Cancer Chemother Pharmacol (2000) 45: 483–488

ORIGINAL ARTICLE

**Ralf Axel Hilger · Gabriele Jacek · Carsten Oberhoff
Susanne Kredtke · Joachim Baumgart · Siegfried Seeber
Max Ernst Scheulen**

Investigation of bioavailability and pharmacokinetics of treosulfan capsules in patients with relapsed ovarian cancer

Received: 28 July 1999 / Accepted: 16 December 1999

Abstract *Purpose*: Treosulfan (L-threitol-1,4-bis-methanesulfonate, Ovastat) is a prodrug of a bifunctional alkylating agent with activity in ovarian carcinoma and other solid tumors. In a pharmacologic study of the bioavailability of treosulfan in a capsule formulation, patients with relapsed ovarian carcinoma were treated with alternating doses of oral and intravenous (i.v.) treosulfan of 1.5 or 2.0 g daily for 5 to 8 days. *Methods*: A sensitive method for the determination of treosulfan in plasma and urine by reversed-phase high-performance liquid chromatography had previously been developed. Pharmacokinetic analyses of treosulfan were carried on plasma and urine samples from 20 i.v. courses and 20 courses of oral administration. *Results*: The bioavailability ratio (*f*) of oral to i.v. administration was calculated as 0.97 ± 0.18 (mean ± SD) using the values $AUC_{oral} = 82.1 \pm 39.4$ μg/ml h and $AUC_{i.v.} = 85.4 \pm 30.3$ μg/ml h. The peak plasma concentration c_{max} (29 ± 14 μg/ml vs 65 ± 23 μg/ml) was significantly ($P < 0.01$) higher after i.v. administration and the t_{max} after oral administration was 1.5 ± 0.34 h. The terminal half-life of treosulfan was about 1.8 h. The mean urinary excretion of the parent compound was about 15% of the administered total dose over 24 h (range 6–26%). *Conclusions*: The high and relatively constant bioavailability of treosulfan indicates that capsules provide a satisfactory noninvasive treatment alternative. A feasible and reliable oral treosulfan formulation could provide the basis for the development of long-term low-dose outpatient treatment of patients with malignant diseases.

Key words Treosulfan · Bioavailability · Dihydroxybusulfan · Pharmacokinetics · Ovarian carcinoma

Supported by Förderverein Essener Tumorklinik, Gemeinnütziger Verein e.V.

R. A. Hilger (✉) · S. Kredtke · S. Seeber · M. E. Scheulen
Innere Klinik und Poliklinik (Tumorforschung),
Universitätsklinikum Essen,
Westdeutsches Tumorzentrum,
Hufelandstrasse 55, 45122 Essen, Germany
e-mail: ralf.hilger@uni-essen.de
Tel.: +49-201-7233157; Fax: +49-201-7233790

G. Jacek · C. Oberhoff
Zentrum für Frauenheilkunde,
Abteilung für Gynäkologie,
Universitätsklinikum Essen,
Westdeutsches Tumorzentrum,
Essen, Germany

J. Baumgart
Medac GmbH, D-20354 Hamburg, Germany

Introduction

Treosulfan (L-threitol-1,4-bis-methanesulfonate), is a prodrug of a bifunctional alkylating agent [6, 7] with a broad spectrum of antitumor activity [9, 10, 17–20, 24–26, 29]. It is a registered cytostatic drug for the treatment of ovarian carcinoma in several European countries. The mono- and diepoxybutane derivatives are the active metabolites formed by a nonenzymatic, pH- and temperature-dependent intramolecular nucleophilic substitution [4, 8, 30]. Although treosulfan is structurally related to busulfan, its mechanism of activation is entirely different. Whereas the methanesulfonyloxy groups of busulfan alkylate nucleophilic centers directly, the introduction of the two hydroxy groups in position 2 and 3 of treosulfan leads to a different activation pathway [3, 27]. Thus, treosulfan undergoes a nonenzymatic internal nucleophilic substitution (S_Ni) to the monoepoxy intermediate (1,2-epoxy-3,4-butanediol-4-methanesulfonate) and to L(+)-diepoxybutane, which are supposed to be the active metabolites producing DNA alkylation of guanine bases and interstrand crosslinks (Scheme 1) [8, 12]. At pH values below 6.0 no transformation of treosulfan occurs [8]. In contrast to busulfan, treosulfan is soluble in water and can be easily infused intravenously (i.v.). Since the early 1980s clinical studies with i.v. formulations of treosulfan have been conducted either with the drug alone or in combination

with other cytostatic agents such as cisplatin [1, 2, 5, 21, 23].

Despite the clinical use of both the oral and the i.v. formulation, pharmacokinetic data are scarce [8, 9, 28]. Our validated analytical method for treosulfan based on separation by reversed-phase high-performance liquid chromatography (RP-HPLC) with refractometric detection [14, 15] was used to determine the bioavailability of treosulfan in this investigation. Prior to this bioavailability study, a dose-escalation trial of i.v. treosulfan was performed. The maximum tolerated dose (MTD) was found to be 10 g/m^2 treosulfan every 4 weeks. The dose-limiting toxicity is thrombocytopenia [11].

Here we report the clinical pharmacokinetics of repeated daily doses of 1.5 g or 2 g treosulfan, depending on the body surface area (BSA) of the patients, administered either i.v. or orally (six to eight capsules) to patients with relapsed ovarian cancer up to a conventional dose of 8 to 10 g/m^2 treosulfan.

Table 1 Patient characteristics (*FIGO* International Federation of Gynecology and Obstetrics)

Number of patients	10
Age (years)	
Median	63
Range	58–71
Tumor type (*n*)	
Ovarian (relapsed)	10
Prior therapy	
Surgery	10
Radiation	4
Chemotherapy	
Two regimens	6
Three regimens	4
Stage (FIGO)	
3	7
4	3
Karnofsky performance index (%)	70–90
Study treatment courses	
1 g/m^2 i.v.	20
1 g/m^2 oral	20

Materials and methods

Materials

Treosulfan (Ovastat) was kindly supplied by medac, Hamburg, Germany. All other chemicals were obtained from Sigma Chemical Co. and were of the highest purity available. Ultrafiltration of plasma samples was carried out with Centrisart I ultrafilters (SM 13239; cut-off 10,000 Da; Sartorius, Göttingen, Germany).

Patients and treatment

Ten pretreated patients with relapsed ovarian cancer not amenable to platinum- or taxane-based salvage chemotherapy were included in the study. The patients' Karnofsky performance status was >70%, and they also had adequate hematologic, hepatic and renal function (creatinine clearance >60 ml/min). The average age of the patients was 63 years, with a range of 58 to 71 years. Tumor stage according to FIGO at the time of therapy was stage III in seven, and stage IV in three patients (Table 1). Written informed consent to the blood sampling was given before chemotherapy.

A randomized individual crossover design with alternating oral and i.v. drug administration between days 1 and 4 was chosen to minimize the intra- and interindividual differences. Depending on the BSA of the patient, treosulfan was administered at doses of 1.5 or 2 g in oral form (six or eight hard-gelatin capsules) or dissolved in sterile water for injection at a concentration of 50 mg/ml and administered i.v. to the same individual (slow bolus infusion over about 15 min; the infusion time had to be documented). To reach the therapeutic objective of conventional 8 to 10 g/m^2 treosulfan, different durations of treatment were necessary (up to 8 days). For the pharmacokinetic objectives, 20 plasma and 10 urine courses were determined after oral administration in comparison with the corresponding courses of the slow bolus infusional regimen (day 1 to 4 of therapy, in one patient to day 6).

Methods

Treosulfan was administered 1 h after a standard breakfast. Blood was drawn (in 3-ml aliquots) via an indwelling venous access at 0 (preadministration), 30, 60, 90, 120 and 150 min, and 3, 4, 5 and 6 h after oral administration and 0, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min after the start of i.v. infusion. Blood samples were adjusted to a final pH of 5.5 by the addition of 300 µl 1 *M* sodium citrate (citric acid disodium salt sesquihydrate) to a 3-ml S-Monovette (Sarstedt, Nümbrecht, Germany) before collection in order to avoid ex vivo degradation of treosulfan. Plasma was separated by centrifugation at 1500 *g* for 10 min at 4 °C followed by microfiltration (cut-off 10,000 Da) and was then applied to the analysis system. Urine was collected from patients into separate containers over 24 h with the addition of crystalline citrate to guarantee a final pH < 6.0. The volume of each urine sample was determined before an aliquot was centrifuged (4 °C, 14,000 *g*, 15 min) and analyzed.

Treosulfan was separated by a validated RP-HPLC method and quantitated by refractometric detection [14, 15]. The calculations for blood and urine concentrations were performed by means of external standard curves of authentic treosulfan diluted in plasma or urine, respectively. The limit of quantification for treosulfan was 1 µg/ml in plasma and 20 µg/ml in urine. Reproducibility was 99 ± 2.8%, recovery 96 ± 4%, and linearity 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 [13]. All pharmacokinetic data were correlated to BSA (per meter squared). Based on the data from our previous study [15], the expected pharmacokinetic parameters for the 1 g/m^2 infusional regimen were simulated using TOPFIT 2.0 [13].

Representative graphs were constructed using individually fitted concentration-time curves of treosulfan in plasma and urine.

Statistical analysis

The differences between the mean values of the pharmacokinetic parameters were analyzed for significance using the Mann-Whitney rank sum test. The analysis of variance models for repeated measures (ANOVA) was used to assess whether the renal excretion or the AUC of the orally administered treosulfan doses differed from the renal excretion or the AUC of treosulfan given i.v., and whether there were differences in the clearance and renal excretion data between this and our previous study [15]. *P*-values < 0.05 were considered to be statistically significant, and < 0.01 to be statistically highly significant.

The bioavailability ratio f was calculated as the mean ± SD of the ten individual bioavailabilities according to the formula:

$$f = \frac{AUC_{p.o.} \times D_{i.v.}}{AUC_{i.v.} \times D_{p.o.}} \quad .$$

Results

Pharmacokinetics of treosulfan

Plasma concentrations of treosulfan were determined in ten patients for 20 alternate courses of orally and i.v. administered treosulfan. Additionally, ten courses of each administration modality were analyzed for urine concentrations. All courses were used for statistical analysis. Plots of typical individual concentration-time curves after oral and i.v. administration are shown in Figs. 1 and 2.

After i.v. and after oral administration the plasma concentration of treosulfan declined exponentially and was best fitted by a first-order elimination process ($r^2 = 0.99 \pm 0.01$; median 0.99, 0.97–1.00). Absorption of treosulfan from the capsule formulation was fitted best by a first-order input with dissolution and absorption ($r^2 = 0.99 \pm 0.01$; median 0.99, 0.96–1.00), leading to a maximum plasma concentration after oral administration (t_{max}) of 1.5 h. Mean peak plasma levels of infused treosulfan were reached at the end of the infusion time of approximately 15 min. The value of c_{max} was significantly ($P < 0.01$) higher after infusion (65 ± 23 μg/ml) compared with oral administration (29 ± 14 μg/ml). A mean plasma distribution half-life $t_{1/2\alpha}$ of 0.2 h was calculated for both administration forms. The mean plasma elimination half-life $t_{1/2\beta}$ of treosulfan was 1.9 h for the oral and 1.7 h for the infusional regimen (Table 2). The values calculated for the $AUC_{0-\infty}$ using a two-compartment disposition model did not differ significantly between the two administration forms ($P = 0.77$; i.v. administration 85.4 ± 30.3 μg/ml h, oral administration 82.1 ± 39.4 μg/ml h). As might be expected, the oral AUC values were more variable (variance AUC_{oral} 1217) than the i.v. AUC values (variance $AUC_{i.v.}$ 830). The calculated pharmacokinetic parameters were similar to those from a simulated i.v. formulation (1 g/m^2 treosulfan, Table 2) based on previous results [14, 15] which led to a predicted $AUC_{sim\ i.v.}$ of 97 ± 23 μg/ml h.

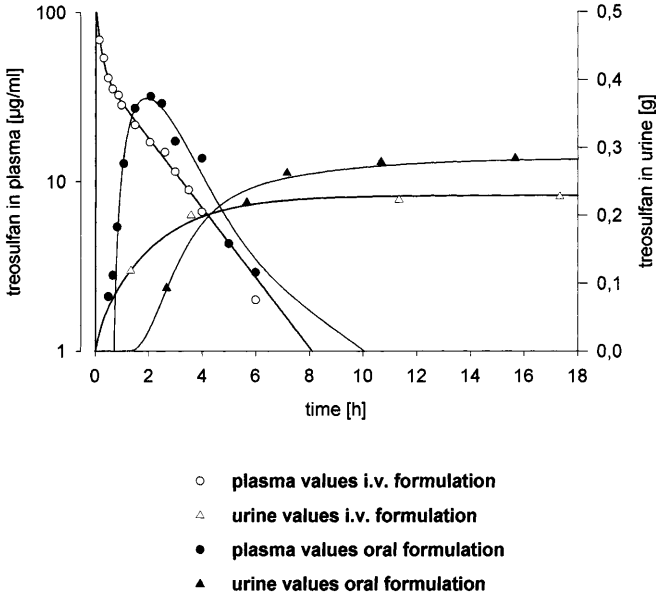

Fig. 1 Time course of plasma concentrations and cumulative renal excretion of treosulfan after slow bolus i.v. infusion and after oral administration of 1.1 g/m^2 treosulfan (patient Q/1, curves were fitted by a two-compartment model, absorption of the capsule by a first-order input with dissolution and absorption in addition to a two-compartment model). Plasma kinetics: ● oral administration, ○ i.v. administration; urine kinetics: ▲ oral administration, △ i.v. administration

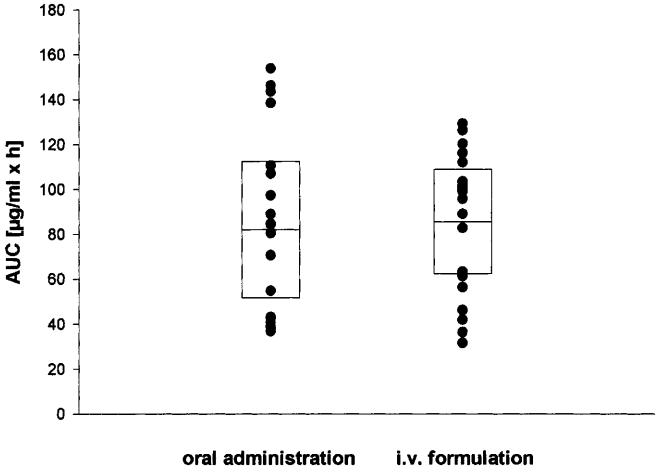

Fig. 2 Plots of the AUC of treosulfan after oral and i.v. administration. The arithmetic means ± SD (*boxes*) and all the measured values (*circles*) are plotted

Urinary excretion of the parent compound was about 15% of the total dose administered during the first 24 h after the start of infusion (range 6–26%; Table 3). More than 90% of the total urinary excretion occurred within the first 6 h after administration without any significant difference between the two groups ($P = 0.98$). Compared to a renal excretion of 24–26% found in our previous study [15], there was a significant decrease in urinary excretion of the parent compound ($P = 0.03$) which was correlated with a significant decrease in renal clearance (CL_{ren}) from a mean CL_{ren} of about 105 ml/min in the previous study to 59 ml/min calculated for the lower doses in this study, possibly as a result of significantly lower creatinine clearance of the patients presented here. The total clearance CL_{tot} of about 233–259 ml/min obtained here was not significantly higher than the mean CL_{tot} values of 176–183 ml/min which we had found earlier [15]. There were no significant differences in clearance and renal excretion between the low-dose oral and the i.v. treosulfan routes of administration.

Calculation of the bioavailability

The bioavailability ratio f was calculated as 0.97 ± 0.18 ($n = 10$ patients). Calculation of f on a per course basis led to the same result but with a wider standard devia-

Table 2 Calculated pharmacokinetic parameters of treosulfan following oral and i.v. administration

Patient	Age (years)	Course	Dosage (g/m^2)		t_{max} (h)		c_{max} (μg/ml)		$t_{1/2\beta}$ (h)		$AUC_{0-\infty}$ (μg/m h)[a]		Bioavailability (f)	
			Oral	i.v.	Oral	i.v.	Oral	i.v.	Oral	i.v.	Oral	i.v.	Per course	Per patient
Q	60	1	1.10	1.10	1.15	0.03[b]	32	112[b]	1.49	1.87	97.3	120.0	0.81	0.72
		2	1.10	1.10	1.93	0.03[b]	28	97[a]	2.72	1.46	84.7	99.1	0.86	
		3	1.10	1.10	1.13	0.03[b]	19	172[b]	1.46	1.46	54.7	111.8	0.49	
LW	65	1	1.14	1.06	2.20	0.33	25	114	2.72	1.73	110.5	95.7	1.15	1.01
		2	1.14	1.06	1.48	0.67	17	40	2.72	1.51	42.6	56.2	0.76	
RB	58	1	0.80	0.80	1.86	0.03[b]	20	156[b]	1.48	1.46	84.3	115.9	0.73	1.16
		2	0.80	0.80	1.16	0.33	74	48	1.46	1.47	153.8	88.9	1.73	
HA	61	1	1.43	1.32	1.87	0.33	21	64	1.48	1.46	43.1	36.3	1.19	0.69
		2	1.43	1.32	1.27	0.33	33	83	1.46	1.46	38.4	82.6	0.46	
MH	63	1	0.78	0.78	1.49	0.03[b]	44	342[b]	1.48	1.46	146.2	103.3	1.41	1.06
		2	0.78	0.78	1.22	0.07[b]	27	113[b]	2.72	1.47	70.5	101.3	0.70	
RU	58	1	1.19	1.10	1.94	0.33	13	42	1.50	1.46	39.0	31.5	1.24	1.03
		2	1.19	1.10	1.38	0.36	13	54	1.47	1.46	40.6	46.1	0.88	
PK	64	1	1.23	1.14	1.63	0.40	18	91	1.46	1.55	36.7	61.2	0.60	0.75
		2	1.23	1.14	1.84	0.33	20	44	1.47	1.46	40.6	41.8	0.97	
SE	71	1	1.00	1.00	1.48	0.33	43	71	1.69	1.69	108.0	98.9	1.08	1.16
		2	1.00	1.00	1.11	0.33	33	51	2.62	1.46	80.4	63.1	1.27	
W	63	1	0.92	0.92	1.12	0.23	34	104	1.78	2.72	143.5	126.1	1.14	1.03
		2	0.92	0.92	1.02	0.50	42	72	2.72	2.45	88.9	100.2	0.89	
WU	67	1	1.07	1.07	1.69	0.03[b]	32	567[b]	2.72	2.72	138.3	129.0	1.07	1.07
Mean			1.07	1.03	1.50	0.35 (0.03[b])	29*	67 (223[b])	1.93	1.69	82.1	85.4	0.97	0.97
SD			0.20	0.16	0.35	0.14 (0.01[b])	19	24 (173[b])	0.59	0.42	40.5	31.1	0.3	0.18
Expected[c]				1		0.34		50		1.93		97		
SD								10		0.3		23		

*$P < 0.01$

[a] Normalized to a dosage of 1 g/m^2

[b] Bolus infusion

[c] Calculated from reference 15

Table 3 Renal clearance of treosulfan following oral and i.v. administration (*n.d.* not done)

Patient	Course	Creatinine clearance (ml/min)	CL_{tot} (ml/min)		CL_{ren} (ml/min)		Renal excretion (%)	
			Oral	i.v.	Oral	i.v.	Oral	i.v.
Q	1	63	171	139	40	40	25.8	23.6
	2		197	168	51	35	n.d.	21.5
	3		305	149	50	44	8.3	8.5
LW	1	65	150	174	n.d.	n.d.	n.d.	n.d.
	2		391	297	n.d.	n.d.	n.d.	n.d.
RB	1	60	189	144	n.d.	n.d.	n.d.	n.d.
	2		109	187	n.d.	n.d.	n.d.	n.d.
HA	1	90	387	459	118	96	19	15.3
	2		434	202	45	63	12	21.8
MH	1	82	114	161	n.d.	n.d.	n.d.	n.d.
	2		236	165	n.d.	n.d.	n.d.	n.d.
RU	1	62	428	528	n.d.	n.d.	n.d.	n.d.
	2		410	362	n.d.	n.d.	n.d.	n.d.
PK	1	60	454	272	57	41	7.8	9.4
	2		411	399	43	66	6.4	13.7
SE	1	74	156	168	80	80	21.8	20.5
	2		207	264	92	113	7.4	8.7
W	1	87	116	133	n.d.	n.d.	n.d.	n.d.
	2		187	166	n.d.	n.d.	n.d.	n.d.
WU	1	61	124	121	14	15	17.3	21.2
Mean		73	259	233	59	59	14.8	16.4
SD		12	127	118	30	30	7.2	6.0
Expected[a]		111*		172		105*		28*
SD		44.6		55		41		13

*$P < 0.05$
[a] Calculated from reference 15

tion (Table 2). The calculated values of f for each patient demonstrate a normal distribution for the bioavailability of orally administered treosulfan around the optimal value of $f = 1$. Additionally, the corresponding intraindividual variation amongst the ten patients is shown in Fig. 2.

Pharmacodynamics of treosulfan – toxic side effects

Treosulfan induced CTC toxicity grade 3 leukocytopenia in one patient, CTC grade 2 thrombocytopenia in one patient and anemia CTC grade 2 in two patients. Treosulfan treatment was not associated with significant nonhematologic side effects.

Discussion

Treosulfan is a prodrug of an alkylating agent [6, 7] with activity in ovarian carcinoma and other solid tumors [9–11, 17–25, 29], given in either oral or i.v. formulations. According to our dose escalation trial [11], the MTD of i.v. treosulfan is 10 g/m^2 with thrombocytopenia as dose-limiting toxicity. Thus, daily repeated doses of 1 g/m^2 treosulfan, administered either i.v. or orally for 8 days, are feasible. Bioavailability estimations are based solely on data of the area under the concentration time curve. Therefore, the drug must be administered i.v. and orally. We applied a simple, sensitive and direct method for the determination and quantification of treosulfan in plasma and urine based on separation by RP-HPLC with refractometric detection [14, 15]. Simulation of an i.v. administration of 1 g/m^2 treosulfan based on the data from a previous phase I/II study [15] led to an expected $AUC_{i.v.}$ of 97 μg/ml h, a c_{max} of 50 μg/ml and a renal clearance of 105 ml/min. These are in good agreement with the results from the i.v. group in this study. The terminal half-life of treosulfan after oral and i.v. infusion was 1.9 h and 1.7 h, respectively, which is in good agreement with clinical results reported previously [15].

Urinary excretion of the parent compound was about 16% of the total dose administered during 24 h of which more than 90% (15% of the total dose) was excreted during the first 6 h after administration. Individual values varied over a wide range from 6% to 26% possibly depending on individual renal function. Moreover, renal excretion of treosulfan per time unit directly correlated with the concentration of treosulfan in plasma as shown by clearance plots (data not shown). Additionally, individual patient characteristics such as large ascites volumes, pleural effusions and edema may have an impact on individual pharmacokinetic parameters. Remarkably, the recovery of treosulfan in the urine is in good agreement with previous findings in dogs and humans [8, 15].

Together with the data from a currently ongoing high-dose protocol, the pharmacokinetics of treosulfan are linear in the range from 1 to 47 g/m^2 [16]. Comparing the data from the i.v. group with those of the oral group, we determined the relative bioavailability of oral

treosulfan to be 97%. This finding indicates that there is no 'first pass' effect for the prodrug treosulfan itself. Furthermore, the intraindividual variability of AUC_{oral} and $AUC_{i.v.}$ for treosulfan was in the same range and the interindividual variability of the bioavailability of treosulfan was low with a standard deviation of only 18%. Thus, according to its high and relatively constant bioavailability oral treosulfan in the form of capsules is a sufficient noninvasive treatment alternative. This therapeutic option of oral treosulfan administration would allow ambulant treatment with a favorable impact on compliance and the patients' quality of life.

A feasible and reliable oral treosulfan formulation could form the basis for the development of long-term low-dose outpatient treatment of patients with malignant diseases such as metastatic cutaneous melanoma [24] or malignant glioma [25].

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