Cancer Chemother Pharmacol (1988) 22: 348–352

Cancer Chemotherapy and Pharmacology

Renal clearance and protein binding of melphalan in patients with cancer*

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Summary. The renal clearance of melphalan and the fraction unbound in plasma were determined after intravenous infusion of 5 mg/m^2 over 5 min in nine patients with cancer to obtain information regarding the mechanism of renal handling of melphalan. Four of the patients underwent bone marrow transplantation and also received an IV dose of 220 mg/m^2. Total melphalan clearance after the 5 mg/m^2 dose ranged from 66.0 to 272 ml/min per m^2; the percentage of the dose excreted unchanged in urine, from 2.5% to 92.8%; renal clearance, from 4.1 to 188 ml/min per m^2; the fraction unbound in plasma, from 0.0598 to 0.460; and $t_{1/2\beta}$, from 39.4 to 84.3 min. Unbound melphalan clearance and renal clearance calculated from the unbound fraction in plasma for each patient ranged from 441 to 3356 ml/min per m^2 and 15 to 961 ml/min per m^2 respectively and were not related to serum albumin, serum creatinine or creatinine clearance. The percentage of the dose exctreted and melphalan renal clearance were not related to urine flow. There was evidence of active secretion of melphalan in the kidney an possible reabsorption. There were no significant paired differences in melphalan disposition between the high- and low-dose studies. Highly variable renal clearance involving active secretion may contribute in part to large interpatient differences in the total plasma clearance of melphalan in patients with cancer.

Introduction

The anticancer drug melphalan (L-phenylalanine mustard) has been reported to have extremely variable total plasma clearance after i.v. infusion in patients with cancer. Estimates ranging from 70 to 1000 ml/min per m^2 have been reported [1–4, 6, 8, 9, 11, 13] and in our own studies the range was 104–694 ml/min per m^2 [9]. There are at least three possible contributors to this variability: differences in the renal clearance of unchanged drug, in the rate irreversible protein binding, or in the rate of drug hydrolysis. Irreversible binding and hydrolysis are non-renal or metabolic contributors to clearance, and the sum of these processes can be determined from the difference between total and renal melphalan clearance. Reversible protein binding, principally to serum albumin [7], does not contribute directly to the clearance of the drug but may alter both renal and non-renal clearance. Binding to albumin has been suggested to stabilise melphalan in plasma [5]. The relationship of the renal clearance of unbound melphalan to the glomerular filtration rate has not been adequately studied.

The aim of the present study was to determine the renal clearance and protein binding of melphalan after its i.v. infusion in patients with cancer to obtain information regarding the mechanism of renal handling of melphalan and to assess the importance of these factors in the variability in total clearance of the drug. The potential effect of the dose on these pharmacokinetic parameters was also examined in four of the patients, who received doses of 5 mg/m^2 and 220 mg/m^2 on separate occasions.

Materials and methods

Patients and drug administration. Nine patients with various neoplastic disorders were studied (Table 1); all gave informed consent before proceeding with the study. They fasted and all other anticancer medication and non-essential therapy were withheld on the day of the study. Melphalan (5 mg/m^2) was infused over 5 min into an arm vein of all patients; 1 week later patients 6–9 also received an i.v. dose of 220 mg/m^2 melphalan infused over 5 min. The latter patients subsequently underwent bone marrow transplantation. To promote adequate urine flow, patients received IV hydration of normal saline at a rate of 100 ml/h.

Specimen collection. Blood specimens (10 ml) were collected via an indwelling Teflon catheter from a forearm vein into ice-cold tubes containing heparin. They were taken pre-infusion and 5, 10, 15, 30 and 45 min as well as 1, 1.5, 2, 3, 4 and 5 h post-infusion. Samples were kept up to 1 h on ice and were then rapidly centrifuged at 4° C and the plasma stored at −20° C until analysis within 1 week. An aliquot of the plasma specimens collected 10 and 15 min post-infusion was centrifugally ultrafiltered at 4° C using the method described by Greig et al. [7]. The ultrafiltrate was stored at −20° C until analysis within 1 week. Urine was collected pre-infusion and as close as possible to the following times post-infusion: 0.5, 1, 2, 4 and 6 h, no mat-

* This study was supported by a grant from The Queen Elizabeth Hospital Research Foundation

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Table 1. Patient characteristics

Patient no.	Sex/Age (years)	Surface area (m^2)	Dose (mg/m^2)	Serum Albumin (g/l)	Serum Creatinine (μmol)	Calculated Creatinine clearance (ml/min per m^2)	Neoplasm
1	M/67	1.89	4.76	35	114	32	Mult. Myel.[a]
2	M/53	1.97	5.00	35	87	55	Mult. Myel.
3	F/76	1.48	5.00	26	87	27	Mult. Myel.
4	F/78	1.60	5.00	39	89	28	CML[b]
5	M/36	1.59	5.03	34	75	60	CML
6	F/36	1.60	5.00	38	61	59	CML
7	F/39	1.45	5.17	37	57	62	ALL[c]
8	M/32	2.18	4.58	36	83	78	CML
9	M/21	1.74	5.17	47	101	53	ALL
Mean	49	1.72	4.97	36	83	51	–
SD	21	0.25	0.18	5	18	17	

[a] Multiple myeloma
[b] Chronic myelotic leukaemia
[c] Acute lymphocytic leukaemia

ter how small the voided volume. The volume was immediately recorded and an aliquot immediately frozen and stored at −20° C until analysis within 1 week.

Assay methods. Plasma levels of melphalan were assayed by high-performance liquid chromatography with fluorescence detection [9]. The only modifications to the originally published method were the use of C18 Extra-Sep extraction columns (200 mg–3.0 ml, Lida Manufacturing Corp, Bensenville, Ill.) and an LS-1 fluorescence detector (Perkin-Elmer, Beaconsfield, England) with a 260-nm band-pass excitation filter and a 340-nm cut-off filter. Plasma ultrafiltrate and urinary melphalan levels were assayed by direct injection of the biological fluid into the chromatograph.

Pharmacokinetic analyses. Plasma levels of melphalan were fitted to a two-compartment model by nonlinear regression analysis using the Nonlinear module of Systat (Systat Inc, Evanston, Ill.) run on a Macintosh PC. Total area under the curve (AUC), clearance (CL), steady-state volume of distribution (V_{ss}) and terminal half-life ($t_{1/2\beta}$) were determined from the parameters of the equation describing the model; all parameters were corrected for the infusion time. The fraction of the dose excreted unchanged in urine (% excreted) was determined from the urinary concentrations of melphalan, and from this the renal clearance of melphalan (CL_R) was estimated. Non-renal clearance (CL_{NR}) was the difference between CL and CL_R. The unbound melphalan fraction in plasma (f_u) was determined from the average of the ratios of unbound to total melphalan plasma concentrations in the two samples collected 10 and 15 min post-infusion. These did not differ by more than 20% in any patient.

Statistical analyses. Paired comparisons between parameters determined for high- and low-dose melphalan within a patient were made using Student's paired *t*-test (two-tailed). Possible relationships between the melphalan disposition and various clinical parameters were determined using either linear or multilinear regression analysis. All statistical analyses were carried out using Statview

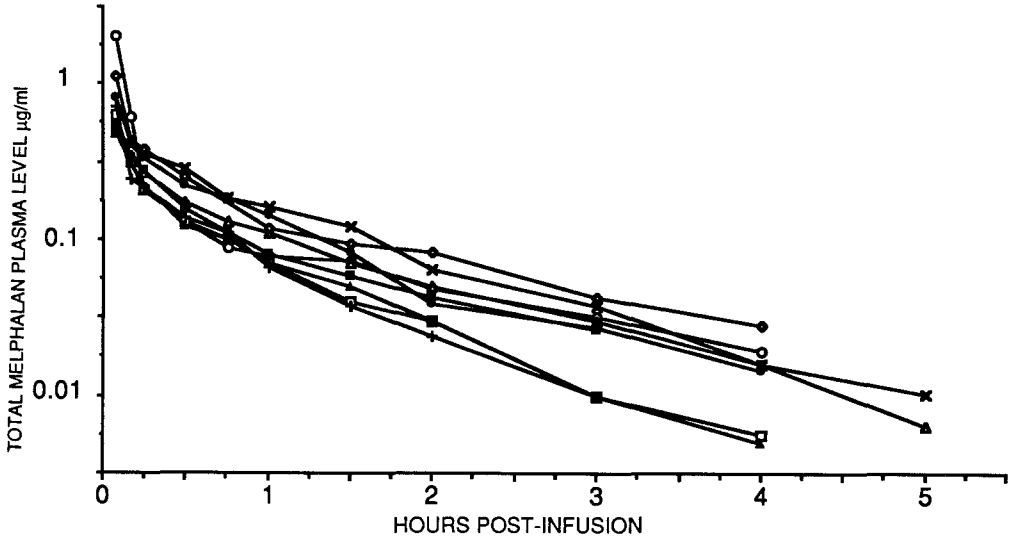

Fig. 1. Melphalan plasma levels in nine patients who received an i.v. dose of melphalan (5 mg/m^2) over 5 min

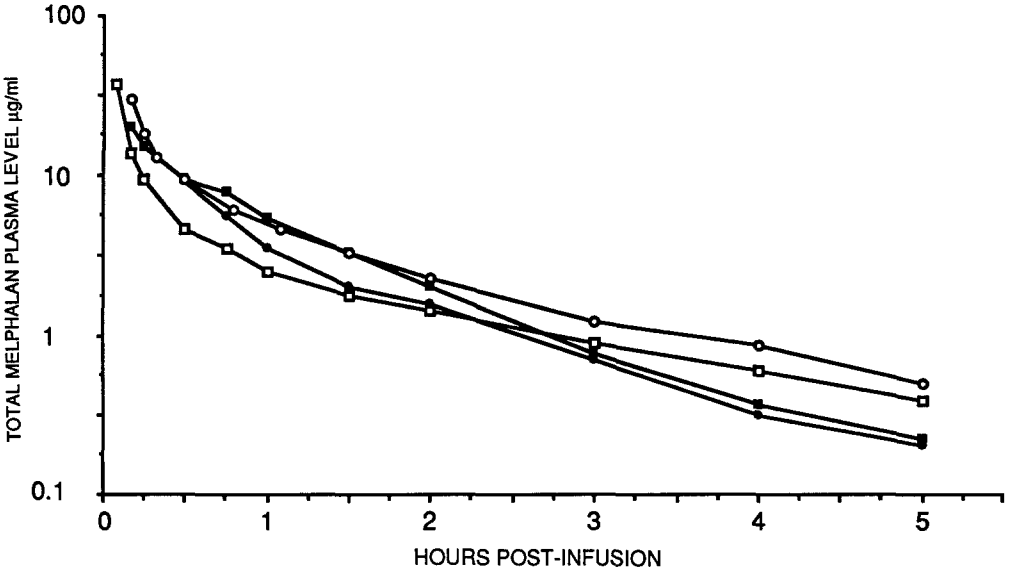

Fig. 2. Melphalan plasma levels in four patients (6–9) who received an i.v. dose of melphalan (220 mg/m^2) over 5 min for bone marrow transplantation

Table 2. Pharmacokinetic parameters for i. v. melphalan (5 mg/m^2) given over 5 min to nine patients with cancer

Patient no.	CL[a] (ml/min per m^2)	% excreted[b]	CL_R[c] (ml/min per m^2)	CL_{NR}[d] (ml/min per m^2)	V_{ss}[e] (l/m^2)	$t_{1/2\beta}$[f] (min)	f_u[g]
1	66.0	13.2	8.7	57.3	15.0	84.3	0.109
2	230	61.4	141	89.0	9.7	47.7	0.226
3	201	4.0	8.1	193	13.8	61.2	0.124
4	122	44.5	54.2	67.6	8.7	76.4	0.204
5	203	92.8	188	14.6	6.0	39.4	0.460
6	164	2.5	4.1	160	12.7	70.9	0.283
7	164	4.0	6.6	158	8.6	52.4	0.301
8	201	20.6	41.2	160	15.0	75.2	0.0598
9	272	64.4	175	97.0	12.9	46.5	0.182
Mean	180	34.2	69.7	111	9.93	61.6	0.217
SD	60.5	32.9	76.7	59.6	4.14	15.8	0.121

[a] Total plasma clearance of melphalan
[b] Percentage of the melphalan dose excreted unchanged in urine
[c] Renal clearance
[d] Non-renal clearance
[f] Steady-state volume of distribution
[g] Terminal half-life
[h] Unbound fraction in plasma

512$^+$ (Brain Power, Calabasas, Calif.) run on a Macintosh Plus PC.

Results

Plasma melphalan levels after the 5 mg/m^2 dose to all patients are shown in Fig. 1, and those after the 220 mg/m^2 dose to patients 6–9, in Fig. 2. Pharmacokinetic parameters for patients receiving the 5 mg/m^2 dose are summarized in Table 2, and those for patients 6–9, who received the 220 mg/m^2 dose, in Table 3. The CL after the low dose ranged from 66.0 to 272 ml/min per m^2; % excreted, from 2.5% to 92.8%; f_u from 0.0598 to 0.460; and $t_{1/2\beta}$, from 39.4 to 84.3 min. Measurement of the f_u in all plasma samples collected from patient 7 indicated that plasma protein binding was linear over the concentration range of 0.1–20 µg/ml in this patient. The average of the f_u determinations in the 10- and 15-min samples in each patient was therefore taken to be representative of the f_u in that patient.

Urine flow at the time of these studies ranged from 0.4 to 3.8 ml/min (mean ± SD, 1.7 ± 1.2 ml/min) and was not significantly related to either the % excreted or the CL_R. Potential multilinear relationships between serum albumin, serum creatinine and f_u and various correlations tested between parameters of melphalan disposition and serum albumin, serum creatinine and creatinine clearance (calculated from serum creatinine) were not significant. The unbound melphalan clearance and renal clearance calculated from the unbound fraction in plasma for each patient ranged from 441 to 3356 ml/min per m^2 and from 15 to 961 ml/min per m^2 respectively and were also not related to serum albumin, serum creatinine or creatinine clearance.

In the four patients (6–9) who also received the high melphalan dose, the CL ranged from 137 to 295 ml/min per m^2; % excreted, from 3.8% to 41.8%; f_u from 0.170 to 0.445; and $t_{1/2\beta}$, from 46.5 to 75.2 min. There were no significant paired differences in any parameter of melphalan

Table 3. Pharmacokinetic parameters for high-dose i. v. melphalan (220 mg/m^2) in four patients undergoing bone marrow transplantation

Patient no.	CL (ml/min per m^2)	% excreted	CL_R (ml/min per m^2)	CL_{NR} (ml/min per m^2)	V_{ss} (l/m^2)	$t_{1/2\beta}$ (min)	f_u
6	198	11.3	22.4	176	12.2	70.9	0.263
7	295	3.8	11.3	284	19.1	52.4	0.170
8	137	28.3	38.7	98.0	9.1	75.2	0.176
9	190	41.8	79.3	110	11.1	46.5	0.445
Mean	205	21.3	37.9	167	12.9	73.3	0.264
SD	66	17.1	29.8	85.0	4.4	12.7	0.128

disposition between the high- and low-dose studies in these patients.

Discussion

The renal clearance and plasma protein binding of melphalan were found to be highly variable between patients in the present study: one patient excreted only 2.5% of the dose, whereas another excreted 92.8%. There were no major differences in the urine flow, creatinine clearance or serum albumin between these two patients. However the patient excreting the highest percentage of the dose also had the highest f_u, which varied over an approximately 8-fold range between patients. Total melphalan clearance varied over a 4-fold range in patients receiving the low melphalan dose. The clearance of unbound melphalan varied over an even greater range, approximately 8-fold. Since unbound drug concentrations are likely to reflect the melphalan concentrations available to enter tumor cells, large differences in tumor concentrations are expected even after i.v. administration. The co-administration of melphalan with food or amino acids is likely to reduce the intracellular melphalan concentration through the inhibition of cellular uptake [10, 12].

The % excreted in urine in the present study differs markedly from that reported by Alberts et al. [1], who reported a value of 13.0%±5.4% (range, 1.5% to 21.6%) in patients given a dose of 30–50 mg/m^2. These workers noted that their value may have been too low, since melphalan undergoes rapid decomposition in urine. In the present study, particular attention was paid to rapidly freezing urine specimens and this may have avoided drug losses. Ninane et al. [8] have reported urinary excretion of 1.7%–12.8% of the dose in 5/9 patients given doses of 150 mg/m^2 or 180 mg/m^2. Total melphalan clearance in the present study (180 ml/min per m^2 for the 5 mg/m^2 dose and 205 ml/min per m^2 for the 220 mg/m^2 dose) was comparable to that reported by Alberts et al. (~170 ml/min per m^2) [1] but lower than that previously reported by us (median, 362 ml/min per m^2) [9]. Ardiet et al. [2] and Gouyette et al. [6] have reported mean values of 373 ml/min per m^2 and 525 ml/min per m^2 respectively in patients receiving forced diuresis. However, in all cases the range of values was remarkably variable between patients. Comparisons with published estimates of the V_{ss} were difficult because V_{ss} has generally not been reported. Ardiet et al. [2] have reported a mean value of 20.6 l/m^2 in patients with forced diuresis, which was higher than the value in the present study (~10 l/m^2).

Melphalan undergoes hydrolysis to mono- and dihydroxy derivatives in aqueous solutions [5]. Concentrations of these metabolites were not determined in the present study. It is possible that variations in the rate of hydrolysis in urine, particularly in the bladder, prior to collection of the specimens may contribute to apparent variations in the CL_R of unchanged melphalan. It would be desirable in future studies to catheterise patients for urine collections to avoid such losses, which may contribute to variability in measured CL_R.

The CL_R of melphalan exceeded the product f_u. GFR in 7/9 patients given the low dose by a factor of 2.5 to 11.7. This was evidence for active renal tubular secretion, possibly by a pathway responsible for the active transport of some of the dietary amino acids. However, patients 6 and 7 had CL_Rs that were substantially less than f_u. GFR, suggesting that the drug may also be reabsorbed in the kidney. The extent of active secretion may vary markedly between patients and could also be inhibited by amino acids. Saturation of the secretory mechanism does not appear likely, given the similar CL_Rs of the four patients receiving both low- and high-dose melphalan. The difference in dose given these four patients was approximately 44-fold, which was reflected in markedly different plasma levels. There were also no differences in protein binding between the two dose levels, suggesting that protein binding was not saturated at the plasma levels attained. Nonlinear protein binding has been reported at very high melphalan plasma concentrations in vitro [7]. The same workers also reported that protein binding ranged from 55%–76% in vitro. This value varied from 54% to 94% in our studies. Ehrsson and Lonroth [5] have reported that melphalan plasma protein binding in vitro was 69%±3.4% (i.e. a mean f_u of ~0.3) and was linear over the concentration range of 5–50 μg/ml. The mean f_u values in the present study were 0.22 for the 5 mg/m^2 dose and 0.26 for 220 mg/m^2 and were therefore comparable with the estimates of Ehrsson and Lonroth [5].

Potentially useful relationships between serum albumin, serum creatinine or calculated creatinine clearance and melphalan clearance were not evident from this study. Ninane et al. [8] and Taha et al. [11] have also reported a lack of correlation between creatinine clearance and total melphalan clearance. Correlations with melphalan $t_{½\beta}$ have limited practical value, since half-life differences need not necessarily reflect differences in drug clearance. The situation regarding melphalan clearance is complex: not only does it bind irreversibly to protein, but it also appears to undergo a complex mechanism of renal clearance probably involving active transport, which varies markedly between patients. It is therefore unlikely that the GFR would be predictive of melphalan clearance.

The high variability in free and total melphalan clearance in the present study suggests that some patients might

have been exposed to subtherapeutic concentrations of the drug despite the fact that it was given i.v.. At this time it is impossible to predict which patients will have high or low clearances. It might be appropriate to undertake a study in a substantial number of patients to establish whether there is a relationship between the free or total melphalan AUC or a single, appropriately timed plasma specimen and the therapeutic or toxic effects to ascertain the role of therapeutic monitoring for the drug.

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Received August 21, 1987/Accepted May 17, 1988