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Note

High-performance liquid chromatographic assay for mexiletine enantiomers in human plasma

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Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane (I, Fig. 1), is an orally effective antiarrhythmic agent possessing the same electrophysiological properties as lidocaine [1, 2]. It has been shown that an important correlation exists between the therapeutic and toxic effects of this agent and its serum concentrations; therapeutic serum concentrations usually fall in the range 0.75—2.0 μg/ml [3, 4]. Mexiletine possesses a chiral centre and is employed clinically as the racemic mixture. To date, neither the pharmacokinetic nor the pharmacological properties of the separate enantiomers have been described. This new antiarrhythmic drug is eliminated mainly by hepatic metabolism, the main metabolites being mexiletine N-glucuronide conjugate, hydroxymethylmexiletine and *p*-hydroxymexiletine [5, 6]. The conjugation of mexiletine with glucuronic acid has been found to be stereo-selective with *R*(—)-mexiletine

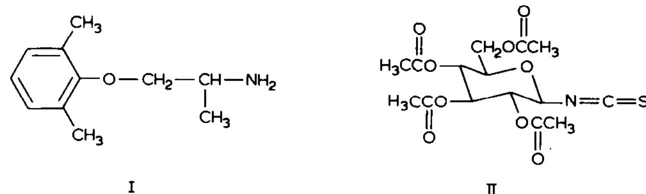

Fig. 1. Chemical structures of mexiletine (I) and GITC (II).

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being more extensively glucuronated than the corresponding $S(+)$-enantiomer [6].

Various methods for the separation of enantiomeric mixtures have been reported [7]. These involve the use of chiral eluents [8], of chiral stationary phases [9, 10] or derivatization with chiral reagents [11–13]. A simple method for the simultaneous analysis of mexiletine enantiomers in serum is described herein. The method involves derivatization of each isomer with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC; II, Fig. 1) followed by high-performance liquid chromatographic (HPLC) separation using ultraviolet (UV) detection.

MATERIALS AND METHODS

Chemicals and reagents

Racemic mexiletine hydrochloride as well as corresponding $R(-)$- and $S(+)$-enantiomers were kindly donated by Boehringer Ingelheim Canada (Ontario, Canada); they were used as received. Silver thiocyanate and α-acetobromoglucose were purchased from Ventron (Danvers, MA, U.S.A.) and from Sigma (St. Louis, MO, U.S.A.), respectively. Anhydrous hydrazine was obtained from Matheson Coleman (Lyndhurst, NJ, U.S.A.); phenylbutylamine from Aldrich (Milwaukee, WI, U.S.A.); HPLC-grade methanol and dimethylformamide from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other chemicals and reagents used were obtained from the usual commercial sources. The chiral reagent GITC was prepared from α-acetobromoglucose and silver thiocyanate as described by Nimura et al. [14]. The yield was 70% and the melting point 109–110°C (reported 113–115°C) [14].

Instrumentation

A Waters chromatographic system consisting of a Model M6000 pump, a U6K injector fitted with a 100-μl loop and a variable-wavelength detector (Model 450) set at 250 nm was used. Separation was performed on a Waters C_{18} μBondapak column (30 cm × 4 mm I.D., 10 μm particle size) using a mobile phase consisting of methanol–10 m*M* phosphate buffer, pH 5.5 (65:35) at a flow-rate of 2 ml/min.

Extraction and derivatization procedure

A 2.0-ml serum or plasma sample was extracted twice with diethyl ether at pH 12–13 after addition of 0.5 ml of an aqueous solution of phenylbutylamine (1.25 μg/ml) as internal reference standard. The combined ethereal extracts were evaporated to dryness in a water bath at 45°C; the residue was dissolved in 50 μl of a freshly prepared solution of GITC in dimethylformamide (0.2%, w/v) on a vortex mixer and the resulting mixture was allowed to stand at room temperature for 10 min. A 10-μl aliquot of a fresh solution of hydrazine in dimethylformamide (0.5%, v/v) was then added, the mixture was again allowed to stand for another 10 min and then injected on to the chromatograph.

Calibration curves

Aqueous solutions of $R(-)$- and $S(+)$-mexiletine hydrochloride and of

phenylbutylamine hydrochloride (0.5 ml) were added to 2.0 ml of blank plasma. The range of concentrations of each enantiomer was 51.9—389.5 ng of free base per ml of plasma and the concentration of phenylbutylamine · HCl was 1.25 μg/ml. All samples were extracted, derivatized and analyzed as above. Calibration curves based on the peak height ratios of each enantiomer to the internal standard were constructed using six different concentrations of each enantiomer analyzed in duplicate. The data were subject to linear regression analysis to give the appropriate calibration factor.

RESULTS AND DISCUSSION

The supplied enantiomers were judged to be pure when the GITC derivative of 2 μg of each enantiomer gave only a single peak using the HPLC conditions developed herein.

Variation of the derivatization time of (*RS*)-mexiletine with GITC in dimethylformamide over the period of 5—240 min showed that derivatization was complete within 10 min. This reaction time is similar to that reported for formation of the GITC derivatives of epinephrine and norepinephrine [14]. Hydrazine is added to the reaction mixture in order to destroy excess reagent [14]; the decomposition products elute at 5.8 min and do not interfere with the assay. GITC has been used for the HPLC resolution of enantiomers of various amino acids [15, 16], of epinephrine and of norepinephrine [14] and of several β-adrenergic antagonists [17]. The isothiocyanate group of GITC reacts rapidly with primary and secondary amino groups to give the corresponding thiourea derivatives [16]. The degree of separation of enantiomers derivatized with a chiral agent depends upon various criteria [13]; amongst these the conformational rigidity around the chiral centres [18], the proximity for a polar or polarizable group to a chiral centre and the distance between the chiral centres of the reagent and the reactant [19, 20] appear to be the most important. The bulky acetylglucosyl moieties of the GITC molecule confer rigidity to the derivative formed and the three-atom distance between the two chiral centres fulfills the distance criteria. HPLC separation of mexiletine can also be affected after derivatization with the chiral agent, N-trifluoroacetyl-*S*-prolyl chloride. However, the derivatives formed do not have the sensitivity required for the UV detection of mexiletine enantiomer serum levels expected after single-dose administration of racemic drug. Thiourea derivatives, on the other hand, are very sensitive to UV detection [15]. This is particularly important in the case of mexiletine which has a low extinction coefficient ($\epsilon = 255$). The minimum measurable amount of underivatized mexiletine using our type of UV detector was 5 μg whilst that of the GITC derivative was 50 ng which is the amount of each enantiomer per ml of serum expected 24 h after administration of a 200-mg dose of racemic mexiletine hydrochloride.

Fig. 2A shows a typical chromatogram obtained after derivatization of an ethereal extract of blank plasma spiked with 311.6 ng of each mexiletine enantiomer and 625 ng of the internal standard, phenylbutylamine, with GITC. Fig. 2B is a chromatogram of a similarly treated blank serum sample. No interference from endogenous products or from mexiletine's major metabolites, *p*-

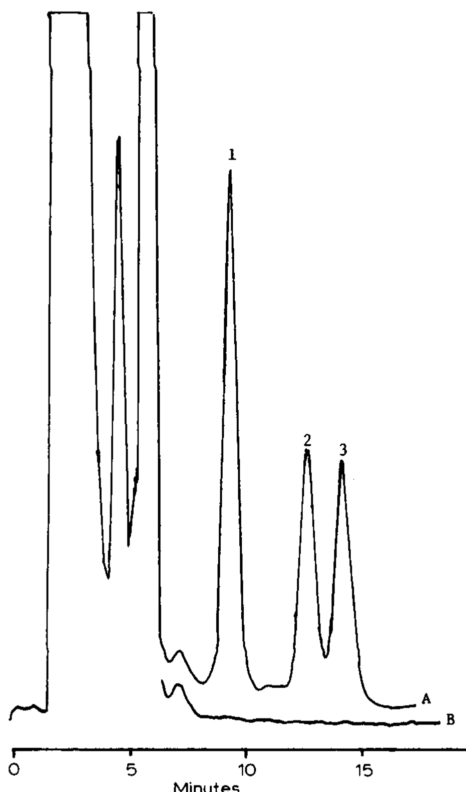

Fig. 2. Representative HPLC chromatograms of (A) serum sample spiked with 311.6 ng each of *R*(—)- and *S*(+)-mexiletine and 625 ng of the internal standard; and (B) blank serum. Peaks: 1 = internal standard, phenylbutylamine; 2 = *R*(—)-mexiletine; 3 = *S*(+)-mexiletine.

hydroxymexiletine and hydroxymethylmexiletine, was observed. The retention times of the GITC derivatives of *R*(—)- and *S*(+)-mexiletine were 12.7 and 14.2 min, respectively, whereas that of phenylbutylamine, the internal reference standard, was 9.4 min. Identification of each enantiomer was made by comparing the retention time with that obtained after separate chromatography of each GITC-enantiomer derivative. Better separation of the enantiomeric pair could be obtained if the methanol concentration in the mobile phase was reduced; for example, when the methanol concentration was 60%, the retention times were increased to 18.2 and 20.7 min, respectively and the peaks were completely separated. However, better sensitivity (narrower and consequently sharper peaks) were preferred to better separation. Methanol was chosen as the organic component of the mobile phase after investigation with the solvents tetrahydrofuran, acetonitrile and ethanol showed that it provided the best separation conditions.

Linear calibration plots were obtained over the range 51.9—389.5 ng/ml of each enantiomer. Typical regression lines were $y = 0.0015x - 0.005$ ($r > 0.99$) for the *R*(—)-enantiomer and $y = 0.0014x - 0.012$ ($r > 0.99$) for the *S*(+)-enantiomer. Intra- and inter-day assay variations for each enantiomer determined at 207, 519 and 779 ng per 2 ml of plasma are shown in Table I. The percentage coefficient of variation (C.V.) for inter- and intra-day analysis was

TABLE I

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

	Amount of S(+)- or R(—)-mexiletine (ng) added to 2 ml of plasma		
	207.0	519.0	779.0
Intra-day variation (n = 4)			
S(+)-Mexiletine found ± S.D.	195.9 ± 8.8	520.9 ± 5.7	778.7 ± 15.7
C.V. (%)	4.5	1.1	2.0
R(—)-Mexiletine found ± S.D.	197.8 ± 6.4	525.8 ± 12.6	769.0 ± 17.5
C.V. (%)	3.2	2.4	2.3
Inter-day variation (n = 5)			
S(+)-Mexiletine found ± S.D.	198.7 ± 20.5	509.6 ± 25.1	748.8 ± 27.1
C.V. (%)	10.3	4.5	3.6
R(—)-Mexiletine found ± S.D.	198.8 ± 20.9	524.4 ± 24.1	749.8 ± 30.5
C.V. (%)	10.5	4.6	4.1

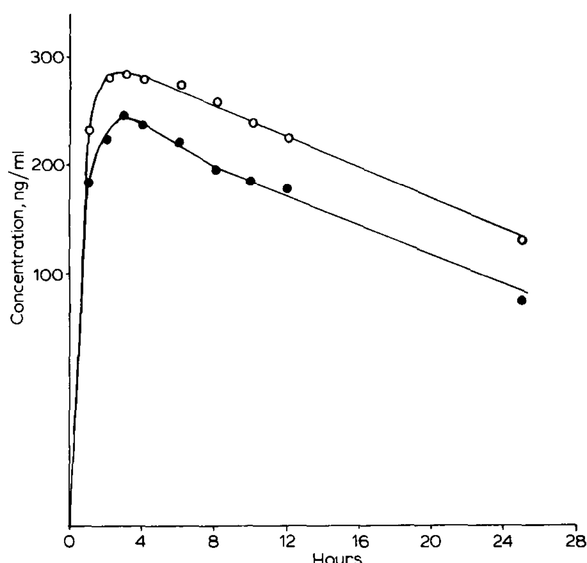

Fig. 3. Plasma concentration—time profile of S(+)-mexiletine (○) and R(—)-mexiletine (•) obtained after oral administration of 200 mg of RS-mexiletine hydrochloride to a healthy young volunteer.

below 5% except for the inter-day variations in the lowest concentration studied which were 10.3 and 10.5% for S(+)- and R(—)-mexiletine, respectively. Extractibility of (RS)-mexiletine with 2 vols. of diethyl ether under alkaline conditions was higher than 90% which agrees with reported values [21, 22].

The method described herein was applied to the determination of the separate enantiomers in the plasma of a healthy volunteer to whom 200 mg of racemic mexiletine hydrochloride had been administered. Fig. 3 shows the concentration—time profile for S(+)- and R(—)-mexiletine obtained in this

subject. The elimination half-lives for the $S(+)$- and the $R(-)$-enantiomer, as calculated from the slope of the terminal linear portion of the ln serum concentration—time curve, were 13.79 and 12.82 h, respectively.

In conclusion, a stereo-specific method for the simultaneous analysis of mexiletine enantiomers in serum is reported. The method is specific and simple and it can be used to study the serum enantiomeric composition of mexiletine after administration of the racemic drug.

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