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Pharmacokinetics of trofosfamide and its dechloroethylated metabolites

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Abstract To contribute to effective and safe outpatient treatment, we investigated the metabolism of trofosfamide (Trofo) after oral administration. We analyzed Trofo metabolism in 15 patients aged from 3 to 73 years who were treated with 150 or 250 mg/m^2 Trofo in combination with etoposide. Serum samples were collected with 13 patients after oral administration, and Trofo and its dechloroethylated metabolites were quantified by gas chromatography. Urine samples were collected from five patients and analyzed by same method. Ifosfamide (Ifo) was the main metabolite in serum and urine (AUC_{Trofo}:AUC_{Ifo} 1:13), whereas cyclophosphamide (Cyclo) was formed in smaller amounts (AUC_{Ifo}:AUC_{Cyclo} 18:1). Ifo and Cyclo were further oxidized in the chloroethyl side chains to form 2- and 3-dechloroethylifosfamide in varying quantities. The urinary excretion of Trofo and its dechloroethylated metabolites amounted to about 10% of the total dose. Our results confirm former in vitro observations about the metabolism of Trofo. The main side-chain metabolites Ifo and Cyclo can be further activated by oxidation and formation of their respective phosphoramide mustards. Hence, Trofo is an interesting agent for oral chemotherapy.

Key words Trofosfamide · Ifosfamide · Cyclophosphamide · Oral chemotherapy

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Introduction

Trofosfamide (Trofo) is an alkylating agent of the oxazaphosphorine group. Although the drug has been commercially available for more than 20 years, it has gained little recognition in clinical application, probably because it is marketed only in a formulation for oral use. Most of the treatment protocols have focused on optimization of ifosfamide treatment. Trofo is considerably more lipophilic than the other oxazaphosphorines. Early clinical trials have reported efficacy and limited toxicity [13, 12]. Whereas high-dose intravenous treatment, especially with ifosfamide, has dominated therapy regimens for a long time, currently there is an increasing need for reappraisal of oral treatment strategies on an outpatient basis. Recently, Trofo has been employed in our group in combination with etoposide for palliative oral treatment in outpatient care [17].

Oxazaphosphorines require metabolic activation by the cytochrome P450 system. Hydroxylation at position 4 leads to unstable metabolites, which react spontaneously to the active phosphoramide-mustard derivatives and acrolein (Fig. 1). Furthermore, ifosfamide (Ifo) and, to lesser extent, cyclophosphamide (Cyclo) are inactivated by side-chain oxidation, which results in the formation of 2-dechloroethylifosfamide (2-D) and 3-dechloroethylifosfamide (3-D) with no cytostatic activity. During this reaction chloroacetaldehyde is formed, which is thought to cause neurotoxic side effects during Ifo therapy [10, 14]. Clinical trials with oral Ifo have shown intensive first-pass metabolism to be associated with unexpected neurologic toxicity at higher doses [6]. Although Trofo has been applied at doses of up to 50 mg/kg (about 1700 mg/m^2) [13], neurotoxicity has not yet been reported as a problem for this drug. In vitro studies of Trofo with rat and human liver microsomes indicate that Trofo is mainly oxidized by side-chain oxidation to Ifo and, to

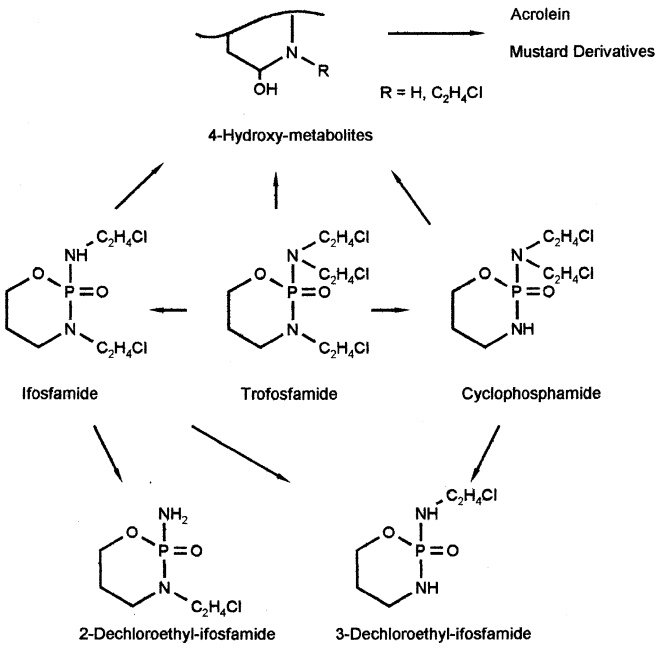

Fig. 1 Metabolism of Trofo

a minor extent, to Cyclo [5]. However, no study has been carried out to investigate the metabolism of Trofo in vivo. As part of a palliative treatment strategy using Trofo and etoposide, we investigated the pharmacokinetics of Trofo and its dechloroethylated metabolites in 15 patients.

Patients and methods

Patients

This study was approved by the local ethics committee. A total of 15 patients aged between 3 and 72 years (median 18 years; 8 females) were included (Table 1). All patients or their parents gave informed consent to the study. All patients showed tumor progression after standard chemotherapy regimens and were treated with palliative intent. They had no renal or hepatic dysfunction according to standard biochemical parameters. The chemotherapeutic regimen consisted of a combination of oral etoposide and Trofo. These two cytostatics were chosen because the combination of an oxazaphosphorine as an alkylating agent with the topo II inhibitor etoposide has been shown to be effective in solid and lymphoblastic malignancies [11, 19]. Both drugs can be taken orally and, thus, can be given on an outpatient basis.

Trofo was given daily at a single oral dose (Ixoten tablets, 50 mg; Asta Medica, Germany) of 150–250 (mean 177) mg/m^2 on the 1st day and at 50–150 (mean 87) mg/m^2 on the following 20 days (Table 1). Etoposide was given at a dose of 25–150 mg/m^2 (mean 78 mg/m^2) on the 1st day and at 15–62.5 mg/m^2 (mean 29 mg/m^2) on subsequent days. Serum samples were collected on day 1 of therapy at 30 min and at 1, 1.5, 2, 4, 6, 9, 12, and 24 h after ingestion. Urine was collected from five patients at intervals of 0–3, 3–6, 6–12, and 12–24 h after oral administration. All samples were stored at − 18 °C until analysis.

Sample preparation and analysis

Serum samples were extracted using Bakerbond C_{18} 500-mg solid-phase extraction columns. After conditioning with 5 ml methanol and 5 ml water, 1 ml serum was applied to the column. Subsequently, serum constituents were washed off with 0.5 ml water. The analytes were eluted with 1 ml methanol. The eluate was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 50 µl methanol. A 1-µl aliquot was applied to the gas chromatography (GC) system. Urine samples were extracted according to a method described elsewhere [15] using liquid/liquid extraction with Extrelut columns.

The chromatographic separation was carried out according to a method described elsewhere [4], with slight modifications. A Shimadzu GC 14A gas chromatograph with an FTD detector was used that was equipped with an NB 1701, 10-m-long fused-silica capillary column. The temperature program was set as follows: from 140 ° to 170 °C, a rise time of 5 °C/min, 170 °C a rise time of 5 °C/min, 170 °C for 4 min, an increase to 200 °C at 15 °C/min, and a hold of that temperature for 10 min. The injector and detector temperatures were set at 200 ° and 250 °C, respectively. Separation was carried out using helium as the carrier gas at a flow rate of 2.5 ml/min and

Table 1 Patients' characteristics (BSA Body surface area, *ALL* acute lymphocytic leukemia, *CML* chronic myelocytic leukemia, *PNET* primitive neuroectodermal tumors)

Patient number	Sex	Age (years)	Weight (kg)	Dose[a] (mg/m^2)	BSA (m^2)	Diagnosis	Samples
1	F	5	28	150	0.8	ALL	Urine
2	M	8	17	150	0.8	ALL	Urine
3	M	25	69	150	1.8	CML	Serum
4	F	73	66	150	1.7	Ovarian carcinoma	Urine, serum
5	F	58	61	150	1.7	Breast cancer	Serum
6	F	21	50	150	1.6	PNET	Urine, serum
7	M	18	64	220	1.8	Ewing's sarcoma	Serum
8	M	18	52	180	1.7	Testicular teratoma	Serum
9	F	18	67	230	1.7	Rhabdomyosarcoma	Serum
10	F	7	25	220	0.9	Wilm's tumor	Serum
11	M	3	17	210	0.7	Hepatoblastoma	Serum
12	M	20	69	170	1.8	Medulloblastoma	Serum
13	F	38	59	140	1.8	Cervical carcinoma	Urine, serum
14	M	15	41	190	0.8	Hybrid leukemia	Serum
15	F	20	64	150	1.7	Ewing's sarcoma	Serum

[a] Trofo dose on day 1 of treatment

synthetic air and H_2 as the detector gas. Peak areas were calculated using a Shimadzu C-R6A integrator. Calibration graphs were prepared with blank serum samples spiked with 5-, 2-, 1-, or 0.5-, and 0.25-μg/ml of each analyte. Serum concentrations of Trofo, Ifo, Cyclo, and the dechloroethylated metabolites 2-D and 3-D were calculated using the external standard method. Correlation coefficients of the daily calibration curves were always ≥ 0.99, and the inter- and intraassay coefficients of variation were less than 13%. Urine samples were quantified in the same way using spiked urine samples for calibration.

Pharmacokinetic calculations

Body surface areas were calculated according to the Dubois method. The parameters peak concentration (C_{max}), peak time (t_{max}), area under the concentration-time curve (AUC), oral clearance (Cl/F), and terminal elimination half-life ($t_{1/2}$), were calculated using Topfit V.2.0. by noncompartmental analysis. C_{max} and t_{max} values were directly obtained from the concentration-time profiles. The determination of plasma half-lives was done using linear regression analysis of the terminal phase. The AUC value was calculated by the trapezoidal rule. There was no indication of nonlinear kinetics in this dosing regimen. Thus, the C_{max} and AUC values recorded for all patients were normalized to a dose of 100 mg/m^2 for a better estimation of variability.

Results

Due to the small number of data sets and the complex metabolic pathway involved, the results could not be fitted to a compartmental model. Serum concentration data could be determined in 12 patients for Trofo and Ifo, in 11 patients for Cyclo, in 9 patients for 2-D, and in 13 patients for 3-D. In three cases for Cyclo and in two cases for 3-D there were not enough data points to determine $t_{1/2}$ from the terminal phase due to incomplete sample collection. Thus, the AUC values could not be calculated. In Table 2 the pharmacokinetic parametrs of all patients are summarized. High interpatient variability was observed, especially in the C_{max} and AUC values recorded for Trofo and its metabolites.

Maximal concentrations of Trofo were observed at 2.3 ± 1.5 h with high interpatient variability. Subsequently, Ifo and Cyclo were formed with t_{max} values of 3.3 ± 1.6 and 3.0 ± 2.4 h, respectively. The secondary metabolites 2-D and 3-D reached their highest concentrations at 3.8 ± 1.9 and 4.5 ± 2.3 h, respectively. For Trofo the $t_{1/2}$ was only 1.5 ± 0.6 h and the Cl/F value was 3036 ± 2283 ml/min, indicating fast hepatic metabolism. In contrast, the declines noted in plasma concentrations of Ifo and Cyclo were much slower. 2-D and 3-D disappeared from plasma even more slowly than did Ifo and Cyclo. However, one should bear in mind that both formation and excretion take place at the same time.

Ifo was the predominant metabolite in all patients. The AUC value recorded for Ifo was 5–23 times (mean 12.7 times) that noted for the parent compound and 3.5–37 times (mean 17.6 times) that found for Cyclo. The C_{max} and AUC values determined for the secondary metabolites 2-D and 3-D were comparable.

Table 2 Pharmacokinetic parameters recorded for all patients. C_{max} and AUC values were normalized to 100 mg/m^2

Trofo	Range	Mean	Geometric mean	SD
C_{max} (nmol/ml)	0.069–3.868	1.424	1.029	1.184
t_{max}(h)	0.5–6	2.38	2.00	1.72
AUC (nmol h ml^{-1})	0.37–15.70	4.464	2.86	4.859
$t_{1/2}$ (h)	0.62–1.47	1.55	1.43	0.62
Cl/F (ml/min)	478–8770	3036	2495	2283
Ifo				
C_{max} (nmol/ml)	0.152–4.654	2.975	2.860	1.503
t_{max} (h)	1–6	3.33	4.00	1.72
AUC (nmol h ml^{-1})	2.95–65.74	33.68	35.33	17.26
Cyclo				
C_{max} (nmol/ml)	0.079–1.859	0.588	0.511	0.591
t_{max} (h)	0.5–9	3.09	2.00	2.51
AUC (nmol h ml^{-1})	1.52–12.66	3.31	1.64	4.10
2-D				
C_{max} (nmol/ml)	0.516–1.06	0.793	0.744	0.203
t_{max} (h)	1–6	3.83	4.00	1.97
AUC (nmol h ml^{-1})	8.49–26.84	13.02	10.27	6.19
3-D				
C_{max} (nmol/ml)	0.219–718	0.539	0.627	0.242
t_{max} (h)	1–9	4.75	4.00	2.42
AUC (nmol h ml^{-1})	3.65–30.33	13.01	12.55	7.70

In five patients the AUC values noted for 2-D were higher than those recorded for 3-D (range of ratio AUC_{2-D}/AUC_{3-D} 0.5–2.5). A typical concentration-time curve generated for Trofo and its metabolites in patient 4 after oral administration is given in Fig. 2. In five patients the concentration-time curves show two maxima, possibly indicating distribution to a second compartment.

In Fig. 3 the urinary excretion of Trofo and its metabolites as determined in patient 4 is shown. Unchanged Trofo was found to a lesser extent. In this patient the Cl_{renal} value noted for Trofo was 50.9 ml/min for the first 24 h, i.e., 3.7% of the total Trofo Cl/F. As hyothesized, Ifo was the main metabolite excreted in urine from all patients, with the Ifo/Cyclo ratio being 45/1. In Table 3 the mean total urinary excretion determined in the five patients measured is shown. The mean overall urinary excretion of Trofo and its dechloroethylated metabolites as determined for the first 24 h was 12.2% (5.8% of the delivered dose).

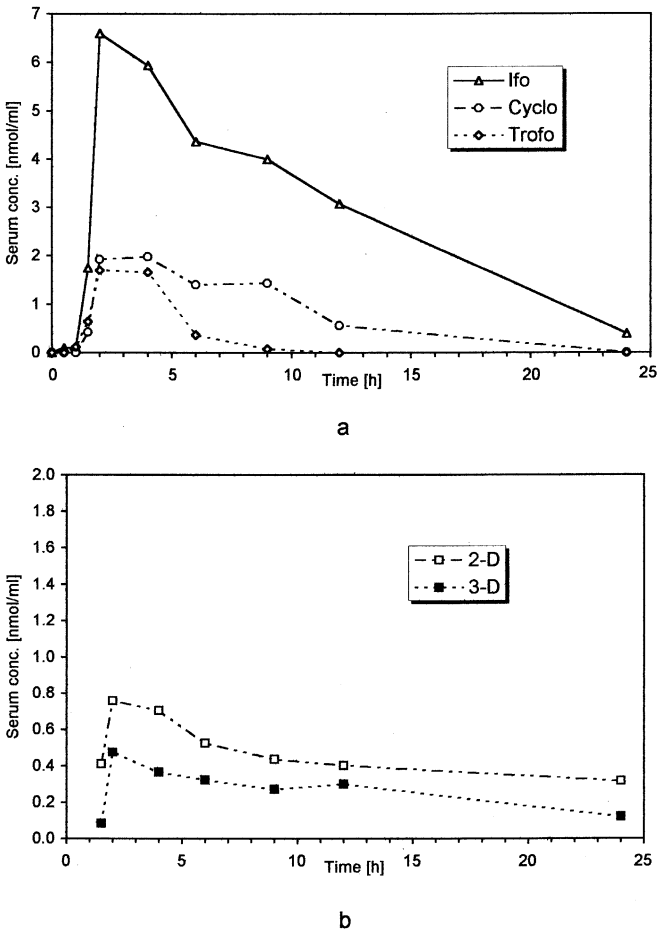

Fig. 2a, b Serum kinetics determined in patient 4 after oral administration of Trofo. **a** Trofo, Ifo, and Cyclo. **b** 2-D and 3-D

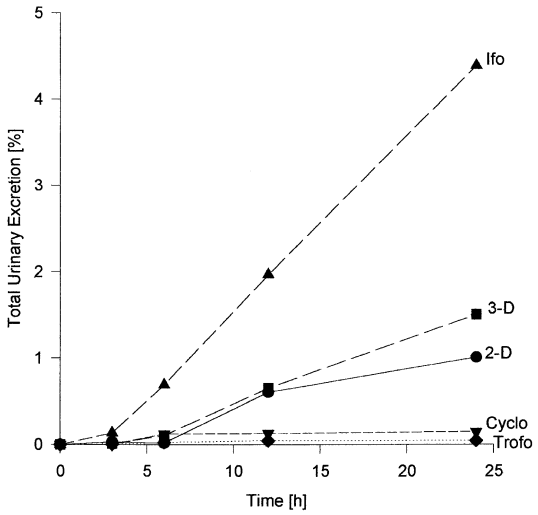

Fig. 3 Cumulative urinary excretion of Trofo and its metabolites (patient 4), expressed as a percentage of the delivered dose of Trofo

Table 3 Cumulative urinary excretion of Trofo and its major metabolites as determined in 5 patiens and expressed as a percentage of the delivered dose (ND Not detectable)

Patient number	2-D	3-D	Ifo	Cyclo	Trofo
1	3.08	3.78	4.66	0.21	0.76
2	2.36	1.63	0.85	0.05	ND
4	1.01	1.50	4.39	0.15	0.05
6	4.12	5.07	10.71	0.09	0.48
13	1.67	1.57	2.00	0.01	0.01
Mean	2.45	2.71	4.52	0.10	0.33
SD	1.21	1.63	3.81	0.08	0.36

Discussion

To our knowledge, this is the pharmacokinetics investigation in humans that involves direct determination of Trofo and its dechloroethylated metabolites. Earlier Trofo studies used only indirect methods, e.g., the determination of the alkylating activity of serum samples by the photometric 4-nitrobenzylpyridine (NBF) method [7], which gives only an estimate of alkylating activity. Furthermore, this method cannot quantify the metabolites of Trofo and does not provide a clear-cut discrimination between active and inactive metabolites [16].

N-Dechloroethylation of Ifo characterizes the metabolic profile and inactivates up to 30% of a given dose in children [2, 3]. This study was carried out to quantify the amount of dechloroethylation occuring after oral administration of Trofo. Trofo was given together with etoposide. In vitro studies conducted by a member of our group with isolated P-450 enzymes indicate that etoposide does not influence the metabolism of the oxazaphosphorines (Boos, unpublished results).

Trofo is rapidly absorbed and shows a short plasma half-life due to its immediate metabolism to the primary metabolites Ifo and Cyclo. Urinary excretion plays only a minor role in the total clearance of Trofo. The metabolic dechloroethylation of Trofo consists of two steps. Side-chain oxidation at the exocyclic nitrogen to Ifo is the predominant metabolic pathway in the first step. In the second step, Ifo and Cyclo are further dechloroethylated to 2-D and 3-D (in varying amounts).

High variability was observed in the AUC and C_{max} data. This might have been due to variations in metabolism as shown by the varying ratios of the AUC values recorded for the parent compound and the metabolites. The sum of the overall AUC values noted for all metabolites and the parent compound did not show such a high degree of variability (63.9 ± 22.4 nmol h ml^{-1}), indicating that metabolism more than absorption was the main reason for the high variability. However, metabolism of Trofo in the gastrointestinal tract before or during absorption cannot be excluded, although this has never been reported for Ifo or Cyclo.

Bioavailability could not be measured for Trofo because an i.v. formulation is not availabe at present. Pain at the injection site and thrombosis have been reported when Trofo is given i.v. [12]. More than 20 years ago, human serum kinetics determined after the oral administration of Trofo were compared with those of Cyclo using the NBP method, showing that the alkylating activity was about 50% higher for Trofo in comparison with Cyclo [7]. For Ifo and Cyclo it has been reported that bioavailability is almost 100% after oral administration [9, 21]. However, from our data the amount absorbed cannot be estimated, because not all metabolites were quantified and only 12% of the delivered dose was recovered in the urine.

Our results confirm former in vitro observations that side-chain oxidation of Trofo mainly results in the formation of Ifo [5]. Dechloroethylation is an inactivation pathway following the administration of Cyclo or Ifo. In contrast, after Trofo administration this process produces drugs that can subsequently be activated. Therpeutic drug monitoring of patients receiving Trofo therefore requires the measurement of Trofo, Ifo, and Cyclo serum levels.

After i.v. administration of Ifo the molecule is preferentially dechloroethylated at the cyclic nitrogen, resulting in high 3-D levels [3]. After oral administration of Trofo, Ifo is the main metabolite, indicating that Trofo is mainly dechloroethylated at the exocyclic nitrogen. From the AUC values we obtained for 2-D and 3-D, no predominant metabolism at a specific position of the molecule can be seen. The formation of 2-D and 3-D and the release of chloroacetaldehyde has been associated with higher neurologic toxicity and renal damage [8, 10] during Ifo therapy. The amounts of 2-D and 3-D formed after Trofo administration are comparatively low, but during the formation of Ifo and Cyclo for Trofo, chloroacetaldehyde is also produced. To date a relationship between toxic side effects and chloroacetaldehyde has never been proven, since different groups have reported highly variable plasma chloroacetaldehyde concentrations [20] and these concentrations do not correlate with the side effects. Neither neurotoxicity nor Fanconi-like syndromes have yet been reported following treatment with Trofo, although in small groups the drug has been given at doses of up to 1700 mg/m^2 [13]. Nevertheless, in clinical trials of Trofo these possible side effects should be considered.

Only 12% of delivered dose was recovered in urine as unchanged Trofo and the dechloroethylated metabolites together. This finding may indicate that a high amount of the drug is metabolized through hydroxylation in position 4 to the active 4-hydroxy metabolites. Dechloroethylation of Trofo leads to formation not only of the inactive metabolites 2-D and 3-D but, mainly, also of Ifo, which can be further activated. This observation can be advantageous for Trofo in comparison with Ifo and Cyclo.

In summary, *N*-dechloroethylation of Trofo mainly results in Ifo and Cyclo. This can be advantageous for oral therapy, because autoinduction of dechloroethylation during repeated administration, which has been reported for Ifo therapy [18], should lead to increased levels of Ifo and Cyclo, which can be further activated. Quantification of the active 4-hydroxy derivatives of Trofo, Ifo, and Cyclo will be an interesting aspect of further studies. However, this requires more sophisticated analytical methodology, because one has to separate the 4-hydroxy derivatives of Trofo, Ifo, and Cyclo, and these metabolites are very unstable.

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