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Note

Simultaneous determination of sulfinpyrazone and four of its metabolites by high-performance liquid chromatography

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Sulfinpyrazone (SO) (Fig. 1), 1,2-diphenyl-4-[2-(phenylsulfinyl)-ethyl]-3,5-pyrazolidinedione, Anturane®, was originally introduced as a uricosuric drug [1]. New investigations [2] concerning the drug have shown it to be effective in the suppression of platelet function in patients suffering from thrombo-embolic disorders. Recently [3], a significant reduction in the incidence of sudden death after myocardial infarction has been reported.

Attention was drawn to the metabolism of SO by the finding of a prolonged and biphasic effect of SO in the rabbit [4]. This led to the identification of two new and very active metabolites, the sulfide (S) and the *p*-hydroxylated sulfide (SOH), in rabbit [5] and in man [6]. Quantitative determination of the new metabolites was carried out by gas chromatography—mass spectrometry. Several papers [7—10] have dealt with the determination of sulfinpyrazone itself and its well-known metabolites — the sulfone (SO_2) and the *p*-hydroxylated sulfinpyrazone (SOOH) — but none of these methods are directly applicable to determination of the new metabolites. This paper presents a rapid, selective and sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous determination of SO and its four metabolites (SO_2, S, SOH and SOOH) in human plasma.

MATERIALS AND METHODS

Chromatography

A Waters Assoc. Model 600 liquid chromatographic pump was equipped with a U6K injection system. Separation was achieved with a Knauer® 250 mm × 4.6 mm I.D. steel column packed with 5 μm Spherisorp ODS particles. The absorbance was measured at 254 nm by a Waters 440 dual-channel photometer. The solvent system was methanol—0.02 *M* phosphate

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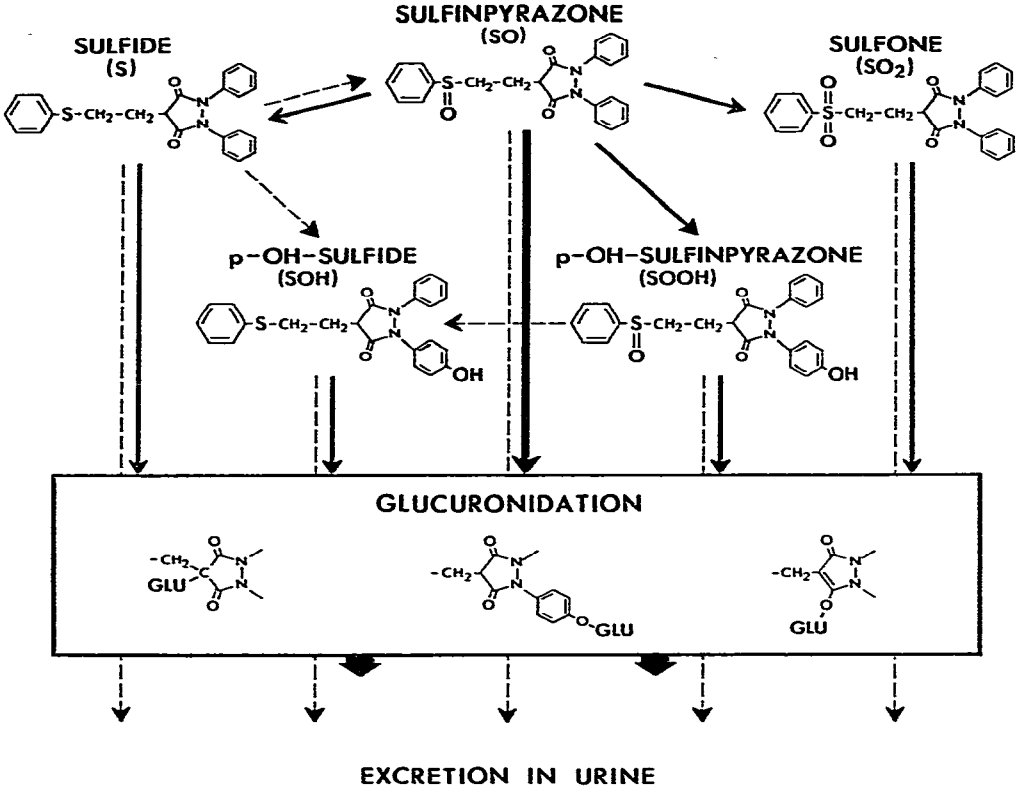

Fig. 1. Reductive and oxidative metabolic pathways of sulfinpyrazone.

buffer (pH 7.0) (40:60) at a flow-rate of 1.3 ml/min. The chromatograms were recorded on an OmniScribe (Houston Instruments) dual-channel recorder.

Reagents

Sulfinpyrazone (SO, 1,2-diphenyl-4-[-2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione) and its metabolites, the sulfone (G 31 442, SO_2), the *p*-hydroxylated sulfinpyrazone (G-32 642, SOOH), the sulfide (G 25671, S) and the *p*-hydroxylated sulfide (G 33378, SOH) were obtained from Ciba-Geigy, Basel, Switzerland. 1-Chlorobutane (Fluka, Bachs, Switzerland), methanol, and dichloromethane (Merck, Darmstadt, G.F.R.) were of analytical grade. The internal standard, naproxen (Nap), was obtained from Astra-Syntex, Södertälje, Sweden. Sodium sulfite (Merck) in water (100 mg per 5 ml) was prepared just before use.

Procedure

A 0.5—1 ml sample of plasma, to which a suitable amount of internal standard was added, 0.5 ml of sodium sulfite and 1 ml of 1 *N* HCl in a 12-ml glass stoppered centrifuge tube were extracted for 30 min on a horizontal shaker with 8 ml of a mixture (1:3) of dichloromethane and 1-chlorobutane.

After centrifugation 7.0 ml of the organic phase were transferred to another glass stoppered tube and washed with 2 ml of 0.05 *M* phthalate buffer (pH 5.0) by vortex mixing for 60 sec. After centrifugation, 6 ml of the organic phase were evaporated to dryness under nitrogen in a tapered centrifuge tube and redissolved in 100 μl of 1 *N* NaOH, 100 μl of sodium sulfite and 200 μl of hexane. After vortex mixing and centrifugation, 25 μl of the aqueous phase were injected on the chromatograph.

Preparation of standard curves

Standard curves were constructed by the addition of 0.5—20 μg of the compounds per milliliter of plasma (internal standard, naproxen: 5 μg/ml). Samples were treated as described under Procedure and the ratios of the peak heights of sulfinpyrazone and its four metabolites to that of the internal standard were plotted against the concentration of sulfinpyrazone and metabolite.

Application

Two healthy male volunteers were given a single oral dose of 200 mg of sulfinpyrazone. The time courses of the plasma levels of SO and metabolites were followed for 48 h. Treatment was then continued with 200 mg of sulfinpyrazone twice for one day (at 8 a.m. and 12 a.m.), followed by 200 mg four times a day during the next five days (at 8 a.m., 12 a.m., 6 p.m. and 10 p.m.). Heparinized blood samples were drawn just before the first morning dose (10 h after the previous dose) on days 4, 6 and 8 (subject 1) or on days 4, 7 and 8 (subject 2). Thereafter the decline of SO and the metabolites was followed for 48 h (subject 1) and 24 h (subject 2). Plasma samples were kept frozen at —20°C until analysis.

RESULTS AND DISCUSSION

The extraction of sulfinpyrazone (SO) and its metabolites was carried out using a mixture (3:1) of 1-chlorobutane and dichloromethane, which gave satisfactory recovery for five of the six compounds (Table I). Extraction with 1-chlorobutane alone did not give sufficient recovery of the polar compounds, while extraction with dichloromethane alone gave low recovery of the very lipophilic sulfide metabolite (S) [6]. Addition of dichloromethane to 1-chlorobutane provided a polar and efficient extraction medium with a density lower than that of plasma, and was thus more easy to handle. The recovery from plasma of the hydroxylated sulfinpyrazone (SOOH) was very low, but it could be increased using the extraction procedure described by Bjornsson et al. [10].

Oxidation of the compounds in the 4-position of the dioxopyrazolidine ring [11] or S-oxidation of the sulfide is possible during the extraction procedure. Initial experiments gave a low recovery for sulfide concentrations of less than 2 μg/ml, but addition of sodium sulfite to the plasma before extraction increased the recovery of the sulfide metabolite considerably, indicating that the sulfite protected the metabolite against oxidation. A small but constant part (about 4%) of the sulfide was converted to sulfinpyrazone during the

extraction procedure. Reduction of the extraction time did not diminish this fraction but reduced the recovery. The fraction could, however, easily be calculated from the standard curves making a correction of the concentration calculations possible. The sulfite did not reduce the sulfone or sulfinpyrazone.

Addition of sulfite to the final sodium hydroxide solution also protects the sulfide metabolite from oxidation, making this solution stable for several days at room temperature. Back-extraction from the extraction mixture into 1 *N* NaOH, as used by Bjornsson et al., in the extraction of SO and the sulfone metabolite (SO_2) was not applicable since the sulfide was not extracted. Instead evaporation to dryness under nitrogen and redissolving the residue in 1 *N* NaOH was preferred. To obtain complete dissolution and a clear liquid to inject into the chromatograph, it was necessary to wash the sodium hydroxide phase with hexane. No loss of compounds was seen during this final step. Also the washing with pH 5 buffer caused no loss, and better chromatograms were obtained when this step was added to the extraction procedure.

Sulfinpyrazone and its four metabolites together with the internal standard could be separated by reversed-phase HPLC using a 10 μm LiChrosorb RP-18 or a 5 μm Spherisorp ODS column. The latter was preferred since the retention time for the less-polar metabolite, the sulfide (S), could be reduced to 11 min without losing separation of the polar compounds; 20—25 min were necessary with the LiChrosorb column.

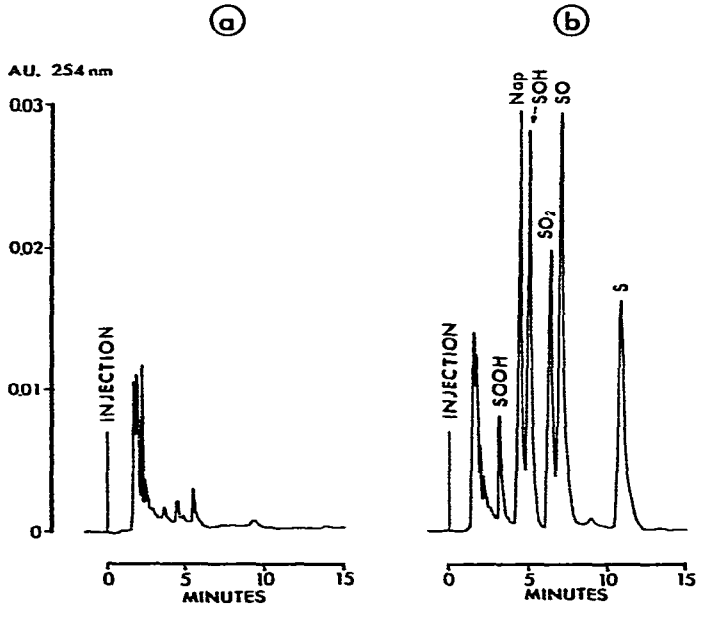

Fig. 2. Chromatograms of HPLC of (a) a plasma blank and (b) a plasma sample spiked with sulfinpyrazone (SO, 2.5 μg/ml), and metabolites (SOOH, 2 μg/ml; SOH, 2 μg/ml; SO_2, 2.5 μg/ml; S, 2.5 μg/ml), with naproxen (Nap, 5 μg/ml) as internal standard. The plasma samples were treated as described under Procedure.

Fig. 2 shows chromatograms of a plasma blank (a) and of a plasma sample spiked with sulfinpyrazone, its four metabolites and the internal standard (b). A small blank peak appears at the same retention time as that of the hydroxylated sulfide metabolite (SOH) equivalent to about 150 ng/ml of this metabolite. Reducing the methanol content in the eluent to 36% separated this interfering peak from the SOH metabolite.

Standard curves for SO and the metabolites were linear ($r > 0.995$) up to 20 μg/ml and with the exception of the SOH metabolite the straight lines intercepted the ordinate very near the origin. The slopes of standard curves, minimum detectable concentrations, extraction recoveries and precision of the analysis are given in Table I.

TABLE I

SLOPES OF STANDARD CURVES, RECOVERY, DETECTION LIMITS AND PRECISION OF THE ANALYSIS FOR SULFINPYRAZONE AND METABOLITES

Compound	Slope of standard curve*	Recovery** (%)	MDC*** (ng/ml)	C.V. (%)§	
				a	b
SO	0.380	85	30	4.5	10.8
SO_2	0.286	85	30	3.9	9.0
S	0.242	88	30	3.2	8.3
SOOH	0.108	16	100	5.4	15.2
SOH§§	0.442	99	100	4.8	12.3

*Ratio of peak height of 1 μg/ml compound to that of 5 μg/ml naproxen (internal standard).
**Recovery corrected for loss of solvent during the extraction procedure.
***MDC = minimum detectable concentration, defined as peak height = two times baseline noise.
§Within-run coefficient of variation ($n = 6$) for 5 μg/ml (a) and 0.5 μg/ml (b).
§§Blank peak subtracted.

Fig. 3 shows the plasma concentrations of sulfinpyrazone and the metabolites after treatment with SO as described under Application. After a single oral dose of SO only small amounts of the metabolites were found. The concentrations of the *p*-hydroxylated metabolites, SOOH and SOH, were below the detection limit of the method. Continuous treatment with sulfinpyrazone causes the level of the metabolites to increase considerably. In vitro the sulfide is more than ten times as active as SO and the sulfone is about five times as active as SO in inhibiting platelet aggregation [12]. Taking these data into consideration the beneficial effect of long-term treatment with sulfinpyrazone upon sudden death after acute myocardial infarction [3] could be assigned to the presence of these two metabolites. The rate of disappearance of the compounds from plasma after cessation of treatment is shown in the right-hand parts of the graphs in Fig. 3. Further investigation of the metabolism and pharmacokinetics of sulfinpyrazone during long-term treatment is in progress.

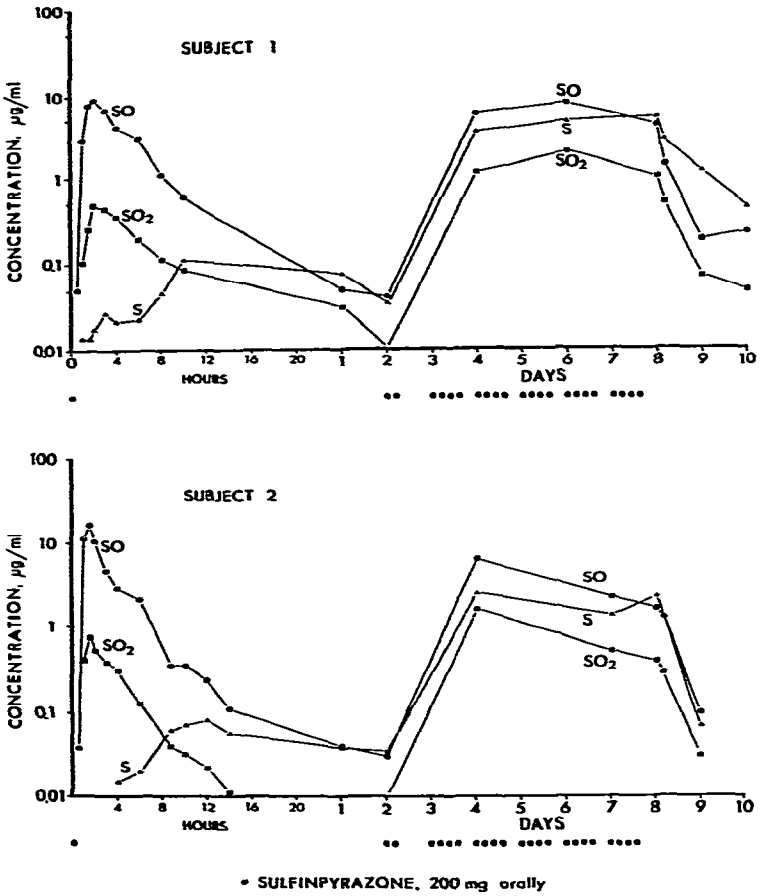

Fig. 3. Plasma concentrations of sulfinpyrazone and metabolites in two subjects after a single dose followed by continuous oral administration of sulfinpyrazone as described under Application.

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