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Clinical report

Urinary excretion of thioTEPA and its metabolites in patients treated with high-dose cyclophosphamide, thioTEPA and carboplatin

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The urinary excretion of *N,N′,N″*-triethylenethiophosphoramide (thioTEPA), and its metabolites *N,N′,N″*-triethylenephosphoramide (TEPA), *N,N′*-diethylene,*N″*-2-chloroethylphosphoramide (monochloroTEPA) and thioTEPA–mercapturate was determined in patients receiving thioTEPA as part of a high-dose combination chemotherapy regimen with cyclophosphamide and carboplatin. The thioTEPA dose was 40 or 60 mg/m^2 in short infusions, twice daily, during 4 days. Urine samples were collected after each voiding on each day of drug administration until 24–48 h after the last thioTEPA infusion. ThioTEPA, TEPA and monochloroTEPA concentrations were determined with gas chromatography and thioTEPA–mercapturate with liquid chromatography–mass spectrometry with direct sample injection. ThioTEPA was present in urine 30 min after infusion and was still excreted 18 h after the last infusion. All metabolites were detected in urine 1 h after infusion. Patients with a creatinine clearance above 140 ml/minl showed higher excretion of TEPA than patients with a creatinine clearance below 140 ml/min (12.8 versus 4.9%, *p*=0.01). The excretion of monochloroTEPA relative to the excreted amount of TEPA increased at lower pH values of the urine. The excretion of thioTEPA–mercapturate relative to the dose was higher in patients treated with 60 mg/m^2. Excretion of thioTEPA and monochloroTEPA both accounted for only 0.5% of the dose, while TEPA and thioTEPA–mercapturate both accounted for 11.1%.

Key words: monochloroTEPA, TEPA, thioTEPA, thioTEPA–mercapturate, urinary excretion.

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Introduction

The alkylating agent *N,N′,N″*-triethylenethiophosphoramide (thioTEPA) has been known to have anti-tumor activity for approximately 40 years.[1] Nowadays thioTEPA is being employed mainly in high-dose combination regimens for breast cancer, ovarian cancer and other solid tumors, because of its broad spectrum of antitumor activity and manageable toxicities.[2–7] The first reported metabolite of thioTEPA is *N,N′,N″*-triethylenephosphoramide (TEPA),[8] which is formed in the liver after oxidative desulfuration (Figure 1). This reaction is catalyzed by cytochrome P450 isoenzymes 2B1 and 2C11.[9,10] In a recent study, two new metabolites of thioTEPA, thioTEPA–mercapturate and *N,N′*-diethylene,*N″*-2-chloroethylphosphoramide (monochloroTEPA), were identified (Figure 1).[11] ThioTEPA–mercapturate is formed after glutathione conjugation, followed by the removal of the glutamate moiety by glutathionase and subsequent removal of glycine by a peptidase. The latter two enzymes are both present in the liver and kidney cytosol. Finally, the amino group of cysteine is acetylated by a hepatic *N*-acetylase, resulting in the mercapturic acid conjugate.[12,13] The conversion of TEPA to monochloroTEPA is influenced by the pH and the chloride concentration.[14] Urinary excretion of thioTEPA and TEPA has been reported to be 1.5 and 4.3% of the administered dose, respectively.[15,16] Data about the excretion of monochloroTEPA and thioTEPA–mercapturate are lacking.

Recently, a specific gas chromatographic (GC) assay for simultaneous determination of thioTEPA, TEPA and monochloroTEPA, and a liquid chromatography–mass spectrometric (LC-MS) assay for thioTEPA–mercapturate were developed and validated.[17,18] We have used

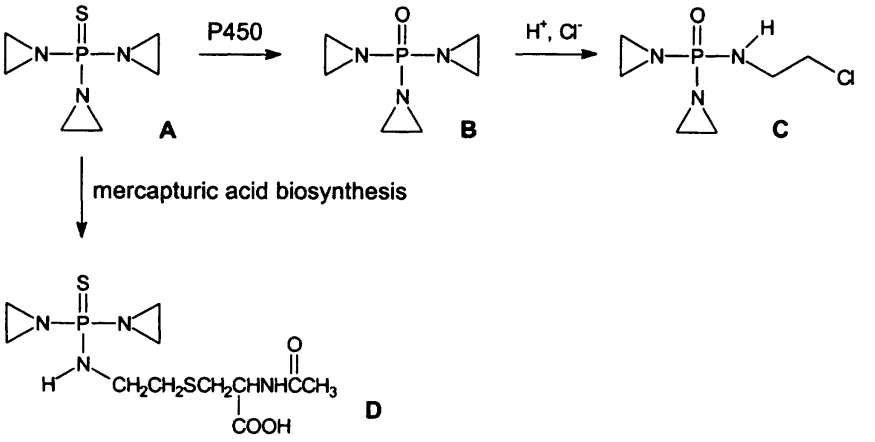

Figure 1. Biotransformation of thioTEPA (A) to TEPA (B), monochloroTEPA (C) and thioTEPA–mercapturate (D).

these methods to characterize the urinary excretion of thioTEPA, TEPA, monochloroTEPA and thioTEPA–mercapturate in eight patients receiving high-dose thioTEPA as part of a high-dose combination chemotherapeutic regimen including cyclophosphamide, thioTEPA and carboplatin.

Materials and methods

Patients and treatment

Six patients suffering from breast cancer and two patients with germ cell cancer were included in this study. At the time of study the patients had WHO performance status 0–1, and adequate renal function (creatinine clearance >60 ml/min) and liver function (bilirubin level <20 μM, and ALAT and ASAT levels <1.5 times the upper limit of normal).

Patients received either a single course of full-dose cyclophosphamide, thioTEPA and carboplatin (CTC) or multiple courses of 'tiny' CTC (tCTC) every 4 weeks.[4] The CTC regimen consisted of cyclophosphamide 1500 mg/m^2 as a 1-h infusion, immediately followed by carboplatin 400 mg/m^2 as a 1-h infusion (both once daily) and thioTEPA 60 mg/m^2 as a 30-min infusion twice daily, during 4 consecutive days. During the tCTC regimen the patient received precisely two-thirds of the dose of each agent as administered during the CTC regimen. Two days after the last administration of thioTEPA, the previously harvested peripheral blood progenitor cells were reinfused. Criteria for treatment with a second or third course of high-dose chemotherapy with CTC were: renal function loss $<20\%$, creatinine clearance >40 ml/min, normal bilirubin levels and ALAT and ASAT levels <2 times the upper limit of normal. Informed consent was obtained from all patients according to institutional guidelines.

Urine sampling

Urine was collected after each voiding on each day of drug administration (day 1–4) and during 24–48 h after the drug administration on day 4. Aliquots were taken from each portion and directly stored at -80°C until analysis.

Analytical procedure

ThioTEPA, TEPA and monochloroTEPA. ThioTEPA and its metabolites TEPA and monochloroTEPA were analyzed as previously described.[18] Briefly, after extraction of the parent drug and metabolites from urine using a 25% (v/v) solution of 1-propanol in chloroform, the compounds were separated by capillary GC, detected using a selective N/P detector and quantified by reference to an internal standard, diphenylamine. Limits of quantification were 25 ng/ml and mean recoveries ranged from 70 to 100%. Both accuracy and precision were less than 15%.

ThioTEPA–mercapturate. Measurement of thioTEPA–mercapturate was performed as previously described.[17] Briefly, urine was directly injected into a LC-MS system and quantified by reference to an internal standard, sulfadiazine. The limit of quantification was 1.0 μg/ml and the recovery was 84%. Both accuracy and precision were less than 20% for the lower limit of quantification, and less than 10% for the other concentration levels.

Results

Eight patients were included in this study, with a median age of 40 years (range 16–52). One patient received two courses of CTC, three patients received a single course of CTC, two patients received three courses of tCTC, one patient received two courses of tCTC and one patient received one course of tCTC. Six patients had breast cancer and two had relapsing germ cell cancer (Table 1). Mild rashes (grade 1–2) occurred in two patients during the first course of tCTC and CTC. One patient developed hypomania during the first course of tCTC and was treated with haloperidol. Epigastric pain occurred in one patient during the first course of CTC and required administration of morphine. During the second course of tCTC one patient developed moderately severe reversible veno-occlusive disease (VOD; bilirubin level up to 71 μmol/l). Tracheitis occurred in one patient during the third course of tCTC.

Individual data on total urinary excretion of thioTEPA and its metabolites after 4 days of thioTEPA administration are given in Table 2. ThioTEPA was detected in urine 30 min after infusion and was excreted up to 18 h after the last infusion. Excretion of thioTEPA reached the highest values within 6 h after infusion. The excretion of thioTEPA accounted, however, for only 0.5% (range 0.2–1.0) of the administered dose. TEPA was detectable 1 h after thioTEPA infusion and was still

Table 1. Patient characteristics

No. of patients	8
Male/female	2/6
Median age [years (range)]	40 (16–52)
Chemotherapeutic courses	14
tCTC[a]	9
CTC[b]	5
Tumor type	
breast	6
germ cell	2
Function tests [median (range)]	
renal	
creatinine clearance	150 (117–186) ml/min
Hepatic	
bilirubine ($N<20$ μM)	7 (6–21) μM
ASAT ($N<48$ U/l)	20 (11–52) U/l
ALAT ($N<75$ U/l)	31 (13–66) U/l

[a]Cyclophosphamide (4 g/m^2), thioTEPA (320 mg/m^2) and carboplatin (1.1 g/m^2) as multiple short infusions of 4 days.
[b]Cyclophosphamide (6 g/m^2), thioTEPA (480 mg/m^2) and carboplatin (1.6 g/m^2) as multiple short infusions of 4 days.

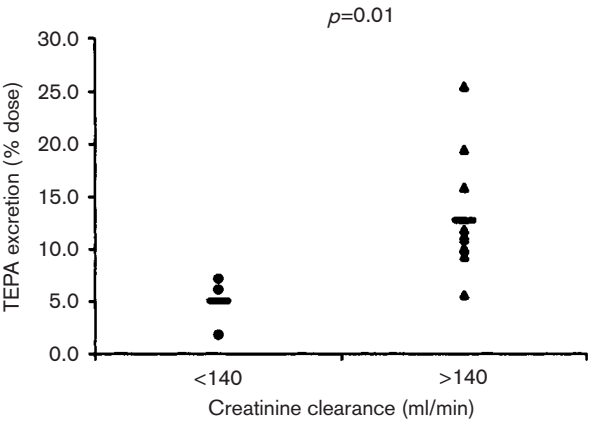

Figure 2. Excretion of TEPA relative to the administered dose of patients with a creatinine clearance <140 (●) and >140 (▲) ml/min. The horizontal dash represents the mean urinary excretion of TEPA.

Table 2. Urinary excretion of thioTEPA, TEPA, monochloroTEPA and thioTEPA–mercapturate in 8 patients after 4 days of thioTEPA administration (patients 1–4 were treated with the tCTC regimen and patients 5–8 were treated with the CTC regimen)

Patient no.	Urinary excretion (% of dose)			
	ThioTEPA	TEPA	MonochloroTEPA	ThioTEPA–mercapturate
1[a]	0.55 (±0.43)	13.0 (±2.5)	0.48 (±0.14)	10.4 (±4.7)
2[b]	0.67 (±0.11)	22.5 (±4.2)	0.53 (±0.10)	10.1 (±3.5)
3[a]	0.43 (±0.20)	5.5 (±4.1)	0.41 (±0.10)	8.2 (±0.9)
4	0.3	9.3	0.6	6.3
5	0.2	6.1	0.8	22.7
6[b]	0.73 (±0.13)	10.1 (±0)	0.38 (±0.02)	11.9 (±0.1)
7	0.4	7.2	0.3	12.3
8	0.8	11.0	0.4	12.8
Mean ± SEM	0.5 ± 0.3	11.1 ± 6.0	0.5 ± 0.1	11.1 ± 4.4

[a]Mean of three courses.
[b]Mean of two courses.

present in the urine 40 h after the last infusion. A significant difference was observed in TEPA excretion between patients with a creatinine clearance lower than 140 and higher than 140 ml/min (p=0.01, Mann–Whitney test). In patients with a creatinine clearance lower than 140 ml/min the excretion of TEPA was 4.9% of the administered dose and at levels higher than 140 ml/min the excretion was 12.8% (Figure 2). Excretion of the highest amount of TEPA (25.5%) was seen in the patient who developed VOD during the second course of tCTC. The amount of TEPA in all patients accounted for 11.1% (range 1.8–25.5) of the administered dose. MonochloroTEPA was detectable in urine within 1 h after thioTEPA administration and was still detectable 40 h after the last infusion. A relation between the urine pH and excreted amount of monochloroTEPA relative to the excreted amount of TEPA was observed. Figure 3 shows this relation for one patient. The excreted amount of monochloroTEPA was approximately 10% of the urinary excretion of TEPA in urine with pH>6.5; in urine with pH<6.5 this percentage amounted up to 50%. The total amount of monochloroTEPA was 0.5% (range 0.3–0.8) of the administered dose. ThioTEPA–mercapturate was detected within 1 h after infusion of thioTEPA and was still excreted 40 h after the last infusion. The highest amount of thioTEPA–mercapturate excretion was observed approximately 6 h after infusion. The excretion of thioTEPA–mercapturate relative to the administered dose was higher in the CTC regimen (p=0.03, Mann–Whitney test; Figure 4). The total amount of thioTEPA–mercapturate excreted was 11.1% (range 6.3–22.7) of the administered dose.

Discussion

The clinical usefulness of thioTEPA was demonstrated almost 50 years ago and it is still applied in clinical oncology, because of its broad spectrum of anti-tumor activity and because of the possibility to dramatically escalate its dose without causing severe extramedullary toxicity.[4,5] The first metabolite of thioTEPA, TEPA, was identified approximately 10 years after the introduction of thioTEPA.[8] The formation of further metabolites of thioTEPA was anticipated,[15,16] but only recently two new metabolites, monochloroTEPA and thioTEPA–mercapturate, were identified.[11] The development of a GC assay for the simultaneous analysis of thioTEPA, TEPA and monochloroTEPA, and a LC-MS assay for the analysis of thioTEPA–mercapturate in urine enabled the investigation of the clinical pharmacology of the two newly identified metabolites. Our study describes the urinary excretion of thioTEPA and its metabolites in patients treated with high-dose thioTEPA in combination with cyclophosphamide and carboplatin.

The metabolism of thioTEPA is very rapid, as its metabolites could be detected in urine within 1 h after infusion. The longer persistence of TEPA than of the parent drug in urine has also been reported by others; however, the excretion of the parent drug was completed after 18 h, which is longer than the reported 8–10 h.[15,16] The excretion of TEPA is dependent on the renal function of the patient. Patients with a low creatinine clearance showed a lower TEPA excretion than patients with a higher creatinine clearance. TEPA is probably excreted by several routes (e.g. urine, feces) and consequently a

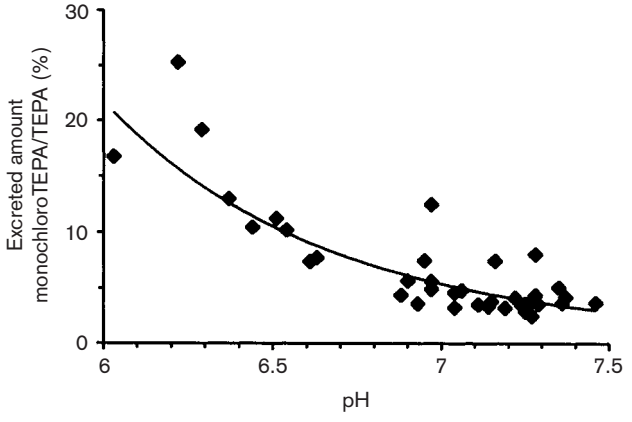

Figure 3. Excreted amount of monochloroTEPA relative to the excreted amount of TEPA as function of the pH of the urine in a patient treated with 60 mg/m^2.

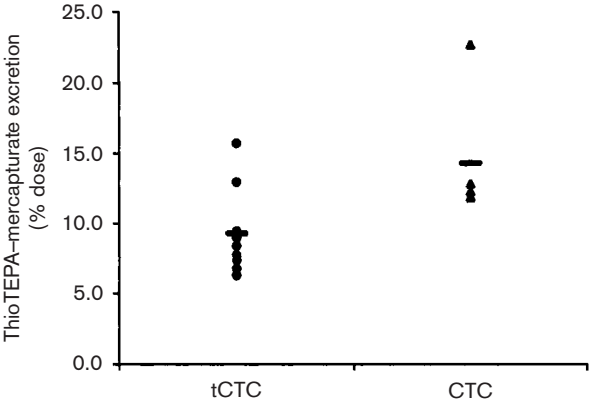

Figure 4. Excretion of thioTEPA–mercapturate relative to the administered dose in patients treated with 40 (●) and 60 (▲) mg/m^2 thioTEPA, twice daily. The horizontal dash represents the mean urinary excretion of thioTEPA–mercapturate.

better renal function results in higher TEPA urinary excretion. One patient developed VOD and had the highest excretion, and probably formation, of TEPA. It is at this point unclear whether any causal relationship between thioTEPA metabolism and the pathogenesis of VOD exists.

The mean urinary recovery of thioTEPA and its metabolites was 0.5% for the unchanged drug and monochloroTEPA, and 11% for TEPA and thioTEPA–mercapturate. The amount of unchanged drug is of the same order of magnitude as found by Cohen *et al.*[15] and Hagen *et al.*.[16] The previously reported urinary excretion of TEPA was in the range of 0.2–4.3% of the administered dose in patients treated with 20 mg or 12 mg/m^2.[15,16] The mean excretion of TEPA found in this study was 11% at dose levels of 40 and 60 mg/m^2. These results indicate that the metabolic conversion of thioTEPA to TEPA might be dose dependent. The high urinary excretion of TEPA found in our study may also be the result of the forced diuresis of the patients. The excretion of thioTEPA–mercapturate equals the excretion of TEPA, which was assumed earlier as the major metabolite of thioTEPA.[8,20,21] With the recently identified metabolites monochloroTEPA and thioTEPA–mercapturate, the urinary recovery of thioTEPA and its metabolites is several fold higher as has been reported for the unchanged drug and TEPA.[15,16] However, in our previous study a gap was still found between the total excreted amount of thioTEPA and its metabolites and the total urinary excretion, indicating the presence of still other alkylating metabolites.[11]

We have already shown that incubation of TEPA in urine at pH 4–6 resulted in the formation of monochloroTEPA.[22] Urine collection in our current study, however, was very strict and designed to prevent *in vitro* formation of monochloroTEPA. Samples were immediately stored at −80°C after collection and analyzed within 1 month; under these conditions there is no *in vitro* formation of monochloroTEPA. The conversion to monochloroTEPA could conceivably occur in plasma after which it is excreted in the urine, but monochloroTEPA was not detected in plasma. This study shows that the excretion of monochloroTEPA relative to the amount of TEPA was dependent on the pH of the urine. Probably TEPA is subjected to degradation during the dwell time in the bladder, resulting in the excretion of higher amounts of monochloroTEPA related to TEPA when the urine pH is lower.

TEPA, monochloroTEPA and thioTEPA–mercapturate are active alkylating agents.[11] What role these metabolites play in the anti-tumor activity of thioTEPA remains to be elucidated. TEPA is known to have cytotoxic activity against various tumors.[1] The chloroethylamine moiety in monochloroTEPA forms the alkylating active part in the cytotoxic nitrogen mustards.[23] However, if monochloroTEPA is formed in the bladder, it must be absorbed from the bladder to exert cytotoxic activity. We could not detect it in plasma—thus this is not very likely to occur. The first step in the mercapturic acid biosynthesis is glutathione conjugation,[12] which is a known mechanism of drug resistance in various tumors.[24,25] Thus, the formation of thioTEPA–mercapturate could play a role in resistance of tumors against thioTEPA.

In summary, this study shows that thioTEPA is extensively metabolized. The identified metabolites TEPA, monochloroTEPA and thioTEPA–mercapturate are all excreted in the urine. TEPA is probably subjected to degradation during the dwell time in the bladder, resulting in the excretion of both TEPA and monochloroTEPA. Besides the presence of TEPA, monochloroTEPA and thioTEPA–mercapturate in urine, other metabolites with alkylating activity are still excreted.

References

1. Sykes M, Karnovsky D, Phillips F, Burchenal J. Clinical studies of triethylenephosphoramide and diethylenephosphoramide compounds with nitrogen mustard-like activity. *Cancer* 1953; **6**: 142–8.
2. Antman K, Eder JP, Elias A, *et al*. High-dose thioTEPA alone and in combination regimens with bone marrow support. *Semin Oncol* 1990; **17**: 33–8.
3. Dimopoulos MA, Alexanian R, Przepiorka D *et al*. ThioTEPA, busulfan, and cyclophosphamide—a new preparative regimen for autologous marrow or blood stem-cell transplantation in high-risk multiple-myeloma. *Blood* 1993; **82**: 2324–8.
4. Rodenhuis S, Westerman A, Holtkamp MJ, *et al*. Feasibility of multiple courses of high-dose cyclophosphamide, thioTEPA and carboplatin for breast cancer or germ cell cancer. *J Clin Oncol* 1996; **14**: 1473–83.
5. Van der Wall E, Beijnen JH, Rodenhuis S. High-dose chemotherapy regimens for solid tumors. *Cancer Treat Rev* 1995; **21**: 105–32.
6. Van der Wall E, Nooijen WJ, Baars JW, *et al*. High-dose carboplatin, thioTEPA and cyclophosphamide (CTC) with peripheral-blood stem-cell support in the adjuvant therapy of high-risk breast-cancer—a practical approach. *Br J Cancer* 1995; **71**: 857–62.
7. Vaughan WP, Reed EC, Edwards B, Kessinger A. High-dose cyclophosphamide, thioTEPA and hydroxyurea with autologous hematopoietic stem-cell rescue—an effective consolidation chemotherapy regimen for early metastatic breast-cancer. *Bone Marrow Transplant* 1994; **13**: 619–24.
8. Mellet LB, Woods LA. The comparative physiological disposition of thioTEPA and TEPA in the dog. *Cancer Res* 1960; **20**: 524–32.

9. Chang TKH, Chen G, Waxman DJ. Modulation of thioTEPA antitumor-activity *in vivo* by alteration of liver cytochrome P450-catalyzed drug-metabolism. *J Pharmacol Exp Ther* 1995; **274**: 270–5.
10. Ng SF, Waxman DJ. Biotransformation of *N,N′,N″*-triethylenethiophosphoramide: oxidative desulfuration to yield *N,N′,N″*-triethylenephosphoramide associated with suicide inactivation of a phenobarbital-inducible hepatic P-450 monooxygenase. *Cancer Res* 1990; **50**: 464–71.
11. Maanen MJ van, Tijhof IM, Damen JMA, *et al*. A search for new metabolites of *N,N′,N″*-triethylenethiophosphoramide. *Cancer Res* 1999; **59**: 4720–4.
12. Chasseaud LF. Conjugation with glutathione and mercapturic acid excretion. In: Arias IM, Jakoby WB, eds. *Glutathione: metabolism and function*. New York: Raven Press 1976: 77–144.
13. Gibson GG, Skett P. *Introduction to drug metabolism*. London: Chapman & Hall 1986.
14. Maanen MJ van, Tijhof IM, Damen MJA, Zwikker JW, Beijnen JH. The degradation of *N,N′,N″*-triethylenephosphoramide in aqueous solutions: a qualitative and kinetic study. *Int J Pharm* 2000; **196**: 85–94.
15. Cohen BE, Egorin MJ, Kohlhepp EA, Aisner J, Gutierrez PL. Human plasma pharmacokinetics and urinary excretion of thioTEPA and its metabolites. *Cancer Treat Rep* 1986; **70**: 859–64.
16. Hagen B, Neverdal G, Walstad RA, Nilsen OG. Long-term pharmacokinetics of thioTEPA, TEPA and total alkylating activity following i.v. bolus administration of thioTEPA in ovarian cancer patients. *Cancer Chemother Pharmacol* 1990; **25**: 257–62.
17. Maanen MJ van, Beijnen JH. Liquid chromatographic–mass spectrometric determination of the novel, recently identified thioTEPA metabolite, thioTEPA–mercapturate, in urine. *J Chromatogr B* 1999; **732**: 73–80.
18. Maanen MJ van, Doesburg Smits K, Beijnen JH Simultaneous determination of thioTEPA, TEPA, and a novel, recently identified metabolite of thioTEPA, monochloroTEPA, in urine using capillary gas chromatography. *J Chromatogr B* 2000; **742**: 335–43.
19. Van der Wall E, Richel RJ, Kusumanto YH, *et al*. Feasibility study of FEC-chemotherapy with dose-intensive epirubicin as initial treatment in high-risk breast cancer. *Ann Oncol* 1993; **4**: 791–2.
20. Craig AW, Fox BW, Jackson H. Metabolic studies of ^{32}P-labeled triethylenethiophosphoramide. *Biochem Pharmacol* 1959; **3**: 42–50.
21. Mellet LB, Hodgson MD, Woods LA. Absorption and fate of C14-labeled *N,N′,N″*-triethylenethiophosphoramide (thioTEPA) in humans and dogs. *J Lab Clin Med* 1962; **60**: 818–25.
22. Maanen MJ van, Doesburg Smits K, Beijnen JH. Stability of thioTEPA and its metabolites, TEPA, monochloroTEPA and thioTEPA–mercapturate in plasma and urine. *Int J Pharm* 2000; **200**: 187–94.
23. Farmer PB. Metabolism and reactions of alkylating agents. *Pharmacol Ther* 1987; **35**: 301–58.
24. Tsuchida S, Sato K. Glutathione tranferases and cancer. *CRC Crit Rev Biochem-Mol Biol* 1992; **27**: 337–84.
25. Waxman DJ. Glutathione S-transferases: role in alkylating agent resistance and possible target for modulation chemotherapy—a review. *Cancer Res* 1990; **50**: 6449–54.

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