Safety of Autotransplants with High-Dose Melphalan in Renal Failure: A Pharmacokinetic and Toxicity Study

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ABSTRACT

Melphalan (MEL) is probably the most effective chemotherapeutic agent in multiple myeloma (MM) with a clear dose-response effect. It can be escalated without excessive toxicity to 200 mg/m², a myeloablative dose requiring hematopoietic stem cell support. Patients with marked renal insufficiency, not an infrequent finding in MM, have either received reduced doses or have been excluded from therapy with high-dose MEL. A prospective study was performed to evaluate the relationship between MEL pharmacokinetics and renal function in 20 patients with MM. Six patients had severe renal insufficiency (creatinine clearance, <40 ml/min), including five on chronic hemodialysis. Three patients with severe renal impairment first received a low test dose of MEL (16 mg/m²) for pharmacokinetic studies. All patients received 200 mg/m² MEL divided into two equal doses of 100 mg/m² i.v. on 2 consecutive days, followed by the administration of peripheral blood stem cells. MEL pharmacokinetics, performed after the first dose of 100 mg/m², was not adversely affected by impaired renal function. The median half-life (t₁/₂), area under the concentration curve, and clearance of MEL were 1.1 h, 5.5 mg h/liter, and 27.5 liter/h, respectively, in patients with a creatinine clearance of <40 ml/min compared to 1.9, 7.9, and 23.6 for the others. Renal insufficiency also had no apparent negative impact on the quality of peripheral blood stem cell collections and did not adversely affect posttransplant engraftment, transfusion requirements, incidence of severe mucositis, or overall survival. However, it was associated with longer durations of fever (P = 0.0005) and hospitalization (P = 0.004). No transplant-related deaths were observed. Plasma t₁/₂ and area under the concentration curve differed by a factor of 10 and MEL clearance by a factor of 5 between patients with the lowest and highest values. These large variations in MEL elimination could not be explained by patient or disease characteristics. We conclude that renal failure does not require dose reduction of MEL in autologous transplant. Due to marked interindividual variation in MEL elimination, pharmacokinetically guided dosing as well as cellular pharmacology studies may be helpful in achieving a more uniform antitumor effect.

INTRODUCTION

MEL, a bifunctional alkylator, is one of the most active agents in the treatment of MM. A steep dose-response effect has been demonstrated, with best results obtained when myeloablative doses of 200 mg/m² were used that require autologous hematopoietic stem cell support (1–9). The low mortality rate associated with autotransplants (3) has encouraged wider application initially in refractory MM and subsequently early in the disease. In newly diagnosed patients, complete remission rates of 40–50% have been observed (4, 7–9), and results of a French randomized study indicate superior event-free and overall survival in patients with transplants when compared to standard chemotherapy (10).

MEL pharmacokinetics appear to be dose and age independent (11). The primary route of MEL elimination is spontaneous degradation rather than renal excretion (11), which constituted only 13–14% in 24 h (12, 13). Others, however, did observe altered pharmacokinetics in patients with impaired renal function (14–16).

Approximately 20% of patients with overt MM present with renal insufficiency, which is reversible in half of these patients as a result of hydration, control of hypercalcemia, and effective chemotherapy (17). Because of conflicting data on altered MEL pharmacokinetics in renal insufficiency, patients with creatinine levels >2 mg/dl have usually been excluded from high-dose MEL treatment (8, 18), and p.o. busulfan with erratic intestinal absorption has been used as a substitute (19).

We report on MEL pharmacokinetics in 20 MM patients receiving two equal doses of 100 mg/m² over 2 days; 6 had severe renal insufficiency with a creatinine clearance of <40 ml/min.

PATIENTS AND METHODS

Twenty patients with MM participated in this pharmacokinetic study. Six patients had severe renal insufficiency (creatinine clearance, <40 ml/min), including five on chronic he-

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3 The abbreviations used are: MEL, melphalan; MM, multiple myeloma; PBSC, peripheral blood stem cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; AUC, area under the concentration curve; CR, complete remission; PR, partial remission.
modiﬁed. They were all enrolled in tandem autotransplant trials, supported by PBSCs which had been mobilized with high-dose cyclophosphamide (4.5 g/m² for four of the six patients with severe renal insufficiency and 6 g/m² for the others) and GM-CSF (250 µg/m²), starting the day after high-dose cyclophosphamide therapy. Pretransplant cytoreduction consisted of MEL at 100 mg/m² administered i.v. over 20 min on 2 successive days followed by PBSC infusion 48 h later. MEL pharmacokinetics was performed with the ﬁrst dose of 100 mg/m². The ﬁrst three patients with renal insufﬁciency received an i.v. test dose of 16 mg/m² with subsequent pharmacokinetic studies. Anticoagulated venous samples were drawn and immediately placed on ice. The plasma was separated by centrifugation at 4°C and stored at −20°C until analysis. MEL was analyzed by reverse-phase high-performance liquid chromatography using a procedure modiﬁed after that of Chang et al. (20). A calibration curve was generated by extracting MEL from spiked, expired plasma. The range of the curve was from 20 to 2000 ng/ml MEL. Each standard was run in duplicate. The regression analysis was generated from the mean of three calibration curve runs on different days. MEL was extracted from preconditioned C₁₈ Varian Bond Elut columns in 0.3 ml 0.01 N-methanolic-HCl. Fifty µl of the eluent was injected directly onto a Waters Radialpak C₁₈ column. Due to the light sensitivity of MEL, the plasma and extract received minimum light exposure in preparation. The Perkin-Elmer ISS-100 autoinjector was used for injection. Waters 510 and 501 Solvent Delivery Systems driven by Waters Automated Gradient controller delivered an isotropic mobile phase. The mobile phase was delivered at a rate of 1.5 ml/min. MEL was separated from other UV-absorbing material using a mobile phase of 55%:0.2 m ammonium acetate (pH 4.0), 45% methanol, and 0.1% triethanolamine. The mobile phase was vacuum filtered with a 0.22-µm nylon ﬁlter purchased from Micron Separations, Inc. A typical elution time for MEL under these conditions is 7.6–7.8 min. Nelson Analytical Software Model 2600 and Nelson 760 Series A/D interface were used for peak analysis, data collection, and storage.

The plasma MEL of patients was extracted and chromatographically analyzed in an identical fashion to the standards described above. Concentrations of patient plasma MEL were calculated from the regression analysis using the external standard method of analysis described above. Standards chromatographed with each set of patient plasma reveal coefﬁcients of variation as follows: 100 ng/ml, 9.08%; 200 ng/ml, 15.29%; 500 ng/ml, 6.40%; and 2000 ng/ml, 12.07%. MEL concentration versus time values for each patient were modeled using nonlinear regression. A two-compartment model with ﬁrst-order input and ﬁrst-order output was initially assumed. If a good ﬁt was not obtained, a one-compartment model with ﬁrst-order input and output was assumed. Ke is not the elimination rate constant from the central compartment but the terminal disposition rate constant. Creatinine clearance was calculated using the equations of Cockcroft and Gault (21) and Jelliffe (22). The Spearman correlation coefﬁcient was used to examine the association of creatinine clearance with melphalan clearance and AUC. Because of skewness in the pharmacokinetic parameter estimates, the nonparametric Mann-Whitney U test was used to determine statistically signiﬁcant differences between patients with and without renal insufﬁciency.

CR was deﬁned by the absence of monoclonal gammopathy in serum and urine using immunoﬁxation analysis in addition to a normal bone marrow aspirate and biopsy. PR required tumor mass reduction by at least 75% and reduction in Bence Jones proteinuria to <100 mg/day; bone marrow aspirate and biopsy could not contain more than 5% plasma cells. These ﬁndings had to be present on at least two occasions, with a minimum interval of 2 months. The treatment protocol was reviewed by the Institutional Review Board. Written informed consent was obtained from all patients.

Overall survival and times to recovery of granulocytes and platolets posttransplantation were estimated from the date of transplantation using the Kaplan-Meier product limit method, and the differences were compared with the log rank test. To compare the differences among the medians of CD34 cells/kg, days of fever, and duration of hospitalization, the NPAR1WAY (SAS Institute Inc.) procedure was applied.

RESULTS

Patient Characteristics. The median age of the 20 patients was 52 (range, 35–69) years. Seventeen of the patients were males and three were females; seven had stage III disease (Durie and Salmon), nine had IgG, two had IgA, eight had only light chain excretion, and one patient had nonsecretory disease. Prior to high-dose cyclophosphamide for PBSC mobilization, standard chemotherapy had been administered for ≤12 months in 13 patients, for 13–24 months in 3, and for more than 24 months in 4 patients. The median serum albumin was 4.1 g/dl (1.5–5.0 g/dl) and was lower (3.3 g/dl) in patients with severe renal insufﬁciency compared to those with creatinine clearance ≥40 ml/min (4.1 g/dl; 0.05 < P < 0.1). The median paraprotein levels were comparable in both groups (P = 0.6) and four patients in each group had only light chain excretion. No patient had hypercalcemia (range, 8.2–10 mg/dl).

Pharmacokinetic Data. Because of the concern for excessive toxicity from high-dose MEL in advanced renal failure, the ﬁrst three patients received a test dose of 16 mg/m². Since MEL pharmacokinetics is dose independent (11), the low test dose should have revealed whether drug elimination was delayed in renal failure. As this was not the case, these patients subsequently received the therapeutic dose of 100 mg/m², again with pharmacokinetics, conﬁrming that clearance and AUC with high-dose MEL were not altered (Fig. 1A). The pharmacokinetic data with the high drug dose were available within 24 h; therefore, the second dose of 100 mg/m² was delayed by only 1 day in these three patients. High-dose MEL pharmacokinetics was similar in patients on hemodialysis and those with normal renal function, as depicted in representative examples in Fig. 1. MEL was not detected in the dialysate of two patients dialyzed within 6 h after the ﬁrst dose of MEL.

The concentration versus time values were ﬁt using a two-compartment model in 19 patients and a single-compartment model in one patient. Table 1 lists the pharmacokinetic parameters according to renal function. Creatinine clearance did not correlate with either MEL clearance or plasma AUC using the Cockcroft-Gault MEL clearance, r = 0.07 (P = 0.8); AUC, r = 0.17 (P = 0.47) or Jelliffe’s prediction equation [MEL clearance, r = 0.02 (P = 0.94); AUC, r = 0.18 (P = 0.44)].
Large interindividual variations in $K_e$, $t_{1/2}$, AUC, and MEL clearance were not explained by differences in patient (age, sex, or body surface) or disease characteristics (stage, paraprotein, or creatinine level). Patients with severe renal insufficiency did not experience a longer half-life (1.1 versus 1.9 h; $P = 0.02$), slower clearance (27.5 versus 23.6 liter/h; $P = 0.2$), or higher MEL AUC (5.5 versus 7.9 mg h/liter; $P = 0.05$) compared to those of patients with creatinine clearance $>40$ ml/min (Fig. 2).

Clinical Data. As a precaution, more PBSCs were collected in patients with renal failure. Thus, the median number of CD34 cells present in PBSC collections after high-dose cyclophosphamide and GM-CSF was $20.3 \times 10^6$/kg for the six patients with severe renal insufficiency compared to $6.4 \times 10^6$/kg for the others ($P = 0.04$; Table 2). Posttransplant hematopoietic recovery, RBC, and platelet transfusion support as well as the frequency of grade $\geq 3$ mucositis were similar in patients

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**Table 1** MEL pharmacokinetics according to renal function

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Body surface area (m²)</th>
<th>$K_e$ (h⁻¹)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC (mg h/liter)</th>
<th>Clearance (liter/h)</th>
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<td>1.50</td>
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$^a$ On chronic hemodialysis.
with and without renal failure, although a trend toward slower platelet recovery to $50 \times 10^9$/liter and longer duration of mucositis was observed with impaired renal function. No early deaths ($\leq 60$ days posttransplantation) occurred. Three patients in each group had a documented infection (septemia/pneumonia) ($P = 0.2$). Four patients with renal insufficiency required i.v. antibiotics compared with 12 in the other group. Patients with renal failure had longer median durations of fever ($>100.5^\circ F$; $6.3$ versus $0$ days; $P = 0.0005$) and hospitalization ($22$ versus $0$ days; $P = 0.004$). All renal insufficiency patients were hospitalized for their transplant, whereas nine patients with adequate renal function were treated entirely in the outpatient setting. A trend toward a lower response rate (CR and PR; $50\%$ versus $81\%$) and an inferior survival (19 months+ versus 39 months+) was observed in the six patients with impaired renal function; however, these differences were not significant.

**DISCUSSION**

In contrast to earlier studies (14–16), MEL clearance in this study was not delayed in renal insufficiency. Thus, renal excretion is not a critical route for MEL elimination (12, 13). The reasons for a somewhat faster elimination in renal failure are not clear. MEL was apparently not removed by dialysis, although the short half-life of MEL in water and its binding to dialysis tubing may have contributed to the lack of detectable levels in the dialysate. MEL undergoes spontaneous hydrolysis in aqueous media and is not significantly metabolized (11).
Chang et al. (20) have shown that, in vitro, MEL hydrolysis is complete within 8 h in water at 37°C, but is slowed down by increasing concentrations of serum albumin and plasma proteins. MEL is extensively bound to plasma proteins (approximately 90%); 60% of this binding is due to interaction with albumin and 20% with acid α1-glycoprotein, while immunoglobulins do not participate in its binding (23). Serum albumin levels were lower in patients with severe renal insufficiency (3.3 g/dl versus 4.1; 0.05 < P < 0.1), which may partially explain the faster drug elimination in these patients.

Our pharmacokinetic data are at variance with a report by Kergueris et al. (24), who observed a significant correlation among creatinine clearance and serum t1/2, AUC, and clearance of MEL in 20 patients with MM, primary amyloidosis, or lymphoma receiving 140 mg/m² MEL as a single dose. However, the decrease in MEL elimination was minor given the large interindividual variations in systemic clearance. These authors, in fact, did not recommend a dose reduction for patients presenting with renal insufficiency, although prolonged and severe mucositis was observed in the three patients with severe renal failure.

The majority of studies on MEL pharmacokinetics in renal insufficiency have failed to correlate pharmacokinetics with clinical toxicity. In our study, renal insufficiency did not compromise PBSC collections after high-dose cyclophosphamide and GM-CSF, although the dose of cyclophosphamide was reduced from 6 g/m² to 4.5 g/m² in four of the five patients on chronic dialysis. Likewise, the frequency of severe mucositis and transfusion requirements was not influenced by renal function, but patients with severe renal impairment received significantly more CD34 cells/kg (P = 0.04). The longer duration of fever (P = 0.0005), despite comparable engraftment kinetics, frequency of documented infections, and requirements of i.v. antibiotics is not easily explained. We speculate that, in renal insufficiency, cytokine levels may remain elevated for a longer period of time after high-dose chemotherapy. However, this needs to be studied. Patients with severe renal insufficiency also spent more days in the hospital (P = 0.004). Although high-dose MEL with PBSC support can be given safely in an outpatient setting when renal function is normal (Ref. 25; 9/14 transplants in the current study), it is prudent to hospitalize patients with severe renal insufficiency to assure proper observation during prolonged duration of fever and mucositis with ensuing slower physical recovery. Yet, with good supportive care, autotransplants were safe even when the full MEL dose was used. The trend toward lower response rate and shortened survival in patients with renal failure is also seen with conventional chemotherapy and probably reflects a higher tumor load (26).

The marked heterogeneity in MEL pharmacokinetics observed in our study could result in large differences in antitumor effect. Because of the relatively small patient population studied and the heterogeneity in their characteristics (age, tumor load, timing of autotransplants, i.e., early versus late in the disease, and renal function), a correlation between MEL pharmacokinetics and outcome could not be addressed appropriately in this study. Plasma t1/2 and AUCs differed by a factor of 10 and MEL clearance by a factor of 5 between patients with the lowest and highest values. These large interpatient variables are probably due to a difference in plasma and tissue levels of glutathione involved in the detoxification of alkylating agents (27–29), as well as to differences in MEL secretion and reabsorption in the kidney (30). In ovarian cancer, pharmacokinetic data obtained after a low test dose were used to reach a specific target serum AUC with the therapeutic dose of MEL (31). The deviation from the target AUC was <15%, and AUC values correlated well with hematological toxicities. MEL is actively transported into the cells by the high-affinity l-amino acid transport system while competing with glutamine and leucine. In addition, sensitivity to MEL decreases with increasing intracellular glutathione concentrations. Therefore, if our aim is to achieve a more uniform antitumor effect of MEL, its direct cytotoxic effect through formation of interstrand and intrastrand DNA cross-links and DNA protein cross-links needs to be assessed along with plasma concentrations versus time.

The terminal phase half-life of MEL is relatively short (median, 69 min), as well as in hemodialysis patients. Since all drug should be cleared within five half-lives, hematopoietic stem cells can be infused 24 h after the last MEL dose without inflicting PBSC damage. Infusion of PBSC after 24 rather than after 48 h will shorten further the duration of myelosuppression and hospitalization.

Since high-dose MEL is one of the most effective therapies for MM and can be administered safely, even in high doses, to patients with end stage renal disease, renal insufficiency should no longer constitute a criterion for dose reduction of or exclusion from such therapy, although patients with renal insufficiency may show a lower response rate and shorter survival.

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