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Note

Simultaneous determination of very low concentrations of haloperidol and reduced haloperidol in human serum by a liquid chromatographic method

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Haloperidol (H) is a neuroleptic drug that is widely used for the treatment of acute and chronic psychotic syndromes. The reduction of H to reduced haloperidol (RH), which was first discovered in humans by Forsman and Larsson [1], is shown in Fig. 1 [1,2]. This compound does have biological activity which should be taken into account when blood concentrations of biologically active H and its metabolites are being measured. Therefore, a routine procedure for the monitoring of therapy with H should include determinations of both H and RH.

A number of techniques are available in the literature for the determination of H in human plasma samples. Some of these procedures involve gas chromatography [3–5], receptor binding assays [6], and high-performance liquid chromatography (HPLC) [7–10]. The simultaneous analysis of H and RH has also been performed using radioimmunoassay [11,12] and HPLC [13,14].

There are several unique clinical situations where low dosages of H are prescribed by the physician. This results in low plasma concentrations of the drug

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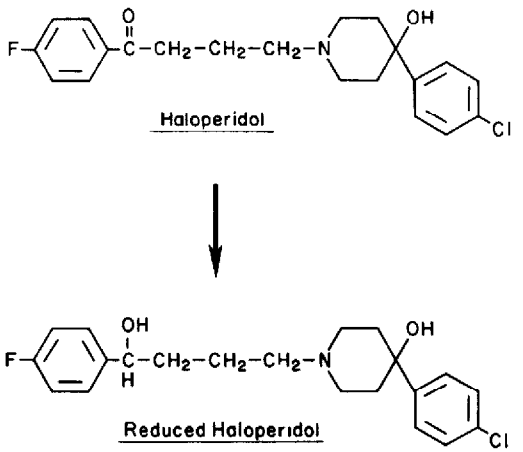

Fig. 1. Formation of reduced haloperidol from haloperidol in humans.

and also possibly its metabolite. The most common among these clinical conditions is the treatment of the elderly patient where small dosages are routinely used. In order to analyze plasma samples from such patients we have developed a liquid chromatographic procedure for the determination of H and RH. This is a modification of the original procedure of Jatlow et al. [7]. Using the procedure described in this report it is possible to analyze H and RH in plasma concentrations as low as 0.3 ng/ml. This report will emphasize the details of the protocol which should be carefully followed in order to achieve such high sensitivity with acceptable degrees of reliability.

EXPERIMENTAL

Apparatus

The liquid chromatographic system was modularly constructed from the following components: Model 2150 pumping system with the Model 2152 HPLC controller from LKB Instruments (Gaitherburg, MD, U.S.A.); WISP 710 B autosampler from Waters Assoc. (Milford, MA, U.S.A.); Model 166 programmable variable-wavelength detector from Beckman Instruments (San Ramon, CA, U.S.A.); Model 3390A electronic integrator from Hewlett Packard (Avondale, PA, U.S.A.). The progress of chromatography was also followed using a strip chart recorder (Model 7127A from Hewlett Packard). A suitable absorbance monitor capable of programmable changes in wavelength during a chromatographic run should be used. The detector should also be selected for the highest sensitivity attainable.

Reagents and standards

The internal standard used was bromperidol (B). H, RH, and B standards were obtained as gifts from McNeil Pharmaceutical (Springhouse, PA, U.S.A.). All solvents were distilled in glass by the supplier, Burdick and Jackson Labs. (Mus-

kegon, MI, U.S.A.). The hexane used for extraction was again redistilled in glass apparatus in the laboratory prior to use. If this was not done, an extra peak which interfered with the quantitation of RH was observed. Isoamyl alcohol (3-methyl-1-butanol) was the Gold Label grade from Aldrich (Milwaukee, WI, U.S.A.). The other chemical reagents such as phosphoric acid, sodium hydroxide, etc., were all of the highest purity from standard chemical sources.

Chromatographic conditions

Water from a glass distillation unit was further purified by pumping through a standard reversed-phase C_{18} column. The potassium phosphate used for preparation of the buffer was produced by neutralization of potassium hydroxide with orthophosphoric acid. The pH of 0.085 *M* potassium phosphate solution was adjusted to about 2.4. For preparing 1 l of the mobile phase, 700 ml of the phosphate buffer (0.085 *M*) were mixed with 300 ml of acetonitrile. The pH of the solution was then adjusted accurately to 3.5 using 50% potassium hydroxide solution. The buffered mobile phase was filtered using Millipore HVHP filters (Millipore, Bedford, MA, U.S.A.) and then degassed under vacuum. The mobile phase was pumped through an Ultrasphere ODS gold star column (Beckman Instruments), with the dimensions 25 cm × 4.6 mm I.D., filled with 5-μm particles. The analytical column was preceded by a 2 cm × 2 mm I.D. guard column containing Perisorb RP18, 30–40 μm particle size, from Upchurch Scientific (Oak Harbor, WA, U.S.A.).

Recommended procedure

To each extraction tube 2 ml of serum and 400 μl of 1 *M* sodium hydroxide were added. An appropriate small volume of an internal standard solution (100 μl of 20 ng/ml B in 0.1 *M* hydrochloric acid) was added to all extraction tubes. The tube was vortex-mixed briefly and 5 ml of hexane containing 1.5% isoamyl alcohol were added. The tubes were placed on the rack on a Big Vortexer (Glascol Apparatus, Terre Haute, IN, U.S.A.), vortex-mixed vigorously for 20 min and then centrifuged at 1200 *g* for 5 min. The aqueous layer was frozen by placing the tubes on dry ice. The organic phase was then poured into another extraction tube which contained 200 μl of 0.1 *M* hydrochloric acid. The original extraction tube was warmed and the contents were thawed. The solution was reextracted for 10 min with 3 ml of hexane containing 1.5% isoamyl alcohol. The second extract was recovered as before and mixed with the first extract. The tube containing the 200 μl of 0.1 *M* hydrochloric acid and about 8 ml of the mixed extracts was vortex-mixed vigorously for 10 min on the Big Vortexer. The tubes were centrifuged at 1200 *g* for 10 min, frozen on dry ice, and then the organic phase was discarded by aspirating it off. The hydrochloric acid extract was thawed and 150 μl were pipetted into the glass limited-volume inserts of the autosampler vials. The solution was partially neutralized by adding 14 μl of 1 *M* sodium hydroxide. The solution was mixed briefly and 100 μl were injected on the column.

Standards of H and B were prepared in 0.1 *M* hydrochloric acid from original stock solutions in methanol. These were added to serum samples from normal

human volunteers and a standard curve was obtained by following the procedure described above.

RESULTS AND DISCUSSION

A number of analyses were done using plasma from patients on H as well as normal human plasma to which H and RH were added. Chromatographic behaviors of both types of samples were identical. The representative chromatogram shown in Fig. 2A was obtained when a sample of normal human plasma containing 0.5 ng/ml H and RH was processed according to the recommended method. This can be compared with the chromatogram obtained from a normal plasma sample containing no H or RH (Fig. 2C). The compounds gave readily quantifiable peaks even at this low level (Fig. 2A). The observed retention times were: RH, 6.03 min; H, 9.26 min; B, 10.87 min.

A standard curve was then obtained by using plasma containing enough H and RH to make the final concentrations of 0.5, 1.0, 1.5, and 2.0 ng/ml. The response is quite linear at these low concentrations (correlation coefficients for linear regression for RH was 0.986 and for H was 0.984). The average ($\pm$S.D.) coefficients of variation at these low concentrations were: RH, $6.21 \pm 1.7\%$; H, $8.31 \pm 4.4\%$.

We have also found that it is possible to conduct reliable analyses of samples with concentrations of drugs as low as 0.3 ng/ml. This is achieved by pooling the

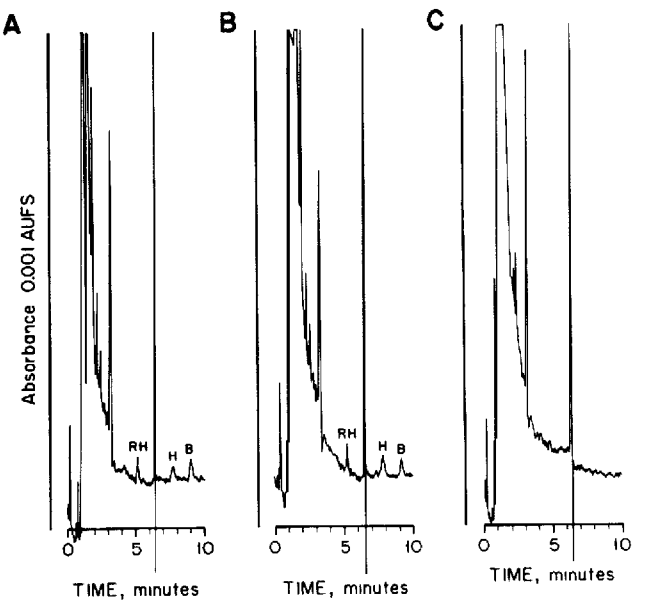

Fig. 2. Chromatograms obtained after processing human plasma samples according to the recommended procedure. (A) Plasma containing haloperidol and reduced haloperidol (0.5 ng/ml of each drug). (B) Plasma containing haloperidol and reduced haloperidol (the extracts from 2×2 ml of plasma containing 0.3 ng/ml of each drug were pooled and analyzed). (C) Plasma with no drugs. Peaks: RH = reduced haloperidol; H = haloperidol; B = bromperidol, internal standard.

extracts as follows. A hexane (with 1.5% isoamyl alcohol) extract is made from 2 ml of plasma and the drugs are back-extracted into 0.2 ml of 0.1 *M* hydrochloric acid as described in *Recommended procedure*. The hexane layer is then discarded. Another 2-ml aliquot of plasma is extracted with fresh solvent and this new hexane extract is poured over the same 0.2 ml of 0.1 *M* hydrochloric acid which was used for back-extraction earlier. The extraction is continued and final analysis done as before. No additional problems such as increases in baseline noise or appearance of extraneous peaks were observed. The standard curves obtained with and without pooling of the extracts were identical. A representative chromatogram obtained after pooling of extracts is shown in Fig. 2B and is essentially similar to the one in Fig. 2A.

The technique was then applied to the analysis of H and RH in plasma from a few selected patients who had been taking different doses of the drug for 8–22 days. The data are given in Table I. It will be noted that the plasma H concentrations are in the 3–15 ng/ml range. In order to ascertain that the current method is sensitive enough to measure lower drug concentrations, the analyses were conducted on low volumes of plasma, e.g., 0.5 ml or less. Significant quantities of RH are present in the plasma even 8 days after beginning drug treatment. In a separate experiment a few samples of plasma containing H and RH in the range of 0.3–1.0 ng/ml were analyzed and the average within-day coefficients of variation for H and RH were 5 and 4%, respectively.

If a single 2-ml sample of blood plasma is analyzed according to the proposed method one can measure H and RH concentrations as low as 0.5 ng/ml. However, if more sample is available, the extracts from two 2-ml plasma samples can be pooled and processed as described under Results and discussion. Concentrations of H and RH as low as 0.3 ng/ml can then be measured. This limit of measure-

TABLE I

CONCENTRATIONS OF HALOPERIDOL AND REDUCED HALOPERIDOL IN PLASMA OF PATIENTS TAKING HALOPERIDOL

Samples of blood were obtained from eight patients who were being treated with haloperidol for periods of 8–22 days. Plasma samples were isolated and analyzed for haloperidol and reduced haloperidol by the recommended method.

Patient No.	Dosage (mg/12 h)	Duration of drug regimen (days)	Plasma concentrations (mean ± S.D.) (ng/ml)	
			Haloperidol	Reduced haloperidol
1	5	8	3.33 ± 0.05	1.15 ± 0.02
2	5	8	3.11 ± 0.08	1.06 ± 0.03
3	5	8	4.97 ± 0.54	1.46 ± 0.22
4	10	8	4.76 ± 0.40	1.67 ± 0.09
5	10	8	4.84 ± 0.32	0.73 ± 0.04
6	10	15	8.53 ± 0.30	2.76 ± 0.14
7	10	22	14.86 ± 0.80	3.57 ± 0.18
8	10	22	5.51 ± 0.16	0.99 ± 0.04

ment is quite low compared with those reported in the literature. A few examples of reported limits of measurement are the following: Jatlow et al. [7], 2 ng/ml; McBurney and George [8], 2 ng/ml; Miyazaki et al. [15], 5 ng/ml; Dhar and Kutt [10], 1 ng/ml; Korpi et al. [13], 0.25–0.5 ng/ml.

It has been generally accepted that RH does possess significant biological activity [12]. As seen in Table I, RH can account for roughly a third of the H concentration as early as a week after the starting of the ingestion of drug. This points to the necessity of measurement of both compounds in studies which deal with correlations of clinical outcome with drug concentrations in plasma. The procedure reported here should prove valuable in achieving this goal in studies of patients receiving very low doses of the drug.

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