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Megabore capillary gas–liquid chromatographic method with nitrogen–phosphorus selective detection for the assay of haloperidol and reduced haloperidol in serum: results of therapeutic drug-monitoring during acute therapy of eight schizophrenics

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Abstract

A gas chromatographic method using a HP-5 megabore capillary and nitrogen–phosphorus selective detection for the quantitative analysis of haloperidol (H) and reduced haloperidol (RH) in human serum or plasma is described. A 3-step liquid–liquid extraction is applied. The extraction yield of this procedure is 63% for haloperidol at 20 ng/ml. The limits of detection are 0.4 ng/ml for haloperidol and 1.0 ng/ml for the metabolite if 2 ml of body fluid are applied. At 10 ng/ml the within-day precision is 4.5% for H and 8.3% for RH. Serum levels of eight schizophrenic patients have been monitored weekly over a therapeutic period of six weeks. Seven patients mainly had metabolite ratios RH/H < 1 over the entire period of investigation. They exhibited a linear correlation between dose and serum concentration of haloperidol. In contrast, one patient had metabolite ratios RH/H > 1 over the entire period of the study. Due to considerable increased serum concentrations this patient did not show a linear correlation between the dose and the serum level of haloperidol.

1. Introduction

Haloperidol (H) is the most widely used neuroleptic drug in acute and maintenance therapy of schizophrenic disorders [1]. Based on the results described in several original papers dealing with the investigation of the correlation between serum levels and clinical effect of haloperidol a therapeutic range of about 4–20 ng/ml is accepted [2–5]. Serum levels < 4 ng/ml are considered to give no effect or only a retarded therapeutic effect, while serum levels > 20 ng/ml are regarded to cause no additional positive effect but an increasing risk of side effects. However, some studies also reported lack of significant correlation between haloperidol serum level and clinical response.

In most of the above mentioned studies the role of the reduced metabolite (RH) is not taken into consideration. This appears to be reasonable, since binding of reduced haloperidol to the dopamine receptor (D_2) is only 1/400 that of the

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parent drug [6]. Furthermore, no effect of RH on the dopaminergic and noradrenergic system was found after direct application to a rat neuron [7]. Nevertheless, a few studies report a negative correlation between the RH/H metabolite ratio and therapeutic effect [8,9]. RH was shown to be 25% as potent as H in the stimulation of prolactin release in female rats [10]. However an indirect effect caused by the considerable reoxidation to haloperidol should be taken into account when discussing the psychotropic effect of reduced haloperidol. Therefore, particularly when RH is present in a great excess, it can be regarded as a reservoir of haloperidol.

Because both the drug and the metabolite occur in very low concentrations, a well established and validated analytical method should be available for pharmacological investigations. Gas–liquid chromatography with nitrogen–phosphorus selective detection (NPD) [11], high-performance liquid chromatography with UV- or electrochemical detection [12,13] and radioimmunoassay (RIA) [14] are reported. For GLC and HPLC a (mainly applied) liquid–liquid or liquid–solid extraction is needed. Limits of detection of 0.2–1.0 ng/ml are achieved. An outstanding method using HPLC and electrochemical (coulometric) detection reports a limit of detection of 0.025 ng/ml [13]. However, it was not shown if this is just the result of the advantageous application of coulometric detection or of the avoidance of adsorption using an excess of internal standard.

Two RIAs have been developed. Because considerable cross-reactivity with RH is reported for the RIA of Rubin et al. [in] this assay yields the total of the serum levels of H and RH. The antibody developed in Janssen Laboratories has a negligible cross-reactivity with the reduced metabolite and is therefore mainly chosen in drug monitoring or clinical investigations of haloperidol. Nevertheless, both RIAs cannot simultaneously asses haloperidol and its reduced metabolite. In this field chromatographic methods should be more suitable.

The aim of the present work was to establish a GC method with nitrogen–phosphorus selective detection for the measurement of haloperidol and its reduced metabolite. In contrast to methods reported in the literature no packed glass column but a megabore capillary with bonded stationary phase is used. The application of this method in therapeutic drug monitoring is shown. The results are discussed with respect to several literature data.

2. Experimental

2.1. Apparatus

The GC system was a Sichromat 2-8 equipped with a nitrogen-selective detector (NSD, Siemens) and a megabore HP-5 capillary (30 m × 0.53 mm I.D., 0.88 μm, Hewlett-Packard). The capillary was introduced into the evaporation tube (single-taper liner) of the split–splitless injector as far as possible to yield by this way a self-made on-column injection system. The injector port and the oven were operated isothermally at 270°C, the NSD at 300°C. Helium was chosen as the carrier gas (20 ml/min), hydrogen and air were the detector gases with flow-rates as recommended by the manufacturer (0.7 and 110 ml/min respectively). Data acquisition and integration of chromatograms were accomplished by an integrator Spectra-Physics SP 4400.

2.2. Chemicals

H, RH and chlorhaloperidol (ClH) were purchased from Research Biochemicals, organic solvents were p.a. from Merck (Darmstadt, Germany). A special water for NPD was applied (Pestanal, Riedel de Haën); 0.1 *M* HCl was prepared by dilution of 37% HCl (p.a., Merck), and for aqueous NaOH solid NaOH (p.a., Merck) and NaCl (p.a., Laborchemie, Apolda) were used.

2.3. Glassware

Glassware was monthly silanized with 5% dichlordimethylsilane in toluene. A special cleaning procedure including a 30-min sonification in 0.001 *M* HCl was applied. Polypropylene

tubes were tested but found to give no advantage; moreover, they are uncomfortable in volume handling because of their opaque nature.

2.4. Sample preparation

A procedure including a 3-step liquid–liquid extraction plus one washing step has been developed to obtain a highly concentrated and very pure extract of the drug and metabolite from body fluid. Serum (2 ml) was mixed in a 10-ml glass tube with 50 μl of standard solution containing 1 μg/ml chlorhaloperidol in 0.02 *M* HCl. A 0.5-ml volume of aqueous NaOH (4 g NaOH + 6 g NaCl per 100 ml) and 4 ml of *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) were added. The first extraction step was carried out by 30 min shaking. After centrifugation at 3000 *g*, 3.5 ml of the organic layer were transferred to 1.25 ml of 0.1 *M* HCl in a 10-ml glass tube and again shaken for 30 min. After 2 min of centrifugation the organic layer was discarded. A 1-ml volume of *n*-hexane–isoamyl alcohol was added, the two phases vortex-mixed for 30 s and separated again by 2–5 min centrifugation. From the lower phase (0.1 *M* HCl) a 1-ml volume was taken away carefully and placed in a 4-ml glass tube. A 150-μl volume of the aqueous NaOH and 100 μl of *n*-hexane–isoamyl alcohol were added and vortex-mixed for 30 s. After centrifugation, as much as possible of the organic layer (ca. 80 μl) was transferred to a tapered 4-ml glass tube. The solution was evaporated to dryness in a vacuum evaporator (5 min) and reconstituted in 20 μl of *n*-hexane–isoamyl alcohol. By standing for 10–15 min (non-stoppered) the solution was evaporated to 5–10 μl. A 3–5 μl aliquot was injected onto the GC system.

2.5. Subjects

Eight newly hospitalized patients (7 female, 1 male) who were diagnosed for schizophrenia or schizoaffective psychosis by DSM-3R were included in the study. All patients gave their informed consent to participate in the study and the protocol was approved by the Ethical Committee of the Medical Faculty. The mean age of the patients was 32.9 ± 10.7 yr (range 20–53), their mean body weight was 65.2 ± 14.0 kg (range 47–84). During the study the oral dosage (7 patients) of haloperidol was adjusted according to the progress of therapy (but constant for one-week periods) until gathering of blood samples. Thus, steady-state values for the concentration of H could be received. One patient received haloperidol as haloperidoldecanoate. To 6 patients biperiden was administered as anticholinergic co-medication. No antidepressants or high-potent neuroleptic drugs were co-administered. Three patients received low doses of the low-potent neuroleptics prothazine or levomepromazine. Serum levels were measured to be < 10 ng/ml and are therefore regarded not to affect the present results concerning haloperidol. Blood samples were drawn in vacutainers once a week at 8:00 a.m.–12 h after the last dose and before the next morning dose. After centrifugation serum was stored at −20°C until assay.

3. Results and discussion

3.1. Analyses

Fig. 1 shows typical chromatograms obtained from blank serum, serum spiked with 10 ng/ml haloperidol and 10 ng/ml reduced haloperidol and a patient serum calculated to have 1.4 ng/ml H and 3.5 ng/ml RH. Haloperidol (5.2 min), the metabolite (5.9 min) and the internal standard chlorhaloperidol (8.6 min) exhibit well separated peaks at the chromatographic conditions described above. Because of aging of the capillary, retention times have been found to be shortened to about 80% of the initial values after six months use. However, this had no negative influence on the qualitative characteristics of the method. In blank serum or plasma of different sources no interference with endogenous compounds was found. Furthermore, several neuroleptics, antidepressants, anticholinergics and benzodiazepines often administered in comedica-

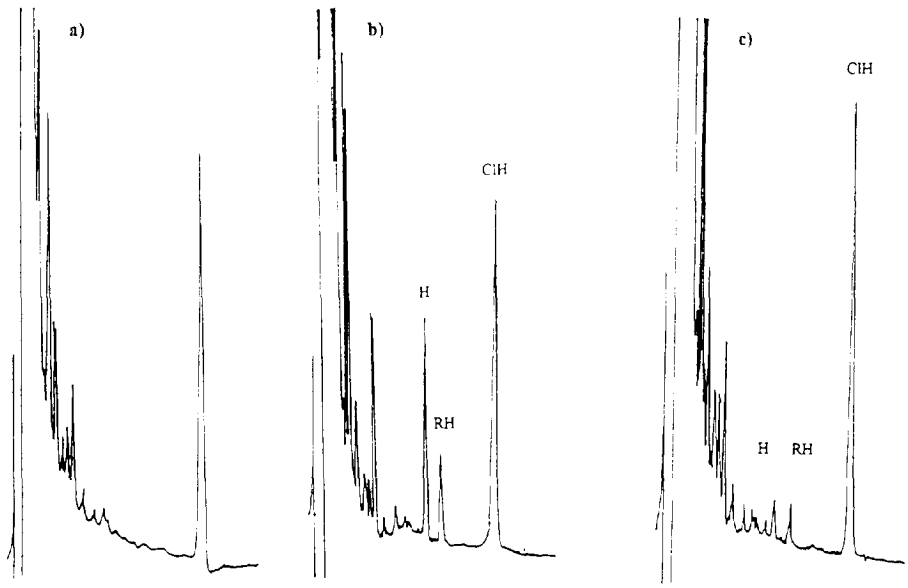

Fig. 1. Typical chromatograms obtained from (a) blank serum spiked with 25 ng/ml I.S. (ClH, $t_R = 8.6$ min), (b) blank serum spiked with 10 ng/ml H ($t_R = 5.2$ min), 10 ng/ml RH ($t_R = 5.9$ min) and 25 ng/ml ClH and (c) a patient serum with 1.4 ng/ml H and 3.5 ng/ml RH.

tion to haloperidol have been tested and shown not to interfere with the analytes of interest.

Quantification of the serum levels of H and RH was based on the peak-height ratio of the analyte and the internal standard H/ClH and RH/ClH. Calibration curves were linear up to 20 ng/ml for both H and RH (H: $y = 0.065x - 0.016$, RH: $y = 0.026x + 0.014$). These functions were also valid for H in the presence of 10 ng/ml of RH and for RH in the presence of 10 ng/ml of H. As has also been described in the literature, non-linear functions $H/ClH = f(c_H)$ and $RH/ClH = f(c_{RH})$ hold for serum levels $c_H > 20$–25 ng/ml and $c_{RH} > 20$–25 ng/ml (increasing slope), where c_H and c_{RH} represent the concentrations of H and RH, respectively. Thus, since serum levels of H and RH are mainly below 20 ng/ml at the doses usually applied, the present method is in our opinion suitable for most cases in clinical praxis and research. If concentrations > 20 ng/ml are detected, a second assay with a smaller sample volume (e.g. 1 ml patient serum + 1 ml blank serum or water) is recommended to get a higher accuracy.

Inter-day and intra-day precision have been determined at various concentrations (Table 1). The limits of detection have been derived from multiple measurements of spiked serum in the low concentration range. The values peak-height ratios H/ClH and RH/ClH found were extrapolated to that point, where, with a confidence of 95% the detector signal is higher than the noise in blank serum extracts: 0.4 ng/ml for H and 1.0 ng/ml for RH (Table 2).

The efficiency of the extraction procedure has been determined with a slightly modified procedure securing the application of constant portions of the extract. For this purpose a fixed volume of 75 μl was separated after the third extraction step (instead of "as much as possible") and without evaporation of the last 20 μl a volume of exactly 3 μl was injected. The peak height of this extract was compared with the peak heights of solutions with a known amount of H. For haloperidol an extraction yield of 63% was calculated for the entire extraction procedure at a concentration of 20 ng/ml.

Analysing the residues from the first and third (both basic) extraction steps, by measurement of H and RH in concentrates from the organic

Table 1
Precision and accuracy for H and RH

Compound	Concentration (ng/ml)	Intra-day			Inter-day		
		Mean ± S.D. (ng/ml)	R.S.D. (%)	*n*	Mean ± S.D. (ng/ml)	R.S.D. (%)	*n*
H	10.0	9.9 ± 0.445	4.5	8	9.1 ± 0.555	6.1	12
	2.0	1.8 ± 0.126	7.0	8	(within 4 months)		
	1.0	0.85 ± 0.14	16.5	8			
RH	20.0	19.7 ± 0.910	4.6	8			
	10.0	10.8 ± 0.896	8.3	6	10.0 ± 1.110	11.1	12
	4.0	3.7 ± 0.356	9.6	6	(within 4 months)		

residues of the second (acidic) extraction and the washing step, it was shown that no substantial improvement can be expected by additional extraction steps; 12% H and RH (compared to the authentic extract) have been found in the residue of the first extraction step, 9.5% H and 7% RH in the residue of the third extraction step; 4% H was found in the organic supernatant concentrate of the acidic extraction and in the organic supernatant concentrate of the washing step, but no RH.

Taking into account the loss of extract during handling of the volumes and the uncertainty in the volume decrease in the last evaporation step, about 10–20% of the initial amount of haloperidol was applied to the GC system. In an experiment with a double first (basic) extraction, peak heights increased to about 125% of those of the authentic extract for both H and RH.

In our opinion the above mentioned method is the best compromise between economic requisites and extraction yield. Only 5.1 ml of organic solvent are needed for one assay. A considerable increase in absolute peak heights is obtained by optimal evaporation and application to the GC of the last 20-μl volume.

3.2. Assay of haloperidol and reduced haloperidol in schizophrenic patients

Metabolite ratio RH/H

Numerous authors reported ratios for the serum levels of RH and H under steady-state conditions or after a single dose of haloperidol. A review shows that metabolite ratios RH/H < 1 seem to be predominant (Table 3).

Metabolite ratios (RH/H) are discussed with respect to polymorphic debrisoquine hydroxylation as a marker also for the metabolization of haloperidol: 5–10% of caucasians are classified as poor metabolizers. For this minority, enhanced serum levels of haloperidol and reduced

Table 2
Calibration data and limits of detection

Compound	Range (n = 10) (ng/ml)	Intercept	Slope	r	Limit of detection (ng/ml)
H	2.0–20.0	−0.016 ± 0.018	0.065 ± 0.0015	0.9979	
	0.5–5.0	0.002 ± 0.007	0.059 ± 0.0022	0.9942	0.4
RH	2.0–20.0	0.014 ± 0.008	0.026 ± 0.0006	0.9976	1.0

Table 3
Number of subjects according to an arbitrary cut-off point of metabolite ratios RH/H < 1 and RH/H > 1 found under steady-state conditions or after a single dose of haloperidol

n (RH/H < 1)	*n* (RH/H > 1)		Ref.
2	0	$AUC_{(0-t)}$ after single dose $t = 5$ days	13
8	0	steady state of H	15
10	5	steady state of H, asiens	16
5	1	steady state of H	17
6	0	$AUC_{(0-t)}$ after single dose $t = 3$ days	18
12	0	steady state of H, asiens	19
1	0	steady state of H, gastric bypass	20
19	11	steady state of H	21
12	3	steady state of H	22
3	14	steady state of H	23
22	<6	RH detectable only in 6 of 28 subjects,	24
37	8	steady state of H, asiens	25
1	4	steady state of H	26
15	0	$AUC_{(0-t)}$ after single dose $t = 1$ day	27
8	7	$AUC_{(t1-t2)}$ after single dose $t_1 = 3$ days, $t_2 = 7$ days	27

metabolite concentrations have been found after haloperidol intake as compared to extensive metabolizers [29]. N-Dealkylation and aromatic hydroxylation of haloperidol seem to be affected by the hepatic P 450 isoenzyme CYP2D6, but contrary data have been reported for the re-oxidation of reduced haloperidol to the parent drug [30,31].

In 55 patients RH/H < 1 was reported within the first two weeks of therapy and RH/H > 1 in the third and fourth weeks [28]. Therefore, RH does not achieve steady-state concentrations during normal acute therapy. This time dependence of the RH/H ratio can be one explanation of the striking results. However, the clinician will mainly encounter RH/H ratios < 1 at least in the first weeks of medication with haloperidol.

This was also found for the eight patients in the present study. Seven patients had an RH/H ratio mainly < 1 over 6 weeks of treatment (0.29 ± 0.19; range 0–0.71). In contrast to the results reported in Ref. [28] no time dependent RH/H ratio with higher values was found after 4 weeks treatment. This may be due to a different dosage management. The dose was lowered continuously over the first four weeks, but during the 5th and 6th week the variance in dosage increased because some patients stopped haloperidol intake and some patients were administered increasing doses again.

One patient (female) had RH/H ratios > 1 over the entire therapeutic period of 6 weeks (1.93 ± 1.12; range 1.0 to 4.1). In agreement with the results reported in Ref. [29] this was the only patient suffering from marked side effects.

Of five additional patients receiving multiple comedication (low potent neuroleptics, antidepressants, carbamazepine) and with a different diagnosis, three had no detectable RH, and two had RH/H ratios of 0.81 ± 0.11 and 0.96 ± 0.13, respectively.

Correlation of dose with serum levels

Because of variable dosage management only those values have been taken into account that

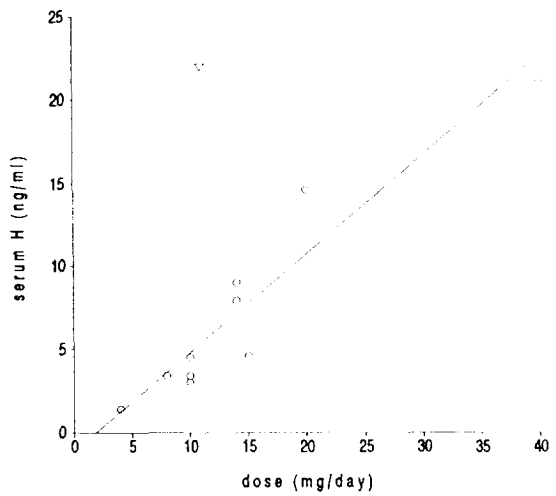

Fig. 2. Dependence of haloperidol serum levels on oral dose of haloperidol ($A = 0.59$, $r = 0.94$), (○) patients with metabolite ratio RH/H < 1, (▽) patient with metabolite ratio RH/H > 1.

were obtained when the dose was constant for at least two weeks. A linear correlation holds for the haloperidol serum levels in the case of the patients with RH/H ratio < 1, which is in agreement with the slope A of numerous literature data (e.g. $A = 0.75$, $r = 0.83$ [32]; $A = 0.6$, $r = 0.99$ [33]). A less significant correlation was found for RH levels ($A = 0.15$, $r = 0.62$) as also reported in Ref. [28]. The patient with RH/H ratio > 1 did not met these correlations. He had considerable higher H and RH levels at a given dose (e.g. 22 ng/ml H and 31 ng/ml RH at 11 mg/day) (Fig. 2).

4. Conclusions

The high-sensitive GC method for the assay of haloperidol and its reduced metabolite described here is suitable for therapeutic drug monitoring and clinical investigation. In contrast to the application of packed columns the use of a megabore capillary enables easy handling by the laboratory staff. It combines the advantages of bonded-phase capillaries (extremely decreased column bleeding also at high temperatures, important particularly in case of nitrogen-selective detection) and packed columns (splitless injection of up to 5 μl of extract possible). Because of the inert surface, priming as described in literature for the assay of haloperidol with GC and packed column [34] can be avoided.

However, a lower system response to the reduced metabolite was found possibly because of adsorption to the glassware or decomposition in the capillary. A method with an improved limit of detection also for RH is desirable for pharmacokinetic investigations after application of single doses of H. Gas chromatography without derivatization does not seem to fulfil this requirement. Additionally, as described in literature also for the application of a packed column [28], the accuracy is lower at serum levels > 20 ng/ml because of the non-linear system response. HPLC with electrochemical detection can provide a better analytical basis.

Nevertheless, the original data of the eight schizophrenic patients provide basic information on the relevance of therapeutic drug monitoring during acute therapy with the high-potent neuroleptic drug haloperidol. Possibly because of polymorphism in drug metabolism two groups of subjects can be distinguished. The majority has metabolite ratios RH/H < 1 and obeys linear–dose serum level correlations. Some subjects have mainly RH/H ratios > 1 and show considerably increased serum levels of H and RH, which are not expected from the dose applied. For such patients an increased risk of side effects and a poorer clinical outcome is discussed. In particular for the screening of this important group of patients in therapeutic drug monitoring the detection limit for RH of the present method is quite sufficient.

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