are expressed in terms of nor-HN2 equivalents.

Pharmacokinetic Analysis. The apparent first-order rate constant of elimination (k) was determined from the regression of ln (plasma melphalan concentration) on time. The area under the plasma melphalan concentration, time curve was approximated by the linear trapezoidal approximation with appropriate extrapolation for the unmeasured infinite portion of the curve. The systemic melphalan clearance (Cl) was determined from Cl = dose/AUC and the apparent volume of distribution (V_d) from V_d = Cl/k. All of these calculations assume a simple one-compartment model for melphalan disposition.

Results

The derived pharmacokinetic parameters are summarised in Table 2. Studies of IV melphalan kinetics in adults, using more intensive blood sampling [1], indicate that a pharmacokinetic model with at least two compartments is appropriate to describe melphalan disposition. Even our limited sampling schedule indicated that in some cases this model might be more appropriate and this is even more apparent in the plot of mean plasma melphalan concentrations with time (Fig. 1). Nevertheless, because of the clinical limitations on more frequent blood sampling in these children, the data points obtained were generally too sparse to characterise such a model rigorously in any individual patient, and the simpler model was therefore adopted. Derivation of the parameters of a pharmacokinetic model from the mean data curve is invalid, however, since it results in biased estimates of pharmacokinetic parameters [6].

Table 2. Derived pharmacokinetic parameters for melphalan following IV bolus administration to patients detailed in Table 1

Patient	Elimination rate constant k (h^{-1})	Half-life $t_{1/2}$ (h)	Systemic clearance Cl ($l/h^1/m^2$)	Apparent volume of distribution V_d (l/m^2)
SH	0.574	1.2	9.1	15.8
LC	0.551	1.8	22.6	41.0
SD	1.022	0.7	11.3	11.1
MG	1.281	0.5	34.4	36.9
HC	0.254	2.7	19.6	77.2
NM	0.279	3.6	4.2	15.1
JP (1)	0.568	1.2	11.1	19.5
JP (2)	1.290	0.5	25.1	19.4
SJ (1)	1.615	0.4	23.3	14.4
SJ (2)	0.999	0.7	31.4	31.4
NH	0.790	0.9	17.4	22.0
MW	0.499	1.4	10.7	21.4
Mean	0.810	1.3	18.4	26.3
SD	0.432	1.0	9.4	18.0

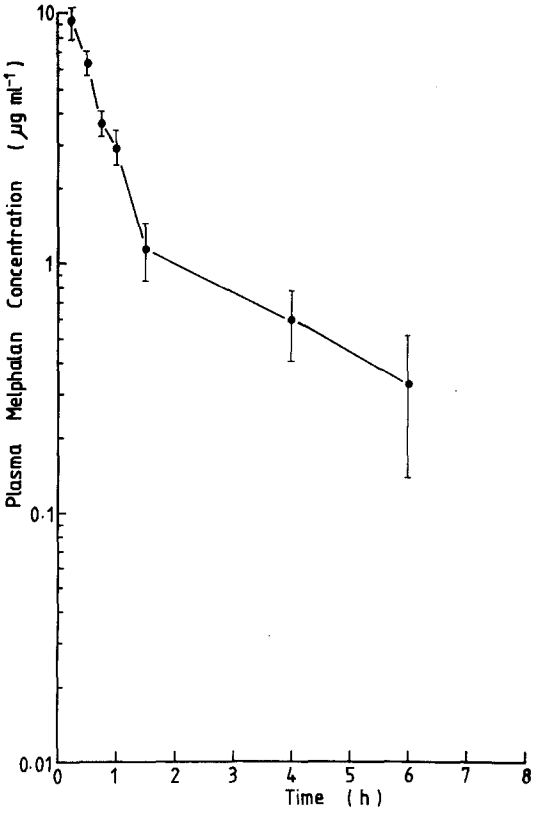

Fig. 1. Mean (SD, indicated by *bars*) melphalan plasma concentration, time profile for all patients studied. Each *point* is mean of three to eight estimates

We, like others [3], have found that melphalan is unreactive in the standard NBP technique devised by Friedman and Boger [11]. The modified NBP assay method described above was therefore devised to produce a sufficient yield of coloured NBP-product to allow spectrophotometric assay of melphalan. A comparison of the estimated concentrations of melphalan in plasma samples by both spectro-

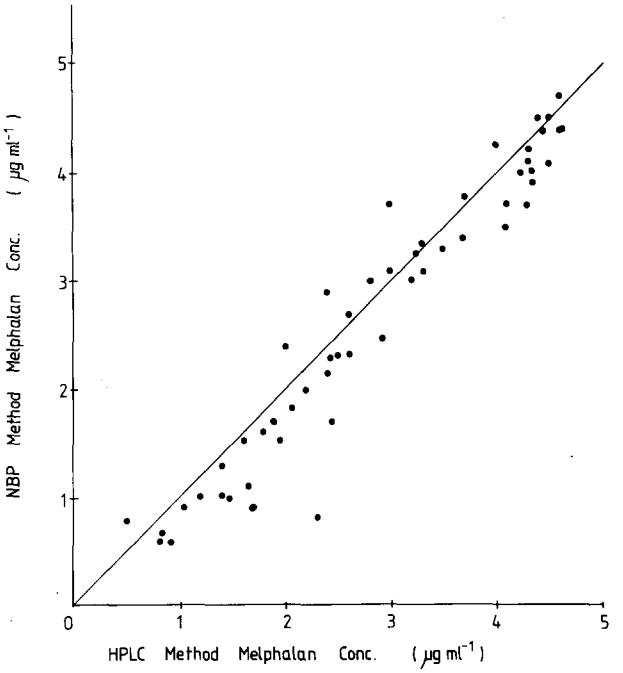

Fig. 2. Comparison between melphalan concentration ($\mu g \cdot ml^{-1}$) measured by HPLC (*abscissa*) and its alkylating activity expressed as $\mu g \cdot ml^{-1}$ melphalan (*ordinate*)

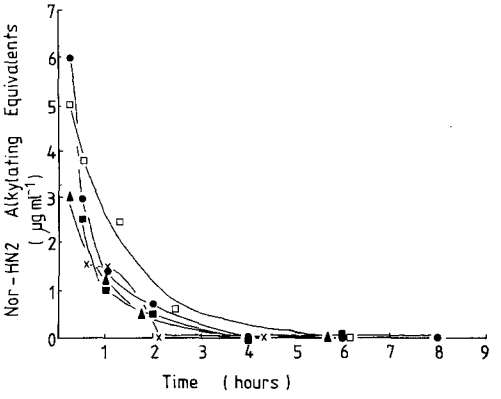

Fig. 3. Plasma L-PAM alkylating activity profiles for paediatric patients (▲ MG; × HC; ● SH; ■ LC; □ SD). Alkylating activity expressed as nor-nitrogen mustard alkylating equivalents ($\mu g \cdot ml^{-1}$): multiply by 0.442 to convert to melphalan concentration ($\mu g \cdot ml^{-1}$)

photometric (using authentic melphalan standards in place of nor-nitrogen mustard) and HPLC methods was made and the results appear in Fig. 2. The solid line in Fig. 2 is the line of identity. The linear regression between melphalan concentration estimated from the alkylating activity (dependent variable) and HPLC (independent variable) had a slope of 1.02 and intercept of 0.19 ($r = 0.97$). The slope was close to unity and shows that the regressions for the estimated melphalan concentration by each method versus the 'true' concentration are parallel. However, the intraclass correlation coefficient [9] was 0.677 and the variance ratio of mean square method/mean square residual in the analysis of variance was 17.3, which on 1.54 degrees of freedom is significant at $P < 0.01$. This indicates poor concordance and significant bias between the methods (as can be seen from Fig. 2) and thus the alkylkating activity measured by this NBP method cannot satisfactorily substitute for direct melphalan estimation, since it generally yields lower estimates of melphalan concentration. In view of these findings the plasma NBP-alkylating activity was estimated after only five of the melphalan administrations studied by HPLC. The individual patient concentration, time profiles are shown in Fig. 3. In view of the few data points a formal pharmacokinetic analysis could not be carried out. Nevertheless, approximate half-lives determined from log linear regression were SH, 0.61 h; LC, 0.70 h; SD, 0.75 h; MC, 0.59 h (there were insufficient data from HC to yield reliable estimates). The half-lives of melphalan and NBP-alkylating activity were therefore of similar magnitude.

Discussion

After IV injection, even in these high doses, melphalan's plasma concentration rapidly declines, apparently in a first-order manner. The mean half-life of 1.3 ± 1.0 h is longer than the published value of 0.36 ± 0.11 h in a series of 15 melphalan administrations (30 mg · m^{-2}) to adults with multiple myeloma [4] but is shorter than the value of 1.78 ± 0.35 h after a dose of 0.6 mg · kg^{-1} in adults with melanomas or uterine or ovarian malignancies [1]. In the former study, however, the blood sampling protocol was apparently of short duration and this could result in underestimation of the half-life. The systemic clearance found in this adult study [4] was 30.1 ± 12.6 l · h^{-1} · m^{-2}, implying a higher total-body clearance than would be expected from the shorter half-life, and the mean apparent volume of distribution was 16.3 ± 8.6 l · m^{-2}. The significance (unpaired Student's *t*-test) of the differences of the means between the present study and these values was 0.08 for clearance and 0.102 for apparent volume of distribution, so that in neither case was a significant difference found for these parameters in the children as against adults. The relatively large dispersion (relative standard deviations were 0.53 for k; 0.51 for clearance, and 0.69 for apparent volume of distribution) may account for the differences in magnitude of the means in these two series and their lack of significant difference. In the present study no significant correlation was demonstrated between melphalan clearance and age. These results therefore show no evidence of a significant difference in the pharmacokinetics of unchanged melphalan between children of 2 years or more and adults.

Melphalan therapy may need to be used in patients with renal insufficiency due to disease such as myeloma or to previous treatment with nephrotoxic drugs such as cisplatin. It has been suggested that melphalan dosage should be reduced in uraemic myeloma patients on the assumption that the rate of melphalan urinary excretion could be decreased in the presence of renal failure [7, 16]. In patients with myeloma and renal insufficiency (BUN > 30 mg/100 ml) there was a significantly higher frequency of severe leucopenia following IV, but not PO, melphalan than after administration to patients with normal renal function [7]. It has also been shown that in dogs with renal insufficiency due to subtotal nephrectomy there was a significant increase in the myelotoxic effects of melphalan [2]. Although a significant decrease in systemic and renal clearance of melphalan was demonstrated in these dog studies, the overall fraction of melphalan clearance due to urinary elimination was only of the order of 5%–6%, and the main change in melphalan clearance must therefore have been in the non-renal component. In man the renal contribution to

systemic clearance is similarly minor, only 13.0% ± 4.5% of an IV dose of 0.6 mg · kg^{-1} appearing in the urine over 24 h [1]. In the six patients in the present study for whom reliable glomerular filtration rates are available, the correlation coefficients for the relationship of glomerular filtration rate and melphalan clearance and elimination rate constant were only 0.61 and 0.36, respectively. Although an increase of melphalan toxicity in the presence of renal insufficiency has been observed in adult patients, the mechanism for this is by no means clearly related to changes in melphalan elimination associated with decreased renal melphalan excretion, and could perhaps be related to changes in the melphalan concentration, time profile [7] or to alterations in the bone marrow's regenerative capacity.

We therefore conclude that melphalan kinetics are similar in children and adults and are not influenced to any significant extent by moderate decreases in renal function. The observations are consistent with the concept that the major route for melphalan elimination is spontaneous hydrolysis rather than enzyme-mediated metabolism [1, 8]. In clinical practice we suggest:

1) melphalan dosage in children can be calculated from regimens found to be effective in adults by appropriate correction for surface area;

2) renal impairment down to a glomerular filtration rate of 30 ml · min^{-1} · 1.73 m^{-2} as seen after cisplatin therapy is not an indication for reduction in melphalan dosage;

3) forced diuresis is not necessary after IV high-dose melphalan.

4) autologous marrow can sefely be reinfused between 12 and 24 h after high-dose melphalan.

Acknowledgements. The authors thank the Nursing Staff and House Officers of the Great Ormond Street Hospital Bone Marrow Transplant Team for their careful collection of blood samples, and Dr Alan Craft for providing samples from patient SD. JP and DWR were supported by the Leukaemia Research Fund.

References

1. Alberts DS, Chang SY, Chen H-SG, Moon TE, Evans TL, Furner RL, Himmelstein K, Gross JF (1979) Kinetics of intravenous melphalan. Clin Pharmacol Ther 26: 73
2. Alberts DS, Chen H-SG, Berg D, Mason DL (1981) Effect of renal dysfunction in dogs on the disposition and marrow toxicity of melphalan. Br J Cancer 43: 330
3. Bramwell V, Calvert RT, Edwards G, Scarffe H, Crowther D (1979) The disposition of cyclophosphamide in a group of myeloma patients. Cancer Chemother Pharmacol 3: 253
4. Brox L, Birkett L, Belch A (1979) Pharmacology of intravenous melphalan in patients with multiple myeloma. Cancer Treat Rev 6 [Suppl]: 27
5. Chang SY, Alberts DS, Melnick LR, Walson PD, Salmon SE (1978) High-pressure liquid chromatographic analysis of melphalan in plasma. J Pharm Sci 67: 679
6. Cocchetto DM, Wargin WA, Crow JA (1980) Pitfalls and valid approaches to pharmacokinetic analysis of mean concentration data following intravenous administration. J Pharmacokinet Biopharm 8: 539
7. Cornwell CG, Pajak TF, McIntyre OR, Kochwa S, Dosik H (1982) Influence of renal failure on myelosuppressive effect of mephalan. Cancer and Leukaemia Group B Experience. Cancer Treat Rep 66: 475
8. Evans TL, Chang SY, Alberts DS, Sipes IG, Brendel K (1982) In vitro degradation of L-phenylalanine mustard (L-PAM). Cancer Chemother Pharmacol 8: 175
9. Fleiss JL (1975) Measuring agreement between two judges on the presence or absence of a trait. Biometrics 31: 651
10. Flora KP, Smith SL, Cradock JC (1979) Application of a simple high-performance liquid chromatographic method for the determination of melphalan in the presence of its hydrolysis products. J Chromatogr 177: 91
11. Friedman OM, Boger E (1961) Colorimetric estimation of nitrogen mustard in aqueous media. Anal Chem 33: 906
12. McElwain TJ, Hedley DW, Burton G, Clink HM, Gordon MY, Jarman M, Juttner CA, Millar JL, Milsted RAV, Prentice G, Smith IE, Spence D, Woods M (1979) Marrow autotransplantation accelerates haematological recovery in patients with malignant melanoma treated with high-dose melphalan. Br J Cancer 40: 72
13. Pritchard J, McElwain TJ, Graham-Pole J (1982) High-dose melphalan with autologous marrow for treatment of advanced neuroblastoma. Br J Cancer 45: 86
14. Rane A, Wilson JT (1976) Clinical pharmacokinetics in infants and children. Clin Pharmacokinet 1: 2
15. Sladek NE, Priest J, Doeden D, Mirocha CJ, Pathre S, Krivit W (1980) Plasma half-life and urinary excretion of cyclophosphamide in children. Cancer Treat Rep 64: 1061
16. Speed DE, Galton DAG, Swan A (1964) Melphalan in the treatment of myelomatosis. Br Med J 1: 1664

Received October 1, 1982/Accepted December 7, 1982