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Long-term pharmacokinetics of thio-TEPA, TEPA and total alkylating activity following i.v. bolus administration of thio-TEPA in ovarian cancer patients*

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Summary. The serum pharmacokinetics of unchanged thio-TEPA and the active metabolite TEPA and the urinary excretion of thio-TEPA, TEPA and total alkylating activity were studied after a single i.v. bolus injection of thio-TEPA in six ovarian cancer patients. TEPA was present in serum as of 5 min after drug administration, and its concentration rapidly reached a plateau in the range of 50–100 ng/ml. After about 3 h the serum concentration of TEPA exceeded that of thio-TEPA, and in five of the six patients the metabolite persisted longer than the parent drug in serum. AUCs of thio-TEPA and TEPA were 822 ±83 and 1,084 ±234 ng h/ml, respectively. The great interindividual variation encountered in the serum pharmacokinetics of TEPA may be of clinical importance and represents a further indication that pharmacokinetically guided dosing of thio-TEPA could be valuable. Urinary recoveries of both thio-TEPA and TEPA were low, together consituting <2% of the delivered dose. A substantial gap existed between this and the total urinary alkylating activity, which averaged 13% of the dose in terms of thio-TEPA equivalents. This gap strongly indicates the presence of other unknown metabolites.

Introduction

Little knowledge is available on the metabolism in humans of the alkylating agent thio-TEPA. It has been assumed that an oxidative desulphuration to TEPA represents the first metabolic step and that TEPA is the main metabolite. This is based on the general recognition that organic thiophosphates are metabolized to their oxygen analogues by liver microsomal enzymes in mammals [16] as well as the demonstration in animal studies of TEPA in plasma after the administration of thio-TEPA. However, these studies have also shown great interspecies differences in the metabolism of thio-TEPA [2, 5, 14].

Two groups have recently reported on the metabolic fate of thio-TEPA in human patients. McDermott et al. [13] published the plasma pharmacokinetics of thio-TEPA and TEPA in three patients after an intramuscular injection of thio-TEPA. At the first point of measurement 1 h after drug administration, the metabolite was demonstrated at concentrations roughly the same as those of the parent drug. A slower elimination of the metabolite as compared with the parent drug was indicated. However, both the clinical and pharmacokinetic data presented were limited, reflecting that this study was mainly a description of methodology for the determination of thio-TEPA and TEPA in plasma.

Cohen et al. [4] published a pharmacokinetic study of thio-TEPA and metabolites in 21 patients with advanced breast cancer who were treated with the VATH regimen (a combination of vinblastine, Adriamycin, thio-TEPA and halotestin). Again, the rapid occurrence of TEPA in plasma after the administration of thio-TEPA was demonstrated. At 2 h after administration, the concentration of TEPA in plasma equaled or exceeded that of the parent drug, and the rate of elimination of TEPA from plasma was claimed to be the slower one. A substantial urinary excretion of TEPA, greatly exceeding that of the unchanged drug, was demonstrated and urinary alkylating activity, assessed by the 4-(*p*-nitro-benzyl) pyridine reaction (NBP), was found to exceed the sum of thio-TEPA plus TEPA, indicating the presence of additional active metabolites. However, in this study one cannot exclude the possibility of interactions with other drugs in the VATH regimen, and several of the patients had impaired liver function. The blood-sampling period after drug administration was very short, only 2 h in the majority of the patients. Two additional studies have recently been published in abstract form, describing the metabolic conversion of thio-TEPA to TEPA [11, 17]. A separate study has shown a similar in vitro cytotoxic activity for thio-TEPA and TEPA [3].

The aim of the present work was to study in more detail the pharmacokinetics of thio-TEPA and TEPA in serum, their urinary excretion and total urinary alkylating activity during a long-term period after a single i.v. administration of thio-TEPA to a homogeneous group of patients with ovarian cancer.

Materials and methods

Patients. Six patients treated for epithelial ovarian cancer were included in the study. All patients initially underwent

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Table 1. Patient characteristics

Patient number	Age (years)	Body surface (m^2)	Clinical stage (FIGO)	Performance status[a] (WHO)	Hb[a] (g/l)	Leucocyte count[a] ($\times 10^9$/l)	Platelet count[a] ($\times 10^9$/l)	Serum creatinine[a] (μmol/l)	Serum bilirubin[a] (μmol/l)
1	62	1.6	III	0	12.0	4.5	273	99	6
2	68	1.9	III	1	11.6	2.7	100	97	12
3	80	1.8	IA	0	14.8	5.0	243	95	12
4	85	1.7	II	1	13.4	4.9	144	101	7
5	72	1.9	IA	0	12.4	4.8	272	102	5
6	72	1.6	IV	1	12.1	4.2	133	90	4

[a] Immediately before the course under study

a laparotomy for maximal tumour clearing prior to single-drug chemotherapy with thio-TEPA. The median age of the patients was 73 years (range, 62–85 years). At the time of study, all patients had normal liver and renal functions as judged by serum bilirubin and creatinine concentrations and the WHO performance status was in the range of 0–1. None of the patients had received cancer chemotherapy prior to the thio-TEPA treatment. The patient characteristics are given in Table 1.

Treatment. Two loading courses of 60 mg thio-TEPA were followed by maintenance courses of 20 mg; no dose correction based on body weight or body surface area was made. All courses were given at 2-week intervals: Loading courses, by intravenous (i.v.) bolus injection and maintenance courses, usually by intramuscular (i.m.) injection. Prior to each thio-TEPA course a haematologic status consisting of haemoglobin (Hb), leucocyte count (lc) and platelet count (pc) was obtained. A Hb of <9.5 g/l, a lc of $<2.5\times10^9$/l or a pc of $<100\times10^9$/l resulted in dose reduction.

Blood and urine sampling. Blood and urine sampling was carried out during one maintenance course in each patient for the purpose of studying the serum pharmacokinetics and urinary excretion of unchanged drug and the TEPA metabolite, together with total urinary alkylating activity. The course under study was given by i.v. bolus injection. Venous blood samples were taken from a Venflon cannula prior to and 1, 5, 10, 20 and 30 min as well as 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 30 and 48 h after the administration of thio-TEPA. The samples were allowed to coagulate for 0.5 h before serum was separated by centrifugation (1,100 *g* for 10 min) and stored at −20° C until analysis. Urine was collected at 2-h intervals during the first 12 h, then 12 h thereafter and, finally, for the remaining 24–48 h after drug administration. Urine was immediately refrigerated and aliquots were taken from the portions and stored at −20° C until analysis. The mean duration of treatment was 46 weeks (range, 22–94 weeks) and the mean accumulated dose was 467 mg (range, 260–805 mg).

During the treatment periods prior to the course under study, myelosuppression made dose reduction necessary in two patients, whereas the haematologic statuses enabled the administration of a full dose to all six patients during the course under study (Table 1).

Estimation of thio-TEPA concentrations. The quantitation of thio-TEPA in serum and urine was done by gas chromatography according to our previously described method [8]. The detection limit was 5 ng/ml.

Estimation of TEPA concentrations. TEPA in serum and urine was quantitated according to a modification of the methods described by McDermott et al. [13] and Egorin et al. [6]. Pure TEPA was obtained from Polysciences Inc. (USA), after which standard solutions in the range of 10–1,000 ng/ml were made using commercial serum and 0.9% NaCl. A 1-ml sample or standard was mixed in a vortex with 50 μl internal standard [diphenylamine (10 μg/ml)] and 100 μl sodium cacodylate buffer (0.5 *M*, pH 7.4). The mixture was passed through a Bond Elut C-18 column that had been preconditioned with 5 ml methanol and 5 ml distilled water.

After being washed with water (2 × 5 ml), the column was eluted with 2.4 ml methanol. The first 0.4 ml eluate was discarded and the remainder was gently evaporated at 37° C under a stream of dried air to approximately 100 μl, 1 μl of which was injected onto the gas chromatograph. The gas chromatographic conditions were similar to those used for quantitation of thio-TEPA. The retention time of TEPA under these conditions was 4.3 min and the detection limit was 10 ng/ml.

Stability experiments using standard solutions of TEPA in serum and 0.9% NaCl revealed a substantial degradation of the compound when it had been stored at 4° C for 24 h and after repeated freezing and thawing. For these reasons, all standards were made fresh from a stock solution of ethanol stored at −20° C, where the stability was satisfactory for up to 6 months. Patient sera stored at −20° C remained stable for at least 3 months, provided that analysis was done rapidly after thawing.

McDermott et al. [13] reported a substantial loss of TEPA during the C_{18}-extraction/evaporation step and ascribed it to the relatively polar nature and high volatility of the compound. Our own recovery experiments with and without the C_{18}-extraction/evaporation step were in agreement with this, showing recoveries of 60%, 74% and 70% for standards of 100, 500 and 1,000 ng/ml, respectively ($n = 10$). However, the standard curve for TEPA was linear in the range of 10–1,000 ng/ml ($r^2 = 0.999$), and the variations in repeated analyses ($n = 10$) of standards of 100, 500 and 1,000 ng/ml were 5.1%, 7.4% and 10.9%, respectively.

Estimation of alkylating activity. Alkylating activity in urine was determined by colorimetry after reaction with 4-(*p*-nitrobenzyl)-pyridine (NBP) according to the method

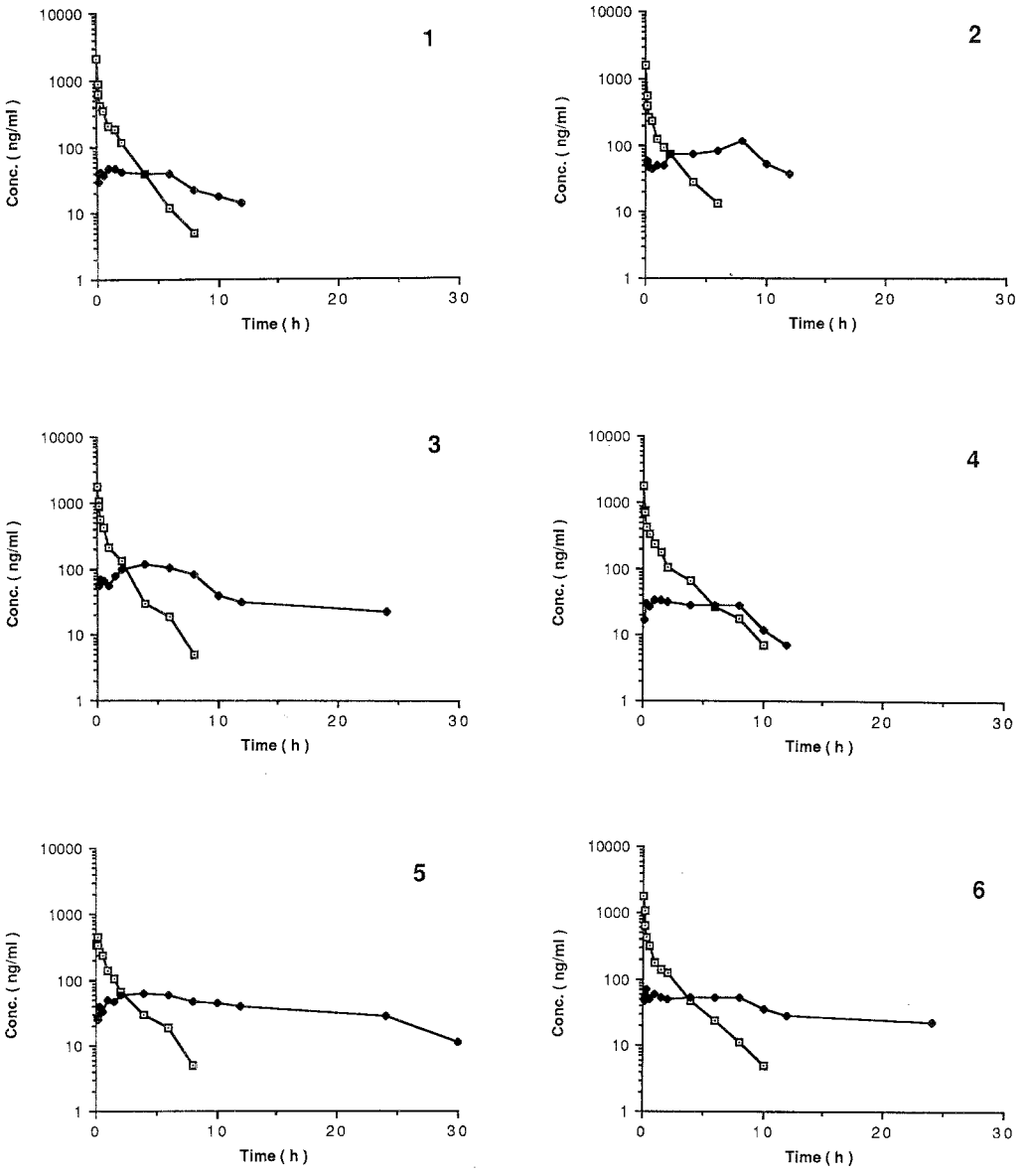

Fig. 1. Individual serum concentration – time relationships of thio-TEPA (□—— □) and TEPA (◆—— ◆) in six patients after i. v. bolus injection of 20 mg thio-TEPA

described by Friedman and Boger [7]. Linearity was demonstrated with thio-TEPA standards in the range of 0.1 – 10.0 μg/ml, and alkylating activity was expressed as thio-TEPA alkylating activity equivalents. Separate experiments with TEPA demonstrated that its intrinsic ability to alkylate NBP was equivalent to that of thio-TEPA. The detection limit of alkylating activity was 0.1 μg/ml.

Pharmacokinetic calculations. Model-independent pharmacokinetic parameters were calculated. The elimination rate constant (K_e) was calculated for thio-TEPA and TEPA by least-squares regression from the terminal linear slope of the semilogarithmic serum concentration-time plots. The elimination half-lives were derived from the equation:

$$t_{1/2} = \ln 2/K_e.$$

The area under the serum concentration-time curve was calculated from 1 min after drug administration to infinite time using the trapezoidal rule:

$$AUC = \sum_{i=1\ \min}^{n-1} (t_{i+1} - t_i) \quad \frac{C_{i+1} + C_i}{2} + \frac{C_n}{K_e},$$

where C_i represents the serum concentration measured at time t_i and C_n denotes the last measurable serum concentration at time t_n.

Results

Semilogarithmic plots of the serum concentration-time relationships for thio-TEPA and TEPA for individual patients are given in Fig. 1. In addition, the average curves of the six patients are shown on a larger time scale in Fig. 2 to show in more detail the initial decay of the parent drug and build-up of the metabolite. In accordance with pre-

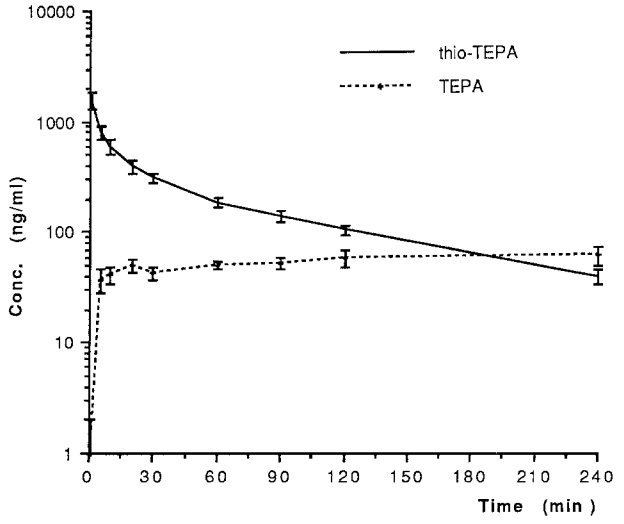

Fig. 2. Serum concentration – time relationships of thio-TEPA and TEPA in six patients during the initial 4 h after i. v. bolus injection of 20 mg thio-TEPA. *Points* and *bars* represent means and SEM, respectively

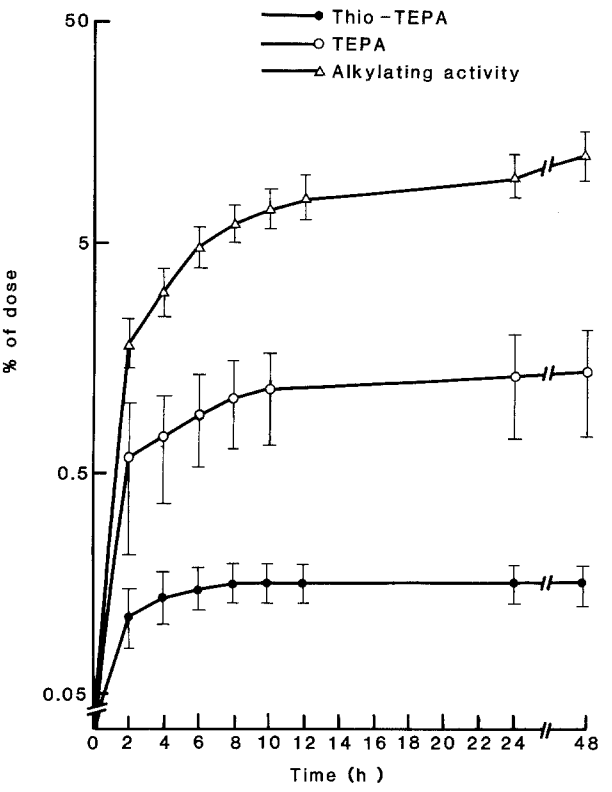

Fig. 3. Cumulative urinary excretion of thio-TEPA, TEPA and total alkylating activity during the 48 h after i. v. bolus injection of 20 mg thio-TEPA. *Points* and *bars* represent means and SEM, respectively

viously reported studies by others [4] and ourselves [9, 10], the curves for unchanged drug were characterized by a short phase of distribution that was completed after about 1 h, followed by a rapid elimination, the elimination half-life being 1.58 ± 0.13 h.

TEPA was identified in serum 5 min after the administration of thio-TEPA in five of the patients, and in all six after 10 min. At 1 min after administration the metabolite was not detected, whereas the peak concentration of thio-TEPA was observed at that time in all but one patient. After an initially rapid increase, the concentration of TEPA reached a plateau in the range of 50–100 ng/ml. The time after which the concentration of TEPA exceeded that of thio-TEPA averaged 3.2 h. In five of the patients the plateau in the concentration of the metabolite persisted until the parent drug had nearly disappeared from the serum, then a slow elimination was observed. When calculated from the point where the concentration of TEPA equalled or exceeded 10 times that of the parent drug, the elimination half-life of TEPA was 11.2 ± 3.36 h among these five patients. In patient 4, a parallel decline of thio-TEPA and TEPA was observed; thus, the elimination half-life of the metabolite in this patient was equal to or shorter than that of the unchanged drug, which was 2.04 h.

Measurable amounts of thio-TEPA in serum were not demonstrated later than 10 h after drug administration, whereas traces of the drug were detected after 12 h in three patients. In contrast, the metabolite could be measured 12 h after drug administration in all six patients and up to 30 h in one patient; in addition, trace amounts of TEPA were present at 30 h in two patients. At 48 h after drug administration, no trace of the metabolite could be detected.

Individual and mean pharmacokinetic data calculated from Fig. 1 are given in Table 2. The interindividual variation of the AUC was most pronounced for the metabolite, with a >5-fold range of variation. Although the peak concentration of TEPA in serum only reached about 5% of that of thio-TEPA, in terms of the AUC, the exposure of the body to the parent drug and metabolite was of the same order of magnitude: 822 ± 83 and $1{,}084 \pm 234$ ng h/ml for thio-TEPA and TEPA, respectively.

Figure 3 shows the cumulative urinary excretion of thio-TEPA, TEPA and total alkylating activity during the 48 h after thio-TEPA administration. Individual data for total urinary excretion are given in Table 3. The urinary excretion of unchanged drug averaged 0.16% of the dose (range, 0.05%–0.27%). All patients had thio-TEPA in their urine until 6 h after administration, and no patient excreted detectable amounts of the drug for longer than 10 h.

The corresponding data for TEPA showed a high degree of interindividual variation. Up to 4.33% of the dose was excreted in the urine; however, in two of the patients no urinary TEPA at all was found. The mean urinary excretion of TEPA was 1.40% of the delivered dose. In patient 6, who exhibited the maximal TEPA excretion, the metabolite was present in all urine samples collected until 48 h after drug administration. In the remaining patients, TEPA was not demonstrated in urine later than 8 h after administration. The two patients who did not excrete TEPA in their urine (patients 1 and 4) had the lowest AUC values for TEPA in serum, whereas the two patients with the highest AUC values (patients 3 and 6) showed the highest urinary excretion of TEPA.

In terms of thio-TEPA equivalents, the mean urinary alkylating activity was 13.0% of the delivered dose (range, 5.8%–28.5%). Alkylating activity was present in urine collected during the first 24 h after drug administration in all patients as well as in the final urine samples (from 24 to 48 h) from four of the patients. Urinary alkylating activity

Table 2. Serum pharmacokinetics of thio-TEPA (tT) and TEPA (T) in six patients after the administration of 20 mg thio-TEPA by i.v. bolus injection

Patient number	Peak concentration: (ng/ml)		tmax: (min)		Elimination rate constant: (h^{-1})		Elimination half-life: (h)		Area under the curve[a] (ng h/ml)			
	tT	T	tT	T	tT	T	tT	T	tT	T	tT + T	T/tT
1	2,161	47	1	30	0.58	0.12	1.20	5.78	919	498	1,417	0.5
2	1,599	116	1	480	0.44	0.18	1.58	3.85	587	1,068	1,655	1.8
3	1,750	119	1	240	0.55	0.06	1.26	11.55	979	1,654	2,633	1.7
4	1,802	34	1	60	0.34	0.34	2.04	≤2.04	996	312	1,308	0.3
5	456	62	5	240	0.41	0.06	1.69	11.55	540	1,316	1,856	2.5
6	1,778	69	1	10	0.40	0.03	1.73	23.10	913	1,657	2.570	1.6
Mean ±SEM	1,591 ±239	75 ±14	2 ±1	177 ±74	0.45 ±0.04	0.13 ±0.05	1.58 ±0.13	9.65 ±3.13	822 ±83	1,084 ±234	1,906 ±233	1.4 ±0.3

a From 1 min after drug administration, extrapolated to infinity

always exceeded the alkylating activity constituted by the sum of urinary thio-TEPA and TEPA, strongly indicating the presence of other metabolites in the urine.

Discussion

The rapid occurrence of TEPA in human plasma after the administration of thio-TEPA has also recently been reported by others [4, 11, 13, 17]. The longer persistence of TEPA than of the parent drug in plasma has previously been described by Cohen et al. [4], based on samples taken up to 4 h after drug administration. Our data, which are based on serum sampling until 48 h after drug administration, give further information about the kinetics of the metabolite. After an initial net increase, the distribution and elimination of TEPA was obviously balanced by its formation from the parent drug, as reflected by the constant serum concentration of TEPA from 1 to 8–10 h. Only when the serum concentration of thio-TEPA was substantially lower than that of TEPA did a net elimination of the metabolite occur. A lower elimination rate for TEPA than for the parent drug could be observed in five of the six patients. This TEPA low elimination rate made a major contribution to its AUC. The mean AUC of TEPA exceeded that of thio-TEPA, although the peak serum concentration of TEPA only reached about 5% of that of thio-TEPA.

As expected, the interindividual variation in pharmacokinetics was more pronounced for TEPA than for thio-TEPA. The AUCs of TEPA for patients 1 and 4 were 498 and 312 ng h/ml, respectively, and differed significantly from the mean of 1,084 ng h/ml. These two patients also showed the lowest peak values for the metabolite in serum, and were also the two with no detectable amounts in their urine. This could be explained by a relative impairment of the formation of TEPA from thio-TEPA, a fast metabolism of TEPA, or a combination of these factors. The corresponding thio-TEPA data for the two patients does not show altered kinetics, which would be expected if formation of the metabolite from the parent drug was impaired and no alternative metabolic pathway existed. Thus, a faster metabolism of TEPA in these patients could be a reasonable explanation, provided that the volumes of distribution of TEPA are relatively equal among patients. The parallel decline of thio-TEPA and TEPA in patient 4 could indicate that the formation of the metabolite from the parent drug was the rate-limiting step for the elimination of the metabolite in this patient.

Urinary recovery of both thio-TEPA and TEPA was low, averaging <2% of the dose, and the total alkylating activity excreted in urine largely exceeded the sum of thio-TEPA and TEPA. The total alkylating activity excreted in urine was 13.0%±3.4% of the dose. However, this relatively low figure, should not be taken literally to indicate a low urinary recovery of thio-TEPA metabolites, because the alkylating activity in this study refers to thio-TEPA equivalents and we do not know the intrinsic ability of potential, non-identified metabolites to alkylate NBP. In addition, the presence of non-alkylating metabolites in urine would further complicate the picture. A study in rats after the administration of radioactively labeled thio-TEPA showed a 24-h urinary radioactive recovery of >90%, with <5% recovered from faeces and expired air [2]. To our knowledge, the only human study using the administration of radiolabeled drug showed urinary radioactive recovery of 63% during the first 24 h in one patient after i.v. administration [15].

Table 3. Urinary excretion of thio-TEPA (tT), TEPA (T) and alkylating activity (aa) in six patients until 48 h after the administration of 20 mg thio-TEPA by i.v. bolus injection

Patient number	Urinary excretion (% of dose):		
	tT	T	aa
1	0.18	0	5.8
2	0.27	1.44	28.5
3	0.21	2.41	12.0
4	0.05	0	10.7
5	0.10	0.22	6.6
6	0.16	4.33	14.3
Mean ±SEM	0.16 ±0.08	1.40 ±0.71	13.0 ±3.4

The urinary excretion of thio-TEPA, TEPA and alkylating activity reported by Cohen et al. [4] amounted to 1.5%, 4.2% and 24% of the dose, respectively, after thio-TEPA had been given in a combination regimen. The reason for the lower excretion in the present study could be due to methodology, but it could also reflect the fact that thio-TEPA was given as a single-drug to patients with normal hepatic and renal function. Apart from this difference, however, a substantial gap between the sum of thio-TEPA and TEPA and the total alkylating activity in urine was demonstrated in both studies. Obviously, the biotransformation of thio-TEPA is not restricted to its conversion to TEPA. A number of metabolites with possible cytotoxic activity may result from the liberation of aziridine rings from thio-TEPA and TEPA and the opening of rings.

We tried to measure total alkylating activity in serum, hoping in that way to clarify the importance of unknown thio-TEPA metabolites. Such a study has been successfully carried out with cyclophosphamide by Juma et al. [12]. Our attempt, however, was hampered by a great loss of sensitivity and much less stability of the colored reaction product as compared with urine, making a pharmacokinetic interpretation of the data unreliable. A similar attempt to determine alkylating activity in plasma after high-dose administration of thio-TEPA has recently been reported by Ackland et al. [1], with the same poor result.

The desirability of further characterization of the metabolism of thio-TEPA is highlighted by the recent study by Teicher et al. [18] of thio-TEPA antigrowth effects on the EMT6 mouse mammary carcinoma cell line in vitro and in vivo. A potentiation of the effect was observed in vivo, suggesting that a metabolic activation of thio-TEPA had occurred.

In conclusion, this study further confirmed that a rapid conversion of thio-TEPA to TEPA occurs in humans. Due to TEPA's slow elimination, its AUC in serum is comparable with that of the parent drug, indicating an important contribution of this metabolite to the total cytotoxic effect. The great interindividual variation in the AUC of TEPA may be ascribed to factors such as alternative metabolic pathways of the parent drug, differences in the volume of distribution and different rates of further metabolism. This variation may contribute to the observed variation in clinical response, and it emphasizes the possible importance of pharmacokinetically guided dosing of thio-TEPA, which we have previously proposed [9, 10]. The urinary excretion of thio-TEPA and TEPA makes only a minor contribution to the total alkylating activity in the urine, pointing towards the presence of other metabolites. However, the origin, structure and function of such metabolites remain unknown.

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