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Note

High-performance liquid chromatographic analysis of N,N′,N″-triethylenethiophosphoramide in human plasma

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The anti-neoplastic drug N,N′,N″-triethylenethiophosphoramide (Thio-TEPA) is a polyfunctional alkylating agent, which, since its clinical introduction [1], has been used in the treatment of meningeal neoplasia [2,3], breast cancer [4,5], and superficial bladder cancer [6,7] Recently, high-dose Thio-TEPA has been used as either a single agent [8,9] or in combination with cyclophosphamide [10], followed by autologous bone marrow transplantation

Early attempts to measure Thio-TEPA utilizing radiolabelled drug [11], paper chromatography [12], and fluorometric [13] or spectrometric assays [14] were too insensitive and non-specific to yield well characterized pharmacokinetic data An effective gas chromatographic (GC) method has been developed [15,16] and successfully used for pharmacokinetic studies of Thio-TEPA [9,10,17] An equally sensitive high-performance liquid chromatographic (HPLC) method has not been reported, although HPLC has been used

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for high-dose (135–1215 mg/m^2 intravenously over three days) Thio-TEPA pharmacokinetics [8]

In this study, a convenient and sensitive HPLC method for the analysis of Thio-TEPA in human plasma from patients treated with a pharmacologic dosage (30 mg/m^2) of Thio-TEPA will be presented The HPLC method approaches the sensitivity demonstrated by GC and yields comparable pharmacokinetic results

EXPERIMENTAL

Chemicals

Parenteral Thio-TEPA (15 mg Thio-TEPA, 80 mg sodium chloride, and 50 mg sodium bicarbonate per vial) was obtained from Lederle Labs (Pearl River, NY, U S A) The chromatography solvents (water, methanol and acetonitrile) were all HPLC-grade and were purchased from Burdick and Jackson (Muskegon, MI, U S A)

Sample preparation

Patient blood samples (5 ml) were obtained at selected times following a 10-min zero-order drug infusion Samples were centrifuged at 4 °C and 1200 *g* for 10 min and the plasma was removed and stored at −70 °C until analysis Human plasma (1 0 ml) was applied to a preconditioned (10 ml methanol followed by 10 ml water) C_{18} Bond Elut disposable column from Analytichem International (Harbor City, CA, U S A) The column was washed with water (10 ml) followed by 20% acetonitrile (1 0 ml) and the Thio-TEPA was eluted with 100% acetonitrile (1 0 ml) The solvent was evaporated to dryness with a stream of nitrogen at room temperature The residue was reconstituted in 20% acetonitrile (500 μl) and filtered through a Millipore (Bedford, MA, U S A) centrifuge filter (Ultrafree-MC, 0 22 μm Durapore) The HPLC injector volume was 100 μl

High-performance liquid chromatography

The chromatographic system consisted of a Hewlett-Packard (Palo Alto, CA, U S A) HP-1090 Series A liquid chromatograph equipped with an autoinjector/autosampler and an HP1040A diode-array UV detector The column effluent was monitored at 200 nm The chromatograph was operated with a Hewlett Packard HP-85B personal computer and data interpreted with a DPU multi-channel integrator Chromatography was performed on an Alltech (Deerfield, IL, U S A) reversed-phase C_{18} analytical column (Spherisorb ODS II, 5 μm, 250 mm × 4 6 mm I D) The isocratic mobile phase of acetonitrile–water (20 80, v/v) was delivered at a flow-rate of 1 0 ml/min Standard curves were plotted as the Thio-TEPA peak area versus concentration of Thio-TEPA, the linear regression lines were calculated by the method of least squares

Pharmacokinetic studies

A standard curve for Thio-TEPA measurements ranging from 25 ng/ml to 10 μg/ml was constructed in plasma and extracted along with the patient samples. The concentration of Thio-TEPA in the patient samples was calculated by comparing the peak area of Thio-TEPA in the unknown sample to the standard curve.

A semilogarithmic plot of plasma Thio-TEPA concentration versus time showed apparent biexponential elimination that conformed to the general equation $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$.

The plasma Thio-TEPA elimination curve was fitted to a two-compartment body model following a zero-order drug infusion by using the NONLIN84 nonlinear curve fitting program [18]. Initial parameter estimates, required for the NONLIN84 program, were obtained using the method of residuals. Output from the NONLIN84 program includes values for the coefficients (A and B) and the exponents (α and β) of the biexponential equation. Other output includes the area under the plasma concentration versus time curve (AUC), volume of the central compartment (V_c), α half-life and β half-life ($t_{1/2}$). Total body clearance (Cl_t) was calculated from dose/AUC and the steady state volume of distribution (V_{ss}) from dose·AUMC/(AUC)^2 where AUMC is the area under the first moment of the concentration versus time curve and is equal to $A/\alpha^2 + B/\beta^2$ [19].

RESULTS AND DISCUSSION

Chromatograms of the blank plasma (pretreatment) and the Thio-TEPA-containing plasma samples (post-treatment) are shown in Fig. 1. The chromatograms show several unidentified peaks which do not interfere with the elution of Thio-TEPA at 11.4 min. Two noteworthy problems were encountered during the development of this method. First, due to the appearance of ghost peaks, the time required for HPLC analysis had to be increased from 13 to 25 min. Secondly, it was found that a preservative in heparin (used to flush the venous catheter) interfered with the analysis, thus preservative-free heparin was substituted into the protocol.

There was a linear relationship between the peak area and the concentration of Thio-TEPA standards (Fig. 2, △). Likewise, the peak area and the plasma concentration of Thio-TEPA standards after C_{18} cartridge extraction was linear (Fig. 2, □). The limits of linear detectability for Thio-TEPA from plasma were 25 ng/ml of plasma up to 10 μg/ml of plasma. The equation obtained from triplicate curves after extraction of plasma spiked with Thio-TEPA was $y = 309x + 0.856$ ($r = 1.0000$). The average recovery of Thio-TEPA from the C_{18} cartridge was 86.1 ± 7.8% over the linear range.

The plasma Thio-TEPA disappearance curve was well described by the two-

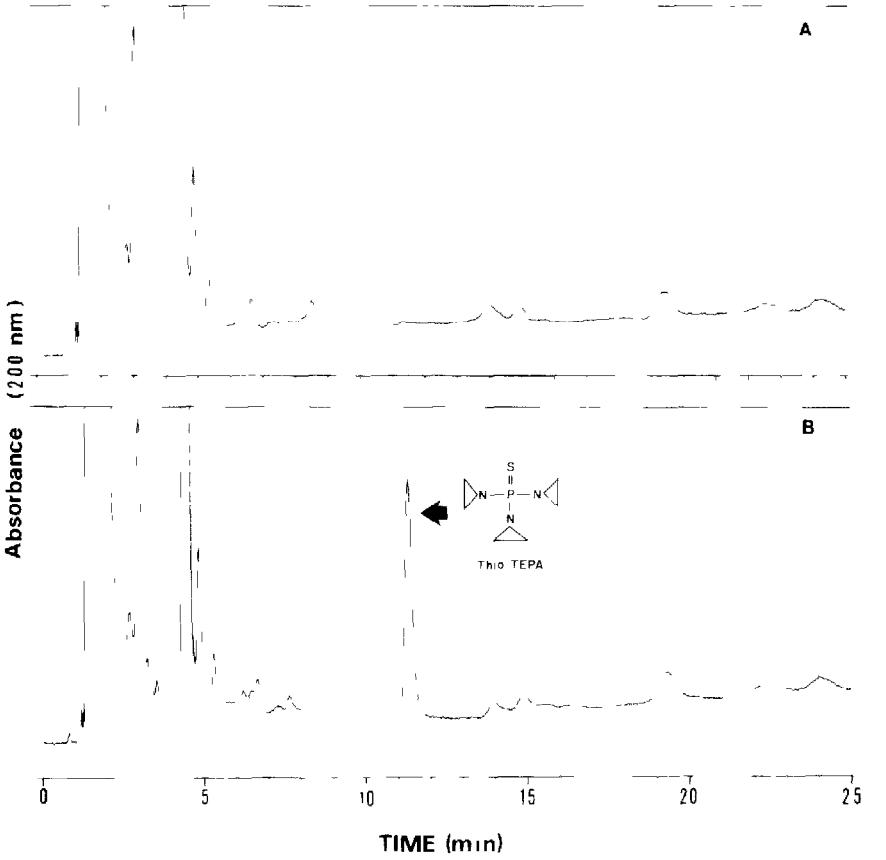

Fig 1 Chromatograms of blank and Thio-TEPA-containing plasma samples (A) Blank, 100 μl injected (B) Plasma containing 0 80 μg Thio-TEPA per ml plasma, 100 μl injected

compartment body model selected for this analysis Following a 10-min infusion, Thio-TEPA was rapidly distributed ($t_{\frac{1}{2}\alpha} = 2\ 8$ min) throughout the body followed by a slower terminal elimination phase ($t\frac{1}{2}\beta = 150$ min) (Fig 3) The initial volume of distribution (V_c) was 5 0 l/m^2 and the V_{ss} was 41 1 l/m^2 Total body clearance was 226 2 ml/min/m^2 These values are in general agreement to those reported using the GC method [9,10,17]

The C_{18} cartridge purification of Thio-TEPA from human plasma followed by isocratic reversed-phase HPLC, monitored at 200 nm, proved to be a convenient, sensitive, and effective method for the detection of Thio-TEPA after treatment at routinely used therapeutic dosages This HPLC method is sensitive to 25 ng/ml of plasma, which is within the range of published values for the sensitivity of the GC method (10–60 ng/ml of plasma) [10,16] This suggests that both methods are equally applicable for use in pharmacokinetic studies of Thio-TEPA in plasma

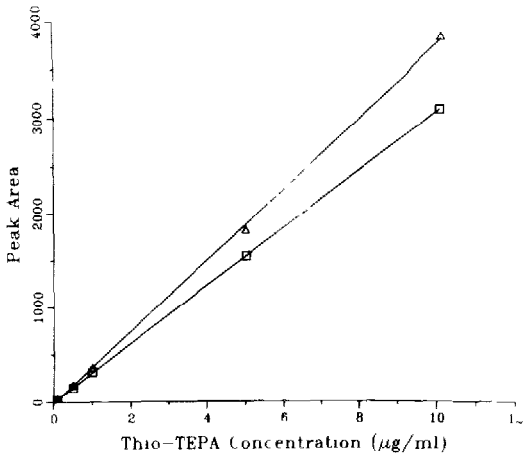

Fig 2 Standard Thio-TEPA curves, as determined by HPLC, of Thio-TEPA standards (△) and plasma samples spiked with Thio-TEPA and extracted with C_{18} cartridges (□)

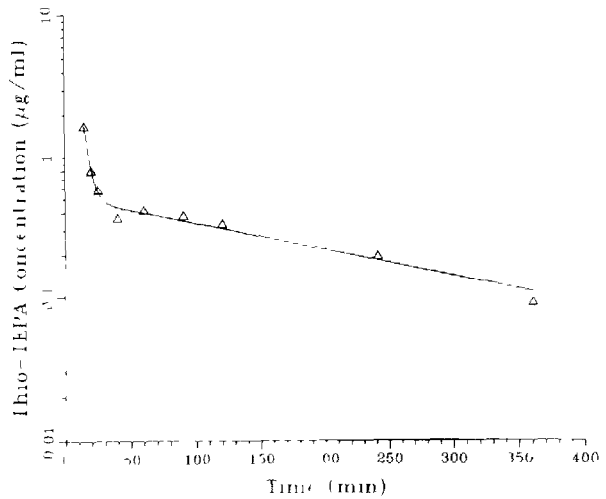

Fig 3 Time course of plasma Thio-TEPA disappearance following a 10-min infusion of 30 mg/m^2 The solid line represents a computer-simulated curve obtained from a non-linear regression analysis

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