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Short communication

Simultaneous determination of serum flecainide and its metabolites by using high performance liquid chromatography

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Abstract

Simultaneous determination of serum flecainide and its oxidative metabolites was carried out by using high performance liquid chromatography (HPLC) equipped with conventional octadecylsilyl silica (ODS) column and fluorescence detector. Flecainide and its metabolites, *m-O*-dealkylated flecainide (MODF) and *m-O*-dealkylated lactam of flecainide (MODLF) in serum were extracted with ethyl acetate. The recoveries of flecainide, MODF and MODLF were greater than 92, 93, and 60% with the coefficient of variations (CVs) less than 3.2, 5.8, and 5.3%, respectively. The calibration curves were linear at the concentration range of 50–1500 ng/mL for flecainide and 10–500 ng/mL for MODF and MODLF ($r > 0.999$). The CVs for intra-day assay were 2.7–5.3% for flecainide, 3.0–4.2% for MODF, and 3.7–4.3% for MODLF, respectively. The CVs for inter-day assay were 7.0–8.4% for flecainide, 3.3–6.7% for MODF, and 4.4–7.7% for MODLF, respectively. This assay method can be used for assessing the metabolic ability of flecainide in the patients with tachyarrhythmia.

Keywords: Flecainide; Metabolite; High performance liquid chromatography (HPLC); Simultaneous determination; Therapeutic drug monitoring

1. Introduction

Flecainide acetate (Fig. 1), a class Ic (Vaughan Williams) antiarrhythmic agent, is used for a variety of supraventricular tachycardias [1]. Since the therapeutic range for serum flecainide is narrow, 200–1000 ng/mL, the serum concentration monitoring is required for dose-adjustment [2,3]. Change in the renal excretion and hepatic metabolism is a factor affecting flecainide clearance and cause for individual variations of blood concentrations [4–7], which closely associate with drug efficacy and adverse reactions [2,3,8,9]. It is known that the hepatic

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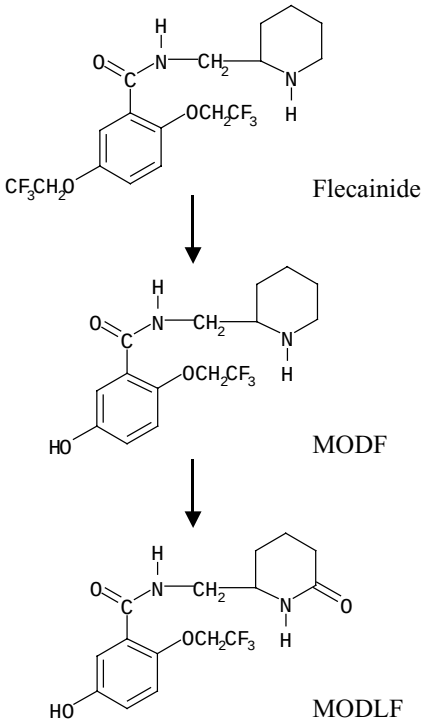

Fig. 1. Chemical structures and metabolic pathway of flecainide and its metabolites, MODF, and MODLF.

metabolism catalyzes the conversion of flecainide to *m*-*O*-dealkylated flecainide (MODF, Fig. 1), which subsequently is oxidized to the *m*-*O*-dealkylated lactam (MODLF, Fig. 1) [10,11]. Since these major metabolites are found in blood and urine, principally as the glucuronide conjugates [11], it might be important for assessing hepatic metabolism of flecainide to determine them as well as flecainide itself.

Simultaneous determination of flecainide and its metabolites by using high performance liquid chromatography (HPLC) was carried out by Munafo and Biollaz [12] and Benijts et al. [13]. They used phenyl column as an analytical column followed by gradient elution. These analytical conditions may not be suitable in terms of routine monitoring of serum flecainide and its oxidative metabolites. In the present study, we developed a HPLC for simultaneous determination of serum flecainide and its metabolites by using conventional octadecylsilyl silica (ODS) column and isocratic elution. The method was applied to routine monitoring of serum flecainide and its metabolites in patients with tachyarrhythmia.

2. Methods

2.1. Chemicals and instruments

Flecainide acetate, *m*-*O*-dealkylated flecainide, *m*-*O*-dealkylated lactam of flecainide and the internal standard [*N*-(2-piperidinylmethyl)-2,3-bis(2,2,2-trifluoroethoxy)benzamide acetate] were kindly supplied by Eisai Co. (Tokyo, Japan). 1-Pentanesulfonic acid sodium salt was purchased from Wako Pure Chemicals (Osaka, Japan). β-Glucuronidase, type HP-2 from *H. pomatia* (137 800 U/mL) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). All other chemicals purchased from Wako Pure Chemicals were of HPLC or analytical reagent grade.

2.2. Preparation of solutions

Alternative human serum (Twin-consera H, Nissui, Tokyo, Japan) was used for preparing the control sera. All standard solutions of flecainide, MODF, and MODLF were prepared by diluting a 1 mg/mL in methanol. The internal standard was prepared as the 20 μg/mL solution in distilled water and was stored at 4 °C until analysis.

2.3. HPLC apparatus and analytical conditions

HPLC apparatus consisted of a pump (CCPD, TOSOH, Tokyo, Japan), a fluorescence detector (FS-8020, TOSOH) and an integrator (C-R4A, Shimadzu, Kyoto, Japan). ODS column (TSK-GEL ODS-80TS, 4.6 i.d. × 250 mm, TOSOH) as the analytical column was maintained at ambient temperature. The excitation and emission wavelengths were set at 300 and 370 nm, respectively. The HPLC mobile phase consisted of 0.1 M 1-pentanesulfonic acid sodium salt, acetonitrile and acetic acid (310:150:2.5, v/v/v) and the flow rate was set at 1.0 mL/min.

2.4. Assay procedures

Conjugated metabolites in serum were enzymatically hydrolyzed by following procedure. Five hundred microliters of serum sample was added to

1 mL of 0.2 M acetate buffer (pH 5.0) and 25 μL of β-glucuronidase solution (3445 U). The resulting mixture was incubated at 37 °C for 24 h. The mixture, to which 100 μL of internal standard solution (20 μg/mL) was added, was alkalinized with 200 μL of 2 M sodium carbonate and then, treated with 1 mL of ethyl acetate three times. The organic layer was evaporated to dryness under a stream of nitrogen at 45 °C. The residue was reconstituted with 100 μL of mobile phase solution and the 20 μL aliquot was injected into the HPLC system.

Pre-treatment of urine sample was performed with similar manner for serum except for diluting the sample to 30-fold with water.

2.5. Calibration and validation

Control serum samples spiked with flecainide at the concentrations of 50, 200, 500, 1000, and 1500 ng/mL, and the metabolites, MODF and MODLF at the concentrations of 10, 30, 80, 200, and 500 ng/mL were used for calibration curve. The recovery of flecainide and its metabolites, MODF and MODLF, was examined at the concentrations of 200 and 1000 ng/mL and the concentrations of 30 and 200 ng/mL, respectively. The assay precision was evaluated by intra- and inter-day validation at the concentrations of 200 and 1000 ng/mL for flecainide, and 30 and 200 ng/mL for MODF and MODLF. For intra-day precision, five sets of each control sample were assayed on the same day. For inter-day assay precision, five sets of each control sample were assayed on 5 different days.

2.6. Subjects

Six out-patients (three males and three females) and one healthy male volunteer were enrolled for this study. Healthy volunteer received single dose (50 mg) of flecainide acetate for pharmacokinetic study. Blood and urine samples were collected after dosing (1, 2, 3, 4, 6, 12 h after for blood, from 0 to 12 h after for urine). The liver and kidney functions for all patients were normal. Blood drawing was carried out at 9:00–11:00 a.m. in out-patients visit. Patients postponed taking morning flecainide until blood drawing. Informed consent was obtained from all subjects and the study was approved by The Ethical Committee of the University of Tsukuba.

3. Results

Typical chromatogram for simultaneous determination of serum flecainide and its metabolites was shown in Fig. 2. The retention times of MODLF, MODF, internal standard and flecainide were 5.7, 6.2, 27.7, and 28.7 min, respectively. Although a minor interfering peak overlapping MODLF was observed in control serum, it did not affect the quantitative determination of MODLF in routine TDM. The detection limits for flecainide, MODF and MODLF were 0.1, 0.7, and 0.1 ng as the injected amounts, respectively.

The calibration curves were linear at the concentration range of 50–1500 ng/mL for flecainide, and 10–500 ng/mL for MODF and MODLF. The equations of the calibration curves calculated by regression analysis were $Y = 0.0051X - 0.0068$ ($r = 0.9996$) for flecainide, and $Y = 0.0035X - 0.025$ ($r = 0.9995$) for MODF, $Y = 0.0054X - 0.0096$ ($r = 0.9997$) for MODLF, where Y were the serum concentration (ng/mL) and X is the peak height ratio of flecainide, MODF or MODLF to internal standard.

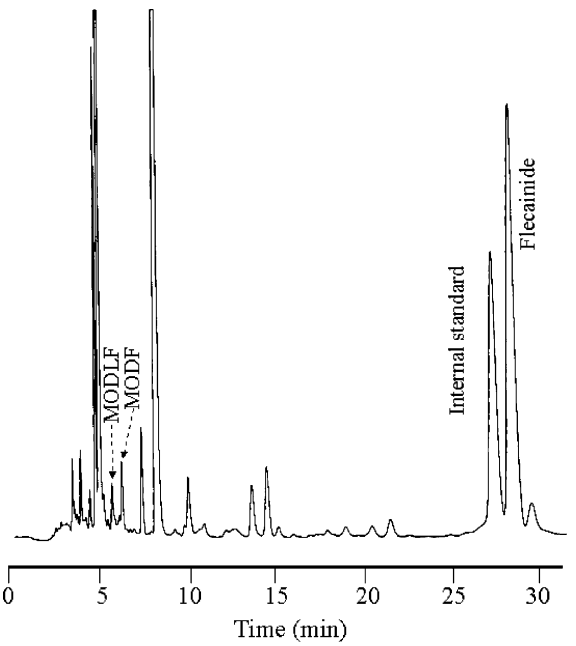

Fig. 2. Typical chromatogram for simultaneous determination of serum flecainide, MODF, and MODLF in patient receiving flecainide acetate. The concentrations of flecainide, MODF, and MODLF were 301.7, 69.3, and 27.0 ng/mL, respectively.

Table 1
Recovery of flecainide, MODF, and MODLF in spiked serum samples

Concentration added (ng/mL)	Recovery ($n = 5$)	
	Mean ± S.D. (%)	CV (%)
Flecainide		
200	97.1 ± 1.4	1.5
1000	92.8 ± 3.0	3.2
MODF		
30	98.1 ± 5.7	5.8
200	93.9 ± 2.6	2.8
MODLF		
30	60.7 ± 3.2	5.3
200	65.4 ± 1.9	2.9

The recoveries of flecainide at concentrations of 200 and 1000 ng/mL, and MODF and MODLF at concentrations of 30 and 200 ng/mL were greater than 92, 93, and 60% with the coefficient of variation (CV) values less than 3.2, 5.8, and 5.3%, respectively (Table 1). The CVs for intra-day assay were 2.7–5.3% for flecainide, 3.0–4.2% for MODF, and 3.7–4.3% for MODLF, respectively (Table 2). The relative errors (bias) of intra-day assay were less than 6.4 for flecainide, 4.7 for MODF, and 7.4 for MODLF, respectively. The CVs for inter-day assay were 7.0–8.4% for flecainide, 3.3–6.7% for MODF, and 4.4–7.7% for MODLF, respectively (Table 2). The biases of inter-day assay were less than 4.3 for flecainide, 3.0 for MODF, and 4.7 for MODLF, respectively.

Serum concentrations of flecainide, MODF, and MODLF were 469.5 ± 298.1 ng/mL (range 230.1–887.0), 83.4 ± 33.1 ng/mL (range 60.5–149.9), and 36.8 ± 20.2 ng/mL (range 15.9–73.7), respectively, in six patients receiving 3.4 ± 1.2 mg/kg flecainide acetate daily (Table 3). Oral clearance of flecainide was 0.30 ± 0.09 L/(h kg) (range 0.21–0.44) and the metabolic ratio of flecainide (MODF + MODLF)/flecainide, was 0.37 ± 0.11 (range 0.15–0.46).

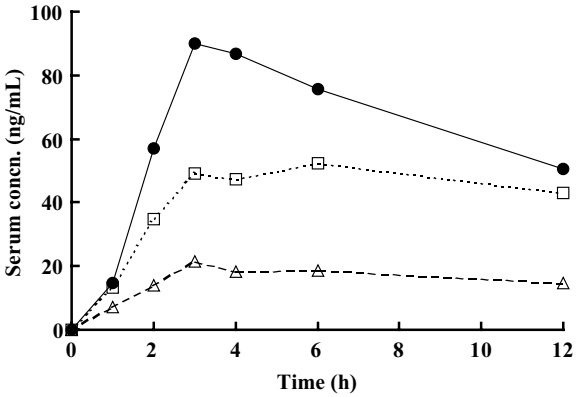

Fig. 3. Serum concentration profiles for flecainide (●), MODF (□), and MODLF (△) after oral administration of 50 mg flecainide acetate in one healthy volunteer.

The assay was applied to illustrating concentration–time profile in one healthy volunteer who received single dose (50 mg) of flecainide acetate (Fig. 3). To determine the low levels of flecainide and MODLF, another calibration curves were employed at the concentration range of 10–100 ng/mL for flecainide ($Y = 0.0054X - 0.0155$, $r = 0.9991$) and 2–30 ng/mL for

Table 2
Intra- and inter-day precision for determination of flecainide, MODF, and MODLF

Concentration added (ng/mL)	Intra-day ($n = 5$)			Inter-day ($n = 5$)		
	Mean concentrations found ± S.D. (ng/mL)	CV (%)	Bias (%)	Mean concentrations found ± S.D. (ng/mL)	CV (%)	Bias (%)
Flecainide						
200	187.2 ± 9.9	5.3	6.4	191.4 ± 16.0	8.4	4.3
1000	973.0 ± 26.4	2.7	2.7	982.9 ± 68.6	7.0	1.7
MODF						
30	29.7 ± 0.9	3.0	1.1	30.0 ± 1.0	3.3	0.1
200	209.5 ± 8.8	4.2	4.7	206.1 ± 13.8	6.7	3.0
MODLF						
30	30.6 ± 1.1	3.7	2.0	30.2 ± 1.3	4.4	0.8
200	214.8 ± 9.3	4.3	7.4	209.3 ± 16.0	7.7	4.7

Table 3
Trough concentrations of flecainide, MODF, and MODLF in patients receiving flecainide acetate

Case	Sex	Age (year)	Weight (kg)	Dose (mg/kg)	Trough concentration (ng/mL)			Flecainide CL (L/(h kg))	Metabolic ratio[b]
					Flecainide	MODF[a]	MODLF[a]		
1	Male	85	40	5.0	887.0	73.7	37.3	0.21	0.15
2	Female	77	42	4.8	814.8	149.9	73.7	0.21	0.34
3	Female	24	52	1.9	230.1	69.1	15.9	0.30	0.46
4	Male	42	75	2.7	301.7	69.3	27.0	0.32	0.39
5	Female	57	71	2.8	322.8	78.0	41.2	0.32	0.45
6	Male	66	64	3.1	260.3	60.5	25.8	0.44	0.41
Mean ± S.D.		58.5 ± 22.7	57.3 ± 14.9	3.4 ± 1.2	469.5 ± 298.1	83.4 ± 33.1	36.8 ± 20.2	0.30 ± 0.09	0.37 ± 0.11

[a] Concentration of conjugated plus unconjugated.
[b] The ratio was calculated by following formula: (MODF + MODLF)/flecainide.

MODLF ($Y = 0.0051X + 0.0086$, $r = 0.9993$). The AUCs of flecainide, MODF, and MODLF were 746.3, 459.8, and 172.5 ng h/mL, respectively. Oral clearance of flecainide was 0.43 L/(h kg). The amounts of flecainide, MODF, and MODLF excreted in the urine from 0 to 12 h after dosing were 5.8, 4.9, and 2.1 mg, respectively. The renal clearance of flecainide was 0.09 L/(h kg). The metabolic clearance of flecainide was estimated to be 0.34 L/(h kg), which corresponded to 78% of total clearance.

4. Discussion

Our method employing liquid–liquid extraction and isocratic HPLC showed a sufficient sensitivity for simultaneous determination of serum flecainide and its metabolites in the TDM. Chromatographic conditions produced satisfactory separation of flecainide, MODF, and MODLF from endogenous compounds and concomitant drugs (Fig. 2). The sensitivity of the assay was enough for routine monitoring and was comparable with the methods of Munafo and Biollaz [12] and Benijts et al. [13]. They used gradient elution with phenyl column for peak separation. We employed isocratic elution with ODS column leading disadvantage of time and cost consuming. Assay precision confirmed by the recovery and intra- and inter-day validations, was also comparable with the previous method [12].

Ethyl acetate was used for simultaneous extraction of flecainide, MODF, and MODLF. We treated the serum with 1 mL of ethyl acetate three times to enhance the recovery of MODF and MODLF because both metabolites were polar compounds. The recovery of MODLF, however, was still low compared with other compounds because of its high polarity. Similar results have been reported in previous study [12].

Since MODF and MODLF are circulating in the plasma primarily as glucuronide conjugates [10,11], we determined total metabolites (both conjugated and unconjugated forms) in order to assess the hepatic metabolism of flecainide. Serum concentrations of MODF and MODLF in six patients were 83.4 ± 33.1 and 36.8 ± 20.2 ng/mL, respectively. Large individual variations in serum concentrations of flecainide and its metabolites were observed in six patients with tachyarrhythmia (Table 3). These results suggest that difference in metabolic capacity is one of the causes for individual variation of flecainide pharmacokinetics through genetic polymorphism for CYP2D6 mediated hepatic metabolism of flecainide, since the liver and kidney functions for all patients were normal in this study.

In one healthy volunteer the metabolic clearance was estimated to be 0.34 L/(h kg), which is lower than the value of extensive metabolizers (0.68 ± 0.23 L/(h kg)) reported in the previous study [14]. Thus, our method could be used for assessing metabolic clearance by determining both serum and urinary flecainide.

In conclusion, simultaneous determination of the flecainide and its metabolites was developed and validated. We confirmed that the present assay method could be used for assessing the impact of metabolic enzyme in the TDM of flecainide.

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