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Note

Determination of diastereoisomeric pairs of thioridazine 5-sulfoxide by high-performance liquid chromatography

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Thioridazine 5-sulfoxide (thioridazine ring sulfoxide) is a major metabolite of thioridazine, a phenothiazine neuroleptic drug used in the management of psychotic disorders. Thioridazine possesses an asymmetric carbon and has been separated into enantiomers [1]. Oxidation of the ring sulfur atom of thioridazine gives rise to an additional chiral center; therefore, thioridazine 5-sulfoxide exists as two diastereoisomeric pairs of enantiomers. Juenge et al. [2] recently reported the chemical synthesis, isolation, and structural analysis of these two stereoisomer pairs.

Wells et al. [3] developed a high-performance liquid chromatographic (HPLC) method utilizing post-column oxidation and fluorometric detection for the determination of thioridazine and its major metabolites in plasma, including the pairs of ring sulfoxides. Applying this method, Poklis et al. [4] demonstrated the presence of the thioridazine 5-sulfoxide stereoisomeric pairs in blood from patients receiving thioridazine. Previous metabolic studies of thioridazine had failed to recognize the presence of the two ring sulfoxide pairs. The pharmacokinetics and pharmacodynamics of these stereoisomeric pairs have not been studied.

This communication presents a normal-phase HPLC procedure for the rapid determination of the two diastereoisomeric pairs of enantiomers of thioridazine 5-sulfoxide in serum. The ring sulfoxide pairs are tentatively identified as thioridazine ring sulfoxide fast eluter (RSF) and slow eluter (RSS). The analysis is performed in a short silica column with an ultraviolet detector. The method is particularly suited for pharmacokinetic studies in animals; studies that require the rapid analysis of a large number of serum or blood samples.

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EXPERIMENTAL

Chemicals and reagents

Thioridazine and mesoridazine were obtained from Sandoz Pharmaceuticals (Hanover, NJ, U.S.A.). 2-Propanol and *n*-butyl chloride (HPLC grade) were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) and diethylamine from Mallinckrodt (St. Louis, MO, U.S.A.). Distilled water was purified for HPLC use with a Millipore filtration unit. All glassware was silanized with 2% dimethyldichlorosilane in benzene (Sigma, St. Louis, MO, U.S.A.).

Standards

Standards of the diastereoisomeric pairs of thioridazine ring sulfoxide were prepared by the method of Juenge et al. [2]. A stock standard solution of the racemic ring sulfoxides (100 μg/ml) was prepared by dissolving 10 mg of the drug in 100 ml of ethanol. An intermediate standard of 10 μg/ml was prepared by dilution of 0.10 ml of the stock standard with 0.90 ml of ethanol. A second intermediate standard of 1 μg/ml was prepared by dilution of 0.10 ml of the 10 μg/ml standard with 0.90 ml of ethanol. Mixed standards in serum were prepared daily by adding the following amounts of intermediate standards to 15-ml reusable culture tubes and diluting to 2.0 ml with serum: 0.10, 0.20, and 0.50 ml of the 1 μg/ml standard and 0.10 ml of the 10 μg/ml standard. The resultant serum standards contained the following concentrations of RSF and RSS (ng/ml serum): 25, 50, 125, and 250.

The internal standard was prepared by dissolivng 300 μg of mesoridazine in 100 ml of ethanol. A 0.40-ml aliquot of this standard was added to each sample before extraction. Although mesoridazine is also an oxidative metabolite of thioridazine, data from additional experiments indicate that after administration of the ring sulfoxides to rats, the compounds are not reduced and then oxidized again on the side-chain sulfur to form mesoridazine. Instead the ring sulfoxides are oxidized further to the disulfoxide and sulfone.

Chromatography

Analyses were performed with a Waters Assoc. (Milford, MA, U.S.A.) HPLC system consisting of a Model M6000A chromatography pump, Model U6K injector and Model 450 variable-wavelength detector. The mobile phase consisted of *n*-butyl chloride—2-propanol—water—diethylamine (92.0:7.9:0.05:0.05). The solvents were mixed and then filtered through a 0.45-μm filter before use. Separations were carried out isocratically in an IBM (Danbury, CT, U.S.A.) 5-μm silica 50 mm × 4.5 mm minicolumn. The mobile phase flow-rate was 1.7 ml/min. The column effluent was monitored at 279 nm for optimal detection of the ring sulfoxides [2]. Chromatograms were recorded using a Hewlett-Packard (Avondale, PA, U.S.A.) 3390A integrator. Instrument operating conditions were maintained for 0.5 h before the first injection was made. Under these conditions, the retention times were 3.7 min for the fast eluting ring sulfoxide, 5.4 min for the slow eluting ring sulfoxide, and 9.8 min for mesoridazine (Fig. 1). Thioridazine and sulforidazine do not interfere with this assay as both compounds elute in the solvent front. Thioridazine disulfoxide, if present, elutes several minutes after mesoridazine.

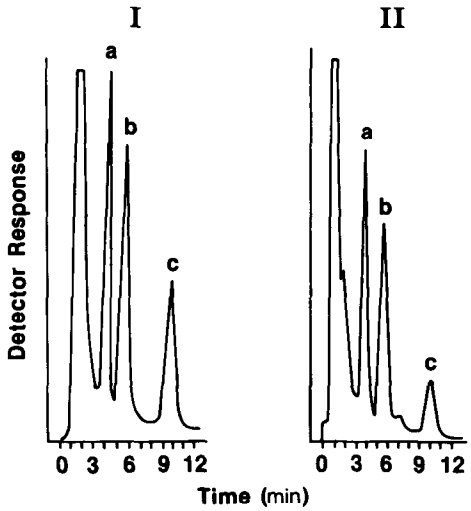

Fig. 1. HPLC chromatograms of (I) a 200 ng/ml serum standard; (II) extracted rat serum 2 h after a 10 mg/kg injection of thioridazine ring sulfoxide. Peaks: a = thioridazine ring sulfoxide fast eluter; b = thioridazine ring sulfoxide slow eluter; and c = internal standard.

Procedure

To 2.0 ml of serum, 3.0 ml of heptane—ethyl acetate (1:1) and 0.40 ml of the internal standard, mesoridazine (3 μg/ml), were added. The mixture was vortexed for 2 min, centrifuged at 1000 *g* for 10 min and the upper (organic) layer aspirated into a new tube. The remaining aqueous phase was reextracted with 3.0 ml of fresh heptane—ethyl acetate. The mixture was again vortexed and centrifuged, and the organic layer drawn off and combined with the organic phase from the first extraction. The combined organic extracts were evaporated to dryness on a steam bath at 40°C under a stream of nitrogen. The residue was dissolved in 0.50 ml of the HPLC mobile phase and 0.10-ml aliquots were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Standard curves from 5 to 250 ng/ml were constructed from analyses of plasma containing a known concentration of the isomeric ring sulfoxides. The least-squares linear regression equations for the fast eluting thioridazine ring sulfoxide and the slow eluting thioridazine ring sulfoxide were: Y (peak area ratio RSF/internal standard) = $0.022X$ (RSF, ng/ml) + 0.018, (r = 0.991); and Y (peak area ratio RSS/internal standard) = $0.024X$ (RSS, ng/ml) − 0.136, (r = 0.997). The within-run coefficient of variation (C.V.) for RSF (target concentration 250 ng/ml) was 6.31% (n = 9) and that for RSS (target concentration 250 ng/ml) was 4.50% (n = 9). The within-run C.V. for RSF (target concentration 50 ng/ml) was 16.8% (n = 21) and that for RSS (target concentration 50 ng/ml) was 15.0% (n = 21).

The absolute, uncorrected, analytical recovery for both RSF and RSS was calculated by comparison of peak areas obtained from supplemented serum samples with those of non-extracted standards. The mean recoveries over the

concentration range of 10—250 ng/ml of RSF and RSS were 103% (n = 22) and 97% (n = 22), respectively.

The ring sulfoxides are the major metabolites found in the plasma of patients chronically treated with thioridazine. Values range from less than 40 ng/ml to 5 μg/ml of plasma depending on the dose and the duration of thioridazine treatment [5]. Plasma concentrations of the ring sulfoxide have also been correlated with electrocardiographic abnormalities in thioridazine-treated patients [6].

As quantitative separation of the diastereoisomeric ring sulfoxides has only recently been realized [3], this selective and sensitive method is particularly suited for the determination of plasma concentrations of each ring sulfoxide and also for evaluation of the disposition and elimination of the ring sulfoxides in animal models.

The relative retention of the ring sulfoxides and mesoridazine was found to be dependent on the concentration of diethylamine present in the mobile phase. Increasing the concentration of diethylamine only slightly caused a decrease in the retention times of all components. This effect may be due to diethylamine's ability to compete with phenothiazines for acidic binding sites on the silica surface of the column [7, 8].

The HPLC procedure of Wells et al. [3] is also capable of separating the ring sulfoxides. However, their method is cumbersome as it requires post-column oxidation before fluorometric detection. The present method does not require oxidizing reagents, mixing coils, or a fluorometric detector. Instead, non-destructive ultraviolet detection at 279 nm is employed, allowing greater recovery of each isomer for further qualitative determinations, e.g. mass spectroscopy.

The absolute limit of detection using the present method is 5 ng/ml, a value similar to the 2 ng/ml obtainable with fluorometric detection. Our method offers the advantages of sensitive ultraviolet detection of the diastereoisomeric ring sulfoxides and short elution times.

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