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Antitumor activity of treosulfan against human breast carcinomas

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Summary. Treosulfan (L-threitol-1,4-bismethanesulfonate, Ovastat) is a bifunctional alkylating agent that shows a formal structural similarity to busulfan and is applied clinically to patients suffering from ovarian cancer. The present study demonstrated the pronounced antitumor activity of this drug against three of five human breast carcinomas xenografted to athymic mice. It was shown that treosulfan is capable of inducing irreversible and complete remission of the heterotransplanted human breast carcinomas MDA-MB-436 and MX-1 within 14 days after drug application and of effecting growth inhibition by more than 90% in the MDA-MB-435S xenograft. In all three carcinomas, treosulfan caused more pronounced growth reduction than did equitoxic doses of the alkylator cyclophosphamide. Adriamycin, an intercalating cytostatic agent that is an important component of clinical nonhormonal chemotherapy of breast carcinomas, induced only partial remission of these three xenografts and inhibited the tumor growth by 80%–90% (MDA-MB-436, MX-1) and by 70%–80% (MDA-MB-435S), respectively. In the M 3 xenograft, treosulfan just led to a retardation and stagnation of tumor growth; it was again more effective than Adriamycin but was clearly less active than cyclophosphamide. The FM 2 breast carcinoma, finally, was the only xenograft whose growth was not influenced by treosulfan at doses up to that which was lethal to 50% of the treated mice (LD_{50} value). These results confirm that treosulfan is effective against human breast carcinomas. Because of this activity as well as the known low toxicity and good clinical compatibility of treosulfan, it should be considered for introduction into nonendocrine chemotherapeutic regimens against human breast carcinomas and investigation in clinical trials.

Introduction

With the exception of a few tumor types, the therapy of solid human carcinomas is rarely sufficient. Since numerous carcinomas have metastasized by the time they are detected and identified, surgery is of only limited value in such cases and is incapable of curing patients who suffer from progressive disease. Radiotherapy and chemotherapy, which are usually given to these patients, may then truly cause stagnation of tumor growth or induce transient, partial, or complete remission. With respect to the long-lasting survival of the patients, however, these strategies mostly fail to cure the patients and to treat their malignant, disseminated disease effectively. Even when patients respond to chemotherapy, a prolongation of the life span is usually achieved at the cost of severe side effects, which often profoundly burden the patients and reduce their quality of life during the remaining survival period. This conflict has induced a deep pessimism during past years and has led to the demand that chemotherapeutic regimens be applied only to patients with progressive cancer if the toxic side effects evoked by the cytostatic drugs can be justified with respect to the expected therapeutic success. Moreover, the search continues for cytostatically active drugs of only minor toxicity that would not reduce the patients' quality of life in such a drastic manner.

Treosulfan is an alkylating agent that inhibits the growth of human ovarian carcinomas without affecting the general condition of the patients to a mentionable extent [1, 8, 24]. When it is given to patients suffering from ovarian cancer, it is usually combined with cisplatin and exerts antitumor activity similar to that of cyclophosphamide. However, in comparison with the latter drug, the tolerability of treosulfan is markedly better, mainly because it produces much less alopecia and only slight gastrointestinal irritation and its use results in a good general condition of the patients during and after therapy [1, 3, 14, 21].

In the present study, we investigated the antitumor activity of treosulfan against human breast carcinomas, a type of gynecological tumor that represents the most frequent cancer occurring in women in the industrial Western world. Although quite different approaches exist for the therapy of disseminated breast carcinomas, such as radiotherapy, endocrine therapy, and polychemotherapy, none of these approaches has thus far fundamentally im-

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proved the prognosis or definitively prolonged the survival of women with advanced breast cancer [20].

Materials and methods

Antitumor agents. Treosulfan (L-threitol-1,4-bismethanesulfonate, Ovastat; Medac, Hamburg), cyclophosphamide (Endoxan, Asta-Werke, Bielefeld), and Adriamycin (doxorubicin hydrochloride, Adriblastin; Farmitalia Carlo Erba, Freiburg) were obtained from the suppliers mentioned above and were handled according to the instructions of the manufacturers.

Animals. Male athymic mice (NMRI, nu/nu) purchased from the Bomholtgard Breeding and Research Centre Ltd. (Ry, Denmark) were kept under a humidified atmosphere at elevated room temperature (25°–27° C) in laminar air-flow benches. Bedding, food (Altromin), and water were autoclaved before being placed in contact with the animals. 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 tumor transplantation, the animals were about 8–12 weeks old and weighed 18–22 g.

Tumors. Five human breast carcinomas that had been serially heterotransplanted into athymic mice were investigated in the present study. The MDA-MB-435S and MDA-MB-436 tumors were gratefully obtained in 1990 from Mr. H. Löhrke (Tumorbank, Deutsches Krebsforschungszentrum, Heidelberg). Both tumors had been primarily established as cell lines growing in vitro at the M. D. Anderson Hospital and Tumor Institute [4, 5], and both derived from pleural effusions, i. e., from metastases of human breast carcinomas. MDA-MB-435S is a cell line that proliferates rapidly in vitro, its approximate doubling time being 1–1.5 days [4, 16]. The doubling time of MDA-MB-436 is much longer, amounting to 6–8 days in vitro [4]. Studies on the hormone receptor status of MDA-MB-436, which had not responded to clinical hormonal therapy [4, 6], revealed only low levels of the nuclear and cytoplasmic 17-β estradiol receptor; the progesterone receptor was not detectable [6]. After we had transferred the MDA-MB-435S and MDA-MB-436 cell lines to our laboratory and grown them as monolayers, we inoculated them subcutaneously into athymic mice and transplanted them serially; the investigations described in the present report were done between passages 4 and 7 and passages 2 and 4, respectively.

The MX-1 carcinoma represents a human breast cancer xenograft that is part of the new sceening panel of the National Cancer Institute (NCI, USA), which is used to evaluate the antitumor activity of newly developed cytostatic drugs [10, 23]. This tumor, which has been shown to be quite sensitive to many established cytostatics [23], was obtained from the NCI (Frederick, Md., USA) in 1988. The experiments described in the present report were performed between passages 34 and 39 after shipment of the tumor.

The human breast cancer xenografts M 3 and FM 2 were generously donated by Dr. J. Mattern (Deutsches Krebsforschungszentrum, Heidelberg). The M 3 xenograft is a rapidly proliferating tumor that was investigated in the present study between its 38th and 41st passage in athymic mice. The FM 2 tumor, which proliferates much more slowly, was tested between its 18th and 20th passage. Both tumors had previously been sporadically used in drug-testing trials [13, 15].

For tumor propagation and substance testing, the tumors were removed from donor animals when they had reached a size of about 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.

Testing procedure. Substance application was done when the tumors had reached a size of 0.6–0.8 cm^3; this volume was attained on day 10 (MDA-MB-435S, MX-1, M 3), day 12 (FM 2), or day 19 (MDA-MB-436), depending on the rate of growth of the tumors. The cytostatic agents treosulfan, cyclophosphamide, and Adriamycin were injected intraperitoneally as single doses immediately after they had been dissolved in distilled water 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.

The animals were weighed on days 3, 7, 10, 14, 21, and 28 after treatment. At the same time, 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 after substance application, were calculated by relating the absolute tumor volumes measured on certain days after treatment to those determined on the day of drug injection. Within all experimental and control groups, mean values for the relative tumor volume and standard deviations were then calculated for the different days. Treated/control (T/C) values were obtained using the equation

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

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

Results

The human breast cancer xenografts MDA-MB-435S, MDA-MB-436, MX-1, M 3, and FM 2 were serially transplanted into athymic mice, in which they grew at a mean doubling time of 4.4, 6.3, 3.2, 2.8, and 6.2 days, respectively, as determined during the experimental growth phase of the heterotransplanted tumors. When the animals bearing these xenografts were treated with equitoxic doses of the cytostatic agents treosulfan, cyclophosphamide, and Adriamycin, they responded in a clearly graduated manner. All applied doses of the cytostatics were sublethal, the highest one being about 20% smaller then the dose that was lethal to 20% of the treated mice (LD_{20}), which amounted to 4.0 mg/kg for treosulfan, 300 mg/kg for cyclophosphamide, and 10 mg/kg for Adriamycin. The results obtained are summarized in Table 1 and illustrated in Figs. 1–4.

The MDA-MB-435S carcinoma responded to treosulfan in a marked and pronounced way, diminishing in size in a clearly dose-dependent manner by 60%–95% in relation to untreated control tumors (Table 1, Fig. 1). The two comparative compounds, the alkylating agent cyclophosphamide and the intercalating drug Adriamycin, both of which belong to the standard regimen used for the clinical nonhormonal chemotherapy of human breast carcinomas, were markedly less effctive than treosulfan. They inhibited tumor growth by only 40%–58% and 50%–79%, respectively, and induced growth delays of about 10 days at the highest dose levels, whereas treosulfan caused growth delays of more than 25 days at the higher doses of 3000 and 3500 mg/kg.

In the case of the more slowly proliferating carcinoma MDA-MB-436, treosulfan provoked a tremendous therapeutic effect and induced complete and irreversible regression of tumors that were treated with the two higher, nonlethal doses of 3000 and 3500 mg/kg within 14–21 days after drug applicaton. Even the lower doses of 2000 and 2500 mg/kg gave rise to growth inhibition of 92%–98%, which nonetheless slightly surpassed the pronounced ther-

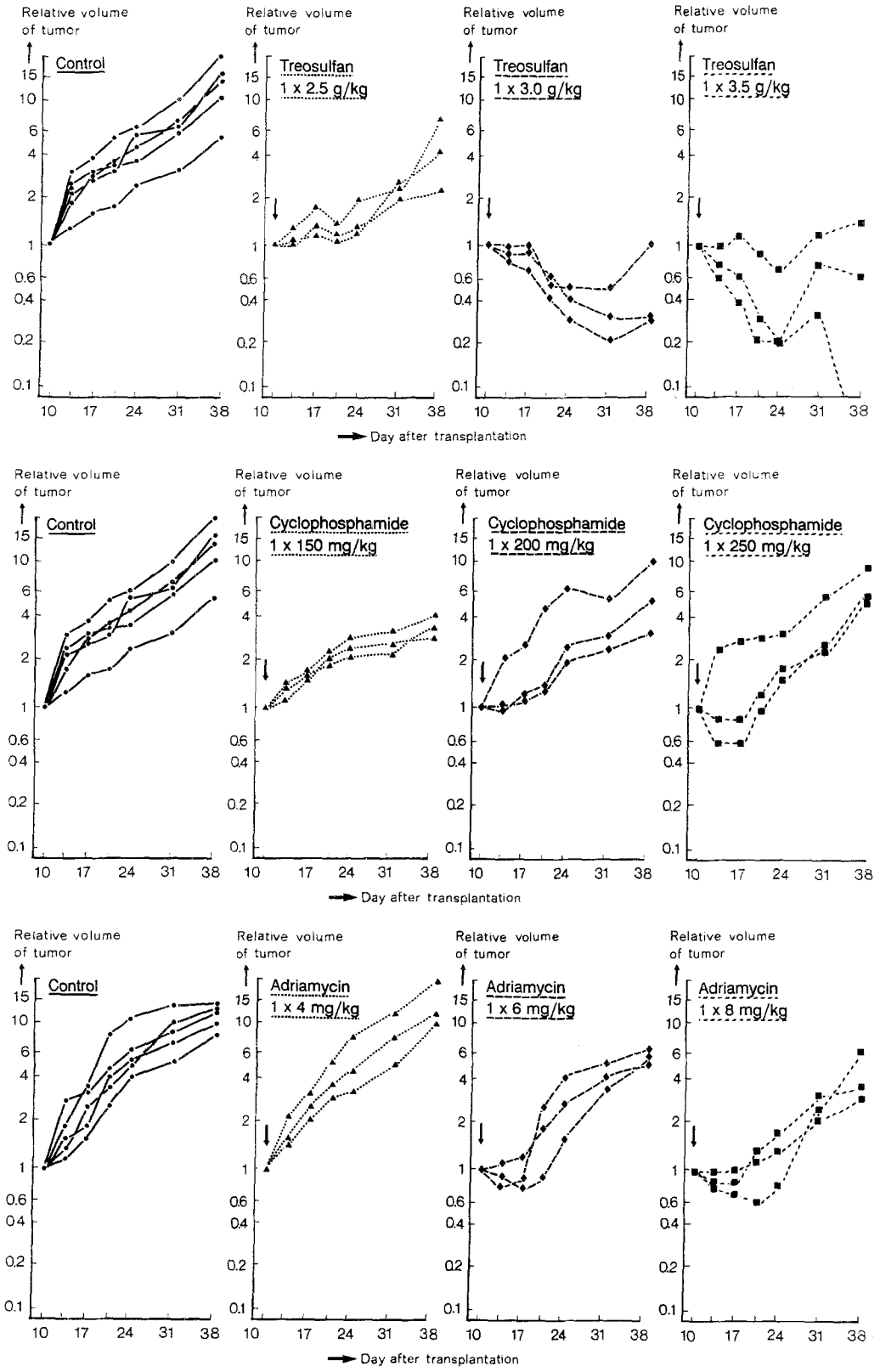

Fig. 1. Growth development of the xenografted human breast carcinoma MDA-MB-435S following treatment with equitoxic doses of treosulfan (2.5, 3.0, and 3.5 g/kg), cyclophosphamide (150, 200, and 250 mg/kg), and Adriamycin (4,6, and 8 mg/kg) given as single injections (*arrows*) on day 10 after tumor transplantation. The graphs show the growth curves generated for individual tumors. *Abscissa*, Days after tumor implantation on day 0; *ordinate*, volume of the tumor relative to that measured on the day of drug administration

apeutic effects of cyclophosphamide and markedly exceeded those of adriamycin (Table 1, Fig. 2).

The NCI standard breast carcinoma MX-1 was also more sensitive to treosulfan than to cyclophosphamide or Adriamycin (Table 1, Fig. 3). After the administration of single doses of 2500, 3000, and 3500 mg/kg, three of four xenografts in the treated groups regressed totally and irreversibly. The corresponding mean values for growth inhibiton amounted to 93%–98%. After application of cyclophosphamide and Adriamycin, no complete remission was observed. The size of tumors treated with cyclophosphamide decreased below the initial value in a clearly

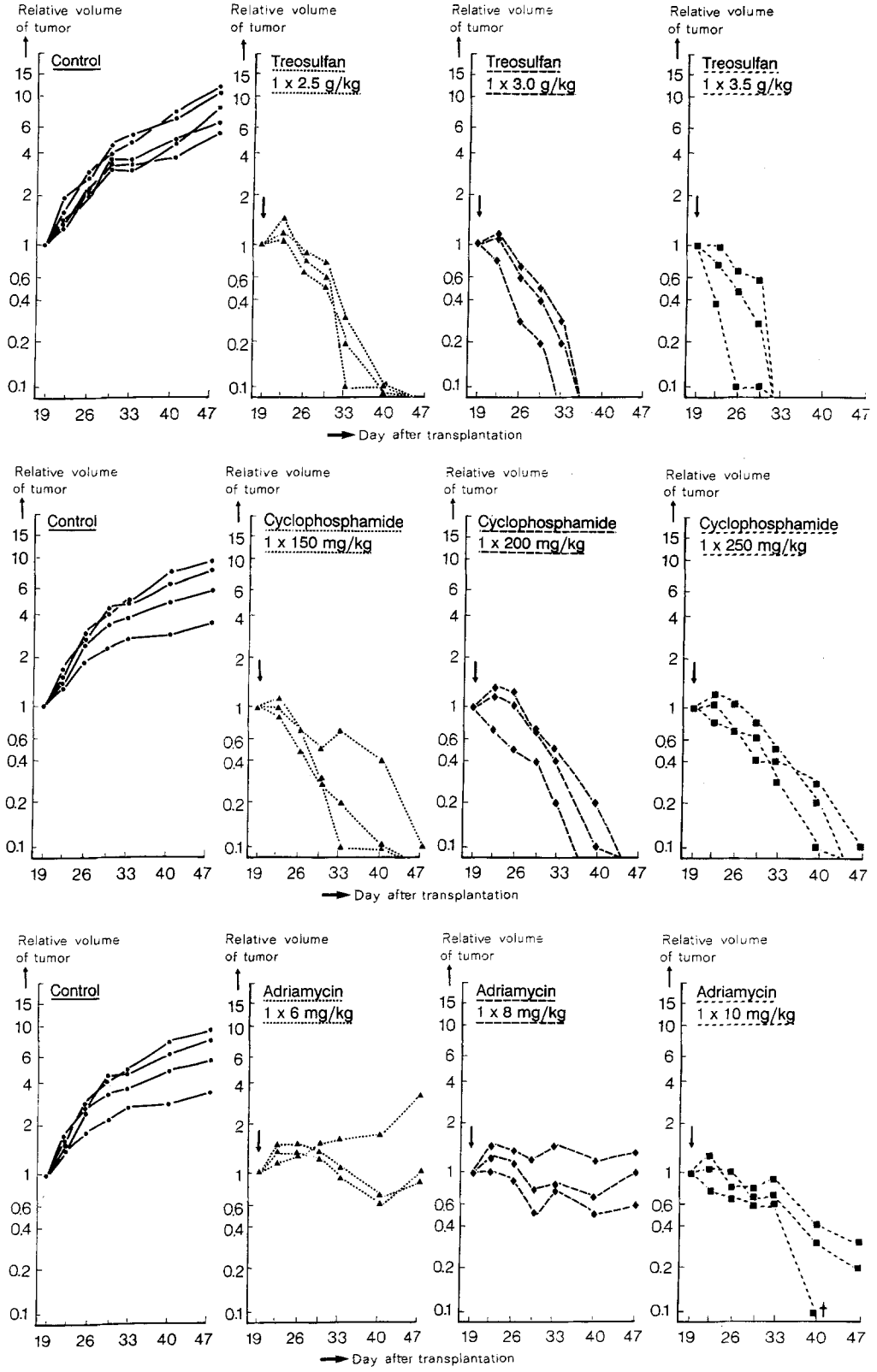

Fig. 2. Growth behavior of the human breast cancer xenograft MDA-MB-436 after the application of treosulfan, cyclophosphamide, and Adriamycin on day 19 after tumor transplantation (for further explanations, cf. legend to Fig. 1). +, Death of an animal on day 42

dose-dependent manner, the growth-inhibition values amounting to 92%–95%. Adriamycin only slowed the growth of these tumors, producing growth-inhibition values of 65% and 85% after the administration of 6 and 8 mg/kg, respectively.

When the M3 carcinoma, which proliferated rapidly in athymic mice, was treated with treosulfan, cyclophosphamide, and Adriamycin, marked differences in the response to therapy were detected. Whereas treosulfan induced only growth retardation and, at the highest dose given (3500 mg/kg), stagnation of tumor development,

Table 1. Growth inhibition effected by treosulfan, cyclophosphamide, and Adriamycin in five human breast carcinomas xenografted into athymic mice[a]

Drug	Dose (mg/kg)	MDA-MB-435S[b]		MDA-MB-436[b]		MX-1[b]		M 3[b]		FM 2[b]	
		Day 14	Day 21	Day 14	Day 21	Day 14	Day 21	Day 14	Day 21	Day 14	Day 21
Treo-	2000	45%	34%	**92%**	**96%**	**96%**	**97%**	**52%**	49%	5%	0%
sulfan	2500	**65%**	**63%**	**94%**	**98%**	**93%**	**93%**	**84%**	**82%**	32%	15%
	3000	**85%**	**96%**	**94%**	**100%**	**97%**	**94%**	**80%**	**79%**	0%	0%
	3500	**94%**	**90%**	**100%**	**100%**	**96%**	**98%**	**97%**	**98%**	0%	0%
Cyclo-	100	36%	34%	**90%**	**94%**	**90%**	**93%**	**71%**	17%	38%	32%
phosphamide	150	46%	42%	**93%**	**97%**	**86%**	**86%**	**81%**	32%	**61%**	**52%**
	200	48%	42%	**90%**	**98%**	**92%**	**95%**	**99%**	**97%**	**65%**	**55%**
	250	**58%**	**58%**	**90%**	**96%**	**92%**	**95%**	**92%**	**88%**	**67%**	**65%**
Adria-	4	18%	0%	34%	25%	**58%**	**64%**	0%	0%	**51%**	25%
mycin	6	**55%**	**53%**	**76%**	**81%**	**64%**	**68%**	10%	8%	48%	26%
	8	**79%**	**70%**	**80%**	–[e]	**81%**	**85%**	3%	0%	**56%**	9%
	10[c]	**62%**	–[e]	**83%**	–[e]	–[d]	–[d]	–[e]	–[e]	**60%**	–[e]

[a] The parameter evaluated is tumor growth inhibition expressed as a percentage of control tumor size and calculated as 100% –T/C

[b] Tumor growth inhibition as determined on days 14 and 21 after drug application (values exceeding 50% are shown in boldface)

[c] This regimen corresponds to an $LD_{20}-LD_{50}$ regimen

[d] Not determined

[e] More than 50% of the animals had died by the day of investigation

cyclophosphamide caused absolute diminution of the tumor size and pronounced, albeit transient, inhibition of growth by 92%–99% at the higher doses of 200 and 250 mg/kg (Table 1, Fig. 4). Adriamycin was inactive against the M 3 xenografts and failed to induce any change in the growth behavior of the tumors.

The FM 2 xenograft, the fifth human breast carcinoma investigated, was the tumor that proved to be least responsive to the cytostatic drugs tested in the present study. The compound most effective against this xenograft was cyclophosphamide, which caused growth retardation and inhibited tumor growth by 50%–67% (Table 1). Adriamycin exerted marginal activity, suppressing growth by 50%–60%, whereas treosulfan was totally inactive and incapable of altering the growth behavior or of reducing the size of the xenografts.

Discussion

Treosulfan is a bifunctional alkylating agent that shows a formal structural similarity to busulfan (Fig. 5) and was introduced into clinical chemotherapy more than 30 years ago. In contrast to busulfan, treosulfan is converted in vivo nonenzymatically to a diepoxide derivative (Fig. 5) that obviously effects alkylation at the nucleophilic centers of biological molecules such as proteins and nucleic acids [7]. Experimental studies have revealed that treosulfan is active against Dunning leukemia [11] and the L2C lymphoblastic tumor [18] and has only a slight inhibitory effect on sarcoma 180 and carcinoma 755, whereas leukemia 1210 does not respond to the drug (Feit, personal communication). Investigations in vitro have confirmed that the sensitivity of human ovarian tumors to treosulfan is similar to their sensitivity to cyclophosphamide and cisplatin [24].

Clinical trials, which have been performed since the 1970s, have actually proved treosulfan to be active against human ovarian carcinomas [1, 8]. Only minor side effects such as slight depressions of the counts of leukocytes, erythrocytes, and thrombocytes in the peripheral blood and a negligible impairment of the general condition of the patients have accompanied the clinical administration of treosulfan [1, 14]. As a consequence, combination therapy of ovarian carcinomas with cisplatin, the most potent cytostatic drug presently known against this tumor, and treosulfan as its alkylating partner has proved to be an effective chemotherapeutic regimen, producing only limited subjective side effects [3, 14, 21]. Because of its higher tolerability and its similar antitumor efficacy, this combination is obviously superior to other combinations containing Adriamycin and/or cyclophosphamide as partners of cisplatin [3, 14, 21].

Besides these data, only little information is available on the pharmacological and pharmacokinetic behavior of treosulfan in animals and humans. Moreover, it is not yet known whether treosulfan additionally exerts antitumor activity against other types of human carcinomas, since clinical phase II trials of treosulfan have thus far been carried out and completed only in ovarian carcinomas [1, 3, 8, 14, 21]. In the present study, we observed remarkable antitumor activity for treosulfan against three of five human breast carcinomas that had been heterotransplanted into athymic mice. In general, xenografts are known to retain the pattern of drug sensitivity shown by the primary tumors throughout many passages in athymic mice [2, 9, 22]. In most of the tumors that were investigated in the present study, including the NCI standard tumor MX-1, the antitumor activity of treosulfan was more pronounced than were the growth-inhibitory effects of cyclophosphamide and Adriamycin. Both of the latter compounds are the main components of clinical nonendocrine chemotherapy of breast cancer and are known to be active against estrogen-receptor-positive and estrogen-receptor-poor mammary carcinomas [12, 17, 19]. The observation that treosulfan was more effective against most of the breast xenografts investigated than was the alkylating agent cyclo-

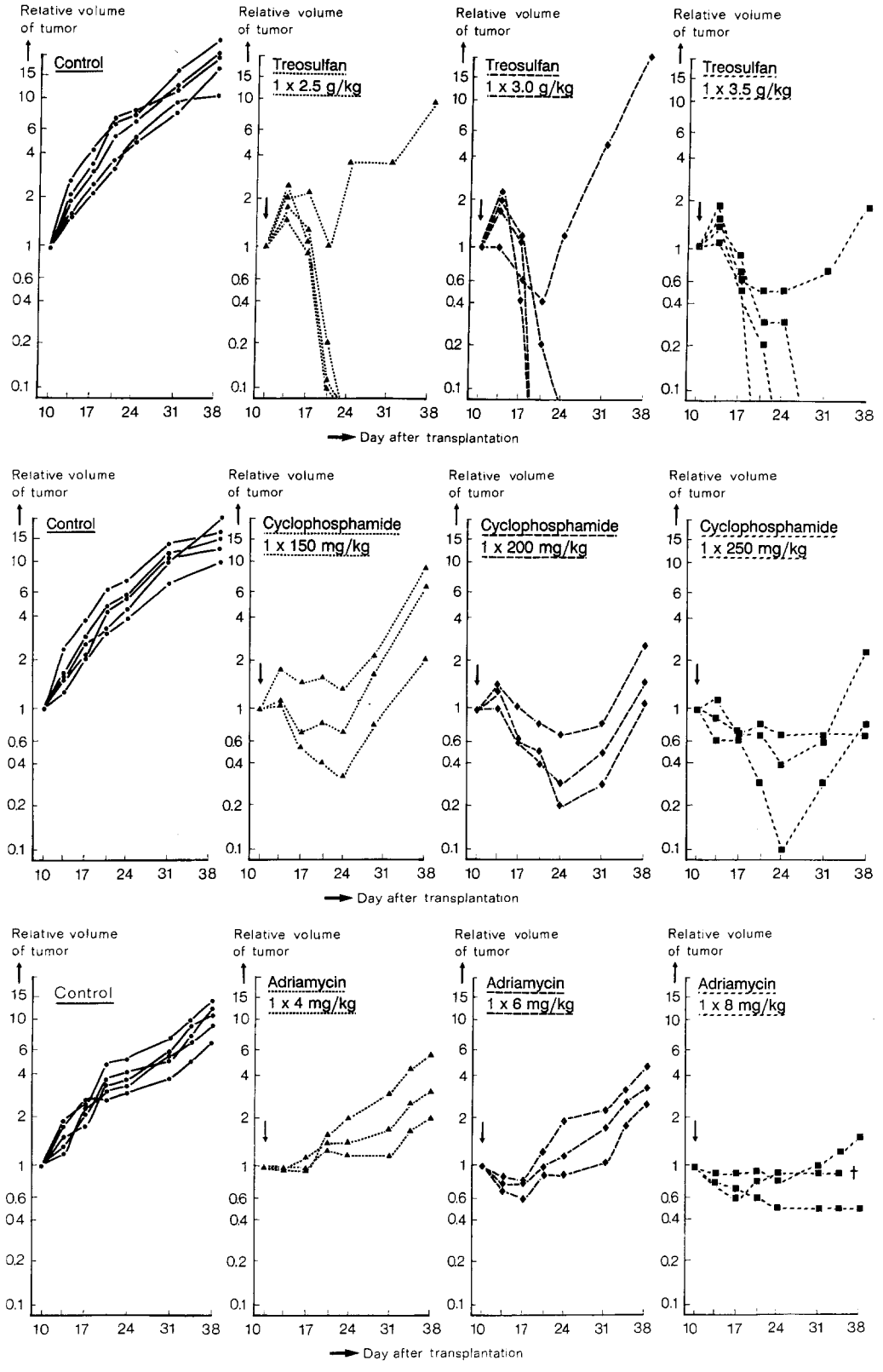

Fig. 3. Growth behavior of the NCI xenograft system MX-1 under the influence of treosulfan, cyclophosphamide, and Adriamycin given as single doses on day 10 after transplantation (for further explanations, cf. legend to Fig. 10). +, Death of an animal on day 36

phosphamide or the intercalating drug Adriamycin should stimulate clinicians to introduce treosulfan into polychemotherapeutic regimens against human breast carcinomas as a substitute for cyclophosphamide.

The doses of treosulfan (2000–3500 mg/kg) that are necessary to induce pronounced cytostatic effects in human breast cancer xenografts are remarkably high in comparison with the effective doses of cyclophosphamide, Adriamycin, or other cytostatic agents, usually ranging between 1 and 500 or 800 mg/kg. Despite this pronounced numerical difference, treosulfan doses of 2000, 2500, and 3000 mg/kg neither reduced the general condition of the

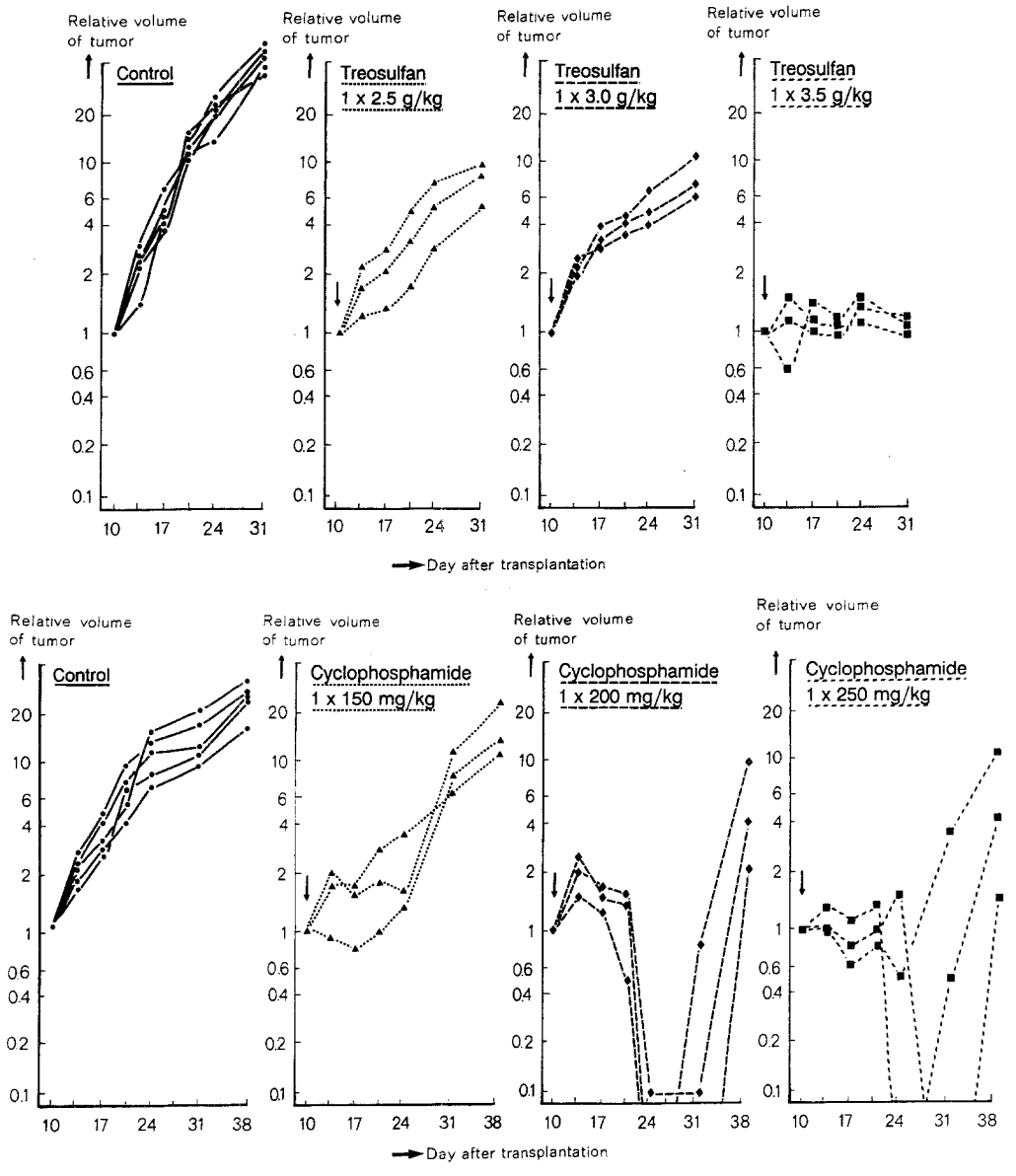

Fig. 4. Growth behavior of the heterotransplanted human breast carcinoma M 3 after treatment with treosulfan and cyclophosphamide given as single doses on day 10 after transplantation (for further explanations, cf. legend to Fig. 1)

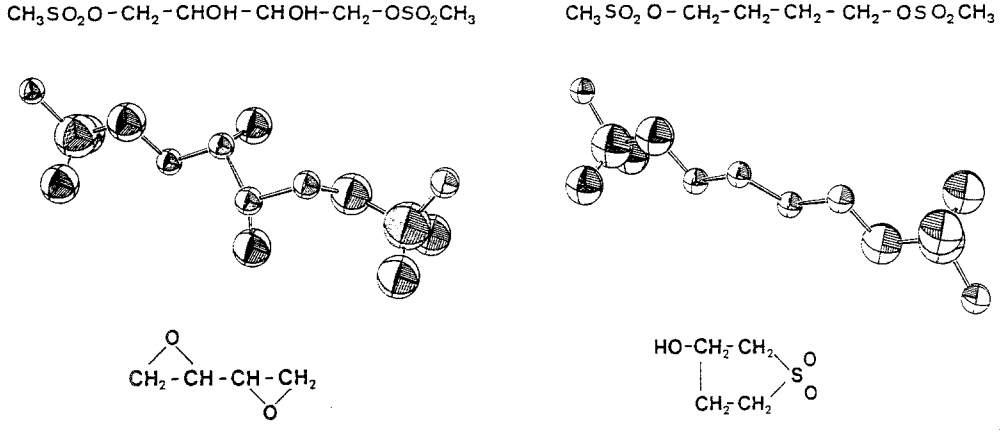

Fig. 5. Comparison of treosulfan (L isomer, *left*) with busulfan (*right*), showing the formulae for both compounds (*upper row*), their molecular structures (*middle row*), and their main metabolites active in vivo (*lower row*). Modified according to Feit [7]

animals nor induced any apparent toxic effect. Only at the dose level of 3500 mg/kg did the animals lose 5%–10% of their body weight. After the application of 4000 mg/kg, about 8%–10% of the animals died due to substance toxicity.

In recent pilot experiments, we examined the cytotoxic activity of treosulfan in organoid cultures of the five human breast carcinomas investigated in the present study and observed a graduated pattern of response by the five tumors similar to that seen in vivo. Interestingly, the effec-

tive cytotoxic concentrations (IC_{50} values) ranged in vitro between 10^{-7} and 10^{-4} mol/l and were comparable with those found for established cytostatics such as cyclophosphamide, cisplatin, carboplatin, Adriamycin, and 5-fluorouracil. This was analogously confirmed in a former in vitro study using monolayer cultures of human ovarian carcinoma cells [18]. This means that the discrepancy in the effective dose levels of treosulfan and other cytostatic drugs observed in nude, athymic mice is not reflected by a similar discrepancy in vitro. Particular conditions of the in vivo situation must be responsible for the unusually high doses of treosulfan that are required to induce growth inhibition in xenografted human breast carcinomas in vivo. Possible reasons could be a highly incomplete absorption of the drug from the peritoneal cavity, an unusually high degree of drug binding to plasma proteins, or an extremely rapid elimination of the drug or its active metabolites from the body. Experimental studies are under way to elucidate the situation and to explore the causes for the unusual in vivo/in vitro discrepancy. On the other hand, the extremely high doses of treosulfan that can be given to athymic mice without inducing mentionable side effects should stimulate clinicians to try to cautiously escalate the doses applied to human patients. On the basis of the results of the present study, it would seem possible to increase the therapeutic efficacy of treosulfan in humans by gradual dose escalation without the danger of inducing severe toxicity or profoundly impairing the general condition of the patients.

Moreover, the results of the present study emphasize not only that treosulfan is effective against ovarian cancer, which is presently the only human tumor that is clinically treated with this drug, but that it is also capable of inhibiting the growth of other types of human tumors. The present investigation confirms its activity against breast carcinomas; this antitumor potency is apparently more pronounced than that induced by standard components of non-endocrine regimens such as cyclophosphamide and Adriamycin. Further studies must reveal whether other types of human carcinoma are also sensitive to treosulfan and, if so, whether their response to this agent is similar to or more pronounced than their response to standard chemotherapeutic 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. Bellet RE, Danna V, Mastrangelo MJ, Berd D (1979) Evaluation of a "nude" mouse-human tumor panel as a predictive secondary screen for cancer chemotherapeutic agents. J Natl Cancer Inst 63: 1185–1188
3. Breitbach GP, Rutt G, Saß G, Bastert G (1988) Patientenzentrierte Weiterentwicklung der cisplatin-haltigen Chemotherapie von fortgeschrittenen Ovarialkarzinomen 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
4. Brinkley BR, Beall PT, Wible LJ, Mace ML, Turner DS, Cailleau RM (1980) Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res 40: 3118–3129
5. Cailleau R, Olive M, Cruciger QVJ (1978) Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro 14: 911–915
6. Clarke R, Van den Berg HW, Kennedy DG, Murphy RF (1985) Oestrogen receptor status and the response of human breast cancer cell lines to a combination of methotrexate and 17-β oestradiol. Br J Cancer 51: 365–369
7. 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 9–17
8. Fennelly J (1977) Treosulfan (dihydroxybusulphan) in the management of ovarian carcinoma. Br J Obstet Gynaecol 84: 300–303
9. Fiebig HH, Schuchhardt C, Henss H, Fiedler L, Löhr GW (1984) Comparison of tumor response in nude mice and in patients. Behring Inst Mitt 74: 343–352
10. Goldin A, Wolpert-deFilippes MK (1979) Nude mouse models as predictors of chemotherapy in man: thymidine and pyrimidines. Bull Cancer (Paris) 66: 61–66
11. Jones R, Kessler WB, Lessner HE, Rane L (1960) New developments in cancer chemotherapeutic agents. Cancer Chemother Rep 10: 99–108
12. Kolaric K (1988) Chemotherapy of mammary cancer with anthracyclines and alkylators. In: Nicolini M (ed) Platinum and other metal coordination compounds in cancer chemotherapy. M. Nijhoff, Boston, pp 436–449
13. Köpf-Maier P, Köpf H (1988) Transition and main-group metal cyclopentadienyl complexes: preclinical studies on a series of antitumor agents of different structural type. Struct Bond 70: 103–185
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 57–72
15. 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
16. McCormack SA, Bearden D, Dennison DK, Egan T, Misra L, Hazlewood CF (1984) Methodological aspects of analysing human breast cancer cell lines by NMR spectroscopy. Physiol Chem Phys Med NMR 16: 359–379
17. Moser K, Stacher A (1984) Chemotherapie maligner Erkrankungen. Deutscher Ärzte-Verlag, Köln, pp 194–209
18. 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
19. Rosen F (1981) Human breast cancer: steroid receptors and response to hormonal manipulation and cytotoxic therapy. In: Mihich E (ed) New leads in cancer therapeutics. G. K. Hall, Boston, pp 51–61
20. Schmähl D (1988) Möglichkeiten und Grenzen der antineoplastischen Chemotherapie. 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 1–6
21. Schwarzenau E, Hilfrich J (1988) Treosulfane in combination with cisplatinum for treatment of advanced ovarian cancer – a phase II trial. J Cancer Res Clin Oncol 114 [Suppl]: S191
22. Shorthouse AJ, Smyth JF, Steel GG, Ellison M, Mills J, Peckham MJ (1980) The human tumour xenograft – a valid model in experimental chemotherapy? Br J Surg 67: 715–722
23. Venditti JM (1981) Preclinical drug development: rationale and methods. Semin Oncol 8: 349–361
24. Wilson AP, Neal FE (1981) In vitro sensitivity of human ovarian tumours to chemotherapeutic agents. Br J Cancer 44: 189–200