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Pharmacokinetics of high-dose melphalan in children and adults

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Summary. Melphalan pharmacokinetics were studied in 20 children with stage IV neuroblastomas or Ewing's sarcomas and in 10 adults with AML, ALL, or small cell lung carcinomas, after IV administration of high doses (140 mg/m^2 with furosemide-induced diuresis and 180 mg/m^2 without induced diuresis) and high fluid intake (3000 ml/m^2/day).

Unchanged melphalan was assayed in plasma and cerebrospinal fluid by means of a high-performance liquid chromatographic procedure. The elimination half-life ($t_{1/2}$ < 80 min) allows autologous bone marrow transplantation 24 h after the drug administration. In some children we were able to detect melphalan in cerebrospinal fluid samples.

Introduction

Melphalan, or phenylalanine mustard, has long been used as an effective alkylating agent in the treatment of multiple myeloma [2] and ovarian cancer [16]. Its clinical pharmacology has recently been studied in man after the development of sensitive analytical assays.

High-performance liquid chromatographic (HPLC) techniques [5, 9] allow the quantitation of melphalan down to 50 ng/ml plasma with UV detection methods. When HPLC is used with a fluorescence detector the limit of detection is below 5 ng/ml [8], in the same range as that obtained with a gas chromotographic-mass spectrometric assay [15]. The same order of sensitivity was also reported when intact radioactive [^{3}H]melphalan was separated from its degradation products by HPLC [3].

Thus, the disposition of melphalan and its elimination in urine were studied in nine patients after IV administration of 0.6 mg/kg (about 20 mg/m^2). The plasma decay was biphasic, with a distribution half-life, $t_{1/2}$ (α), of 12.6 ± 8.8 min and an elimination half-life, $t_{1/2}$ (β), of 86.5 ± 48.8 min [1].

At the time our study was initiated, high-dose melphalan protocols had just been proposed by McElwain et al [13]. However, melphalan is known to be myelotoxic, even at conventional doses of 0.25 mg/kg/day for 4 days every 6 weeks [2]. To prevent severe aplasia in our patients, autologous bone marrow transplantation was necessary. Therefore, we studied the pharmacokinetics of melphalan in children and adults following IV administration of 140 or 180 mg/m^2 under high fluid intake with or without furosemide-induced diuresis. Melphalan was also analyzed in the cerebrospinal fluid in 16 of 22 courses in 20 children and in 6 of 12 courses in 10 adults.

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Patients, materials and methods

Materials. Pure melphalan was obtained from the Laboratoires Wellcome SA (Principality of Monaco) through the courtesy of Dr C. L. Benet. Melphalan powder (Alkeran, from the Laboratoires Wellcome S. A., Paris) was supplied in 100-mg vials along with 1 ml acidic ethanol and 9 ml K_2HPO_4 in propylene glycol and water. Dansyl-DL-proline, piperidinium salt (Koch-Light Laboratories) was used as an internal standard for melphalan quantitation. Glacial acetic acid (Merck) 99%–100% was extra pure. Methanol (Carlo Erba) was spectrophotometric grade. Water was obtained through a Milli-Q water purification system (Millipore Corporation).

Apparatus. The HPLC system consists of two pumps (6000 A and M45), a UV detector (M480), and a reversed-phase column (μ-Bondapak C18) equipped with a precolumn filled with C18 Corasil (all from Millipore/Waters Associates). The peak areas, computed by a Sigma 10 integrator (Perkin-Elmer Co.), were used to quantitate the amout of melphalan in biological fluids with reference to standard calibration curves.

Melphalan analysis. Throughout our study, we used the HPLC assay described by Chang et al. [5] to monitor the melphalan concentrations in biological samples (limit of detection 50 ng/ml).

Calculations. The experimental data were fitted to a two-compartment body model using the ADAPT program [6]. This program allows the determination of the pharmacokinetic parameters after IV infusion of a drug without the need of correction for the duration of the infusion. The areas under the curves (AUC) were computed by the logarithmic trapezoidal method [10], and the total clearance was calculated according to the equation: Cl = dose/AUC

Patients. Our patients were 20 children with stage IV neuroblastomas or Ewing's sarcomas and 10 adults with acute

Table 1. Characteristics of patients and MPH dosage

Patients	Tumor Type[a]	Stage	Age years	months	Clinical status[b]	Melphalan mg/m²	Other concomitant drugs
Children							
1	N	III	14		PR		Furosemide
2	N	IV	10		CR		Furosemide
3 (1st course)	N	IV	4	1	PR		Furosemide
3 (2nd course)							Furosemide
4	N	IV	2		CR		Furosemide
5	E	IV	11	7	PD		Furosemide
6	N	IV	1	10	PR		Furosemide
7	N	IV	4	5	PD	140	Furosemide
8	N	IV	5		PR		Furosemide
9	R	IV	5		CR		Furosemide
10	N	IV	2	6	PR		Furosemide
11	N	IV	7	7	PR		Furosemide
12	N	IV	3	3	PR		Furosemide
13	N	IV	3		CR		Furosemide
14	N	IV	2	7	PR		Furosemide
15	E	IV	3	6	PR		BCNU, PCZ[c]
16	E	IV	10		PR		BCNU, PCZ[c]
17 (1st course)	N	IV	11		CR		No
17 (2nd course)						180	BCNU, VP-16[d]
18	N	IV	2	3	CR		No
19	E	IV	6	4	PR		BCNU, PCZ[c]
20	R	IV	4	4	CR		BCNU, VP-16, PCZ[d]
Adults							
21	SCL		32		PD		Furosemide
22 (1st course)	SCL		35		PD		Furosemide
22 (2nd course)							Furosemide
23	SCL		38		PD		Furosemide
24 (1st course)	AML		29		PD	140	Furosemide
24 (2nd course)					CR		Furosemide
25	Testis		27		PD		Furosemide
26	SCL		42		PD		+ BCNU, PCZ[c]
27	ALL		22		PD		+ BCNU, PCZ[c]
28	SCL		62		PD		BCNU, VP-16, PCZ[d]
29	SCL		45		CR	180	BCNU, VP-16, PCZ[d]
30	SCL		35		CR		BCNU, VP-16, PCZ[d]

[a] N, neuroblastoma; E, Ewing's sarcoma; R, rhabdomyosarcoma; SCL, small cell lung carcinoma; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia

[b] At the start of the study: CR, complete response; PR, partial response; PD, progressive disease

[c] BCNU (300 mg/m², day 1), procarbazine (PCZ: 200 mg/m², days 2, 3, 4), MPH (day 5)

[d] BCNU (300 mg/m², day 1), VP-16 (200 mg/m², days 1, 2, 3), PCZ (400 mg/m², days 2, 3, 4), MPH (day 5)

myeloid or lymphoblastic leukemias and small cell lung carcinomas. Their characteristics are given in Table 1.

Protocol. The protocol used was a modification of that described by McElwain et al [13]. In short, after harvesting of bone marrow under anesthesia, the required dose of melphalan (140 or 180 mg/m²) was injected IV over 5 min. Blood samples were collected prior to the administration, then 5, 10, 15, 30, 45, 60, and 90 min and 2, 3, 4, and 6 h after the end of the injection. After rapid centrifugation at 4 °C plasma was frozen at −70 °C until assay. In some patients, cerebrospinal fluid was obtained through lumbar

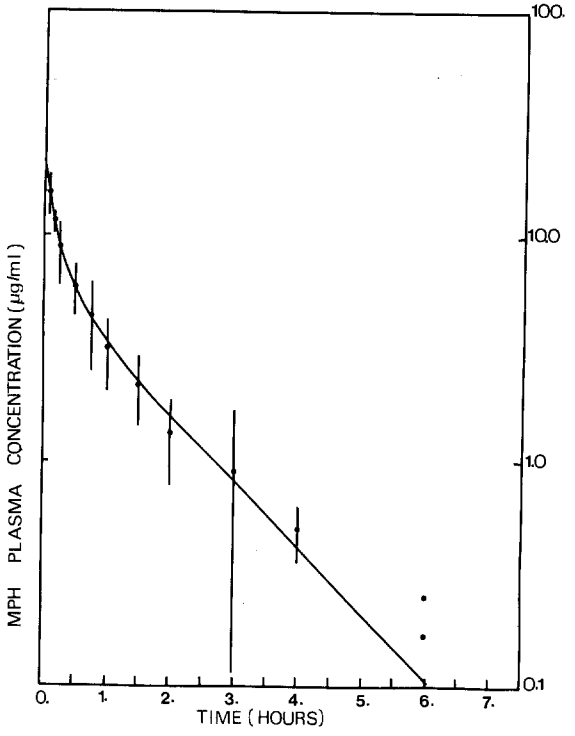

Fig. 1. Mean melphalan concentrations and 95%-confidence interval measured as a function of time after IV injection of 140 mg/m^2 in children

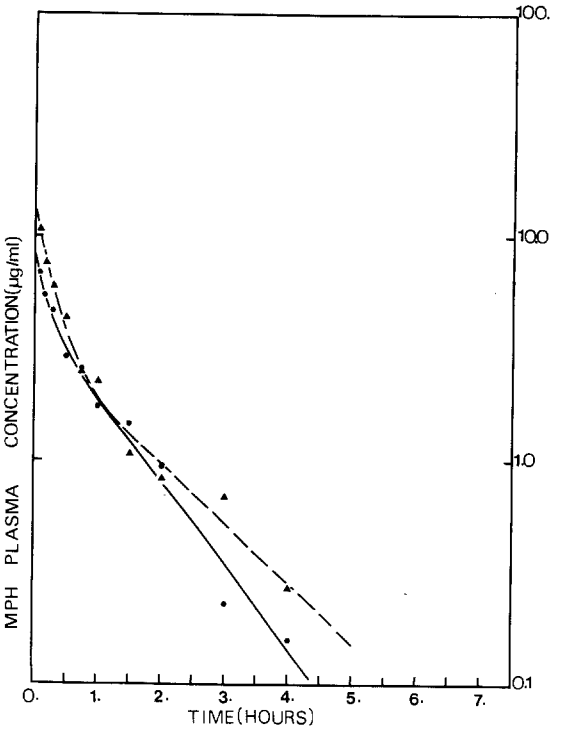

Fig. 2. Mean melphalan concentrations measured in adults after IV injection of 140 mg/m^2 (●) and 180 mg/m^2 (▲)

puncture. Bone marrow was reinfused 8 or 24 h after melphalan had been administered.

At the beginning of this study, our patients were given furosemide (1 mg/kg) 15 min prior to the melphalan administration. Diuresis was compensated by an equivalent amount of saline as an IV infusion for the first 3 h. Then, the patients were given 3000 ml/m^2 as an IV infusion over the next 24 h (induced diuresis protocol). Later, this hydration protocol (3000 ml/m^2/day) was used without furosemide (see Table 1).

Results

The HPLC assay is specific for unchanged melphalan, and no interaction was detected with other drugs given to our patients. The mono- and dihydroxy metabolites were not analyzed, since they have shown no evidence of cytotoxicity. The recovery (90%) and precision (±7%) are quite easily maintained, since the procedure requires no extraction.

In 19 of 34 courses, plasma concentration profiles versus time were biphasic (Figs. 1 and 2): the data can be fitted and correspond to a two-compartment model. However, in 9 studies the distribution phase was not clearly distinguishable from the elimination phase; in these cases the elimination half-life was calculated according to a one-compartment model. In six cases (2, 9, 21, 22a, 22b, and 23), we obtained only one or two experimental points for the characterization of the distribution phase. Then, to avoid underestimation of AUCs and overestimation of clearances, the corresponding AUCs have not been included in Table 2. However, the elimination half-life was calculated by means of nonlinear regression.

In children, the distribution half-life, $t_{1/2}$ (α), is 8.8 ± 6.9 (SD) min and 10.5 ± 5.8 min after administration of 140 and 180 mg/m^2, respectively. The elimination half-life, $t_{1/2}$ (β), is 43 ± 17 min (range: 17–74 min) and 48 ± 16 min (range: 27–75 min), respectively.

In adults, the elimination half-life, $t_{1/2}$ (β), is 50 ± 7 min (range: 45–65 min) after administration of 140 mg/m^2 and 41 ± 12 min (range: 27–50 min) after injection of 180 mg/m^2.

The total plasma clearance is calculated from the AUCs. Therefore, the systemic clearance in children is 257 ml/min/m^2 at doses of 140 mg/m^2 with furosemide and 498 ml/min/m^2 at doses of 180 mg/m^2 without furosemide. Although, in adults, there is no difference between the two modes of administration, the corresponding figures are 525 ml/min/m^2 at 140 mg/m^2, and 532 ml/min/m^2 at 180 mg/m^2.

After collection of cerebrospinal fluid samples through lumbar puncture at different times following melphalan administration no drug was detectable in adult cerebrospinal fluid samples. However, melphalan was detected in 6 out of 16 children (see Table 2).

Discussion

In our patients, we monitored unchanged melphalan in plasma after IV administration of 140 mg/m^2 with furosemide-induced diuresis and no other concomitant drug. The dosage was later increased to 180 mg/m^2 without furosemide administration, and in most cases, melphalan was injected on day 5 of a combination chemotherapy including BCNU, procarbazine, and in some cases, VP-16 (see Table 1). The main objective of our study was to determine the elimination half-life of melphalan after high-dose administration and the lag-time required between infusion of

Table 2. Pharmacokinetic parameters of high-dose melphalan

Patients		$t^{1}/_{2}$ (α) (min)	$t^{1}/_{2}$ (β) (min)	AUC (μg × min/ml)	Cl total (ml/min/m²)	CSF melphalan	
						Time	C (μg/ml)[a]
Children	1		46	642	218		NS
140 mg/m²	2		55			3 h	ND
	3a	4.3	17	328	427		NS
	3b		29	365	384		NS
	4		24	714	196		NS
	5	3.6	33	362	387	3 h	ND
	6	2.8	48	522	268	6 h	ND
	7	18.7	74	630	222		NS
	8		25	500	280	3 h	0.62
	9		50			4.5 h	ND
	10	19.6	62	1515	92	3 h	1.0
	11	6.5	35	573	245	6 h	0.47
	12	3.8	41	582	240	6 h	1.1
	13		39	1099	127	1.75 h	ND
	14	11.2	69	546	257	6 h	0.31
Mean		8.8	43	644	257		
SD		6.9	17	327	97		
Children	15		46	382	472	5 min	ND
180 mg/m²	16		53	645	279	5 min	0.85
	17a	8.1	49	416	433	5 h	ND
	17b		27	192	938	3 h	ND
	18	18.4	75	643	280	6 h	ND
	19	4.9	32	249	723	2 h	ND
	20	10.7	52	503	358		NS
Mean		10.5	48	433	498		
SD		5.8	16	177	246		
Adults							
140 mg/m²	21		45				NS
	22a		48				NS
	22b		58			6 h	ND
	23		50			4 h	ND
	24a	6.9	50	146	961		NS
	24b	8.1	47	261	536		NS
	25		64	555	252		NS
	26	4.2	42	588	238		NS
	27	8.3	50	220	636	2 h	ND
Mean		6.9	50	354	525		
SD		1.9	7	203	300		
Adults							
180 mg/m²	28	1.2	27	358	503	2 h	ND
	29	5.0	45	631	286	6 h	ND
	30	11.4	50	223	806	3 h	ND
Mean		5.9	41	404	532		
SD		5.2	12	208	261		

[a] NS, no sample; ND, not detected (< 50 ng/ml)

the drug and effective autologous bone marrow transplantation.

Plasma pharmacokinetics generally follow a biphasic pattern, as previously described in the literature for conventional doses (about 20 mg/m^2). The distribution and elimination phases are characterized by their corresponding half-lives, $t_{1/2}$ (α) and $t_{1/2}$ (β). After IV administration of 8–28.5 mg melphalan, Bosanquet and Gilby [3] reported a $t_{1/2}$ (α) of 7.7 ± 3.3 min and a $t_{1/2}$ (β) of 83 ± 14 min. Alberts et al. [1] found $t_{1/2}$ (α) = 12.6 ± 8.8 min (range: 4.7–29.9 min) and $t_{1/2}$ (β) = 86.5 ± 48.8 min (range: 20.1–291.5 min). But in some patients, the plasma concentration decayed monoexponentially. This might be attributable by a fast distribution process, which could take place within the very first minutes following the end of the infusion, so that we do not have enough data points to evaluate the half-life of the distribution process confidently. In their study, Bosanquet and Gilby [3] calculated a very short distribution half-life of 1.1 and 1.4 min in two of their nine patients.

Moreover, our results are in good agreement with other reports. Tattersall et al. [18] found a mean elimination half-life of 67 min in patients given 20–23 mg/m^2 [^{14}C]melphalan by the IV route. And Davis et al. [7] calculated $t_{1/2}$ (α) at 6.2 min and $t_{1/2}$ (β) at 53.5 min in a patient given 140 mg/m^2 IV over 5 min.

In the cited literature, however, the authors did not report the value of systemic clearance for melphalan, except Taha et al [17]. Most of the drug is hydrolyzed to its mono- and dihydroxy derivatives by a chemical reaction [4]. In plasma, at 37 °C, the in vitro chemical half-life is about 2.1 ± 0.1 h [8], in agreement with the findings of Chang et al [4]. In man, the renal contribution to plasma melphalan clearance is minor: only 13.0% ± 5.4% of an IV dose of 0.6 mg/kg appears in the urine over 24 h [1]. From Table 3 of the paper published by Alberts et al [1] we estimated a total plasma clearance of 182 ± 95 ml/min/m^2 with a range of 61–397 ml/min/m^2. In another paper, the systemic clearance was evaluated at 5.26 ± 1.76 ml/min/kg in adults receiving 8 to 285 mg melphalan [3]. In one patient given 31 mg [18] the clearance was estimated at 423 ml/min.

In our two groups of adults, there was no significant change in clearance according as whether the drug was given alone or with other cytotoxic agents. But in children who received 140 mg/m^2 with furosemide the mean plasma clearance was lower than in the group of children given 180 mg/m^2 in a combination chemotherapy but without furosemide, although we do not have any rationale to reconcile this difference among these two groups of children.

We did not detect any melphalan in CSF of adults or in children given 180 mg/m^2 (except in one who had low clearance). But in the lower clearance group of children we found melphalan in five of ten CSF samples. Hedley et al. [11] found a CSF melphalan concentration of less than 0.39 µg/ml after a lumbar puncture performed 35 min after IV administration of 140 mg/m^2. At the same time the plasma concentration was 3.6 µg/ml, indicating that in this case the drug did not cross the intact blood-brain barrier to any significant extent. However, since we first submitted the present paper for publication, Lazarus et al [12] have reported that in three of four patients with intravesicular Rickham reservoir the CSF concentration was about 10% of the corresponding plasma concentration after IV administration of 60 mg/m^2/day over 3 days. The peak concentration occurred 30–60 min after the infusion. Some other clinical observations made in our institution also clearly indicate that melphalan must cross the blood-brain barrier after high-dose melphalan treatment (data to be published).

Finally, this pharmacokinetic study indicates that on the assumption that there is no more circulating melphalan after seven elimination half-lives, it may be possible to reinfuse autologous bone marrow 8–10 h after drug administration: in our patients the longest half-life was 75 min. Our assumption seems to be valid, since Lazarus et al. [12] state that "the drug did not appear to undergo sequestration and redistribution, since drug concentrations were undetectable at 24 h after injection." This is also supported by McElwain et al [13], who did not detect melphalan in plasma after 2 h or in urine after 6 h when using a sensitive mass spectrographic analytical method. Last, Davis et al. [7] reported a detection limit of 10 ng/ml and did not find a prolonged elimination phase that could not have been detected in our study using an HPLC assay.

A more recent report indicates that bone marrow can be preserved in the nonfrozen state for up to 48 h [14]. Thus, the final protocol we use for high-dose melphalan chemotherapy is infusion of 140–180 mg/m^2 IV over 5 min with hydration (3 l/m^2/day), followed by autologous bone marrow transplantation 24 h after injection of the drug. We no longer give furosemide, since we did not observe any clinical difference as far as gastrointestinal toxicitiy and hematologic recovery were concerned (data to be published). This protocol is in good agreement with the results and the statements of Taha et al. [17].

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