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Clinical pharmacokinetics of intravenous treosulfan in patients with advanced solid tumors

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Abstract 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. For a clinical and pharmacology study, patients with advanced, refractory, or resistant solid tumors were treated with a single-dose intravenous 30-min infusion of 8 or 10 g/m^2 treosulfan. A sensitive method for the determination of treosulfan in plasma and urine by reverse-phase high-performance liquid chromatography was developed. A total of 14 plasma and urine treosulfan pharmacokinetics determinations were analyzed in the 8-g/m^2 group and 7 were analyzed in the 10-g/m^2 group, the maximum tolerated dose for this group of pretreated patients. The terminal half-life of treosulfan was in the range of 1.8 h. AUC and C_{max} values were significantly ($P < 0.01$) higher in the 10-g/m^2 group (AUC 708 ± 168 versus 977 ± 182 μg ml^{-1} h, C_{max} 465 ± 98 versus 597 ± 94 μg/ml). The mean urinary excretion of the parent compound was about 25% of the total dose delivered over 48 h (range 5–49%), and about 20% was excreted during the first 6 h after administration. Currently, a clinical phase I pharmacokinetics and dose-escalation trial with autologous blood stem-cell support has been started at 20 g/m^2 treosulfan using a 2-h infusion protocol.

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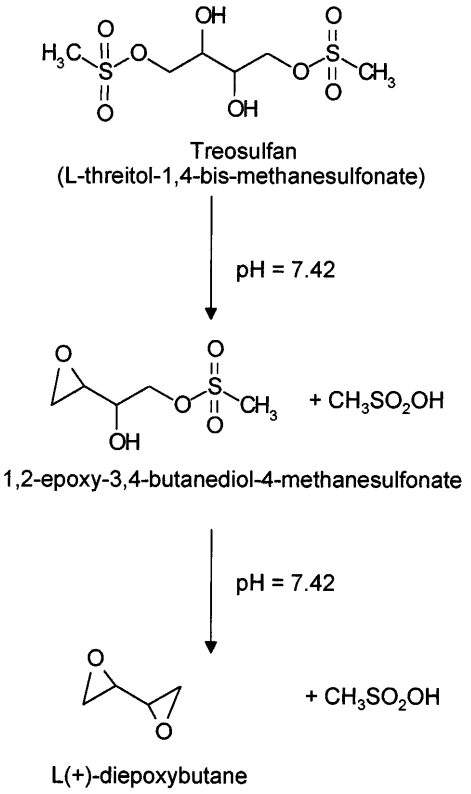
Introduction

Treosulfan (L-threitol-1,4-bis-methanesulfonate), which was first synthesized in 1961 [6, 7], is a prodrug of a bifunctional alkylating agent with activity in ovarian carcinoma and other solid tumors [9, 15–18, 24, 27]. The mono- and diepoxybutane derivatives are the active metabolites formed by a nonenzymatic pH- and temperature-dependent intramolecular nucleophilic substitution [4, 8, 28]. 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 positions 2 and 3 of treosulfan leads to a different activation pathway. 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 that produce DNA alkylation of guanine bases and interstrand cross-linking [8, 11, 23] (Scheme 1). At pH values below 6.0, no transformation of treosulfan occurs [8].

Both alkylating agents, busulfan and treosulfan, were grouped into the class of S_N2 reactors according to the mechanism by which they interacted with nucleophilic centers. However, the S_N2 reactors were further grouped into four classes on the basis of their chemical reactivity and the spread in their affinity toward different nucleophils. Dependent on this classification, treosulfan was grouped into the "epoxides, ethyleneimines, and β-lactones," whereas busulfan belongs to the "primary alkyl methanesulfonates." This difference demonstrates molecular evidence of the potency of epoxides against the alkylate guanine of DNA, whereas busulfan demonstrates much less reactivity toward guanine. Furthermore, a greater spread has been found in the affinity of



Scheme 1 Schematic illustration of the pH-dependent transformation of treosulfan to the corresponding epoxybutane derivatives (according to Feit [7])

busulfan for nucleophils, and its reactivity toward thiol groups is far greater than that toward amines or acids as compared with the epoxides [3, 25]. Interestingly, in contrast to busulfan, treosulfan is soluble in water and can easily be applied intravenously. Since the early 1980s, clinical studies with intravenous formulations of treosulfan have been conducted using the drug either as a single agent or in combination with other cytostatic agents such as cisplatin [1, 2, 5, 19, 21].

Despite the clinical use of treosulfan for more than 30 years, pharmacokinetic data are scarce [8, 9, 26], perhaps because no valid method for the direct determination of treosulfan has been available. To date, treosulfan has been indirectly determined by a gas chromatography method after ex vivo derivatization to L(+)-diepoxybutane by alkaline treatment of samples. Recently we introduced an analytical method based on separation by reverse-phase high-performance liquid chromatography (RP-HPLC) with refractometric detection (Hilger RA, Baumgart J, Scheulen ME (1998) Validated analysis of treosulfan and its corresponding epoxide species in human plasma and urine by reversed phase HPLC. (submitted)) which allows the determination of treosulfan.

Prior to this phase I/II study, dose escalation of treosulfan was studied in patients with lung cancer. In this study the maximum tolerated dose was found to be

Table 1 Patients' characteristics (*KPI* Karnofsky performance index, *SCLC* small-cell lung cancer)

Number of patients	18
Gender (M/F)	10/8
Age (years):	
Median	60
Range	44–77
Tumor type:	
SCLC	11
Ovarian carcinoma	7
Prior surgery	10
Prior radiation	4
Prior chemotherapy:	
1 regimen	4
2 regimens	6
>2 regimens	8
Performance status:	
KPI	70–100%
Study treatment:	
8 g/m^2 × 0.5 h	14 courses
10 g/m^2 × 0.5 h	7 courses
8 g/m^2 × 2 h	4 courses
10 g/m^2 × 2 h	3 courses

10 g/m^2 treosulfan given every 4 weeks. The dose-limiting toxicity was thrombocytopenia [10]. Herein we report on the clinical pharmacokinetics of treosulfan after a single-dose intravenous 30-min infusion of 8 or 10 g/m^2 in patients with advanced or resistant solid tumors.

Patients and methods

Materials

Treosulfan (Ovastat) was kindly supplied by medac GmbH, 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 Sartorius ultrafilters Centrisart I (SM 13239, cutoff 10,000 Da; Sartorius AG, Goettingen, Germany).

Patients and treatment

A total of 18 patients (10 men and 8 women) aged a mean of 60 years (range 44–77 years) were included in the study according to standard phase I criteria such as an advanced progressive solid tumor not amenable to any standard salvage chemotherapy or radiotherapy, a Karnofsky performance status of ≥70%, and normal hepatic and renal function (creatinine clearance >60 ml/min). In all, 11 patients had heavily pretreated small-cell lung cancer (SCLC) and 7 patients suffered from advanced refractory ovarian carcinoma (Table 1). Written informed consent to the study protocol, which was approved by the ethics board of Essen University Medical School, was obtained prior to chemotherapy.

Treosulfan was dissolved in sterile water for injection at a concentration of 50 mg/ml and was intravenously infused at the two different doses of 8 and 10 g/m^2. For the single-dose 30-min infusion protocol, 14 plasma and urine pharmacokinetics determinations were analyzed in the 8-g/m^2 group and 7 were analyzed in the 10-g/m^2 group. Furthermore, the duration of the intravenous infusion was extended to 2 h in an additional five patients (four kinetics determinations in the 8-g/m^2 group and three in the 10-g/m^2 group).

Table 2 Pharmacokinetic parameters of patients treated with a 0.5-h intravenous infusion of 8 g/m^2 treosulfan (data included in the calculation of *P* values shown in Table 3)

Patient	Age (years)	Sex	BSA (m^2)	Total dose (g)	C_{max} (μg/ml)	$t_{1/2}$ (h)	V_{ss} (l)	$AUC_{0-\infty}$ (μg ml^{-1} h)	Cl_{tot} treosulfan (ml/min)	Clearance S-creation (ml/min)	Renal excretion	
											(g)	(%)
EP	61	M	2.0	16	460	1.43	21	631	211	87	4.0	25
MW/2	69	M	1.88	15	477	1.21	18	654	204	253	6.3	42
LG/1	44	M	1.63	13	413	1.92	26	776	172	83	4.9	33
LG/3	44	M	1.63	13	651	1.36	16	870	153	95	4.4	32
LA/1	67	M	1.75	14	412	1.93	24	668	200	113	2.5	18
LA/2	67	M	1.75	14	556	1.84	20	699	191	90	3.4	25
HR	44	M	2.0	16	455	1.49	22	637	209	170	5.1	32
KH/1	59	M	2.0	16	378	1.14	19	631	211	120	5.5	35
RT	55	F	1.63	13	324	1.33	18	653	204	95	4.1	31
AH/1	73	M	2.0	16	540	1.58	20	782	170	69	4.7	29
ES	74	M	1.88	15	546	3.53	21	1,100	122	75	5.5	37
EH/1[a]	77	F	1.75	14	270	4.73	81	566	235	110	0.2	1
AF[a]	66	F	1.88	15	379	1.88	35	378	352	125	2.2	15
KR	69	M	1.88	15	505	1.76	19	865	154	48	0.8	6
Mean	72.3		1.83	15	465	1.94	26	708	199	110	3.8	26
±SD	5.7		0.08	1	98	0.99	17	168	53	51	1.8	12

[a] Patient with documented edema prior to therapy

Methods

Aliquots of blood were drawn via an indwelling venous access at time zero (preadministration); at 2, 5, 15, 25, 30, 35, 45, 60, and 90 min; and at 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 42, and 48 h after the start of infusion. Blood samples were adjusted to a final pH of 5.5 by citrate immediately after collection to avoid artificial ex vivo degradation of treosulfan, and plasma was separated by centrifugation at 4 °C and 1,000 *g* for 10 min, processed by microfiltration (cutoff 10,000 Da), and applied to the analysis system. Urine was collected by patients into separate containers over a time course of 48 h under the addition of crystalline citrate to guarantee a pH of < 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 quantified by refractometric detection [14]. A Nucleosil C_{18} column (25 cm × 4.6 mm, particle size 5 μm), a Knauer refractometric detector, a Spectra-Physics SP-8800 ternary HPLC gradient pump, and a Waters Millennium Chromatography Manager were used. The solvent system was phosphate buffer [1 g K_2HPO_4 A 3-H_2O + 0.1 g ethylenediaminetetraacetic acid (disodium salt, dihydrate) per 1,000 ml H_2O adjusted to pH 5.0 with H_3PO_4 (85%)]. Analysis was carried out at a flow rate of 1 ml/min at room temperature. Samples were injected via a Waters 717 plus autosampler. The injection volume was 200 μl for plasma samples and 10 μl for urine samples. The calculation of blood and urine concentrations was performed by means of external standard curves of authentic treosulfan diluted in plasma or urine, respectively.

Treosulfan was found to be stable in stored plasma and urine samples for up to 9 days at 4 °C (without ultrafiltration) and for up to several months at −20 °C (after ultrafiltration). The limit of quantification for treosulfan was 1 μg/ml in plasma and 20 μg/ml in urine. The reproducibility was 99 ± 2.8%; the recovery, 96 ± 4%; and the linearity, from 1 μg/ml to 50 mg/ml treosulfan (correlation coefficient 0.99). Increasing the injection volume of urine samples to 200 μl led to a limit of quantification of 1 μg/ml. However, by this measure the reproducibility decreased to 92 ± 7%; the recovery, to 95 ± 4.5%; and the correlation coefficient of linearity, to 0.95. As the concentration of treosulfan in urine is high, its analysis in an injection volume of 10 μl was sensitive enough and led to more valid results.

Individual pharmacokinetic parameters were evaluated by two-compartment disposition modeling using the data analysis system TOPFIT 2.0 [12]. All pharmacokinetic data are correlated to body surface area (BSA; liters per square meter of BSA). Mean graphs were constructed using individually fitted concentration-time curves generated for treosulfan in plasma and urine.

Statistical analysis

Differences between the mean values recorded for the pharmacokinetic parameters were analyzed for significance using the Mann-Whitney rank-sum test; *P* values of < 0.05 were considered to be statistically significant and those of < 0.01, to be highly statistically significant.

Results

Pharmacokinetics of treosulfan

Plasma and urine concentrations of treosulfan were determined in 13 patients for 14 courses of 8 g/m^2 and 7 courses of 10 g/m^2 treosulfan. In one course of 8 g/m^2 (patient RT, Table 2) and one course of 10 g/m^2 treosulfan (patient AH/2, Table 3) the time of infusion of 30 min was exceeded to 60 min; therefore, the C_{max} values recorded for these courses were excluded from statistical analysis. Plots of the mean concentration-time curves (±SD) from each dose group treated on the 30-min infusion protocol are shown in Fig. 1.

After intravenous administration the plasma concentration of treosulfan declines exponentially best fitted for a first-order elimination process. Mean peak plasma levels of treosulfan were reached at the end of the infusion time at 30 min. C_{max} was significantly ($P < 0.01$) lower in the 8-g/m^2 group (465 ± 98 μg/ml) as compared with the 10-g/m^2 group (597 ± 94 μg/ml). For treosulfan a mean plasma distribution half-life ($t_{1/2\alpha}$) of 0.2 h was calculated for both dose groups. The mean plasma elimination half-life ($t_{1/2\beta}$) of treosulfan was 1.87 h for the 8-g/m^2 group (Table 2) and 1.94 h for the 10-g/m^2 group (Table 3). The values calculated for the

Table 3 Pharmacokinetic parameters of patients treated with a 0.5-h intravenous infusion of 10 g/m^2 treosulfan (calculation of *P* values included the data shown in Table 2)

Patient	Age (years)	Sex	BSA (m^2)	Total dose (g)	C_{max} (μg/ml)	$t_{1/2}$ (h)	V_{ss} (l)	$AUC_{0-\infty}$ (μg ml^{-1} h)	Cl_{tot} treosulfan (ml/min)	Clearance S. creatinine (ml/min)	Renal excretion	
											(g)	(%)
WP/1	57	M	1.7	17	439	1.62	27	711	234	125	8.3	49
WP/2	57	M	1.7	17	727	2.15	21	1,130	148	125	7.4	44
MW/1	69	M	1.9	19	642	1.3	18	911	183	174	6.3	33
LG/2	44	M	1.6	16	583	1.69	22	964	173	95	2.9	18
KH/2	59	M	2.0	20	607	0.98	16	825	202	115	2.5	12
EH/2	77	F	1.7	17	581	3.44	27	1,240	134	100	1.0	6
AH/2	73	M	2.0	20	491[a]	1.94	21	1,060	157	69	1.0	5
Mean	60		1.8	18	597	1.87	22	977	176	115	4.2	24
±SD	16.6		0.2	1.6	94	0.79	4	182	34	33	3.1	18
Significant:				**	**			**				
P value				0.001	0.015	>0.1	>0.1	0.005	>0.1	>0.1	>0.1	>0.1

*$P < 0.05$; **$P < 0.01$

[a] Excluded from calculation of the arithmetic mean value because the time of infusion was exceeded

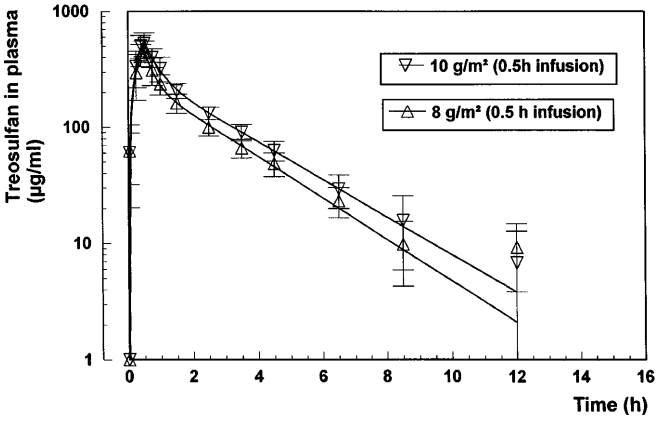


Fig. 1 Time course of plasma concentrations of treosulfan as determined after 0.5-h intravenous infusion of 8 and 10 g/m^2 treosulfan (arithmetic means ± SD; according to Tables 2 and 3)

$AUC_{0-\infty}$ by means of a two-compartment disposition model were significantly ($P < 0.01$) higher in the 10-g/m^2 group (977 ± 182 μg ml^{-1} h) than in the 8-g/m^2 group (708 ± 168 μg ml^{-1} h).

Urinary excretion of the parent compound was about 25% of the total delivered dose during the first 48 h after the start of the infusion (range 5–49%). Approximately 90% of the total urinary excretion occurred within the first 6 h of administration, without a significant difference being observed between the two groups.

To make the pharmacokinetic data for the dose levels of 8 and 10 g/m^2 treosulfan comparable with the higher dose levels achieved in the course of a planned dose-escalation study with autologous blood stem-cell support the infusion time was extended from 30 min to 2 h because of the expected infusion volumes of more than 1,000 ml (Table 4). Plasma and urine concentrations of treosulfan recorded for six patients (four courses at a

Table 4 Pharmacokinetic parameters of patients treated with a 2-h intravenous infusion of 8 and 10 g/m^2 treosulfan, respectively

Patient	Age (years)	Sex	BSA (m^2)	Total dose (g)	C_{max} (μg/ml)	$t_{1/2}$ (h)	V_{ss} (l)	$AUC_{0-\infty}$ (μg ml^{-1} h)	Cl_{tot} treosulfan (ml/min)	Clearance S-creatinine (ml/min)	Renal excretion	
											(g)	(%)
Dose level: 8 g/m^2:												
CS/1	57	F	1.75	14	199	1.7	26	590	226	120	1.0	7
VH	51	F	2.00	16	200	1.74	25	636	210	150	2.8	18
HJ[a]	57	F	1.63	13	126	1.84	42	390	342	80	1.8	14
HS	53	F	1.75	14	197	1.71	25	549	243	100	2.4	17
Mean	55		1.79	14	181	1.75	30	541	255	113	2.0	14
±SD	3.5		0.19	1	36	0.06	8	107	59	30	0.8	5
Dose level: 10 g/m^2:												
CS/2	57	F	1.7	17	346	1.31	20	946	176	110	2.3	13
AH/3	73	M	2.0	20	199	2.47	40	644	259	68	2.1	11
BM	60	F	1.8	18	374	2.19	19	1,230	136	90	4.7	26
Mean	63		1.83	18	306	1.99	26	940	190	89	3.0	17
±SD	8.5		0.15	1	94	0.61	12	293	63	21	1.5	8
Significant:					*							
P value				0.057	0.036	>0.1	>0.1	0.057	>0.1	>0.1	>0.1	>0.1

*$P < 0.05$; **$P < 0.01$

[a] Patient with documented edema prior to therapy

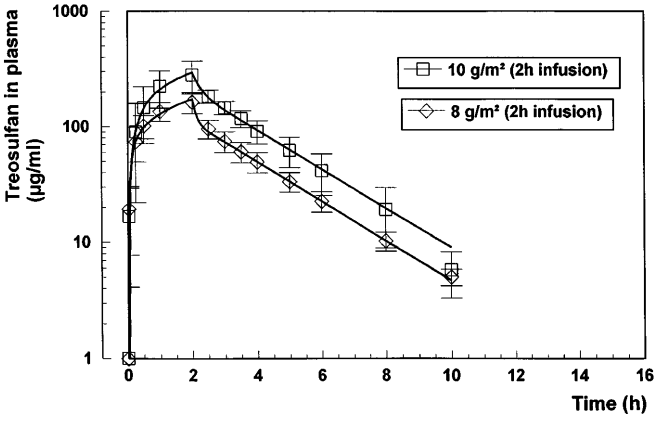


Fig. 2 Time course of plasma concentrations of treosulfan as determined after 2.0 h intravenous infusion of 8 and 10 g/m^2 treosulfan (arithmetic means ± SD; according to Table 4)

dose level of 8 g/m^2 and three courses at 10 g/m^2) are shown in Table 4. Mean graphs plotting the concentration-time curves generated for treosulfan in plasma and urine are shown in Fig. 2.

Due to the prolongation of the infusion time the mean peak plasma levels of treosulfan were reached at the end of the 2-h infusion time. C_{max} was significantly ($P < 0.05$) lower relative to that obtained on the 30-min infusion protocol in both dose groups, being 181 ± 36 (2 h) versus 465 ± 98 µg/ml (30 min) in the 8-g/m^2 group and 306 ± 94 (2 h) versus 597 ± 94 µg/ml (30 min) in the 10-g/m^2 group, respectively. In contrast, there was no significant difference in AUC or terminal half-life (1.8 versus 1.9 h). Urinary excretion did not differ significantly between the two groups (Table 4).

Three patients had a documented generalized edema prior to therapy (patients EH1 and AF at 8 g/m^2 – 30 min; patient HJ at 8 g/m^2 – 2 h). With respect to the high water solubility of treosulfan, it is assumed that the volume of distribution was higher in these patients because of the "third space" and that the total clearance was higher as treosulfan distributed in greater amounts. However, the number of patients with edema was too low for statistical analysis.

Pharmacodynamics of treosulfan

Toxic side effects

Treosulfan induced WHO grade 3 thrombocytopenia in 3 of 28 chemotherapy courses (11%), including 2 of 18 courses at 8 g/m^2 and 1 of 10 courses at the 10-g/m^2 level. Treosulfan treatment was not associated with significant nonhematologic side effects exceeding WHO grade 1.

Therapeutic response

We documented 1 partial remission, 2 minor remissions, 5 cases of stable disease, and 3 cases of progression in the group of 11 heavily pretreated patients with progressive SCLC. In the group of seven patients with progressive ovarian cancer, six cases of stable disease and one case of progression were documented.

Discussion

Treosulfan was synthesized in 1961 [6, 7] and represents a prodrug of an alkylating agent with activity in ovarian carcinoma and other solid tumors [9, 16–18, 27]. Although treosulfan is structurally related to busulfan, alkylation proceeds via epoxybutane derivatives formed by a temperature- and pH-dependent nonenzymatic chemical reaction with a $t_{1/2}$ value of about 2 h in human plasma ex vivo [6, 7, 11] (Fig. 3). Therefore, the measurement of treosulfan as the prodrug for the active mono- and diepoxybutane derivatives allows a direct correlation with its alkylating potency.

Due to its lack of significant nonhematological side effects [10], treosulfan is an interesting candidate for inclusion into high-dose chemotherapy regimens with autologous blood stem-cell support. Determination of the pharmacokinetic parameters is mandatory for clinical phase I dose-escalation studies exploring doses beyond the conventional maximum tolerable doses [10]. To date, pharmacokinetic data reported for treosulfan [8, 9, 26] have been limited. They are based on its indirect determination by a gas chromatography method after ex vivo derivatization to L(+)-diepoxybutane at alkaline pH, which does not allow simultaneous determination of the active metabolites, the monoepoxybutane derivative and L(+)-diepoxybutane. Thus, we developed 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]. Up to a dose of 10 g/m^2 treosulfan, no epoxy derivative is detectable in plasma and urine by the method described. Nevertheless, treosulfan correlates directly with the alkylating species, as the formation of the epoxy derivatives is only pH-dependent and is independent of individual enzymatic activity.

The terminal half-life of treosulfan was in the range of 1.8 h, which is in good agreement with previously reported clinical results [26]. When the two treosulfan doses of 8 and 10 g/m^2 were compared, AUC ($P < 0.01$) and C_{max} values ($P < 0.01$) were found to be significantly higher in the 10-g/m^2 group. Urinary excretion of the parent compound was nearly 30% of the total dose delivered over 48 h, with about 25% being excreted during the first 6 h after administration. Individual values varied over a wide range (5–45%), depending on individual renal function. Moreover, the renal excretion of treosulfan per unit of time correlated directly with the concentration of treosulfan measured in plasma as shown by clearance plots (data not shown). Additionally, individual patient characteristics such as large ascites volumes, pleural effusions, or edema may

have had an impact on individual pharmacokinetic parameters. Interestingly, the urinary recovery of treosulfan was in good agreement with the results previously described in dogs [8].

In a dose range of up to 10 g/m^2, no epoxybutane derivative was detected by the method described. It has previously been shown that pure L(+)-diepoxybutane injected intravenously in dogs disappears completely from the plasma within 10 min [26]. Therefore, it is assumed that the epoxy metabolites of treosulfan are rapidly cleared from plasma as a consequence of their lipophilic character and by reaction with nucleophilic sites.

Prolonged intravenous infusion (2 h) was performed in a number of patients to make the pharmacokinetic data comparable with those to be obtained during an ongoing dose-escalation study using a starting dose of 20 g/m^2 treosulfan with autologous blood stem-cell support [13]. As shown in Table 4, infusion of 8 or 10 g/m^2 treosulfan over 2 h instead of 30 min did not change the pharmacokinetic parameters except for C_{max} and treosulfan recovery in urine, which were lower after the 2-h infusion. A correlation between serum creatinine clearance and renal excretion of treosulfan was not evident in the few patients in whom the drug was infused over 2 h. However, the glomerular filtration rate in these patients was in a normal range of 60–150 ml/min. Therefore, the 2-h infusion regimen is recommended for high-dose therapy using total administration volumes of at least 1,000 ml treosulfan solution, depending on the low degree of solubility of treosulfan (50 mg/ml).

In summary, determination of the clinical pharmacokinetics of treosulfan in plasma and urine is feasible. Further investigations in the course of a multicenter phase I dose-escalation study with autologous blood stem-cell support have started at a dose level of 20 g/m^2. They will show whether the pharmacokinetic behavior of treosulfan is linear or nonlinear and whether levels of epoxybutane derivatives can be quantified in correlation with the treosulfan dose delivered.

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