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GAS CHROMATOGRAPHIC ASSAY FOR THE NEW ANTITUMOR AGENT SULFAMIC ACID DIESTER (NSC 329680) AND ITS STABILITY IN BUFFER, BLOOD AND PLASMA

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SUMMARY

A sensitive gas chromatographic assay with electron-capture detection has been developed for sulfamic acid diester (sulfamic acid 1,7-heptanediyl ester, NSC 329680) based on its conversion to 1,7-diiodoheptane in the presence of excess sodium iodide. The assay is linear up to 1 μg/ml sulfamic acid diester and has a lower limit of detection of 25 ng/ml from 0.5 ml plasma. The coefficient of variation of the assay is 6.4% at 1 μg/ml and 8.0% at 100 ng/ml. Sulfamic acid diester is relatively stable in 0.9% sodium chloride and 0.1 *M* sodium phosphate buffers, pH 7.0–9.0, with half-lives greater than 38 h. The major breakdown product of sulfamic acid diester is sulfamic acid 1,7-heptanemonoyl ester. When added to whole blood sulfamic acid diester shows concentration-dependent breakdown. At 50 and 100 μg/ml sulfamic acid diester, the half-time in whole blood is 6.9 h and 65% of the drug is sequestered by the blood cells. At 10 μg/ml sulfamic acid diester in blood, there is no detectable breakdown of the drug over 24 h and all of the drug is sequestered by the blood cells. Protein binding of sulfamic acid diester in human plasma is 82% at 10 μg/ml and 68% at 100 μg/ml.

INTRODUCTION

Sulfamic acid diester (sulfamic acid 1,7-heptanediyl ester), whose structure is shown in Fig. 1, was synthesized to determine if a more polar leaving group could enhance and broaden the antitumor activity of busulfan [1,4-

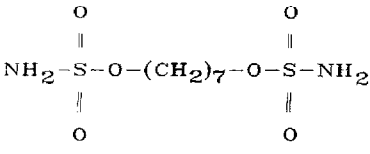

Fig. 1. Structure of sulfamic acid diester.

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bis(methanesulfonoxy)butane] [1]. Sulfamic acid diester administered intraperitoneally to mice has been found to have activity against transplanted murine B16 melanoma, L1210 and P388 leukemias and human MX-1 mammary tumor [1]. Sulfamic acid diester is currently being considered by the National Cancer Institute, U.S.A., for eventual clinical trial.

A sensitive assay for sulfamic acid diester has been developed based on capillary gas chromatography (GC) and electron-capture detection (ECD) following conversion of sulfamic acid diester to 1,7-diiodoheptane in the presence of excess sodium iodide. The assay has been used to determined the stability of sulfamic acid diester in buffers and various biological media.

EXPERIMENTAL

Drugs

Sulfamic acid diester (NSC 329680) was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD, U.S.A.). Sulfamic acid diester was formulated immediately before use as a 30 mg/ml solution in ethanol–propylene glycol–0.05 *M* sodium phosphate buffer, pH 7.4 (10:40:50). Sulfamic acid 1,7-heptanemonoyl ester was prepared from sulfamic acid diester by the method of Paborji et al. [2]. Busulfan was purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were reagent grade.

Preparation of samples

Samples (0.5 ml) of buffer, urine, plasma or heparinized blood containing sulfamic acid diester were mixed with 0.5 ml of 8 *M* sodium iodide and 0.1 μg busulfan as an internal standard. The mixture was heated in a sealed tube at 70°C for 60 min. After cooling to room temperature, 0.8 ml heptane was added and the tube shaken at a 45° angle for 10 min on a mechanical shaker. The tube was centrifuged for 10 min at 3000 *g*, the upper organic layer removed and 2 μl were taken for GC analysis.

Gas chromatography

1,7-Diiodoheptane formed from sulfamic acid diester was assayed using a Hewlett-Packard 5880A gas chromatograph with a ^{63}Ni electron-capture detector and an automatic sample injector. The output from the detector was fed into a Hewlett-Packard 5880A gas chromatograph terminal and peak areas were integrated. Heptane, 2 μl, containing 1,7-diiodoheptane and 1,4-diiodobutane (the internal standard formed from busulfan) was injected at a 1:10 split ratio onto a 25 m × 0.32 mm I.D., 0.17 μm film thickness, cross-linked 5% phenyl methyl silicone capillary column (Hewlett-Packard, Avondale, PA, U.S.A.) with helium as the carrier gas. The oven temperature was maintained at 145°C, the injector temperature at 200°C and the detector temperature at 250°C. The gas flow-rate was for helium, the carrier gas, 2 ml/min and for nitrogen, the make up gas, 30 ml/min.

Mass spectral analysis of the chromatographic peaks employed a Kratos MS50 gas chromatograph–mass spectometer and 70-eV electron-impact ionization. For

structural confirmation of 7-iodo-1-heptanol formed from sulfamic acid 1,7-heptanemonoyl ester, the 7-iodo-1-heptanol was converted to the trimethylsilyl ether derivative by silylation with N,O-bis(trimethylsilyl)trifluoroacetamide for 10 min at room temperature.

Stability studies

The stability of sulfamic acid diester in various media was determined by incubating drug concentrations of 10, 50 and 100 μg/ml at 4, 23 (room temperature) and 37°C. Stability was studied over 72 h in 0.9% sodium chloride and 0.1 *M* phosphate buffers, pH 5.0, 7.0 and 9.0, and over 24 h in fresh citrated human blood and plasma. Samples (0.5 ml) of buffer or biological media were taken for analysis of sulfamic acid diester. Sulfamic acid diester concentration data were subjected to non-linear least-squares regression analysis using the NONLIN computer program [3]. Plasma protein binding of sulfamic acid diester in human and dog plasma was measured at drug concentrations of 100, 50 and 10 μg/ml using Amicon C550A ultrafiltration cones (Amicon, Danvers, MA, U.S.A.) at 23°C. The concentration of sulfamic acid diester in the plasma and ultrafiltrate was determined and the percentage plasma protein binding calculated.

RESULTS

The GC assay developed was based on conversion of sulfamic acid diester to 1,7-diiodoheptane in the presence of sodium iodide and heat. Reaction of sulfamic acid diester with sodium iodide at 70°C for 60 min gave 48% of the theoretical yield of 1,7-diiodoheptane. Busulfan, the internal standard, was converted to 1,4-diiodobutane. Extraction of 1,7-diiodoheptane and 1,4-diiodobutane into heptane from plasma was 90% in both cases. The hydrolysis product of sulfamic acid diester, sulfamic acid 1,7-heptanemonoyl ester, was identified by the formation of 7-iodo-1-heptanol. Spontaneous formation of 7-iodo-1-heptanol during derivatization of sulfamic acid diester was less than 10% of the 1,7-diiodoheptane over the linear range of the assay. Typical chromatograms for sulfamic acid diester added to human plasma and urine are shown in Fig. 2.

Positive identification of 1,7-diiodoheptane as the major product formed by derivatization of sulfamic acid diester was provided by mass spectral analysis of the chromatographic peak (Fig. 3). The electron-impact mass spectrum contained a molecular ion at *m/z* 352. The other fragment ions shown indicated fragmentation along the hydrocarbon backbone. In addition, the ion at *m/z* 97 resulted from loss of HI_2. The base peak at *m/z* 55 was the hydrocarbon fragment C_4H_7. The second derivatization product, 7-iodo-1-heptanol, was identified both by comparison to the peak formed by derivatization of sulfamic acid 1,7-heptanemonoyl ester and by mass spectral analysis. The electron-impact mass spectrum of trimethylsilylated 7-iodo-1-heptanol contained a small molecular ion at *m/z* 314 with an intense $(M-CH_3)^+$ ion at *m/z* 299, which is expected for trimethylsilyl ether derivatives. Other major ions (e.g., *m/z* 97, 103, 185) in the spectrum were consistent with the trimethylsilylated derivative of 7-iodo-1-heptanol.

The GC assay was linear up to 1 μg/ml sulfamic acid diester and showed only

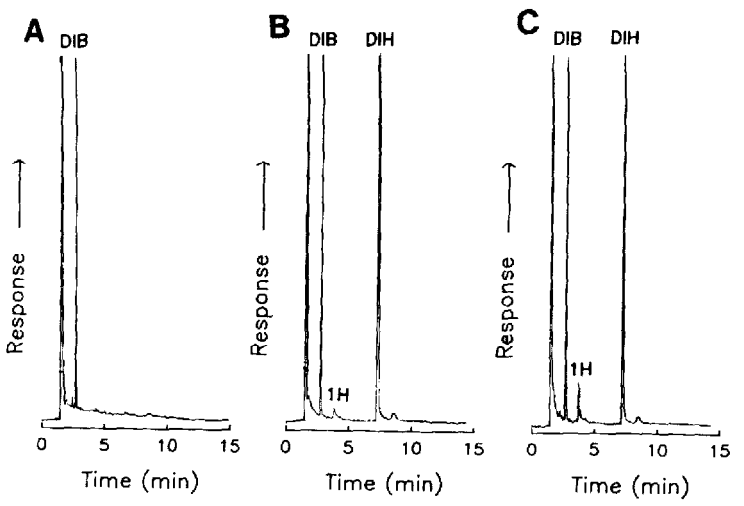

Fig. 2. Gas chromatograms of derivatized sulfamic acid diester from 0.5 ml human plasma and urine. All the samples contained 1 μg/ml busulfan as an internal standard which gave peak DIB (1,4-diiodobutane). Peak DIH (1,7-diiodoheptane) and peak 1H (7-iodo-1-heptanol) were both derived from sulfamic acid diester. (A) Blank human plasma; (B) human plasma containing 1 μg/ml sulfamic acid diester; (C) human urine containing 1 μg/ml sulfamic acid diester.

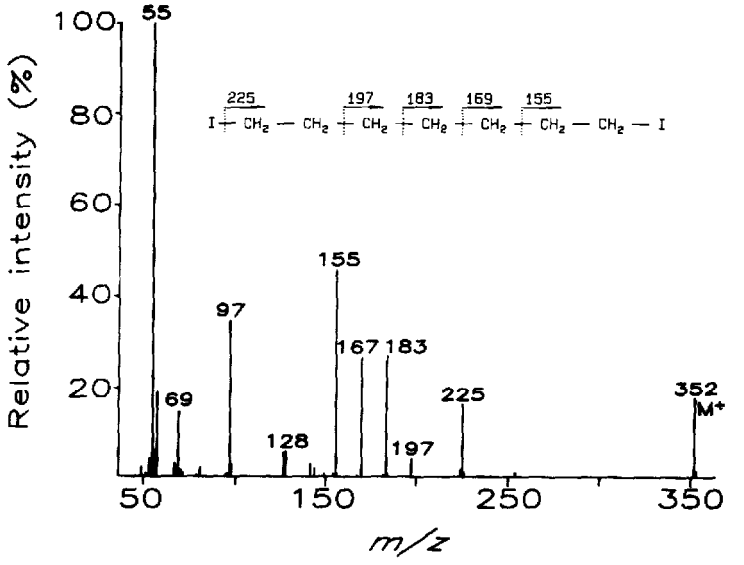

Fig. 3. Electron-impact mass spectrum (70-eV) of the gas chromatographic peak of 1,7-diiodoheptane formed by incubation of 4 *M* sodium iodide with sulfamic acid diester. The predominant molecular ion $M^{+\cdot}$ has a mass of 352 representing the 1,7-diiodoheptane. There are major fragment ion peaks at *m/z* 127 and 254 being I and I_2, respectively.

slight non-linearity up to 10 μg/ml. The assay had a lower limit of detection (peak more than 3× background) for sulfamic acid diester from 0.5 ml plasma of 25 ng/ml. The coefficient of variation of ten repeated assays was 8.7% at 10 μg/ml, 6.4% at 1 μg/ml, 8.0% at 100 ng/ml and 9.9% at 50 ng/ml.

The stability of sulfamic acid diester was studied under a variety of conditions using the GC assay developed. Sulfamic acid diester was found to be relatively stable in 0.9% sodium chloride at 4 °C and room temperature with less than 10% degradation over 72 h in both cases. Sulfamic acid diester in fresh human plasma or urine showed less than 10% degradation at 4 °C or room temperature over 24 h. At 37 °C, there was a slow but measurable monoexponential breakdown of sulfamic acid diester in 0.9% sodium chloride, 0.1 *M* sodium phosphate (pH 5.0, 7.0, 9.0) and human urine with a half-life in all cases greater than 38 h (Table I). The breakdown of sulfamic acid diester showed an apparent pH dependency with a half-life of 72.3 h at pH 9.0 compared to 41.2 h at pH 7.0 (Table I). In fresh human plasma there was less than a 20% breakdown of sulfamic acid diester over 24 h at 37 °C. The hydrolysis product sulfamic acid 1,7-heptanemonoyl ester was produced by the spontaneous breakdown of sulfamic acid diester in these media (Fig. 4). By 48 h, 1,7-heptanemonoyl ester accounted for 58% of the sulfamic acid diester initially present in 0.9% sodium chloride. Sulfamic acid 1,7-heptanemonoyl ester was present at a lower concentration in the other media. After 48 h incubation at 37 °C, the monoester accounted for 30% of the sulfamic acid diester initially present in 0.1 *M* phosphate buffer, pH 7.0, and 37% of the sulfamic acid diester initially present in urine.

When sulfamic acid diester was added at 50 and 100 μg/ml to fresh citrated whole human blood at 37 °C, non-linear decay curves were observed both in the whole blood and in the plasma from the whole blood (Fig. 5). The half-time of disappearance of sulfamic acid diester from the whole blood was 7.2 h at 100 μg/ml and 6.6 h at 50 μg/ml. Sulfamic acid diester exhibited a greater rate of disappearance from the plasma separated from the whole blood; the half-time of disappearance being 3.3 h at both 100 and 50 μg/ml. In contrast to the degradation seen with sulfamic acid diester at 100 and 50 μg/ml, there was no degradation of sulfamic acid diester at 10 μg/ml in whole blood incubated at 37 °C for 24 h. Also, the plasma from the whole blood at the 10 μg/ml concentration contained no detectable sulfamic acid diester. This suggests that at 10 μg/ml sulfamic acid diester all of the drug is sequestered and stabilized by blood cells. At concentra-

TABLE I

STABILITY OF SULFAMIC ACID DIESTER IN VARIOUS MEDIA AT 37 °C

Medium	Half-life* (h)
Sodium phosphate buffer, 0.1 *M*	
pH 5.0	46.0 ± 2.7
pH 7.0	41.2 ± 5.4
pH 9.0	72.3 ± 15.6
Sodium chloride, 0.9%	49.5 ± 1.3
Human urine	38.7 ± 0.7
Human plasma	< 20% loss in 24 h

*Values are the mean ± S.D. of three concentrations of sulfamic acid diester (100, 50, 10 μg/ml) measured over 72 h incubation. Human plasma was incubated for 24 h.

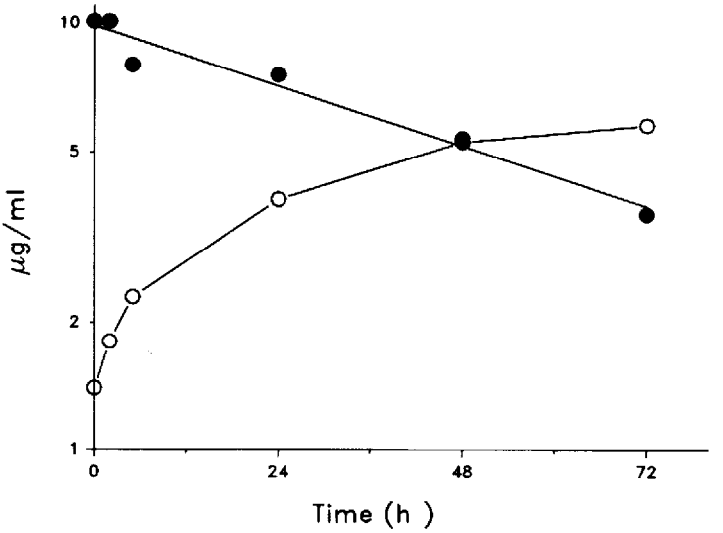

Fig. 4. Breakdown of sulfamic acid diester in 0.9% sodium chloride at 37°C. Sulfamic acid diester was added to the 0.9% sodium chloride at an initial concentration of 10 μg/ml. (●) Sulfamic acid diester; (○) sulfamic acid 1,7-heptanemonoyl ester.

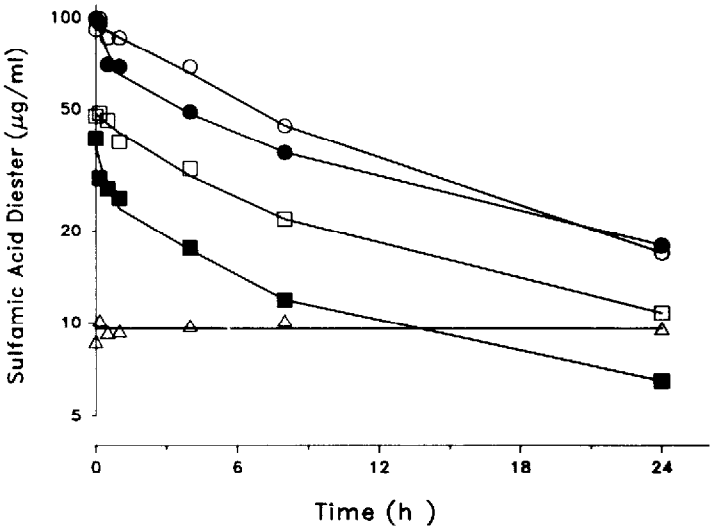

Fig. 5. Breakdown of sulfamic acid diester in fresh citrated human blood (open symbols) and in plasma separated from the whole blood (closed symbols) at 37°C. Sulfamic acid diester was added to the whole blood at an initial concentration of 100 μg/ml (◑), 50 μg/ml (◨) and 10 μg/ml (△).

tions of sulfamic acid diester greater than 10 μg/ml the drug is taken up by the blood cells but is also broken down. The percentage of sulfamic acid diester sequestered by the red blood cells at different concentrations is shown in Table II. This was calculated by extrapolating to zero time the concentration of sulfamic acid diester in whole blood and plasma in Fig. 5. The hematocrit of the blood used for the studies was 50%. The results show that the accumulation of sulfamic acid

TABLE II

ACCUMULATION OF SULFAMIC ACID DIESTER BY RED BLOOD CELLS

Initial blood concentration (μg/ml)	Percentage in blood cells*
100	63.5
50	66.5
10	100.0

*Calculated from the extrapolated zero-time blood and plasma sulfamic acid diester concentrations in Fig. 5.

TABLE III

PLASMA PROTEIN BINDING OF SULFAMIC ACID DIESTER

Sample	Initial concentration (μg/ml)	Percentage protein bound*
Human plasma	100	68 ± 1
	50	74 ± 2
	10	82 ± 2
Dog plasma	100	61 ± 6
	50	65 ± 0
	10	79 ± 3

*Values are the mean ± range of two separate determinations.

diester by blood cells increased as the concentration of the drug decreased reaching 100% at 10 μg/ml.

Binding of sulfamic acid diester to human dog plasma protein was determined by ultrafiltration at 23°C (Table III). The mean plasma protein binding of sulfamic acid diester was 75% for human plasma and 68% for dog plasma. Some saturation of plasma protein binding was seen as the concentration of sulfamic acid diester increased from 10 to 100 μg/ml.

DISCUSSION

Sulfamic acid diester is an analogue of busulfan which was synthesized in an attempt to improve the antitumor activity of busulfan [1]. A sensitive GC assay for sulfamic acid diester has been developed based on conversion of sulfamic acid diester to 1,7-diiodoheptane in the presence of excess sodium iodide. The nucleophilic substitution of methanesulfonate esters by sodium iodide in aqueous solution has previously been reported [4]. Sulfamic acid diester gave a 48% theoretical yield of 1,7-diiodoheptane which is similar to the reported yield of 1,4-diiodobutane formed from 1,4-bis(methanesulfonoxyl)butane (busulfan) [4]. Sulfamic acid diester also gave a minor derivatization product identified as 7-iodo-1-heptanol. This derivative is formed from sulfamic acid 1,7-heptanemonoyl

ester. Paborji et al. [2] have reported that sulfamic acid 1,7-heptanemonoyl ester is an intermediate in the hydrolysis of sulfamic acid diester to 1,7-heptanediol.

Sulfamic acid diester was relatively stable in 0.1 *M* sodium phosphate buffers, pH 7.0–9.0, 0.9% sodium chloride and plasma. The half-life at 37°C was greater than 38 h in all cases. Under these incubation conditions most of the sulfamic acid diester appeared to be converted to sulfamic acid 1,7-heptanemonoyl ester rather than being completely hydrolyzed to 1,7-heptanediol. This differs from the breakdown of busulfan. Hudson et al. [5] reported that 1,4-butanediol was the only breakdown product of busulfan, while Feit and Rastrup-Andersen [6] and Hassan and Ehrsson [7] reported only the formation of tetrahydrofuran. The half-lives measured for sulfamic acid diester in our study show that it is a more stable compound than busulfan. Ehrsson and Hassan [8] have reported a half-life for busulfan of 16.0 h in 0.1 *M* phosphate buffer, pH 7.0, at 37°C.

When sulfamic acid diester was added to whole blood at concentrations above 10 μg/ml, it disappeared with a half-time of 6.9 h. Ehrsson and Hassan [8] reported a half-life for busulfan in whole blood at a concentration of 70 μg/ml of 8.7 h. Thus, at relatively high concentrations in whole blood sulfamic acid diester does not appear to be more stable than busulfan. However, at a sulfamic acid diester concentration of 10 μg/ml in whole blood there was no detectable degradation of sulfamic acid diester. This appears to be related to the extensive sequestration of sulfamic acid diester by the blood cells at this concentration. At higher concentrations of sulfamic acid diester there was less complete sequestration of sulfamic acid diester by blood cells. A possible explanation for this observation is that there is a binding site for sulfamic acid diester in blood cells which protects the drug from degradation. At higher concentrations of sulfamic acid diester this site may become saturated allowing sulfamic acid diester to react at other sites in the blood cells and to be degraded. It is possible that sulfamic acid diester reacts with thiol groups in the blood cells since busulfan has been reported to react with glutathione and cysteine [9].

In summary, we report a sensitive GC assay for sulfamic acid diester in biological fluids. Sulfamic acid diester is relatively stable in plasma, urine and 0.1 *M* sodium phosphate buffer, but in whole blood at high concentrations it is more rapidly degraded. At low concentrations in whole blood sulfamic acid diester is stable, apparently because of sequestration by the blood cells. The results of these stability studies may provide insight into the pharmacokinetics and mechanism of action of this alkylating agent.

ACKNOWLEDGEMENTS

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