Determination of the Antitumor Agent SOAz 
(1,3,3,5,5 Pentakis(Aziridino)-1λ6,2,4,6,3 λ5,5λ5 
Thia-Triazadiphosphorine-1-Oxide)
by a Gas Chromatographic Assay Suitable 
for Pharmacokinetic Studies in Man

Maria H. Beneken genaamd Kolmer 1, Sjoerd Rodenhuis 2, Johan C. van de Grampel 3, and Donald R. A. Uges 1

1 Laboratory for Toxicology and Clinical Pharmacy, Department of Pharmacy, State University Hospital, P.O. Box 30.001, NL-9700 RB Groningen
2 Division of Medical Oncology, Department of Internal Medicine, State University Hospital, P.O. Box 30.001, NL-9700 RB Groningen
3 Department of Inorganic Chemistry, State University of Groningen, The Netherlands

Summary. A sensitive method, based on capillary gas chromatography using a thermionic detector, has been developed for the new antitumor agent pentakis(aziridino)-thiatriazadiphosphorine-oxide, (NPAz2)2NSOAz ('SOAz'), in order to obtain pharmacokinetic data from patients receiving this drug IV in clinical trials. A structural analog of SOAz, (NPAz2)2NSOPh ('SOPh'), was used as an internal standard.

The detection limit of SOAz with this method was 0.01 mg/l for serum and 0.04 mg/l for urine.

The coefficient of variation (n = 10) was 6.0% at 1.5 mg/l in serum and 1.6% at 75.0 mg/l in urine.

Analytical recoveries averaged 89.9% from serum and 86.7% from urine. In two patients treated with subtoxic doses of SOAz (55 mg/m2), serum levels were found ranging from 3.0 to 0.16 mg/l at 10 min and 12 h, respectively, after administration.

This assay seems to be useful for determining SOAz in samples from patients receiving subtoxic doses of SOAz.

Introduction

The thiatriazadiphosphorine oxides constitute a new class of compounds characterized by a heterocyclic ring system consisting of sulfur, nitrogen, and phosphorus [6].

Amongst the numerous compounds the aziridino derivatives [7] have been shown to display cytotoxic activity both in in vitro systems (H. B. Lamberts et al. 1983, unpublished work) and in tumor-bearing mice (L1210 and P388 leukemia and B16 melanoma) [2].

The first of this group of compounds to enter clinical studies is 1,3,3,5,5-pentakis(aziridino)-1λ6,2,4,6,3 λ5,5λ5-thia-triazadiphosphorine-1-oxide, (NPAz2)2NSOAz (SOAz) (Fig. 1). A phase I trial is currently in progress at our institute, and further trials using SOAz and other derivatives are planned.

To obtain pharmacokinetic information in the course of those studies a sensitive and reliable assay is needed for quantification of SOAz and related substances in body fluids.

Detection of SOAz after separation by high-performance liquid chromatography (HPLC) seems to be problematic as this compound shows neither absorption of UV or visible light nor fluorescence; furthermore, SOAz lacks electrochemical activity. A derivation procedure using γ-(4-nitro-benzyl)pyridine has been carried out in analogy to a method for the estimation of thiotepa [1]; this method proved to be unreliable and insensitive.

Matsushima and co-workers developed an HPLC method with refractometer detection; by this method they claimed a detection limit for SOAz of 0.5 mg/l. No data concerning reproducibility were reported [5].

In the gas chromatographic assay for SOAz described by Uchida and co-workers [5], separation is carried out by means of a packed column that has to be primed with SOAz prior to each determination. No internal standard is used. The resulting assay is rather insensitive, with a claimed detection limit of 0.5 mg/l.

In our hands this procedure was inadequate because linearity of the calibration curves could not be achieved even at serum levels considerably higher than those typically found in patients treated with subtoxic doses.

Since a more sensitive procedure was required we decided to develop a capillary gas chromatographic assay for the determination of SOAz in body fluids.

Materials and Methods

Reagents. All chemicals used were of reagent grade. Sodium hydrogen carbonate, sodium hydroxide, 1,2-dichloroethane,
and dichloromethane were purchased from Merck, Darmstadt, FRG, and were used without further treatment.

SOAz was supplied by Otsuka Chemical Co., Tokushima, Japan.

SO Ph[(NP Az)3NSOPh] (Fig. 1) was synthesized at the Department of Inorganic Chemistry, State University of Groningen, The Netherlands.

Newborn bovine serum was purchased from the Flow Laboratories, Irvine, United Kingdom.

**Apparatus.** A Model 5730A gas chromatograph was used in combination with a Model 18740B capillary column control module and a Model 7671A automatic sampler.

After detection by a Model 18789A N-P-FID, automatic registration and integration of the chromatograms was performed by a laboratory automatic system (32 K 21 MX E Computer) (all from Hewlett Packard Co., Avondale, PA, USA).

The traces were plotted by a standard dual-line compact recorder, with input 10 and 100 mV (PM 8252, Philips N.V., Eindhoven, The Netherlands).

A wall-coated open tubular column, fused silica, 3.3 m x 0.225 mm (inside diameter), liquid phase CP Sil 5, film thickness 0.12 μm with a height equivalent equivalent to a plate of 0.23 mm, was used (Chrompack B.V., Middelburg, The Netherlands).

The split injection method was used. When the column flow of carrier gas (He) was 1 ml/min the column pressure was 0.66 kg/cm². A split ratio of 50 : 1 was maintained. The septum vent was 3 ml/min, the bypass flow 30 ml/min, the detector air flow 50 ml/min, and the hydrogen flow 3.1 ml/min. The column temperature was fixed at 170 °C, while the injection port and detector temperature were both kept at 250 °C.

For identification of the gas chromatographic peaks we used a gas chromatograph-mass spectrometer combination consisting of a Model 3700 gas chromatograph and a MAT 212 mass spectrometer (Finnigan Mat, Bremen, FRG).

The column was identical to the one used for the capillary gas chromatographic assay; the column length was 25 m. The splitless method was used. The initial oven temperature was 100 °C, maintained during 3 min and was programmed to rise 20 °C/min to a final temperature of 250 °C maintained over 6 min. The temperature of the injection port was 270 °C, that of the interface 270 °C, and that of the separator 350 °C (direct combination). The carrier gas was He, the column pressure 1.0 kg/cm². The source temperature was 300 °C, the emission 6 A, and the ion accelerating voltage 3 kV.

**Patients.** All patients were part of a phase I clinical trial approved by the local ethical committee, and informed consent was obtained from every patient.

Patient A, a 66-year-old male, suffered from disseminated large cell bronchial carcinoma. He had not received any previous chemotherapy. Patient B, a 43-year-old male, had skeletal metastases from a nasopharyngeal carcinoma; previous chemotherapy included cis-DDP, bleomycin, and vindesine. Both had normal renal and hepatic function as measured by standard biochemical tests. After rapid IV infusion of SOAz 55 mg/m² dissolved in 100 ml 0.9% sodium chloride solution (patient A: 95 mg, and patient B: 100 mg), blood samples were taken 10 min, 2 h, and 12 h after the end of the infusion. From patient A urine was collected from 0 to 180 min and from 3 to 24 h. Patient C, a 63-year-old male suffering from an adenocarcinoma of the lung, received SOAz 55 mg/m² dissolved in 100 ml 0.9% sodium chloride solution (105 mg) by IV infusion over 30 min. Three hours after discontinuation of the infusion, blood and pleural fluid samples were taken simultaneously.

Patient D, a 54-year-old female, had ascites due to hepatic metastases of breast carcinoma. She was treated by rapid IV infusion of SOAz 75 mg/m² dissolved in 100 ml 0.9% sodium chloride solution (145 mg); 3.5 h later abdominal paracentesis was performed and blood was taken at the same time.

**Treatment of Specimens.** Serum, ascites and pleural fluid were stored at −20 °C until analysis. Urine samples were stored at 4 °C after alkalinization to at least pH 7.5 with sodium hydrogen carbonate.

**Assay**

**Procedure for Determining SOAz in Serum, Ascites and Pleural Fluid:** To 0.5 ml serum, ascites or pleural fluid were added 25 μl sodium hydroxide 4 M and 100 μl internal standard solution containing SOPh in demineralized water (40 mg/l). The mixture was vortexed with 5 ml dichloromethane for 30 s and centrifuged at 3,000 rpm for 5 min. The water layer was discarded and the organic layer was decanted into another test tube and evaporated under reduced pressure at 25 °C (Vortex Evaporator, Buchler Instruments Inc., Fort Lee, NJ 07024, USA). The residue was dissolved in 200 μl 1,2-dichloroethane. Of this solution, 1 μl was injected into the gas chromatograph.

**Procedure for Determining SOAz in Urine:** An aliquot of 3 ml urine was centrifuged at 3,000 rpm for 5 min. To 0.1 ml of the clear supernatant was added 0.4 ml demineralized water, and this solution was treated exactly the same as the 0.5-ml serum samples.

**Calibration.** To calibrate the assay of SOAz in serum, sufficient blank bovine serum was pipetted to 0, 10, 30, 50, 100, 300, and 500 μl of spiked bovine serum (15.0 mg SOAz/l) to give 500 μl; following this procedure these samples contained 0, 0.3, 0.9, 1.5, 3.0, 9.0, and 15.0 mg SOAz/l of serum. To calibrate the assay of SOAz in urine, sufficient blank urine, alkalinized with sodium hydrogen carbonate (pH 7.5) was pipetted to 0, 10, 30, 50, and 100 μl alkalinized spiked urine (pH 7.5, 75 mg/l) and to 10, 30, and 50 μl alkalinized spiked urine (pH 7.5, 750 mg SOAz/l) to give 100 μl; following this procedure these samples contained 0, 7.5, 22.5, 37.5, 75, 225, and 375 mg SOAz/l of urine. The following procedures for serum and urine were followed and the calibration curves were plotted, the volumes of spiked serum or urine added being related to the concentrations measured.

The calibration curve slopes and correlation coefficients were calculated by a least-squares procedure.

**Recovery Studies.** Analytical recoveries for different concentrations of SOAz and of the internal standard SOPh were determined by adding the internal standard of SOAz after the extraction and before the evaporation step and assaying. The relative peak height ratios were calculated (R1) and compared with the ratio obtained by injection of a mixture of a known amount of SOAz and internal standard onto the column (R2). The recovery is the ratio between R1 and R2 (× 100%).