Clinical Phase I Dose Escalation and Pharmacokinetic Study of High-Dose Chemotherapy with Treosulfan and Autologous Peripheral Blood Stem Cell Transplantation in Patients with Advanced Malignancies[1]

Max E. Scheulen,[2] Ralf A. Hilger, Carsten Oberhoff, Jochen Casper, Matthias Freund, Klaus M. Josten, Martin Bornhäuser, Gerhard Ehninger, Wolfgang E. Berdel, Joachim Baumgart, Andreas Harstrick, Peter Bojko, Hans-Heinrich Wolf, Adolf E. Schindler, and Siegfried Seeber

Department of Internal Medicine (Cancer Research) and Department of Gynecology and Obstetrics, West German Cancer Center, University of Essen Medical School, D-45122 Essen [M. E. S., R. A. H., C. O., A. H., P. B., A. E. S., S. S.]; Department of Hematology and Oncology, University of Rostock, D-18055 Rostock [J. C., M. F.]; Deutsche Klinik für Diagnostik, D-65191 Wiesbaden [K. M. J.]; Department of Hematology and Oncology, Technical University Dresden, D-01307 Dresden [M. B., G. E.]; Department of Internal Medicine, University of Münster, D-48149 Münster [W. E. B.]; Medac GmbH, D-20354 Hamburg [J. B.]; Department of Hematology and Oncology, University of Halle-Wittenberg, D-06097 Halle [H-H. W.], Germany

ABSTRACT

A Phase I dose escalation and pharmacokinetic study of the alkylating cytotoxic agent treosulfan was conducted to evaluate the maximum tolerated dose and the dose-limiting toxicities in patients with advanced malignancies rescued by autologous peripheral blood stem cell transplantation. Twenty-two patients (15 ovarian and 7 other carcinomas/lymphomas) with a median age of 48 years were treated with 28 high-dose courses. Treosulfan was infused over 2 h at escalating doses from 20 to 56 g/m^2, and pharmacokinetic parameters were analyzed. At 56 g/m^2, three of six patients experienced dose-limiting toxicities: diarrhea grade III/IV in three patients; mucositis/stomatitis grade III in one patient; toxic epidermal necrolysis in one patient; and grade III acidosis in one patient. Other low-grade side effects, including erythema, pain, fatigue, and nausea/vomiting, were recorded. Two patients died within 4 weeks after treatment because of rapid tumor progression and fungal infection, respectively. Plasma half-life, distribution volume, and renal elimination of treosulfan were independent of dose, whereas the increase in area under the curve was linear up to 56 g/m^2 treosulfan. The maximum tolerated dose of high-dose treosulfan is 47 g/m^2. A split-dose or continuous infusion regimen is recommended for future high-dose trials. In consideration of antineoplastic activity and limited organ toxicity, inclusion of high-dose treosulfan in combination protocols with autologous peripheral blood stem cell transplantation seems worthwhile.

INTRODUCTION

Treosulfan (L-threitol-1,4-bis-methanesulfonate; dihydroxybusulfan; NSC 39069; Ovastat) is a prodrug of a bifunctional alkylating cytotoxic agent (Fig. 1; Ref. 1). It is indicated for oral or i.v. treatment of patients with ovarian carcinoma in several European countries (2–6). In addition, preclinical and clinical activity was demonstrated against a broad range of other solid tumors and hematological malignancies (7–14). More recently, efficient stem cell toxicity against committed and primitive stem cells was evident when fractionated, and escalated doses of treosulfan were administered before allogeneic bone marrow transplantation (15, 16). The mono- and diepoxybutane derivatives of treosulfan are considered to be the active cytotoxic species formed by a nonenzymatic, pH-dependent, and temperature-dependent intramolecular nucleophilic substitution (17). Although treosulfan is related structurally to busulfan, its mechanism of alkylation is entirely different (18–20). Because of the introduction of the two hydroxy groups in positions 2 and 3 of treosulfan, the nonenzymatic activation pathway (Fig. 1) leads to the formation of a monoepoxy intermediate (1,2-epoxy-3,4-butanediol-4-methanesulfonate) and, subsequently, to L-(+)-diepoxybutane under the release of 2 mol of methanesulfonic acid. Both epoxides are supposed to be responsible for producing DNA alkylation, interstrand cross-links, and chromosomal aberrations (21, 22).

At pH <6, almost no treosulfan is metabolized (17, 20). This results in the stabilization of the renally eliminated inert parent drug because of concomitant elimination of methanesulfonic acid that is released during activation of treosulfan in the plasma. It is noteworthy that, in contrast to busulfan, treosulfan is soluble in water and can be applied i.v. easily after reconstitution of the crystalline powder in water for injection.

Since the early 1980s, clinical studies with the i.v. formulation of treosulfan have been conducted predominantly in patients with ovarian cancer either as a single drug or in combi-

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[2] To whom requests for reprints should be addressed, at Department of Internal Medicine (Cancer Research), University of Essen Medical School, Hufelandstrasse 55, D-45122 Essen, Germany. Phone: 49-201-723-3152; Fax: 49-201-723-3790; E-mail: max.scheulen@uni-essen.de.

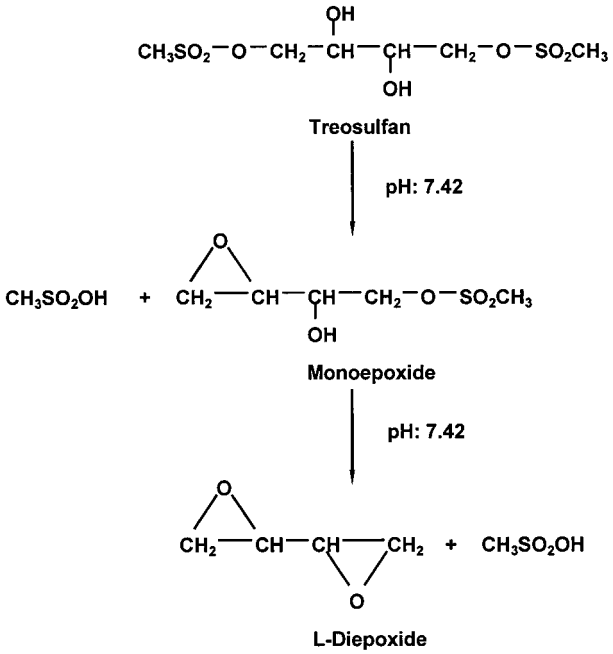

Fig. 1 Nonenzymatic, pH-dependent, and temperature-dependent activation of treosulfan (according to Feit *et al.* 17).

nation with other cytotoxins such as cisplatin or gemcitabine (23, 24).

In general, conventional doses of treosulfan administered i.v. (5–8 g/m^2) are well tolerated subjectively. Hematological toxicity is observed commonly and was confirmed recently to be a DLT,[3] resulting in a MTD of 10 g/m^2 treosulfan without autologous PBSCT (25). Mild nonhematological toxicities such as gastrointestinal or cutaneous side effects have been reported sporadically.

Because of the lack of significant nonhematological toxicity, treosulfan was considered to be an appropriate candidate for further dose escalation in combination with autologous PBSCT. The escalation of high-dose treosulfan with PBSCT was accompanied by pharmacological investigations using a recently developed analytical method based on treosulfan separation by validated RP-HPLC with refractometrical detection of treosulfan in plasma and urine samples (26). The clinical and pharmacokinetical results of a high-dose treosulfan Phase I dose escalation trial combined with autologous PBSCT are reported.

PATIENTS AND METHODS

The Phase I trial was conducted between March 1996 and October 1998. All patients gave written informed consent, and the study protocol was approved by the local ethics committees. The trial was conducted on the basis of the German Drug Law and under consideration of the International Conference of Harmonization consolidated guideline "Good Clinical Practice."

Eligibility. Inclusion criteria for the study were as follows: (*a*) patients of either sex with histologically proven relapsed and advanced carcinomas or lymphomas no longer amenable to standard therapy; (*b*) patients with a sufficient number of frozen stored autologous PBSCs available; (*c*) patients aged 18–60 years with a WHO performance status of 0–1 and with a life expectancy of at least 3 months; (*d*) patients with $\geq 100 \times 10^9$ platelets/l and $\geq 3.5 \times 10^9$ WBC/liter; and (*e*) patients with adequate hepatic (bilirubin <2.0 mg/100 ml; transaminases and lactate dehydrogenase $<2\times$ upper normal limit), cardiac (normal blood pressure and electrocardiogram), lung (normal diffusion capacity and acid-base balance), and kidney (creatinine clearance >60 ml/min) functions.

Exclusion criteria were any acute infectious diseases, secondary malignancies, CNS metastases, and persisting toxicity from previous treatments with the exception of alopecia.

Pretreatment and Follow-Up Evaluations. Patient history, physical examination, body weight, WHO performance status, blood pressure, cardiac and lung function, and routine laboratory tests were evaluated before high-dose treatment. After treosulfan administration, daily checks of hematological parameters and weekly checks of standard laboratory parameters were performed. In addition, blood pressure and cardiac function were monitored hourly up to 4 h after termination of high-dose treosulfan infusion, and a blood gas analysis was done to monitor a possible metabolic acidosis because of the formation of methanesulfonic acid during treosulfan activation.

In cases of measurable disease, assessment of tumor response was performed ~4 weeks after therapy. Tumor markers (*e.g.*, CA 125 plasma levels) were used as surrogate parameters in patients with nonmeasurable disease (*e.g.*, ovarian carcinoma patients with peritoneal carcinomatosis).

Drug Administration. Treosulfan was supplied by Medac (Hamburg, Germany) and was dissolved in sterile 0.45% saline at a concentration of 50 mg/ml. The total dose was administered i.v. over 2 h using a peripheral venous access.

Stem Cell Acquisition. Most patients (all ovarian cancer patients) were treated with 2 g/m^2 cyclophosphamide and hematopoietic growth factors for stem cell mobilization before high-dose therapy. Sufficient numbers of peripheral blood progenitor cells were collected using the apheresis system COBE Spectra Vision 5.1.

Study Design. At least three patients/dose level were enrolled in this multicenter dose escalation Phase I trial. Dose escalation was started at a dose of 20 g/m^2 treosulfan (Table 1). This dose was chosen on the basis of a previous conventional dose escalation trial without autologous PBSCT. In this former protocol, a maximum of 12.5 g/m^2 treosulfan was administered, and prolonged CTC grade IV thrombocytopenia was found to be dose limiting (25). In addition, one pilot patient was treated with 16 g/m^2 treosulfan and PBSCT without any complications (data not shown). Dose increments of 100 (start dose, level 1), 30, 25, and 20% were chosen for a safe treosulfan escalation within this Phase I protocol (Table 1).

No dose escalation was performed within individual patients; however, a second treatment cycle was allowed. Toxic-

[3] The abbreviations used are: DLT, dose-limiting toxicity; MTD, maximum tolerated dose; PBSCT, peripheral blood stem cell transplantation; RP-HPLC, reversed-phase high-performance liquid chromatography; PBSC, peripheral blood stem cells; CTC, common toxicity criteria; AUC, area under the curve.

Table 1 Patient characteristics (n = 22) and dose levels (six) of treosulfan within the high-dose treosulfan Phase I protocol

Patient no.	Age (yr)	WHO-PS[a]	Dose (g/m^2)/ courses	Tumor type	Pretreatments (no. of regimens)		
					Surg.	Chemo.	Rad.
1	51	0	20.0/1	Ovarian	8	11	0
2	57	1	20.0/1	Ovarian	1	4	0
3	35	1	20.0/1	Yolk sac	0	3	0
4	29	0	20.0/1	Hodgkin's lymphoma	0	4	1
5	43	1	26.0/1	Breast	1	2	1
6	60	1	26.0/2	Ovarian	1	5	0
7	56	0	26.0/2	Ovarian	2	3	0
8	53	0	32.5/2	Ovarian	2	3	0
9	45	0	32.5/1	Ovarian	1	2	0
10	19	1	32.5/1	NHL[a]	0	5	0
11	39	0	39.0/1	Ovarian	1	4	0
12	48	0	39.0/1	Ovarian	1	3	0
13	45	0	39.0/2	Ovarian	2	4	0
14	58	1	47.0/1	Ovarian	1	6	0
15	59	2	47.0/1	Ovarian	2	3	0
16	54	0	47.0/2	Ovarian	3	4	0
17	51	0	56.0/1	Ovarian	2	4	0
18	47	0	56.0/2	Ovarian	3	3	3
19	39	1	56.0/1	Ovarian	1	4	1
20	44	1	56.0/1	Multiple myeloma	0	6	1
21	36	0	56.0/1	Sarcoma	2	3	0
22	56	0	56.0/1	NHL[a]	0	3	0
Median	48	0			1	4	0

[a] WHO-PS, WHO performance status; Surg., surgery; Chemo., chemotherapy; Rad., radiation; NHL, non-Hodgkin's lymphoma.

ities were evaluated according to the CTC of the National Cancer Institute (version 1.0). The DLT was defined as any of the following toxicities: nephrotoxicity ≥ CTC grade II, neurotoxicity ≥ CTC grade II, other nonhematological toxicity ≥ CTC grade III (excluding alopecia), delayed engraftment (granulocytes $<0.5 \times 10^6$/liter for >14 days or thrombocytes $<50 \times 10^9$/liter for >21 days), or graft failure despite transfusion of $\geq 1.5 \times 10^6$/kg body weight of $CD34^+$ autologous PBSCs.

The MTD was defined as one dose level below the level at which greater than or equal to two of three or three of six patients experienced DLT, respectively.

Concomitant Therapy. Standard antiemetic therapy was given prophylactically with class 3 5-hydroxytryptamine receptor antagonists and dexamethasone. In addition, standard prophylactic antibiotic and antimycotic therapies were given at higher dose levels. After treosulfan infusion, posthydration was performed with 2 liters of sterile saline administered within ~12 h. In addition, granulocyte-colony stimulating factor was given at a dose of 5 μg/kg/day s.c. beginning 1 day after PBSCT and was continued until WBC counts reached 2.0×10^9/liter.

Pharmacokinetic Studies. Aliquots of blood were drawn via a separate indwelling venous access at 0 (pre-administration), 30, 60, 90, 120, and 150 min and 3, 4, 6, 8, 12, 24, 36, and 48 h after the start of infusion. Blood samples were adjusted to a final pH of 5.5 by citrate to avoid artificial *ex vivo* degradation of treosulfan. Plasma was separated by centrifugation at 4°C and $1,000 \times g$ for 10 min, proceeded by microfiltration (cutoff M_r 10,000), and then applied to the HPLC analysis system. Spontaneous urine was collected from patients into separate containers over 48 h under the addition of crystalline citrate to guarantee a pH of <6.0. The volume of each urine sample was recorded before an aliquot was centrifuged (4°C; $14,000 \times g$ for 15 min) and analyzed.

Treosulfan was separated by a validated RP-HPLC method and quantified by refractometrical detection as described (26). The limit of quantification for treosulfan was given to 1 μg/ml in plasma and 20 μg/ml in urine. Reproducibility was 99 ± 3%, recovery was 96 ± 4%, and linearity was from 1 μg/ml to 50 mg/ml treosulfan (correlation coefficient, 0.99).

Individual pharmacokinetic parameters were evaluated by two-compartment disposition modeling using the data analysis system TOPFIT 2.0 (27). All pharmacokinetic data are normalized to body surface area ($1/m^2$).

Statistical Analysis. The difference between the mean values of the pharmacokinetic parameters were analyzed for significance using the Mann-Whitney rank sum test. $P < 0.05$ were considered to be statistically significant; $P < 0.01$ were considered to be statistically highly significant.

RESULTS

Patient Characteristics. Characteristics of all 22 patients who received a total of 28 treatment courses of high-dose treosulfan are given in Table 1. All patients were pretreated with at least two chemotherapy regimens (median, 4; range, 2–11) before they entered this protocol. Most patients with ovarian cancer were pretreated additionally with ≥ 2 g/m^2 cyclophosphamide and granulocyte-colony stimulating factor to mobilize PBSCs. All patients but one (patient 3; early death because of rapid progression of relapsed stage IV yolk sac tumor) were evaluable for toxicity. A limited number of patients had measurable disease because of frequently diagnosed peritoneal carcinomatosis of relapsed ovarian cancer.

Hematotoxicity. All patients received a mean of 3.3×10^6/kg body weight $CD34^+$ autologous PBSC (range, 1.1–11.2) 2 days after treosulfan infusion and engrafted. However, a dose

Table 2 Hematological toxicity: worst toxicity per patient at each dose level

Dose level (g/m^2)	No. of patients	No. of CD34+ cells reinfused (no./kg b.w.); mean (range)	WBC nadir[a] below 0.5×10^9/liter		Thrombocyte nadir[a] below 25×10^9/liter	
			Median (days)	Range (days)	Median (days)	Range (days)
20.0	4	2.15 (1.7–2.8)	0	0	0	0–1[b]
26.0	3	2.50 (1.6–4.6)	0	0–1	0	0–1
32.5	3	5.33 (2.3–11.2)	2	1–3	3	1–10[c]
39.0	3	1.80 (1.1–2.7)	2	2–3	3	0–3
47.0	3	5.90 (3.4–8.4)	4	3–4	2	1–3
56.0	6	2.59 (1.3–3.4)	3	3–10[d]	2	1–7[d]

[a] Transplantation of autologous PBSC at day 0.
[b] Patient 3 died 10 days after autologous PBSCT because of rapid progression of metastatic yolk sac tumor (18×10^9/liter platelets at day 10).
[c] Patient 10 (Hodgkin's disease) died 19 days after autologous PBSCT because of fungal sepsis despite hematological recovery.
[d] Patient 20 (multiple myeloma) was heavily pretreated including radiotherapy and previous autologous PBSCT.

Table 3 Drug-related, nonhematological toxicities: worst toxicity per patient and dose level

Dose level (g/m^2)	No. of patients	CTC grade	Diarrhea	Mucositis/Stomatitis	Acidosis	Skin	Vomiting	Pain	Fever/ Infection
20.0	4	II		1			1	1	
		III							
		IV							
26.0	3	II							
		III							
		IV							
32.5	3	II							1
		III							1
		IV							1[a]
39.0	3	II							
		III							
		IV							
47.0	3	II	2			2		1	1
		III							
		IV							
56.0	6	II	1		2	3	2	3	2
		III		1	1				1
		IV	2	1		1			

[a] Patient 10 (Hodgkin's disease) died 19 days after autologous PBSCT because of fungal sepsis despite hematological recovery.

dependency of onset and duration of hematological toxicity was evident with regard to leukocytopenia and thrombocytopenia (Table 2).

At dose level 3 (32.5 g/m^2 treosulfan), the nadir of WBC and platelet counts was reached comparatively late on days 6 and 7 and days 9 and 10 after PBSCT, respectively. A rapid recovery of WBC counts and platelet counts was evident within a few days (Table 2). Patient 10 (relapsed Hodgkin's lymphoma with multiple pretreatments including high-dose chemotherapy and autologous PBSCT) entered the treosulfan Phase I trial with 3.9×10^9 WBC/liter and only 36×10^9 platelets/liter. His WBC counts recovered to 2.6×10^9/liter and 4.1×10^9/liter on days 10 and 11, respectively. However, his platelets did not recover fully until day 18 (25×10^9/liter). Unfortunately, this patient died because of *Candida* sepsis despite recovery of WBC counts on day 19 after PBSCT.

With further dose escalation (39, 47, and 56 g/m^2 treosulfan), WBC nadir was reached already on day 5 after PBSCT, and platelet nadir also was reached earlier on days 7 and 8. Nevertheless, the cell counts of all of these patients recovered rapidly except in patient 20 (heavily pretreated multiple myeloma patient; 56 g/m^2 treosulfan).

In general, a comparatively short cytopenia was evident, and transfusion of platelets or RBC preparations was necessary only one to three times/patient (except patient 20).

Nonhematological Toxicities. Drug-related toxicities are listed in Table 3. Diarrhea and mucositis/stomatitis are the nonhematological DLTs of high-dose treosulfan. Onset of diarrhea was comparatively late, beginning about 6–10 days after PBSCT. One patient (patient 19; ovarian carcinoma, 56 g/m^2 treosulfan) developed CTC grade IV mucositis and diarrhea as well as toxic epidermal necrolysis 8–10 days after PBSCT. At the same time, intestinal atonia-related emesis occurred, together with aspiration and subsequent cardiac arrest. Cardiopulmonary resuscitation was performed successfully, and the patient was treated further at the intensive care unit. The patient recovered but died 7 weeks after treatment because of rapid tumor progression.

Laboratory tests at dose level 6 (56 g/m^2 treosulfan) revealed acidotic changes up to CTC grade III in patient 18. These

Table 4 Pharmacokinetic parameters of high-dose treosulfan

Treosulfan dose (g/m^2)	Patient no./course	AUC ($\mu g/ml \times h$)	Clearance (ml/min)	Terminal half-life (h)	Total dose (g)	Renal excretion (g)	Renal elimination (%)	c_{max} ($\mu g/ml$)[a]	V_{ss} (liter)[a]
20.0	01	2530	132	1.70	40	5.8	14	876	14
	02	2120	157	1.75	33	11	32	643	20
26.0	05	2410	180	2.53	49	12	25	788	26
	06/1	2170	200	1.56	46	8.0	18	562	23
	06/2	3860	112	2.11	46	6.0	13	1050	18
	07	3360	129	2.44	54	11	20	860	23
32.5	08/1	4070	133	1.46	59	15	25	1430	13
	08/2	2640	205	1.71	59	23	40	996	24
	09	2860	162	1.72	55	17	30	933	19
39.0	11	4350	149	1.77	66	20	29	1390	17
	12	4130	158	1.66	68	29	42	1290	17
	13	6580	99	2.37	76	26	34	1660	18
47.0	14	5970	131	2.60	78	22	28	1180	24
	15	6370	123	2.15	71	12	17	1640	21
	16	5600	140	1.74	83	28	33	1070	16
56.0	17	8040	116	3.06	95	29	31	1520	19
Mean	$n = 16$		145.4	2.02			26.9		19.5
±SD			30.3	0.46			8.7		3.8

[a] c_{max}, maximum concentration; V_{ss}, distribution volume.

changes occurred a few hours after treosulfan infusion and were considered to be related to the release of substantial amounts of methanesulfonic acid during the spontaneous activation process of treosulfan in the plasma (see also "Pharmacokinetic Results").

Several patients, all at dose level 56 g/m^2 treosulfan, suffered from reversible pruritus, erythema (up to CTC grade II), neutropenic fever, and permanent hyperpigmentation of skin. Other non-DLTs are listed in Table 3. Frequently, mild headache was reported during or shortly after treosulfan infusion.

Pharmacokinetic Results. Analysis of treosulfan was performed in plasma and urine of 14 patients and for 16 individual courses at the various dose levels administered. At higher dose levels, the projected infusion time of 2 h was sometimes exceeded because of the high total infusion volume of ≥2 liters. Therefore, C_{max} values were comparatively low in these patients. Pharmacokinetic parameters of each individual treatment course are given in detail in Table 4. Plasma concentrations of treosulfan declined exponentially and were described by a first-order elimination process best fitted by a two-compartmental model (Fig. 2). Mean peak plasma levels of treosulfan were reached at the end of the infusion time, and terminal half-life of treosulfan in plasma was ~2.0 h. Similarly, total clearance (145.4 ml/min), renal elimination (26.9% of total dose), and distribution volume (19.5 liters) were obviously independent of dose. Regression analysis of $AUC_{0-\infty}$ *versus* treosulfan dose indicated a linear correlation (Fig. 3). However, at dose level 6 (56 g/m^2 treosulfan), half-life and $AUC_{0-\infty}$ of treosulfan were suggested to be somewhat increased, possibly because of acidotic changes in plasma, slowing down treosulfan cleavage, and total clearance (Fig. 2, patient 17).

Renal elimination of treosulfan, however, was independent of dose. A significant amount of the unchanged parent compound (~25% of total treosulfan dose) was found in the urine within the first 6 h after administration (Table 4).

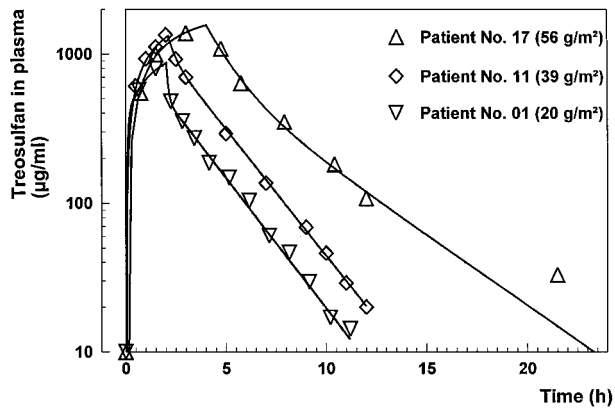

Fig. 2 Representative individual concentration time curves of treosulfan in plasma after infusion of 20, 39, and 56 g/m^2 treosulfan to patients 1, 11, and 17, respectively.

Antitumor Activity. Despite heavy pretreatment and acquired chemoresistance, there was evidence of antitumor activity of high-dose treosulfan in several of the patients. Four patients experienced tumor regression >50% (ovarian carcinoma in patients 6 and 16; Hodgkin's lymphoma in patient 10; and non-Hodgkin's lymphoma in patient 22). Patient 22 showed a complete resolution of his mediastinal tumor mass.

Tumor marker analysis revealed three additional ovarian carcinoma patients (patients 8, 13, and 15) who showed a significant (>50%) decline of tumor marker values (CA 125 and CA 72-4) after treosulfan treatment despite their disease progression under previous paclitaxel- and platinum-based treatments.

Patient 20 (relapsed multiple myeloma with multiple pretreatments including high-dose therapy with autologous PB-SCT) showed a normalization of his disease-related monoclonal

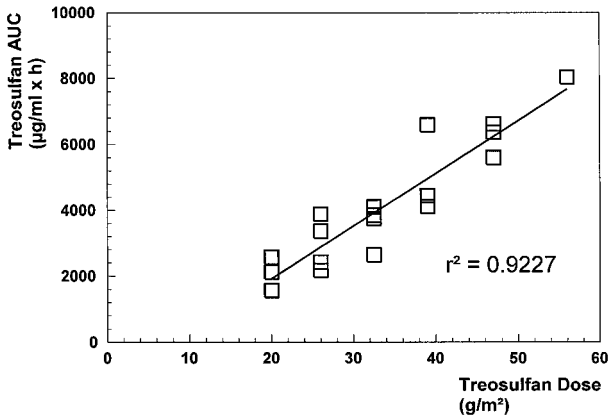

Fig. 3 Correlation of treosulfan dose and $AUC_{0-\infty}$ (linear regression analysis, $r^2 = 0.9227$).

gammopathy for 6 weeks after treatment. Eight weeks after high-dose treosulfan, total plasma protein and γ-globulins reached pathological values again.

DISCUSSION

Treosulfan is a bifunctional alkylating cytotoxin with a broad spectrum of antitumor activity (7–14). For many years, it has been used for oral and i.v. treatment of patients with ovarian carcinoma in several European countries (2–6, 24). More recent publications report activity of treosulfan in other indications such as cutaneous and choroidal melanoma, small cell lung cancer, and malignant glioma (11–14).

Myelosuppression with predominant thrombocytopenia is the DLT when conventional doses of 10–12.5 g/m^2 treosulfan are administered i.v. (12, 23, 25). However, autologous PBSCT 2 days after treosulfan infusion allowed substantial dose escalation up to 56 g/m^2, as reported here. At this dose level, nonhematological DLTs such as diarrhea, mucositis, stomatitis, skin toxicity, and metabolic acidosis were evident. However, at the MTD of 47 g/m^2, these toxicities, in general, were only mild (Table 3). In addition, several low-grade drug-related side effects such as erythema, pruritus, neutropenic fever, hyperpigmentation of skin, and headache were observed. Neither severe nephrotoxicity, bladder toxicity, cardiotoxicity, nor severe central nervous system toxicity that had been reported after high-dose treatments with other alkylators such as ifosfamide or cyclophosphamide (28–30) was evident after high-dose treosulfan.

In contrast to busulfan (31, 32), high-dose treosulfan did not induce severe hepatotoxicity or veno-occlusive disease in the nine patients treated at or above MTD of 47 g/m^2. This might be considered a consequence of the different mode of alkylation and the reliable i.v. infusion of high-dose treosulfan. However, sites of DNA alkylation of both busulfan and treosulfan are obviously identical (21). Therefore, and because of the toxicity reported recently to primitive and committed hematopoietic stem cells of mice (15), treosulfan is considered an alternative conditioning cytotoxin before allogeneic stem cell transplantation (16).

Thus far, high-dose therapy with treosulfan and autologous PBSCT is well tolerated comparatively and shows a rapid recovery of peripheral blood cell counts. However, because of the release of two moles of methanesulfonic acid during the nonenzymatic activation of one mole of treosulfan, the development of metabolic acidosis has to be taken into account when maximum doses of treosulfan are infused within a short period of time.

At dose level 6 (56 g/m^2), at least one patient experienced drug-related CTC grade III acidosis, which was obviously the result of the formation of methanesulfonic acid. Alkalinization of patients, *e.g.*, by infusion of sodium bicarbonate solution, is nevertheless not recommended. This might increase the pH-dependent activation of treosulfan in plasma and might induce bladder injury from activation of the drug in alkaline urine. However, this process should be self-limiting because of rapid acidification of urine by concomitantly excreted and released methanesulfonic acid in the bladder. To avoid any risk of methanesulfonic acid induced acidosis, a split-dose or continuous i.v. infusion regimen of high-dose treosulfan is recommended.

Meanwhile, two treosulfan-based high-dose combination therapy protocols have been conducted in patients with advanced breast or ovarian carcinomas (33, 34). Treosulfan was infused on 3 consecutive days and cumulative doses of 49 and 42 g/m^2 were found to be tolerable despite the combination with high-dose dacarbazine/melphalan or carboplatin/etoposide and autologous PBSCT, respectively.

Plasma analysis of treosulfan as the prodrug of active mono- and diepoxybutane derivatives may allow a direct correlation to its alkylating potency. Thus, a simple, sensitive and direct method for the determination and quantification of treosulfan in plasma and urine was used based on separation by RP-HPLC and refractometrical detection (26).

Calculation of the pharmacokinetic parameters of treosulfan at the various dose levels of 20–56 g/m^2 resulted in a linear correlation of treosulfan dose and AUC (Fig. 3) as well as C_{max}. However, at the highest dose level of 56 g/m^2, linearity seemed to be compromised by a metabolic acidosis caused obviously by the formation of methanesulfonic acid, thus possibly leading to an increased AUC because of a decreased pH-dependent activation of treosulfan in plasma (Figs. 1 and 2). The extension of the infusion time from 2 to 24 h may be suitable to avoid the development of metabolic acidosis because of the limited formation of methanesulfonic acid per unit of time.

Extensive toxicity experienced by patient 19 (ovarian carcinoma at 56 g/m^2 treosulfan) might be related to ascites and edema already present at accrual. This circumstance might be responsible for a somewhat increased exposure to treosulfan because of the presence of an additional compartment for distribution of treosulfan in this patient.

All pharmacokinetic parameters presented are in agreement with results described previously using an indirect analytical method of treosulfan based on the gas chromatographic detection of L-diepoxybutane (35).

Although not a primary objective of the Phase I dose escalation trial, several objective tumor remissions were documented in patients with relapsed and refractory ovarian cancer and Hodgkin's and non-Hodgkin's lymphoma. Because of the

fact that all these patients were pretreated heavily including previous high-dose combination chemotherapy in some of them, high-dose treosulfan appears to be an active treatment and should be further investigated in patients with chemosensitive malignancies. Therefore, high-dose treosulfan might be considered an attractive candidate for combination treatment of primary or relapsed ovarian cancer patients to increase remission rates and, possibly, survival in comparison with conventionally dosed chemotherapy (36–44).

In this context, the limited organ toxicity of high-dose treosulfan was confirmed by combination of treosulfan with other dose-escalated cytotoxins such as carboplatin, melphalan, dacarbazine, and etoposide (33, 34). Moreover, dose-escalated treosulfan is investigated for conditioning therapy before allogeneic transplantation of patients with hematological malignancies (15, 16).

In conclusion, this Phase I trial demonstrates that a substantial 5-fold dose escalation of the alkylating cytotoxic treosulfan is feasible when combined with autologous PBSCT. A split-dose or continuous infusion regimen is recommended for future high-dose trials with treosulfan. The nonenzymatic activation, simple i.v. administration, linear correlation between dose and AUC, and limited nonhematological toxicity profile make treosulfan a promising candidate for combination or sequential high-dose chemotherapy protocols.

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Max E. Scheulen, Ralf A. Hilger, Carsten Oberhoff, et al.

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