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Short Communication

Improved high-performance liquid chromatographic assay for the stereoselective determination of mexiletine in plasma

Zohreh Abolfathi, Pierre-Maxime Bélanger, Marcel Gilbert, Jacques R. Rouleau and Jacques Turgeon

Institut de Cardiologie de Québec, Hôpital Laval, and École de Pharmacie, Université Laval, Ste-Foy, Quebec (Canada)

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ABSTRACT

A simple and sensitive high-performance liquid chromatographic procedure for resolution of mexiletine enantiomers has been developed. Proteins from plasma samples containing *RS*-mexiletine were precipitated with a mixture of barium hydroxide and zinc sulphate before extraction under alkaline conditions with diethyl ether. Organic extracts were evaporated to dryness, and the residues reconstituted with 0.03 *M* hydrochloric acid (20 μl). Derivatization with *o*-phthalaldehyde N-acetyl-L-cysteine reagent was performed after alkalinization with 0.1 *M* sodium borate. An aliquot of the resulting solution was injected onto a reversed-phase C_{18} column and resolution of mexiletine diastereoisomeric derivatives was achieved with a mobile phase consisting of methanol–50 m*M* sodium acetate (65:35), at a flow-rate of 1 ml/min. The retention times of *S*-(+)- and *R*-(−)-mexiletine diastereoisomeric peaks were 14 and 15 min, respectively. Product elution was monitored by fluorescence detection using excitation and emission wavelengths fixed at 350 and 445 nm, respectively. Calibration curves were linear over the concentration range 2.5–500 ng/ml for each enantiomer ($r > 0.99$). The assay is shown to be suitable for pharmacokinetic studies after administration of a single oral dose of 200 mg of *RS*-mexiletine hydrochloride to healthy volunteers.

INTRODUCTION

Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane (Fig. 1), is an orally effective type IB antiarrhythmic agent with electrophysiological properties similar to those of lidocaine [1,2]. Mexiletine contains an asymmetric carbon atom and the drug is used clinically as the racemate. Disposition of mexiletine is stereoselective in humans, and the enantiomers exhibit different pharmacological properties [3–6]. Moreover, some mexiletine metabolic pathways are stereoselective and mediated by different cytochrome P450 isozymes [7]. Therefore, determination of mexiletine enantiomers in biological fluids may be clinically relevant [8].

Two methods have been reported previously for the stereoselective analysis of mexiletine in human biological fluids [6,9]. We first described a stereoselective high-performance liquid chro-

Correspondence to: Dr. Jacques Turgeon, Centre de Recherche, Hôpital Laval, 2725 Chemin Ste-Foy, Ste-Foy, Quebec G1V 4G5, Canada.

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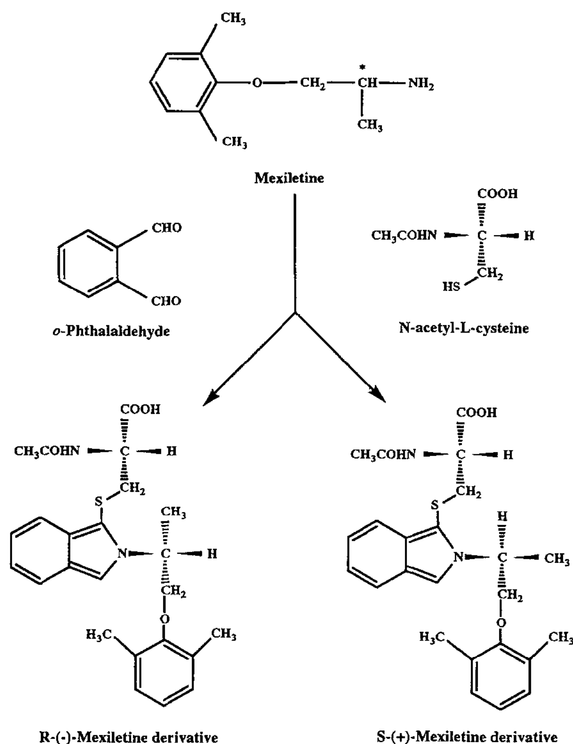

Fig. 1. Structures of mexiletine, *o*-phthalaldehyde and N-acetyl-L-cysteine, and structures of the diastereoisomeric derivatives; the asymmetric carbon is marked with an asterisk.

matographic (HPLC) method using the chiral reagent 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) to resolve the diastereoisomers on a reversed-phase column [9]. Later, resolution of mexiletine enantiomers on a chiral column was reported [6]. The first assay lacks the sensitivity required for complete single-dose pharmacokinetic studies, and the second produces significant interfering peaks at low concentrations of the drug.

Therefore, to assess stereoselective drug–drug interactions involving mexiletine enantiomers, we developed a new sensitive and simple stereoselective HPLC assay. This new assay involves derivatization with *o*-phthalaldehyde N-acetyl-L-cysteine, combines high sensitivity and specificity of fluorescence detection and is performed with a standard reversed-phase column.

EXPERIMENTAL

Chemicals and reagents

Hydrochloride salts of racemic mexiletine and 1-(2,4,6-trimethylphenoxy)-2-aminopropane (methylmexiletine; internal standard) were kindly supplied by Boehringer Ingelheim Canada (Burlington, Canada). *o*-Phthalaldehyde was purchased from Sigma (St. Louis, MO, USA) and N-acetyl-L-cysteine and HPLC-grade methanol were obtained from Fisher Scientific (Montreal, Canada). All other chemicals and solvents used were obtained from usual commercial sources.

Apparatus

The chromatographic system consisted of a Waters Model M510 pump, a Rheodyne injector fitted with a 100-μl loop and a Shimadzu Model RF-535 fluorometer. The excitation and emission wavelengths of the fluorometer were set at 350 and 445 nm, respectively. Separation was performed on an Apex C_{18} column (ODS, 250 mm × 4.6 mm I.D., 5 μm, Jones Chromatography, Littleton, CO, USA) using methanol–50 m*M* sodium acetate (65:35, v/v) as the mobile phase (pH 7.3) at a flow-rate of 1 ml/min.

Stock solutions

Racemic mexiletine stock solution was prepared by dissolving 60.2 mg of *RS*-mexiletine hydrochloride (equivalent to 50 μg/ml mexiletine base) in 1000 ml of distilled water. A 10-ml aliquot of the stock solution was diluted to 500 ml with distilled water to obtain a final concentration of 1 μg/ml mexiletine base. Solutions of the internal standard were prepared similarly by dissolving 40 mg of methylmexiletine hydrochloride in 1000 ml of distilled water (4 μg/ml) and diluting this solution 1:10 to obtain a final concentration of 0.4 μg/ml.

Barium hydroxide (0.15 *M*) and zinc sulphate (0.17 *M*) solutions were prepared as described previously [6]. The chiral reagent, *o*-phthalaldehyde N-acetyl-L-cysteine, was prepared daily by dissolving 40 mg of *o*-phthalaldehyde and 50 mg of N-acetyl-L-cysteine in 5 ml of methanol [10].

Calibration curves

Aqueous solutions of *RS*-mexiletine and of methylmexiletine (100 μl) were added to 1.0 ml of blank plasma. Because of the wide range of mexiletine plasma concentrations to be studied, two standard curves were constructed. For the first curve, *RS*-mexiletine concentrations were varied from 5 to 50 ng/ml and the concentration of methylmexiletine was fixed at 40 ng/ml. For the second curve, mexiletine concentrations were varied from 50 to 1000 ng/ml and that of methylmexiletine was 400 ng/ml.

Extraction and derivatization procedure

Plasma proteins from 1-ml samples were precipitated with 1 ml of barium hydroxide and 1 ml of zinc sulphate solutions. Following vortex-mixing, the pH was rendered alkaline (>12) by the addition of 0.5 ml of 2 *M* sodium hydroxide [6]. The resulting mixture was extracted twice with 5 ml of diethyl ether, and the combined ethereal extracts were evaporated to dryness in a water-bath at 45°C. The residue was dissolved in 20 μl of 0.03 *M* hydrochloric acid, and 60 μl of 0.1 *M* sodium borate and 60–80 μl of *o*-phthalaldehyde N-acetyl-L-cysteine reagent were added [10]. The resulting mixture was kept at 4°C before analysis.

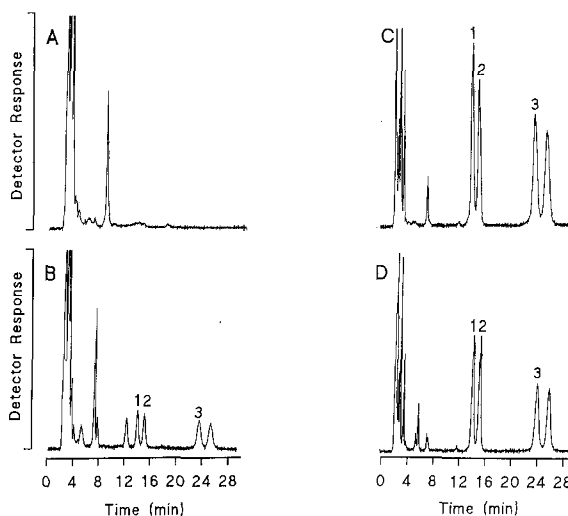

Fig. 2. Representative chromatograms of (A) blank plasma, (B) blank plasma spiked with 20 ng of *RS*-mexiletine and 40 ng of methylmexiletine, (C) plasma spiked with 300 ng of *RS*-mexiletine and 400 ng of methylmexiletine and (D) plasma obtained after oral administration of 200 mg of *RS*-mexiletine hydrochloride to a healthy volunteer. Peaks: 1 = *S*-(+)-mexiletine; 2 = *R*-(−)-mexiletine; 3 = internal standard.

RESULTS AND DISCUSSION

Baseline resolution of mexiletine enantiomers was achieved on a C_{18} reversed-phase column after derivatization with the chiral reagent *o*-phthalaldehyde N-acetyl-L-cysteine. Fig. 2A represents a chromatogram of a treated blank plasma sample. Fig. 2B and C show typical chromatograms obtained after derivatization with *o*-phthalaldehyde N-acetyl-L-cysteine reagent of ethereal extracts of blank plasma samples spiked with 20 and 300 ng of *RS*-mexiletine and 40 and 400 ng of methylmexiletine, respectively. A chromatogram obtained after analysis of a treated plasma sample from a subject who received a single oral dose of *RS*-mexiletine hydrochloride (200 mg) is shown in Fig. 2D. The retention times of the *o*-phthalaldehyde N-acetyl-L-cysteine derivatives of *S*-(+)- and *R*-(−)-mexiletine were 14 and 15 min, whereas those of methylmexiletine were 23 and 24 min, respectively. The first diastereoisomeric peak of *RS*-methylmexiletine (retention time of 23 min) was used as the internal standard throughout the analysis. No interfering peaks from endogenous products or from mexiletine's major metabolites, *p*-hydroxymexiletine and hydroxymethylmexiletine, were observed.

o-Phthalaldehyde has been widely used for the fluorometric analysis of amino acids and biologically active amines [10]. Under alkaline conditions, *o*-phthalaldehyde can react with optically active thiol compounds such as N-acetyl-L-cysteine and primary amines (Fig. 1) to yield fluorescent active diastereomeric isoindole derivatives of the corresponding chiral amines [10].

Therefore, this reagent has been used as a chiral reagent for resolution of amino acid enantiomers [10]. With minor modifications, we were able to apply a similar derivatization and chromatographic procedure to the resolution of mexiletine enantiomers. It is conceivable that this assay could be applicable to the resolution and

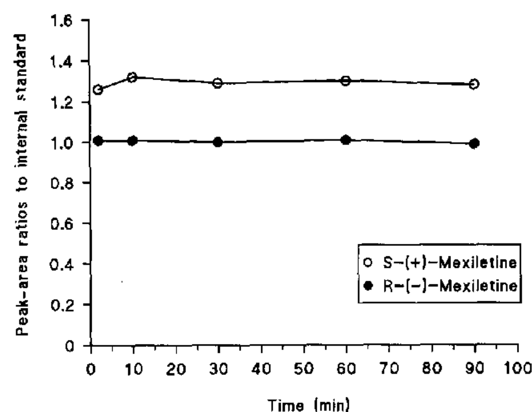

Fig. 3. Assessment of derivatization time of *RS*-mexiletine with *o*-phthalaldehyde N-acetyl-L-cysteine reagent over a period of 2–90 min.

sensitive detection of several other primary alkylamine enantiomers.

The derivatization time of *RS*-mexiletine with *o*-phthalaldehyde N-acetyl-L-cysteine reagent over a period of 2–90 min showed that derivatization was completed within 2 min or less (Fig. 3). Repeated analysis of the same extract also suggested that derivatives were stable for at least nine days at 4°C, and samples can be processed without degradation or racemization on a large scale with the use of an autoinjector.

Linear calibration plots were obtained over the ranges 2.5–25 and 25–500 ng/ml of each enantiomer. Typical regression lines were $y = 0.0 + 0.0868x$ ($r > 0.999$) and $y = 0.0055 + 0.0079x$ ($r > 0.999$) for the *S*-(+)-enantiomer and $y = 0.0379 + 0.0839x$ ($r > 0.99$) and $y = 0.0066 + 0.0074x$ ($r > 0.99$) for the *R*-(−)-enantiomer. Intra- and inter-day assay variations for each enantiomer determined at 10, 25 and 200 ng of each enantiomer per 1 ml of plasma are shown in Table I. Coefficients of variation (C.V.) for inter- and intra-day analysis were below 10%, demonstrating good precision of the assay.

The limit of detection (detector response greater than three times the noise) of the assay was 1.5 ng/ml for each enantiomer using 1 ml of plasma. This limit is lower than plasma concentrations usually measured 48 h after administration of a single oral dose of the drug.

The method was used to determine concentrations of mexiletine enantiomers in the plasma of a subject who received a single oral dose of racemic mexiletine hydrochloride (200 mg). Fig. 4 shows the concentration–time profile for *S*-(+)-and *R*-(−)-mexiletine obtained. The elimination half-lives for the *S*-(+)- and the *R*-(−)-enantiomers, as calculated from the slope of the terminal linear portion of the curve, were 6.9 and 7.5 h, respectively.

In conclusion, a stereoselective HPLC proce-

TABLE I

INTRA- AND INTER-DAY VARIATIONS IN THE SIMULTANEOUS ANALYSIS OF *S*-(+)- AND *R*-(−)-MEXILETINE IN PLASMA

Values in parentheses are coefficients of variation (%).

Amount of *S*-(+) or *R*-(−)-mexiletine added (ng)	Amount found (ng)	
	S-(+)-Mexiletine	*R*-(−)-Mexiletine
Intra-day variation (n = 6)		
10	9.4 (2.8)	10.0 (6.8)
25	20.5 (4.1)	21.7 (5.7)
200	204 (4.1)	205 (3.7)
Inter-day variation (n = 6)		
10	10.2 (2.9)	10.2 (2.7)
25	23.3 (4.4)	23.0 (8.3)
200	211 (6.9)	203 (9.3)

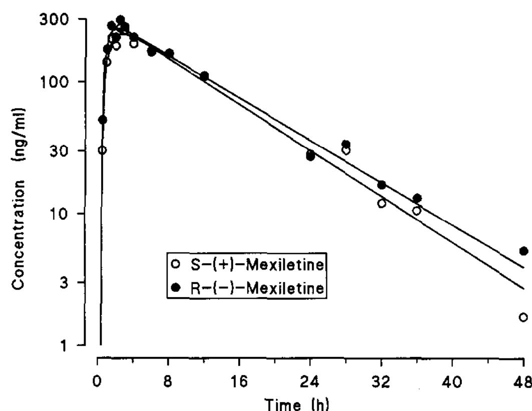

Fig. 4. Plasma concentration–time profile of *S*-(+)-mexiletine and *R*-(−)-mexiletine obtained after oral administration of 200 mg of *RS*-mexiletine hydrochloride (Mexitil) to a healthy young volunteer.

dure for the similtaneous analysis of mexiletine enantiomers in plasma is reported. We selected *o*-phthalaldehyde N-acetyl-L-cysteine as the chiral reagent for the stereoselective analysis of mexiletine because of the high sensitivity and high selectivity of fluorescence detection and because resolution of the diastereoisomers can be achieved on non-chiral columns. The method is sensitive and simple, and can be used to quantify mexiletine enantiomers in plasma for up to 48 h after administration of a single oral dose of the racemic drug.

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