ELSEVIER

Journal of Chromatography B, 698 (1997) 209–216

JOURNAL OF CHROMATOGRAPHY B

Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotropic drugs

Ole Vendelin Olesen*, Kristian Linnet

Institute for Basic Research in Psychiatry, Department of Biological Psychiatry, Clinical Biochemical Laboratory, Psychiatric Hospital, Aarhus University Hospital, DK-8240 Risskov, Denmark

Received 28 January 1997; received in revised form 12 May 1997; accepted 20 May 1997

Abstract

A HPLC method was developed for determination of risperidone and its therapeutically active main metabolite 9-hydroxyrisperidone in serum. After a single-step liquid–liquid extraction the analytes were separated on a C_{18} column and measured by UV detection at 280 nm. Inter-day coefficient of variation was <7% for both compounds at serum levels occurring in patients treated with ordinary doses. Studies of analytical interference showed that the most commonly coadministered antidepressants and benzodiazepines did not interfere. Some conventional low dose neuroleptics and clozapine did interfere, but this is of minor importance, because risperidone is intended as an alternative to these drugs.

Keywords: Risperidone; 9-Hydroxyrisperidone

1. Introduction

Risperidone belongs to a new generation of neuroleptic drugs used in the treatment of schizophrenia. Risperidone has affinity to both dopaminergic and serotonergic receptors, and extrapyramidal side effects should be less pronounced than observed for classical neuroleptic drugs [1,2]. Clinical studies indicate that daily doses of 4–6 mg are effective in most patients [3]. The main metabolite of risperidone, 9-hydroxyrisperidone (Fig. 1), has a similar activity as the parent compound, and the serum concentration of active moiety is thus the sum of the serum concentrations of risperidone and 9-hydroxyrisperidone [4,5]. Linear kinetics have been described for the active moiety [6], but among individuals given an equal dose, the concentration of the active moiety may vary by factor 40 [7]. Therefore attempts to establish a therapeutic range for serum concentrations of risperidone and 9-hydroxyrisperidone might be of relevance. Further, measurement of serum concentrations of the drug may be useful for control of non-compliance and to disclose extremely high or low serum concentrations in patients with atypical metabolic rates.

An early method for the quantitative determination of risperidone and 9-hydroxyrisperidone in serum was a radioaminoassay with specific antibodies against the active compounds [8]. Later on high-performance liquid chromatography (HPLC) assays

*Corresponding author.

0378-4347/97/$17.00
PII S0378-4347(97)00302-2

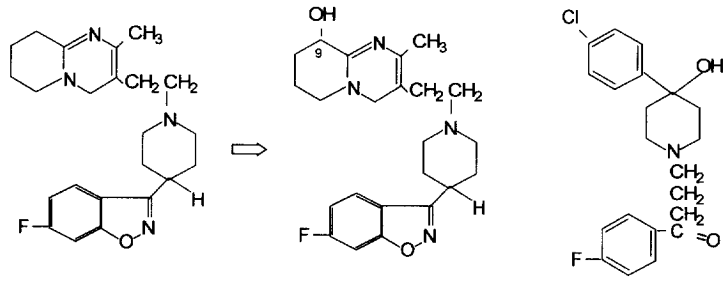

Fig. 1. Molecular structure of risperidone and its main metabolite. The structure of haloperidol, used as internal standard, is also given.

have been developed, and using electrochemical detection Aravagiri et al. [9] measured risperidone itself, but 9-hydroxyrisperidone, which normally accounts for about two thirds of the active moiety at steady-state, was not determined. Le Moing et al. [10] also developed an HPLC method with electrochemical detection and claimed that this detection principle was more specific in the context of polymedicated patients than a previously described method using UV detection [11]. However, preliminary studies in our laboratory showed that many commonly used psychotropic drugs and their metabolites are oxidized at the same potential settings (0.5–0.8 V) as used for determination of risperidone. Therefore, in many cases analytical interference from comedication can only be avoided by creating differences in retention times, and the use of electrochemical detectors in a routine setting is more demanding than using UV principle. Furthermore, with a few exceptions, the list of drugs studied for analytical interference by Le Moing et al. [10] did not include metabolites known to be present in serum in considerable concentrations. The major problem in routine therapeutic drug monitoring (TDM) at a psychiatric hospital is that only a small percentage of the patients are in monotherapy. More than 50% of all patients are comediated with sedatives, often benzodiazepines, but also small doses of other neuroleptics, e.g., methotrimeprazine and chlorprothixen are extensively used [12]. About 10% receive tricyclic antidepressants as comedication, and at present an increasing number is given the selective serotonin reuptake inhibitors citalopram, fluoxetine, paroxetine or sertraline in addition to neuroleptic drugs. Finally, we found the multi-step extraction procedures used in previous assays rather laborious [10,11]. Woestenborghs et al. [11] extracted 1 ml plasma twice with ethyl acetate, and the combined extracts were then cleaned up by back extraction into dilute sulphuric acid, and after alkalinization the analytes were reextracted. Le Moing et al. [10] used an almost similar laborious extraction and clean-up procedure and besides, the use of electrochemical detector also required extensive care with respect to cleaning of glassware and apparatus.

The aim of the present study was to develop a simple and specific method for the quantitative determination of risperidone and 9-hydroxyrisperidone in serum excluding interference from commonly used psychotropic drugs and their metabolites in order to make the method suitable for routine TDM.

2. Experimental

2.1. Chemicals

Risperidone, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one, 9-hydroxyrisperidone and haloperidol (internal standard) were donated by Janssen Pharmaceutica (Beerse, Belgium). HPLC-grade heptane, isoamylalcohol, methanol and acetonitrile were from Fisons Scientific Equipment (Loughborough, UK). Ammonium acetate, sodium carbonate and sodium bicarbonate, all of analytical-grade, were obtained from Merck (Darmstadt, Germany). Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

All stock solutions were prepared by dissolving pure substances in ethanol:risperidone, 9-hydroxyrisperidone or haloperidol in a concentration of 2.5 mmol/l (1.026, 1.063 or 0.940 mg/ml). For further dilution of stock solutions ethanol–water (50:50, v/v) was used for risperidone and the metabolite, whereas methanol was used for dilution of haloperidol.

Serum standards and controls containing known amounts of risperidone and 9-hydroxyrisperidone were prepared by spiking serum from healthy drug-free donors.

2.3. Extraction

In 12 ml centrifuge tubes, 1.0 ml of serum was mixed with 0.5 ml 0.6 *M* sodiumcarbonate/bicarbonate buffer, pH 10, and 50 μl haloperidol solution, 3.76 mg/ml (10 mmol/l) as internal standard. 8 ml heptane–isoamylalcohol (98:2, v/v) was added and the mixture was shaken for 5 min in the horizontal position at 250 shakings/min on a HS 500 (Janke and Kunkel, Staufen, Germany) shaking apparatus. After centrifugation at 1500 *g* for 10 min, the aqueous layer was frozen by immersing the tubes into a cooling bath consisting of dry ice and ethanol. The heptane layer was decanted into centrifuge tubes and evaporated to dryness at 60°C in a gentle stream of nitrogen. The residue was dissolved in 75 μl of mobile phase, of which 65 μl was injected into the chromatograph.

2.4. Chromatography and calculations

The chromatographic analysis was performed on a Perkin-Elmer (Norwalk, CT, USA) system consisting of a LC Model 250 pump, ISS-200 autosampler, LC 90 UV photometer set at 280 nm, and chromatograms were recorded by Turbochrom Navigator software (PE Nelson). The analytical column was a 250×4.6 mm LicChroCart (Merck). The mobile phase was 40 m*M* ammonium acetate buffer pH 7.0–methanol (100:900, v/v). The flow-rate was 1.0 ml/min.

From recorded peak heights, the ratios of drug to internal standard were calculated. The results obtained from serum standards spiked with different known amounts of risperidone and 9-hydroxyrisperidone were used to calculate the factor for multiplying the ratios between heights of unknown and internal standard peaks.

3. Results

3.1. Chromatography and recovery

A chromatogram of a serum blank from a healthy drug-free blood donor is shown in Fig. 2A. After the solvent front no interfering peaks were detected, and Fig. 2B shows that 9-hydroxyrisperidone, risperidone and the internal standard were eluted within 6 min. In serum from patients treated with risperidone an unidentified peak, presumable a risperidone metabolite, was found at a retention time of about 6.5 min (Fig. 2C). For routine use it was necessary to use 10 min for each chromatogram in order to avoid that late eluting peaks from comedicated drugs appeared in the next chromatogram (Fig. 2D). The latter chromatogram also shows that the tricyclic antidepressant drugs amitriptyline and nortriptyline, which are often used in combination with neuroleptic drugs in the treatment of psychiatric disorders, did not disturb the quantitation of risperidone and 9-hydroxyrisperidone.

The recovery was calculated by comparing the peak heights after injection of risperidone and 9-hydroxyrisperidone dissolved in mobile phase with the peak heights obtained after extraction of the same amount of the compounds from serum. The recoveries of risperidone and 9-hydroxyrisperidone were (mean±C.V., $n=4$) 59.6±2.8% and 45.4±2.9%, respectively and that of the internal standard haloperidol was 73.9±1.5%.

3.2. Serum concentrations in patients

Serum concentrations of risperidone and 9-hydroxyrisperidone were determined in fifty-two patients referred to our TDM service for the first time (Fig. 3). The patients are presumed to be in steady-state. The risperidone dose (mean±S.D.) given was 6.9±2.7 mg/24 h, and the mean concentration of the active moiety was 48.2±32.8 ng/ml. There was a

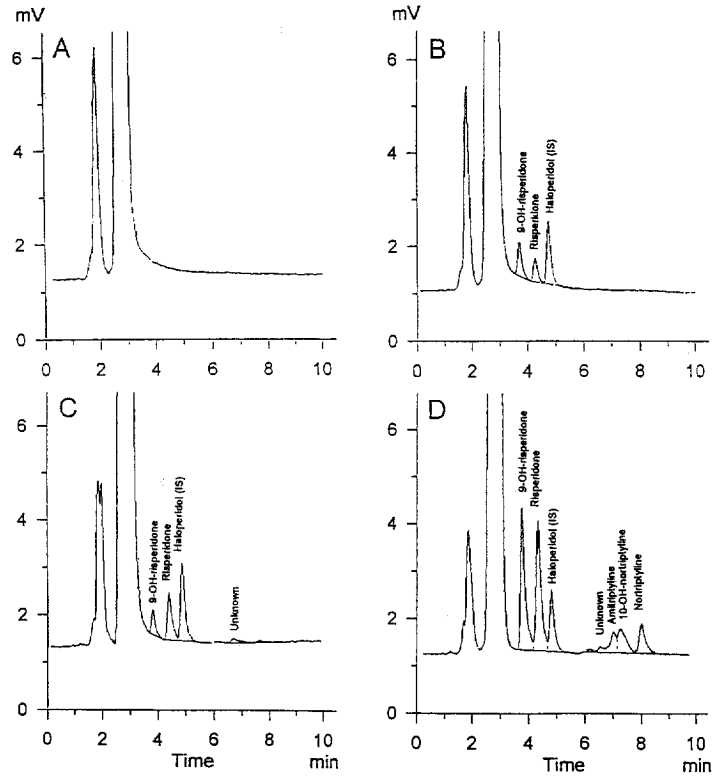

Fig. 2. (A) Chromatograms of a 1-ml blank serum sample. (B) Serum blank spiked with 21.3 ng/ml hydroxyrisperidone, 8.2 ng/ml risperidone and the internal standard (haloperidol). (C) Serum from a patient treated with risperidone 6 mg/day. The measured concentrations of risperidone and 9-hydroxyrisperidone were 15 and 14 ng/ml, respectively. (D) Serum from a patient treated with 6 mg/day of risperidone and 75 mg/day of amitriptyline. The measured concentrations of risperidone and 9-hydroxyrisperidone were 46 and 88 ng/ml, respectively.

significant correlation ($r=0.53$, $p<0.01$) between the 24 h doses given and the sum of the concentrations of the two active compounds in serum, but there was a large inter-individual variation in the concentration of the active moiety when equal doses was given to different patients. The serum concentration of the active moiety corrected for 24 h dose (C/D) was 7.25±3.26 ng/ml/mg with a total range from 0.87 to 18.33 and the ratio was not correlated to dose ($r=0.01$). The mean risperidone concentration in serum was 14.8±14.8 ng/ml and the mean concentration of the metabolite was twice as high as the concentration of the parent compound, 33.4±33.8 ng/ml.

Blank serum spiked with 0–400 ng/ml of risperidone or 9-hydroxyrisperidone was analyzed and the detector response was linear for both substances ($r=0.999$) up to 200 ng/ml, i.e., including the range observed in the fifty-two patients. At higher serum concentration there was a tendency towards a lowering of the recovery of both analytes which could be ascribed to an increase of the peak height of the internal standard due to an overlap from the risperidone peak. If accurate quantitation of concentrations exceeding 200 ng/ml of risperidone or 9-hydroxyrisperidone is required, the serum sample in question should be diluted with blank serum and reanalyzed.

3.3. Precision and accuracy

The within-day and day-to-day precision and accuracy were evaluated by analyzing blank serum spiked with different amounts of risperidone and

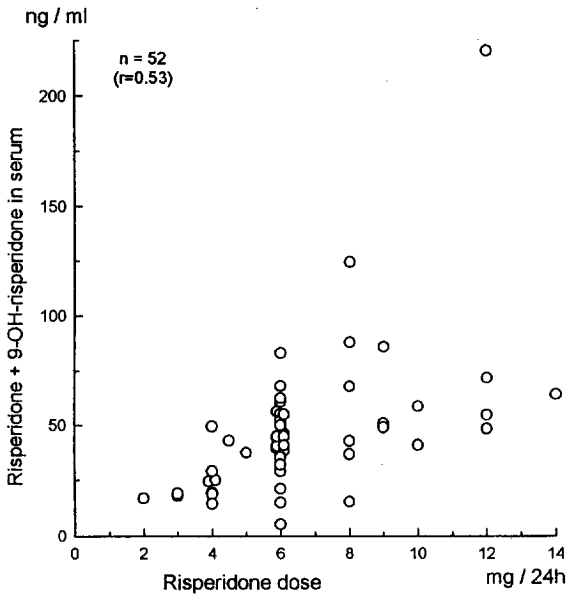

Fig. 3. Sum of risperidone and 9-hydroxyrisperidone concentrations (active moiety) in serum from fifty-two patients referred to TDM service for the first time as a funtion of dose.

9-hydroxyrisperidone. The results are given in Table 1 which shows that the day-to day variations for both compounds were less than 7% within the concentration range found in the fifty-two patients. The lower levels of quantitation were 1.2 and 2.1 ng/ml, respectively. At these low levels, the C.V. was about 12% for both analytes. The accuracy (corrected recovery) ranged from 95 to 118% in the experimental series with an overall average of 104%.

3.4. Analysis for interference

Serum specimens from patients receiving drugs which may be used in combination with risperidone, were analyzed in order to study interference with regard to risperidone and 9-hydroxyrisperidone determination. Absolute retention times and retention times relative to haloperidol are given in Table 2. It appears that none of the commonly used antidepressant drugs and sedatives are supposed to interfere, but the method does not allow determination of risperidone in the presence of the most commonly used conventional antipsychotic drugs and the atypical neuroleptic clozapine. However, since risperidone is supposed to replace these types of drugs, the interference should not be of clinical relevance.

4. Discussion

The recovery of risperidone from serum was 60% and that of the more polar hydroxy metabolite only 45%. Recovery is improved if the more polar ethyl acetate is used as organic phase instead of heptane–isoamylalcohol. However, if ethyl acetate is used it is

Table 1
Precision and accuracy of the determination of risperidone and 9-hydroxyrisperidone in spiked serum

Concentration (ng/ml)	*n*	Intra-day		Inter-day	
		C.V. (%)	Accuracy (%)	C.V. (%)	Accuracy (%)
Risperidone					
1.85	5			11.2	118
4.11	18			3.60	106
4.11	9	3.59	108		
16.4	18			3.74	104
16.4	10	1.78	107		
61.5	6			6.79	98.3
9-Hydroxyrisperidone					
3.19	5			11.8	116
10.64	21			4.34	97.1
10.64	8	3.44	105		
42.55	21				
42.55	10	2.40	99.0		
127.5	6			4.12	95.3

Table 2
Absolute retention times (in min) and retention times relative to haloperidol (internal standard) of non-interfering and interfering drugs and metabolites

Compound	Retention time	Relative retention time
Risperidone	4.42	0.87
9-Hydroxyrisperidone	3.90	0.76
Haloperidol (internal standard)	5.10	1.00
Non-interfering drugs		
Tricyclic antidepressants (TCAs)		
Amitriptyline	7.35	1.44
10-Hydroxyamitriptyline	6.60	1.29
Clomipramine	7.32	1.43
8-Hydroxyclomipramine	6.50	1.27
Desmethyl-clomipramine	8.42	1.65
8-Hydroxydesmethyl-clomipramine	7.50	1.47
Imipramine	8.16	1.60
Desipramine	8.61	1.69
Nortriptyline	8.50	1.67
10-Hydroxynortriptyline	7.40	1.45
Selective serotonin reuptake inhibitors (SSRIs)		
Citalopram	7.40	1.45
Desmethyl-citalopram	7.88	1.55
Fluoxetine	7.13	1.40
Nor-fluoxetine	6.63	1.30
Paroxetine	8.05	1.58
Sertraline	6.26	1.23
Demethyl-sertraline	6.00	1.18
Benzodiazepines		
Clonazepam	<3	<0.6
Flunitrazepam	<3	<0.6
Nitrazepam	<3	<0.6
Oxazepam	<3	<0.6
Neuroleptics used as sedatives		
Methotrimeprazine	6.76	1.33
Demethyl-methotrimeprazine	7.62	1.49
Methotrimeprazine-sulfoxide	9.07	1.78
Chlorprothixen	6.06	1.19
Chlorprothixene metabolite	8.18	1.60
Antiepileptic drugs		
Carbamazepine	<3	<0.6
Oxcarbazepine	<3	<0.6
Oxcarbazepine metabolite	<3	<0.6
Interfering drugs		
Neuroleptics		
Clozapine	3.89	0.76
Fluphenazine	3.73	0.73
Perphenazine	3.95	0.77
Zuclopenthixol	3.79	0.74
Other drugs		
Hydroxizine	3.80	0.75
Diltiazem	3.92	0.77
Mianserine	4.13	0.82

not possible to separate the organic and aqueous phases by freezing because too much water is dissolved in ethyl acetate. Freezing of the aqueous phase and subsequent decanting of the organic layer into new tubes for evaporation is a relatively quick, and more important, a much less tiring procedure for separating phases than to remove the organic layer quantitatively by aspiration. By using a rather laborious extraction procedure, Woestenborghs et al. [11] obtained a recovery of risperidone and 9-hydroxyrisperidone of 75 and 70%, respectively, and thus only 25–30% higher than our values. Le Moing et al. [10] claimed that the coulometric detection improves the limit of quantitation by a factor 5 compared to UV detection. However, the high sensitivity of the detector did not result in a lower level of quantitation than 2 ng/ml for both risperidone and 9-hydroxyrisperidone, which is the same level found by Woestenborghs et al. [11] and in the present method. Aravagiri et al. [9] also used electrochemical detection and found a lower level of quantitation of 0.1 ng/ml for risperidone, but 9-hydroxyrisperidone which is responsible for about 66% of the antipsychotic effect at steady-state was not mentioned. Furthermore, these authors [9] found that the mean concentration of risperidone in plasma from patients given 6 mg/24 h was 0.78 ng/ml or 10–20 times less than the mean concentration reported here and by other investigators [7,10]. For a routine TDM method, the demand for sensitivity can only be estimated by analyzing real samples from patients treated with the drug in question. Of the fifty-two patients referred to TDM for the very first time, 44% received a dose of 6 mg/24 h, which according to the clinical trials should be effective in most patients. In the group given 6 mg/24 h, the concentration of the active moiety, i.e., the sum of the risperidone and 9-hydroxyrisperidone concentrations in serum, ranged from 5.3 to 83.1 ng/ml, but about 90% of the patients had serum levels within the range 30–60 ng/ml. It is likely that this range is close to the therapeutic range, and a lower level of quantitation of 2 ng/ml for risperidone and 9-hydroxyrisperidone is therefore satisfactory for TDM. The reproducibility of the present method is also sufficient for clinical use.

In the development of a routine HPLC method, it is of course impossible to take into account all possible drugs which may interfere with the determination of risperidone and 9-hydroxyrisperidone. In order to obtain maximum benefit of TDM, blood samples used for determination of drug concentrations are always drawn during steady-state in the morning 8 to 12 h after the last dose intake. It is also mandatory that information about comedication is given. Risperidone treatment is an alternative to treatment with the older low-dose neuroleptic drugs like perphenazine [13] and haloperidol [14] and not a supplement to treatment with these drugs. Since treatment of psychotic symptoms with a combination of risperidone and haloperidol is irrational or at least not suited for TDM, we found no problems in using haloperidol as an internal standard, although methyl-risperidone used by Woestenborghs et al. [8] might seem a more obvious choice due to its close chemical relationship to the measured compounds. Unfortunately, methyl-risperidone had a retention time of 8 min, which coincided with retention times of several tricyclic antidepressants and/or their metabolites. For use in a clinical laboratory, this internal standard is thus unsuitable. With respect to analytical interference, we have focused on antidepressant drugs, benzodiazepines and other sedative acting drugs often used in combination with neuroleptic treatment, and the results indicate that these drugs do not disturb the quantitation of risperidone and hydroxyrisperidone.

Acknowledgments

The authors are grateful to the excellent technical assistance of Mrs. Birgit Poulsen. This work has been supported by The Lundbeck Foundation, The Novo Nordic Foundation and The Psychiatric Research Foundation.

References

[1] G. Chouinard, W. Arnott, Can. J. Psychiatry 38 (1993) 89.
[2] A. Schotte, P.F.M. Janssen, A.A.H.P. Megens, J.E. Leysen, Brain Res. 631 (1993) 191.
[3] G. Chouinard, B. Jones, G. Remington, D. Bloom, D. Addington, W. MacEwan, A. Labelle, L. Beauclair, W. Arnott, J. Clin. Psychopharmacol. 13 (1993) 25.
[4] L. Ereshefsky, S. Lacombe, Can. J. Psychiatry 38 (1993) 80.

[5] M.-L. Huang, A. Van Peer, R. Woestenborghs, R. De Coster, J. Heykants, A.A.I. Jansen, Z. Zylicz, H.W. Visscher, J.H.G. Jonkman, Clin. Pharmacol. Ther. 54 (1993) 257.
[6] E. Snoeck, A. Van Peer, M. Sack, M. Horton, G. Mannens, R. Woestenborghs, R. Meibach, J. Heykants, Psychopharmacology 122 (1995) 223.
[7] C. Anderson, J. True, L. Ereshefsky, A. Miller, Psychopharmacol. Bull. 30 (1994) 88.
[8] R. Woestenborghs, I. Geuens, D. Van Roosbroeck, L. Cornelissen, F. Van Rompaey, F. Knaeps and J. Heykants, Janssen Preclinical Research Report R 64766/24, Janssen, Beerse, August 1990.
[9] M. Aravagiri, S.R. Marder, T. Van Putten, K.K. Midha, J. Pharm. Sci. 82 (1993) 447.
[10] J.P. Le Moing, S. Edouard, J.C. Levron, J. Chromatogr. 614 (1993) 333.
[11] R. Woestenborghs, W. Lorreyne, F. Van Rompaey, J. Heykants, J. Chromatogr. 583 (1992) 223.
[12] M. Gex-Fabry, A.E. Balant-Gorgia, L.P. Balant, Ther. Drug Monit. 19 (1997) 1.
[13] O.J. Høyberg, C. Fensbo, J. Remvig, O. Lingjærde, M. Sloth-Nielsen, I. Salvesen, Acta Psychiatr. Scand. 88 (1993) 395.
[14] A. Claus, J. Bollen, H. De Cuyper, M. Eneman, M. Malfroid, J. Peuskens, S. Heylen, Acta Psychiatr. Scand. 85 (1992) 295.