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High dose melphalan in children with advanced malignant disease

A pharmacokinetic study

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Summary. Nine children with poor-prognosis malignancies – seven with advanced neuroblastoma and two with metastatic Ewing's sarcoma – were given high doses of melphalan (HDM), 150 mg/m^2 (3 patients) and 180 mg/m^2 (6 patients), as a 'late intensification' agent combined with noncryopreserved autologous bone marrow transplants. Melphalan levels in the plasma decreased biphasically, with mean half-lives of 6.6 min and 3.0 h. At the time of marrow reinfusion (12–21 h after HDM) the melphalan plasma level was generally below 0.1 μg/ml. The renal contribution to melphalan clearance was low, a mean of 5.8% of the injected dose being found in patients' urine over the 12 h following HDM administration. No significant difference was seen in pharmacokinetic parameters between patients undergoing and not undergoing forced diuresis.

Introduction

In spite of the undeniable progress made in pediatric oncology, there are two tumors known to be drug-sensitive that conventional chemotherapy is still unable to cure once they have formed metastases: neuroblastoma [9, 12] and Ewing's sarcoma [10]. Several new therapeutic approaches have therefore been attempted, including high-dose chemotherapy combined with autologous or allogenic bone marrow grafting [4, 7] following standard-dose 'induction' chemotherapy with or without surgery and/or radiotherapy.

The OPEC regimen (vincristine, platinum, cyclophosphamide, and VM-26) is active as induction therapy against neuroblastoma [16], whilst vincristine, adriamycin, cyclophosphamide, and actinomycin D remain first-choice drugs for the induction treatment of Ewing's sarcoma [15].

Melphalan is one of the drugs used at high doses as a late intensification agent for these two tumors [6, 14]. The advantages of this alkylating agent, whose therapeutic effect is indeed related to the dose given, are that its acute toxicity is essentially hematologic and to a lesser extent gastrointestinal, and that its plasma half-life is short. Therefore, although bone marrow autografting accelerates the recovery of hematopoiesis in children receiving high-dose melphalan (HDM), the bone marrow does not need to be cryopreserved but only kept at 4 °C and reinfused within 24 h after its collection, when more than 50% of the stem cells (CFU-C, CFU-E, BFU-E) are still likely to be viable [13]. In this study, the pharmacokinetics of melphalan have been determined in nine children for at least four reasons: (i) To correct the paucity of pharmacokinetic data recorded in children following high-dose IV injection [17]; (ii) to analyse the influence of renal function on the drug elimination; (iii) to verify the usefulness of hyperhydratation on the drug elimination; and (iv) to establish the plasma melphalan concentration at the time of bone marrow reinfusion.

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Patients and methods

Patients. Nine children, all boys aged from 18 months to 10 years (median: 4 years), received high dose melphalan (HDM) combined with non cryopreserved autologous bone marrow transplants. Melphalan was given as late intensification chemotherapy to seven children with advanced neuroblastoma – two stage III and five stage IV with bone metastases [8] and to two children with metastatic Ewing's sarcoma. All patients had been given previous chemotherapy and five had undergone surgery; each of the Ewing's sarcoma patients had had radiotherapy delivered to the primary tumor site. Renal function was slightly impaired in three patients. A forced diuresis regimen was used in the first three cases but not subsequently (Table 1).

Chemotherapy. Melphalan was given as in IV push without cyclophosphamide pretreatment at 150 mg/m^2 for the first three patients and escalated to 180 mg/m^2 for the other six.

Blood and urine sampling. Blood samples (2–3 ml) were taken before and 1, 5, 10, 15, 30, and 60 min and 3, 12, and 24 h after the end of melphalan administration. Urines were collected every 2 h up to 24 h after HDM. Samples were kept at 4 °C prior to analysis.

Estimation of plasma and urine melphalan concentration. The quantitative determination of melphalan was made using a UV detector after separation of the drug from its hydrolysis products by high-pressure liquid chromatography according to Chang et al. [5]. For this, 500 μl either plasma or urine was mixed with 1 ml chilled methanol, cooled for 3 min in isopropanol-dry ice, and centrifuged for 3 min in a bench centrifuge at full speed. The clear su-

Table 1. Patients' details at the time of high-dose melphalan

Patient	Sex	Age (years)	Diagnosis[a]	Stage	Previous treatment[b]	Status[c]	Serum urea (mg/100 ml)	Serum creatinine (mg/100 ml)	Creatinine clearance (ml/min/1.73 m²)	Forced[d] diuresis
G.L.	M	$1^{1}/_{12}$	NBL	III	OPEC × 6 Surgery	CR	35	.4	48	No
L.H.	M	$2^{11}/_{12}$	NBL	III	CVA-dacarbazine OPEC × 6 Surgery	CR	30	.6	72	Yes
J.L.	M	$4^{1}/_{12}$	NBL	IV	CVA-dacarbazine OPEC × 6 Surgery	CR	42	1.0	144	No
F.D.	M	$1^{6}/_{12}$	NBL	IV	OPEC × 6	GPR	43	.5	82	Yes
H.C.	M	7	NBL	IV	OPEC × 8	CR	38	.5	94	No
D.D.	M	$7^{6}/_{12}$	NBL	IV	OPEC × 8 Surgery	CR	45	1.2	60	No
F.X.S.	M	$3^{5}/_{12}$	NBL	IV	OPEC × 6	CR	38	.4	92	No
O.T.	M	10	EW	IV	CVA-actinomycin D Radiotherapy	CR	36	.4	184	No
J.F.D.	M	$4^{6}/_{12}$	EW	IV	CVA-actinomycin D Radiotherapy	CR	40	.5	112	Yes

[a] NBL, neuroblastoma; EW, Ewing's sarcoma
[b] OPEC, vincristine, platinum, cyclophosphamide and VM-26 [19]; CVA, cyclophosphamide, vincristine and adriamycin
[c] CR, complete response: GPR, good partial response
[d] No, 1500 cc/m² for 24 h, furosemide to maintain diuresis of 8 ml/kg per h for 24 h; Yes, 2500 cc/m² for 24 h

pernatant was injected into a Hewlett-Packard 1084 B liquid chromatograph (Hewlett-Packard GMBH, Böblingen, FRG). Chromatography was carried out isocratically at ambient temperature using a mixture of water-acetic acid-methanol (590 : 10 : 400 by volume) as eluent at a flow rate of 2 ml/min. The column (250 × 4.6 mm) was packed with 10 µ bonded phase (µ Bondapack C18 phenyl from Waters). Peak detection was performed with a Pye-Unicam model LC-UV spectrophotometer set at 263 nm and quantitation, with the aid of a calibration curve plotted after the addition of melphalan, 0.1–50 µg/ml, to human plasma.

Pharmacokinetic analysis. Plasma concentrations (C) versus time (t) data obtained from each separate patient were fitted to a biexponential equation and the pharmacokinetic parameters were calculated assuming an open two-compartment model for melphalan disposition, as follows [1]:

$\ln C = \ln (A.e^{-\alpha.t} + B.e^{-\beta.t})$

V1 = volume of central compartment = dose/(A + B)

k_{21} = transfer constant from peripheral to central compartment: $(A.\beta + B.\alpha)/(A + B)$

k_{el} = elimination constant = $\alpha.\beta/k_{21}$

k_{12} = transfer constant from central to peripheral compartment = $(\alpha + \beta) - (k_{21} + k_{el})$

V2 = volume of peripheral compartment = $V1 . k_{12}/k_{21}$

AUC = area under the C versus t curve = $(A/\alpha) + (B/\beta)$

Cl = systemic clearance = dose/AUC

Vd_{area} = apparent volume of distribution = Cl/k_{el}.

Results

The melphalan plasma levels (Fig. 1), and therefore the pharmacokinetic parameters deduced from them (Table 2), exhibited great variability from one individual to the other. The initial plasma levels were generally greater than 15 µg/ml, except for one patient (L.H.), and decreased biphasically with mean half-lives of 6.6 min (range: 1.4–18 min) and 3.0 h (range 1–5 h). More than 90% of the drug was cleared from the plasma during the first hour after the injection. In one case (F.D.) the plasma levels first rose during the first hour and then declined; this was apparently due to injection of the drug into the venous line distally instead of very close to the vein.

At the time of marrow reinfusion (12–21 h after HDM), plasma levels of melphalan were very low (mean 0.06 µg/ml) (range: < 0.01–0.15 µg/ml). The renal contribution to melphalan clearance is low, since a mean of 5.8% of the injected melphalan dose (range 2%–13%) was eliminated intact during the first 6 h in the urine with little further excretion thereafter (Fig. 2). In four patients there were enough data points to allow determination of the urinary elimination half-life. The values, given in Table 2, were not significantly different from those for the second phase half-life of plasma melphalan disappearance.

The mean plasma exposure to melphalan, expressed as the area under the C versus t curve (AUC) was 68.5 µg.h.l^{-1} for the five surviving children who had neuroblastoma, while it was 23.1 µg.h.l^{-1} in the other cases. This difference is significant at $P = 0.05$ (t-test). The difference was more pronounced in neuroblastoma patients, the

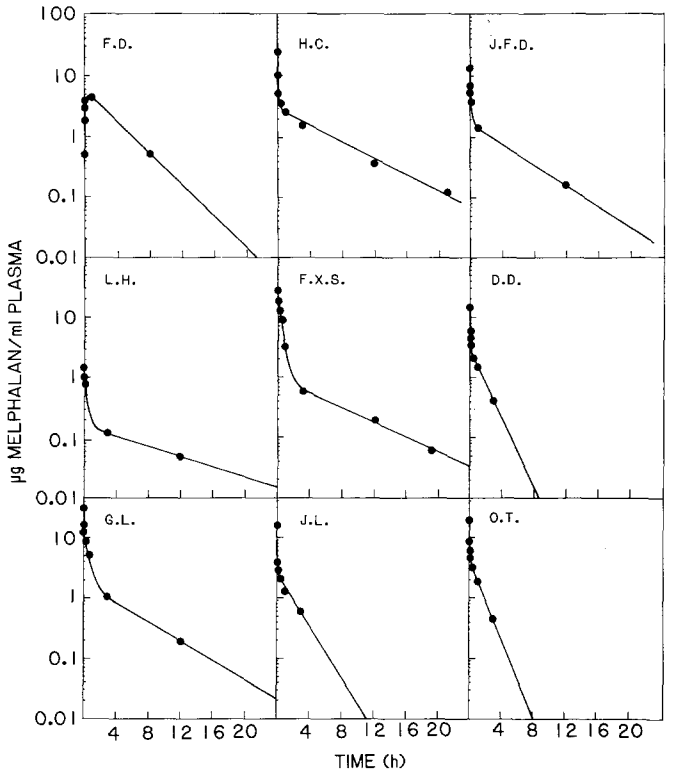

Fig. 1. Plasma concentration of melphalan (●) in nine children after high-dose therapy. *Continuous lines* represent the computer-generated best-fit curves to the data assuming an open two-compartment model

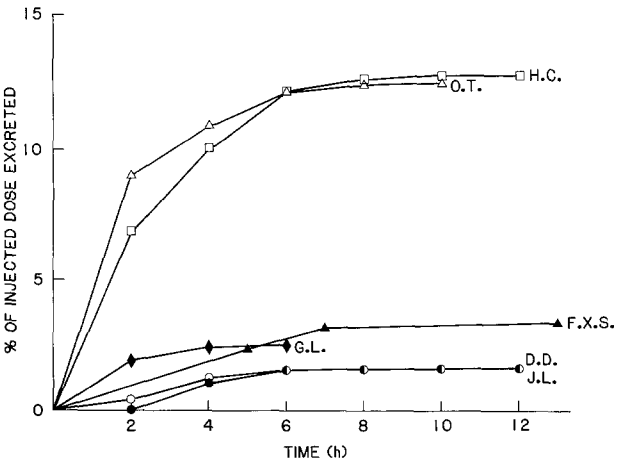

Fig. 2. Cumulative urinary excretion of intact melphalan in five patients, expressed as percentages of the injected dose

patient dying of his disease (L.H.) having an AUC 9 times lower than the mean value for survivors while the systemic clearance was 5 times greater in this patient. The mean plasma clearance (Cl) was lower (12.1 $l.h^{-1}.m^{-2}$) in the five surviving children than in the other children (32.9 $l.h^{-1}.m^{-2}$), but the difference was not significant at $P=0.05$ (*t*-test). These observations await further studies to allow more confident assessment, and their relevance remains to be established.

Forced diuresis had no significant influence on the plasma melphalan disposition, no significant difference in the various pharmacokinetic parameters deduced from the open two-compartment model being observed (Table 3).

To assess the influence of renal function on the melphalan disposition, linear regression analyses between creatinine clearance and the elimination constant (k_{el}), plasma clearance (Cl), or percentage of injected dose excreted in the urine were carried out. The correlation coefficients were, respectively, 0.506 ($n=8$), 0.025 ($n=9$), and 0.499 ($n=6$). At a significance level of 0.05 none of these correlation was significant [11]; thus, melphalan disposition could not be related to renal function.

Discussion

Though the sampling schedule used in this study was limited, it is evident that the plasma levels of melphalan decreased at least biphasically in each patient. Therefore, the pharmacokinetic parameters were calculated by best-fit curve to the data assuming an open two-compartment model. In agreement with previously published work [17], after high-dose administration by IV push melphalan plasma concentrations decreased rapidly, resulting in very low levels of drug by the time of bone marrow reinfusion, at which time a great number of GM-CFU were still present. However, the mean elimination phase of 3.0 ± 1.9 h is longer than previously published values obtained in children [17] or in adults [3] with single-compartment models for melphalan disposition.

Table 2. Pharmacokinetic parameters

	From plasma data											From urine data	
Patient	C_o (µg/ml)	V_1 ($1/m^2$)	V_2 ($1/m^2$)	$V_{d\,area}$ ($1/m^2$)	k_{12} (h^{-1})	k_{21} (h^{-1})	k_{el} (h^{-1})	AUC (µg h l^{-1})	Cl ($l\,h^{-1}\,m^{-2}$)	$t_{1/2}$ (α) (min)	$t_{1/2}$ (β) (h)	% Dose eliminated	$t_{1/2}$ (h)
G.L.	21.4	4.2	12.8	16.9	21.4	7.0	1.4	112.0	5.7	1.4	2.1	2.6	–
L.H.	1.4	89.1	381.1	470.2	2.0	0.5	0.7	7.5	61.4	13.4	6.5	–	–
J.L.	19.8	10.4	41.0	51.4	8.1	2.0	2.7	23.2	27.8	3.4	1.6	1.7	2.0
F.D.	–	–	–	27.5	–	–	–	67.8	7.9	6.5	2.3	–	–
H.C.	25.9	7.0	40.1	47.1	7.7	1.3	1.2	77.5	8.4	4.1	4.4	12.8	–
D.D	16.5	10.9	27.0	37.8	8.1	3.3	2.9	20.4	31.7	3.1	1.0	1.7	1.2
F.X.S.	24.4	7.4	25.4	32.8	0.8	0.2	1.4	61.8	10.6	18.4	5.0	3.5	4.4
O.T.	21.3	8.4	18.2	26.7	7.1	3.3	2.9	26.5	24.5	3.3	0.9	12.5	0.9
J.F.D.	14.1	10.6	48.9	59.5	5.0	1.1	1.3	38.0	14.2	5.8	3.4	–	–
Mean	18.1	18.5	74.3	85.5	7.5	2.3	1.8	48.3	21.4	6.6	3.0	5.8	2.1
± SEM	2.8	10.1	44.1	48.3	2.2	0.8	0.3	11.2	5.9	1.9	0.6	2.2	0.8

Table 3. Mean pharmacokinetic parameters

	Neuroblastoma[a]			Ewing's sarcoma	Patients alive	Patients died	Forced diuresis	
From plasma data	A.W.D. ($n=5$)	D.O.C. ($n=1$)	D.W.D. ($n=1$)	D.W.D. ($n=2$)	($n=5$)	($n=4$)	Yes ($n=3$)	No ($n=6$)
C_o (μg/ml)	22.9 ± 1.4	16.5	1.4	17.5 ± 3.6	22.9 ± 1.4	13.3 ± 6.0	7.8 ± 6.4	21.6 ± 1.4
V_d (l/m^2)	35.1 ± 6.3	37.8	470.2	43.1 ± 16.5	35.1 ± 6.3	148.6 ± 107.4	185.7 ± 142.5	35.5 ± 5.2
k_{el} (h^{-1})	1.7 ± 0.4	2.9	0.7	2.1 ± 0.8	1.7 ± 0.4	2.0 ± 0.6	1.0 ± 0.3	2.1 ± 0.3
AUC (μg.h.l^{-1})	68.5 ± 14.3	20.4	7.5	32.8 ± 5.7	68.5 ± 14.3	23.1 ± 6.4	37.8 ± 17.4	53.6 ± 15.1
Cl ($l.h^{-1}.m^{-2}$)	12.1 ± 4.0	31.7	61.4	19.4 ± 3.7	12.1 ± 4.0	33.0 ± 10.2	27.8 ± 16.9	18.1 ± 4.6
$t_{1/2}$ (α) (min)	6.8 ± 3.0	3.1	13.4	4.6 ± 0.9	6.8 ± 3.0	6.4 ± 2.4	8.6 ± 2.4	5.6 ± 2.6
$t_{1/2}$ (β) (h)	3.1 ± 0.7	1.0	6.5	2.2 ± 0.9	3.1 ± 0.7	3.0 ± 1.3	4.1 ± 1.3	2.5 ± 0.7
From urine data	($n=4$)	($n=1$)	N.D.	($n=1$)	($n=4$)	($n=2$)	N.D.	($n=6$)
$t_{1/2}$ (β) (h)	3.2 ± 0.9	1.2	–	0.9	3.2 ± 0.9	1.1 ± 0.1	–	2.1 ± 0.8
% Dose eliminated	5.2 ± 2.6	1.7	–	12.5	5.2 ± 2.6	7.1 ± 5.4	–	5.8 ± 2.2

[a] A.W.D.: alive without disease; D.O.C.: died of complications; D.W.D.: died with disease

After IV administration, melphalan was distributed in a volume greater than the body volume, suggesting that the drug is segregated and concentrated in concealed compartments. In vitro, melphalan is taken up by an active carrier-mediated process and concentrated 5- to 10-fold in various cell lines [2, 18].

The effect of hyperhydration on drug elimination has been studied with reference to pharmacokinetic parameters deduced from the plasma values rather than from urinary data. The elimination constant k_{el} and the plasma exposure to the drug were lower, though not significantly, in patients undergoing forced diuresis, and the systemic clearance was somewhat higher in those patients. Therefore, from a pharmacokinetic point of view forced diuresis is not necessary in children treated with HDM given by the IV route, as already suggested by Taha et al. [17].

No significant relationship could be found between creatinine clearance and such pharmacokinetic parameters as k_{el}, Cl or percentage of injected dose found in patients' urine, and therefore in this study the melphalan disposition does not seem to be influenced by renal function. This could be due to the fact that melphalan is relatively unstable and is eliminated mainly by way of spontaneous hydrolysis or by biliary excretion [17].

The possibility that patients with relatively slow melphalan plasma clearance fare better than those who eliminate the drug more quickly deserves further study.

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