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Note

Measurement of haloperidol and reduced haloperidol in human plasma using reversed-phase high-performance liquid chromatography

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There is increasing interest in monitoring plasma concentrations of psychoactive drugs as an aid to improving optimal therapeutic effects while avoiding incipient side-effects. This has been a difficult exercise with the most extensively used class of antipsychotics, the phenothiazines, because their complicated metabolic profile results in the generation of multiple metabolites, some of which are pharmacologically active. An example of an antipsychotic compound which has a relatively simple metabolic profile is haloperidol, therefore, making it attractive for studying the relationship between the plasma drug concentrations and clinical effects. A high-performance liquid chromatographic (HPLC) procedure for the quantitation of haloperidol concentrations in plasma was previously described by one of us [1]. The method has a sensitivity of 2 ng/ml and has proven suitable for plasma concentration monitoring of haloperidol.

Recently, the reduced metabolite of haloperidol has been found to possess dopamine blocking activity of approximately 20% or more of the potency of the parent compound [2, 3]. There is a need to assess the contribution of this active metabolite to the overall effects of administered haloperidol.

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Although several HPLC methods exist for analysis of haloperidol in plasma [4—6], few methods exist for analysis of reduced haloperidol. They include radioimmunoassay (RIA) preceded by thin-layer chromatography to separate haloperidol from reduced haloperidol [7] and HPLC with ultraviolet [8] or electrochemical detection [9]. The purpose of this report is to describe a modified procedure whereby reduced haloperidol may also be quantitated by the previously published HPLC method for haloperidol [1].

EXPERIMENTAL

Apparatus

Chromatography was performed with a Waters (Milford, MA, U.S.A.) Model 6000A pump fitted with a Rheodyne 7126 injection valve (Rheodyne, Cotati, CA, U.S.A.) and a 100-μl sample loop. The column was a 25 cm × 4.6 mm reversed-phase octadecyl, 10.0 μm particle size (C_{18}) column (Alltech Assoc., Deerfield, IL, U.S.A.). An IBM (IBM Instruments, Wallingford, CT, U.S.A.) Model 9523 variable-wavelength UV detector was operated at 196 nm. An IBM CS9000 laboratory computer was used to record, store and analyze chromatograms.

Reagents and chemicals

Analytical- or reagent-grade hexane, acetonitrile, isoamyl alcohol, hydrochloric acid, phosphoric acid, sodium hydroxide and monobasic potassium phosphate were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) while haloperidol, chlorohaloperidol and reduced haloperidol were gifts from the McNeil Pharmaceutical (Springhouse, PA, U.S.A.). Water was passed through a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.) before use.

Stock standard solutions of haloperidol, chlorohaloperidol and reduced haloperidol (calculated as 1 mg/ml of free base) were prepared in 10 m*M* hydrogen chloride in methanol.

Chromatographic conditions

The mobile phase consisted of 55% acetonitrile in 100 m*M* monobasic potassium phosphate adjusted to pH 3.8—4.0 with phosphoric acid. The flow-rate was 2.0 ml/min.

Plasma standards for calibration curves were prepared by spiking 1.0-ml aliquots of plasma with diluted stock solutions of haloperidol and reduced haloperidol to make standards ranging from 2.5 to 50.0 ng/ml. The concentration of the internal standard (chlorohaloperidol) was 50 ng/ml in each plasma sample.

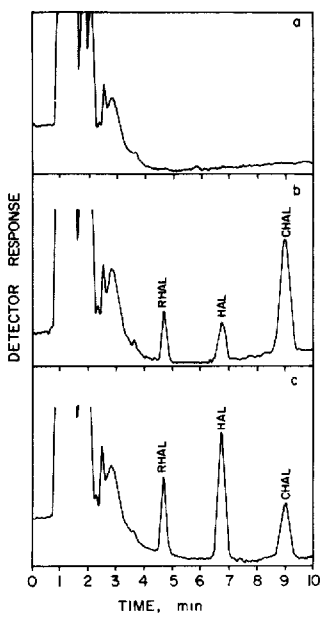
The extraction procedure was similar to that previously described [1]. A 1-ml volume of sample (standard, control or patient plasma) was pipetted into a polypropylene tube and exactly 10 μl (50 ng) of internal standard (chlorohaloperidol), 100 μl of 5 *M* sodium hydroxide and 6 ml of hexane containing 2% isoamyl alcohol were added. Each sample was mixed briefly following each addition using a vortex-type mixer. The tubes were then stoppered and mixed on a reciprocating shaker (Eberbach, Ann Arbor, MI, U.S.A.) for 10 min. After

centrifuging for 10 min at 220 *g*, the hexane (upper phase) was transferred with a pasteur pipet into a clean test tube and 100 μl of 0.1 *M* hydrochloric acid were added. After 10 s vortex-mixing, the samples were again centrifuged and the hexane layer was aspirated and discarded. A 90-μl aliquot of the remaining aqueous phase was injected onto the HPLC column.

RESULTS AND DISCUSSION

Peak height ratios of both haloperidol and reduced haloperidol to internal standard showed a linear relationship to serum concentration over a range of at least 2 to 100 ng/ml (highest concentration tested). Calibration curves were consistently linear and passed through the origin. The within-day and between-day coefficients of variation at a concentration of 10 ng/ml (n = 6) were 5.7 and 6.5%, respectively, for haloperidol and 6.1 and 4.3%, respectively, for reduced haloperidol. Absolute recoveries of haloperidol and reduced haloperidol were 92 and 86%, respectively, at a concentration of 10 ng/ml.

The retention times for reduced haloperidol, haloperidol and chlorohaloperidol were 4.6, 6.7 and 9.0 min, respectively. Fig. 1 shows the absence of interfering peaks from extracted blank plasma (Fig. 1a) and the elution of reduced haloperidol, chlorohaloperidol and haloperidol from spiked plasma (Fig. 1b) and from a 1-ml plasma obtained from a patient chronically taking haloperidol (Fig. 1c).



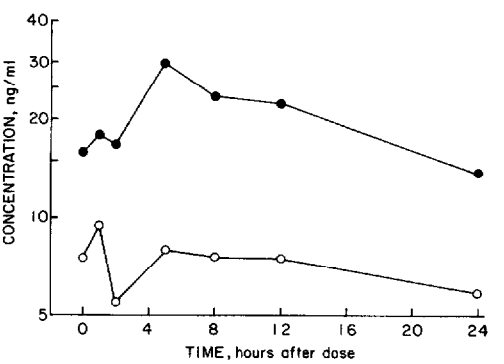


Fig. 1. Chromatograms of the extracts from 1 ml of (a) blank plasma; (b) plasma which had been spiked with 10 ng/ml haloperidol (HAL), 50 ng/ml chlorohaloperidol (CHAL) and 10 ng/ml reduced haloperidol (RHAL); (c) plasma obtained from a woman chronically taking 20 mg per day of haloperidol orally. The plasma concentration was found to be 65 ng/ml haloperidol and 33 ng/ml reduced haloperidol.

Fig. 2. Concentration versus time curves of haloperidol (•) and reduced haloperidol (○) during a 24-h dosage interval in a patient receiving 20 mg of haloperidol daily.

The assay was utilized to assess haloperidol absorption in a psychotic woman following her gastric bypass surgery for obesity [10]. Fig. 2 shows the patient's concentration versus time profile of haloperidol and reduced haloperidol over a 24-h dosage interval while receiving 20 mg of haloperidol by mouth per day.

The present method represents an improvement of the previous assay [1] by being able to quantitate the reduced metabolite along with haloperidol from the same injection. It has the advantage of greater sensitivity than a previously described HPLC procedure by monitoring UV absorbance at 196 nm rather than at 254 nm [8] and does not require the evaporation step for sample preparation which is present with electrochemical detection methods [9]. In conclusion, the present method requires only a simple sample preparation, has a rapid elution time of less than 10 min and is suitable for studying haloperidol pharmacokinetics in humans (Fig. 2).

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