Plasma Pharmacokinetics of High-Dose Oral Melphalan in Patients Treated with Trialkylator Chemotherapy and Autologous Bone Marrow Reinfusion

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ABSTRACT

The pharmacokinetics of melphalan following high-dose p.o. administration were determined in 17 patients with various malignancies for the purpose of assessing interpatient and intrapatient pharmacokinetic variability. All patients underwent bone marrow harvest on day −8 (relative to bone marrow reinfusion). On days −7, −6, and −5, melphalan was given p.o. and the dose was escalated on each cohort consisting of at least 3 patients (beginning at 0.75 mg/kg). On days −6, −4, and −2, cyclophosphamide at 2.5 g/m² and thiotepa at 225 mg/m² were given i.v. On day −7 the peak melphalan concentration was 1.64 ± 0.89 (SD) µM with a terminal half-life of 1.56 ± 0.86 h. The area under the plasma concentration time curve (AUC) and oral clearance were 217.9 ± 115.1 µM/min and 30.2 ± 14.2 ml/min/kg. There was only a moderate correlation between the melphalan dose and both the peak concentration (r = 0.50, P < 0.05) and AUC (r = 0.64, P < 0.01) over the dosage range of 0.75−2.5 mg/kg. There was a trend towards greater interpatient variability in peak concentration, AUC, and oral clearance observed at the higher doses of melphalan. Analysis of intrapatient pharmacokinetic variability in 8 patients showed a significant difference between the doses given on days −7 and −5 in the peak concentration (2.09 versus 1.07 µM, P = 0.02), AUC (264.9 versus 134.8 µM/min, P = 0.01), and oral clearance (25.1 versus 53.1 ml/min/kg, P = 0.05) but no significant difference in the time to peak and terminal half-life. We conclude that there is marked interpatient and intrapatient variability in melphalan pharmacokinetics following high-dose p.o. administration. The data are consistent with saturable absorptive pathways for melphalan, which might be especially sensitive to concurrent high-dose chemotherapy.

INTRODUCTION

Melphalan (NSC 8806), l-phenylalanine nitrogen mustard, is an alkylating agent that has been used for many years for the treatment of a variety of malignancies including multiple myeloma, ovarian carcinoma, and breast carcinoma (1−3). Although melphalan is administered both p.o. and i.v. in Europe, it is usually given p.o. in the United States because its i.v. formula is still under investigation. A number of clinical pharmacokinetic studies have shown that melphalan has a short half-life in the plasma, and its absorption from the gastrointestinal tract is extremely variable (4−7). Following standard p.o. doses, a reduction in melphalan absorption with concurrent ingestion of food has been demonstrated by Reece et al. (7).

In conjunction with a clinical trial of high-dose cyclophosphamide, thiotepa, and p.o. melphalan (trialkytator chemotherapy), with autologous bone marrow reinfusion (8), we have undertaken a prospective study to determine the pharmacokinetics of melphalan following high-dose p.o. administration and assess both interpatient and intrapatient pharmacokinetic variability.

MATERIALS AND METHODS

Patient Population. A total of 17 patients (10 male, 7 female) with a variety of malignancies were studied. The major characteristics of the patients are summarized in Table 1. Eligibility criteria included no evidence of bone marrow involvement, Cancer and Leukemia Group B performance status ≤2, marrow cellularity greater than 30%, measurable or evaluable disease, and no significant other medical illness. Patients with renal insufficiency, serum creatinine greater than 2.5 mg/dl, or creatinine clearance less than 40 ml/min or with severe liver dysfunction, bilirubin greater than 6.0 mg/dl, or prothrombin time longer than 16 s were excluded. In most patients, creatinine clearance (Clcr) was directly measured; however, in two patients, it was calculated using the following formula developed by Cockcroft and Gault (9):

\[
Clcr (\text{ml/min}) = \frac{(140 - \text{age}) \times \text{body wt}}{k \times Scr (\text{mg/dl})}
\]

where k = 72 for males and 85 for female patients and Scr is serum creatinine. Following signed informed consent, all patients underwent bone marrow harvest on day −8 of the study period. The harvested bone marrow was stored as described elsewhere (10). On days −7, −6, and −5, p.o. melphalan was given in six equally divided doses over 0.5 h, each dose taken 5 min apart. An escalating dose schedule of melphalan was used with a modified Fibonacci scheme: 3 patients were treated at 0.75 mg/kg/day; 6 patients at 1.5 mg/kg/day; 3 patients at 2.0 mg/kg/day; and 5 patients at 2.5 mg/kg/day. On days −6, −4, and −2, cyclophosphamide at 2.5 g/m² and thiotepa at 225 mg/m² were given i.v. after dilution in 250 ml of 5% dextrose and 500 ml of normal saline, respectively. All patients received p.o. melphalan on an empty stomach and were then kept fasting for 2 h after dosing. Bone marrow was then reinfused on day 0, 48 h after the last dose of chemotherapy.

Sample Collection. Serial samples of venous blood (5−7 ml) were obtained in heparinized tubes just prior to drug treatment; at the end of the 0.5-h p.o. administration (t0); and at 10, 20, and 30 min and 1, 1.5, 2, 4, 6, and 8 h after the completion of p.o. dosing on days −7 (first p.o. dose) and −5 (third p.o. dose).

Blood samples were centrifuged immediately after collection, and the plasma was separated and stored at −70°C until the time of analysis. Chemicals. Melphalan powder was provided by Burroughs Wellcome (Research Triangle Park, NC), and dansyl-L-proline as internal standard was purchased from Sigma (St. Louis, MO). All water used for HPLC was deionized, distilled, and filtered with a Milli-Q water purification system (Millford, MA).

Sample Preparation. Frozen plasma was rapidly thawed and vigorously vortexed over 1 min prior to solid-phase extraction. Plasma, 1 ml (standard or patient samples), was spiked with 100 µl of a 20-µg/ml solution of dansyl-L-proline (internal standard). To determine proteins, 100 µl of 60% HClO4 were added and centrifuged at 15,600 × g for 2

The abbreviations used are: HPLC, high performance liquid chromatography; AUC, area under the plasma concentration-time curve; CLR/F, apparent p.o. clearance.

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### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient/Sex</th>
<th>Age (yr)</th>
<th>Wt (kg)</th>
<th>Tumor type</th>
<th>Melphalan dose (mg/kg p.o.)</th>
<th>( C_c ) (mg/dl)</th>
<th>( \text{CLR/F} ) (ml/min)</th>
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<td>NHL</td>
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</table>

* NSCL, non-small cell lung carcinoma; NHL, non-Hodgkin's lymphoma.

### RESULTS

Pharmacokinetics on Day —7 (First Dose). The pharmacokinetic parameters are summarized in Table 2. Peak plasma melphalan concentrations exceeded 0.33 \( \mu \text{M} \) in all but one patient with a hepatoma. This patient had no detectable melphalan in the plasma up to 8 h after p.o. administration. The peak plasma concentrations on day —7 ranged from 0.65 to 3.61 \( \mu \text{M} \) with the time to peak of 1.2 ± 0.5 (SD) h. Fig. 1 shows the relationship between melphalan dose and peak concentration on day —7. There was only a moderate correlation between the dose and both the peak concentration (\( r = 0.50, P < 0.05 \)) and AUC (\( r = 0.64, P < 0.01 \)) in the dosage range studied. The \( \text{CLR/F} \) ranged from 49 to 155 ml/min with a mean of 95.1 ml/min, and was not significantly correlated with either the CLR/F (\( r = -0.21 \)) or \( t_\text{o} \) (\( r = -0.38 \)) of melphalan.

There was a trend towards greater interpatient variability in peak concentration, \( t_\text{o} \), AUC, and CLR/F observed with higher p.o. doses of melphalan. When our patients were stratified into two groups by p.o. melphalan dosage (Table 3), the mean peak concentration was 1.42 \( \mu \text{M} \) at doses <2.0 mg/kg and 1.87 \( \mu \text{M} \) at doses ≥2.0 mg/kg. There was, however, no statistically significant difference in the peak concentration between these two groups due to marked interpatient variability observed at doses ≥2.0 mg/kg. Although the time to peak showed no significant difference between the two groups, there was a tendency for melphalan absorption to be somewhat more delayed (longer time to peak) at higher doses. Delayed absorption of the drug from the gastrointestinal tract may cause apparent prolongation of the \( t_\text{o} \), which is supported by the finding that there was a statistically significant difference in the \( t_\text{o} \) between the two groups. The mean AUC and CLR/F were also higher at the increased dose by 60 and 34%, respectively, but the differences were not statistically significant.

Pharmacokinetics on Day —5 (Third Dose). Eight patients were evaluable for p.o. melphalan pharmacokinetics following high-dose administration on day —5 (Table 2); blood samples were not obtained from the other 8 patients, and the hepatoma patient was also excluded for analysis because no plasma melphalan was detected. The peak concentrations on day —5 ranged from 0.37 to 1.52 \( \mu \text{M} \) with the time to peak of 1.1 ± 0.7 h. The mean peak concentration after the 2.5-mg/kg dose was not significantly different from that after the 1.5-mg/kg dose [1.31 ± 0.22 versus 0.93 ± 0.39 \( \mu \text{M} \) (Fig. 1)]. In contrast to the results on day —7, there was a poor correlation (\( r = 0.35 \)) between the dose and the AUC. The \( \text{CLR/F} \) was only slightly correlated with both the CLR/F (\( r = 0.42 \)) and \( t_\text{o} \) (\( r = 0.24 \)) of melphalan. As shown in Table 3, at the higher melphalan dose, there was greater interpatient variability observed in the AUC and CLR/F, but not in the peak concentration. There was no significant difference between the two groups in the peak concentration, time to peak, \( t_\text{o} \), AUC, and CLR/F (Table 3).

Intrapatient Pharmacokinetic Variability. Eight patients were evaluable for analysis of intrapatient pharmacokinetic variability. As shown in Fig. 2, there were marked intrapatient differences in the peak concentration, AUC, and CLR/F between the two doses, given on days —7 and —5, in those 8 patients. Following the third dose of p.o. melphalan administered on day —5, the mean peak concentration was significantly lower, when compared to that observed on day —7 (1.07 ± 0.37 versus 2.09 ± 1.05 \( \mu \text{M} \), \( P = 0.02 \)) (Fig. 2a). There was no statistically significant difference between the two doses in the \( t_\text{o} \) or time to peak. The mean AUC determined on day —5 was significantly smaller than that determined on day —7 (134.8 ± 70.8 versus 119.8 ± 46.7 ml/min).

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**min in an Eppendorf microcentrifuge. The supernatant was separated, loaded onto a prewet 3 ml Supelclean LC-18 cartridge (Supelco, Inc., Bellefonte, PA), and washed with 5 ml of water. After the LC-18 cartridge was dried under vacuum, melphalan and the internal standard were eluted with 7 ml of methanol. The entire methanol eluate was collected in a 16 × 125-mm borosilicate tube and evaporated to complete dryness under nitrogen at room temperature. The residue was reconstituted with 200 µl of the mobile phase and 150 µl were injected onto the HPLC system. The extraction efficiency of melphalan from plasma was 85.3% and the interassay and intraassay precisions were 7.6 and 5.3%, respectively.**

**HPLC Analysis. Melphalan and the internal standard in plasma were analyzed using a modification of the HPLC assay reported by Reece et al. (7). The HPLC system consisted of a Waters Model 510 HPLC pump, a Model 720 Waters Intelligent Sample Processor, and a Waters Model 481 variable wavelength detector set at 254 nm (Waters Associates, Milford, MA). The separation was achieved isocratically using a reverse phase 10-µm C18-µBondapak column (Waters Associates) and a mobile phase consisting of 52% methanol in 10 mM sodium phosphate buffer (pH 3.0). The flow rate was maintained at 1.0 ml/min and the retention time was 8.0 min for melphalan and 16.8 min for the internal standard. The detection limit of this assay was 0.08 µM and interassay and intraassay precisions were 7.6 and 5.3%, respectively.**

**The CLR/F was calculated as**

\[
\text{CLR/F} = \frac{\text{Dose}}{\text{Total AUC}}
\]

**Statistical Analysis. All statistical analyses were performed on a Professional 350 system (Digital Equipment Corp., Waltham, MA), using the RS/1 software package (Bolt Beranek and Newman, Cambridge, MA).**

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Table 2 Summary of pharmacokinetic parameters in patients receiving high-dose p.o. melphalan

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/kg)</th>
<th>Peak (µM)</th>
<th>Day -7</th>
<th>Day -5</th>
<th>Time to peak (h)</th>
<th>t1/2 (h)</th>
<th>AUC (µM/min)</th>
<th>CLR/F (ml/min/kg)</th>
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<tr>
<td>1</td>
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<td>0.81</td>
<td>0.81</td>
<td>1.5</td>
<td>1.5</td>
<td>1.4</td>
<td>100.9</td>
<td>116.0</td>
</tr>
<tr>
<td>2</td>
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<td>1.11</td>
<td>*</td>
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<td>*</td>
<td>1.36</td>
<td>*</td>
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<tr>
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<td>0.82</td>
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<td>1.24</td>
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<td>0.5</td>
<td>0.96</td>
<td>0.91</td>
<td>223.1</td>
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<td>6</td>
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<td>0.37</td>
<td>0.5</td>
<td>1.0</td>
<td>1.14</td>
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<td>1.18</td>
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<td>8</td>
<td>1.5</td>
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<td>1.3</td>
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<td>0.72</td>
<td>*</td>
<td>161.2</td>
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</table>

Mean ± SD 1.64 ± 0.89 1.07 ± 0.37 1.2 ± 0.5 1.1 ± 0.7 1.55 ± 0.87 1.33 ± 0.5 217.9 ± 115.1 134.8 ± 70.6 30.1 ± 14.7 53.1 ± 34.0

* Blood samples not obtained.

Excluded from statistical analysis.

Fig. 1. Relationship between melphalan dose and peak concentration on days -7 and -5. Bars, mean values.

264.9 ± 136.0 µM/min, P = 0.01 (Fig. 2b). The mean CLR/F following the day -5 dose was significantly greater than that following the day -7 dose (53.1 ± 34.0 versus 25.1 ± 10.2 ml/min/kg, P = 0.05) (Fig. 2c).

DISCUSSION

We have shown that there is marked interpatient and intrapatient pharmacokinetic variability following high-dose p.o. melphalan (≥0.75 mg/kg). Our data demonstrate that there is a 3- to 6-fold variation between patients in the pharmacokinetic parameters on both day -7 and day -5, which is in good agreement with previous reports by Alberts et al. (5) and Reece et al. (7) who used melphalan dosages lower than or equal to 0.6 mg/kg. Unfortunately, we could not relate pharmacokinetic variability to clinical events since all patients became aplastic and life-threatening nonhematological toxicity (mucositis/enteritis, dermatitis) occurred only in a few patients treated at the highest dose level (8). Furthermore, interpatient differences in toxicity might also be attributable to interpatient variability in cyclophosphamide (11) or thiopeta (12) pharmacokinetics during high-dose therapy, which were not measured in the current study.

One of 17 evaluable patients who was treated with p.o. melphalan at 1.5 mg/kg did not have measurable melphalan in plasma up to 8 h on both day -7 and day -5. There was no remarkable finding other than liver function abnormalities. The patient had had a resection of the right lobe of the liver at the time of diagnosis (about 2 years ago). Following the p.o. melphalan on day -7, the patient had severe nausea and vomiting but experienced no further nausea and vomiting on subsequent days. It has also been noted by several investigators that in some patients following p.o. melphalan at conventional dosages, the drug could not be detected in the plasma at any time point (5, 7, 13). In contrast to the report by Adair et al. (14), no correlation between the renal function assessed by measured Cr, and the elimination rate constant was found in our patients.

The analysis of intrapatient pharmacokinetic variability in the 8 patients revealed that following the third dose of p.o. melphalan, there was a significant decrease in both the peak concentration and AUC and a marked increase in CLR/F, as compared to the first dose on day -7. When the first dose of p.o. melphalan was given on day -7, the patients had not yet received any other chemotherapeutic agent, but the third p.o. dose was given approximately 24 h after administration of high-dose thiopeta and cyclophosphamide. All of the patients re-
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It is also possible that similar intrapatient variability may occur at more conventional doses of melphalan (11–13, 21), or even with other oral antineoplastic agents, such as chlorambucil, cyclophosphamide, or etoposide. Clinicians should be aware of these possibilities and should consider poor drug absorption as a possible explanation for treatment failure for regimens in which oral chemotherapy is used.

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REFERENCES


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