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Note

Haloperidol determination in serum and cerebrospinal fluid using gas—liquid chromatography with nitrogen—phosphorus detection: application to pharmacokinetic studies

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Haloperidol, a widely used major tranquilizer of the butyrophenone class [1] has been measured by gas—liquid chromatography (GLC) using electron-capture detection [2], GLC using mass spectroscopy [3] and nitrogen—phosphorus detection [4—6], liquid chromatography with electrochemical detection [7] and by radioreceptor assay [8, 9]. With the exception of radio-receptor assays, these techniques are specific but are limited by the number of analyses that can be performed in a working day. Furthermore, many of the methods lack adequate sensitivity to detect very low haloperidol serum or plasma levels. This is of importance since steady-state concentrations are often in the 1—10 ng/ml range, and studies of pharmacokinetics and bioequivalence may require sensitivity of less than 1.0 ng/ml [10, 11]. Here we describe a method that is selective, separating haloperidol from metabolites and plasma contaminants, and sensitive to 0.5 ng/ml using GLC with nitrogen—phosphorus detection. When coupled with an automated injection system, up to 60 samples per 24 h may be analyzed after a straightforward plasma extraction which requires only 3—4 h technical time.

EXPERIMENTAL

Apparatus and chromatographic conditions

The analytic instrument is a Hewlett-Packard Model 5840A gas

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chromatograph equipped with a nitrogen—phosphorus detector and electronic integrator, and fitted with an optional logic board (Hewlett-Packard No. 07670-60840) to permit injection of small volume samples with alternate wash vials. The column is coiled glass, 1.22 m × 2 mm I.D., packed with 3% SP-2250 on 80—100 mesh Chromosorb WHP (Packing 1-1767, Supelco, Bellefonte, PA, U.S.A.). The carrier gas is ultra high purity helium (Matheson Gas Products, Gloucester, MA, U.S.A.) at a flow-rate of 30 ml/min. The detector purge is ultra high purity hydrogen (Matheson) at a flow-rate of 3 ml/min mixed with dry air (Matheson) at a flow-rate of 50 ml/min. Operating temperatures are: injection port, 310°C, column, 270°C, detector, 275°C. Before being connected to the detector, a new column is conditioned at 280°C for 48 h with carrier flow-rate of 30 ml/min.

At the beginning of each work day, the column is primed with 2 μg of phospholipid (asolectin) in benzene.

Reagents

The following reagents are used: certified 99% pure *n*-hexane (Mallinckrodt, St. Louis, MO, U.S.A.), analytical reagent-grade toluene (Fisher Scientific, Fairlawn, NJ, U.S.A.), certified isoamyl alcohol (Fisher), HPLC grade methanol (Fisher), analytical reagent grade concentrated hydrochloric acid (Mallinckrodt), and analytical reagent-grade sodium carbonate and sodium bicarbonate (Mallinckrodt). Isoamyl alcohol is glass-distilled prior to use. Other organic solvents are used without further distillation. All aqueous solvents (0.25 *M* sodium hydroxide, 0.1 *M* hydrochloric acid, 1 *M* carbonate—bicarbonate buffer at pH 11.5) are washed five times with hexane—isoamyl alcohol (98:2) prior to use.

Reference standards

Pure standards of haloperidol and the internal standard McN-JR-1854 (chlorohaloperidol, Fig. 1) were kindly provided by McNeil Labs. (Ft. Washington, PA, U.S.A.). Standards of each are prepared by dissolving 100 mg in 100 ml methanol. Sequential dilutions to 1 μg/ml are made. The solutions are stored in the dark in glass-stoppered bottles at 4°C and are stable for at least twelve months.

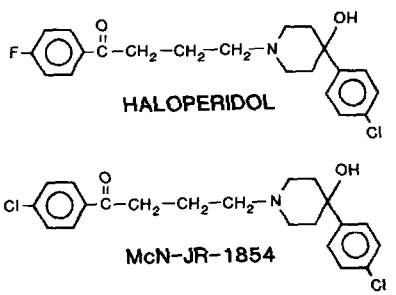

Fig. 1. Structural formulae of haloperidol and the internal standard, McN-JR-1854.

Preparation of samples

McN-JR-1854 (chlorohaloperidol) is used as the internal standard for all

analyses. A 100-μl volume of stock solution (1 μg/ml), containing 100 ng of McN-JR-1854, is added to a series of 15-ml round-bottom glass culture tubes, with PTFE-lined screw-top caps. A 0.25—2.0 ml sample of unknown serum or plasma is added to each tube. Calibration standards for haloperidol are prepared by adding 1, 2.5, 5, 10, 25, 50, and 100 ng of drug to consecutive tubes. Drug-free control serum or plasma is added to each of the calibration tubes. One blank sample, taken from the experimental animal prior to drug administration, is analyzed with calibration standards and each set of unknown samples.

Extraction procedure

To each tube 1 ml of 0.25 *M* sodium hydroxide solution is added, followed by 5 ml hexane—isoamyl alcohol (98:2). The tubes are agitated gently in the upright position on a vortex mixer for 15 min, then centrifuged at room temperature for 5 min at 400 *g*. The organic layer is transferred to another 15-ml glass culture tube which contains 1.2 ml of 0.1 *M* hydrochloric acid. This mixture is agitated gently in the upright position on a vortex mixer for 10 min, and again centrifuged at room temperature for 5 min at 400 *g*. The upper organic layer is discarded. The aqueous layer is transferred by a 23-cm pipet to a conical 13-ml screw-top centrifuge tube. Great care is taken to transfer only the aqueous layer uncontaminated with organic residue. To this 0.5 ml of 1 *M* carbonate—bicarbonate buffer (pH 11.5) is added. The final organic extraction is done by adding 80 μl toluene—isoamyl alcohol (85:15) to the conical centrifuge tube. This mixture is agitated gently in the upright position on a vortex mixer for 15 min. The samples are again centrifuged at room temperature for 5 min at 400 *g*. After freezing in a dry ice—acetone bath to break emulsions formed, with a 23-cm disposable pipet passed through the organic layer, the entire aqueous layer is removed leaving the small volume (80 μl) of organic phase containing haloperidol and internal standard. This is transferred to a 0.3-ml Wheaton automatic sampling micro vial (Wheaton Scientific, Millville, NJ, U.S.A.). A 6-μl aliquot is injected into the gas chromatograph using the automatic injection sampling system.

Animal study

An adult mongrel dog (17 kg) was anesthetized with intravenous pentobarbital (30 mg/kg), intubated, and ventilated with a Harvard respirator to maintain arterial oxygen tension within normal limits [12, 13]. Body temperature was maintained with a heating pad, and fluid losses were approximately replaced with intravenous 0.9% sodium chloride solution. A 7.5-cm spinal needle (19 gauge) was inserted into the cisternum magnum to allow repeated sampling of cerebrospinal fluid (CSF). A single 5-mg intravenous bolus of haloperidol (McNeil) was administered through a glass syringe. Multiple venous blood (4—5 ml) and CSF (1—2 ml) samples were drawn into additive-free tubes over the following 8 h. Concentrations of haloperidol in all samples were determined by the method described above. CSF samples were extracted and analyzed identically to serum.

Serum haloperidol concentrations were analyzed by iterative weighted nonlinear least-squares regression analysis [14]. The following serum pharmaco-

kinetic variables were determined: distribution half-life, elimination half-life, central compartment volume, total volume of distribution, and total clearance. CSF disappearance half-life was determined by least-squares regression analysis.

RESULTS

Evaluation of the method

Under the described conditions, retention times for haloperidol and McN-JR-1854 were approximately 3.5 and 7.0 min, respectively (Fig. 2). The relation between haloperidol concentrations and the peak height ratio (versus internal standard) is linear at least to 100 ng/ml. Analysis of ten standard curves over a 4-month period indicates that the correlation coefficient is always 0.99 or greater. Day-to-day coefficient of variation in the slopes of the calibration curves was 7.8%.

The sensitivity limit of the method is 0.5 ng/ml for a 2-ml sample. Within-day coefficients of variation for identical samples were: at 1 ng/ml, 9.6%; 2.5 ng/ml, 13.6%; 5 ng/ml, 7.6%; 10 ng/ml, 6.7%; and 25 ng/ml, 3.6%. Residue

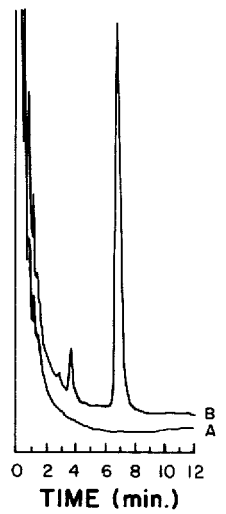

Fig. 2. Gas—liquid chromatograms of (A) extract of 1 ml serum obtained from dog prior to receiving haloperidol and (B) calibration standard spiked with 5 ng haloperidol (3.5 min) and 100 ng internal standard, MCN-JR-1854 (7.0 min).

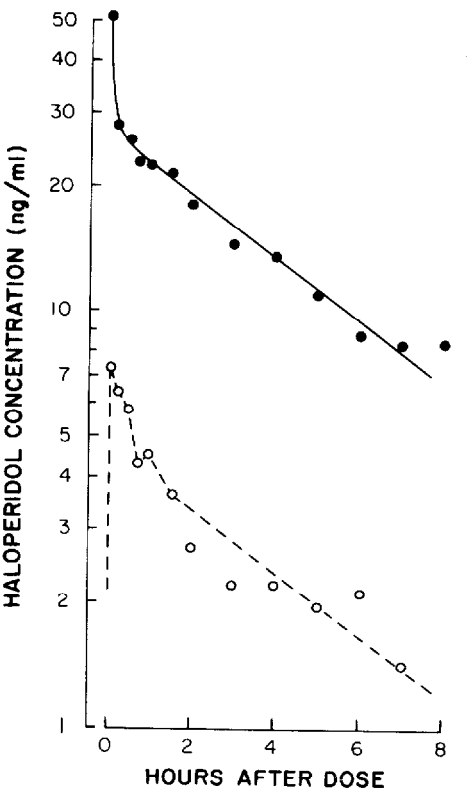

Fig. 3. Serum (•—•) and CSF (○— — —○) haloperidol concentrations and pharmacokinetic functions following intravenous haloperidol (5 mg) administration to a mongrel dog. See Table I for derived kinetic variables.

analysis indicated the extraction efficiency of haloperidol is greater than 95% at 10 ng/ml plasma concentrations.

The retention time of haloperidol was considerably longer than that of a number of other commonly used psychotropic drugs including benzodiazepines (diazepam, desmethyldiazepam, desalkylflurazepam, oxazepam, lorazepam) and antidepressants (imipramine and metabolites, desipramine and metabolites). Trazodone, on the other hand, had considerably longer retention than haloperidol. Therefore these compounds did not interfere with the assay.

Pharmacokinetic study

Fig. 3 shows serum and CSF haloperidol concentrations and pharmacokinetic functions for the experimental animal. Derived pharmacokinetic parameters are listed in Table I. Haloperidol was extensively distributed, with a total volume of distribution of more than 10 l/kg. Haloperidol rapidly entered CSF, and was eliminated in parallel from both serum and CSF after attainment of distribution equilibrium. The mean CSF to total serum haloperidol concentration ratio averaged only 0.17, probably due to serum protein binding of haloperidol.

TABLE I

DERIVED HALOPERIDOL SERUM AND CSF PHARMACOKINETIC PARAMETERS AFTER A 5-mg INTRAVENOUS DOSE ADMINISTERED TO A 17-kg MONGREL DOG

Parameter	Plasma	CSF
Distribution half-life (min)	3.0	—
Elimination half-life (h)	3.9	3.9
Central compartment volume (l/kg)	2.8	—
Total volume of distribution (l/kg)	10.6	—
Total metabolic clearance (ml/min/kg)	31.6	—
Mean CSF/total plasma concentration ratio*	0.17	

*Mean of values 1—7 h after the dose.

DISCUSSION

This report describes a reliable, selective method for the quantitation of haloperidol in serum or plasma and in CSF using GLC with nitrogen—phosphorus detection. Sensitivity is adequate to carry out single-dose pharmacokinetic studies and evaluate entry of haloperidol into CSF. A basic extraction from serum or plasma, acidic back-extraction, subsequent adjustment of the aqueous phase to a basic pH, and final organic extraction into a small volume for direct injection into the gas—liquid chromatograph is the method employed. This method produces blank serum and CSF samples that are consistently free of contaminants in the areas corresponding to the retention times for haloperidol and the internal standard. Dihydrohaloperidol, a metabolite of haloperidol [7], also is resolved from both haloperidol and the internal standard under these conditons. However we did not attempt to quantitate dihydrohaloperidol since a pure reference standard was not available.

The value of this method includes the reasonable time required for sample preparation, as well as its sensitivity which is adequate for single-dose serum and CSF pharmacokinetic studies. The method is potentially applicable to monitoring of steady-state haloperidol concentrations during clinical use of haloperidol, and we have analyzed a large number of clinical samples using this procedure.

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