A Phase I and Pharmacokinetic Study of Melphalan Using a 24-hour Continuous Infusion in Patients with Advanced Malignancies

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ABSTRACT

The objectives of the present study were to determine the following: (a) the maximum tolerated dose (MTD) of melphalan using a 24-h continuous infusion; (b) the clinical toxicity; and (c) the pharmacokinetic characteristics of melphalan at each dose level. Twenty-one patients with refractory solid tumors were enrolled in the study. Melphalan, packaged in 3% sodium chloride, was administered i.v. over a 24-h period. Patients were assigned to one of three escalating dose levels of melphalan: (a) 20 mg/m² (n = 5); (b) 30 mg/m² (n = 7); and (c) 40 mg/m² (n = 6). Each patient underwent pharmacokinetic evaluation during the first cycle of treatment. Melphalan concentrations in plasma were determined by high-performance liquid chromatography. Toxicity was evaluated after each course of chemotherapy. All of the patients were assessable for toxicity and pharmacokinetics, and 20 patients were assessable for response analysis. A total of 50 courses of melphalan was studied. The MTD was 30 mg/m². The dose-limiting toxicity was neutropenia and thrombocytopenia. Hematotoxicity was reversible (nadir, 14–15 days; recovery, 3.5 and 12.5 days for 30 and 40 mg/m², respectively), cumulative, and related to the administered dose and to the history of previous therapy. There were six episodes of neutropenic sepsis. Individual pharmacokinetic parameters were estimated using a Bayesian approach and linear elimination kinetics. Data were compatible with a one-compartment model. Relationships have been found between the area under the plasma concentration-time curve and doses and between Cₘₚ and doses. Moreover, clearance, t₁/₂ elimination, and volume of distribution did not change statistically with dose, which suggests linear kinetics. Two partial responses were observed in patients with ovarian carcinoma or adenocarcinoma of unknown primary origin, and another patient had stabilization disease. In conclusion, melphalan MTD was determined to be 30 mg/m² when administered as a 24-h infusion. Hematological toxicity was the dose-limiting toxicity. The most important nonhematological toxicity encountered was nausea and vomiting. The recommended dose for Phase II studies was 30 mg/m².

INTRODUCTION

Melphalan was introduced into clinical use in the late 1950s and has since been established as an agent with a wide spectrum of antitumor activity (1, 2). It is extensively used in the treatment of multiple myeloma, ovarian cancer, breast cancer, and neuroblastoma (3–5). Melphalan is an alkylating agent of the bischloroethylamine type that exerts a cytotoxic effect through the formation of interstrand or intrastrand DNA cross-links or DNA-protein cross-links via its two chloroethyl groups (1).

Founded on the pharmacokinetic principles of phase-specific, plasma half-life, and stability in solution, infusional schedules for chemotherapy administration represent a rational method for the delivery of many antineoplastic agents. For several antimitotic drugs, long-term continuous infusion increased therapeutic activity or improved the therapeutic index by decreasing toxicity (6).

Bosanquet and Bird (7) studying in vitro degradation of melphalan reported that continuous exposure of melphalan in chemosensitivity assays is probably preferable to the arbitrary 1-h exposure commonly used. Moreover, Teicher et al. (8) indicated that in vitro continuous administration of melphalan could be at least as cytotoxic as bolus administration of the same dose on MCF7 breast cancer cells. In a recent in vitro study, we have shown that protracted infusion of melphalan had a higher cytotoxic effect than bolus administration on 8226 (myeloma) and A2780 (ovarian) cancer cell lines (9).

On the basis of in vitro study and pharmacokinetic (2) considerations (short half-life, small Vd,2 and low protein-binding capacity), melphalan is a good candidate for continuous

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2 The abbreviations used are: Vd, volume of distribution; MTD, maximum tolerated dose; ECOG, Eastern Cooperative Oncology Group; Cl, clearance; t₁/₂ elim, elimination half-life; AUC, area under the plasma concent-
infusion. Significant intra- and interpatient variabilities in pharmacokinetic parameters have been shown. After short i.v. infusion, the distribution half-life ranged from 5 to 15 min, and the $t_{1/2}\text{elim}$ from 17 to 75 min (10–12) or from 2 to 4 h (13). Total plasma Cl ranged from 92 to 961 ml/min/m$^2$. The Vd was found to be greater than the total-body water (35.5–185.7 liters/m$^2$), although lower values have been reported (8 and 50 liters/m$^2$; 1, 10–16).

However, in clinical practice, melphalan must be prepared extemporaneously before administration because its rapid degradation in conventional 0.9% sodium chloride at ambient temperature prohibits its use in continuous infusion (17). Thus, the manufacturer recommends use of this drug within 1.5 h after constitution. Degradation has been reported to be a function of sodium chloride concentration, temperature, and pH. In a previously published work (17), we have optimized the stability conditions of melphalan. In 3% sodium chloride, the drug was stable for up to 6 h at room temperature, which allowed a longer infusion time.

Here, we conducted a Phase I trial in adult patients who had various types of cancer that were refractory to conventional therapy and who received continuous constant infusion of melphalan for 24 h. The objectives of this study were to determine the MTD of melphalan and to evaluate its toxicity and its antitumor activity as well as its pharmacokinetic characteristics at each dose.

PATIENTS AND METHODS

Requirements for Patient Enrollment. Twenty-one patients, ages 18–74 years, with histologically documented malignancies, admitted to the Medical Oncology Service of Anti-cancer Center (Montpellier, France), were considered eligible for this study. They had a performance status of 0–2 on the ECOG scale. Each patient underwent an initial evaluation, including (a) history and physical examination; (b) histopathological review; (c) chest X-ray; (d) pulmonary function test; (e) computerized tomodensitometry of the brain, thorax and abdomen; (f) and bone scan. Laboratory tests included: (a) complete hematology; (b) liver function (serum bilirubin, aminotransferases, γ-glutamyl-aminotransferase, alkaline phosphatase, and serum proteins); (c) renal function (serum urea, serum creatinine, creatinine clearance calculated according to Cockcroft and Gault; Ref. 18); and (d) serology (HIV, hepatitis B and C virus, and cytomegalovirus). All of the subjects were negative for HIV virus and hepatitis B and C. Patients with granulocytes < 1500/mm$^3$, platelets < 100,000/mm$^3$, significant renal dysfunction (creatinine clearance < 50 ml/min), significant hepatic dysfunction (serum bilirubin > 2.5 times normal or alanine aminotransferase, aspartate aminotransferase > four times normal), significant pulmonary dysfunction (forced expiratory volume in 1 s, forced vital capacity, or transfer factor for carbon monoxide < 65% of the predicted values), or significant cardiac dysfunction (isotopic left ventricular ejection fraction < 50%) were excluded from the study. A total of six cycles per patient was planned.

The study protocol was reviewed and approved by the institutional review board. It was performed in accordance with the Declaration of Helsinki, and with current European Community and United States Food and Drug Administration guidelines for good clinical practice. The patients were fully informed about the procedure and the purpose of the experiment and gave written consent.

Treatment Regimen. Melphalan was administered over a 24-h period. The drug was dissolved in four syringes of 60 ml of 3% sodium chloride; each syringe was administered i.v. over 6 h through a central venous catheter using a two-way portable infusion pump. Before administration, syringes were stored at +4°C (in these conditions melphalan was stable for 48 h). The first dose-step of melphalan consisted of 20 mg/m$^2$. Dose escalations were 10 mg/m$^2$ and were to proceed after at least five patients had successfully completed therapy at a given dose level. Chemotherapy was repeated every 3 weeks. If WHO grade 4 toxicity occurred in one patient at a given dose, at least four additional patients were enrolled at that level before dose escalation. If grade 4 toxicity occurred in five patients at a given dose, escalation was stopped.

Response Criteria. Tumor measurements were repeated once every two to three treatment cycles. Moreover, at the end of treatment, patients were evaluated for response by physical examination, chest X-ray, and computerized axial tomography of the abdomen and/or chest when appropriate. Tumor response definition was based on WHO criteria. Patients designated complete responders (CR) attained complete resolution of all disease lasting for at least 4 weeks. A partial response (PR) was defined as a ≥50% reduction in the sum of products of bidirectionally measurable tumors without the appearance of new lesions. Patients with stable disease had tumor measurements within 25% of on-study measurements lasting for at least 4 weeks without development of new lesions. Progressive disease (PD) was considered as the appearance of any new lesions or a more than 25% increase in the sum of products.

Toxicity Evaluation. Patients were assessed after each course of chemotherapy by clinical examination and by serum and urinary parameters studies to evaluate possible bone marrow, renal, or liver toxicity. Toxicity was defined according to the Cancer Therapy Evaluation Program’s common toxicity criteria and graded 1 to 4. Dose-limiting toxicity was defined as irreversible grade 2 nonhematological toxicity, reversible grade 3 nonhematological toxicity, or grade 4 hematological toxicity. The recommended MTD dose was defined as the dose below that causing dose-limiting toxicity.

Pharmacokinetic Analysis. During the first cycle of treatment each patient underwent pharmacokinetic evaluation. Blood samples for determination of melphalan concentrations were obtained during the infusion at 2, 8, 23, 23.25, 23.5, 23.75, and 24 h and, after the end of the infusion, at 15, 30, 45, 60, and 75 min and 2 and 4 h. Blood was collected in heparinized tubes and immediately centrifuged. Plasma was removed and frozen at −20°C until assay. The plasma concentration versus time data for each patient were subjected to pharmacokinetic analysis using the P-PHARM software (19). Individual pharmacokinetic parameters were estimated using a Bayesian methodology that
combines the prior knowledge of the mean and dispersion of the pharmacokinetic parameters in the population to which the selected individual belongs and the individual samples. Such an approach avoided a possible bias in the estimation of the elimination half-life. Indeed, at the low dose (20 mg/m²) for the last sampling times, melphalan concentrations were below the limit of quantitation of the analytical method. Preliminary analysis revealed that the data were best fitted by a one-compartment model (on the basis of the examination of the Akaike criterion, the objective function, and the residuals distribution) and that the residuals distribution showed that the error variance was better described by a heteroscedastic (proportional to the squared value of the predictions) model. The structural model was parametrized in terms of Vd and Cl. From the resulting individualized parameter values, the t₁/₂ elim and the AUC were calculated as follows:

\[ t_{1/2} \text{ elim} = \frac{V_d \times 0.693}{C_l} \]

and

\[ \text{AUC} = \frac{\text{dose}}{C_l} \]

respectively. Cₘₜ is the observed plasma concentration at steady-state. Cₘₜ and AUC were normalized to a 20 mg/m² administered dose.

**Determination of Melphalan Concentration in Plasma Samples by HPLC.** Melphalan concentrations in plasma were assayed by HPLC with UV detection (254 nm) using the method described previously (20). The procedure involves the addition of an internal standard (propylparaben) followed by treatment of the samples with methanol. The HPLC column, Ultrasphere C18 (250 × 4.6 mm, 5 μm), was equilibrated with an eluent mixture consisting of methanol/water/acetic acid (49.5:49.5:1). Calibration standards were prepared in the range of 0.020 to 50 μg/ml. Within- and between-day variabilities of the method were <8%. The limit of quantitation was 10 ng/ml; at this level the analytical error averaged 20%.

Quality-control samples were included in each analytical sequence to verify the stability of samples during storage and the accuracy and precision of analysis. Each determination was performed in duplicate.

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 analyzed because they have shown no evidence of cytotoxicity.

**Statistical Analysis.** The results are expressed as mean ± SD.

Plasma concentrations and AUC were plotted against dose. Linear regression was performed using unweighted least-squares analysis of the data. The significance of the regression was confirmed using the F test.

A Kruskal-Wallis test was performed to compare normalized (to a 20 mg/m² dose) AUC and Cₘₜ, Cl, Vd, and the t₁/₂ elim across doses.

**RESULTS**

**Patient Characteristics.** The main clinical characteristics of the patients entering this study are listed in Table 1. All of the patients had advanced-stage disease at the time of the initiation of treatment. All of them had received conventional chemotherapy protocols previously, and most of them had received prior radiotherapy. A total of 50 courses of melphalan were given, with a median of 2.4 courses per patient (range, one to six). The starting dose level was 20 mg/m² (five patients, 14 courses); the dose was escalated to 30 mg/m² (nine patients, 23 courses), 40 mg/m² (seven patients, 13 courses) in cohorts of newly enrolled patients.

All of the patients were assessable for toxicity, and 20 patients were assessable for response.

**Toxicity**

**Hematological Toxicity.** Prestudy neutrophil, platelet, and hemoglobin levels were within the normal range. As expected, neutropenia was the main hematological toxicity (Table 2). The median time to leukocyte and neutrophil nadirs occurred at 15 days (range, 13–18) and 14 days (range, 9–21 for the 30- and 40-ng/ml dose level, respectively. The median time to recovery to pretreatment values was 3.5 days (range: 1–9) at 30 mg/m² and 12.5 days (range: 1–23) at 40 mg/m². Grade 4 neutropenia developed in 38% (19 courses) of the cycles, and the median duration of neutropenia lower than 0.5 × 10³/μl was 3 (range, 1–8) days and 11 (range, 1–20) days after administration of 30 and 40 ng/ml, respectively. Neutropenia showed a high degree of interpatient variability. It seemed to be dose-related; grade 3–4 neutropenia was observed in 0, 55, and 71% of the cycles at 20, 30, and 40 mg/m², respectively. Episodes of neutropenic fever were reported in six patients (2 at 30 mg/m² and 4 at 40 mg/m²). In five cases, fever episodes were bacteriologically documented: *Escherichia coli* in two cases, *Enterococcus faecalis* in one case, *Streptococcus D* in one case, and *Proteus mirabilis* in one case.

Thrombocytopenia was always associated with grade 3–4 neutropenia. Grade 4 thrombocytopenia was observed in four

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics</th>
</tr>
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<tbody>
<tr>
<td>Characteristics</td>
<td>No. of patients</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Total patients</td>
<td>21</td>
</tr>
<tr>
<td>Female/Male</td>
<td>10/11</td>
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<td>Age, years</td>
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<tr>
<td>Median: 54</td>
<td></td>
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<td>Range: 18–74</td>
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<tr>
<td>ECOG 1</td>
<td>10</td>
</tr>
<tr>
<td>ECOG 2</td>
<td>11</td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>21</td>
</tr>
<tr>
<td>Radiotherapy</td>
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<td>Primary tumors</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Unknown primary</td>
<td>3</td>
</tr>
<tr>
<td>Ovary</td>
<td>3</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>2</td>
</tr>
<tr>
<td>Myeloma</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
</tr>
</tbody>
</table>

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patients and two patients treated at doses of 40 mg/m² and 30 mg/m², respectively. Grade 3 thrombocytopenia was observed in three patients treated at 30 mg/m². At the dose level of 20 mg/m², no grade >1 was observed. Twenty-six cycles (52%) were associated with thrombocytopenia of at least grade 3, and packed thrombocyte infusions were given on 2, 7, and 16 occasions after the administration of 20, 30, and 40 mg/m², respectively. Recovery occurred at 5.5 (range, 4–7), 2.1 (range, 1–6), and 5.4 (range, 1–37) days at 20, 30, and 40 mg/m², respectively.

There were cumulative myelo- and thrombocyto-suppressions. Indeed, neutropenia and thrombocytopenia were more pronounced after the first courses of melphalan. The severity and the incidence of toxicity varied according to the history of previous cancer therapy.

Anemia was dose related. In the first course of chemotherapy, four grade 3 (two at 30 mg/m² and two at 40 mg/m²) and two grade 4 were observed. Thirty-four cycles (68%) were associated with anemia of at least grade 2, and packed red cell infusions were given on 4, 13, and 21 occasions for 20, 30, and 40 mg/m² dose level, respectively.

**Extramedullary Toxicity.** Toxicities of the three dose levels of melphalan are listed in Table 3. They were moderate, and their incidence and severity were not dose related. Indeed, there were only three grade 3 and one grade 4 effects noted for the 50 courses of chemotherapy. The main toxicities included gastrointestinal and hepatic complications.

Melphalan was administered 15 min after i.v. conventional antiemetic therapy (granisetron, granisetron and methylprednisolone, granisetron and alizapride, or alizapride). Despite this treatment, nausea and vomiting were observed in 4 of 50 courses. In most of the patients, these gastrointestinal complications were brief in duration and occurred during the treatment or a few hours after the infusion.

During this study, mucositis was not observed.

Mild elevations of alkaline phosphatase (WHO grade 1, three patients) and γ-glutamyl-aminotransferase (WHO grade 2, four patients) were observed. Alkaline phosphatase elevation of greater than 130 times normal (WHO grade 4) occurred in one patient treated with 20 mg/m² of melphalan; for this patient the other liver tests were in the normal range. Prior chemotherapy with high-dose methotrexate (about 2 years ago) might explain such results.

**MTD**

The MTD was 30 mg/m² (Table 2). At this dose, at the first course, four patients (40%) of seven had grade 4 thrombocytopenia and neutropenia, and only 10% of patients showed anemia; these percentages did not increase over the successive courses of chemotherapy. The 30 mg/m² dose was tolerable for two to six courses according to the patient. The study was closed to enrollment after the development of significant toxicity at highest dose (40 mg/m²), and no additional patients were enrolled.

**Pharmacokinetic Characteristics of Melphalan**

The population database consisted of 130 melphalan concentrations. The population parameters (fixed effect: CI and Vd and random effect: sigma(Cl), sigma(Vd) were as follows: Cl = 168 ml/min/m² (CV = 30.6%) and Vd = 15 liters/m² (coefficient of variation = 50.4%). Fig. 1 shows the plasma decay curves of melphalan (plasma concentration versus time curve) of three representative patients. The mean (± SD) values of the main pharmacokinetic parameters are reported in Table 4. Large interindividual variability of the pharmacokinetic parameters was observed. For the majority of patients, a steady state concentration was reached about 5 h after the start of infusion (extreme values: 0.039–0.122 μg/ml at 20 mg/m²; 0.084–0.15 μg/ml at 30 mg/m²; and 0.123–0.265 μg/ml at 40 mg/m²). A linear relationship was found between the dose and Cₜ₅₀ (r = 0.57; P = 0.0068) and between the dose and AUC (r = 0.60, P = 0.0038). Normalized to a 20-mg/m² dose, mean AUC values showed considerable interindividual variability and overlapped between dose levels. They were 1.91, 1.96, and 2.54 μg·h/ml, after administration of 20, 30, and 40 mg/m², respectively. Despite an increase in the mean values with the doses, no significant difference occurred (P = 0.148; NS). The t₁/₂₅₀ e l i m (mean values: 40.2 min at 20 mg/m², 55.2 min at 30 mg/m² and 86.4 min at 40 mg/m²) and Vd (mean values: 10.8 liters/m² at 20 mg/m², 17.8 liters/m² at 30 mg/m² and 16.6 liters/m² at 40 mg/m²) increased with dose, whereas CI (mean values: 192 ml/min/m² at 20 mg/m², 178 ml/min/m² at 30 mg/m², and 141

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**Table 2** Hematological toxicity according to dose level of melphalan

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of patients</th>
<th>Anemia grade</th>
<th>Neutropenia grade</th>
<th>Thrombocytopenia grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n¹</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>23</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

¹ n, number of courses.

**Table 3** Nonhematological toxicity for all dose levels (N = 50)

<table>
<thead>
<tr>
<th>Grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea/Vomiting</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pain</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>γ-glutamyl-aminotransferase</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Confusion, hallucination</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
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</table>
ml/min/m² at 40 mg/m²) decreased; however, statistical comparison of the pharmacokinetic parameters revealed no significant difference in the $t_{1/2}$ elim ($P = 0.0997$), Cl ($P = 0.148$), and Vd ($P = 0.203$; Table 4). No significant relationship was found between AUC or $C_{ss}$ and the hematologic toxicity.

Tumor Response

Disease response was determined in 20 patients. Two patients (10%) had a partial response (>50% reduction) of an ovarian carcinoma and an adenocarcinoma of unknown primary origin that has been sustained for >4 months in both patients. One patient with thymoma had stable disease for a period of up to 4 months. Disease progression was observed in the other patients.

DISCUSSION

The mechanism of action (phase-specificity), pharmacokinetics (plasma half-life, Vd, and protein binding), stability in solution, and available preclinical data predict that many cytotoxic agents will have schedule-dependent antitumor activity and toxicity. Refinement i.v. access options and ambulatory infusion pumps have stimulated increased interest in infusional delivery because of the decreased logistic complexity with attendant increased patient convenience. Particularly compelling is the schedule dependency of cytarabine cytotoxicity, in which single large bolus injections have modest antitumor activity, whereas continuous exposure or multiple smaller bolus infusions display markedly enhanced activity. Selected trials of continuous infusion of bleomycin in cervical carcinoma, vinblastine in breast carcinoma, 5-fluorouracil in colon carcinoma, and methotrexate in acute lymphocytic leukemia have suggested increased antitumor activity compared with conventional bolus infusion (21–25). In addition to therapeutic activity, several practical aspects of infusional chemotherapy provide potential advantages over bolus chemotherapy. Infusional administration may improve the therapeutic index by decreasing gastrointestinal side effects and cardiotoxicity associated with doxorubicin, as well as pulmonary toxicity, associated with bleomycin (26–29).

The alkylating agents represent a chemically heterogeneous group of compounds, with a broad spectrum of applications in cancer treatment (4, 30, 31). Infusional schedules for these agents demonstrate a clinical substantial experience and increasing application (cyclophosphamide and ifosfamide; Ref. 32). For melphalan, no study has been published with systemic infusional chemotherapy. The difficulty in using melphalan

**Fig. 1** Representative concentration-time curve (nonlinear regression fit of the data) after 24-h continuous infusion of melphalan; plasma decay curves from three representative patients. Subject 1: □, 20 mg/m²; Subject 10: ◇, 30 mg/m²; and Subject 15: ●, 40 mg/m².

![Representative concentration-time curve](image)

Table 4 Mean (±SD) plasma pharmacokinetic parametersa by dose level of melphalan

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of patients</th>
<th>$C_{ss}$ (µg/ml)</th>
<th>AUC (µg·h/ml)</th>
<th>$t_{1/2}$ elim (h)</th>
<th>Cl (ml/min/m²)</th>
<th>Vd (liters/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5</td>
<td>0.080 ± 0.034</td>
<td>1.91 ± 0.64</td>
<td>0.67 ± 0.31</td>
<td>192 ± 63</td>
<td>13.4 ± 4.8</td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>0.121 ± 0.046</td>
<td>2.94 ± 0.85</td>
<td>0.92 ± 0.67</td>
<td>178 ± 38</td>
<td>12.3 ± 6.9</td>
</tr>
<tr>
<td>40</td>
<td>7</td>
<td>0.206 ± 0.045</td>
<td>5.09 ± 1.38</td>
<td>1.44 ± 0.58</td>
<td>141 ± 40</td>
<td>17.1 ± 5.2</td>
</tr>
</tbody>
</table>

$P < 0.05$, $C_{ss}$ and AUC values between brackets are values normalized to a 20-mg/m² administered dose.

$^c$ Comparison between doses.
during long-term infusion was its low stability in 5% dextrose and 0.9% sodium chloride fluids. Therefore, the manufacturer recommended a use not exceeding 1.5 h after reconstitution in 0.9% sodium chloride. However, we have recently shown that the stability of melphalan can be increased using 3% sodium chloride (17). Consequently, it becomes possible to consider the administration of melphalan by continuous infusion.

Preclinical studies suggested that prolonged infusion of melphalan might be more active than short infusion. Indeed, Bosanquet and Bird (7), using in vitro chemosensitivity assays, attempted to reproduce a system that mimics the action of this drug in vivo. This physicochemical study showed that for melphalan a continuous drug incubation should be optimal. These authors suggest that an in vitro pharmacokinetic study should be undertaken to determine whether continuous exposure of melphalan would be preferable to the i.v. bolus administration commonly used. In a recent study, we have confirmed the interest of continuous infusion by in vitro experiments using human cancer cell lines (9). Moreover, the pharmacokinetic parameters and the fact that alkylating agents are phase-specific support continuous infusion for this drug. Brox et al. (33) studied the effect of concentration and duration of exposure on the extent of melphalan-induced cross-linking in a human lymphoblastoid cell line, RPMEL6410. The S-phase block became irreversible if cells were exposed to 1 μg/ml melphalan for 4–6 h. Similarly, Ross et al. (34) have shown that, in murine L1210 leukemia, the extent of DNA cross-linking increased for 5 h after exposure to melphalan.

We undertook a Phase I study of i.v. melphalan, given for 24 h, with the aim of identifying the MTD, the dose-limiting toxicities, and the pharmacokinetic characteristics of this drug in patients with refractory cancers. In our trial, performed on a small group of patients, the toxicity profile of melphalan after long-term infusion did not differ greatly from that reported after 1-h infusion (2). However, for the same total-administered-dose, hematological and digestive toxicities were more pronounced after long-term infusion than after bolus administration. Long-term melphalan infusion caused dose-limiting neutropenia and thrombocytopenia at the MTD of 30 mg/m²; there were four grade 4 neutropenia (57%) at this dose level. Without autologous bone marrow support or peripheral-blood progenitor cells, this MTD dose was lower than that determined after 1-h infusion (90 mg/m²; Ref. 1). The main nonhematological toxicities associated with 24-h infusion melphalan were mild nausea and vomiting. In opposite to bolus injection, mucositis was not observed. These symptoms occurred during 8–16% of treatment courses but were usually of grade 1–2 severity and were not life-threatening. The median time-to-recovery of blood counts did not exceed 8 days, which suggests that, on average, a 3-week administration schedule may be feasible in these patients.

This study demonstrates the presence of significant interpatient pharmacokinetic variability. This variability has been demonstrated previously for melphalan after short-term infusion and after chronic oral administration (2). We were, however, unable to identify clinical or biological parameters (age, performance status, serum albumin, and creatinine clearance) that could predict this interpatient variability. Cmax of melphalan (0.08, 0.12, and 0.21 μg/ml after 20, 30, and 40 mg/m², respectively) were equal or higher than the concentrations inhibiting human tumor-cell proliferation in in vitro studies. Indeed, the melphalan concentration that inhibited 50% of growth in human myeloma 8226 cell line was 0.11 μg/ml for a 12-h exposure (9). In our study, this concentration was exceeded in the plasma of all of the patients after 30 and 40 mg/m². By comparison with the AUC observed by several authors after bolus administration of 15–20 mg/m² dose, the AUC obtained after long-term infusion of 20 mg/m² was about 1.5 times higher (11, 14, 35).

Relationships have been found between AUC and doses and between Cmax and doses. Moreover, Cl, t1/2 elim, and Vd did not change statistically with dose, suggesting linear kinetics.

The short t1/2 elim of melphalan, its mechanism of action, and the first results from in vitro studies allow us to think that continuous venous infusion of melphalan may be superior to the short infusion in cancer patients, but its use in clinical practice remains to be determined. However, although the oral route of administration could offer greater flexibility in terms of schedule manipulation than the infusion and could increase the quality of life, systemic levels are very variable following oral administration (bioavailability, 20–80%; Refs. 3, 36, 37), and the absorption of melphalan may be affected by food intake and associated drugs (14, 38). Moreover, the amount of drug intake and tablets per day and the increased risk of nausea and vomiting at doses of about 30 mg/m² make this administration route difficult to perform.

As a conclusion to this study, we recommended a dose of 30 mg/m² for Phase II trials of melphalan in 24-h continuous infusion.

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A Phase I and Pharmacokinetic Study of Melphalan Using a 24-hour Continuous Infusion in Patients with Advanced Malignancies

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