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Single and repeated dose pharmacokinetics of thio-TEPA in patients treated for ovarian carcinoma*

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Summary. Triethylenethiophosphoramide (thio-TEPA) pharmacokinetics were studied in 15 patients being treated for epithelial ovarian carcinoma. Unchanged thio-TEPA was assayed in serum and urine by means of a gas chromatographic procedure.

No accumulation or alteration of the pharmacokinetics occurred during therapy, which was continued for up to 7 months with biweekly administrations of 20 mg, after two initial loading courses with 20 mg daily for 3 consecutive days 2 weeks apart. No significant difference in the pharmacokinetics between i.m. and i.v. administration was demonstrated. However, three patients showed a reduced absorption ability from the i.m. injection site to the systemic circulation and an apparent increase in the elimination half-life (3.86 ± 0.97 h), which could be of clinical relevance.

A first-order elimination process with a short elimination half-life (~ 1.5 h) was demonstrated for thio-TEPA in all patients after i.v. administration. The apparent volume of distribution averaged 50 l. The renal clearance was below 1% of the total-body clearance, which averaged 412 ml/min. The urinary excretion of unchanged thio-TEPA was complete within 8 h after administration, with an average urinary recovery of 0.14% of the dose. Calculation of the area under the serum concentration vs time curve revealed wide variation between patients (range 517–1480 ng/h ml^{-1}), indicating the need for drug monitoring during therapy.

Introduction

Triethylenethiophosphoramide (thio-TEPA) was introduced into cancer treatment in the early 1950s and is one of the oldest alkylating agents still in clinical use. Besides epithelial ovarian cancer, the drug is used for adjuvant treatment of bladder carcinoma [13], metastatic breast carcinoma [7], and meningeal carcinomatosis [14]. In the treatment of ovarian cancer thio-TEPA is used both as single agent [3, 16] and in combination therapy [1].

Due to acid instability the drug has to be injected; both the i.m. and the i.v. route have been widely used. Thio-TEPA causes little local irritation in biologic tissue and has also been given by intracavitary administration (abdomen, pleura, subarachnoid space). Alkylating agents, including thio-TEPA, are known to be myelotoxic. Apart from that, the drug is very well tolerated, causing little or no deterioration in the patients' quality of life.

A close dose – response relationship of thio-TEPA has been found following administration to monolayer cultures of human ovarian cancer cells [16]. The same relationship for thio-TEPA has been demonstrated in studies of in vitro models with human bladder tumor cells [9] and mammary tumors induced in rats [5]. In a study of the dose – response relationship in cancer patients, it was concluded that a direct positive correlation existed [8]. However, in clinical work, the dosing of thio-TEPA has often been based on the development of myelotoxicity. Dosing up to bone marrow depression has been advocated. The scientific basis for this, however, is scanty. In a study of the relation between drug response and toxicity in 144 patients with ovarian cancer no positive correlation was found, and it was concluded that the development of myelotoxicity was not a good indicator for correct dosing of thio-TEPA [15].

A better knowledge of the clinical pharmacology of thio-TEPA is therefore needed. The aim of the present study was to examine the pharmacokinetics of thio-TEPA in ovarian cancer patients.

Materials and methods

Patients. Fifteen patients with epithelial ovarian carcinoma were included in the study. The median age at the start of therapy was 65 years (range 43–75 years). All patients underwent laparotomy for the purpose of surgical tumor clearing and staging of disease prior to chemotherapy. All patients had normal renal and hepatic functions. The patient characteristics are given in Table 1.

Prior to loading and maintenance treatment a hematologic status, with hemoglobin concentration (Hgb), white blood cell count (WBC), and platelet count (PC), was obtained. Limits were: Hgb, 9.5 g/100 ml; WBC, $2.5 \cdot 10^9$/l, and PC, $100 \cdot 10^9$/l. Values below these limits were taken to indicate dose reduction.

Treatment. Thio-TEPA (Lederle Laboratories) was dissolved in 0.9% sterile saline to a concentration of 1 mg/ml. Two loading courses of 20 mg thio-TEPA daily for 3 con-

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Table 1. Patient characteristics

Pt no.	Age (years)	Body weight (kg)	Stage (FIGO)	Postoperative status[a]	Histologic diagnosis
1	70	56	III	C	Papillomatous adenocarcinoma
2	70	90	III	C	Serous cystadenocarcinoma
3	57	66	III	C	Low differentiation, adenocarcinoma
4	75	41	III	C	Low differentiation, adenocarcinoma
5	74	50	IA	A	Mucinous cystadenocarcinoma
6	69	48	III	C	Mixed adenocarcinoma (serous/endometroid)
7	43	55	III	C	Serous cystadenocarcinoma
8	52	84	III	C	Serous papillomatous adenocarcinoma
9	67	55	IV	C	Low differentiation, adenocarcinoma
10	75	50	II	B	Mucinous cystadenocarcinoma
11	73	55	III	C	Papillomatous adenocarcinoma
12	66	76	III	C	Serous papillomatous adenocarcinoma
13	73	65	IC	A	Mucinous cystadenoma (borderline lesion)
14	64	80	IV	A	Low differentiation, adenocarcinoma
15	62	51	III	C	Serous cystadenocarcinoma

[a] A, no macroscopic residual tumor; B, residual tumor, diameter ≤ 2 cm; C, residual tumor, diameter > 2 cm

secutive days were given, with a 2-week period between the two courses followed by maintenance therapy with 20 mg once every 2 weeks. The drug was administered i.m. or by i.v. bolus injection (i.v.). No dose correction based on the body surface area was made.

Six patients received the two loading courses according to a crossover design for the mode of administration. Three were given the first 3-day loading course by i.m. administration and the second 3-day course 2 weeks later by i.v. administration, and for the other three the order was the opposite. The remaining nine patients received most of the treatment by i.m. injection. All patients were given injections of vitamins and anabolic steroids as supportive therapy.

Blood and urine sampling. From a Venflon cannula 5 ml blood was taken before, and 0.5, 1, 1.5, 2, 4, 6, 8, and 24 h after administration of thio-TEPA. The samples were allowed to coagulate at 4 °C. Within 2 h serum was separated by centrifugation (1100 *g* for 10 min at room temperature) and stored at −20 °C until analysis, which was performed within 1 week. Urine was collected at intervals of 2 h for 8 h after administration of thio-TEPA. An additional urine sample was taken 24 h after administration. Aliquots were immediately placed at 4 °C and within 2 h were moved storage at −20 °C. The urine samples were analyzed within 1 week.

The six patients in the crossover study underwent the sampling procedures after the first and third doses in both loading courses. For the remaining nine patients sampling was performed after the first dose during loading and/or during maintenance therapy.

Drug assay. In a previous article we described a gas chromatographic method for quantitation of thio-TEPA in serum and urine [6]. Thio-TEPA was extracted from 500 µl serum or urine into 300 µl ethylacetate, and 1 µl of the organic layer was injected onto the gas chromatograph.

Diphenylamine was used as internal standard. The retention times for thio-TEPA and diphenylamine were 1.9 and 2.5 min, respectively. The detection limit of thio-TEPA in serum and urine was 5 ng/ml. All analyses were performed in duplicate.

Pharmacokinetic calculations. The following model-independent parameters were calculated. The apparent first-order elimination rate constant (K_e) of thio-TEPA was calculated from the slope of the serum concentration – time curve in the linear phase of the semilogarithmic plot. The slope was computed as the least-square regression line, with equal weight for each point. The correlation (r) between the experimental points and the straight line was always better than 0.980.

The elimination half-life ($t^1/_2$) was derived from the equation:

$$t^1/_2 = \ln 2/K_e$$

The area under the serum concentration – time curve from zero to infinite time after a single dose ($AUC_{0-\infty}$) and after repeated doses ($AUC_{0-24\,h}$) was calculated by the trapezoid rule:

$$AUC = \sum_{i=0}^{n-1} (t_{i+1} - t_i) \frac{C_{i+1} + C_i}{2} + \frac{C_n}{K_e},$$

where C_i represents the the serum concentration measured at time t_i and C_n denotes the last measurable serum concentration on the serum concentration – time curve at time t_n. The term C_n/K_e was only used for the determination of $AUC_{0-\infty}$.

For patients receiving thio-TEPA i.v. and i.m. the fraction of the dose entering the systemic circulation (F) was calculated as:

$$F = AUC_{i.m.}/AUC_{i.v.}$$

The apparent volume of distribution (V_d) was obtained from the equation:

$$V_d = D/AUC \times K_e$$

where D is the dose administered i.v.
Total body clearance (Cl_t) was calculated as:

$$Cl_t = V_d \times K_e$$

and renal clearance (Cl_r) as:

$$Cl_r = X_u/AUC$$

where X_u is the total amount of thio-TEPA excreted in the urine after a single dose or the amount excreted during a dosage interval; AUC represents the corresponding area under the serum concentration – time curve.

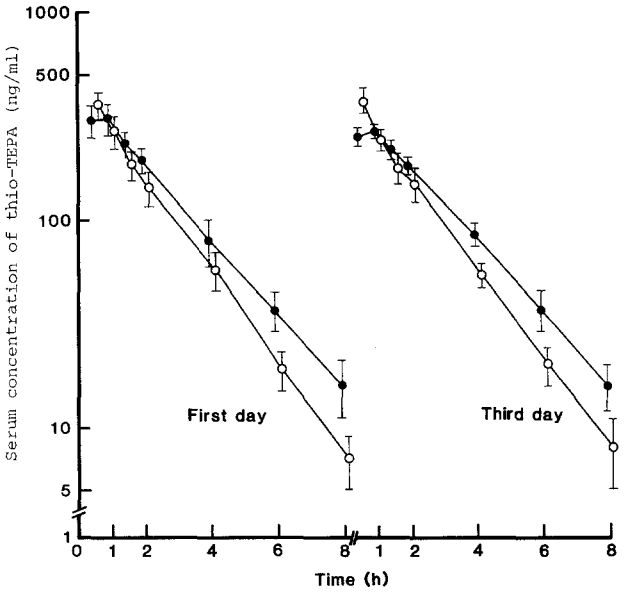

Fig. 1. The serum concentration – time relationship of thio-TEPA in six patients after the first and third daily doses (20 mg) by the i.m. route (●———●) and as i.v. bolus injections (○———○) *Points* and *bars* represent means ± SEM

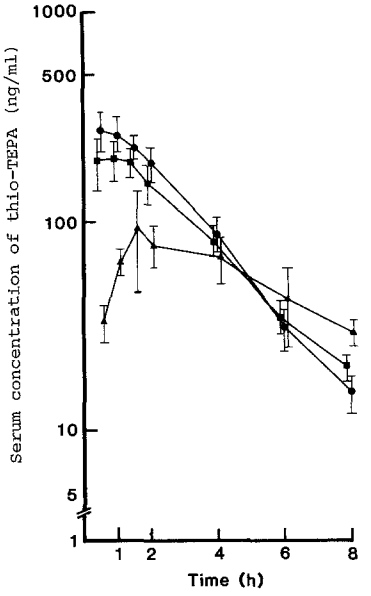

Fig. 2. Serum concentration – time relationship of thio-TEPA in nine patients (■———■) after a single dose (20 mg) i.m. during loading or maintenance therapy. ▲———▲, curve for three patients with diverging pharmacokinetics; ●———●, curve for the remaining six patients. *Points* and *bars* represent means ± SEM

K_e, $t_{1/2}$ and AUC were calculated for all patients. In addition, V_d, Cl_t, and Cl_r were calculated for patients receiving thio-TEPA by i.v. administration.

Statistics. The results are presented individually or as means ± SEM. Differences between groups of observations have been evaluated statistically by the Wilcoxon rank sum test.

Results

Figure 1 shows the serum concentration – time relationship in six patients after the first and third doses of thio-TEPA according to the i.m./i.v. crossover design. The curve after i.v. administration was nearly linear from the first point measured 0.5 h after injection, indicating a fast initial distribution. After i.m. injection the absorption in to the systemic circulation seemed to be complete within 1 h after injection. The elimination process was found to be a first-order process after both modes of administration in all patients.

Table 2 gives the pharmacokinetic parameters of thio-TEPA. The area under the serum concentration – time curves after i.m. and i.v. administration were not significantly different, demonstrating that the fraction of the dose entering the systemic circulation after i.m. administration (F) was approximately 100% in these patients. No significant differences between the first and third doses were found with respect to the area under the curve or the elimination half-life, indicating that there was no accumulation potential of the drug with the dosage regimens used. The difference observed in the elimination half-life between i.m. and i.v. administration did not reach a statistically significant level.

Figure 2 shows the serum concentration – time relationship in nine patients after single doses of thio-TEPA injected i.m. Three patients in this group demonstrated kinetics which differed substantially from the others investigated, with a serum concentration – time profile that was also different from that observed in the crossover study. The average curve for these three patients (Fig. 2) was characterized by a prolonged absorption phase and a decreased rate of elimination, with an apparent elimination

Table 2. Pharmacokinetic parameters of thio-TEPA in six patients after the first and third consecutive daily doses (20 mg) i.m. and i.v.[a]

Administration regimen	Elimination half-life (h)		Area under the curve (ng h ml^{-1})	
	i.m.	i.v.	i.m.	i.v.
First dose	1.59 (±0.13)	1.37 (±0.09)	961 (±128)	904 (±145)
Third dose	1.65 (±0.15)	1.47 (±0.13)	889 (±82)	895 (±109)

[a] Figures shown represent mean (±SEM) in each case

Table 3. Pharmacokinetic parameters of thio-TEPA in nine patients after a single dose (20 mg) i.m. during loading or maintenance therapy[a]

Patient subgroups	Apparent elimination half-life (h)	Area under the curve (ng h ml^{-1})
$n = 9$	2.34 (±0.48)	738 (±107)
$n = 6$[b]	1.57 (±0.07)	809 (±152)
$n = 3$[b]	3.86 (±0.97)	597 (±83)

[a] Figures shown represent mean (±SEM) in each case

[b] Data observed in the three patients with divergent pharmacokinetics are displayed separately from the rest

Table 4. Pharmacokinetic parameters of thio-TEPA in three patients after the first dose (20 mg) of loading therapy and after a dose (20 mg) given after more than 10 previous doses (late dose)

Patient no.	Mode of administration	Elimination half-life (h)		Area under the curve (ng h ml^{-1})	
		First dose	Late dose	First dose	Late dose
6	i.m.	1.93	1.82	825	600
7	i.m.	1.24	1.36	509	585
12	i.v.	1.51	1.16	432	555

half-life of 3.86 ± 0.97 h. Table 3 gives the pharmacokinetic data corresponding to Fig. 2. When the data from the three patients with atypical kinetics are treated separately, the elimination half-life for the remaining patients is about equal to the half-life found for the six patients in the crossover study group after i.m. administration (1.57 ± 0.07 and 1.59 ± 0.13 h, respectively). The differences in elimination half-life and AUC between the three patients with atypical and the remaining six in the present group are statistically significant ($P < 0.05$).

It was possible to obtain supplementary data from two of the three patients with diverging pharmacokinetics during the treatment regimen, after one i.m. and one i.v. injection in each. After i.m. injection the prolonged absorption phase and reduced elimination rate was found again in both patients. The elimination half-lives this time were 4.62 h and 3.15 h, respectively. After i.v. administration of the drug, elimination half-lives of 1.44 and 1.87 h were found. The fraction of the dose entering the systemic circulation after i.m. administration (F) for the two patients were calculated at 71% and 56%.

In Table 4 $t_{1/2}$ and AUC after the first dose in the first loading course and after a dose given during the maintenance therapy when more than 10 doses had been previously given are compared. Data from three patients were available, and no obvious sign of alterations in the kinetics were demonstrated.

In Table 5 supplementary pharmacokinetic parameters are given for the six patients in the crossover study group after i.v. administration of the drug (20 mg). A volume of distribution of about 50 l and urinary clearance that was always below 1% of the total-body clearance was demonstrated. When a correction for body weight was made, the dose variation between patients was relatively minor (0.34 ± 0.02 mg/kg).

In Fig. 3 the urinary excretion of thio-TEPA after the third dose in the loading therapy after i.m. and i.v. administrations is shown. The urinary excretion seemed to be

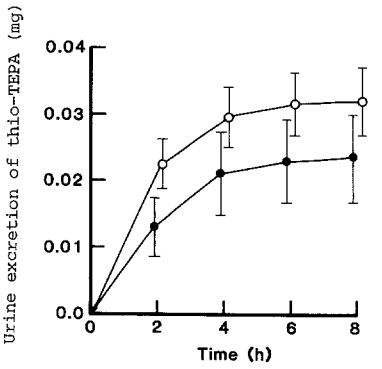

Fig. 3. Urinary excretion of thio-TEPA in six patients after the third consecutive daily dose (20 mg) i.m. (●———●) or as i.v. bolus (○———○). *Points* and *bars* represent means ± SEM

complete within 8 h. The drug was never detected in urine samples taken 24 h after administration. The urinary recovery of thio-TEPA (% of dose excreted in the urine) was 0.12% (range 0.04%–0.26%) and 0.16% (range 0.08%–0.21%) after i.m. and i.v. administration, respectively. The difference in urinary excretion between i.m. and i.v. administration was not statistically significant.

Discussion

Animal experiments have revealed wide variation between species in the distribution, metabolism, and elimination of thio-TEPA [2, 11]. Therefore, data from human patients are needed for the evaluation of its clinical pharmacology. The patients included in this study were homogeneous with respect to sex, disease and treatment (single-drug therapy for all patients).

We detected no drug in the blood or urine samples taken 24 h after the administration of thio-TEPA, which is in keeping with its short elimination half-life. Furthermore, no differences in the pharmacokinetics were found between the beginning and end of loading therapy or between the very first dose and a dose given after more than 3 months of therapy. This demonstrates a low accumulation potential of the drug and no induced alteration in the pharmacokinetics of the drug during therapy.

For 3 of the 15 patients the pharmacokinetics after i.m. administration was characterized by a prolonged phase of absorption followed by an apparent reduction in elimination rate. Two of these patients were also studied after i.v. administration of the drug. This revealed incomplete absorption into the systemic circulation after i.m. administration in these patients (F = 56% and 71%). The kinetics

Table 5. Pharmacokinetic parameters of thio-TEPA in 6 patients after intravenous administration (20 mg) the first and the third consecutive day of loading therapy[a]

	Elimination rate constant (h^{-1})	Elimination half life (h)	Area under the curve (ng h ml^{-1})	Apparent volume of distribution (l)	Total body clearance (ml/min)	Renal clearance (ml/min)
First day	0.54 (±0.05)	1.37 (±0.09)	904 (±145)	48 (±8)	421 (±67)	0.9 (±0.3)
Third day	0.51 (±0.04)	1.47 (±0.13)	895 (±109)	51 (±8)	403 (±51)	0.7 (±0.1)

[a] Figures given represent mean (± SEM) in each case

after i.v. administration showed 'normal' elimination rates, that is; the elimination half-life after i.v. administration was within the range found for the other patients studied after i.v. administration. We believe, therefore, that the reduced elimination rate observed in the three patients was secondary to the slow absorption rate from the i.m. injection site to the systemic circulation, with the drug at the injection site acting as a depot, thereby leading to a situation where the correct value for the elimination half-life could not be estimated. The additional pharmacokinetic trial in two of the three patients following a later i.m. injection revealed that the inherent factors for altered kinetics were stable in these patients.

Apart from the fact that a positive dose – response correlation exists for thio-TEPA, little is known about the penetration of the drug into cancer tissue and the mode of action at the subcellular level. However, the exposure of the target tissue to the drug, expressed by the area under the serum concentration versus time curve (AUC), is generally though to be of importance for the effect of cytostatics [12]. The data in Tables 2, 3, and 5 reveal considerable variation in the AUC at both modes of administration, with a three-fold range. This could explain, in part, the wide variation in response to treatment that is observed clinically.

The very low urinary recovery of thio-TEPA is in keeping with observations recorded in other studies [4, 11]. Mellett et al. [11] also found a high urinary recovery of isotope (up to 85%) when patients were treated with ^{14}C-labeled drug. This indicates that metabolites are excreted in the urine.

Two groups have recently reported the pharmacokinetics of thio-TEPA in human patients. McDermott et al. [10] utilized a capillary gas chromatographic method to assay thio-TEPA and reported first-order elimination of the drug in only one of three patients after a dose of 30 mg i.m. This is in contrast to our study, where first-order elimination kinetics have been demonstrated in all 15 patients. The lack of patient characteristics in the article makes closer analysis of the difference difficult.

Egorin et al. [4] have reported the pharmacokinetics in one patient after i.v. bolus injection of thio-TEPA using a gas chromatograpic method for assay of the drug. The elimination process was of first order, with an elimination half-life of 1.23 h which is in accord with our results. In Egorin et al.'s study the distribution phase was studied after i.v. injection, and a distribution half-life of 3.4 min was demonstrated, which agrees well with our results.

Oxidative desulfuration of thio-TEPA to TEPA (triethylenephosphoramide) has been thought to be the first step in the metabolism of the drug. Animal studies have revealed wide variation between species in the levels of TEPA in serum and urine [2, 11]. Egorin et al. [4] and McDermott et al. [10] both examined the content of TEPA in human serum after the administration of thio-TEPA and reported substantial amounts of the compound. A slower elimination of the metabolite than of the parent drug was indicated. TEPA concentrations 1–3 μg/ml in the urine were recorded in the first 12 h after the administration of thio-TEPA by Egorin et al. [4]. This, however, is in contrast to the results of Mellett et al., who were able to detect only trace amounts of TEPA in the urine from human patients [11]. In the present study we made no attempt to determine TEPA in blood or urine. It has been shown that TEPA cannot be extracted from serum or urine by means of ethylacetate [4], and in our determination of thio-TEPA we therefore did not codetermine TEPA. In our laboratory we are currently working on problems with separation of TEPA from the parent drug and its quantitation, in order to examine the metabolism and kinetics of thio-TEPA furhter in human patients.

In conclusion, this study has demonstrated a relatively rapid first-order elimination of thio-TEPA in all patients studied. No accumulation or alteration of the pharmacokinetics seems to occur during therapy. In most of the patients only minor differences in the pharmacokinetics were found between i.m. and i.v. administration, which were probably of no clinical importance. However, 3 of the 15 patients had diverging pharmacokinetics after i.m. administration, probably resulting from reduced absorption ability for the drug from the injection site to the systemic circulation. This disability to absorp the drug after i.m. injection might well have clinical implications, in that the response could be reduced, at least in cases where incomplete absorption of the dose occurs ($F \ll 100\%$). To overcome the problem of uncertain absorbtion after i.m. administration, we advocate that the drug should be given by the i.v. route.

The demonstration of a considerable variation in the area under the serum concentration – time curve might justify routine monitoring of the serum drug concentration to optimize dosing with respect to both response and toxicity.

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