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Note

High-performance liquid chromatography of haloperidol in serum at the concentrations achieved during chronic therapy

A. McBURNEY* and S. GEORGE

Department of Clinical Pharmacology, Groby Road Hospital, Leicester, LE3 9QE (U.K.)

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Haloperidol, 4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone, is a widely prescribed neuroleptic which is used in both adults and children for the treatment of acute and chronic psychotic syndromes and abnormal movements. Therapeutic monitoring of haloperidol is indicated for the following reasons; (1) there is no direct relationship between daily doses and serum levels at steady state; (2) saturation kinetics may apply at higher doses; (3) commonly associated drugs or disease states may alter the drugs pharmacokinetic parameters; (4) drug disposition is faster in children than adults; (5) optimum serum concentrations vary between pathological syndromes and age groups, and (6) side effects and adverse reactions are clearly related to haloperidol serum concentrations [1].

To date all the accepted methods for monitoring haloperidol have used gas chromatography (GC) and large volumes of serum [2]. A recent electron-capture GC method required only 1 ml of plasma or serum, but the selectivity of the assay was not reported [3]. The present method overcomes the use of such high sample volumes, is simpler to perform and is of equal or higher sensitivity. It has been used to monitor haloperidol serum concentrations in various psychiatric patients and the results of these determinations in 28 patients are presented.

EXPERIMENTAL

Materials and reagents

Haloperidol and the internal standard, fenethazine [10-(2-dimethylamino) ethylphenothiazine·hydrochloride], were obtained from Janssen Pharmaceuticals (Marlow, U.K.) and Rhone-Poulenc (Paris, France), respectively. The inter-

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nal standard was used as a 1 mg/l solution in methanol. The haloperidol metabolites 4-(*p*-chlorophenyl)-4-hydroxypiperidine and 4-fluorobenzoyl propionic acid were obtained from Aldrich (Gillingham, U.K.). Heptane, methanol and acetonitrile were all HPLC grade (Fahrenheit Lab. Supplies, Rotherham, U.K.). Sulphuric acid, isoamyl alcohol, sodium hydroxide and potassium bromide were all analytical reagent grade (BDH, Poole, U.K.).

High-performance liquid chromatography (HPLC)

The solvent delivery system was a constant-flow reciprocating pump (Laboratory Data Control, ConstaMetric III pump) and sample injection was performed using a Rheodyne Model 7125 syringe-loading valve fitted with a 100-μl sample loop. Stainless-steel tubing (0.25 mm I.D.) was used to connect the outlet port of the valve to the analytical column, a stainless-steel tube 30 × 0.5 cm I.D., packed with LiChrosorb Si 60 (5 μm particle size) (Analytical Supplies, Derby, U.K.), which was used at ambient temperature (normally 22°C). The

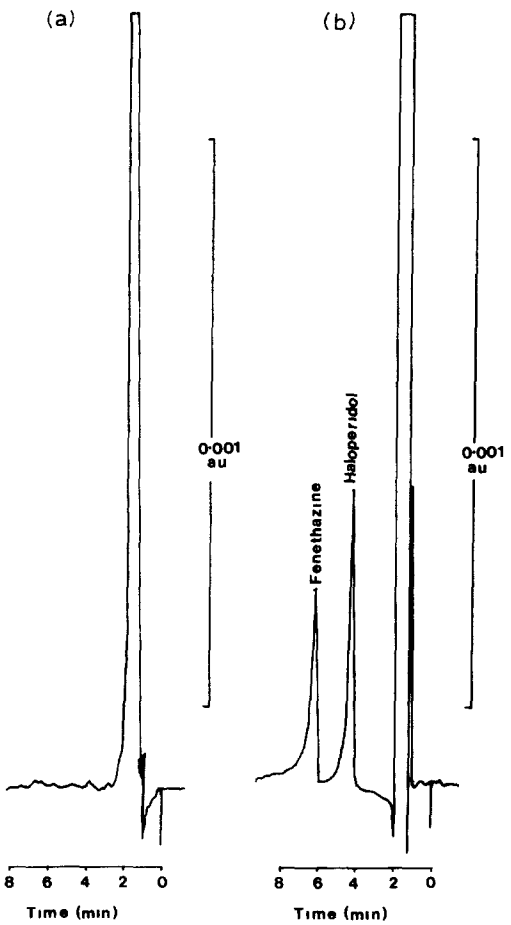

Fig. 1. Chromatograms obtained on analysis of an extract of (a) drug-free human plasma without internal standard; (b) serum from a patient treated with haloperidol, 30 mg/day; the serum haloperidol was found to be 28.7 ng/ml. Conditions were as described in the text.

TABLE I

RETENTION TIMES RELATIVE TO FENETHAZINE OF HALOPERIDOL AND OTHER COMPOUNDS ON A MICROPARTICULATE SILICA COLUMN

Compound	Relative retention time
Amitriptyline	1.41
Nortriptyline	1.89
Imipramine	1.66
Desipramine	1.97
Protriptyline	2.25
Phenytoin	0.20
Phenobarbitone	0.17
Ethosuximide	0.18
Primidone	0.20
Carbamazepine	0.23
Procyclidine	2.03
Chlorpromazine	1.40
Fenethazine	1.00
Haloperidol	0.66
Mianserin	0.74
Trimipramine	1.00
Sulthiame	0.20
Benztropine	>3.00
Metabolite 1*	>3.00
Metabolite 2**	>3.00

*4(*p*-Chlorophenyl)-4-hydroxypiperidine.
**4-Fluorobenzoyl propionic acid

column effluent was monitored at 244 nm at a sensitivity of 0.002 a.u.f.s. (Laboratory Data Control SpectroMonitor III, Model 1204A). The mobile phase was methanol—acetonitrile (10:90) containing 2 m*M* potassium bromide and this was degassed by sonication for 15 min before use. The flow-rate was 2 ml/min.

Using this system the separation of haloperidol and fenethazine obtained is illustrated in Fig. 1. The relative retention times of these compounds and some additional drugs on this system are given in Table I.

Extraction from serum

Serum (2 ml) was pipetted into a 10-ml conical centrifuge tube and internal standard solution added (30 μl of 1 μg/ml fenethazine in methanol). The tube was vortex-mixed for 30 sec, the contents were made alkaline with 2 *M* sodium hydroxide (1 ml) and 5 ml heptane—isoamyl alcohol (98.5:1.5) added. The contents of the tube were vortex-mixed for 10 sec and the tube was centrifuged at 1000 *g* for 10 min. The organic phase was transferred to a 15-ml centrifuge tube and the extraction repeated. The combined organic extracts were acidified with 0.005 *M* sulphuric acid, (2 ml) and vortex-mixed for 60 sec. On separation of the phases, the organic phase was aspirated off and 1.8 ml of the sulphuric acid were transferred to a 5-ml centrifuge tube. The acid extract was made alkaline with 1 *M* sodium hydroxide (200 μl) and extracted with 500 μl heptane—isoamyl alcohol (98.5:1.5) by vortex-mixing for 30 sec. Following

separation of the organic and aqueous phases by centrifugation at 1000 *g* for 5 min, 150 μl of the organic phase was transferred to a small (Dreyer) test-tube (R.W. Jennings, Nottingham, U.K.), and evaporated to dryness under nitrogen at 60°C. The extract was reconstituted in methanol (50 μl) and 40 μl injected into the chromatograph. Analyses were performed in duplicate and the mean result taken.

Instrument calibration

Standard solutions containing haloperidol at 5, 10, 20, 30 and 50 ng/ml were prepared in expired blood bank plasma by dilution of a 1 mg/ml solution of this compound in methanol.

On analysis of these solutions, the ratio of the peak height of drug to the peak height of the internal standard, when plotted against drug concentration, was linear between 5 and 50 ng/ml and passed through the origin of the graph.

RESULTS AND DISCUSSION

Reproducibility and accuracy

The intra-assay coefficients of variation (C.V.) measured from replicate analyses (n=6) of standard solutions prepared in blood bank plasma were as follows; at 2 ng/ml haloperidol, C.V. = 10.0%; at 20 ng/ml, C.V. = 8.8%.

Overall recovery of haloperidol was determined over the concentration range 0—30 ng/ml using concentrations of 0, 5, 10, 20 and 30 ng/ml in duplicate estimations. Overall recovery of drug relative to the internal standard, fenethazine, was 104.6%.

Selectivity

No endogenous sources of interference have been observed. The chromatogram obtained on analysis of a specimen of drug-free human plasma is illustrated in Fig. 1a and that from a patient treated with haloperidol in Fig. 1b. Analyses of specimens with and without the internal standard fenethazine have not revealed any endogenous compound which could co-elute with this standard.

As haloperidol is frequently given together with other centrally acting drugs, the potential interference from a number of such drugs was investigated. The retention times of these compounds are given in Table I. Of the compounds examined, only trimipramine was found to pose a problem as it co-eluted with the internal standard, fenethazine.

Sensitivity

The limit of sensitivity of the assay was 2 ng/ml. The intra-assay C.V. at this concentration was 10.0% (n=6).

Haloperidol could be detected using a fixed-wavelength UV detector at 254 nm but at this wavelength sensitivity was reduced by almost 35%.

Serum concentrations in psychiatric patients

Haloperidol serum concentrations were determined in 28 patients receiving the drug for a variety of psychiatric conditions. Blood samples were drawn be-

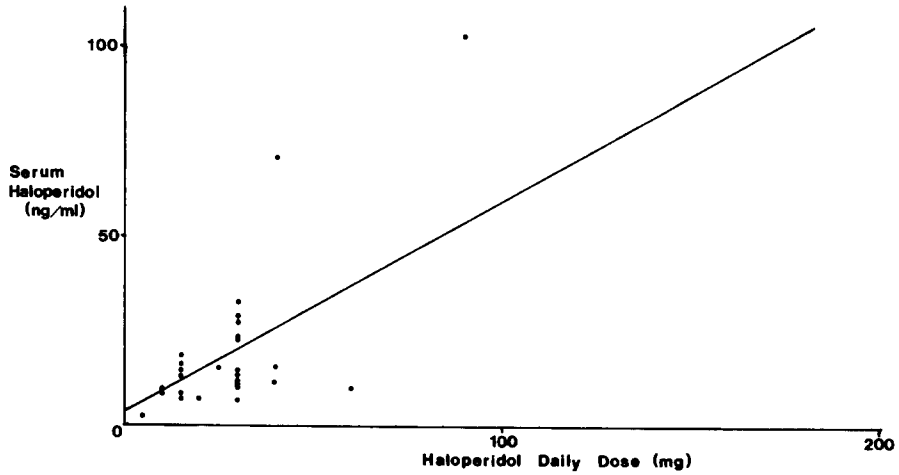

Fig. 2. The relation between steady-state serum concentrations of haloperidol (Y) and daily dose of haloperidol (X) in 28 psychiatric inpatients. The regression line was $Y = 0.56\ X + 3.84$ and the correlation coefficient $r = 0.793$ ($p<0.001$).

TABLE II

SERUM HALOPERIDOL CONCENTRATIONS IN PATIENTS RECEIVING THE DRUG FOR TREATMENT OF MANIA AND SCHIZOPHRENIA

Subject	Mania		Subject	Schizophrenia	
	Dose (mg/day)	Serum concentration (ng/ml)		Dose (mg/day)	Serum concentration (ng/ml)
1	25	15.8	13	30	15.1
2	30	11.6	14	10	9.2
	20	7.0			
			15	15	15.5
3	40	12.1			
	60	10.5	16	30	28.7
4	15	7.6	17	15	13.4
5	15	7.7	18	30	10.6
	30	28.5			
	30	33.0			
6	30	7.2			
7	4.5	2.6			
8	40	16.0			
9	15	18.9			
10	30	23.2			

tween 1 and 4 h post dose. The results as the average of duplicate estimations are shown in Fig. 2 in relation to the total daily dose of haloperidol. Although there was a good correlation between the two variables (r=0.793, $p<0.001$), serum haloperidol concentrations varied up to five-fold in patients given the

same daily dose. For patients given 15 mg/day the serum concentrations ranged from 7.6 to 18.9 ng/ml and for patients receiving 30 mg/day the range was 7.2 to 33.0 ng/ml. It was observed that the greatest variation occurred in samples drawn during the first 2 h post dose, suggesting individual variations in absorption of drug may be responsible for this wide range in serum concentration.

The results of haloperidol determinations in patients given the drug for the control of mania and schizophrenia are shown in Table II. No attempts were made to assess the clinical effect of the drug in relation to the serum concentration.

CONCLUSIONS

The method described here has been found to be suitable for the measurement of serum haloperidol concentrations achieved during therapy and may be useful in single-dose pharmacokinetic studies. Only 2 ml of serum are required and the method is simpler than GC methods to perform in that it is not necessary to silanize glassware, the calibration is linear and passes through zero, the extraction procedure is faster and the level of sensitivity is equal to GC methods which require 5 ml of serum. A recent GC method [3] quoted a limit of sensitivity of 0.5 ng/ml based on the extraction of 1 ml plasma. However, the selectivity of this method was not assessed and the assay protocol involved heating the sample on a boiling-water bath for 1.25 h in order to release protein-bound drug. The current HPLC method is a significant improvement on a previous HPLC method described for the analysis of pure drug solutions only which had a limit of sensitivity of 50 ng/ml [4]. Further HPLC methods using a reversed-phase mode of operation have been described [5,6]. These methods gave equivalent limits of sensitivity to the method described here but one report [5] had a longer analysis time, did not utilise an internal standard during the extraction procedure and gave no indication of the selectivity of the assay whilst the second method [6] employed two internal standards in order to circumvent problems associated with the selectivity of the assay. The HPLC methodology described here using normal-phase liquid chromatography avoids the use of buffered eluents and the concomitant problems associated with the effects of pH variations on the separation parameters. It has been shown to be a rapid, reliable and selective method for the analysis of haloperidol in patients receiving chronic therapy.

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