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Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography: application to therapeutic drug monitoring in Japanese patients with schizophrenia

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Abstract

A high-performance liquid chromatographic method has been developed for the simultaneous determination of risperidone and its major active metabolite 9-hydroxyrisperidone in plasma. Risperidone and 9-hydroxyrisperidone in plasma were extracted using a CN bonded-solid phase cartridge, followed by, C4 reversed-phase HPLC separation. Risperidone, 9-hydroxyrisperidone and trazodone as an internal standard were detected by ultraviolet absorbance at 280 nm. It was possible to determine risperidone in the concentration range of 1.0–100.0 ng ml^{-1} and 9-hydroxyrisperidone at a range of 2.0–200.0 ng ml^{-1}. The detection limits of risperidone and 9-hydroxyrisperidone were 0.5 and 1.0 ng ml^{-1}, respectively. The mean recoveries of risperidone and 9-hydroxyrisperidone added to plasma were less than 92.0 and 92.6%, with a coefficient of variation of less than 10.6 and 10.5%, respectively. This method has been used for the simultaneous determination of steady-state plasma concentration (Css) of risperidone and 9-hydroxyrisperidone in schizophrenic patients treated with 3-, 6-, and 12-mg risperidone oral doses per day. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Risperidone; 9-Hydroxyrisperidone; High-performance-liquid-chromatography; Schizophrenia; Steady-state plasma concentration

1. Introduction

Risperidone, a benzisoxazole derivative (Fig. 1a), is a new atypical antipsychotic agent with serotonin-5-HT2 and dopamine-D2 antagonist activity [1,2]. In clinical trails, resperidone is effective in the treatment of both positive and negative symptoms of schizophrenia, with a low potential for inducing extrapyramidal symptoms [3]. Risperidone is extensively metabolized in the human liver by cytochrome P-450 2D6 to form the princi-

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pal metabolite, 9-hydroxyrisperidone (Fig. 1b), which has biologic activity approximately equal to the parent compound. Risperidone may cause serious interactions such as increased plasma level with competition of drugs metabolism by the cytochrome P-450 2D6, notably tricyclic antidepressants, some neuroleptics, and some antiarrhythmics. Furthermore, the extent of the metabolism depends on genetic polymorphism in the cytochrome P-450 2D6 enzyme, so that individuals are classified as either poor metabolizers (PMs) or extensive metabolizers (EMs).

It is well known that plasma levels of risperidone and 9-hydroxyrisperidone exhibit a great deal of variability [4]. Some HPLC methods have been described for the quantitative determination of risperidone and 9-hydroxyrisperidone in plasma [5–8]. However, these methods are time-consuming because of tedious liquid–liquid extraction procedure. [5–7]. On the other hand, Martin and Douglas [8] reported the HPLC method with coloumetric detection coupled with solid-phase extraction of risperidone and 9-hydroxyrisperidone in serum. However, the apparatus of coloumetric detector is expensive, and the applicability to the real samples from patients was not reported. A highly sensitive and simple assay method is required to monitor of risperidone and 9-hydroxyrisperidone because the recommended risperidone dose may achieve therapeutic steady state levels at about 1.0–2.0 ng ml^{-1} in risperidone in Japanese patients. However, the steady state level of 9-hydroxyrisperidone was not described in previous papers. Therefore, an efficient extraction method and an excellent chromatography system without interfered peaks was needed for clinical monitoring of risperidone levels in patients.

The present paper, describes an HPLC method with UV-detection combined with a simple and selective solid-phase extraction method for the determination of risperidone and 9-hydroxyrisperidone levels in human plasma, which is simpler than the previous method. The present method was applied to the monitoring of steady-state concentrations (Css) of risperidone and 9-hydroxyrisperidone in Japanese schizophrenic patients.

2. Materials and methods

2.1. Chemicals and reagents

Risperidone and 9-hydroxyrisperidone (Fig. 1) were kindly donated by Janssen Pharmaceutical (Tokyo, Japan). Trazodone was kindly donated by Kanebo Pharmaceutical (Tokyo, Japan). The Sep-Pak® CN cartridge was purchased from Millipore Co. (Bedford, MA). The solvents used were of HPLC grade (Wako Pure Chemical Industries,

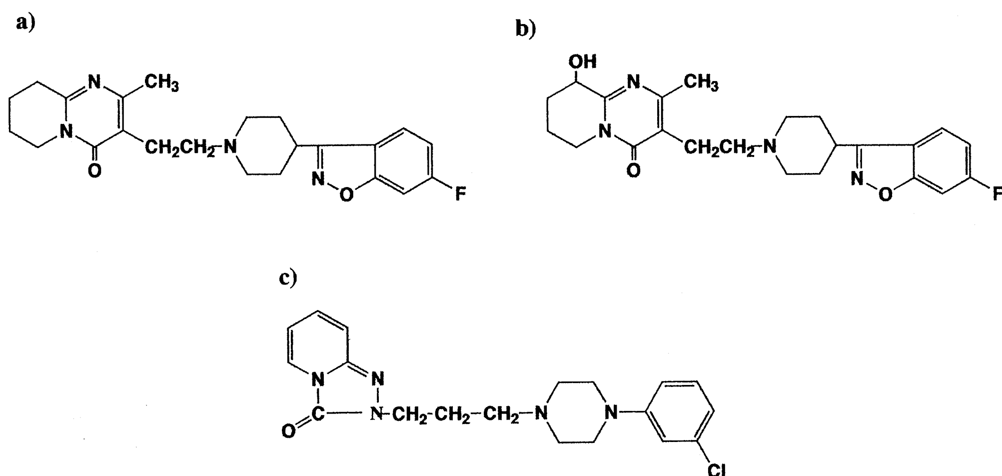

Fig. 1. Chemical structures of (a) risperidone, (b) 9-hydroxyrisperidone and (c) trazodone.

Osaka, Japan). Other reagents and chemicals were purchased from Wako Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan).

2.2. Preparation of standard solutions

The standard stock solutions were prepared by dissolving accurately weighed amount of risperidone, 9-hydroxyrisperidone and trazodone (I.S.) in methanol to make 1 mg ml^{-1} of each concentration. These solutions could be stored stably in −20°C for up to 6 months. The working solutions were prepared by diluting the stock solutions with methanol.

2.3. Instruments and chromatographic conditions

The apparatus used for HPLC was a Model PU-880 chromatography pump (JASCO, Tokyo, Japan) equipped with a JASCO Uvidec 980 ultraviolet detector set at 280 nm wavelength, and a Rheodyne Model 7120 injector (loop volume 100 μl). The HPLC column (150 × 4.6 mm i.d.) was packed with Develosil C4-5 of 5 μm particle size, (Nomura Chemical, Seto, Japan). The mobile phase consisted of K_2HPO_4 (pH 6.5; 0.5% w/v)-acetonitrile (75:25 v/v). A flow-rate of 1 ml min^{-1} was used at ambient temperature. Before mixing, the pH of K_2HPO_4 (0.5% w/v) was adjusted with 50% phosphoric acid and mobile phase was degassed ultrasonically before use.

2.4. Assay procedure

Trazodone (300 ng) in methanol (10 μl) was added to the plasma sample (1 ml) as an internal standard, then the plasma samples were diluted with 5 ml of K_2HPO_4 (pH 10; 0.5 %w/v) and the solution was briefly mixed. The mixture was applied to a Sep-Pak® CN cartridge that had been activated previously with 5 ml acetonitrile, K_2HPO_4 (pH 10; 0.5 %w/v) and water. The cartridge was then washed with 10 ml of water and 5 ml of 10% acetonitrile in water. The desired fraction was eluted with 5 ml of 80% acetonitrile in water. The eluate was evaporated to dryness in vacuum at 60°C by a rotary evaporator (Iwaki, Tokyo, Japan). The residue was dissolved in a 100 μl portions of the mobile phase and injected into the HPLC apparatus.

2.5. Calibration graphs

From the recorded peak heights, the ratios of drug to internal standard were calculated. The results obtained from spiked plasma samples containing known amounts of drug were calculated on the basis of liner regression analysis.

2.6. Recovery and assay validation

The samples were prepared by spiking 1.0, 2.0, 5.0, 10.0, 25.0, 50.0 and 100.0 ng ml^{-1} risperidone and 2.0, 5.0, 10.0, 20.0, 50.0, 100.0 and 200.0 ng ml^{-1} 9-hydroxyrisperidone to blank plasma. Sample extraction and subsequent HPLC analyses were carried out as described above. Intra-day and inter-day variability was assessed by extracting six spiked samples for reproduction in the same day or repeatedly over several assay days.

2.7. Application

Seven Japanese patients diagnosed as suffering from schizophrenic disorders were treated with risperidone (3–12 mg day^{-1}) for at least 14 days. Blood samples from patients were collected by venipuncture into heparinized blood collection tubes in the morning before breakfast and before the morning dose. The blood was immediately centrifuged (1900 × g) at ambient temperature, and the plasma layer separated was stored at −40°C until analysis.

3. Results

The chromatograms of blank plasma, spiked plasma and plasma sample from a patient who received risperidone 6 mg per day are shown in Fig. 2. These chromatograms indicated that no endogenous substances interfered with the detection of 9-hydroxyrisperidone, risperidone or I.S. at their retention times. Nor did other neuroleptics or antidepressants interfere with the peak of risperidone, 9-hydroxyrisperidone and I.S. on the

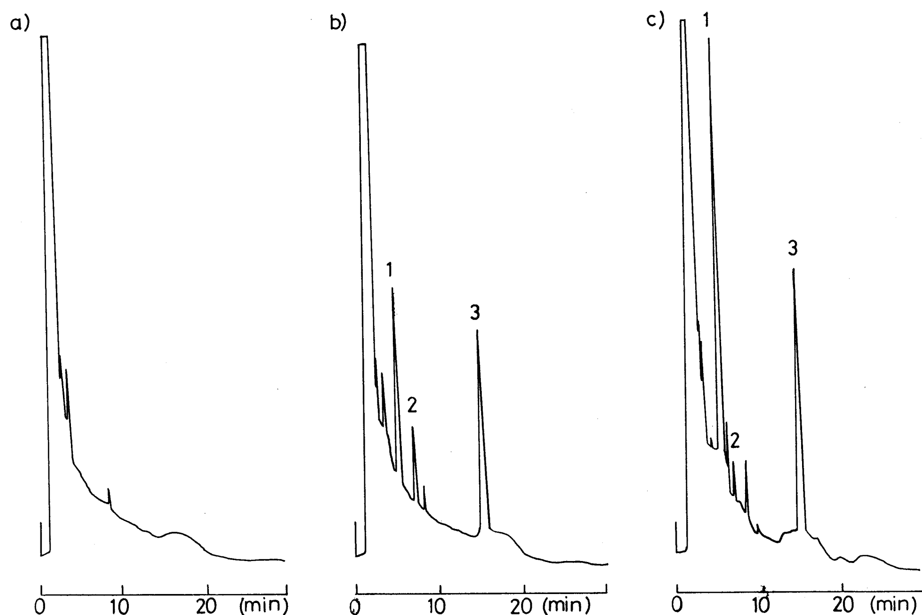

Fig. 2. Typical chromatograms of (a) plasma blank, (b) spiked with risperidone (5 ng ml^{-1}) and 9-hydroxyrisperidone (10 ng ml^{-1}) in plasma and (c) plasma sample from a patient (risperidone oral dose: 6 mg day^{-1}). Peaks: 1 = 9-hydroxyrisperidone, 2 = rispeidone, 3 = I.S.

chromatogram (data not shown). The retention times of 9-hydroxyrisperidone, risperidone and I.S. were 9.0, 13.5 and 17.5 min, respectively. The chromatographic run time was less than 20 min. The standard curves for risperidone and 9-hydroxyrisperidone were liner over the range of 1.0–100.0 ng ml^{-1} ($r = 0.973$), and 2.0–200.0 ng ml^{-1} ($r = 0.962$), respectively. The detection limits of determination for risperidone and 9-hydroxyrisperidone were 0.5 and 1.0 ng ml^{-1}, respectively.

The intra-day and inter-day assay variances are shown in Table 1. The intra-day assay variance was determined by analyzing 1 ml aliquots from six spiked plasma samples containing risperidone at 2.0, 5.0, 10.0, 25.0, 50.0 and 100.0 ng ml^{-1} and 9-hydroxyrisperidone at 5.0, 10.0, 20.0, 50.0, 100.0 and 200.0 ng ml^{-1}, and by calculating their concentrations with a standard curve analyzed on the same day. The inter-days assay variance was determined by 1 ml aliquots of spiked plasma samples containing risperidone and 9-hydroxyrisperidone at the same concentrations as above on 6 different days, and the concentrations were calculated from the standard curve analyzed on the respective days. In both cases, the coefficients of variation for risperidone and 9-hydroxyrisperidone were less than 10.6 and 10.5%. The recovery values for risperidone and 9-hydroxyrisperidone were 92.0–97.5%, and 92.6–97.8%, respectively.

The present method was applied to the monitoring of risperidone and 9-hydroxyrisperidone in Japanese patients diagnosed as suffering from schizophrenia. Seven clinical samples were assayed by the present method to determine the steady state plasma concentrations (Css) of risperidone and 9-hydroxyrisperidone (active moiety: risperidone plus 9-hydroxyrisperidone) in patients treated with oral doses of 3, 6 and 12 mg risperidone per day. These results are shown in Table 2.

4. Discussion

Our goal in conducting this study was to develop a highly efficient chromatographic method for the determination of risperidone and 9-hydroxyrisperidone levels in human plasma that

would be clinically useful for monitoring of drug effect in patients who are being treated with risperidone. During treatment for schizophrenia, patients are sometimes treated with multiple medications; therefore, a reliable extraction technique is needed that can completely separate interfering medications, as well as endogenous interfering substances, from the chromatographic system. Furthermore, in order to study the pharmacokinetics of risperidone and 9-hydroxyrisperidone in Css, and also to correlate the drug dosage and plasma levels with the patient's clinical response to these drugs, a simple and dependable assay method is needed.

Several papers have described liquid–liquid extraction methods [5–7], however such procedures are tedious and time-consuming. D.C. Martin and H.W. Douglas have reported solid phase extraction method using Bond-Elut® Certify LRC, as a mixed-bed solid phase extraction column [8]. It has been described that an efficient extraction technique is necessary because other prescribed medicines may be present at much higher concentrations than those attained by risperidone itself and may cause significant interference. In previous papers, we have described a simple solid phase extraction method for various drugs coupled with specific chromatographic separation [9–11]. This efficient combination system in HPLC determination method could remove interference substances. Therefore, we have examined solid-phase extraction procedures in detail for the purpose of eliminating such endogenous interference peak.

The extraction method of risperidone, 9-hydroxyrisperidone and trazodone (I.S.) in plasma was established using Sep-Pak® CN cartridge and K_2HPO_4 (pH 10; 0.5% w/v) as an applied solution. Lower interference from endogenous components of plasma was achieved by using Sep-Pak® CN rather than for Sep-Pak® C18. By testing several kinds of analytical columns, we found that a reversed-phase C4 (150×4.6 mm^2 i.d., 5 μg) column was the most efficient for separation of risperidone, 9-hydroxyrisperidone and I.S. from interfering endogenous substances in human plasma with suitable analytical time (Fig. 2).

Inter-day variations for risperidone and 9-hydroxyrisperidone were in the range of 6.3–10.6%, and 6.7–10.5%, respectively. These results indicated that the present method has satisfactory precision and is acceptable for monitoring of risperidone in patient's plasma.

Table 1
Accuracy and precision of determination of risperidone and 9-hydroxyrisperidone in human plasma ($n = 6$)

Added	Found (mean ± SD)	Recovery (%)	C.V. (%)	
			Inter-day	Intra-day
Risperidone (ng ml^{-1})				
1.0	0.92 ± 0.01	92.0	10.6	9.4
2.0	1.9 ± 0.02	93.5	10.3	7.3
5.0	4.8 ± 0.13	95.4	8.4	4.3
10.0	9.6 ± 0.35	96.3	6.3	3.9
25.0	23.4 ± 1.05	93.4	7.2	5.1
50.0	48.8 ± 1.78	97.5	7.6	6.3
100.0	97.2 ± 2.86	97.2	8.7	7.2
9-Hydroxyrisperidone (ng ml^{-1})				
2.0	1.93 ± 0.18	96.5	9.7	8.9
5.0	4.6 ± 0.17	92.6	8.7	6.9
10.0	9.3 ± 0.43	93.2	7.8	5.2
20.0	19.5 ± 1.24	97.5	7.2	5.8
50.0	46.8 ± 2.13	93.5	6.7	5.4
100.0	96.7 ± 2.67	96.7	9.2	8.2
200.0	195.6 ± 5.12	97.8	10.5	7.3

Table 2
Steady-state plasma concentrations (Css) of risperidone, 9-hydroxyrisperidone and active moiety (9-hydroxyrisperidone plus risperidone) in risperidone-treated schizophrenic patients

Patient no.	Dose (mg day^{-1})	Gender[a]	Age (years)	Steady-state plasma concentration (Css) (ng ml^{-1})		
				9-Hydroxyrisperidone	Risperidone	Active moiety
1	3.0	F	47	8.0	N.D.	8.0
2	6.0	F	65	31.5	2.5	34.0
3	6.0	F	32	15.5	2.0	17.5
4	6.0	F	52	15.0	37.0	52.0
5	6.0	M	23	29.0	2.5	31.5
6	6.0	F	55	11.2	44.5	55.7
7	12.0	M	63	75.0	9.5	84.5

[a] M, male; F, female.

The method was then applied to the determination of Css of risperidone and 9-hydroxyrisperidone in Japanese schizophrenic patients treated with oral doses of 3, 6 and 12 mg of risperidone per day for at least 14 days. These results are shown in Table 2. There were individual variations in the Css of risperidone and 9-hydroxyrisperidone in seven Japanese patients. The Css of 9-hydroxyrisperidone/risperidone ratio in five patients was in the range of 7.8–12.6, but there were two patients who were outside this usual range. In patient no. 1, the Css of risperidone could not be detected, however, 9-hydroxyrisperidone could be detected at range of 8.0 ng ml^{-1}. To the best of our knowledge, there is only one report in the literature [8] that describes the ratio of 9-hydroxyrisperidone to risperidone in patients; they reported 9-hydroxyrisperidone/risperidone ratio range of 6–8:1. In this investigation, we found the ratio range of 9-hydroxyrisperidone/risperidone to be 7.8–12.6:1, which is different than previously reported. However, in our study, two of seven patients (no. 4, no. 6) had a Css of 9-hydroxyrisperidone and risperidone that resulted in a 9-hydroxyrisperidone/risperidone ratio of between 0.25 and 0.5:1. This low ratio was due to a high Css of risperidone, rather than the Css of 9-hydroxyrisperidone.

Some neuroleptics, such as tricyclic antidepressants, which are metabolized by CYP 2D6, cause drug-drug interactions with risperidone, which may higher than the Css of risperidone. This is one possible explanation for the exceptionally high Css of risperidone in these two patients. These two patients had been treated with fixed doses of risperidone for at least 3 months, and they were not being treated with any other neuroleptic drugs. On the other hand, risperidone is extensively metabolized to 9-hydroxyrisperidone in the liver by CYP 2D6.

May-Lynn Huang et al. [4] has reported that the formation of 9-hydroxyrisperidone is related to the dextromethorphan oxidative status of the subjects. They classified the patients as EMs, intermediate, and PMs by phenotyping of dextromethorphan metabolic ratios. In their studies, they have reported that the half-life of risperidone was about 3 h in EMs and 22 h in PMs. Furthermore, by measuring the area under the plasma-concentration/time curve with time extending from zero to infinity, the average ratio of 9-hydroxyrisperidone to risperidone in the EMs was 3 when the drug was administered intravenously or intermuscular and 6 when the drug was administered orally, and the average ratio in the PMs was 0.2, regardless how the drug was administered. In a previous paper, Dahl et al. [12] have reported a significant relationship between the CYP 2D6 genotype and the Css of nortriptyline in 21 depressed Swedish patients (20 EMs, 1 PMs). The PMs patient had the highest Css. Among 20 EMs, the genotype (homozygous for the wt allele versus heterozygous for the wt and defect alleles) and gender significantly influenced the variance in the

Css. Nortriptyline is metabolized predominantly by CYP 2D6 [13]. The patients in this study did not have the genotype and phenotype of CYP 2D6, however, it is possible that patient no. 4 and patient no. 6 may have had a mutant of this allele, or be PMs of CYP 2D6, because these two patients had a smaller ratio of 9-hydroxyrisperidone/risperidoene than the other five. Therefore, polymorphism of CYP 2D6 may contribute to the variations in the Css of risperidone and 9-hydroxyrisperidone that were observed in different individuals.

In conclusion, a solid-phase extraction method using HPLC with UV detection has been described in this report to determine Css of risperidone and 9-hydroxyrisperidone in schizophrenic patients. This method is simple, accurate, and sensitive enough to be used on a routine basis to measure Css of risperidone and 9-hydroxyrisperidone. Furthermore, we analyzed the relationship between the CYP 2D6 genotype and the Css of risperidone in Japanese patients receiving this drug for schizophrenia. At the present time, we are planning further applications of this method to investigate pharmacokinetic interactions between risperidone and co-administered drugs.

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