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Note

Gas chromatographic assay of triethylenethiophosphoramide in serum and urine

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The alkylating agent triethylenethiophosphoramide (thiotepa) has been used for about 30 years in cancer treatment. However, better knowledge of the pharmacokinetic and penetration properties of the drug in patients during therapy is necessary for the determination of individual optimal dosing regimens. Side-effects dependent on dose or serum concentration may also be evaluated and perhaps abolished by drug monitoring. Therefore, a method for the quantitation of the drug in human biological material is needed. Several reports on thiotepa assays using radiolabelled drug [1, 2], fluorometry [3] or spectrophotometry [4—6] have been published. However, the methods described are either time-consuming, insensitive or require a large sample volume. They are, consequently, not suitable for advanced pharmacokinetic studies or for clinical drug monitoring. Our attempt to determine thiotepa by means of high-performance liquid chromatography failed because the drug is very volatile and disappears when extracts of serum or urine are evaporated to dryness. Taking into consideration the chemical structure and volatility of thiotepa, a single extraction procedure without any evaporation step, followed by a gas chromatographic (GC) separation and a nitrogen—phosphorus-sensitive detection of the drug, was carried out.

MATERIALS AND METHODS

Instrumentation

A gas—liquid chromatograph (Carlo Erba 2351) equipped with a nitrogen—phosphorus (NP)-specific detector and an Ombiscribe recorder (Houston

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Instruments) was used. The glass column was packed with 3% OV-17 on 100—120 mesh Gas Chrom Q (Supelco). Helium was used as the carrier gas. The column temperature was 195°C while injector and detector temperatures were 250°C. The gas flow-rates were 33, 37 and 225 ml/min for helium, hydrogen and air, respectively.

Chemicals and standards

Ethyl acetate (Merck, p.a.) was used for the extraction of the drug from serum or urine. Standard solutions of thiotepa (Lederle Laboratories) in the range 5—500 ng/ml were made in commercial serum and 0.9% sodium chloride for serum and urine analyses, respectively. Diphenylamine was used as internal standard and 50 μl of the standard solution (10 μg/ml) was added to all samples and standards. The standard solutions were stored at —70°C.

Procedure

Internal standard (50 μl) and ethyl acetate (300 μl) were added to 500-μl samples or standards in micro test-tubes (Eppendorf 3810). The tubes were whirlmixed for 30 s and then centrifuged at 600 *g* for 5 min. The organic layer was transferred to new microtubes and 1 μl was injected into the gas chromatograph. The internal standard method was used for the determination of drug concentration. Peak-height ratios of drug to diphenylamine were plotted versus the concentration of drug added to blank samples of commercial serum and 0.9% sodium chloride. The standards and samples were kept on ice and protected from light after thawing. All the microtubes were capped and sent for destruction after use according to the laboratory routines for handling cytotoxic drugs.

Pharmacokinetics

Blood (5 ml) was taken 0.5, 1, 1.5, 2, 4, 6, 8 and 24 h after intramuscular injection of thiotepa in patients treated for ovarian cancer. Urine was collected at 2-h intervals for the first 8 h after injection, thereafter urine was collected until 24 h had passed. Serum and urine were stored at —70°C and analysed within one week.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of serum and urine samples from a patient with ovarian cancer, before and after intramuscular injection of 20 mg of thiotepa. The retention times for thiotepa and the internal standard were 1.9 and 2.5 min, respectively. The drug-free sample showed no interfering peaks in the chromatogram at the place where thiotepa was eluted from the column. In addition, it should be noted that chromatograms obtained from blank serum and urine samples showed no peaks interfering with the internal standard peak.

Linearity and sensitivity

The GC system response (ratio of height of thiotepa peak to the height of internal standard peak) for thiotepa in commercial serum and 0.9% sodium chloride after extraction was linear from 5 to 500 ng/ml; the straight lines

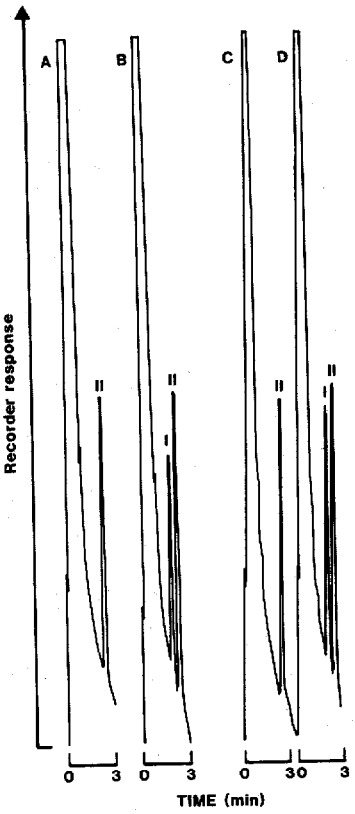

Fig. 1. Chromatograms of thiotepa (I) and internal standard (II) in serum (A, B) and urine (C, D) extracts from a patient with ovarian cancer before (A, C) and after (B, D) an intramuscular injection of 20 mg of thiotepa.

passed through the origin and were reproducible from day to day. The results of linear regression analyses (mean ± S.D., n = 10) for standards in 0.9% sodium chloride were: slope = 0.0161 ± 0.0017, y intercept = −0.0138 ± 0.0713 and r = 0.9999; for standards in serum: slope = 0.0165 ± 0.0015, y intercept = 0.0273 ± 0.1138 and r = 0.9994. The slope value for aqueous standards is in agreement with that for serum standards. The limit of detection was 5 ng/ml, allowing a signal-to-noise ratio of 2.

Precision

The precision of the method was assessed by repeated analyses of three different thiotepa concentrations in serum. Within-day variation and day-to-day variation were evaluated ranging from 4.6 to 10.8% and from 5.4 to 11.1%, respectively (Table I).

Recovery

The recovery was investigated by comparing peak-height ratios of standard solutions after extraction, with peak-height ratios after direct injection of standards with the same concentrations in methanol. The internal standard was added to the thiotepa serum standards after extraction. Recovery ranged

TABLE I

PRECISION STUDY

Sample No.	Thiotepa concentration (ng/ml)	Day-to-day precision (n = 10)		Within-day precision (n = 10)	
		Peak-height ratio (mean ± S.D.)	C.V. (%)	Peak-height ratio (mean ± S.D.)	C.V. (%)
1	25	0.36 ± 0.04	11.1	0.38 ± 0.04	10.8
2	100	1.49 ± 0.08	5.4	1.52 ± 0.12	7.6
3	500	7.82 ± 0.65	8.3	8.25 ± 0.38	4.6

TABLE II

RECOVERY STUDY

Sample No.	Thiotepa concentration (ng/ml)	Peak-height ratio			Recovery (%)	
		Extracted from 0.9% NaCl	Extracted from serum	Without extraction	NaCl	Serum
1	25	0.30	0.36	0.38	79	95
2	50	0.58	0.62	0.68	85	91
3	75	1.06	1.00	1.10	96	91
4	100	1.44	1.46	1.46	99	100
5	200	3.24	2.90	3.26	99	89
6	500	8.46	8.28	8.48	100	98

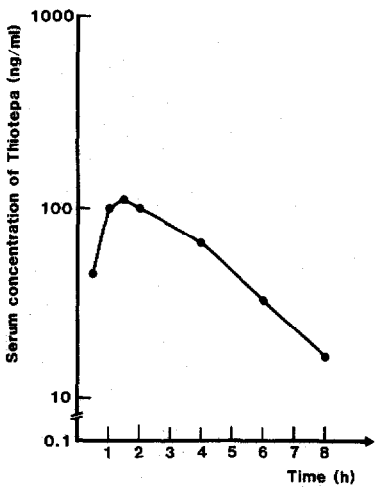

Fig. 2. Serum concentration—time relationship in a patient with ovarian cancer after intramuscular injection of 20 mg of thiotepa.

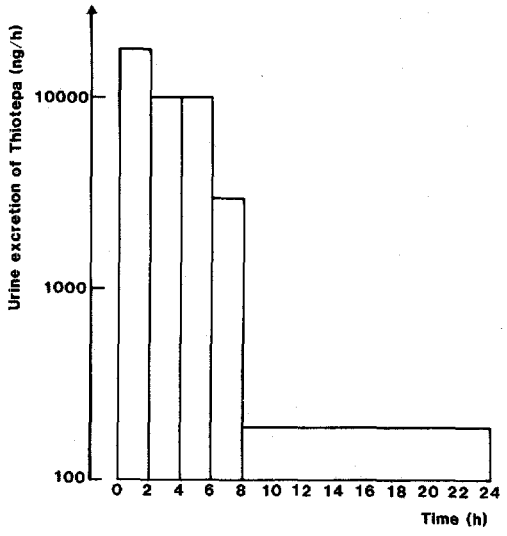

Fig. 3. Urinary excretion in the first 24 h following the intramuscular injection of 20 mg of thiotepa in a patient with ovarian cancer.

from 79 to 100% with no dependence on drug concentration within the range studied (Table II).

Clinical results

The results of plasma and urine concentration analyses in a 42-year-old woman treated for epithelial ovarian carcinoma are illustrated in Figs. 2 and 3. The patient received a single intramuscular dose of 20 mg of thiotepa. The plasma concentration—time curve is consistent with a linear first-order pharmacokinetic process. The elimination half-life was 1.2 h, calculated between 2 and 8 h after drug administration. Corresponding half-life values were found in the urine. However, the urinary recovery of thiotepa was very low (< 1%), indicating that the main route of elimination is metabolism.

In conclusion, the present GC method is simple, requires only small sample volumes and is highly sensitive compared to previously reported methods. The selectivity of the method is demonstrated by the fact that there are no interfering peaks in the chromatograms of blank patient samples at the place where thiotepa is eluted from the column. The method is therefore convenient for drug monitoring in patients treated with thiotepa in conventional doses, and for all sorts of research situations dealing with low thiotepa concentrations. A method which seems rather similar to ours has been published in abstract form [7]. However, a full description of the methodology is missing.

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