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Antitumor activity of treosulfan in human lung carcinomas

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Abstract Treosulfan (L-threitol 1,4-bismethanesulfonate, Ovastat) is an alkylating agent and a structural analogue of busulfan. It has been established in the clinical chemotherapy of human ovarian carcinomas for several years and has additionally been shown to be effective against xenografted human breast carcinomas. No other human carcinoma is yet known to be sensitive to treosulfan. The present study confirms the pronounced and significant antitumor activity of treosulfan against heterotransplanted human lung carcinomas of both the small-cell and the non-small-cell type. Treosulfan reduced the growth of all four small-cell lung carcinomas that were investigated in a significant manner. It was even more active than equitoxic doses of the clinically approved cytostatics ifosfamide, cisplatin, and etoposide toward three of them and induced long-lasting growth reductions (60–98% of control tumor size) corresponding to partial and nearly complete remissions. In the case of the nine non-small-cell lung carcinomas investigated, treosulfan effected significant growth inhibition of more than 50%, again in all of them, and was more active than the comparative compounds ifosfamide, mitomycin C, and cisplatin at least in one of four epidermoid lung carcinomas, one large-cell carcinoma, and one of three lung adenocarcinomas. These results are remarkable and unexpected, and the present study should be followed rapidly by phase II clinical trials of treosulfan against human lung carcinomas of both the small-cell and the non-small-cell type.

Key words Treosulfan · Alkylating agent · Antitumor activity · Small-cell lung carcinomas · Non-small-cell lung carcinomas

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Introduction

Treosulfan (L-threitol 1,4-bismethanesulfonate, Ovastat; Fig. 1) is an antitumor drug that was synthesized more than 30 years ago, but it has been characterized only insufficiently as far as antitumor properties in experimental systems are concerned [5, 6, 10, 21, 24]. It was analyzed mechanistically during the late 1960s and the 1970s and was shown to be a structural analogue to the alkylator busulfan, but to differ from busulfan in its mechanism of biochemical action. It has been confirmed that treosulfan is the prodrug of L-diepoxybutane and converts non-enzymatically to the diepoxide (Fig. 1) via the corresponding monoepoxide under physiological conditions. This L-diepoxybutane species obviously effects alkylation at the nucleophilic centers of biological molecules such as proteins and nucleic acids, whereas busulfan alkylates as a primary methanesulfonate [6, 7].

As early as during the 1970s, treosulfan was introduced as an alkylating agent into the clinical therapy of ovarian carcinomas [8, 13]. It was well tolerated by human patients, induced only a negligible impairment of the general condition of the patients, and lacked the capacity to induce pronounced and troublesome side effects such as alopecia or gastrointestinal irritation [1, 2, 8, 13, 14, 22]. Because of its high clinical compatibility combined with its respectable antitumor activity in human ovarian carcinomas, treosulfan has proved to be especially appropriate for combination with platinum-based cytostatic drugs [2, 4, 14, 18, 19, 22], which are highly effective against ovarian carcinomas but are burdened by severe and troublesome side effects such as nephrotoxicity, gastrointestinal irritation, and hair loss. Thus, treosulfan is increasingly replacing the alkylating agent cyclophosphamide in platinum-based combination regimens for the treatment of ovarian cancer [3, 15].

More detailed studies into the spectrum of antitumor properties of treosulfan at the preclinical level have

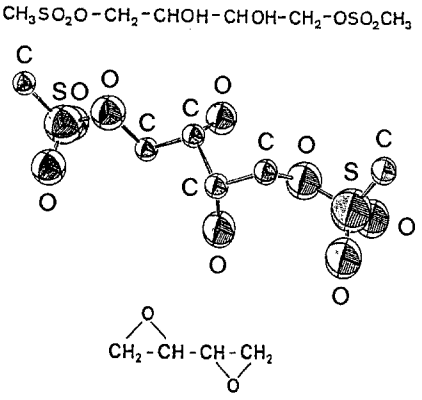

Fig. 1 Formula (*top*) and molecular structure (*center*, H atoms omitted) of treosulfan and its main active metabolite in vivo (*bottom*)

been done within the past few years. These investigations have revealed that the antitumor activity of treosulfan is not confined to ovarian carcinomas but that the organic compound also induces significant growth reduction in human breast carcinomas that have been xenografted to athymic mice [12]. In the present study we continued these trials by investigating lung carcinomas as another important type of human cancer, and we report on the significant growth-inhibitory activity of treosulfan in human small-cell and non-small-cell lung carcinomas.

Materials and methods

Antitumor drugs

Treosulfan (L-threitol 1,4-bismethanesulfonate, Ovastat; medac, Hamburg) and the comparative compounds ifosfamide (Holoxan, Asta-Werke, Bielefeld), mitomycin C (Mitomycin medac, medac, Hamburg), cisplatin [*cis*-diamminedichloroplatinum(II), Alfa, Karlsruhe], and etoposide (Vepesid, Bristol Arzneimittel, München) were obtained from the suppliers mentioned above and were handled according to the instructions of the manufacturers.

Human carcinoma strains

Four small-cell lung carcinomas (LX 1, L 182, L 215, and H 69) and nine non-small-cell lung carcinomas (epidermoid carcinomas L 3, L 2917, L-Heck 6, and L-Heck 8; large-cell carcinoma L 2906; and adenocarcinomas L 27, L 261, HTB 53, and CCL 185) that had been serially heterotransplanted into athymic mice were investigated in the present study (Table 1). The xenografts L 3, L 27, L 182, L 215, and L 261 were generously provided by Dr. J. Mattern (Deutsches Krebsforschungszentrum, DKFZ, Heidelberg) in 1987; the small-cell lung carcinoma strains LX 1 and H 69, by H. Löhrke (Tumorbank, DKFZ, Heidelberg); and the non-small-cell lung carcinomas L 2906 and L 2917, by Dr. I. Fichtner, Max-Delbrück-Centrum, Berlin-Buch. The non-small-cell lung carcinomas HTB 53 and CCL 185 were received from the American Type Culture Collection (ATCC, Rockville, Md., USA). The tumor strains L-Heck 6 and L-Heck 8 were obtained as surgically removed specimens from Prof. Dr. D. Kaiser, Krankenhaus Berlin Zehlendorf, Heckeshorn, and were established as xenografts in our laboratory.

Table 1 Characteristics of the xenografted human lung carcinomas investigated in the present study

Tumor designation	Histopathological classification	Mean doubling time (days)[a]	Passage number[b]
LX 1	Small-cell lung carcinoma	4.2	51–59
L 182	"	5.3	39–51
L 215	"	7.4	26–35
H 69	"	11.0	13
L 3	Lung epidermoid carcinoma	10.0	21–29
L 2917	"	5.2	14–19
L-Heck 6	"	11.1	10
L-Heck 8	"	13.0	10, 11
L 2906	Undifferentiated large-cell lung carcinoma	9.1	14–16
L 27	Lung adeno-carcinoma	6.2	28–44
L 261	"	8.0	36–45
HTB 53	"	5.1	22,23
CCL 185	"	10.5	11

[a]Determined during the phase of exponential growth
[b]Given are the passage numbers when the experiments of the present study were performed

The LX 1 carcinoma represents a human small-cell lung carcinoma [20] that is one of the standard tumors of the National Cancer Institute (NCI, USA) and is used to evaluate the antitumor potency of newly developed cytostatic drugs [9, 23]. It has been shown to be quite insensitive to established cytostatic drugs [23]. The xenografts L 182 and L 261 were also used in previous preclinical trials concerned with determination of the antitumor activity of established and newly developed cytostatic agents [11, 16].

The general characteristics of the human lung carcinomas under investigation, such as the histological classification, the mean doubling time, and the passage number during the testing experiments, are summarized in Table 1.

Animals

Male athymic mice (NMRI, nu/nu) were purchased from the Bomholtgard Breeding and Research Centre Ltd. (Ry, Denmark) and were kept under a humidified atmosphere at 25–27°C in laminar air-flow benches. They obtained food (Altromin) and tap water ad libitum. The drinking water was adjusted to pH 2.5 by the addition of hydrochloric acid to prevent gastrointestinal infections. Antibiotics were not applied. At the time of transplantation, the animals were about 8–12 weeks old and weighed 18–22 g.

Tumor propagation and drug testing

For tumor propagation and drug testing, the tumors were removed from donor animals when they had reached a size of 3–5 cm^3. They were minced mechanically, pressed through injection needles, and suspended in equal volumes of Hanks' balanced salt solution. Volumes of 0.3 ml tumor suspension were then injected subcutaneously into the right flank of athymic mice. Thereafter, the animals were randomized into control and treated groups, each group consisting

of 3–5 animals. The day of tumor inoculation was defined as day 0 of the experiment.

Drug application was done when the tumors had reached a size of 0.4–0.7 cm^3. This volume was attained on days 8–9 (LX 1, L 27, L 2917), days 10–11 (L 3, L 215, L 261), days 13–14 (L 2906, CCL 185), day 15 (L 182, L 2906, L-Heck 8, HTB 53), or day 35 (H 69, L-Heck 6). Treosulfan, ifosfamide, mitomycin C, and cisplatin were applied intraperitoneally in single doses, whereas etoposide was given via the same route in a fractionated manner, i.e., as five injections every 2 days. The days of drug application are indicated by arrows in Figs. 2–4. Except for cisplatin, which was dissolved in saline, all cytostatics were dissolved in distilled water immediately before injection such that volumes of 0.4–0.5 ml/mouse, corresponding to 0.02 ml/g body weight, were given. Control animals received 0.4 ml of the vehicle fluid only.

Evaluation of the experiments

The animals were weighed on the days of drug application and at 2, 4, 7, 10, 12, 14, 21, and 28 days thereafter, in some cases also at 35 and 42 days after the last drug injection. At the same days, two perpendicular diameters (length, a; breadth, b) of the tumors were measured with a graduated caliper. Absolute tumor volumes were calculated according to the formula $V = a \times b^2/2$. Thereafter, relative tumor volumes, expressing the changes in the volume of individual tumors following substance application, were calculated by relating the absolute tumor volumes measured on certain days after treatment to those determined on the (first) day of drug injection. Within all experimental and control groups, mean values ($\pm$ SD) for the relative tumor volume were then calculated for the different days. Treated/control (T/C) values were obtained by determining the quotient

$$\frac{\text{Mean relative tumor volume of treated tumors}}{\text{Mean relative tumor volume of control tumors}} \times 100\%.$$

Growth inhibition, expressed as a percentage of control tumor size, was then calculated as 100% - T/C.

Results

The 13 lung carcinomas investigated in the present study differed from one another not only in histological type, but also in their mean doubling time and their passage number in nude mice when the testing experiments were performed (Table 1). There were both xenografts of high passage number that generally proliferated rapidly with a short doubling time, such as the LX 1 and L 182 tumors, and xenografts of low passage number that generally grew slower, such as the L-Heck 6 and L-Heck 8 carcinomas. The mean doubling times ranged from 4.2 days in the case of the LX 1 tumor to 11.1 and 13.0 days for the L-Heck 6 and L-Heck-8 xenografts respectively (Table 1). All five cytostatics investigated were applied on optimal dose schedules, i.e., either in single doses or, in the case of etoposide, in five fractionated doses. They were given at sublethal equitoxic dose levels, the highest dose being about 20% smaller than the dose that was lethal to 20% of the mice of the same strain (LD_{20}). At the highest dose level applied, the animals lost about 10–20% of their initial body weight within 8 days of drug injection. At the dose level beneath that the body weight remained unchanged for 4–6 days after substance application and increased continuously thereafter, whereas at lower doses the weight development of treated animals did not differ from that of control animals that had received the vehicle fluid only (without drug addition).

The results of the testing experiments with treosulfan, ifosfamide, cisplatin, and etoposide at equitoxic dose levels in xenografted human small-cell lung carcinomas are summarized in Table 2. The growth

Table 2 Growth inhibition[a] effected by treosulfan and comparative compounds in human small-cell lung carcinomas heterotransplanted to athymic mice (*ND* Not determined)

Drug	Dose[b] (mg/kg)	Small-cell lung carcinomas LX 1 day 7	LX 1 day 14	LX 1 day 21	H 69 day 7	H 69 day 14	H 69 day 21	L 182 day 7	L 182 day 14	L 182 day 21	L 215 day 7	L 215 day 14	L 215 day 21
Treosulfan	1 × 2,500	**57**	45	–	37	24	25	**81**	**79**	48	39	38	28
	1 × 3,000	**58**	43	–	37	**64**	**71**	**90**	**92**	**85**	**60**	**70**	**68**
	1 × 3,500	**54**	**55**	–	25	**51**	**60**	**93**	**98**	**98**	**65**	**70**	**69**
Ifosfamide	1 × 200	0	7	5				**57**	47	0	0	11	25
	1 × 300	26	24	22		ND		**76**	**76**	**70**	0	21	40
	1 × 400	21	48	38				**81**	**82**	**65**	12	44	45
Cisplatin	1 × 6	6	5	40				27	32	35	42	**59**	39
	1 × 9	40	30	19		ND		**54**	**50**	**53**	44	**55**	43
	1 × 12	33	20	5				**75**	**64**	**62**	38	47	32
Etoposide	5 × 5	0	0	0				6	12	29	0	0	0
	5 × 10	0	0	0		ND		42	15	31	14	1	9
	5 × 15	0	0	19				48	**56**	**66**	16	0	0

[a]The parameter evaluated is the tumor growth inhibition expressed as a percentage of control tumor size and calculated as 100% - T/C. The values determined on days 7, 14, and 21 after the last substance application are indicated. Growth inhibition values of $\geq$ 50% (**boldface**) are significant

[b]The doses investigated correspond to equitoxic dose levels. Treosulfan, ifosfamide, and cisplatin were applied in single doses; the etoposide dose was fractionated into five doses given every other day (q2d × 5 regimen)

development of the L 182 and L 215 tumors under treatment with the two higher doses of treosulfan and the comparative compounds (only in the case of L 182) are illustrated in Figs. 2 and 3. A significant growth inhibition of more than 50% was observed after administration of treosulfan in the case of all four small-cell lung carcinomas. In the H 69 and L 215 xenografts, treosulfan reduced the tumor growth by about 70%; in the L 182 xenograft it achieved a growth reduction of 90–98%. This means that the mean size of the treated tumors amounted to about 30% (H 69, L 215) or 2–10% (L 182) of the size of untreated control tumors. There was an absolute reduction in the initial tumor size (1.0) of more than 50% to a relative tumor volume of 0.5 and 0.4 within 6–14 days of the application of treosulfan to the L 215 and H 69 xenografts and an absolute reduction of more than 90% to a relative tumor volume of 0.1 and even less in the case of the L 182 xenograft.

In a comparison of these results with the growth development of the small-cell lung carcinomas LX 1, L 182, and L 215 under the influence of the clinically established cytostatic drugs ifosfamide, cisplatin, and etoposide, these compounds were clearly less effective than treosulfan and induced a significant growth inhibition of more than 50% in the case of just two of three (cisplatin) or one of three (ifosfamide, etoposide) xenografts (Table 2; also see Table 5). In addition to this, the effects of these drugs on growth reduction were smaller, even in the case of the more sensitive xenografts, amounting to only 60% (L 215, cisplatin), 60–65% (L 182, cisplatin and etoposide), and 80–82% (L 182, ifosfamide), respectively (Table 2). This means that in the case of the three small-cell lung xenografts LX 1, L 182, and L 215, treosulfan was clearly more active than the reference compounds ifosfamide, cisplatin, and etoposide both when the mean values for growth inhibition (Table 2; also see Table 5) and when the tumor volume of individual xenografts (Figs. 2, 3) were considered.

Non-small-cell lung carcinomas are a quite heterogenous group of carcinomas with respect to their histological classification. They are represented by either epidermoid carcinomas, large-cell carcinomas, or adenocarcinomas, all of them being derived from the bronchial epithelium. All three subtypes of non-small-cell lung carcinomas were investigated in the present study, the epidermoid carcinoma type being represented by the four xenografts L 3, L 2917, L-Heck 6, and L-Heck 8, the large-cell carcinoma type, by the L 2906 xenograft; and the adenocarcinoma type, by the four tumors L 27, L 261, HTB 53, and CCL 185. All these xenografts were treated with treosulfan, and most of them were additionally treated with equitoxic doses of the comparative compounds ifosfamide, mitomycin C, and cisplatin.

In the case of epidermoid lung carcinomas, treosulfan effected a significant growth inhibition of more than 50% in all four xenografts investigated, whereby the recently established xenograft L-Heck 6 was the most sensitive one. It was inhibited in growth by treosulfan by more than 90% in comparison with control tumors (Table 3; also see Table 5). The analysis of the development of individual tumor growth showed an absolute growth reduction of more than 80% in relation to the initial value of 1.0 for relative tumor volume to less than 0.2 on days 14–28 after the application of 3,500 mg/kg treosulfan (Fig. 3). The comparison with other clinically established cytostatic drugs, which were applied at equitoxic dose levels, confirmed that only mitomycin C was more effective than treosulfan in the xenografts L 3, L 2917, and L-Heck 8 but not in the L-Heck 6 tumor (Table 3; also see Table 5).

Regarding the large-cell lung carcinoma L 2906, the only one of this histological type that was investigated in the present study, it was more sensitive to treosulfan than to the comparative compounds ifosfamide and cisplatin, whereas mitomycin C effected a similar growth reduction. Both treosulfan and mitomycin C induced a remarkable growth inhibition of 80–89% (Table 4), corresponding to absolute diminutions in the initial tumor volume (1.0) of 70–80% to relative tumor volumes of 0.3–0.2 within 12–14 days of drug application (Fig. 3); ifosfamide inhibited the tumor growth by only 50–60% to relative tumor volumes of 0.6–0.5; and cisplatin was totally inactive (Table 4).

A third and clinically important type of non-small-cell lung carcinoma is reflected in adenocarcinomas, represented by the xenografts L 27, L 261, HTB 53, and CCL 185. All four xenografts were inhibited significantly by more than 50% of control tumor size by intraperitoneal single-dose application of treosulfan at higher dose levels (Table 4). Pronounced growth reduction of more than 80–90% was observed with treosulfan, especially in the L 261 tumor (Table 4, Fig. 4), the long-term growth reduction effected by treosulfan in this xenograft clearly surpassing that of ifosfamide, mitomycin C, and cisplatin (Table 4, Fig. 4). On the other hand, regarding the adenocarcinoma xenografts L 27 and HTB 53, treosulfan was certainly active and inhibited the tumor growth by more than 50%; the comparative compound mitomycin C, however, was more effective at least in one (L 27) of the two xenografts (Tables 4, 5).

Table 5 summarizes the results of the antitumor testing experiments, obtained with treosulfan and the comparative compounds ifosfamide, mitomycin C, cisplatin, and etoposide in human small-cell and non-small-cell lung carcinomas. It documents that treosulfan is highly active both in small-cell and in most non-small-cell lung carcinomas investigated in the present study, whereby the growth inhibition induced by treosulfan clearly surpassed the growth reductions caused by most of the comparative cytostatic drugs, all of which have been approved as cytostatic drugs in the clinical therapy of human lung carcinomas (Table 5).

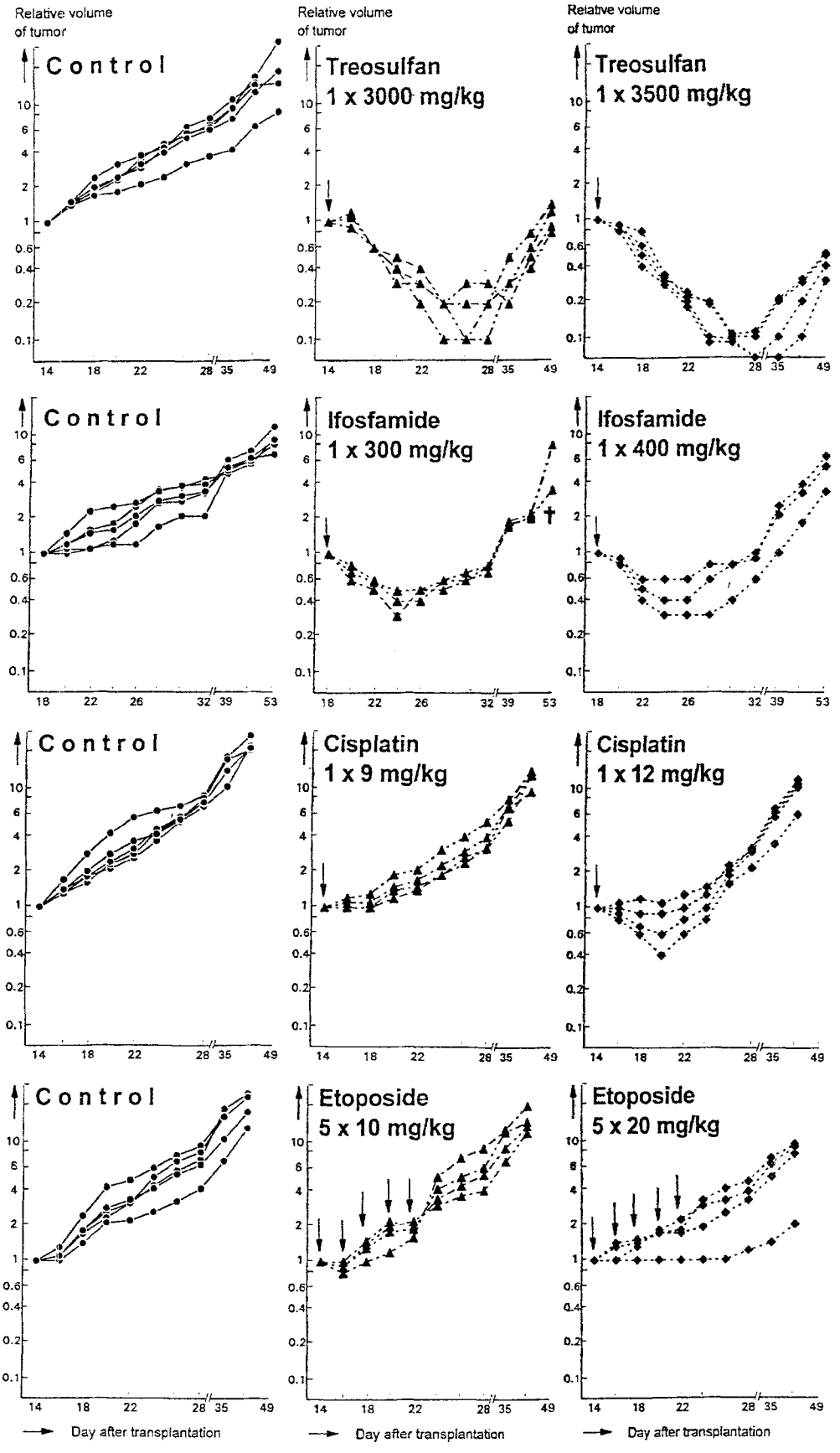

Fig. 2 Growth development of the xenografted human small-cell lung carcinoma L 182 growing in the 39th, 51st, 42nd, and 48th passage, respectively, and treated with equitoxic doses of treosulfan, ifosfamide, cisplatin, and etoposide applied in a single dose or in 5 fractionated doses (*arrows*), respectively. The graphs show the growth curves generated for individual tumors (*Ordinate* Tumor volume related to the volume measured on the [first] day of drug administration; *crosses*†, deaths occurring during the experimental period)

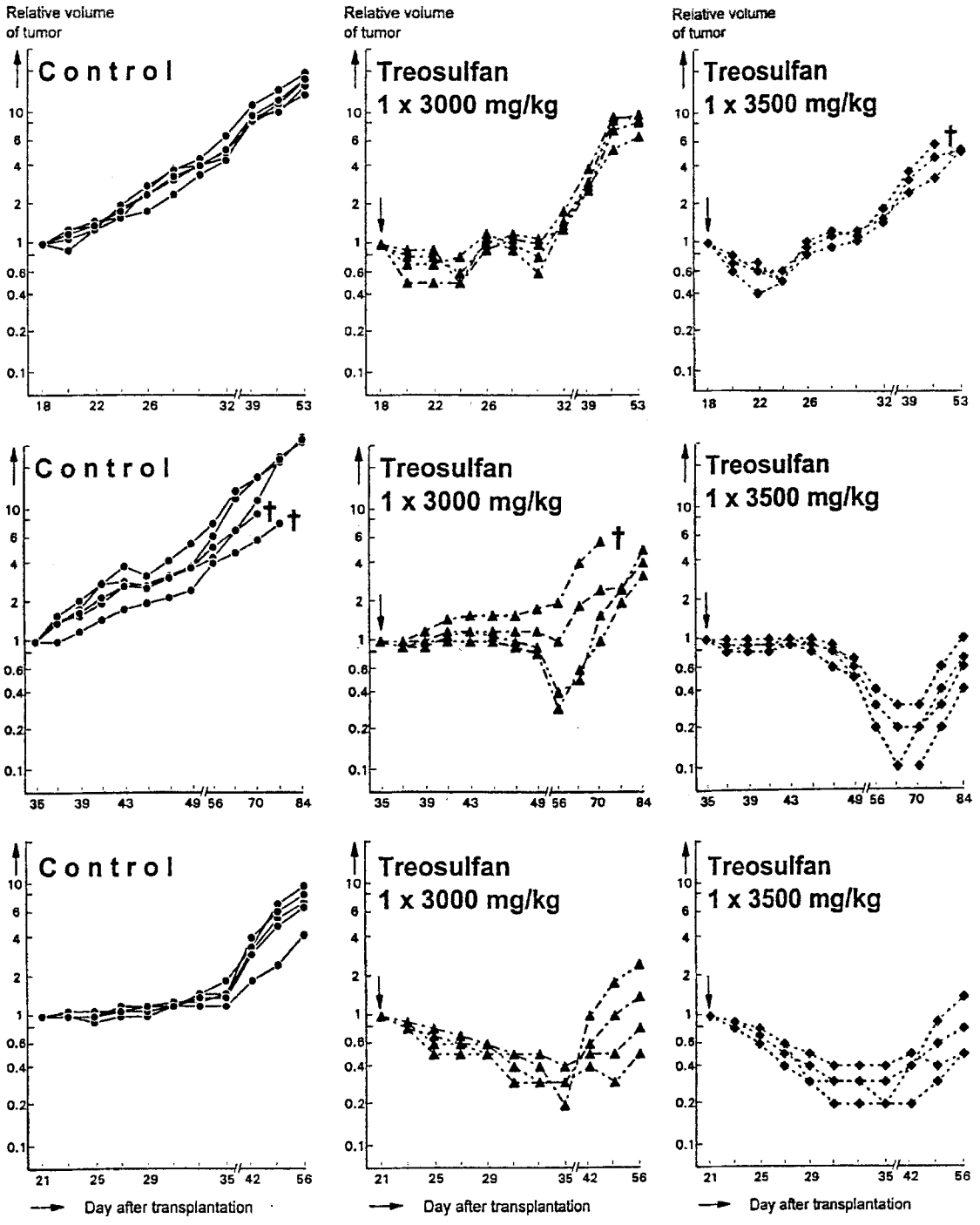

Fig. 3 Growth development of the xenografted human small-cell lung carcinoma L 215 in the 28th passage (*top*), the epidermoid lung carcinoma L-Heck 6 in the 10th passage (*center*), and the undifferentiated large-cell lung carcinoma L 2906 in the 14th passage (*bottom*) after single-dose application of treosulfan. Further details are explained in the legend of Fig. 4. (*crosses*†, Deaths occurring during the experimental period)

Discussion

Although treosulfan was synthesized as a chemical compound and recognized to be cytostatically active more than 30 years ago [10, 24], it has thus far been introduced into clinical chemotherapy only in the case of advanced ovarian carcinoma and is increasingly being combined with cisplatin or carboplatin instead of other alkylating agents such as cyclophosphamide for the treatment of ovarian cancer [2–4, 8, 13, 18, 19, 22]. Moreover, since treosulfan does not show significant cross-resistance to cisplatin in cisplatin-resistant sublines of human ovarian carcinoma cell lines (Harstrick, personal communication), it should be considered as a "second-line" drug to be applied to patients with advanced ovarian carcinomas

Table 3 Growth inhibition[a] effected by treosulfan and comparative compounds in human epidermoid lung carcinomas heterotransplanted to athymic mice

Drug	Dose[b] (mg/kg)	Epidermoid lung carcinomas											
		L 3			L 2917			L-Heck 6			L-Heck 8		
		day 7	day 14	day 21	day 7	day 14	day 21	day 7	d14	day 21	day 7	day 14	day 21
Treosulfan	1 × 2,500	23	45	**55**	30	32	30	**55**	**58**	**68**	31	**62**	**62**
	1 × 3,000	23	30	46	**53**	**63**	**62**	**52**	**68**	**81**	22	**55**	**58**
	1 × 3,500	31	**55**	**60**	**60**	**69**	**61**	**63**	**84**	**96**	17	**52**	**69**
Ifosfamide	1 × 200	0	12	17	45	**65**	**55**	10	0	0	0	0	0
	1 × 300	9	19	25	43	**76**	**69**	0	0	0	18	22	12
	1 × 400	27	31	25	**63**	**80**	**80**	25	29	26	19	34	30
Mitomycin C	1 × 6	38	48	**55**	**52**	**77**	**67**	42	**74**	**89**	**64**	**93**	**94**
	1 × 8	**50**	**61**	**73**	**62**	**92**	**87**	**53**	**76**	**85**	**68**	**93**	**97**
	1 × 10	41	**67**	**76**	**66**	**94**	**93**	**64**	**72**	**80**	**73**	**97**	**99**
Cisplatin	1 × 6	0	0	0	38	**60**	–	16	36	26	30	29	39
	1 × 9	0	0	0	31	**58**	–	24	46	44	39	46	**51**
	1 × 12	32	19	14	46	**71**	–	**52**	**75**	**80**	**57**	**66**	**70**

[a]The parameter evaluated is the tumor growth inhibition expressed as a percentage of control tumor size and calculated as 100% - T/C. The values determined on days 7, 14, and 21 after the last substance application are indicated. Growth inhibition values of ≥ 50% (**boldface**) are significant

[b]The doses investigated correspond to equitoxic dose levels. All compounds were applied in single doses

Table 4 Growth inhibition[a] effected by treosulfan and comparative compounds in some non-small-cell lung carcinomas heterotransplanted to athymic mice

Drug	Dose[b] (mg/kg)	Undifferentiated large-cell lung carcinoma			Lung adenocarcinomas											
		L 2906			L 27			L 261			HTB 53			CCL 185		
		day 7	day 14	day 21	day 7	day 14	day 21	day 7	day 14	day 21	day 7	day 14	day 21	day 7	day 14	day 21
Treosulfan	1 × 2,500	42	**79**	**71**	42	**50**	27	**57**	**71**	**76**	29	43	43	47	**55**	**65**
	1 × 3,000	40	**77**	**73**	40	40	25	**60**	**76**	**88**	35	49	**51**	**53**	**55**	**55**
	1 × 3,500	**52**	**79**	**86**	**52**	**53**	34	**69**	**83**	**92**	**59**	**61**	**61**	**60**	**77**	**75**
Ifosfamide	1 × 200	33	38	48	5	0	0	**52**	**62**	**57**	**52**	**59**	**71**	12	17	7
	1 × 300	38	**55**	**60**	15	0	0	48	**60**	**58**	48	**60**	**75**	10	0	0
	1 × 400	32	37	**52**	25	32	0	**57**	**62**	**60**	**58**	**66**	77	7	0	0
Mitomycin C	1 × 6	36	**73**	**79**	**56**	**74**	**66**	**63**	**76**	**54**	30	33	–	29	**34**	40
	1 × 8	45	**78**	**76**	**57**	**65**	**68**	**79**	**84**	**84**	26	40	–	27	**54**	**50**
	1 × 10	36	**78**	**79**	**69**	**90**	**98**	**68**	**74**	**64**	30	30	–	**50**	40	**57**
Cisplatin	1 × 6	0	0	0	32	0	0	16	3	4	0	2	37	32	32	36
	1 × 9	0	0	0	32	13	1	**61**	**59**	**63**	18	25	38	**53**	**56**	**68**
	1 × 12	11	12	12	43	31	0	**67**	**64**	**61**	41	46	49	**68**	**74**	**70**

[a]The parameter evaluated is the tumor growth inhibition expressed as a percentage of control tumor size and calculated as 100% - T/C. The values determined on days 7, 14, and 21 after the last substance application are indicated. Growth inhibition values of ≥ 50% (**boldface**) are significant

[b]The doses investigated correspond to equitoxic dose levels. All compounds were applied in single doses

after the failure of "first-line" therapy regimens with cisplatin [17].

Recent experimental investigations have confirmed that beyond this, treosulfan effects pronounced antitumor activity in human breast carcinomas that have been heterotransplanted to athymic mice, whereby the growth inhibition observed in most of the investigated breast carcinoma xenografts has clearly surpassed the growth regressions induced by the alkylator cyclophosphamide and the intercalator doxorubicin hydrochloride [12]. Since treosulfan is characterized by a clinical compatibility much higher than that of other alkylating cytostatics and since it lacks the capacity to induce severe and troublesome side effects such as gastrointestinal irritation and alopecia and does not impair the patients' general condition to a mentionable extent [1–3, 14, 19, 22], clinical trials should be initiated in which cyclophosphamide and other alkylators are replaced by treosulfan in chemotherapeutic regimens for the clinical treatment of hormone-independent breast carcinomas.

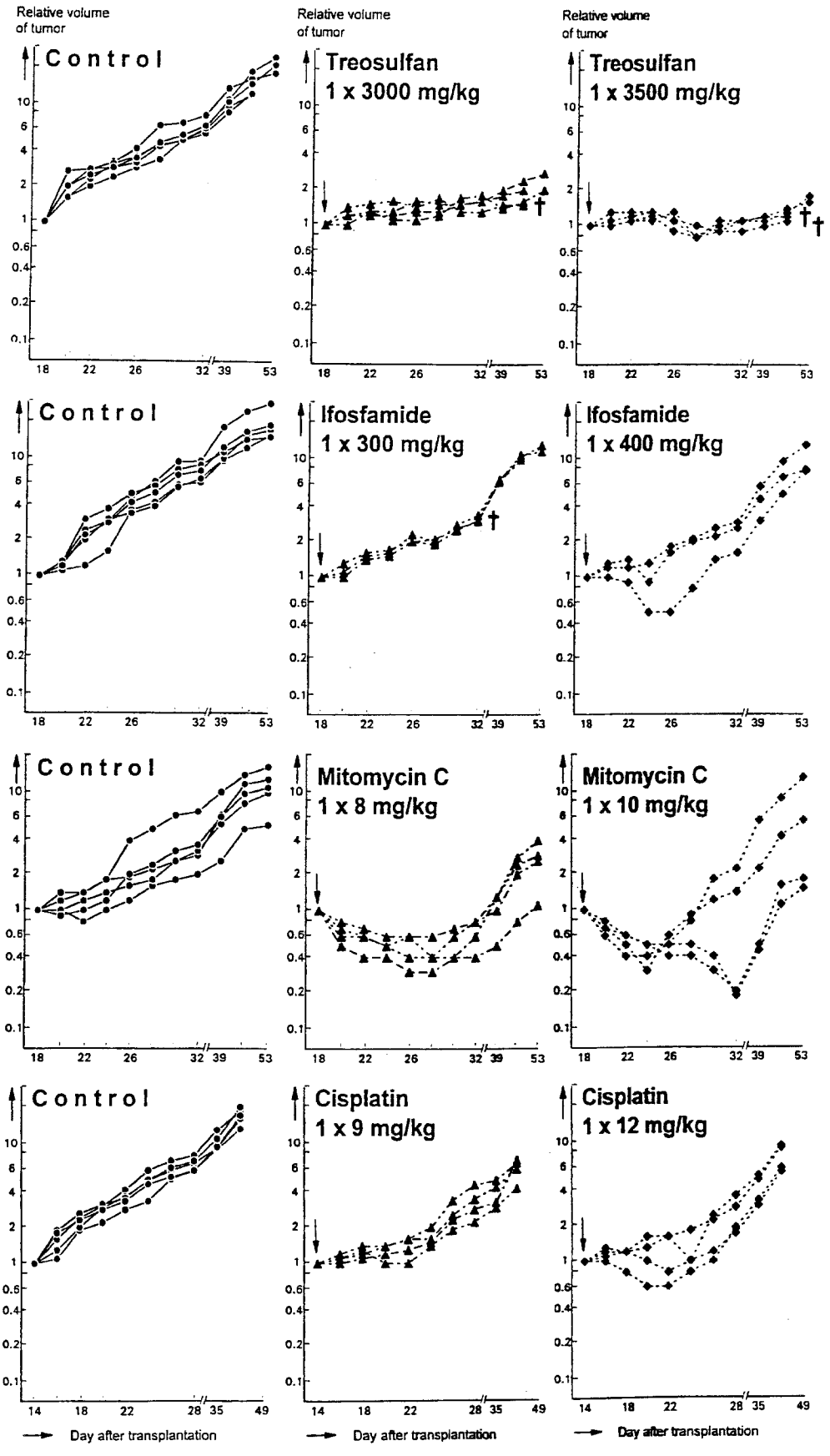

Fig. 4 Growth development of the xenografted human lung adenocarcinoma L 261 growing in the 41st, 36th, 39th, and 45th passage, respectively, and treated with single and equitoxic doses of treosulfan, ifosfamide, mitomycin C, and cisplatin, respectively. The graphs show the growth curves generated for individual tumors. (*Arrows* Day of drug application, *ordinate* tumor volume related to the volume measured on the day of drug administration, *crosses*†, deaths occurring during the experimental period)

Table 5 Survey of the maximal growth inhibition[a] effected by treosulfan and comparative compounds[b] in xenografted human lung carcinomas (*ND* Not determined)

	Treosulfan	Ifosfamide	Mitomycin C	Cisplatin	Etoposide
Small-cell carcinomas:					
LX 1	+	(+)	ND	(+)	–
H 69	++	ND	ND	ND	ND
L 182	+++++	+++	ND	+++	++
L 215	++	(+)	ND	+	–
Epidermoid carcinomas:					
L 3	+	(+)	+++	–	ND
L 2917	++	+++	+++++	++	ND
L-Heck 6	+++++	(+)	++++	+++	ND
L-Heck 8	++	(+)	+++++	++	ND
Undifferentiated large-cell carcinomas:					
L 2906	++++	+	++++	–	ND
Adenocarcinomas:					
L 27	+	–	+++++	(+)	ND
L 261	+++++	++	++++	++	ND
HTB 53	+	+++	(+)	(+)	ND
CCL 185	+++	–	+	+++	ND

[a]The parameter evaluated was growth inhibition in comparison with untreated control tumors. Key: + + + + +, inhibition by more than 90%; + + + +, inhibition by 80–90%; + + +, inhibition by 70–80%; + +, inhibition by 60–70%; +, inhibition by 50–60%; (+), inhibition by 25–50% (minor response); –, inhibition by less than 25% (no effect)
[b]Treosulfan, ifosfamide, mitomycin C, and cisplatin were applied in single doses; etoposide was injected in 5 fractionated doses according to the q2d × 5 regimen

In the present study, a strong growth-inhibitory activity was confirmed for treosulfan in most of the investigated xenografted human lung carcinomas of both the small-cell and the non-small-cell type. Treosulfan proved to be even more active than cisplatin or etoposide at equitoxic doses in three of four small-cell lung carcinomas, the fourth one having thus far been treated only with treosulfan, not with the comparative compounds. Against non-small-cell lung carcinomas, treosulfan was significantly more effective than ifosfamide, mitomycin C, or cisplatin, at least in the case of three of nine xenografts (one of four epidermoid carcinomas, one large-cell carcinoma, and one of four adenocarcinomas). This antitumor activity of treosulfan against human small-cell and non-small-cell lung carcinomas is remarkable and highly surpasses the expectations for a compound that has been known as a cytostatic drug for more than 30 years [24] and has been used clinically against the quite limited spectrum of just one type of human carcinoma for many years [2, 14, 18]. Since the growth-inhibitory activity of treosulfan against heterotransplanted human lung carcinomas is comparable to or even higher than that of clinically approved, standard chemotherapeutic drugs, phase II clinical trials should rapidly follow to investigate the clinical activity of treosulfan in both small-cell and non-small-cell lung carcinomas. In case the antitumor activity should be confirmed under clinical conditions, treosulfan should be introduced into polychemotherapeutic regimens for the treatment of human lung cancer and substitute for other alkylating agents. This would be favored especially by the proven low subjective toxicity and good clinical compatibility in human patients of treosulfan in comparison with other alkylating antitumor drugs [1–3, 14, 18, 19].

In the present study, treosulfan was applied by single intraperitoneal injection. In this connection it is worth mentioning that recent experimental studies with athymic mice bearing the breast carcinomas MDA-MB 436 and M 3 confirmed that after intravenous application of single doses of treosulfan, the growth regressions were as pronounced as those seen after intraperitoneal administration of the drug (Köpf-Maier, unpublished results), whereby optimal growth inhibition was observed after intravenous treatment with single doses of 250–500 mg/kg treosulfan. Since the first toxic deaths did not occur until the administration of doses higher than 3,000 mg/kg, a therapeutic index (T.I., LD_{50}/ED_{90}) of 16 was determined after intravenous administration of treosulfan to tumor-bearing athymic mice (Fig. 5). In the case of cyclophosphamide applied intravenously under the same conditions, the T.I. value was found to amount to 13 (Fig. 5), thus being a little lower than that of treosulfan (Köpf-Maier, unpublished results).

In this connection it should be mentioned that the dose-limiting toxicity of treosulfan in both animals and human patients has been found to be hematoxicity and that the counts of leukocytes and thrombocytes drop markedly (WHO grades 1–3) within 3 weeks of the

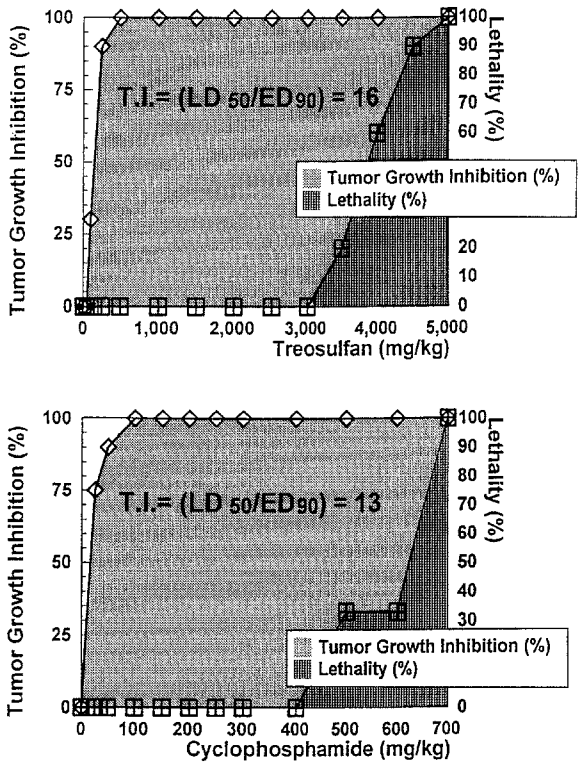

Fig. 5 Dose-activity (*left graphs, diamonds*) and dose-lethality (*right graphs, squares*) relationships of treosulfan and cyclophosphamide. (T.I., Therapeutic index – defined as the relation LD_{50}/ED_{90})

application of doses higher than 7 g/m^2 in human patients [4, 13, 17]. Although no mentionable additional organ toxicity is observed after the administration of treosulfan, this side effect actually limits the possibilities of combining treosulfan with other organic cytostatic drugs in clinical polychemotherapeutic regimens, since most other organic cytostatics similarly induce hematoxicity as their main and dose-limiting side effect. Thus, a phase I clinical study is currently under way with the aim of investigating whether the combination of treosulfan with G-CSF (granulocyte colony-stimulating factor) might reduce the hematoxicity caused by treosulfan, which would allow a further increase in the dose of treosulfan in human patients and facilitate the combination of treosulfan with other organic cytostatic drugs. A positive result of this study would be especially beneficial with respect to the clinical chemotherapy of human lung carcinomas of both the small-cell and the non-small-cell type with treosulfan combined with other organic cytostatic drugs.

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References

1. Aabo K, Hald I, Hrbov S (1985) A randomized study of single agent versus combination chemotherapy in FIGO stages IIB, III and IV ovarian adenocarcinoma. Eur J Cancer Clin Oncol 21: 475–481
2. Breitbach GP, Rutt G, Saß G, Bastert G (1988) Patientenzentrierte Weiterentwicklung der Cisplatin-haltigen Chemotherapie von fortgeschrittenen Ovarialkarzionomen durch Einführung von Treosulfan. In: Bastert G, Lang N, Hilfrich J, Breitbach GP, Saß G (eds) Treosulfan i.v. – Mehr Lebensqualität in der Therapie des Ovarialkarzinoms. W. Zuckschwerdt, München, pp 46–56
3. Breitbach GP, Saß G, Bastert G (1993) Treosulfan/cis-platinum vs. cyclophosphamide/cis-platinum – a phase III study in advanced ovarian carcinoma. Proceedings of the 2nd European Congress on Gynecologic Endoscopy and New Surgical Techniques, Heidelberg, October 21–23, 1993
4. Buser K, Jurgi FW, Joss R, Brunner KW (1988) Chemotherapie des fortgeschrittenen Ovarialkarzinoms mit intravenös verabreichtem Dihydroxybusulfan (Treosulfan). In: Bastert G, Lang N, Hilfrich J, Breitbach GP, Saß G (eds) Treosulfan i.v. – Mehr Lebensqualität in der Therapie des Ovarialkarzinoms. W. Zuckschwerdt, München, pp 19–28
5. Feit PW (1964) 1,4-Bismethanesulfonates of the stereoisomeric butanetetraols and related compounds. J Med Chem 7: 14–17
6. Feit PW (1988) Treosulfan, eine Spezialform aus der Gruppe der Alkylantien. In: Bastert G, Lang N, Hilfrich J, Breitbach GP, Saß G (eds) Treosulfan i.v. – Mehr Lebensqualität in der Therapie des Ovarialkarzinoms. W. Zuckschwerdt, München, pp 9–18
7. Feit PW, Rastrup-Andersen N, Matagne R (1970) Studies on epoxide formation from (2S,3S)-threitol 1,4-bismethanesulfonate. The preparation and biological activity of (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate. J Med Chem 13: 1173–1175
8. Fennelly JJ (1977) Treosulfan in the management of ovarian carcinoma. Br J Obstet Gynecol 84: 300–303
9. Goldin A, Wolpert-deFilippes MK (1979) Nude mouse models as predictors of chemotherapy in man: thymidine and pyrimidines. Bull Cancer (Paris) 66: 61–66
10. Jones R, Kessler WB, Lessner HE, Rane L (1960) New developments in cancer chemotherapeutic agents. Cancer Chemother Rep 10: 99–108
11. Köpf-Maier P (1987) Tumor inhibition by titanocene complexes: influence upon two xenografted human lung carcinomas. J Cancer Res Clin Oncol 113: 342–348
12. Köpf-Maier P, Saß G (1992) Antitumor activity of treosulfan against human breast carcinomas. Cancer Chemother Pharmacol 31: 103–110
13. Lundvall F (1976) Treosulfan in the treatment of ovarian cancer. Acta Obstet Gynecol Scand [Suppl] 47: 55
14. Masding JE (1988) Treosulfan zur Behandlung fortgeschrittener Ovarialkarzinome. In: Bastert G, Lang N, Hilfrich J, Breitbach GP, Saß G (eds) Treosulfan i.v. – Mehr Lebensqualität in der Therapie des Ovarialkarzinoms. W. Zuckschwerdt, München, pp 57–74
15. Masding J, Sarkar TK, White WF, Barley VL, Chawla SL, Boesen E, Rostom AY, Menday AP (1990) Intravenous treosulfan versus intravenous treosulfan plus cisplatinum in advanced ovarian carcinoma. Br J Obstet Gynecol 97: 342–351
16. Mattern J, Keppler B, Volm M (1984) Preclinical evaluation of diethoxy(1-phenyl-1,3-butanedionato)titanium(IV) in human tumour xenografts. Arzneimittelforschung 34: 1289–1290

17. Meier W, Gropp M, Hepp H (1994) Treosulfan as an effective second-line chemotherapy in recurrent ovarian cancer (abstract 829). Proc Am Soc Clin Oncol 13: 261
18. Merkle E (1988) Behandlung des metastasierten Ovarialkarzionoms mit Cisplatin und Treosulfan. In: Bastert G, Lang N, Hilfrich J, Breitbach GP, Saß G (eds) Treosulfan i.v. – Mehr Lebensqualität in der Therapie des Ovarialkarzinoms. W. Zuckschwerdt, München, pp 35–45
19. Merkle E, Bähr I, Bühner M, Martus P, Lang N (1993) Therapie des fortgeschrittenen Ovarialkarzinoms mit Cisplatin und Treosulfan. Tumordiagn Ther 14: 175–180
20. National Cancer Institute (1984) In vivo cancer models. NIH Publ 84–2635: 19–20
21. Preece AW, Wells-Wilson M (1982) Enhancement of response of a lymphoblastic tumour by combination of the cycle specific drugs cisplatin and treosulfan. Br J Cancer 46: 498
22. Schwarzenau E, Hilfrich J (1988) Treosulfan in der Behandlung des Ovarialkarzinoms. In: Bastert G, Lang N, Hilfrich J, Breitbach GP, Saß G (eds) Treosulfan i.v. – Mehr Lebensqualität in der Therapie des Ovarialkarzinoms. W. Zuckschwerdt, München, pp 29–34
23. Venditti JM (1981) Preclinical drug development: rationale and methods. Semin Oncol 8: 349–361
24. White FR (1962) L-Threitol dimethanesulfonate. Cancer Chemother Rep 24: 95–97