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Short communication

Rapid simple high-performance liquid chromatographic determination of paroxetine in human plasma

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Abstract

A rapid, simple method for the measurement of paroxetine in human plasma by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection is described. This method includes only one-step extraction of paroxetine and dibucaine, an internal standard, with chloroform. Their recoveries were around 90%. The mobile phase, 10 m*M* phosphate buffer–acetonitrile (40:60, v/v) was eluted isocratically. Between- and within-day coefficients of variation were in the range of 1.9–9.4% and 2.3–13.3%, respectively. The detection limit was 0.2 ng/ml. The method we describe can be easily applied to the measurement of plasma paroxetine concentration for pharmacokinetic studies as well as for therapeutic drug monitoring in patients taking paroxetine. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Paroxetine

1. Introduction

Paroxetine, a selective serotonin-reuptake inhibitor (SSRI), was recently approved in South Korea for the treatment of depression [1]. It is also effective for the management of obsessive–compulsive disorder and panic disorder [2,3]. Paroxetine is known to be metabolized mainly by cytochrome P450 2D6 isoform (CYP2D6) which exhibits genetic polymorphism in drug metabolism [4–6], which can cause wide inter-individual variation of steady-state plasma drug levels (25-fold difference in 13 extensive and three poor metabolizers of spartein) after same dose [4]. The measurement of plasma paroxetine concentration, therefore, seems to give a clue to increase dose in a patient who is resistant to the therapy due to its pharmacokinetic characteristics. In addition, it has a strong inhibitory effect on CYP2D6 in vitro [7,8] and in vivo [9,10].

Despite extensive studies on the pharmacokinetics and the possible indication of therapeutic drug monitoring of paroxetine, a few chromatographic assay methods have been introduced to measure plasma paroxetine concentration [11–14]. Column liquid chromatographic assay including liquid–liquid

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extraction and pre-column preparation of a dansyl chloride derivative for the sensitive fluorescent detection of paroxetine [12] has been widely used [4–6,15]. More recently, Gupta [13] and Hätter et al. [14] introduced a high-performance liquid chromatography (HPLC) method using solid-phase extraction (SPE) and a HPLC method using automated column switching, respectively. However, these methods are not easily applicable due to their complicated and labor-intensive preparation of plasma samples or use of expensive extraction columns.

The method we describe here has advantages that the pretreatment of plasma samples is easy and simple and the cost is less expensive compared to the previously reported HPLC methods [12–14].

2. Experimental

2.1. Reagents and preparations

Paroxetine hydrochloride hemihydrate (87.4% free base) was a generous gift from SmithKline Beecham (Seoul, South Korea). Dibucaine hydrochloride was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA) and both were HPLC grade. Chloroform (Merck, Darmstadt, Germany) and other reagents used were of analytical grade.

A 0.12 *M* phosphate buffer for paroxetine extraction was prepared by dissolving 3.4 g of Na_2HPO_4 in 180 ml of highly purified water, bringing to pH 12.0 with 4 *M* NaOH and making up to the final volume of 200 ml with water. A 10 m*M* phosphate buffer, for the aqueous component of mobile phase, was prepared by dissolving 1.36 g of KH_2PO_4 in 1000 ml of water. Stock solutions of paroxetine and dibucaine, an internal standard, were prepared at the concentrations of 100 μg/ml (calculated as the pure free base) in methanol and stored at −20°C. The working solutions of paroxetine (20–800 ng/ml) and dibucaine (600 ng/ml) were obtained each day from the dilution of stock solution with purified water for the preparation of the calibration samples. The stock solutions remained stable for one month if stored at −20°C.

2.2. Pharmacokinetic study

Paroxetine, 40 mg, was administered orally to two normal healthy volunteers who were phenotyped as an extensive metabolizer (EM) and poor metabolizer (PM) of cytochrome P450 2D6(CYP2D6) by metoprolol, a probe drug, as described previously [15]. Seventeen blood samples were serially drawn up to 10 days after single dose of paroxetine, and were collected into heparin tubes. These tubes were centrifuged as soon as possible after collection and plasmas separated were stored at −20°C until assayed. Paroxetine concentrations in the plasma were measured within one month of sampling. Paroxetine concentrations were represented as mean of duplicates in each time point of samples.

2.3. Sample preparations

In a glass tube (12 cm×75 mm), a 1-ml aliquot of control human plasma was mixed with 50 μl of working internal standard solution (equivalent to 30 ng of dibucaine) and appropriate working paroxetine solutions to yield concentrations in the range 1–40 ng/ml, and 500 μl of 0.12 *M* phosphate buffer (pH 12.0) was added. Finally, 5 ml of chloroform was added for the extraction.

Authentic plasma samples were extracted with a similar method as described above. To 1 ml of plasma sample in a glass tube were added 500 μl of 0.12 *M* phosphate buffer, 50 μl of internal standard solution and 50 μl of purified water, and 5 ml of chloroform. The samples were mixed with full speed Vortex mixing for 3 min and then centrifuged for 10 min at 1000 *g*. The organic phase was transferred into a conical shape glass tube and evaporated to dryness under the flow of oxygen free nitrogen using concentrator. The residue was redissolved in 100 μl of methanol with vigorous vortex mixing for 30 s, and 70 μl sample was injected onto the HPLC column.

2.4. Chromatography

Chromatographic systems consisted of Gilson Model 307 pump (Villiers Le Bel, France), a Gilson Model 122 fluorescence detector (Tokyo, Japan),

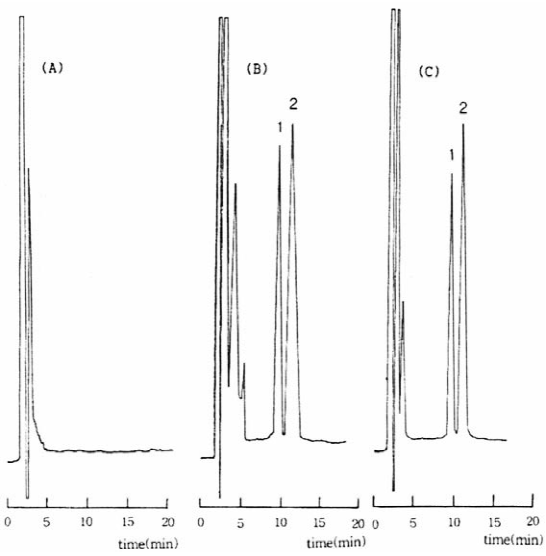

Fig. 1. Chromatograms of plasma extracts obtained from (A) blank plasma, (B) plasma spiked with 20 ng/ml paroxetine and 30 ng/ml dibucaine, (C) plasma obtained from a subjects after single oral administration of 40 mg paroxetine (paroxetine concentration: 17.9 ng/ml at 18 h after dose). Peaks: 1=paroxetine; 2=internal standard (dibucaine).

αTECH chromatocorder 12 integrator (Shimadzu, Kobe, Japan) and Rheodyne 7161 loop injector (Cotati, CA, USA).

Chromatographic separation was achieved isocratically on LiChrosorb RP-8 column (250×4 mm, 10 μm particle size; Merck). The mobile phase, 10 m*M* potassium phosphate buffer–acetonitrile (40:60, v/v) adjusted to pH 3.2 with 80% phosphoric acid, was delivered into the HPLC system at a flow-rate of 1.2 ml/min. Excitation and emission wavelength at 295 and 350 nm was used for the fluorometric detection of paroxetine and dibucaine.

3. Results

Typical chromatograms obtained using the method described above are shown in Fig. 1. Paroxetine and the internal standard (dibucaine) gave well separated peaks.

3.1. Recovery, precision, accuracy and sensitivity

The extraction recovery was determined by comparing peak heights of paroxetine extracted from human plasma with that of the unextracted standard containing same amount. The mean recovery of plasma paroxetine ranged from 86.2 to 93.4% in the concentration range 1–40 ng/ml, and that of dibucaine at 30 ng/ml was 78.5±7.7% (Table 1). The peak height ratio of paroxetine/dibucaine was linear between 1 and 40 ng/ml concentration range of paroxetine. The correlation coefficient (*r*) of the standard curves was consistently higher than 0.997.

The within-day coefficient of variation (C.V.) and bias assessed using quality control samples (2, 20, 40 ng/ml of paroxetine) were less than 9.4% and −6.6%, respectively (Table 1). The between-day C.V. and bias ranged from 2.3 to 13.3% and from −1.9 to −11.7%, respectively. From this method, the limit of quantification was 0.5 ng/ml of parox-

Table 1
Extraction efficiency, within-day and between-day coefficient of variation (C.V.) and bias as determined with a 2, 20 and 40 ng/ml paroxetine concentration in human plasma

Concentration (ng/ml)	Extraction recovery (%)	Within-day		Between-day	
		C.V. (%)	Bias (%)	C.V. (%)	Bias (%)
Paroxetine					
2	86.2±7.2	9.4	−6.6	13.3	−11.7
20	93.4±8.9	3.6	−0.9	4.2	−2.2
40	92.8±5.6	1.9	−1.8	2.3	−1.9
Dibucaine					
30	78.5±7.7				

Values for each concentration are mean±S.D. of six measurements.

etine in plasma. The detection limit, determined as a concentration resulting in a signal-to-noise ratio of 3, was 0.2 ng/ml.

3.2. Selectivity

The drug-free plasma did not show any peaks that interfere with those of internal standard and paroxetine (Fig. 1a). A number of drugs being used in psychiatric field, including amitriptyline, nortriptyline, imipramine, desipramine, fluoxetine, haloperidol, chloropromazine, risperidone, lithium and several benzodiazepines (diazepam, lorazepam, flurazepam, triazolam and alprazolam) were evaluated for the possible interference with internal standard and paroxetine. Desipramine among tested drugs was the only compound co-extracted and showed the peak around the retention time (8.7 min) of paroxetine. However, we could separate the peaks of paroxetine and desipramine when we used a different mobile phase, acetonitrile–methanol–0.56 *M* ammonium acetate–1 *M* ammonium hydroxide (810:10:45:26), pH 10.4 adjusted with 4 *M* NaOH and added 20 μl of triethylamine, at a flow-rate of 1.2 ml/min. However, the detection limit of this method was higher than the original one (3 ng/ml).

3.3. Pharmacokinetic study

After a single oral dose of paroxetine 40 mg to two normal healthy volunteers, time courses of plasma drug concentrations are represented in Fig. 2.

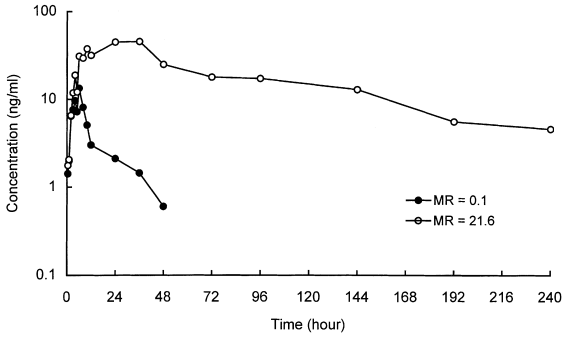

Fig. 2. Representative plasma paroxetine concentration–time profiles after single oral administration of 40 mg paroxetine in a CYP2D6 EM (metoprolol MR=0.1) subject and a PM subject (metoprolol MR=21.6).

With the method described here, plasma paroxetine concentrations could be measured up to 48 h at the concentration 0.6 ng/ml in a high EM subject (metoprolol MR=0.1). Plasma paroxetine concentrations were remained extreme high (10.5 ng/ml) up to 240 h after oral dose 40 mg in a PM subject (metoprolol MR=21.6).

4. Discussion

This paper describes a rapid and simple assay method to quantify paroxetine concentration in human plasma using HPLC with fluorescence detection and describes its possible application on pharmacokinetic study. Brett et al. [12] first introduced the HPLC method to measure paroxetine concentration in human plasma. Although this method has been widely used [4–6,16], it requires complicated, labor-intensive derivatization of paroxetine with dansyl chloride to increase sensitivity of the assay. With using more sensitive fluorescence detectors, however, it has obviated the need for the preparation of the dansyl derivative of paroxetine. Gupta [13] described a HPLC method which used SPE and measured directly the fluorescence of paroxetine and dibucaine, a native fluorescent internal standard, without derivatization. Gupta used a 5–6-times more sensitive fluorescence detector than that used by Brett et al. [12]. We also used a highly sensitive fluorescence detector which was more recently developed.

The SPE of Gupta [13] also has a problem. The dibucaine, an internal standard, is well adsorbed into the SPE column, which leads to the requirement of excess amount of dibucaine (500 ng) in sample preparation and the possible inconsistent recovery of dibucaine. Our method can minimize these problems from using liquid–liquid extraction with chloroform. Therefore, only 30 ng/ml of dibucaine was enough to obtain chromatogram with high sensitivity (Fig. 1) and the mean recovery of dibucaine was 78.5% (Table 1). However, in the process of extraction with chloroform, the exposure of chloroform to the laboratory persons should be minimized due to its toxic potential. For this, the use of chemical hood is absolutely required during adding, transferring and drying of chloroform.

Some of psychiatric agents may be dissolved in chloroform and/or may have a native fluorescence. Among commonly prescribing psychiatric agents tested in the present study, desipramine, an active metabolite of imipramine, interfered with the peak of paroxetine and was co-extracted with paroxetine. We developed a different mobile phase [acetonitrile–methanol–0.56 *M* ammonium acetate–1 *M* ammonium hydroxide (810:10:45:26, v/v), pH 10.4 adjusted with 4 *M* NaOH and addition of 20 μl triethylamine] in order to prevent the interference of desipramine peak with the peak of paroxetine. With using this method, the peaks of paroxetine and desipramine were well separated and the retention times were 12.3 and 17.7 min, respectively (data not shown). Due to its good resolution, this method allows simultaneous measurement of plasma concentrations of tricyclic antidepressants and paroxetine in the patients taking both drugs.

From the pharmacokinetic study, this method showed good sensitivity enough to measure the paroxetine concentration at 48 and 240 h in CYP2D6 EM and PM subjects after oral administration of 40 mg paroxetine (Fig. 2). This method also has an advantage that it requires only one-step extraction with chloroform during the preparation of plasma samples and does not require derivatization, which enables rapid, simple and less expensive assay of plasma paroxetine concentrations compared to previous several HPLC methods.

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