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MEASUREMENT OF SULINDAC AND ITS METABOLITES IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A sensitive high-performance liquid chromatography assay for simultaneous measurement of sulindac and its major metabolites was developed. The extraction methods provided greater than 89% recovery of sulindac and its sulfone and sulfide metabolites from both plasma and urine. Complete resolution and accurate detection of the three compounds was achieved with a reversed-phase column, UV detection at 254 nm and a methanol—acetate buffer mobile phase. Levels of sulindac and its metabolites were determined in plasma and urine from four volunteers after oral administration of 200 mg Clinoril. Glucuronide conjugates in urine were measured after alkaline hydrolysis.

INTRODUCTION

Sulindac is a nonsteroidal, anti-inflammatory agent efficacious in the acute and long-term treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and gouty arthritis [1—5]. When administered orally, sulindac is five to ten times more potent than aspirin in diminishing painful inflammation and, yet, causes significantly less gastrointestinal irritation and fecal blood loss than therapeutically equivalent doses of aspirin [6—9]. The sulfoxide moiety of sulindac (Fig. 1) can be reversibly reduced and irreversibly oxidized in vivo to form sulfide and sulfone metabolites, respectively [10, 11]. In animals, the sulfide metabolite is substantially more potent than sulindac as an anti-inflammatory agent, while the sulfone metabolite is pharmacologically inactive [12]. Sulindac theoretically functions as a prodrug in man and induces less gastrointestinal toxicity because its active metabolite is not present in large quantities in the intestinal lumen. In man, sulindac is eliminated primarily in the urine as intact sulindac, sulindac sulfone and

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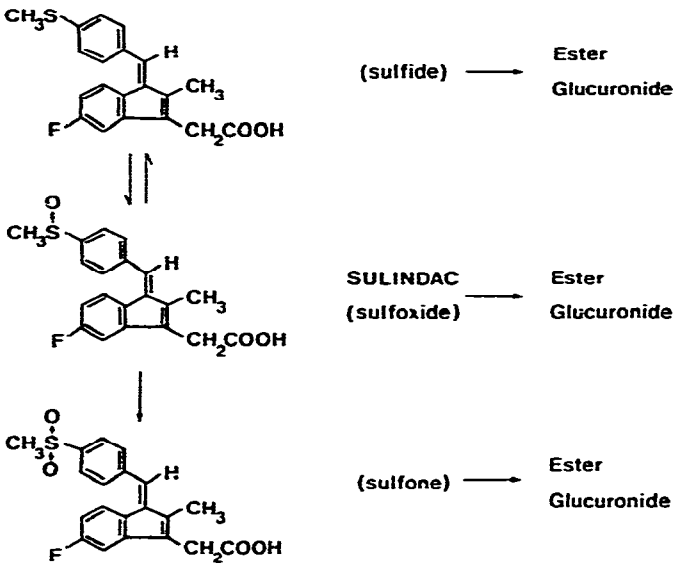

Fig. 1. Metabolism of sulindac in man.

their respective glucuronides [11]. Studies on sulindac pharmacokinetics should properly include measurement of all three oxidative forms of sulindac. Previously reported assay methods have often been too tedious and/or impractical for routine analysis of clinical samples. The mass spectrometric method of Walker et al. [13] offers insufficient accuracy at lower concentrations (< 0.5 μg/ml). The radioimmunoassay procedure of Hare et al. [14] requires lengthy extraction and paper chromatography steps in order to achieve assay specificity. Gas chromatography with electron-capture detection has been employed for measuring sulindac and its metabolites, but this method requires pre-column derivatization and does not adequately resolve sulindac from its sulfone metabolite [15]. Dusci and Hackett [16] have utilized high-performance liquid chromatography (HPLC) with UV detection (254 nm) for simultaneous quantitation of sulindac and its metabolites in serum. However, their method includes a time-consuming evaporation step and is not applicable to assay of urine samples.

Our objective has been to develop a sensitive HPLC assay for quantitation of sulindac and its metabolites in both plasma and urine.

MATERIALS AND METHODS

Chemicals

Sulindac, sulindac sulfone, sulindac sulfide and the *cis*-5-chloro analogue of sulindac sulfide were all obtained from Merck Sharp & Dohme Research Labs. (West Point, PA, U.S.A.). These compounds were found to be > 99% pure by HPLC with UV detection at 254 nm except for a minor impurity (about 3%) in the 5-chloro sulfide analogue. Standard solutions were prepared in methanol and were found to be stable at 4°C. Methanol and 1-chloro-

butane were both HPLC grade solvents (Fisher Scientific, Pittsburgh, PA, U.S.A.).

Drug administration

Four male volunteers (ages 30—45 years) each received a 200-mg Clinoril® tablet (MSD) from the same lot after overnight fasting. Blood samples were periodically withdrawn from a forearm vein through an indwelling catheter and immediately placed in a heparinized centrifuge tube (Venoject, Kimble-Terumo, Elkton, MD, U.S.A.). Samples were centrifuged and plasma was stored at −20°C. Urine was also collected at specified intervals, beginning with a pre-drug sample. The volume of each urine sample was measured promptly after collection and then stored at −20° C.

Extraction of plasma

One ml plasma, 100 μl methanol (containing standards when preparing standard curve) and 50 μl internal standard solution (0.03 mg/ml, 5-chloro sulfide analogue in methanol) were each placed in a 40-ml glass extraction tube and thoroughly mixed. 1-Chlorobutane (25 ml) and 1.0 *N* hydrochloric acid (150 μl) were then added, and the tube shaken for 10 min on a mechanical shaker. After phase separation by centrifugation at 1500 *g* for 10 min, 20 ml of the 1-chlorobutane layer were transferred to a second 40-ml centrifuge tube containing 250 μl 0.4 *N* sodium hydroxide. This tube was shaken mechanically for 10 min and then centrifuged at 1500 *g* for 10 min. The final 1-chlorobutane layer was discarded by aspiration and 50 μl of the aqueous phase was injected into the chromatograph.

Extraction and hydrolysis of urine

For measurement of unconjugated compounds, 200 μl urine, 100 μl methanol (containing standards when preparing standard curve) and 50 μl internal standard solution were thoroughly mixed in a 40-ml glass extraction tube. 1-Chlorobutane (25 ml) and 0.5 *N* sodium citrate buffer (pH 3.0, 1.0 ml) were then added. After mechanical shaking for 10 min and centrifugation for 10 min at 1500 *g*, 20 ml of the organic phase were transferred to another 40-ml extraction tube containing 250 μl 0.4 *N* sodium hydroxide. This tube was again shaken and centrifuged, and the 1-chlorobutane layer was discarded. Fifty microliters of the aqueous phase were injected into the chromatograph.

For measurement of conjugated compounds, 4.0 ml urine were first mixed with 600 μl 1 *N* hydrochloric acid and washed twice with 10-ml portions of 1-chlorobutane. Two milliliters of solvent-washed urine were then alkalinized with 200 μl 4 *N* sodium hydroxide and kept at room temperature for 30 min. A 150-μl aliquot of 5 *N* hydrochloric acid was then added, and aglycones were extracted from 200 μl of this solution according to the method for unhydrolysed urine.

Reversed-phase chromatography

Instrumentation included a Spectra-Physics Model 8000 high-performance liquid chromatograph equipped with a UV detector (254 nm), oven (29°C), electronic peak integrator and a Spherisorb 10-μm ODS column (Applied

Science, State College, PA, U.S.A.). The mobile phase consisted of methanol—0.4 *N* sodium acetate buffer (pH 4.0) with a flow-rate of 1.2 ml/min. An initial volume ratio of 63:37 was maintained for 6 min to isocratically elute sulindac and its sulfone metabolite, and then increased to 80:20 to elute sulindac sulfide and the sulfide internal standard.

RESULTS

Evaluation of assay methods

Typical chromatograms for extracts of human plasma and urine, with and without standards, are shown in Figs. 2 and 3. Small peaks in plasma interfered somewhat with the sulindac peak. This interference was equivalent to 0.05—0.25 μg/ml (mean value, about 0.10 μg/ml) sulindac in plasma from six persons who had not received Clinoril and was found to remain constant throughout the day for any one individual. Thus, even low concentrations (0.1—0.2 μg/ml) can be reported for sulindac in plasma provided a pre-drug blank is obtained and subtracted from post-drug values. No background peaks in plasma interfered with the assay of the two metabolites; hence, the lower limit of sensitivity for these compounds was less than 0.1 μg/ml in plasma. Peaks found in normal urine did not interfere with any of the four standard peaks, and lower limits of assay sensitivity were less than 0.2 μg/ml for each compound in urine.

Extraction recovery was determined by comparing detector response to standards injected directly on the column with the response to standards extracted from plasma and urine. Mean recoveries for sulindac, sulindac sulfone and sulindac sulfide were 89, 92 and 93% from biological samples, respective-

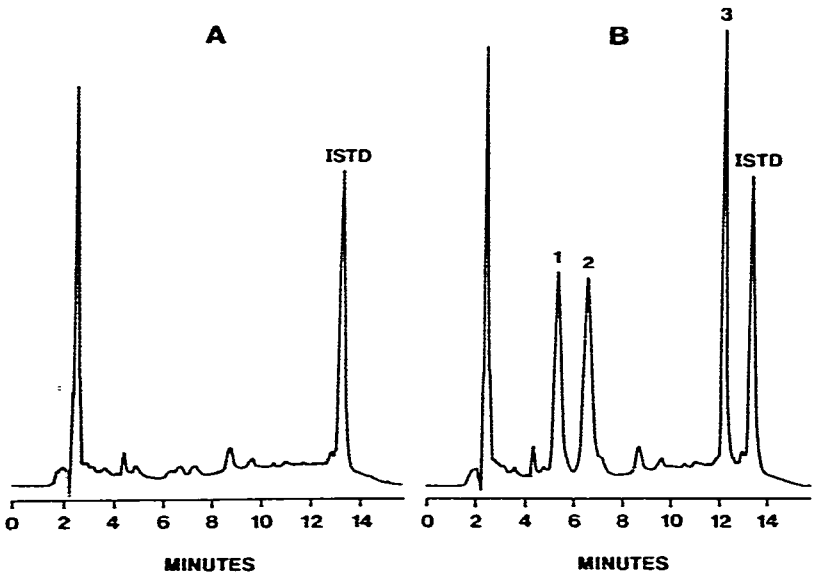

Fig. 2. Chromatograms of plasma extract for blank plasma with internal standard (ISTD) (A) and for plasma spiked to 2 μg/ml each of sulindac sulfone (1), sulindac (2) and sulindac sulfide (3) plus 1.5 μg/ml internal standard (B).

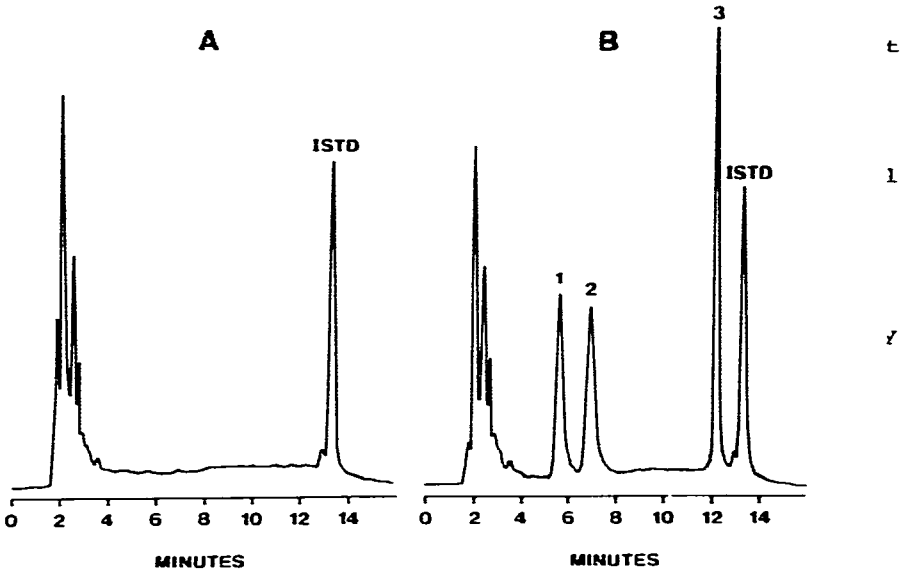

Fig. 3. Chromatograms of urine extract for blank urine with internal standard (ISTD) (A) and for urine spiked to 10 μg/ml each of sulindac sulfone (1), sulindac (2) and sulindac sulfide (3) plus 7.5 μg/ml internal standard (B).

ly. Recoveries for sulindac and sulindac sulfone were constant over the entire concentration range of investigation; therefore, peak areas without internal standardization were used as the basis of quantitation. Recovery of the sulfide metabolite was more variable; however, the sulfide internal standard compensated well for this variation. Standard curves for the various compounds were linear up to at least 40 μg/ml in plasma and 100 μg/ml in urine. Correlation coefficients (least squares method) for plasma standard curves (0.1, 0.4, 1.0, 2.0, 5.0 and 10.0 μg/ml) were typically 0.9998, 0.9997 and 1.000 for sulindac, sulindac sulfide and sulindac sulfone, respectively. Correlation coefficients were equally good for urine standard curves (0.2, 1.0, 2.0, 5.0, 10.0, 25.0 and 50.0 μg/ml). Random variability for the assay methods was

TABLE I

VARIATION IN REPLICATE STANDARDS EXTRACTED FROM HUMAN PLASMA AND URINE

Type of sample	Concentration (μg/ml)	Coefficient of variation (%)		
		Sulindac	Sulindac sulfone	Sulindac sulfide
Plasma ($N = 4$)	0.4	2.5	6.3	11
	2.0	2.4	1.0	6.9
	10	3.8	3.4	0.7
Urine ($N = 6$)	5.0	3.7	4.1	
	50	2.7	2.7	

assessed by preparing in replicate high and low standards in both urine and plasma (Table I).

Aspirin and indomethacin (at concentrations up to 50 μg/ml) did not interfere with the assay for sulindac and its metabolites.

Application of assay to clinical samples

The concentrations of sulindac and its two major metabolites were measured in plasma after a single 200-mg dose of Clinoril (Fig. 4). The magnitude

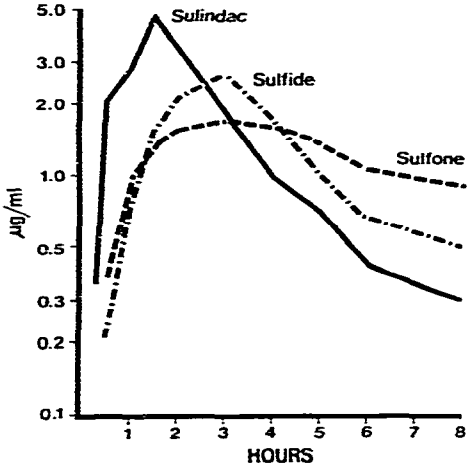

Fig. 4. Concentrations of sulindac, sulindac sulfone and sulindac sulfide in plasma after oral administration of 200 mg Clinoril. Mean values for four subjects are presented.

and shape of the concentration—time curves are similar to that of previously published work [11]. Table II indicates the urinary concentration and total excretion of sulindac and its various metabolites for 8 h after a 200-mg dose of Clinoril. A comparison between this table and historical results is not possible, since earlier work on the urinary excretion of sulindac after a 200-mg dose did not distinguish between glucuronide metabolites and unconjugated sulindac and sulindac sulfone at early time intervals.

DISCUSSION

We have described a sensitive HPLC method which can be readily applied to studies on the bioavailability, metabolism and clearance of sulindac in man. The advantages of this method over a previously published HPLC technique [16] can be attributed to an improved extraction procedure. Thus, the previous method is not applicable to urine samples, includes a time-consuming evaporation step and, due to excessive UV-absorbing contaminants in the solvent front, elutes sulindac and sulindac sulfone prior to achieving a level detector baseline. In addition, the previous assay did not recommend daily extraction of standards from serum, despite intersubject variation in pre-drug serum blanks. For the present work, we have utilized the most universally available UV detector wavelength (254 nm). However, we have recent-

TABLE II

EXCRETION OF SULINDAC AND ITS METABOLITES AFTER 200 mg CLINORIL, ORALLY

Values represent means for 4 subjects. Only trace amounts of sulindac sulfide and its glucuronide were found in urine.

	0—1 h	1—2 h	2—3 h	3—4 h	4—6 h	6—8 h	Total 0—8 h
Sulindac (μg/ml)	42.5	148	100	43.5	13.6	6.44	
(mg)	2.11	6.42	3.72	1.40	1.29	0.66	15.6
Sulindac glucuronide* (μg/ml)	56.1	177	126	67.7	21.3	8.45	
(mg)	1.74	5.63	3.56	1.75	1.54	0.52	14.7
Sulfone (μg/ml)	1.49	7.02	8.18	9.79	3.89	2.55	
(mg)	0.07	0.23	0.25	0.30	0.37	0.25	1.47
Sulfone glucuronide* (μg/ml)	13.4	59.5	62.3	63.3	36.3	24.4	
(mg)	0.55	1.78	1.90	1.78	2.88	2.15	11.0

*Conjugate values are reported as mg or μg/ml of aglycone measured after hydrolysis.

ly found that detection at 280 nm can provide more accurate measurement for low concentrations of sulindac, since, at this wavelength, background peaks from plasma do not interfere with any of the drug assay peaks. While detector sensitivity for sulindac sulfide is diminished at 280 nm relative to 254 nm, the assay is still fully reliable down to 0.1 μg/ml for each compound in plasma.

Automation of the assay with an autosampler (Waters Intelligent Sample Processor Model 710B) allows one worker to process over 60 samples (including standards) each day. We now routinely use sodium metabisulfite solution (0.5% in 0.4 *N* sodium hydroxide, prepared daily) instead of 0.4 *N* sodium hydroxide for the back-extraction procedure, as this completely abolishes the slow oxidation of sulindac and sulindac sulfide that can occur during overnight exposure of these compounds to dilute alkali.

In conclusion, we have found that by introducing an improved extraction procedure, HPLC with UV detection can be utilized for measuring sulindac and its metabolites in both plasma and urine. In fact, no other method presently offers the same high degree of assay sensitivity, ease of execution and adaptability.

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