High-Dose Cyclophosphamide or Melphalan with Escalating Doses of Mitoxantrone and Autologous Bone Marrow Transplantation for Refractory Solid Tumors


Abstract

As the dose-limiting toxicity of mitoxantrone is hematological, the drug is suitable for dose escalation and use in intensive chemotherapy followed by autologous bone marrow rescue. Adult patients with therapy-resistant solid tumors received a regimen of high-dose cyclophosphamide (7 g/m²) and escalating doses of mitoxantrone in dose steps of 30, 45, 60, and 75 mg/m². Both drugs were given i.v. on 3 consecutive days. Despite the addition of mesna (3.5 to 7 g/m²), hemorrhagic cystitis occurred on the second day in four of eight patients, irrespective of the mesna or mitoxantrone dose. Therefore, the cyclophosphamide in the combination regimen was replaced by high-dose melphalan (180 mg/m²). Mucositis was dose limiting at 75 mg/m² of mitoxantrone.

Responses were seen in eight of ten evaluable patients with four complete responses. Three responders received, after the autologous bone marrow transplantation program, radiotherapy or surgery on pretreatment bulky tumor localizations. Five patients still have disease-free survival after 9 to 36 mos.

Pharmacokinetic studies of mitoxantrone were performed by high-performance liquid chromatography with UV detection. The plasma disappearance of mitoxantrone fitted into a three-compartment model with a mean t₁/₂ of 10 min, a mean t₂, of 96 min, and a slow elimination phase of 172 h. The mean distribution volume was 4294 ± 3836 liters.

We conclude that the high-dose cyclophosphamide-mitoxantrone regimen led to unexpected bladder toxicity, but the combination of melphalan (180 mg/m²) and mitoxantrone (60 mg/m²) can probably be given without major extramedullary toxicity. However, more patients should be evaluated at this dose before definite conclusions can be drawn about toxicity.

Introduction

Extensive experimental and clinical evidence (1, 2) supports the notion that higher doses of cytostatics can kill a larger fraction of the tumor. Among the active cytostatic agents suitable for delivery in high dosages are a number of alkylating agents, notably cyclophosphamide and melphalan (3, 4). One of the most widely active cytostatic drugs when applied in normal dosages is doxorubicin, but dose escalation is limited because of mucositis and cardiotoxicity. Mitoxantrone, an anthracycenederivative, is an alternative for doxorubicin with less nonhematological side effects (5, 6) and cross-resistance between these drugs is not absolute (7). Mitoxantrone has shown activity in vitro and in vivo in leukemia, lymphoma, and advanced breast cancer (8–13). In vitro experiments have indicated a dose-response relationship for this drug. In Phase I trials, myelosuppression was the dose-limiting toxicity of mitoxantrone (14). No extramedullary toxicity was seen in adult patients with leukemia treated with doses up to 24 mg/m² (15). Provided the bone marrow function is safeguarded by bone marrow transplantation, mitoxantrone seems an excellent candidate for dose escalation.

In the current clinical trial adult patients with disseminated solid tumors received a regimen of escalating doses of mitoxantrone in combination with high-dose cyclophosphamide on three consecutive days followed by ABMT.2

The objectives of our study were: determination of the maximum tolerated dose of mitoxantrone that can be given in combination with a fixed high-dose of cyclophosphamide; determination of the extramedullary toxicity; assessment of the antitumor activity of the regimen; and determination of the pharmacokinetic characteristics of mitoxantrone following high-dose i.v. administration.

Because of unexpected extramedullary toxicity of the combination, cyclophosphamide was replaced by high-dose melphalan. The same objectives were studied for the regimen of mitoxantrone plus melphalan.

Materials and Methods

Patients between 16 and 60 yr of age were eligible when they had histologically confirmed malignant tumors for which established curative or palliative treatment modalities were nonexistent. Other entry criteria were: no history of congestive heart failure or persistent arrhythmia; serum bilirubin levels < 30 µmol/liter and serum creatinine levels < 120 µmol/liter; a Zubrod performance status of 0–1, and a life expectancy of at least 4 wk. Patients with tumor infiltration of the bone marrow, chemotherapy or radiotherapy within the past 3 wk, or treatment with mitomycin or nitrosoureas within the past 6 wk were excluded. Informed consent was obtained from all patients, and the study was approved by the institutional medical ethical committee.

Chemotherapy

Mitoxantrone was dissolved in 100 ml of 0.9% NaCl solution and administered over 30 min on Days 1, 2, and 3. The first dose step consisted of a 30-mg/m² total dose. Dose escalations were 15 mg/m² and were to proceed in the absence of unacceptable (Grade 3 or higher) extramedullary toxicity at that level. Two h before the start of each mitoxantrone infusion, cyclophosphamide was given in a total dose of 7 g/m² divided over 3 days. Immediately prior to, 4, and 8 h after the cyclophosphamide infusion, mesna was administered i.v. in a total dose equal to 50 or 100% of the cyclophosphamide dose. The administered i.v. volume on the days of chemotherapy was 4 liters/day.

Because of unexpected extramedullary toxicity, cyclophosphamide was replaced by melphalan (180-mg/m² total dose), divided and given on Days 1, 2, and 3 two h before each mitoxantrone infusion. Melphalan was given in 250 ml of 2.5% glucose-0.45% NaCl solution in 1 h.

Supportive Care

Patients were treated in a single-person bedroom. All patients received p.o. prophylactic antibiotic treatment for selective decontamination of the gut to prevent gram-negative or mycotic infection (16). Empiric or specific parenteral antibiotics were given for suspected or documented infection, after appropriate culturing. Platelets were transfused when bleeding was present or prophylactically if the platelet count...
was below $15 \times 10^9$/liter. A number of patients received cryopreserved autologous platelets; otherwise allogeneic single-donor platelets were used. Patients were fed by parenteral nutrition alone or in combination with enteral tube feeding.

**Bone Marrow Collection Cryopreservation, and Reinfusion**

The technique of bone marrow collection without general anesthesia and of cryopreservation has been reported previously (17). On Day 7 bone marrow with a minimum of $1 \times 10^9$ nucleated cells/kg of body weight was reinfused after rapid thawing without washing through a Hickman catheter.

**Toxicity**

Toxicity was assessed daily according to the WHO criteria (18). Pretreatment investigations included chest X-ray, full blood count, biochemistry profile including renal and hepatic function tests, ECG, and a baseline radionuclide ejection fraction or echocardiography. Other investigations were performed if appropriate. Cardiac status was reassessed using repeat ECG and follow-up ejection fraction or echocardiography after 4 wk, or when indicated on clinical grounds. During treatment in the ABMT program, physical examination and full blood cell counts were done daily and liver and renal functions 3 times a wk. Microscopic investigation of the urine sediment was done daily from Day 1 to Day 7 and thereafter 3 times a wk. During mitoxantrone infusions the cardiac rhythm was monitored.

**Response**

A CR was defined as complete disappearance of all signs and symptoms of detectable tumor. A PR was defined as a decrease of at least 50% of the sum of the products of the largest perpendicular diameters of measurable lesions. If a decrease in the level of established tumor marker (e.g., α-fetoprotein or β-human chorionic gonadotropin) of >90% was seen in patients without measurable or evaluable lesions, this was also considered a PR. Stable disease was defined as a decrease or increase of measurable lesion < 25%. Disease progression was defined as the growth of measurable lesions. Response duration was measured from the first day of treatment in the ABMT program until progression of the tumor or rise of the markers. Survival time was also measured from Day 1 until death.

**Pharmacological Study**

**Sampling.** Blood samples for analysis of mitoxantrone concentrations were obtained in heparinized polypropylene tubes before drug treatment, at the end of the 30-min mitoxantrone infusion, and at 5, 10, 15, 30, and 60 min and 2, 4, 8, and 24 h after the end of the infusion. Nadir levels were obtained immediately before each subsequent dose. After the third day of treatment, daily blood sampling was performed at least until Day 8. Samples were immediately centrifuged, and the plasma was separated and frozen in polypropylene tubes containing 0.2 ml of 0.5% ascorbic acid in citrate buffer (0.1 M, pH 3). Where feasible, urine was collected in plastic vials containing ascorbic acid in 12-h intervals on Days 1 to 3 and in 24-h intervals on Days 4 to 10. Plasma and urine aliquots were protected from daylight and stored at -20°C until analysis.

**Analysis.** The mitoxantrone concentrations in plasma and urine were measured by HPLC and UV detection according to Peng et al. (19), with slight modifications (20). The detection limit of mitoxantrone in plasma and urine was 0.5 μg/liter.

**Pharmacokinetics.** The plasma concentration time data for each patient were subjected to pharmacokinetic analysis using a computer analysis program (21). The program includes a correction for infusion time (22), a statistical analysis comparing the accuracy of models containing different numbers of phases (23). Equations for distribution constants, elimination constants, total-body clearance, and apparent central and peripheral distribution volumes have been described (24).

**RESULTS**

From September 1985 to May 1988, 11 patients were included in this study. Patient characteristics are given in Table 1. Toxicity. No deaths due to treatment occurred. The duration of leuko- and thrombocytopenia, number of platelet transfusions, and extramedullary toxic side effects are shown in Table 2. Median duration of leukocytopenia (leukocytes $\leq 1.0 \times 10^9$/liter) and of thrombocytopenia (platelets $\leq 40 \times 10^9$/liter) was 22 days.

Patient 11 had a delayed hematopoietic recovery. From Day 30 until Day 50 she was treated with granulocyte-macrophage colony-stimulating factor i.v. in a dose of 8 μg/kg of body weight/24 h (Sandoz, Basel, Switzerland). On Day 49 the leukocyte count was 1 $\times 10^9$/liter. Thrombocytopenia, however, persisted until Day 114, without signs of hemorrhagic diathesis. During this period the patient was treated with oxymetholone, an androgenic steroid, and blood transfusions.

Four of eight patients developed acute sterile hemorrhagic cystitis Grade 3 within 24 h after the start of the treatment with high-dose cyclophosphamide plus mitoxantrone. In the first five patients the uroprotective regimen consisted of i.v. 4000 ml of fluid/day, and i.v. administration of mesna in a dose equal to 50% of the cyclophosphamide dose. The last three patients received 7 g/m² of mesna. Usually the hematuria subsided on Day 7 when thrombocytopenia became apparent. Patient 5, however, needed platelet transfusions because of hematuria and a platelet count below 15 $\times 10^9$/liter. Hemorrhagic cystitis appeared with the first dose step of mitoxantrone and persisted after doubling of the mesna dose. Patient 8 got a prophylactic urine catheter during the days of high-dose chemotherapy.

Because bladder damage could not be prevented by optimal protective measures, the alkylating agent in the combination with mitoxantrone was changed to high-dose melphalan.

All patients in this study experienced nausea and vomiting and self-limiting diarrhea during the first week of treatment (Table 2). Mucositis was present in all dose steps of mitoxantrone. Oropharyngeal soreness and erythema started characteristically at Day 9 and recovered within 2 wk. Mucositis became severe and dose limiting at 75 mg/m².

No clinical signs of heart failure were observed. During mitoxantrone infusion, no arrhythmias were seen. Six patients had been treated previously with chemotherapy containing cumulative doses of doxorubicin between 100 and 300 mg/m². Patient 7 had prior mediastinal irradiation with 2100 rads. Eight patients are evaluable for cardiotoxicity by echocardiography or radionuclide ejection fraction. Patients 9 and 10 showed, after mitoxantrone treatment, a decrease in radionuclide ejection fraction of 16% and 6%, respectively. Patient 2 complained on Day 15 of chest pain and developed pericarditis as evidenced by echocardiography and low voltage on the ECG. The symptoms resolved spontaneously in 4 days.

Fever due to infection was seen in 8 patients (Table 3). In 7 patients a causative bacterial microorganism was found. Patients 1 and 2 had also reactivation of oropharyngeal herpes simplex virus infection.

Response. Tumor response to the ABMT program, response duration, and survival are shown in Table 1. In Patients 1 to 10, response could be evaluated. The response rate was 80%, and in 4 of 10 patients, a CR was achieved. The 4 PRs were of short duration except in Patient 2 whose tumor became operable. Longlasting bone marrow aplasia precluded laparotomy for evaluation of ovarian cancer in Patient 11. Clinically she had no evidence of disease.

Patient 1 had bulky ovarian cancer with palpable progressive disease immediately after treatment.

Patient 2 had a bulky inoperable Sertoli Leydig cell tumor. After treatment, the tumor could successfully be removed.
evidenced a relapse. After local treatment (radiotherapy and containing polychemotherapy. After a CR of 8 mo following high-dose chemotherapy, relaparotomy showed a PR of 8 mo. Thirteen mo later a small pelvic relapse was also extirpated.

Patient 3 had advanced ovarian cancer with massive ascites, pleural effusion, and liver metastases. Laparoscopy after treatment showed a PR. After 3 mo the ascites returned.

Patient 4 had been treated because of metastatic adenocarcinoma of unknown origin, and later gastric cancer was detected. The a-fetoprotein level decreased more than 90% after high-dose treatment. She received local irradiation on the involved lung field, and she is without evidence of disease 11 mo after ABMT.

Patient 5 had disseminated inflammatory breast cancer with bone metastases. She had persistent intraperitoneal metastases prevented further surgery.

Patient 6 had disseminated adenocarcinoma of the lung with rapidly progressive disease despite high-dose chemotherapy. She has no evidence of disease now 36 mo after treatment.

Patient 7 had been irradiated for a large mediastinal germ cell tumor. The a-fetoprotein level decreased more than 90% after high-dose treatment.

Patients 8 and 10 achieved a pathologically confirmed CR of ovarian cancer. Nine mo after ABMT, Ca-125 levels increased in Patient 8.

Patient 9 had disseminated breast cancer and a single lung metastasis persisting after doxorubicin-containing chemotherapy. After high-dose treatment she had a CR. She received local irradiation on the involved lung field, and she is without evidence of disease 11 mo after ABMT.

**Pharmacokinetic Characteristics of Mitoxantrone.** Plasma mitoxantrone concentrations on Days 1, 2, and 3 of mitoxantrone infusion were measured in 6 patients. After the third day, levels were determined until Day 8 in four patients, and until Day 23 in one patient.

Pharmacokinetic results are summarized in Table 4. Plasma mitoxantrone concentrations measured by HPLC decayed in a triexponential fashion with a mean t½ of 0.17 ± 0.04 h (mean ± SD). In one patient, no cumulation occurred.

**Prophylactic Measures.** The doses of mitoxantrone in the urine was impossible. Combined peak plasma concentrations measured by HPLC decayed in a triexponential fashion with a mean t½ of 0.17 ± 0.04 h (mean ± SD), and t½ of 1.6 ± 0.6 h. The mean terminal phase half-life was 172 h. The mean apparent volume of distribution was 4294 ± 3836 liters. The mean plasma clearance was 0.79 ± 0.3 liter/min. The cumulative recovery of mitoxantrone in the urine was 8.7% after 6 h. In case of hematuria, determination of mitoxantrone concentrations on Days 1, 2, and 3 of mitoxantrone infusion were measured in 6 patients. After the third day, levels were determined until Day 8 in four patients, and until Day 23 in one patient.

Pharmacokinetic results are summarized in Table 4. Plasma mitoxantrone concentrations measured by HPLC decayed in a triexponential fashion with a mean t½ of 0.17 ± 0.04 h (mean ± SD, n = 14), peak concentrations on daily doses of 15 mg/m² on 3 consecutive days were 550 ± 227 µg/liter (mean ± SD, n = 4), on daily doses of 20 mg/m² 526 ± 308 µg/liter (mean ± SD, n = 9), and on daily doses of 25 mg/m² 718 ± 242 µg/liter (mean ± SD, n = 3). No cumulation occurred.

In all patients plasma mitoxantrone was detectable before subsequent doses of mitoxantrone. Low plasma levels of mitoxantrone could still be found on Day 7 (day of marrow reinfusion). A complete pharmacokinetic study is shown in one representative patient (Fig. 1).
Combination mitoxantrone/cyclophosphamide/mesnum could have been competition of mitoxantrone and acrolein for the binding by thiol groups. However, in vitro equimolar mitoxantrone and acrolein did not react at room temperature over a period of 24 h as evidenced by thin-layer chromatography.

Hemorrhagic cystitis in only two patients (17, 28-30).

A possible explanation for the increased incidence after the administration of mitoxantrone is somewhat less than with cyclophosphamide, it has a broad spectrum of clinical activity when applied in ablative regimens (4, 31).

The dose limit of mitoxantrone appeared to be 75 mg/m² due to mucositis Grade 4 of the oropharyngeal region.

No clinical evidence of congestive heart failure occurred in this trial, in agreement with data in the literature that, with cumulative doses of mitoxantrone below 100 mg/m², little cardiotoxicity is seen in patients pretreated with anthracyclines (32, 33). In two patients treated with high-dose melphalan and mitoxantrone, the ejection fraction decreased by, respectively, 16 and 6%. The decrease of 16% turned out to be transient because, after 6 wk, a repeated examination showed a reduction of only 6%.

No severe hepatic dysfunction was noted, but five patients had temporary elevations of hepatic enzymes or bilirubin after treatment with mitoxantrone; this side effect is in accordance with findings by Paciucci et al. (34).

The pharmacokinetic data following mitoxantrone i.v. administration in 30 min on 3 consecutive days showed a three-compartment model, in accordance with previous reports (35). Also the half-time elimination parameters in this high-dose regimen were within the limits found in the literature (36-39). The pharmacokinetic data following mitoxantrone i.v. administration in 30 min on 3 consecutive days showed a three-compartment model, in accordance with previous reports (35). Also the half-time elimination parameters in this high-dose regimen were within the limits found in the literature (36-39). The small amount of mitoxantrone found in the urine confirms that renal elimination is not a significant pathway. The large mean distribution volume, together with the long terminal half-life, is consistent with the reports of detection of mitoxantrone in human autopsy tissues long after the last gift of the drug (40, 41). The slow terminal elimination, also found by Ehninger et al. (39), resulted in the drug being detectable in the plasma on the day of marrow reinstitution. However, this concentration of 1 µg/liter is well below the concentration found to kill human cells in vitro experiments (42).

Another possibility might be that mitoxantrone induces the cytochrome P-450 system resulting in the generation of more 4-hydroxy-cyclophosphamide giving rise to bladder toxicity. As the hemorrhagic cystitis was not avoidable and occasionally even platelet transfusions were required to ameliorate the bleeding, the cyclophosphamide in the combination regimen was replaced by high-dose melphalan. Although the dose escalation with this alkylator is somewhat less than with cyclophosphamide, it has a broad spectrum of clinical activity when applied in ablative regimens (4, 31).

The main toxicity in this study was infection. Its chief cause was the prolonged period of severe granulocytopenia. Earlier infusion of bone marrow does not seem desirable in view of the slow elimination of mitoxantrone.

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Table 4: Summary of mitoxantrone pharmacokinetics

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<th>Mx dose (mg/m²)</th>
<th>Comedication</th>
<th>Day</th>
<th>Peak level (µg/liter)</th>
<th>AUC (µg/h/liter)</th>
<th>Plasma Mx concentration, Day 4</th>
<th>Plasma Mx concentration, Day 7</th>
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Mean ± SD (all patients) 557 ± 301 0.17 ± 0.4 1.6 ± 0.6 172 ± 223 1,102 ± 1,013 2.7 ± 0.4 1.2 ± 0.7 32.6 ± 8.9 4,295 ± 3,837

* Mx, mitoxantrone; AUC, area under the plasma concentration × time curve; V₅, central compartment; V₆, steady-state volume of distribution; C, cyclophosphamide; Me, melphalan.

* Mx dose is the total dose divided and given on 3 consecutive days.

Another possibility might be that mitoxantrone induces the cytochrome P-450 system resulting in the generation of more 4-hydroxy-cyclophosphamide giving rise to bladder toxicity. As the hemorrhagic cystitis was not avoidable and occasionally even platelet transfusions were required to ameliorate the bleeding, the cyclophosphamide in the combination regimen was replaced by high-dose melphalan. Although the dose escalation with this alkylator is somewhat less than with cyclophosphamide, it has a broad spectrum of clinical activity when applied in ablative regimens (4, 31).
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