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Note

Simple and rapid high-performance liquid chromatographic method for the analysis of sulfinpyrazone and four of its metabolites in human plasma

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Sulfinpyrazone (SO), a derivative of phenylbutazone with pronounced uricosuric properties [1, 2], has been mainly used in the treatment of gout. Following the demonstration of its antiplatelet action [3], it has been shown that SO has probable beneficial effects in thromboembolic disorders [4]. Recently, in two major clinical trials, it has been demonstrated that SO reduces the recurrence of myocardial infarction [5] and sudden cardiac deaths [6]. Since the antiplatelet action of SO could be attributed to the active circulating metabolites [7], new interest in studying the pharmacokinetics of SO has arisen.

In the investigation of the cardiac electrophysiological effects of SO in human, it is necessary to have a sensitive and specific assay method to quantitate SO and its metabolites in plasma. There are nine high-performance liquid chromatographic (HPLC) methods [8—16] and two gas chromatographic methods [17, 18] published to date on SO and its metabolites. All of the published methods required extensive and time-consuming sample treatment ranging from single extraction to multiple extractions. There are only two methods reported [15, 16] which are capable of quantitating SO and most of its metabolites. Baseline resolution of all the components has not been achieved [15] using isocratic solvent systems and a gradient solvent system is required

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for total separation [16]. The purpose of this manuscript is to report a simple and rapid chromatographic method to quantitate SO and its metabolites in plasma.

MATERIALS AND METHODS

Reagents

Sulfinpyrazone {SO, 1,2,-diphenyl-4-[-2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione} and its metabolites, the sulfone (G31442, SO_2), the *p*-hydroxylated sulfinpyrazone (G-32642, SOOH), the sulfide (G-25671, S) and the *p*-hydroxylated sulfide (G33378, SOH) were kindly supplied by Ciba-Geigy (Basel, Switzerland). The internal standard, naproxen, was obtained from Astra Pharmaceuticals. Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Fairlawn, NJ, U.S.A.).

Chromatography

A Waters Assoc. chromatographic system which consists of two M-45 pumps, an M-480 variable-wavelength UV detector, automatic sampler (WISP), integrator (Model 730) and system controller (Model 721) was used. Separation of SO and its metabolites was achieved using a 5-μm C_{18} reversed-phase Radial-Pak cartridge (11.5 cm × 8 mm I.D.). A precolumn packed with C_{18} packings (Waters Bondapak C_{18}/Corasil) was connected in front of the column. The absorbance of individual components was measured at 254 nm. The solvent system was acetonitrile—0.02 *M* phosphate buffer pH 7.0 (26:74). The solvent flow-rate was 2 ml/min.

Plasma sample treatment

To a 0.5-ml plasma sample containing SO and its metabolites were added 0.2 ml of naproxen (42 μg/ml) and 0.5 ml of acetonitrile. The mixture was gently shaken and centrifuged at 10,000 *g* for 2 min. The supernatant was removed and an equal volume of water was added. The sample (50—200 μl) was injected into the HPLC system.

Human studies

Two-stage infusion. To test the hypothesis that SO has cardiac electrophysiologic effects, SO was administered intravenously to subjects undergoing invasive electrophysiological testing. SO was given by bolus (4 or 8 mg/kg over 2—3 min) and constant intravenous infusion (0.015—0.045 mg/kg/min) in two stages 45 min apart. Blood samples were taken at 10-min intervals 15 min into the infusion. The blood samples were collected into heparinized vacutainers and were immediately centrifuged; plasma was separated and stored at —20° C until analysis.

Single oral dose study. A subject was fasted overnight and two 100-mg anturan tablets were administered with 150 ml water. Food was not allowed until 2 h after drug administration. Venous blood (5 ml) was collected at appropriate time intervals. The blood samples were immediately centrifuged; plasma was separated and stored at —20° C until analysis.

RESULTS

Fig. 1a shows a chromatogram of blank human plasma. There is no interference observed. Fig. 1b is a representative chromatogram of blank human plasma spiked with SO and its metabolites. All the peaks are baseline-resolved. Fig. 1c is the chromatogram of the plasma sample collected from a subject after oral route of administration. The peak at 8.13 min, which did not interfere with the analysis, was inherent in the subject's plasma (Fig. 1c). S was not detected after a single oral dose of SO. The minor *p*-hydroxy sulfone metabolite was separated but not observed in any of the patient samples; therefore, attempt to quantitate this species was abandoned.

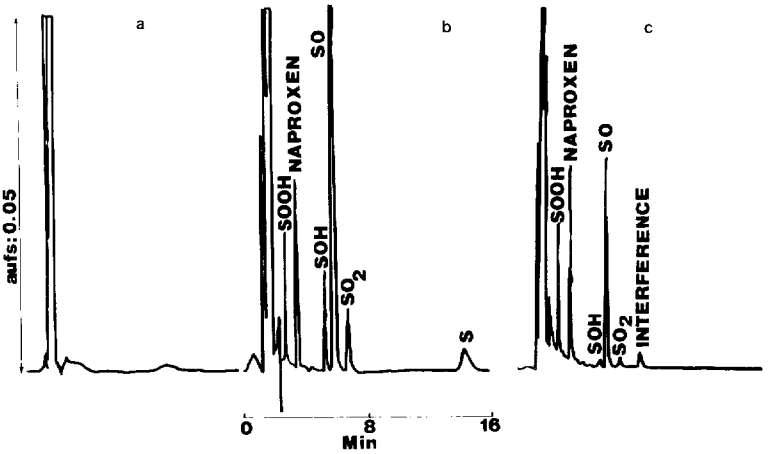

Fig. 1. a, Blank plasma. b, Blank plasma spiked with sulfinpyrazone (SO, 27.2 μg/ml) and metabolites (SOOH, 3.87 μg/ml; SOH, 4.5 μg/ml; SO_2, 5 μg/ml; S, 3.92 μg/ml), with naproxen (4 μg/ml) as internal standard. An 85-μl sample was injected. c, Plasma sample collected 4.0 h after 200 mg of anturan were administered orally. The peak with a retention time at 8.13 min was an inherent peak which was present in the blank plasma sample. This peak did not interfere with the assay.

Some of the subjects recruited into the study were on nitroglycerin and isosorbide dinitrate. These medications were stopped at least twelve days prior to the SO study. Plasma samples collected just before the study did not show any interfering peaks. Since the study was designed to measure the electrophysiological effects of SO and its metabolites, the assay method has not been subjected to an exhaustive test of cross-contamination by drugs which may be given during SO therapy. However, it has been found that lidocaine and its metabolites, propranolol and metoprolol did not interfere with the assay. Ibuprofen interfered with SOOH indicating the quantitation of this metabolite in subjects on ibuprofen has to be treated with caution.

Two sets of calibration curves were prepared. One has SO concentrations ranging from 0.2 to 100 μg/ml and another has SO concentrations from 0.2 to 25 μg/ml. The metabolite concentrations range from 0.2 to 10 μg/ml in both cases. Linearity has been observed in all cases and the results are summarized in Table I. Each standard curve has five points and each point was determined in triplicate. The coefficient of variation in each point was less than 9%. The

TABLE I

EQUATIONS OBTAINED FROM LINEAR REGRESSION ANALYSIS AND PRECISION OF SAMPLE ANALYSIS FOR SO AND METABOLITES

Compound	Linear regression equation	C.V. (%)	
		At 0.5 μg/ml	At 10 μg/ml
SO	$Y = 0.0610X - 0.0089$	5.0*	1.9
SOOH	$Y = 0.0649X - 0.0087$	6.2	1.6
SO_2	$Y = 0.0641X - 0.00942$	8.8	1.2
SOH	$Y = 0.0466X - 0.00424$	1.7	3.7
S	$Y = 00.806X - 0.0143$	2.7	2.2

*At 1 μg/ml.

sensitivity for all the species were 0.1 μg/ml except for S which has a sensitivity of 0.2 μg/ml. Since the plasma samples were not extracted, recovery of the drug and metabolites after protein precipitation was evaluated by comparing the peak area ratios between the spiked plasma samples and an aqueous solution which contained the same concentration (5 μg/ml) of each species. Recovery was found to be higher than 99% in all cases. The stability of SO and its metabolites in plasma after treatment with acetonitrile was observed to be more than 24 h. This was substantiated by the fact that the peak area ratios of the drug and metabolites to internal standard were changed no more than 1%.

DISCUSSION

There are a number of HPLC assays reported in the literature [8—16] to quantitate SO and its metabolites in biological fluids. In the earlier studies [8—15], the biological samples have to be treated with extensive extraction procedures to isolate SO and its metabolites. When a single extraction step was used, the recovery of one or more metabolites would be significantly diminished [12, 14]. Recently, De Vries et al. [16] reported a solvent system which was capable of extracting all of the components with high yield (> 90%) except SOOH (60%) in plasma and urine. The present method required no extraction. The only sample treatment was deproteinization of plasma protein with acetonitrile. The yield of all the components was 100%. It was observed that if water was not added to the supernatant of the deproteinized plasma samples, injection of more than 50 μl of the sample would result in skewed peaks and loss of separation. This phenomenon could be attributed to the high percentage of acetonitrile present in the sample [19]. The addition of equal volume of water to the sample allowed higher volume of sample (> 200 μl) to be introduced into the HPLC system without any loss of separation efficiency and sensitivity.

Chromatographically, SO and its metabolites have not been completely separated (base-line separation) using an isocratic solvent system and a suitable stationary phase. De Vries et al. [16] have recently separated SO and its metabolites with a gradient solvent system and a reversed-phase (C_{18}) column.

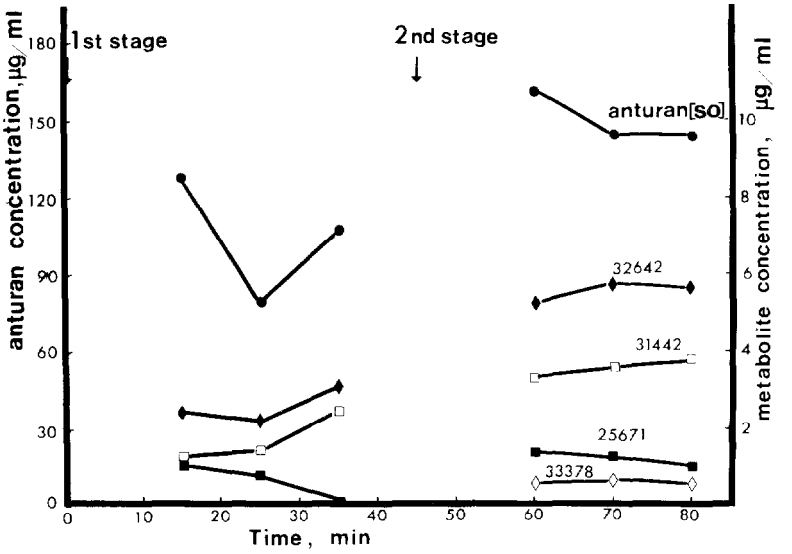

Fig. 2. Representative plasma profiles of sulfinpyrazone SO (•) and its metabolites [SOOH (♦), SOH (◇), SO_2 (□) and S (■)] for a patient who was on a two-stage intravenous study. The first loading dose was 8 mg/kg and the infusion-rate was 0.03 mg/kg/min. The loading dose at the second stage was 4 mg/kg and the infusion-rate was 0.045 mg/kg/min.

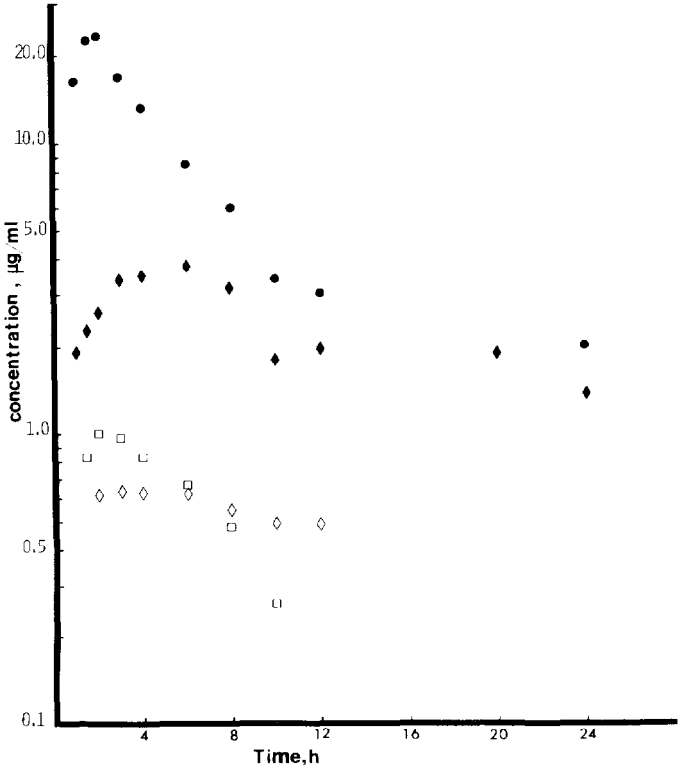

Fig. 3. Plasma profile of SO (•), SOOH (♦), SOH (◇) and SO_2 (■) after a subject has taken 200 mg sulfinpyrazone orally.

The present method has the advantage that SO and four of its metabolites could be separated by an isocratic solvent system and a reversed-phase (C_{18}) column. The analysis time was approximately the same as reported in the method of De Vries et al. [16]. The sensitivity of this assay method was compatible with the published methods except that the procedures were highly simplified. The applicability of this method was demonstrated by measuring SO and its metabolites in human plasma after different routes of administration.

Figs. 2 and 3 are plasma profiles of SO and its metabolites during a two-stage intravenous infusion and after 200 mg oral administration of anturan, respectively. The *p*-hydroxy (SOOH) and the sulfone (SO_2) metabolites were found to be the major species in plasma besides the drug itself. The low level of S observed during intravenous infusion and the absence of S after oral administration were consistent in all the experiments (n = 24). The relatively high SO level at 24 h after oral administration of anturan seemed to be unique in this study. No SO could be detected at 24 h in another study. It is realized that SOOH and SOH have been reported as minor metabolites and S as major metabolite after single and multiple oral doses [16, 20]. The reason for the quantitative difference in the metabolic profiles between this study and the literature is not readily apparent.

With our experience, approximately eighty samples can be processed in a day. Since a pre-column was employed, the column can tolerate a high number of injections (> 500 to date) into the HPLC system.

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