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ORIGINAL ARTICLE

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Population pharmacokinetics of melphalan, infused over a 24-hour period, in patients with advanced malignancies

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Abstract *Purpose*: The objective of the present study was to characterize the population pharmacokinetics of melphalan infused over a 24-h period in patients with advanced malignancies. *Methods*: Enrolled in the study were 64 patients (144 courses). The population pharmacokinetic analysis was performed using NONMEM through the graphical interface Visual-NM. Population characteristics were computed from an initial group of 43 patients (99 courses), and 21 additional patients (45 courses) were used for model validation. With the use of a one-compartment model, the influence of demographic and biological characteristics was examined. The basic parameters were total clearance (CL) and volume of distribution (V). The interoccasion variability was taken into account in the model. The drug exposure was estimated for each patient and correlated with markers of efficacy and toxicity. *Results*: Data analysis was performed using a three-step approach. In step 2, a close relationship was found between creatinine clearance, gender and melphalan CL. The inclusion of this second stage model significantly improved the fit. Melphalan CL was higher in male patients (14.3 ± 4.5 l/h per m^2) than in female patients (12.3 ± 4.5 l/h per m^2). CL was also reduced somewhat in patients with decreased creatinine clearance. Large interindividual variability in pharmacokinetic parameters occurred (CL varied from 4.4 to 30.6 l/h per m^2). The percentage intrapatient variability in clearance between courses was 25.4%. For determining melphalan AUC in clinical routine from one sample drawn at steady state, Bayesian methodology allowed a more accurate estimation of CL than the classical formula. Neutropenia and thrombocytopenia were the main haematological toxicities encountered; grade 4 was observed in 34 and 22 courses over a total of 144 courses, respectively. No significant relationship between AUC and haematological toxicity was found. In patients with prostatic cancer a weak relationship was observed between the decrease in PSA levels and AUC ($P = 0.0457$), while in patients with ovarian cancer no relationship was found between AUC and CA125 levels. *Conclusion*: The population pharmacokinetic approach developed in this study should allow dosage to be individualized in order to decrease toxicity while maintaining good efficacy.

Keywords Melphalan · Continuous infusion · Population analysis · Advanced malignancy

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Introduction

Melphalan was first synthesized in 1953 [4] and has since been extensively used in the treatment of patients with multiple myeloma, ovarian cancer, breast cancer and neuroblastoma [15, 22, 23, 27, 28]. Although the oral form is commercially available in several countries and can offer greater flexibility in terms of schedule manipulation than the infusion and can increase quality of life, the intravenous form is widely used. Indeed, large interindividual variability in bioavailability has been observed following oral administration (20–80%) [27, 30], and the bioavailability of melphalan may be affected by food intake and coadministered drugs [6, 11].

Melphalan is a bifunctional alkylating agent that affects cytotoxicity by forming interstrand, intrastrand, or DNA-protein crosslinks [22]. After short intravenous infusion, melphalan rapidly disappears from the plasma. Most of the published data have shown that plasma

concentration versus time curves are compatible with a two-compartment model, the distribution half-life ranges from 5 to 15 min, and the elimination half-life from 17 to 75 min [2, 10, 29] or from 2 to 4 h [14]. Total plasma clearance ranges from 92 to 961 ml/min per m^2. The volume of distribution has been found to be greater than the total body water (35.5 to 185.7 l/m^2), although lower values have been reported (8 and 50 l/m^2) [2, 6, 10, 12, 14, 22, 26, 29].

Preclinical studies have indicated that prolonged infusion of melphalan might be more active than short infusion [5, 19]. Moreover, based on pharmacokinetic considerations (short half-life, small volume of distribution and low protein-binding capacity), melphalan is a good candidate for continuous infusion. However, due to its low stability in 5% dextrose and 0.9% sodium chloride fluids, melphalan is usually administered as a short infusion. A recent study has shown that the stability of melphalan can be increased using 3% sodium chloride [16]. Consequently, it becomes possible to consider the administration of melphalan by continuous infusion. Recently, Pinguet et al. [20] carried out a phase I trial in adult patients with various types of cancer refractory to conventional therapy who received continuous constant infusion of melphalan for 24 h. The authors recommended a 30 mg/m^2 dose for further studies.

The objectives of this study were (1) to characterize the population pharmacokinetics of melphalan infused over a 24-h period in patients with advanced malignancies, (2) to evaluate the influence of various covariates on melphalan pharmacokinetic parameters, and (3) to further explore the relationships between drug exposure and treatment activity on markers and between drug exposure and side effects.

Patients and methods

Eligibility

Data from 64 patients, aged from 19 to 80 years, with histologically documented malignancies were collected for the population pharmacokinetic analysis. There were 42 female patients in the study sample. All patients were followed-up in the Medical Oncology Service of Anticancer Centre (Montpellier, France). The criteria for patients to be entered into the trial included age more than 18 years and performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale. Exclusion criteria were granulocytes lower than $1500/mm^3$, platelets lower than $100{,}000/mm^3$, bilirubin more than 2.5 times the normal limit, transaminases (AST and ALT) more than three times the upper normal limit or more than five times the upper limit in the presence of liver metastases, and significant renal (creatinine clearance, CL_{CR}, calculated according to Cockcroft and Gault [8], < 30 ml/min), pulmonary (FEV_1, FVC, or DLCO $< 65\%$ of the predicted values), or cardiac (isotopic left ventricular ejection fraction $< 50\%$) dysfunction.

Prior to entry, all patients gave written informed consent to participate in the trial, after reading an information leaflet and having the opportunity to ask questions relating to the trial. This study was conducted in accordance with the Declaration of Helsinki as amended in the 41st World Medical Assembly (Hong Kong 1989) and was reviewed and approved by the regional Ethics Committee (Montpellier, France).

Pretreatment evaluation and follow-up

Evaluation prior to treatment consisted of detailed clinical history and physical examination, full blood counts with differential white cell count and platelets, blood chemistry including electrolytes, albumin, renal and hepatic function tests, electrocardiogram, abdominal echography and chest radiography. During the treatment period, patient monitoring included weekly assessment of toxicity and blood counts, blood chemistries during each course and physical examination before each course.

Treatment regimen

Melphalan was infused intravenously by an automatic infusion pump over a 24-h period. The infusion was started at 8 p.m. The drug was dissolved in four syringes of 60 ml 3% sodium chloride; each syringe was administered intravenously over 6 h through a central venous catheter. Before administration, syringes were stored at $+4°C$ (under these conditions melphalan is stable for 48 h [16]). The administered dose ranged from 20 to 30 mg/m^2 according to the patient. Courses were repeated every 28 days. All patients received prophylactic antiemetic premedication ($5HT_3$-receptor antagonists, alizapride, methylprednisolone) before melphalan infusion. The number of courses per patient ranged from one to six.

Toxicity evaluation

Toxicity was assessed weekly according to the Cancer Therapy Evaluation Program's common toxicity criteria and graded 1 to 4. A total of 144 courses were studied.

Sample collection

Patients were divided into two groups according to the sampling time schedule. The first group included 14 patients with rich data for whom the pharmacokinetic behaviour of melphalan was assessed only during the first chemotherapy course. In this group of patients, blood samples were drawn at the following times: (1) immediately before administration, (2) during the infusion period at 2, 8 (11), 23 and 24 h, and (3) 15, 30, 45, 60 and 75 min after the end of infusion. The second group included 50 patients with sparse data. Blood samples were collected during each chemotherapy course (one to six courses depending on the patient), prior to drug administration, then 11, 14 and 25 h after the start of the infusion. Blood samples were collected into heparinized tubes, and immediately centrifuged. Plasma was removed and frozen at $-20°C$ until assay.

Determination of melphalan in plasma samples by HPLC

Melphalan concentrations in plasma were assayed by HPLC with UV detection (261 nm) using a method derived from that of Pinguet et al. [17]. The procedure for assay of this drug in plasma involves the addition of an internal standard (propylparaben) followed by solid-phase extraction (Bond Elut C2; Varian, Les Ulis, France). Chromatographic conditions involved separation on a Kromasil column packed with 5-μm particles (150×4.6 mm) using a mobile phase composed of water/methanol/acetic acid (51:48:1, v/v) at a flow rate of 1.5 ml/min. Calibration standards were prepared in the range of 10 to 250 ng/ml. Precision and accuracy of the method were $\leq 6\%$ and 99–101%, respectively. The limit of quantitation was 10 ng/ml, and at this level the analytical error averaged 5%. Quality control samples were included in each analytical sequence to verify the stability of the study samples during storage and the accuracy and precision of the analysis. The HPLC

assay is specific for melphalan, and no interaction was detected with other drugs given to the patients. The mono- and dihydroxy metabolites were not analysed, since they have shown no evidence of cytotoxicity.

Pharmacokinetic analysis

The subjects included were allocated into a model building set (population group: data from 10 patients in group 1 and from 33 in group 2) and a test data set (validation group: data from 4 patients in group 1 and from 17 in group 2). Potentially explanatory patient characteristics (covariates) such as age, weight, height, gender, body surface area (BSA), CL_{CR}, AST, ALT, alkaline phosphatases and albumin were included in the original data files.

The population pharmacokinetic analysis was performed with the computer program NONMEM (version 5.1) developed by Beal and Sheiner [3] through the graphical interface Visual-NM (version 5.1) [21]. The population characteristics of the pharmacokinetic parameters (fixed and random effects) were estimated using the subroutines ADVAN-1 and TRANS-2 from the library of programs provided with the NONMEM-PREDPP package.

Pharmacostatistical model

Melphalan plasma concentration-time data were fitted by one- and two-compartment structural pharmacokinetic models to find the model that fitted the data best. Model discrimination was performed using the Akaike information criterion (AIC). AIC is proportional to the objective function value estimated by NONMEM, but adds a penalty proportional to the number of parameters in the model. A smaller AIC value is associated with a better model [7]. Preliminary analyses (data not shown) revealed that the one-compartment model led to a significant decrease in the AIC (difference 9.02). Thus, this model was subsequently used for all the analyses presented in this article. First-order (FO) and first-order conditional estimation (FOCE) methods were used to estimate population pharmacokinetic parameters. The estimation was markedly improved by the use of the FO method.

The basic parameters (θ) considered in the population analysis were total clearance ($\theta_1 = CL$, l/h) and volume of distribution ($\theta_2 = V$, l). The interpatient variability of pharmacokinetic parameters was modelled as (e.g. for CL): $CL_j = \hat{C}L_j.\exp(\eta_{jCL})$, where η_{jCl} denotes the (proportional) difference between the true parameter (CL_j) of individual j and the typical value in the population ($\hat{C}L_j$). A combination model including additive and proportional term components was used for residual variability. This model was as follows:

$$C_{ij} = \hat{C}_{ij} \cdot \exp\left(\varepsilon_{1ij}\right) + \varepsilon_{2ij}$$

in which C_{ij} and $\hat{C}_{ij}$ are the measured and predicted plasma melphalan concentrations, respectively, for individual j, and the random variables ϵ_{1ij} and ϵ_{2ij} denote the residual departure of the model from the observations and contain contributions from intraindividual variability, assay error and model misspecification.

Interoccasion variability was clearly apparent in the melphalan concentration profiles for patients studied on more than one occasion. Variability in clearance and the volume of distribution therefore included terms to describe the interoccasion variability [13]. The inclusion of terms for interoccasion variability in CL and V decreased the overall variability in CL from 54.3% to 45.3% and in V from 68.2% to 65.8%.

The predicted concentrations were computed for each individual using the empirical Bayes estimate of the pharmacokinetic parameters using the POSTHOC option in the NONMEM program. Several secondary pharmacokinetic parameters were calculated from the individual (Bayesian estimates) primary pharmacokinetic parameters: the elimination half-life ($t_{1/2elim}$) and the total area under the plasma concentration-time curve (AUC).

Estimation of population parameters

Data analysis was performed using a three-step approach. In step 1, the population parameters (fixed and random effects) together with the individual posterior estimates were computed assuming that no dependency existed between the pharmacokinetic parameters and the covariates. In step 2, the influence of covariates was first assessed by plotting individual empirical Bayesian pharmacokinetic estimates against all the preselected potential covariates. Then, each selected covariate was added to the model and tested for statistical significance. The change in the NONMEM objective function, which is estimated as a -2 times the log likelihood of the data, produced by the inclusion of a covariate term was used to compare alternative models (χ^2 test). If the objective function did not vary significantly, the relationship between the covariate and the parameter was ignored. At the end of the analysis, all patient characteristics that showed an influence on the parameters were evaluated again by comparison of the full model (with all factors included) with a model from which each of the factors was deleted sequentially. In step 3, accepted covariates were added to the model and the population pharmacokinetic parameters were estimated.

Performance Bayesian individual parameter estimates

The performance of the Bayesian estimation was evaluated using 21 patients not included in the calculation of population parameters. Individual pharmacokinetic parameters were computed using the Bayesian estimation procedure, combining the prior knowledge of mean and dispersion of pharmacokinetic parameters in the population to which the selected individual belonged and the individual sample(s). From the resulting individualized pharmacokinetic parameter values, melphalan concentrations in plasma at each sampling time (IPRED) were calculated for each patient.

Pharmacokinetic/pharmacodynamic analysis

During each chemotherapy course, the percentage decreases in red blood cells (RBC) count, white blood cells (WBC) count, and platelet count were plotted against AUC. The percentage decrease is defined as follows: percent decrease $= 100\times(HV_0 - HV_{nadir})/HV_0$, where HV_{nadir} is the value of the haematological variable at the nadir and HV_0 is the basal value.

The relationship between drug exposure and treatment outcomes (response markers) was also explored. Thus, the percent decrease in markers was correlated with AUC.

Statistical analysis

Model acceptance (population group)

The adequacy of the final model to the data was judged by using graphics and descriptive statistics. Model-predicted concentrations based on population parameter estimates (PRED) and model-predicted concentrations based on individual parameter estimates (IPRED) were plotted versus observed concentrations (DV). Weighted residuals were plotted versus time and versus predicted concentrations. Then, the *t*-test was used to compare the average of residuals with zero. The model was accepted when, on the one hand, plots showed no systematic pattern and, on the other hand, descriptive statistics did not show any systematic deviation from the initial hypothesis (mean of residuals supposed to be 0).

Performance of Bayesian estimation (validation group)

The performance of Bayesian estimation was assessed by comparing the observed concentrations (DV) to the ones estimated using

the Bayesian approach (IPRED and PRED) using bias and precision [24, 25].

The bias or mean predictor error was calculated as follows:

$$\text{Bias} = \frac{1}{N}\sum_{i=1}^{i=n}[DV - IPRED]$$

The precision or root mean square error was calculated as follows:

$$\text{Precision} = \sqrt{\frac{1}{N}\sum_{i=1}^{i=n}[DV - IPRED]^2}$$

In these expressions the index i refers to the concentration number and n is the sample size. Confidence interval for bias was computed and the *t*-test was used to compare the bias to 0.

Results

Patient characteristics

Patients were included in the study from March 2001 to March 2003. All of them had advanced-stage disease at the time of initiation of treatment. The primary tumour types were ovarian cancer (34 patients), prostate cancer (12 patients), kidney cancer (6 patients), non-small-cell lung cancer (2 patients), osteosarcoma (2 patients), multiple myeloma (2 patients), rectal cancer (1 patient), thymoma (1 patient), small round cell desmoplastic tumour (1 patient), and head and neck cancer (1 patient). Two patients had adenocarcinoma of unknown primary. The patient characteristics are listed in Table 1. Hormonotherapy and intraperitoneal paclitaxel administration were not considered as a chemotherapy line.

Table 1 Patient characteristics

Characteristic	Mean (range)	Number of patients
Total patients		64
Males		22
Females		42
Age (years)	60 (19–80)	
Weight (kg)	66 (35–102)	
Height (cm)	164 (152–185)	
Body area (m^2)	1.73 (1.37–2.21)	
Performance status (WHO)		
0		14
1		18
2		32
Line of chemotherapy		
First		5
Second		17
Third		31
Fourth		6
Fifth		3
Sixth and seventh		2
Previous treatment		
Hormonotherapy		14
Immunotherapy		6
Platin salts		43
Taxanes		33
Cyclophosphamide or ifosfamide		34
5-Fluorouracil		8
Anthracyclines		17
Topotecan		11
Vinca alkaloids		12
Surgery		50
Radiotherapy		27
Neutrophil count ($/mm^3$)	8,511 (3,400–36,900)	
RBC count ($/mm^3$)	3.62×10^6 ($2.3–7.1\times10^6$)	
Platelet count ($/mm^3$)	310,406 (100,000–720,000)	
Creatinine clearance (ml/min)	80 (30–195)	
AST (mU/ml)	22.6 (9–110)	
ALT (mU/ml)	16.8 (5–150)	
Alkaline phosphatases (mU/ml)	408 (103–4492)	
Albumin (g/l)	35.7 (21–51)	

Toxicity

Haematological toxicity

Prestudy neutrophil, platelet and RBCs were within the normal range (Table 1). Neutropenia and thrombocytopenia were the main haematological toxicities encountered. The median time to neutrophil nadir occurred at 14 days (range 9 to 21 days). The median time to recovery to pretreatment values was 12 days (range 6 to 19 days). Grade 4 neutropenia was observed in 34% of patients (34 courses). Episodes of neutropenic fever occurred in 7 patients (7 courses). Thrombocytopenia was frequently associated with neutropenia. Grade 4 thrombocytopenia was observed in 19 patients (22 courses) and packed thrombocyte infusions were given on eight occasions. The nadir was between day 9 and 20 and recovery occurred at 11 days (6 to 16 days). Anaemia was much less severe. The haematological toxicities are summarized in Table 2.

Extramedullary toxicity

Melphalan was administered 15 min after intravenous conventional antiemetic therapy (ondansetron, ondansetron and methylprednisolone, ondansetron and alizapride or alizapride). In spite of this treatment, nausea and vomiting were observed in 39 of 144 courses. Only one patient (one course) experienced mucositis.

Population pharmacokinetic parameters

The pharmacokinetic database consisted of 274 melphalan concentrations from 43 patients (99 chemotherapy courses). The basic pharmacokinetic parameters (before inclusion of covariates) are shown in Table 3. In step 2, a linear correlation was found between CL_{CR} and CL ($r = 0.495$, $P < 0.0001$), between BSA and CL (0.38, $P = 0.0054$) and between gender and CL ($r = -0.4125$, $P < 0.0001$). The main steps of the building process, the differences in objective function and the associated *P*

Table 2 Haematological toxicity

Course	No. of patients	Neutropenia (grade)					Thrombocytopenia (grade)					Anaemia (grade)				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	64	12	9	14	15	14	27	4	12	12	9	31	17	8	7	1
2	37	4	5	7	10	11	15	4	6	7	5	13	8	10	5	1
3	21	4	3	4	6	4	7	6	3	1	4	8	7	0	3	3
4	11	1	1	3	4	2	5	2	1	1	2	3	3	2	3	0
5	7	0	1	3	1	2	2	1	3	1	0	1	2	3	1	0
6	4	1	0	1	1	1	0	0	1	1	2	0	2	1	1	0

Table 3 Population pharmacokinetic parameters of melphalan. Values in parentheses are the standard error of estimates expressed as coefficient of variation

Parameter	Population group (43 patients)				All patients (64 patients)	
	Without covariate		With covariate		With covariate	
	Population mean	Interindividual variability (CV%)	Population mean	Interindividual variability (CV%)	Population mean	Interindividual variability (CV%)
CL (l/h)	20.1	45.3	$\theta_1 = 0.914$ (25.1%) $\theta_2 = 21.9$ (11.3%) $\theta_3 = 7.98$ (18.5%)	37.5 (17.7%)	$\theta_1 = 1.37$ (28.4%) $\theta_2 = 18.9$ (12.7%) $\theta_3 = 7.01$ (22.7%)	35.1 (15.9%)
V (l)	21.2	65.8	22.9 (11.4%)	69.4 (22.4%)	25.9 (8.65%)	61.0 (22.9%)
Residual intraindividual coefficient of variability	$\sigma_{\epsilon 1}$ 7.80%; $\sigma_{\epsilon 2}$13.5		$\sigma_{\epsilon 1}$ 8.4%; $\sigma_{\epsilon 2}$11.3		$\sigma_{\epsilon 1}$ 8.9%; $\sigma_{\epsilon 2}$15.6	
Objective function	2003.3		1975.9		2917.7	

With $CL = \theta_1 \cdot CL_{CR} - \theta_2(\text{gender} - 1) + \theta_3$

Table 4 Summary of model building. Differences in objective function and estimates of pharmacokinetic parameter variability (CV%) values from different models (gender: male = 1; female = 2)

Model building steps		Interindividual variability on CL	Interindividual variability on V	Objective function	Difference in objective function
Basic model (model 1) before covariate inclusion (step 1)	$CL = \theta_1$; $V = \theta_2$	45.3	65.8	2003.3	–
Alternative models					
Model 2	CL_{CR}effect on CL $CL = \theta_1 \cdot CL_{CR} + \theta_2$	42.9	63.3	1994.7	8.6
Model 3	Gender effect on CL $CL = -\theta_1(\text{gender}-1) + \theta_2$	38.9	72.3	1981.6	21.7
Model 4	BSA effect on CL $CL = \theta_1 \cdot BSA + \theta_2$	43.5	67.6	1997.8	5.5
Model 5	$CL_{CR}$and gender effects on CL $CL = \theta_1 \cdot CL_{CR} - \theta_2(\text{gender}-1) + \theta_3$	37.5	69.4	1975.9	27.4
Final model	$CL = \theta_1 \cdot CL_{CR} - \theta_2(\text{gender}-1) + \theta_3$; $V = \theta_4$				

values are summarized in Table 4. At the end of the model building, a final model was obtained that recognized only gender and CL_{CR} as significant covariates for CL (Fig. 1). These patient covariates were combined in the following full regression model for CL as follows:

$$CL = \theta_1 \cdot CL_{CR} - \theta_2(\text{gender} - 1) + \theta_3$$

The inclusion of this second stage model significantly improved the fit of the basic model and provided a substantial decrease in unexplained clearance interindividual variability when compared with the baseline model (Table 4). Consideration of the above two covariates during modelling also improved the relationship between model-predicted and observed concentrations

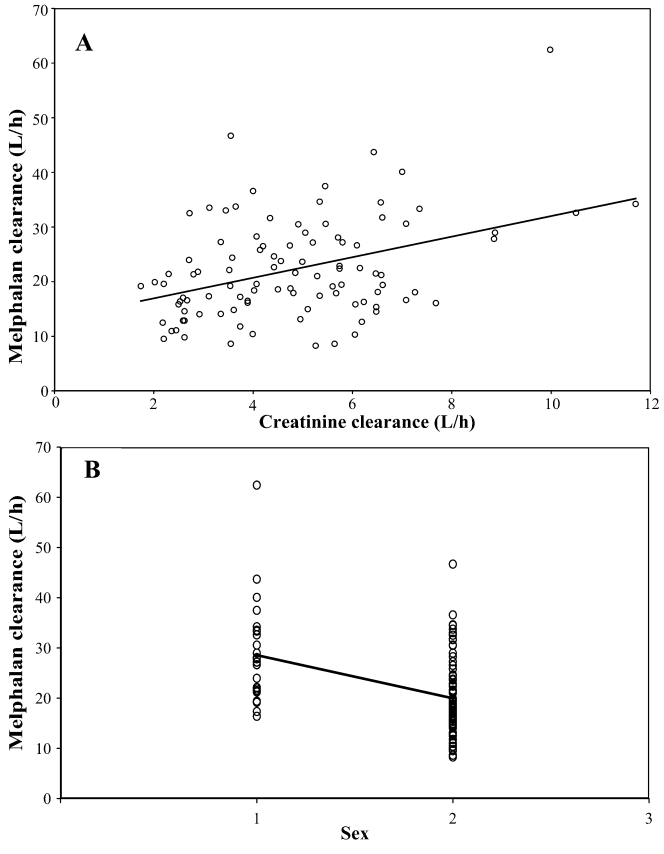

Fig. 1A, B Linear dependency between melphalan clearance and creatinine clearance (**A**) and between melphalan clearance and gender (**B**)

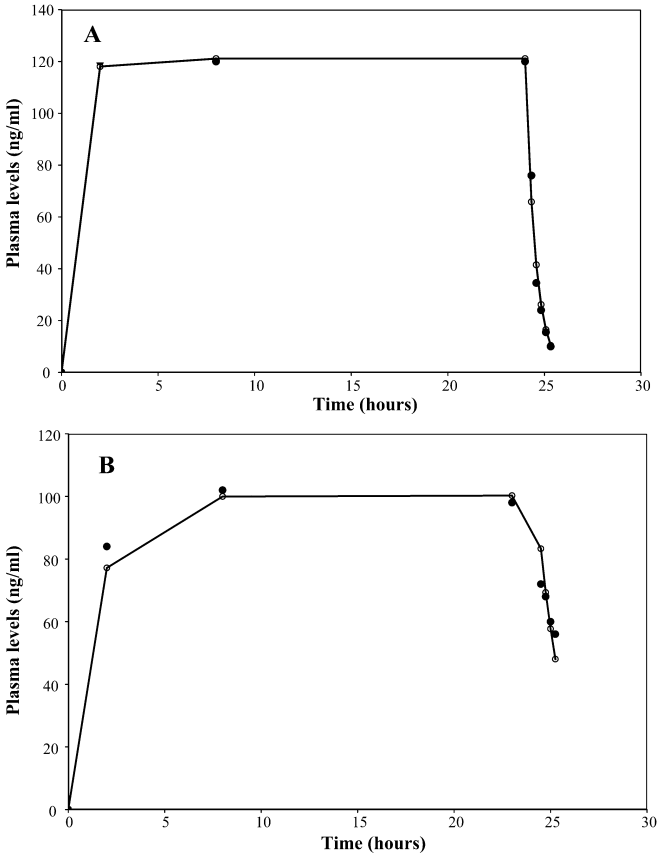

Fig. 2A, B Plasma concentration-time profile of melphalan in representative patients (**A** population group, **B** validation group; ● observed plasma concentrations, ○ individual predicted plasma concentrations). Lines were obtained from individual predicted values connected point by point

and weighted residuals versus model-predicted concentrations and versus time. The parameter estimates calculated from the final model are reported in Table 3 together with their coefficients of variation, which indicate the precision of the estimates. Mean population clearance was 22.2 l/h.

The mean values of secondary pharmacokinetic parameters such as AUC and $t_{1/2elim}$ calculated from the primary pharmacokinetic parameters were 2.12 ± 0.89 mg·h/l (normalized to a 40 mg administered dose) and 0.95 ± 0.52 h, respectively. A typical posterior individual fitting is displayed in Fig. 2A. The interoccasion variability for clearance was 25.4%; it was 27.5% for V.

Model acceptance

The plot representing individual predicted concentrations versus observed data for the final model did not show either any substantial or systematic deviations from the line of identity (Fig. 3A). A plot of model-predicted versus observed concentrations for the final model based on population parameter estimates is shown in Fig. 4A. Plots of weighted residuals versus predicted concentrations and versus time are displayed in Fig. 4B, C, respectively. The vast majority of the weighted residuals lay within two units of perfect agreement and were systematically distributed around the zero ordinate. Moreover, the ability of the model to describe the data well was confirmed as coefficients of variation of final estimates ranged between 8% and 25% (Table 3).

Performance of Bayesian individual parameter estimates

In the validation group, individual pharmacokinetic parameters (137 melphalan concentrations from 21 patients, 45 chemotherapy courses) were estimated using the population characteristics. Mean ($\pm$SD) CL and V values were 22.9 ± 8.1 l/h and 28.7 ± 12.3 l, respectively. The regression line between individual predicted and observed melphalan concentration values did not differ significantly from the reference line of slope = 1 and intercept = 0 (Fig. 3B). Bias (−0.41 ng/ml) was not significantly different from zero (*t*-test) and the 95% confidence intervals included the zero value (−1.9/1.15). Furthermore, the precision of the concentration prediction (7.95 ng/ml; 95% confidence intervals

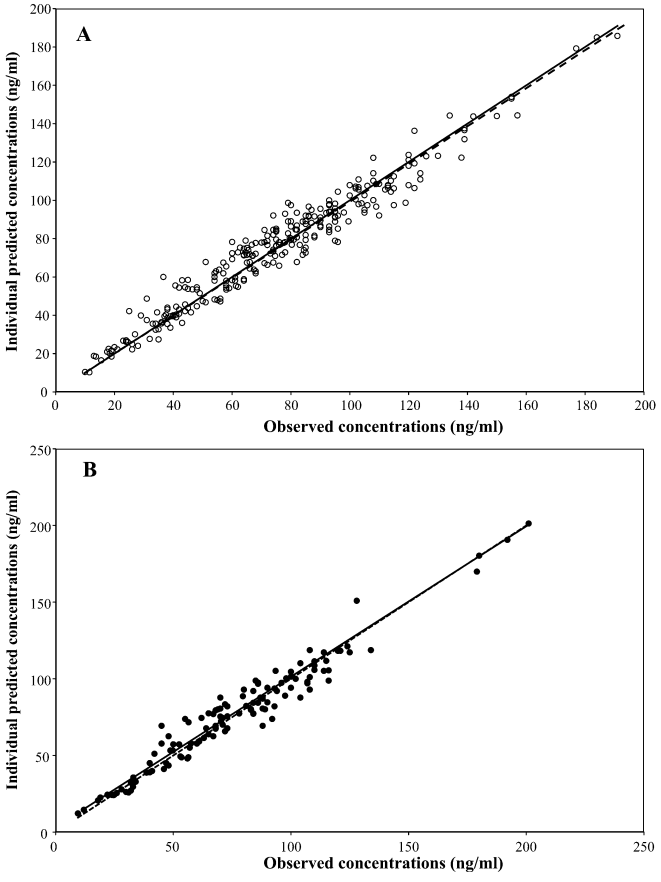

Fig. 3A, B Relationship between individual predicted (IPRED) and observed (DV) plasma concentrations (**A** population group, **B** validation group; *dashed line* line of identity, *solid line* linear regression line)

−9.9/25.8) remained lower than the interindividual standard deviation of the observed concentrations (36.4 ng/ml). Moreover, in order to detect a possible model misspecification, bias and precision of PRED versus observed concentrations were computed. Bias (3.8 ng/ml) was not significantly different from zero (*t*-test) and the 95% confidence intervals included the zero value (−4.9/12.4); the precision of the concentration prediction was 29.6 ng/ml.

A typical posterior individual fitting is displayed in Fig. 2B. In this validation group, for each chemotherapy course, three to nine blood samples per patient were available. In a last step, we compared CL estimated from the full sampling schedule (Bayesian estimation) to that (1) estimated using the population characteristics and only one blood sample drawn at steady state, 11 h after the start of infusion ($C_{ss(11\ h)}$, i.e. 1 h before the change of the second syringe) and (2) computed from the formula $k_0/C_{ss(11\ h)}$ (where k_0 is the perfusion rate). The results are presented in Fig. 5. The best results were obtained when CL was estimated by Bayesian approach and one blood sampling (23.1 ± 7.9 l/h); when compared with the reference value (22.9 ± 8.1 l/h), the bias, −0.14 l/h, was not significantly different from zero (*t*-test) and the 95% confidence interval included the

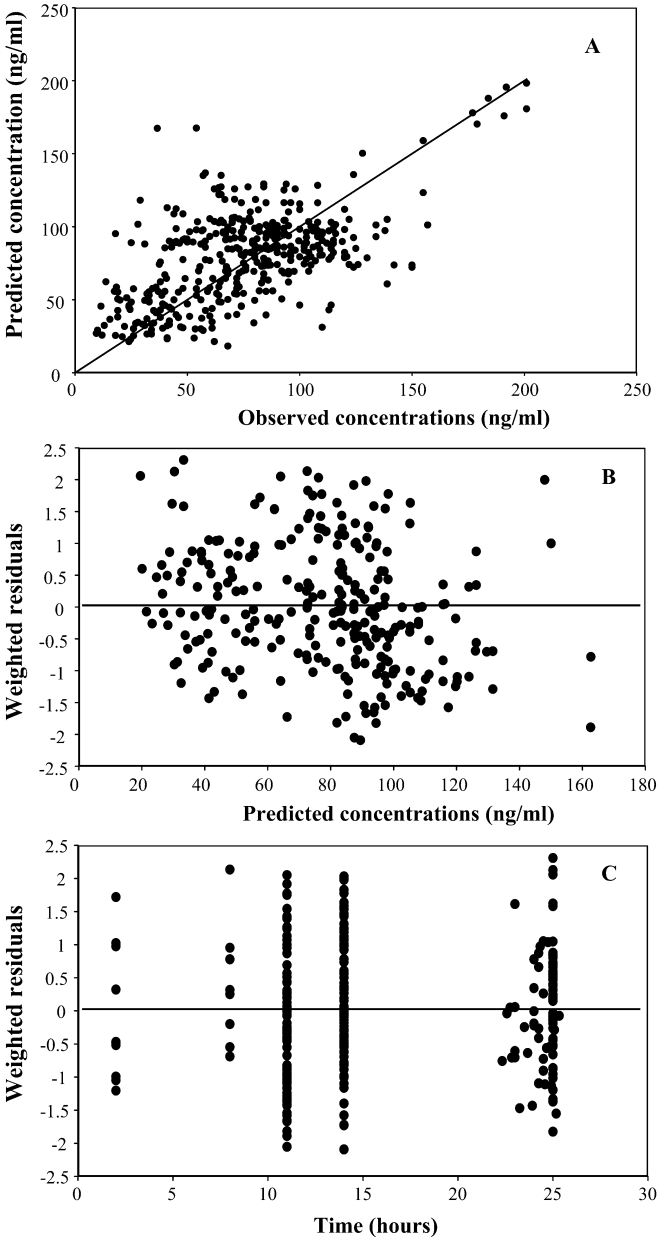

Fig. 4 **A** Model-predicted versus observed melphalan concentrations obtained from final model based on population parameter estimates. The solid line represents the line of identity. **B** Weighted residuals versus predicted concentrations; **C** weighted residuals versus time

zero value (−0.43/0.15). The use of the formula led to biased results (mean CL value 23.6 ± 9.1 l/h, bias −0.68 l/h, 95% confidence interval −1.15/−0.21).

Final population pharmacokinetic parameters

Finally, population pharmacokinetic parameters (including the standard error of estimates expressed as coefficient of variation) were re-estimated using all individuals (64 patients and 144 chemotherapy courses; Table 3). The mean CL value was 22.3 l/h. Mean values

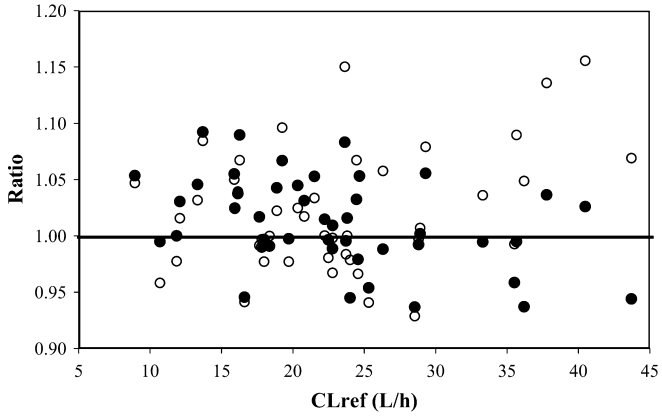

Fig. 5 Ratio plot of CL determined by considering all data concentrations (CL_{ref}, Bayesian estimation) to that (1) determined from one blood sampling taken 11 h after the start of infusion (Bayesian estimation) (●) or (2) computed from $CL = k_0/C_{ss(11\ h)}$ (○)

of the secondary parameters calculated by Bayesian estimation were AUC (normalized to a 40 mg administered dose) 2.03 ± 0.71 mg·h/l and $t_{1/2elim}$ 0.96 ± 0.48 h, respectively. The population parameters were very similar to the ones computed from the patients of the population group (43 patients).

Pharmacokinetic pharmacodynamic analysis

The relationship between dose-limiting toxicities and AUC was studied during first course. Different models including linear, log-linear, exponential and sigmoidal E_{max} were tested. No relationship was found. We also explored the relationship between melphalan exposure and the decrease in markers (PSA in patients with prostatic adenoma, CA125 in patients with ovarian cancer). Of patients with prostatic adenoma, 42% failed treatment, and of these patients PSA levels increased after two chemotherapy courses, and the treatment was stopped. For the other patients, a weak relationship was found between PSA levels and AUC ($P = 0.0457$). A mean of 27% decrease in PSA was observed for AUCs in the range 1500–1600 mg·h/l, while a 58% decrease was observed for AUCs in the range 2100–2500 mg·h/l. Of 29 assessable patients with ovarian cancer, CA125 levels increased in 9 after two chemotherapy courses, and the treatment was stopped. For the other patients, a decrease in CA125 levels occurred during the treatment (from 12% to 95% depending on the patient), but no relationship was found between AUC and CA125 levels ($P = 0.095$).

Discussion

The short elimination half-life of melphalan, its mechanism of action and results from in vitro studies [5, 19] are in favour of continuous infusion for this drug. In a recent phase I study, carried out after continuous melphalan infusion over a 24-h period, the maximum tolerated dose was estimated at 30 mg/m^2 [20]. At this dose, the AUC averaged 2.94 mg·h/l, and the major toxicities of melphalan (neutropenia and thrombocytopenia) were limited.

The present study was designed to assess the population characteristics of melphalan administered over a 24-h period in patients with advanced malignancies. The infusion was started at bedtime. Melphalan displays a biphasic profile with a distribution half-life averaging 16.3 min [18]. However, findings from the present study failed to support a two-compartment structural model. Indeed, samplings within the first half hour of dose administration would have been necessary to detect this rapid distributive phase. Accordingly, as previously reported [20], a one-compartment model was retained for this population pharmacokinetic analysis; this model led to a significant decrease in the AIC.

Melphalan population characteristics were estimated from 43 patients (99 chemotherapy courses) using NONMEM. Covariate model building was performed using NONMEM and supportive exploratory regression analyses based on individual estimates. Both analyses led to very similar findings: melphalan total clearance and therefore drug exposure was shown to be significantly correlated with creatinine clearance and gender. Thus, melphalan clearance was higher in male patients (14.3 ± 4.5 l/h per m^2) than in female patients (12.3 ± 4.5 l/h per m^2), and systemic drug exposure (normalized to a 40 mg administered dose) was accordingly increased in female patients (2.33 mg·h/l versus 1.62 mg·h/l). CL was also reduced somewhat in patients with decreased creatinine clearance, an effect presumably related to impairment of renal function. This result is in accordance with the findings of Cornwell et al. [9] and Adair et al. [1]; indeed, these authors propose reducing the dose in patients with renal insufficiency. The other covariates did not significantly explain part of the interindividual variability in pharmacokinetic parameters. It is interesting to note that BSA was not a covariate significantly retained in the final model, although BSA is currently used for melphalan dosing.

The performance of the Bayesian estimation was evaluated using plasma concentration-time data from 21 patients (45 chemotherapy courses, validation group) not included in the calculation of population parameters. The low values of the bias (−0.41 ng/ml with the confidence interval which included zero) and of the precision (7.95 ng/ml, lower than the interindividual standard deviation 36.4 ng/ml) of the concentration prediction showed that the population characteristics allowed a good prediction of individual pharmacokinetic parameters. For determining melphalan AUC in clinical routine from one sample drawn at steady state, the Bayesian methodology allowed a more accurate estimation of CL than the classical formula (Fig. 4).

Indeed, using a Bayesian approach, the data available for the patient are weighted by the knowledge of population characteristics.

The present study confirmed the large interpatient variability in pharmacokinetic parameters observed during the phase I study [20]. We observed a ratio of 7–9 between the extreme values. Such a variability has also been demonstrated for melphalan after short-term infusion and after chronic oral administration [23, 27, 30]. In addition to the population approach used for analysing the melphalan data, the originality of the present work consists in the careful evaluation of intraindividual pharmacokinetic variability; the interoccasion variability for clearance was 25.4%. In this study, the main haematological toxicities encountered were neutropenia and thrombocytopenia. Neutropenia and thrombocytopenia WHO grade 4 occurred, in a mean of 26% and 18% of patients, respectively.

Pharmacodynamic data relative to melphalan are scarce. It has been reported that after melphalan administration, both the severity and duration of myelosuppression are dose-dependent [23]. However, in the present study, no significant relationship between AUC and haematological toxicity was found. Most of the patients received at least three lines of chemotherapy that could increase the side effects of the treatment. In patients with prostatic cancer, promising results were observed since 7 out of 12 patients experienced a decrease in PSA levels. A weak relationship was found between PSA level and AUC ($P = 0.0457$), but these results must be considered with caution due to the small number of patients. Among patients with ovarian cancer, only one was still in complete remission at the time of this report after 2 years. No relationship was found between AUC and CA125 levels. The large heterogeneity in the study population, including third to seventh chemotherapy lines may mask a clear association with therapy outcome.

In conclusion, drug monitoring of melphalan is planned to be systematically performed at our anticancer centre with dose individualization. Drug adjustment could be made during the infusion period, after the 12th hour, in order to constrain the overall AUC within the range 2–2.5 mg·h/l. This target AUC has been chosen according to the first results obtained during the phase I study [20]. According to the haematological toxicity encountered, the administered dose of the next course might be decreased to achieve an AUC of 1.5–2 mg·h/l, increased to achieve an AUC of 2.5–3 mg·h/l or maintained. In order to confirm the effectiveness of drug monitoring in maintaining the efficacy of therapy and decreasing toxicity, phase II trials with minimally pretreated patients are planned.

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