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Note

High-performance liquid chromatography of mexiletine in plasma

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Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane, is an anti-arrhythmic drug, useful for therapeutic treatment of ventricular arrhythmias. Therapeutic levels in plasma are in the range 0.5–2.0 μg/ml [1–3]. Although the effectiveness of this drug as an antiarrhythmic agent has been well established, its clinical use has been complicated by the narrow therapeutic range and the side-effects that appear when plasma concentrations exceed 2 μg/ml [4,5].

Various methods have been published for the determination of mexiletine in plasma: gas chromatography [6–11] and high-performance liquid chromatography (HPLC) without derivatization [12–14]. The purpose of this study was the determination of mexiletine in plasma by HPLC, following the formation of a pre-column derivative with dansyl chloride [15–20]. The method was used to determine the plasma levels of mexiletine in patients with cardiomyopathies with severe ventricular arrhythmias, and to optimize the oral dose.

EXPERIMENTAL

Chemicals and reagents

Mexiletine hydrochloride and 4-methylmexiletine hydrochloride [1-(2,4,6-trimethylphenoxy)-2-aminopropane hydrochloride] used as internal standard were supplied by Boehringer, (Ingelheim, F.R.G.). A 1 mg/ml solution of dansyl chloride (Pierce, U.S.A.) was prepared in acetone and stored in the dark under refrigeration. A buffer (pH 6.0) was prepared by dissolving 5 g of sodium acetate in 13 ml of water and adjusting the pH to 6.0 with 85% H_3PO_4. Solutions of 2 *M* and 0.5 *M* NaOH, 0.05 *M* H_2SO_4 and 1 *M* $NaHCO_3$ were prepared. Ethyl acetate, hexane, acetone, acetonitrile, methanol and carbon tetrachloride were reagent grade.

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Standard solutions

Standard solutions (20 μg/ml) of mexiletine and 4-methylmexiletine were made by dissolving the mexiletine and 4-methylmexiletine in 0.05 *M* H_2SO_4.

Equipment and conditions

Glass screw-capped vials (20 and 7 ml) were obtained from Kimble (U.S.A.), and tapered reaction vials (2 and 0.1 ml) and a thermostatically controlled heating unit with aluminium block from Pierce. The HPLC system consisted of a Waters Model U6K injector system, a Model 660 equipment for gradient elution with a Model 6000 A solvent-delivery system and M-45 pumps, a Model M 730 integrator and a Model 420 fluorometric detector with excitation and emission filters of 338 and 455 nm, respectively. Analyses were performed on a Waters μBondapak C_{18} column (30 cm × 3.9 mm I.D., 10 μm particle size).

The mobile phase was methanol–acetonitrile–water (50:20:30), the flow-rate 1.5 ml/min, the chart speed 0.5 cm/min, and the injection volume 20 μl.

General procedure

A 1-ml volume of plasma from a patient was transferred by pipette to a glass screw-capped vial (7 ml), together with 100 μl of the internal standard (2 μg), 200 μl of 2 *M* NaOH and 3 ml of ethyl acetate. The samples were extracted by mixing. The aqueous phase was discarded, and 1.5 ml of ethyl acetate were transferred to a tapered reaction vial (2 ml), containing 50 μl of 0.05 *M* H_2SO_4. After mixing and centrifugation, the ethyl acetate phase was discarded, and 1 ml of hexane was added to the acidic extract. After mixing, the vial was immersed in a dry ice–acetone bath to freeze the aqueous phase, and the hexane phase was decanted and discarded. The 50 μl of 1 *M* $NaHCO_3$ and 200 μl of dansyl chloride solution were added to the aqueous acid solution, which was heated for 30 min at 40°C. Next 100 μl of 0.5 *M* NaOH were added to the reaction vial and stirred for 15 min (heating at 40°C). Buffer (pH 6) was added (1 ml) together with 200 μl of carbon tetrachloride, to extract the derivatives of mexiletine and 4-methylmexiletine, and 20 μl of the carbon tetrachloride phase were injected into the chromatograph.

Plasma level study

Mexiletine tablets were administered to 29 patients with ventricular arrythmias, between 24 and 69 years old. The therapeutic plan was: placebo and mexiletine alone with oral dose, 400–1000 mg per 24 h or in combination with quinidine oral dose, 550–900 mg per 24 h; amiodarone oral dose, 200–400 mg per 24 h; atenolol oral dose, 50 mg per 24 h; diphenylhydantoin oral dose, 300 mg per 24 h. The plasma concentrations of mexiletine were determined in each patient after three more days with the therapeutic plan assigned: mexiletine alone or in combination with quinidine, diphenylhydantoin or atenolol, and more than seven days after the treatment with amiodarone. The blood was taken from the patient 3 or 4 h after ingestion of the drug.

RESULTS AND DISCUSSION

Preliminary work showed that to obtain a good sensitivity it was necessary to make fluorescent derivatives of mexiletine and 4-methylmexiletine with dansyl

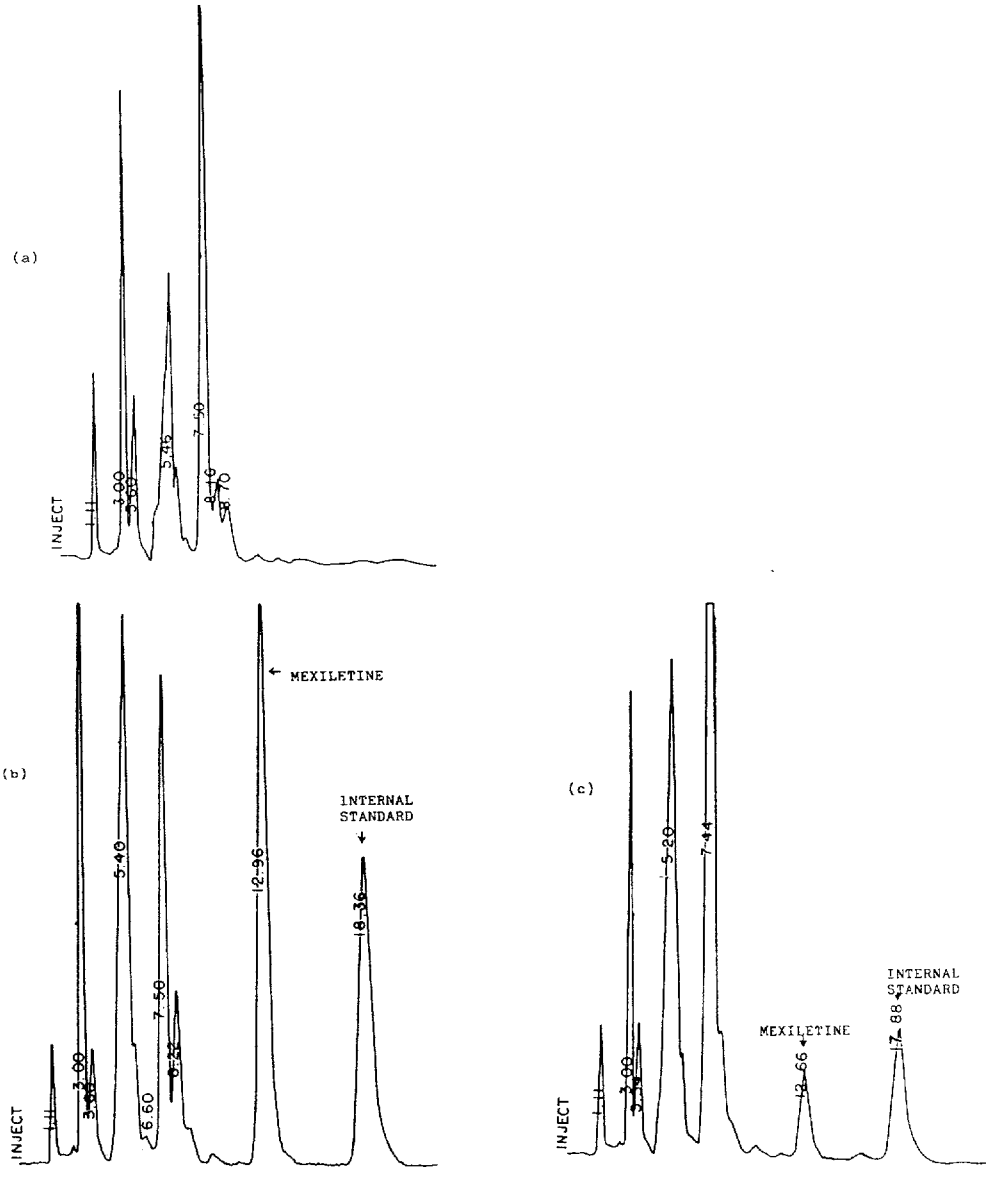

Fig. 1. Chromatograms of (a) drug-free plasma containing dansyl chloride, (b) plasma spiked with mexiletine (2 μg/ml) and internal standard (2 μg/ml) and (c) plasma sample of a patient receiving mexiletine (mexiletine plasma concentration 0.80 μg/ml).

chloride. The excitation and emission maxima of the peaks of the derivatives were 335 and 450 nm. The conditions for the HPLC separation of the derivatives were studied, and a good resolution was achieved with a reversed-phase system with a μBondapak C_{18} column and methanol–acetonitrile–water (50:20:30) as mobile phase. Typical chromatograms from a drug-free human plasma with dansyl chloride, a human plasma spiked with known amounts of mexiletine (2 μg/ml) and internal standard (2 μg/ml), and a plasma of a patient receiving mexiletine are shown in Fig. 1.

Fig. 2 shows a linear calibration curve plotted from the peak-area ratio of mexiletine and 4-methylmexiletine in the final solutions after completing the general procedure, in which there is a concentration process. The final analytical range of 0.5–8.0 μg/ml corresponds to an original plasma sample range of 0.2–3.2 μg/ml (slope, 0.1344; *y*-intercept, 0.3111; *r*, 0.9934).

The absolute mean recovery of mexiletine from plasma was 75.1 ± 1.8% ($n=7$) at 0.5–2.0 μg/ml; the absolute mean recovery of 4-methylmexiletine was 73.4 ± 2.5% ($n=7$) at 2.0 μg/ml. The effect of plasma volume on the peak-height ratio of mexiletine to the internal standard was examined, and the results indicated that the accuracy and precision were independent of the volume of plasma added between 0.05 and 2 ml of plasma (all recoveries were mean ± S.D.). The conditions for the reaction of mexiletine with dansyl chloride for pH, reagent conditions, solvent composition, temperature and time were taken from published data on the reaction of dansyl chloride with primary aliphatic amines.

The limit of quantitation of this assay was 0.05 μg/ml (measured at the maximum working sensitivity).

The method has the sensitivity, specificity, precision and accuracy to measure plasma levels of mexiletine in the range 0.2–3.2 μg/ml.

The within-run precision was established in pooled drug-free plasma, which contained 4-methylmexiletine (2 μg/ml) and mexiletine at concentrations of 0.2,

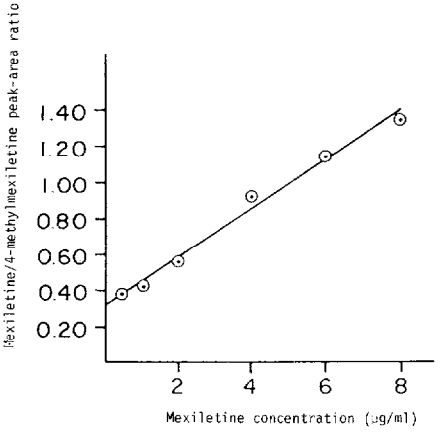

Fig. 2. Calibration curve for the determination of mexiletine in plasma.

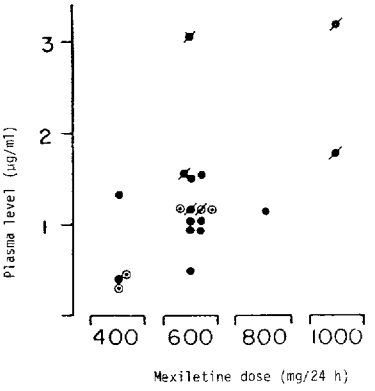

Fig. 3. Distribution of the plasma levels of mexiletine in twenty patients receiving an oral dose of 400–1000 mg per 24 h. (●) Treatment effective; (⊙) treatment not effective: (/) side-effects observed.

0.4, 0.8, 1.6, 2.4 and 3.2 µg/ml. For each concentration the within-run precision of six consecutive runs was determined with a coefficient of variation of 4.0, 4.5, 8.4, 2.6, 5.8 and 3.2%.

The method can be applied to the determination of mexiletine in the presence of other antiarrthymic drugs: amiodarone, quinidine, atenolol and diphenylhydantoin.

The method was applied to the determination of 60 plasma samples from 29 patients: 6 from patients treated with a placebo, 35 from patients treated with mexiletine alone (400–1000 mg per 24 h) and 19 from patients treated with mexiletine and another antiarrhythmic drug [21]. Fig 3 shows the distribution range of the mexiletine plasma levels obtained from patients with the same oral dose. Generally, it was shown that with higher plasma levels of mexiletine there was a lower grade of complexity and less frequent ventricular arrythmia, with a higher frequency of side-effects. To obtain adequate plasma levels it was necessary to carry out several determinations on the same patient.

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