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Review paper

Trofosfamide: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the oral treatment of cancer

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During the last decade, the oxazaphosphorine trofosfamide was underestimated partly due to its unsuitability for i.v. use. Oral daily doses of 150 mg were tolerated well and showed appreciable response rates in the treatment of lymphoma. Furthermore, activity in sarcoma and cancers sensitive to oxazaphosphorines in general seems probable, because ifosfamide is the main metabolite of trofosfamide. Due to its oral mode of application and good tolerance, trofosfamide will be an important option in view of the increasing demand for treatment regimens suited for an outpatient basis. Results of the major *in vitro*, *in vivo* and clinical studies are reported for evaluation of its significance in chemotherapy today.

Key words: Lymphoma, metabolism, oral chemotherapy, pharmacodynamics, trofosfamide.

Introduction

Trofosfamide, like cyclophosphamide and ifosfamide, is an alkylating agent from the group of oxazaphosphorines. As compared to cyclophosphamide, trofosfamide has a distinct chemical structure with a third chloroethyl group in the position of cyclic nitrogen. Whereas ifosfamide and cyclophosphamide have emerged as two of the most effective drugs in cancer chemotherapy during the last decade, trofosfamide has been underestimated, partly due to the fact that it is not water soluble.

Trofosfamide was sporadically employed in the clinical treatment of malignant lymphomas and various solid tumors. Most studies are flawed by small patient numbers and heterogeneity of entities treated. Single cases of testicular, ovarian, breast, lung, bladder carcinoma and soft tissue sarcoma as well as glioma have been reported to respond to various trofosfamide-containing combinations. These experiences have demonstrated trofosfamide to be of superior cytotoxic efficacy when administered orally. The oral formulation is well tolerated at a continuous low-dose schedule and is suited for outpatient care.

Therefore, trofosfamide will be part of the amended regimen of the German Cooperative Soft Tissue Sarcoma Study (GPOH-CWS) and of the Multicenter Study for Childhood Malignant Glioma (HIT-GBM).

This review reports the results of the major *in vitro*, *in vivo* and clinical studies of this little-known oxazaphosphorine, in order to evaluate its significance in chemotherapy today.

Pharmacodynamics

Antineoplastic activity

The basic mechanism of action of trofosfamide is presumed to be identical to that of the alkylating agents ifosfamide and cyclophosphamide. Nevertheless, subtle differences in the molecular pharmacology are evident due to the different location and number of the chloroethyl side chains. Oxazaphosphorines have to be activated by oxidation in position 4 and subsequent release of acrolein and a mustard derivative, the ultimate alkylating agent. Thus, the three oxazaphosphorines produce mustard derivatives with different chemical structures, which may show different alkylating activities depending on the substrate. Despite these differences in chemical structure, most experts ascribe differences in activity mainly to the specifics of metabolic degradation.[1]

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Alkylating agents are highly reactive compounds which are able to substitute alkyl groups for the hydrogen atoms of certain organic compounds. Although many cellular substances can be alkylated in this way, it is the alkylation of nucleic acids, primarily DNA, which is cyctotoxic with most of these compounds.

The antineoplastic activity of trofosfamide was first demonstrated in the Yoshida ascites sarcoma of the rat.[2] The assessment of the curative dose and the LD_{50} showed trofosfamide to be more effective than cyclophosphamide. Further, trofosfamide has shown activity in the L1210 ascites leukemia model of the mouse,[3] an osteosarcoma mouse model and the Ehrlich ascites sarcoma of the mouse.[4] However, these older studies are not well documented and the number of animals is not always given. Trofosfamide was found to be active against Walker-256 carcinosarcoma growing on the chorioallantois in embryonated eggs.[5] Tumor models resistant against cyclophosphamide were shown to be sensitive to trofosfamide treatment.[2,6] This effect may be due to a better cellular uptake of the active trofosfamide metabolites which are more lipophilic. The effect of the oxazaphosphorines on resting and proliferating mouse stem cells has been compared.[7] In contrast to cyclophosphamide and ifosfamide, trofosfamide was active not only against proliferating but also against resting stem cells. In another study, all oxazaphosphorines induced a decrease in the number of granuloid progenitor cells of the mouse[8] after a single dose. Stimulation of granulocytic and monocytic progenitor cells was observed on day 4 after treatment.

In one approach to clarify the mechanism of action, trofosfamide was incubated with rat liver microsomes, and the subsequent reaction of the active metabolites with DNA, nucleosides and nucleotides was assessed.[9] The authors found alkylation of the orthophosphate groups of the DNA which produced phosphotriesters and only to a minor extent direct alkylation of the nucleotides. They concluded that intermolecular cross-links between DNA and chromosomal proteins are the main mechanism of action.

Toxicity

The LD_{50} in the rat was shown to be 72 mg/kg.[2] This finding means that trofosfamide is about twice as toxic as cyclophosphamide and ifosfamide when given i.p. Another study[10] reported an LD_{50} in newborn rats of 55 mg/kg, while sensitivity to trofosfamide decreased to an LD_{50} of 210 mg/kg when the rats were 30 days old. When given i.v. the toxicity is higher due to convulsive effects. Animal studies on chronic toxicity are not available. Trofosfamide has shown immunosuppressive properties both *in vitro* and *in vivo*, but the effects were less severe than after cyclophosphamide.[11]

Mutagenicity

Unlike cyclophosphamide, trofosfamide induced chromosomal aberrations in human leukocytes *in vivo*.[12,13] The concentrations in these experiments were rather high (0.1–1 mg/ml) and only trofosfamide proper was investigated, not its active metabolites. Mitotic gene conversion in *Saccharomyces cerevesiae* was observed after incubation with the urine of trofosfamide-treated rats. Similar results were obtained when yeasts were injected into the peritoneal cavity of rats treated orally with trofosfamide.[14] When yeasts were directly incubated with high concentrations of the oxazaphosphorines, trofosfamide showed higher genotoxic activity than ifosfamide and cyclophosphamide. Such high concentrations, however, cannot be achieved *in vivo*. Experiments with *Salmonella typhimurium* have shown that a phenobarbitone-inducible fraction of cytochrome P450 is responsible for the activation to the mutagenic metabolites.[15] Another group compared the mutagenic activity of the oxazaphosphorines after incubation with rat and mouse liver microsomes in *Escherichia coli* and *S. typhimurium*.[16] In all experiments activated trofosfamide was shown to be more mutagenic than the other oxazaphosphorines.

Male mice were treated i.p. with doses of 25–100 mg/kg of trofosfamide. At the highest dose level sperm abnormalities have been observed.[3] The same effects were seen after high doses of ifosfamide and cyclophosphamide. When male mice were treated with 50 mg/kg trofosfamide, dominant lethal and specific-locus mutations were observed in the offspring.[17] Trofosfamide was shown to be clastogenic and induced heritable translocations in the same test system.[18] The risk of heritable translocations after trofosfamide is thought to be slightly lower than after cyclophosphamide treatment.

Teratogenicity

In one study,[10] newborn rats were treated with trofosfamide s.c. At a dose of 30 mg/kg, the rats

failed to develop normal auricles and became deaf later in life. Those effects were similar for all three oxazaphosphorines investigated. As regards cyclophosphamide, the teratogenic effects have been related to the active metabolites. Cysteine was unable to inhibit these effects. Tests in embryonated chicken eggs showed trofosfamide to be significantly more embryotoxic than the other oxazaphosphorines.[5]

Cancerogenicity

Six out of 153 rats surviving treatment with teratogenic to lethal doses of the oxazaphosphorines developed a malignant tumor at some time during their lifespan.[10] Unfortunately, the author did not distinguish between the three oxazaphosphorines tested. Overall, the number of rats that developed a tumor after long-term treatment with high-dose oxazaphosphorines was about 10%.

Pharmacokinetics and metabolism

Initial studies were focussed on investigating the alkylating activity after i.p. administration of trofosfamide in the serum of rats by the reaction with 4-(4'-nitrobenzyl)pyridine and photometric quantification (NBP test).[2] This method gives only a rough estimate of the alkylating activity in serum, since some inactive metabolites of ifosfamide have also been found to produce a positive reaction.[19] Compared to cyclophosphamide, trofosfamide showed higher levels of NBP activity. Pretreatment with phenobarbitone increased the serum levels, indicating that an inducible fraction of cytochrome P450 is responsible for the metabolic degradation of both oxazaphosphorines to the alkylating forms.

Brock[2] compared the NBP activity of trofosfamide and cyclophosphamide in man after oral administration. Both the C_{max} and the t_{max} values of trofosfamide exceeded the levels after cyclophosphamide ingestion. However, neither the number of patients nor the number of data points for each observation were reported.

Due to the low solubility of trofosfamide in water, the drug is not available as an i.v. formulation. When given i.v., phlebitis and other adverse effects were more frequent.[19] Pharmacokinetic studies comparing oral and i.v. administration have not been published. For ifosfamide and cyclophosphamide, a bioavailability of almost 100% has been reported.[20] The leucopenic response to trofosfamide tablets (Ixoten®) as compared to a trofosfamide formulation in capsules has been tested in beagles.[2] The results only show that the two formulations are bioequivalent.

To date, only few data have been published on the metabolism of trofosfamide. The oxazaphosphorines are activated by hydroxylation in position 4 and subsequent release of acrolein to the mustard derivatives[20] (Figure 1). However, studies to detect 4-hydroxy-trofosfamide and trofosfamide-mustard in biological samples have so far been lacking. Dechloroethylation of the side chains is an inactivating pathway for ifosfamide and cyclophosphamide. In contrast, when trofosfamide is dechloroethylated, ifosfamide and cyclophosphamide are formed, which can be further activated.

An *in vitro* model[21] using liver microsomes of different species showed ifosfamide to be the main metabolite of trofosfamide while the cyclophosphamide fraction was much smaller. Compared to the results obtained with ifosfamide in the same model, the amounts of the active metabolites, determined indirectly by quantification of acrolein, were higher.

The pharmacokinetics of [^{3}H]trofosfamide has been investigated in tumor-bearing rats.[22] The radioactivity was located on the exocyclic side chain. In serum, 10 metabolites were determined by thin layer chromatography with radiochemical detection. Three of those metabolites could be visualized by the NBP method. Identification is difficult, as the conditions of the procedure were not given. According to reports, the mustard derivatives, carboxy-ifosfamide, 2- and 3-dechloroethyl-ifosfamide, aldo-ifosfamide and 4-hydroxy-ifosfamide give a positive reaction at alkaline conditions.[51] The highest radioactivity was found in the liver and kidneys, 67% of the given dose was excreted in the urine and 8% of the radioactivity was found in the feces.

The literature review yields only one report on the pharmacokinetics of trofosfamide in patients, obtained by the direct determination of the parent compound and its dechloroethylated metabolites by gas chromatography with nitrogen–phosphorous detection.[24] In all patients ifosfamide was the main metabolite and cyclophosphamide was found only in smaller amounts. Both 2- and 3-dechloroethyl-ifosfamide, which were detected in patients' plasma and urine, were not found in *in vitro* experiments with liver microsomes.[21] Trofosfamide has a short plasma half-life ($t_{1/2}$ about 1.5 h) due to fast hepatic metabolism. The overall AUC ratio for trofosfamide/ifosfamide/cyclophosphamide was 1/13/1.5. Further studies of trofosfamide are needed which determine

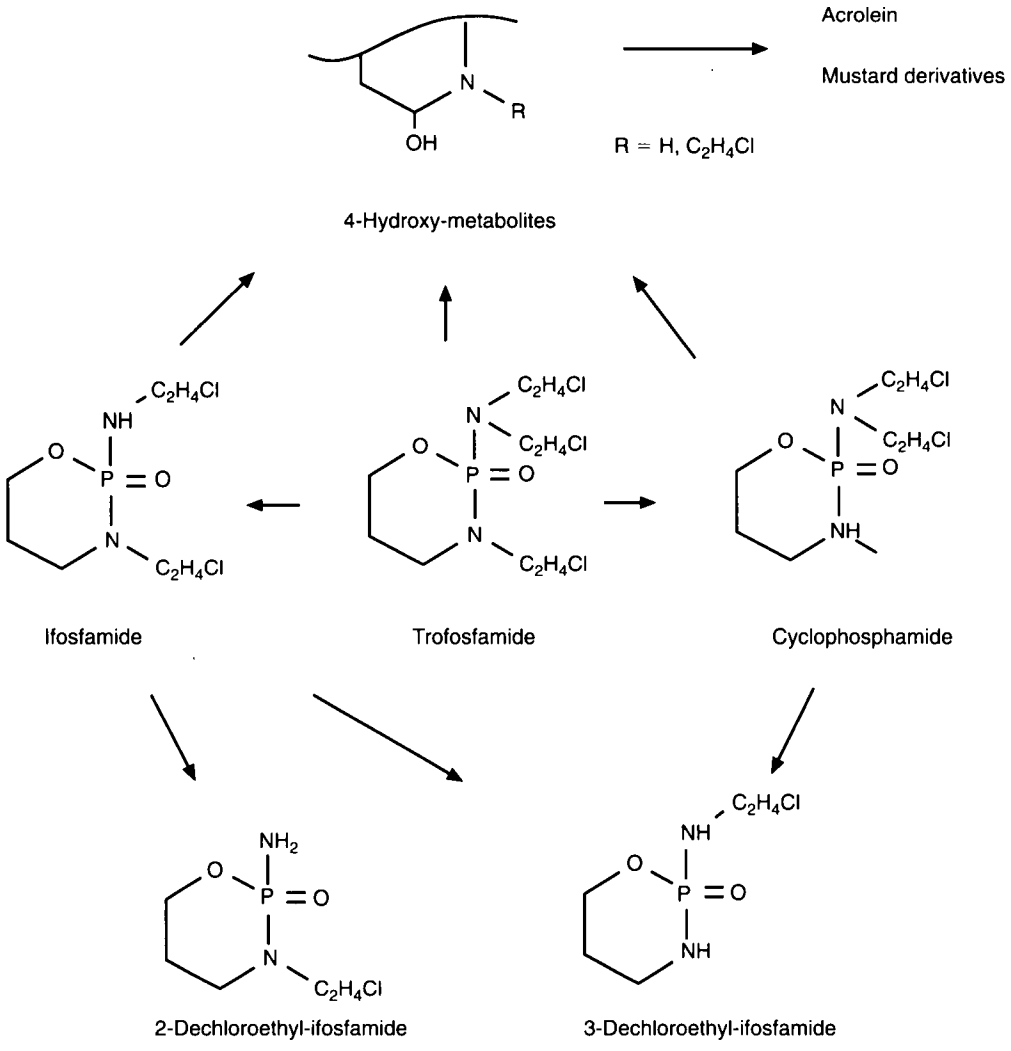

Figure 1. Metabolism of trofosfamide.

the active metabolites in samples from patients and are focussed on the amount of activation.

Therapeutic potential

Lymphomas

Monotherapy. Phase I trials for the evaluation of the tolerance and efficacy of trofosfamide at various dosage regimens in patients with lymphomas have not been published. However, the drug has been the subject of several mostly non-comparative phase II trials as either a first- or second-line agent. The first studies in the 1960s were not performed according to WHO guidelines with regard to response and toxicity. Moreover, trofosfamide was sometimes administered by the i.v. or intramuscular route, and those adverse effects due to the mode of application are irrelevant with regard to current procedures. In addition to confirming that oral treatment is well tolerated, these studies' main conclusions were that the myelotoxic effect is probably dose-dependent and cross-resistance to cyclophosphamide can be excluded, since many patients responding to trofosfamide have had prior chemotherapy including cyclophosphamide. Table 1 summarizes the most important clinical trials of trofosfamide in lymphomas. The definitions of response criteria according to WHO are presented in Table 2.

It is noteworthy that the response was dependent on the histological definition and the grade of malignancy of the treated lymphoma. The response rate in patients with CLL was superior to the response in patients with ALL[25] (87 versus 28%), although the CLL group even received the lower,

Table 1 Summary of non-comparative trials evaluating trofosfamide in the treatment of lymphomas, as monotherapy

Reference	Patients (*n*)	Dosage (mg/day)	Duration of treatment	Prior chemotherapy (%)	CR (%)	PR (%)	SD (%)	PD (%)	Duration of response (months)
Paulisch[43]	89	67.8	87.7 weeks	38	19	57	7	17	23.3
Falkson and Falkson[25]	24	300	??	100	20	66	??	??	> 3
Pötzi *et al.*[49]	29	300	4 weeks–3.5 years	> 50	44	38	9	8	3–42
Wist and Risberg[29]	23	150	??	100	22	39	13	26	1.5–15
Marinsson *et al.*[26]	28	150	1 year	44	7	25	42	25	> 11
Salminen *et al.*[27]	17	150	11 months	> 50	36	20	18	24	7

Response criteria according to WHO or comparable, the daily dose was given continuously. ?? = no information available.

Table 2. WHO criteria CLL[48]

Complete response (CR)	disappearance of lymph nodes, liver or spleen not palpable, good general condition, ≤ 4000 lymphocytes/μl for longer than 2 months
Partial response (PR)	≥ 50% decrease of lymph nodes and liver/spleen size and/or ≥ 50% decrease of lymphocytes for longer than 2 months, without appearance of new lesions
Stable disease (SD)	no 50% decrease of lymph nodes/liver and spleen mass or 25% increase in the number of lymph nodes or size of liver/spleen
Progressive disease (PD)	≥ 50% increase of lymph nodes and liver/spleen size or appearance of new lesions, ≥ 50% increase of lymphocytes

though continuous dose of trofosfamide, i.e. 3 × 100 mg/day for 34 days and 3 × 300 mg/week continuously; the ALL patients had a high dose regimen of 50 mg/kg (corresponding to 3500 mg in a patient of 70 kg body weight) for 48 h. While it seems likely that the high-dose administration of trofosfamide has no advantage over the continuous application of lower doses, the results of both regimens are not comparable due to the different diagnoses of patients included.

The doses used in the reported studies were usually reduced later on, without affecting the response rates, but with a remarkable reduction of toxic effects. The overall response rates (complete and partial remission) ranged from 64 to 82% in the high dose group (300 mg/day) and from 32 to 61% in the low dose group (150 mg/day). The poor response of 32% in Martinsson *et al.*[26] is probably due to the high proportion of high-risk NHL in this population (45 versus 6%) in Salminen *et al.*[27]

The median duration of response in both regimens is comparable; however, recent studies have often been evaluated before they were terminated, when remission status and the end-point were not yet precisely known.

One case report documented successful treatment of a patient with high-risk refractory grade III lymphoma. The patient, who had undergone several prior chemotherapy courses, was administered 3 × 50 mg/day trofosfamide for 10 months. He reached complete remission for a duration of several months. The tolerance of oral trofosfamide treatment was excellent.[28]

Regarding the outcome of studies (Figure 2) which employed daily doses of 150 mg/day trofosfamide, the question of schedule dependence cannot

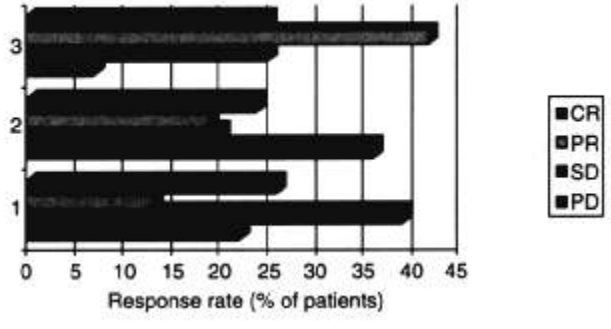

Figure 2. Response rates according to phase II trials of Martinsson *et al.*[26] in 28 patients (1), Salminen *et al.*[27] in 17 patients (2), and Wist and Risberg[29] in 23 patients (3). Patients with refractory NHL received trofosfamide 1 × 150 mg/day (1 + 2) or 3 × 50 mg/day (3) p.o. for a minimum of 1 year.

be finally answered. Obviously the schedule of 3×50 mg/day[29] is associated with a higher overall response rate than one single application of the full daily dose.[27,26] On the other hand, the median duration of response in Wist and Risberg[29] was only 4 months as compared to 7[27] and 11[26] months in the other studies. However, those studies which reported a short duration of response included patients who were in a very poor general condition before treatment and a very high proportion of patients with high-grade lymphoma.

Combination therapy. Although it is widely believed that combination therapy is more effective than monotherapy in lymphoma, treatment approaches with trofosfamide-containing combinations in lymphoma patients have been lacking.

Comparative trials. The first clinical trial using trofosfamide was planned as a multicenter, prospective, comparative phase II study in 273 patients with hematological malignancies and solid tumors.[19]

Trofosfamide monotherapy at various schedules with dosages five times higher than in the trials cited above was compared with ifosfamide monotherapy. Both substances were applied at the same dosage. Unfortunately, there was no randomized allocation to the treatment groups, 234 patients received trofosfamide and 39 patients ifosfamide. The results should be interpreted with caution, as the treatment groups are not at all comparable.

The overall response rate in patients with hematological malignancies was higher after trofosfamide than after ifosfamide treatment (trofosfamide 94/170 = 55% versus ifosfamide 4/18 = 22%). The ratio of complete remissions to partial remissions was identical in the two groups. In solid tumors the response was generally lower, with a slight difference between the treatment regimens (trofosfamide 10/64 = 15% versus ifosfamide 2/21 = 10%). Nevertheless, two complete remissions were observed after trofosfamide in patients with small cell lung cancer and sarcoma of the lower jaw.

The hematotoxicity resulting in grade II–III leukopenia and grade II–III thrombopenia and side effects like alopecia were more severe after trofosfamide, but hemorrhagic cystitis occurred only in the ifosfamide group. When this trial was activated preventive measures against urotoxicity under oxazaphosphorine treatment were not customarily done.

The trial may be valuable as a source of preliminary information for later studies. No further comparative trials about the activity of trofosfamide have been published.

Soft tissue sarcomas

Monotherapy. Data are available from a Finnish phase I study involving a total of 23 evaluable patients with advanced and/or recurrent soft tissue sarcomas.[31] Half of the patients had received prior chemotherapy. Leiomyosarcoma was the predominant diagnosis ($n = 9$), but liposarcoma, Ewing's sarcoma and synovial sarcoma were also included. Trofosfamide was given as continuous oral treatment starting at a daily dose of 150 mg/day. The dose was escalated by 50 mg every third week until grade II leukopenia occurred, which corresponds to the definition of the maximum-tolerated dose (MTD). In the event of grade III leukopenia reduction of the daily dose by 50 mg was mandatory. In patients receiving trofosfamide as first-line therapy the response rate was 25%, as compared to 13% in all evaluable patients. There was no complete remission and stable disease was observed in 26% of the patients. However, the time to progression ranged from 2 weeks to 18 months (median 3 months) and the survival time ranged from 1 to 20 months (median 8 months). The times until progression in those three patients with partial response were 5.8, 17.9 and 8.7 months.

Considering the poor prognosis of advanced metastatic sarcomas, the reported progression-free and survival times give cause for optimism. The MTD was reached at 200–250 mg daily (43% respectively 22% of patients with grade II leukopenia at those dose levels). There was no difference between first-line and second-line treatment in terms of MTD.

The results suggest activity of trofosfamide against soft tissue sarcoma. In an earlier phase I trial tumor response was proven in one of three evaluable sarcoma patients who received 400 mg trofosfamide i.v. daily.[31]

Comparative trials. Comparative trials of trofosfamide in sarcoma patients are unavailable.

Other cancers

Metastatic breast cancer. In the 1980s trofosfamide was used for the first time in adjuvant combination therapy against breast cancer. A total of 190 patients with primary breast carcinoma and lymph node involvement, T_{1a-3a}, N_{1a-b}, M_0, were post-operatively treated with trofosfamide (100 mg/m^2), methotrexate (40 mg/m^2) and 5-fluorouracil (600 mg/m^2) for a period of 12 months.[32] Trofosfamide was given orally, the other substances i.v. After an average

follow-up time of 41 months the lymph node positive patients on post-operative chemotherapy showed no difference in relapse rate from the lymph node negative patients who had surgery alone. Survival of treated patients seemed to depend on tumor stage, independent of menopausal status. Compared to historical trials of adjuvant chemotherapy containing cyclophosphamide instead of trofosfamide, the trofosfamide regimen obtained significantly better results.[33] The reported treatment regimens were associated with serious toxicity, i.e. hematotoxicity and gastrointestinal side effects. In addition, 8% of patients with cystitis have been documented, but the documented adverse effects are not representative for trofosfamide.

The relevant piece of information gained from this study is that trofosfamide may in fact be used along with methotrexate and 5-fluorouracil in the polychemotherapy of breast cancer.

Another study combined trofosfamide (100 mg/day) and methotrexate (25 mg/week) for adjuvant chemotherapy in 66 breast cancer patients T_{1-4}, N_{0-3}, M_0, given by continuous oral administration.[7] After 2 years of therapy and 44 months of total follow-up time the relapse rate was slightly lower (13.6 versus 16.2%) than in patients after 'TMF' therapy as mentioned above.[32] Drug tolerance in both trials was comparable. Subsequent retrospective observations showed similar results.[34]

Retrospective data were obtained on trofosfamide in the high-dose monotherapy of advanced breast cancer.[35] The treatment consisted of three cycles of 25 mg trofosfamide/kg body weight daily on two consecutive days. The objective response rate was 57.1% and the median duration of remission was 3 years. Promptly reversible grade IV leukopenia (60% of patients) with the WBC nadir on day 16 and transitory alopecia were frequently seen. When focussing on a subgroup of 22 patients with advanced stage disease and affected lymph nodes the remission rate and duration remain remarkable, especially when the easy way of application is considered.

Further clinical trials seem warranted to prove the relevance of trofosfamide in the treatment of breast cancer. Results after high-dose trofosfamide monotherapy and trofosfamide combined with methotrexate and/or 5-fluorouracil after mastectomy and/or induction chemotherapy are impressive; however, the reports are mainly based on retrospective surveys. Due to the incomparability of treatment groups, a rationale governing dosage and scheduling cannot be deduced yet and needs further investigations.

Ovarian cancer. A multicenter, randomized phase II trial included 99 patients with advanced ovarian cancer.[37] Adjuvant therapy consisted of either ifosfamide combined with vincristine and 5-fluoro-1-(tetrahydro-2-furfuryl)-uracil or trofosfamide combined with 5-fluorouracil, methotrexate and melphalan. The evaluation of survival rates showed no differences between the treatment groups, but the trofosfamide-containing combination was associated with less severe side effects than the ifosfamide combination. The results could not be interpreted to confirm a clear advantage of trofosfamide, because the therapy arms differed with regard to the other components. Similar to trials in breast cancer the findings seem to indicate an advantage for the combination with methotrexate and 5-fluorouracil.

Lung cancer. Several trials evaluating the activity of trofosfamide in various solid tumors included patients with lung cancer. Often, the results were not specified with regard to the individual entities.[25,38] In a prospective trial, five of 11 (45%) evaluable patients with lung cancer showed tumor response after 400 mg i.v. trofosfamide daily.[32] The authors reported the advantages of trofosfamide in lung cancer without specifying the reference trials. They also omitted information on patients' characteristics, status or duration of remission, or survival time. The results have merely historical value with regard to the mode of application.

Twelve patients, including four with oat cell carcinomas, received trofosfamide, either 200–400 mg continuously or more than 500 mg daily (i.v. or orally), mainly as first-line therapy. One patient was in complete remission, defined as complete dissappearance of symptoms, for more than 4 weeks, three patients achieved partial remission corresponding to disappearance of more than 50% of symptoms for 4 weeks. No response was detectable in 6 patients.

Polychemotherapy combining trofosfamide (250 mg/day), adriamycin (75 mg/m^2/day), methotrexate (15 mg/m^2/day) and vincristine (1 mg/day) in 45 patients with advanced inoperable epidemoid cell lung cancer resulted in an overall long-term response rate of 29%.[38] The median survival rate was 166 days with significantly longer survival times in the group with non-keratizing epidermoid cell cancer. However, survival times were similar in responders and non-responders. The treatment was accompanied by the typical side effects in all patients. There was no apparent advantage of the reported polychemotherapy over established treatment regimens.

Testicular cancer. In a prospective clinical trial,[37] five patients with testicular cancer, i.e. seminoma, received average daily trofosfamide doses between 88 and 137 mg. One of five patients reached complete remission, another one partial remission. The remaining patients were not evaluable for unreported reasons.

Eight patients with seminoma and testicular teratoma received daily trofosfamide doses of 3–5 mg/m^2 body surface area.[29] The treatment was well tolerated. Two patients achieved complete remission and four patients partial remission, which corresponds to an overall response rate of 75%; however, this result has to be interpreted with caution considering the limited number of patients. Remission lasted 3–14 months.

Several trials have employed trofosfamide to treat testicular cancer, among other entities, but the outcome cannot be ascertained. Testicular teratoma ($n = 3$) was, for example, treated with high-dose trofosfamide (50 mg/kg/day orally for 48 h) according to the regimen of the South African trial.[25] The results, however, were drowned out by the overall results for the total group of solid tumors, i.e. 28% remissions longer than 3 months. Activity of trofosfamide against testicular cancer may be assumed and needs further investigations.

Moreover, trofosfamide was used in chemo-immunotherapy regimens in patients with advanced stage IV testicular cancer.[41] A total of 26 patients (12 testicular teratomas, eight embryonic tumors and six seminomas) received chemotherapy cycles of bleomycin + vinblastin and adriamycin + cisplatin for a period of 1 year, followed by maintenance therapy consisting of trofosfamide, vinblastin and OK-432, an immunomodulating agent. Half of the 18 patients who entered remission after induction therapy were observed. Seven of nine patients showed no signs of relapse within the follow-up time of 20 months. The tolerance was excellent and the outcome similar to results after established polychemotherapy regimens.[40]

Case reports of other cancers. Response of mycosis fungoides after 3–5 mg/m^2 body surface area/day trofosfamide monotherapy is reported.[29] Nevertheless, one of four patients achieved complete remission and two of four achieved partial remission.

Other solid tumors such as melanoma ($n = 5$), colon carcinoma ($n = 4$) and pancreatic carcinoma ($n = 4$) were also treated with high doses of trofosfamide.[25] Response rates have not been specified for each individual entity, the overall response rate for the total group was 28%.

Four patients with hypernephroma showed no response after trofosfamide monotherapy at daily doses averaging 88–137 mg.[37] These individual trials have yielded no information about the potential cytotoxic activity against other cancers.

Recently, a case of a second temporal remission in a malignant childhood glioma with trofosfamide and etoposide was reported.[38] Oral chemotherapy with increasing doses of trofosfamide from 10 to 225 mg/m^2 and of etoposide from 10 to 100 mg/m^2 was initiated 8.5 months after diagnosis. The clinical symptoms improved rapidly and the tumor volume decreased. Side effects appeared only at the highest doses used and included nausea, low white cell count and stomatitis. Preliminary results of intensive and prolonged oral treatment with trofosfamide 150–200 mg/m^2 and etoposide 75–100 mg/m^2 in patients with different refractory malignancies ($n = 18$, 3–73 years) show that stable disease could be achieved.[39]

Tolerance

The predominant adverse effect in phase II clinical trials of trofosfamide monotherapy at different doses and schedules was myelotoxicity. Leukopenia was the main, most frequent, dose-dependent and dose-limiting toxic effect. Thrombopenia and anemia were also induced (see below). However, trofosfamide seems to be less urotoxic than other oxazaphosphorines (see below).

Nearly 40% of patients developed transitory alopecia after various trofosfamide treatment regimens[25,32] Phlebitis and gastrointestinal side effects have been rare after the shift to oral application.[19,37] Vomiting and hepatic toxicity were reported in some cases, as well as individual cases of allergic reactions and neurotoxicity, but detailed descriptions are lacking.[43]

Other adverse effects included unspecific symptoms like loss of appetite, amenorrhoea, anorexia and vertigo (see Table 3).

Hematological toxicity

The most common hematotoxic effect observed with trofosfamide is dose-dependent leucopenia.

According to one phase I trial in 23 patients (age 21–79 years) with soft tissue sarcomas, the MTD, defined as the dose associated with grade II leukopenia, was reached at 200–250 mg trofosfamide daily.[30] At that dose level, 15 patients (65%) devel-

Table 3. Adverse effects (besides hematological toxicity) after different treatment regimens of trofosfamide

Reference	Diagnosis	Schedule	Patients (*n*)	Adverse effects
Paulisch[43]	lymphomas	1 × 88–137 mg p.o.	113	*n* = 18 alopecia *n* = 5 hematuria *n* = 2 allergic reactions *n* = 2 neurotoxicity *n* = 4 hepatic toxicity
Drings *et al.*[19]	lymphomas + solid tumors (170:64)	1 × 200–400 mg i.v./ p.o. (*n* = 180), q 17 w (solid tumors)–26 w (lymphoma) > 1 × 500 mg i.v./p.o. (*n* = 34) *q* 4–5 d	234	*n* = 42 alopecia *n* = 39 hematuria *n* = 81 vomiting *n* = 23 phlebitis (i.v.)
Willems and Mainzer[31]	lymphomas + solid tumors (34:27)	1 × 400 mg i.v./p.o.	61	*n* = 14 vomiting *n* = 17 alopecia *n* = 5 hematurie *n* = 7 phlebitis (i.v.) >*n* = 3 amenorrhoe, nausea, appetite loss

oped counts between 2.0 and 3.0×10^9/l leucocytes. Three patients (13%) had an MTD of 100 mg daily and two patients (9%) had an MTD of 150 mg trofosfamide. However, another three patients tolerated doses of 300 mg daily and more with grade II leukopenia. Other adverse effects were negligible. The MTD was independent of prior treatment. The definition of MTD in this study has been related to the daily dose. The relationship between the cumulative dose and the hematotoxic effects could not be established. Trials to study that relationship have not been performed.

Another treatment regimen with a 1× 150 mg/day schedule in 36 patients with NHL was associated with grade III–IV leukopenia in 14 patients (50%), grade III–IV thrombopenia in seven (25%) and grade III–IV anemia in two patients (7%).[26]

As a consequence, treatment was discontinued in 16 patients and the dose was reduced in seven patients.

The schedule dependency of toxic effects remains unclear as controlled trials with different schedules in comparable treatment groups are lacking. With oral application the hematotoxicity of 1 × 150 and 3 × 50 mg/day seems to be similar (see Table 4).

Urotoxicity

Urotoxicity is a serious toxic side effect of the oxazaphosphorines. One patient with advanced NHL IIa maintained continuous complete remission after radiochemotherapy throughout the follow-up time.[44] After 7 years of maintenance therapy with trofosfamide (100 mg/day, p.o.) he developed a highly aggressive bladder carcinoma. The case report presented no answers regarding the relationship between long-term therapy with an oxazaphosphorine and secondary malignancies of the urinary tract.

Both symptoms of hemorrhagic cystitis and secondary bladder carcinomas have been attributed to the primary 4-hydroxy metabolites, which are excreted in the urine. The damage to the kidney and bladder seems to be concentration dependent. This fact was confirmed by several experimental and

Table 4. Schedule and myelotoxicity

Reference	Diagnosis	Schedule	Patients (*n*)	Myelotoxicity (WHO I–IV)
Martinsson *et al.*[26]	refractory NHL	1 × 150 mg/day p.o.	28	2 (7%) anemia III–IV 14 (50%) leucopenia III–IV 7 (25%) thrombopenia III–IV
Salminen *et al.*[27]	refractory NHL	3 × 50 mg/day p.o.	17	10 (58%) leucopenia II–III
Blomquist *et al.*[30]	refractory soft tissue sarcoma	1 × 50 mg/day p.o.	23	13 (56%) leucopenia II 6 (26%) leucopenia III

clinical trials using ifosfamide and cyclophosphamide.[8,40] Compounds containing free sulfhydryl groups may protect from the urotoxic effects by their interaction with activated metabolites.[30,45] Whether or not acrolein or further metabolites are responsible has remained uncertain.[46] Both extensive hydration and the administration of sodium-2-mercaptoethane sulfonate (mesna) have become standard clinical practice for uroprotection in ifosfamide and cyclophosphamide regimens.[18,47] Acetylcysteine, as a protective agent, interacts with tumor response and should be avoided.[46] As yet, uroprotective measures in trofosfamide regimens have not been established.

Side effects of combinations with other antineoplastic agents

According to our own experiences the assessment of trofosfamide toxicity in a trofosfamide–etoposide combination is feasible. Leucopenia was the major side effect in 15 evaluable patients (age 3–73 years) with various pretreated cancers who received cumulative doses of 906–2866 mg/m^2 per cycle trofosfamide and 125–821 mg/m^2 etoposide; 38% of cycles ($n = 46$) were discontinued for this reason. On average, the WBC nadir was reached on day 16 ± 7 (median: day 19).

A total of three cycles were interrupted due to grade II thrombopenia. Adverse events like nausea, alopecia and stomatitis were rarely observed. One patient with hepatoblastoma and another one with PNET without hepatic involvement showed increasing transitory GPT values up to 40 U/l.

The side effects of combinations with other cytostatics are summarized in Table 5.

Dosage and administration

A daily dose of 150 mg seems to be suitable for oral trofosfamide application. Conclusions on the schedule dependency of tumor response cannot be given since the question has not been asked in a randomized two-arm trial in comparable patient groups.

In the comparison of phase II trials,[26,27] differences in the distribution of high grade lymphomas between the two groups have to be taken into account. According to the data obtained with trofosfamide treatment in lymphomas,[29] the response rate in low-grade lymphoma is expected to

Table 5. Selected reports on adverse effects of trofosfamide in combination with other antineoplastic agents (MTX = methotrexate, 5-FU = 5-fluorouracil)

Reference	Diagnosis	Schedule	Patients (*n*)	Adverse effects
Sagaster *et al.*[41]	testicular carcinoma	*Trofosfamide* 1 × 150 mg q day 1 *Vinblastine* 1 × 10 mg q day 2–5 *OK-432* 2KE q day 10, 17, 24 cyc 3 w–1 w interval	26	*n* = 26 alopecia *n* = 6 hematuria *n* = 15 fever *n* = 8 polyneuropathy
Kokron[40]	lung carcinoma	*Trofosfamide* 1 × 250 mg q day 12, 99, 16, 22 *Adriamycin* 75 mg/m^2 q day 1 + 22 *Vincristine* 1 mg q day 1, 8, 15, 22 *MTX* 15 mg/m^2 q day 1, 8, 15, 22 cyc 4 w–6 w interval	45	*n* = 31 leukopenia III–IV *n* = 18 anemia III–IV *n* = 17 vomiting *n* = 12 stomatitis *n* = 11 lost of appetite *n* = 35 alopecia *n* = 12 drowsiness
Albrecht *et al.*[32]	mamma carcinoma	*Trofosfamide* 100 mg/m^2 q day 1–14 *MTX* 40 mg/m^2 q day 1 + 8 *5-FU* 600 mg/m^2 q day 1 + 8 cyc day 1–14, day 14–29 interval	190	*n* = 114 nausea *n* = 114 leucopenia II *n* = 38 thrombopenia II *n* = 76 alopecia *n* = 36 diarrhoea *n* = 26 stomatitis *n* = 26 paresthesia *n* = 15 cystitis *n* = 47 vomiting

be higher than in high grade lymphoma. With respect to hematoxicity, however, the application of 1×150 and 3×50 mg seems to be equivalent (see above)

Conclusion: place of trofosfamide in therapy

The efficacy against lymphoma can be concluded from several clinical phase II trials.[26–29] After trofosfamide monotherapy with an oral daily dose of 150 mg the overall response rate (CR + PR according to WHO) ranged between 32[26] and 61%[29] at a mean follow up of 7–11 months (Table 6).

The difference of results was due to different starting conditions of the patients and the grade of malignancy.

Response rates in low grade lymphomas exceeded those in high grade lymphomas (73 versus 38%).[29]

In half of the patients the dose had to be reduced due to hematotoxicity. Otherwise therapy was well tolerated.

Furthermore, activity in soft tissue sarcoma seems probable. Unfortunately only a single clinical trial with few patients has been reported so far (see below). A dose of 150 mg trofosfamide induced partial response and extended the survival time in heavily pretreated patients.

Recent prospective studies about the use of trofosfamide monotherapy in other cancer types are lacking. Combinations with other agents such as 5-fluorouracil, methotrexate, melphalane and progesterone have been employed in breast, ovarian, lung and testicular cancer (data are summarized in Table 7).

The relevance of these approaches has to be confirmed by further clinical investigations.

In summary, reports in the literature have demonstrated the antitumor activity of trofosfamide in the treatment of lymphoma and probably soft tissue sarcomas as well. The observation that ifosfamide is the main metabolite of trofosfamide[21] leads to the conclusion that trofosfamide may be active in cancers sensitive to oxazaphosphorines in general. However, this conclusion has not yet been confirmed by published evidence.

Due to the lipophilic structure, the oral mode of application and good tolerance, trofosfamide will be an important option in view of the increasing demand for treatment regimens suited for outpatient care.

Table 6. Scheduling and response of trofosfamide (note the differences in the distribution of high and low grade NHL)

Reference	Patients (*n*)	Grade of malignancy (NHL)	Treatment regimen	Response (WHO)
Martinsson *et al.*[26]	28	55% low 45% high	1×150 mg/day p.o.	CR 7% PR 25% SD 42% PD 26%
Salminen *et al.*[27]	17	64% low 29% intermediate 6% high	3×50 mg/day p.o.	CR 35% PR 25% SD 17% PD 23%

Table 7. Summary of combination chemotherapies including trofosfamide

Reference	Diagnosis	Trofosfamide in combination with	Kind of therapy
Brachetti *et al.*[50]	breast	methotrexate	maintenance therapy
Kokron[40]	lung	adriamycin, vincristine, methotrexate	polychemotherapy, low dose maintenance
Sagaster *et al.*[41]	testicular	vinblastine, OK-432	chemoimmunotherapy
Albrecht *et al.*[32]	breast	methotrexate, 5-fluorouracil	induction therapy, TMF regimen
Franz and Lietz[36]	ovarian	5-fluorouracil, methotrexate melphalane and progesterone	induction therapy maintenance therapy
Krümpelmann *et al.*[39]	refractory tumors	etoposide (oral)	maintenance therapy, palliative therapy

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