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Note

Gas chromatographic analysis of underivatized tocainide

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Tocainide hydrochloride (Fig. 1) is an oral form of an analogue of lidocaine which has recently been marketed for the suppression of symptomatic ventricular arrhythmias. Its mechanism of action and electrophysiologic effects are similar to those of lidocaine and since it can be administered orally, it is useful for the long-term treatment of ventricular arrhythmias [1–4].

Gas chromatography (GC) [4–11] and high-performance liquid chromatography (HPLC) [12–17] have been used to quantitate tocainide in plasma, but many GC and HPLC procedures are time-consuming, require derivatization following solvent extraction, and some are not sensitive enough to be useful in pharmacokinetic studies. The present report describes an improved GC methodology using nitrogen-selective detection (i.e., nitrogen–phosphorus detection, NPD) which can quantitate tocainide in plasma without derivatization or sample clean-up.

EXPERIMENTAL

Apparatus and chromatographic conditions

The analytic instrument was a Hewlett-Packard Model 5840A gas chromatograph equipped with a nitrogen-selective detector, electronic data processor integrator and automatic sampler (Model 7672A). The column was coiled glass, 1.83 m×2 mm I.D., packed with 3% SP-2250, consisting of methyl–phenyl (50:50) substituents, on 80–100 mesh Supelcoport (Packing 1-1767, Supelco, Bellefonte, PA, USA). The carrier gas was ultra-high-purity helium (Matheson Gas Products, Gloucester, MA, USA) at a flow-rate of 30 ml/min. The detector purge was

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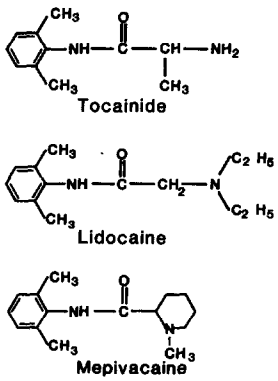

Fig. 1. Chemical structures of tocainide and the closely related antiarrhythmic lidocaine. Also shown is the internal standard, mepivacaine.

ultra-high-purity hydrogen (Matheson) at a flow-rate of 3 ml/min mixed with dry air (Matheson) at a flow-rate of 50 ml/min. Operating temperatures were: injection port, 310°C; column, 230°C; detector, 300°C. Before being connected to the detector, a new column was conditioned at 290°C for 48 h with a carrier flow-rate of 10 ml/min. At the beginning of each work day the column was primed with 2–4 μg of purified soy phosphatides (asolectin) in benzene (1 mg/ml) (Asolectin, Associated Concentrates, Woodside, NY, U.S.A.) [18].

Reagents

The following reagents were used: analytic-reagent-grade toluene, certified isoamyl alcohol and HPLC-grade methanol, all obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.); analytic-reagent-grade sodium hydroxide (Mallinckrodt, St. Louis, MO, U.S.A.); and Baker-analyzed acetone and Baker-analyzed benzene (J.T. Baker, Phillipsburg, NJ, U.S.A.).

Reference standards

Pure standards of tocainide hydrochloride and mepivacaine hydrochloride were kindly provided by Astra Pharmaceutical Products (Worcester, MA, U.S.A.) and Sterling-Winthrop Research Institute (Rensselaer, NY, U.S.A.), respectively (Fig. 1). Standards of tocainide were prepared by dissolving 119 mg of the hydrochloride salt in 100 ml water, to yield the equivalent 100 mg of free base. On each day of assay sequential dilutions were made to 5 μg/ml. Standards of mepivacaine were prepared by dissolving 114.8 mg of hydrochloride salt in 100 ml of methanol to yield the equivalent of 100 mg of free base. Solutions were stored in the dark in glass-stoppered bottles at 4°C. Standard solutions of mepivacaine were stable for at least two months and standard solutions of tocainide were stable for at least one month after preparation.

Analytical method

Extraction tubes were 15-ml round-bottomed glass culture tubes with PTFE-lined screw-top caps. Tubes were rinsed with acetone and air-dried prior to use.

To each tube was added 1 μg (100 μl of the 10 μg/ml) of mepivacaine as the internal standard. Calibration standards for tocainide were prepared by adding varying amounts (0.05–5 μg) to consecutive tubes. Drug-free control plasma (1 ml) was added to each calibration tube, and 1 ml of test sample plasma added to all other tubes containing only internal standard.

To each tube 200 μl of 5 *M* sodium hydroxide and 1 ml toluene–isoamyl alcohol mixture (85:15) were added. The tubes were capped and agitated gently in the upright position on a vortex mixer for 20–30 s. Agitation for longer than 30 s did not improve the extraction yield but increased the likelihood of the formation of an emulsion. The samples were centrifuged at room temperature for 5 min at 400 *g*. The organic layer was transferred to standard 2-ml Wheaton automatic sampling vials (Wheaton Scientific, Millville, NJ, U.S.A.) and capped with aluminum foil. The automatic sampler was programmed to inject 6 μl of each sample.

Pharmacokinetic study

A 32-year-old healthy female volunteer participated. After an overnight fast she received a 600-mg oral dose of tocainide hydrochloride (Tonocard, Merck, Lot K1211), equivalent to 504 mg of tocainide base, with 180 ml of tap water. She remained fasting until 3 h after dosage. Venous blood samples were drawn into heparinized Venoject tubes before drug administration and at the following times after dosage: 15, 30 and 45 min, and 1, 1.5, 2, 3, 4, 8, 12, 24, 34, 48 and 58 h. Blood samples were centrifuged, and the plasma was separated and frozen until the time of assay. Concentrations of tocainide in all samples were determined by the method described above.

RESULTS AND DISCUSSION

Approach to analysis

This assay procedure was developed with the objective of providing a rapid, sensitive, automated method for quantitation of tocainide concentrations in plasma which would be suitable for pharmacokinetic studies. Although solvent evaporation steps are often used to increase analytical sensitivity, losses due to evaporation, degradation or adsorption of drug by glass may occur [14]. No solvent evaporation steps were used in the present method. We utilized a toluene–isoamyl alcohol mixture (85:15) as the organic solvent for initial extraction instead of dichloromethane, since toluene–isoamyl alcohol yields similar recovery, is less likely to form emulsions and is easier to transfer since it constitutes the upper layer when mixed with plasma. As in the case of lidocaine [19], recovery was increased when the biological sample is alkalinized prior to extraction.

At the beginning of each work day the column was primed with 2–4 μg of purified soy phosphatides (asolectin) in benzene (1 mg/ml) [18]. This procedure improves the chromatographic properties of many drugs analyzed by GC [18–29], probably because the lipoidal substance coats the column packing in a non-specific fashion, which results in a reduction of column adsorption of drug, improvement in peak shape and an increase in sensitivity especially at low drug concentrations [18].

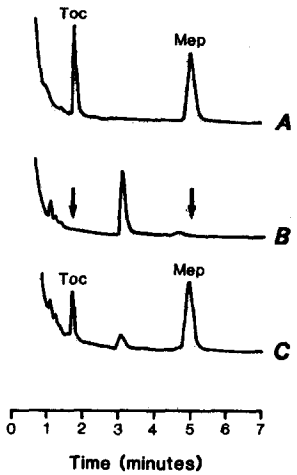

Fig. 2. Chromatograms of plasma extracts. (A) Calibration standard containing 1 μg/ml tocainide and 1 μg/ml mepivacaine. (B) Plasma sample from subject prior to ingestion of tocainide. (C) Plasma sample (to which was also added the internal standard) drawn from a subject 24 h after a single 600-mg dose of tocainide hydrochloride. Peaks: Toc = tocainide; Mep = mepivacaine.

Evaluation of the method

Under the described chromatographic conditions, tocainide and the internal standard gave well resolved chromatographic peaks (Fig. 2). Drug-free blank plasma samples were free of contaminating peaks. Plasma concentrations of tocainide were linearly related from 0.1 to 5.0 μg/ml to the peak-height ratio of tocainide versus the internal standard. The sensitivity limit is approximately 0.05 μg of tocainide per ml of plasma. Table I shows replicability of spiked samples at various concentrations. At each concentration, eight different spiked samples were extracted and assayed.

After extraction, tocainide is stable at room temperature for at least 24 h. Upon evaporation to dryness or exposure to room temperature for more than 24 h, tocainide appears to degrade which results in the appearance of a second chromatographic peak, that increases in peak height or area reciprocally with the decrease in magnitude of the tocainide peak. The extent of degradation increases with time. During initial attempts to extract tocainide from plasma, the extract

TABLE I

REPLICABILITY OF SPIKED SAMPLES AT VARIOUS CONCENTRATIONS

At each concentration, $n = 8$.

Plasma tocainide concentration (μg/ml)	Coefficient of variation (%)
0.05	4.4
0.1	1.7
1.0	5.3
5.0	2.9

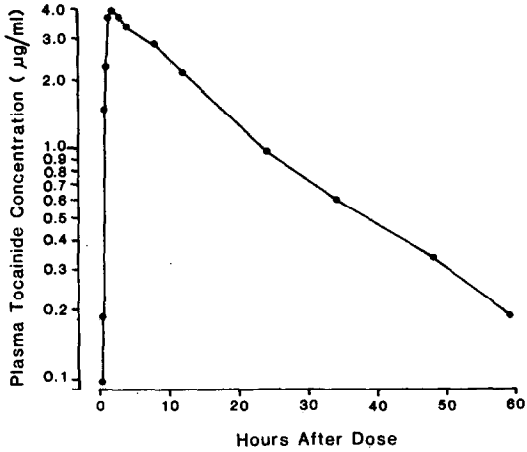

Fig. 3. Plasma concentrations of tocainide in the volunteer subject who participated in the pharmacokinetic study.

was separated and evaporated to dryness under mildly reduced pressure at 40°C. However, degradation occurred whether the sample was evaporated under nitrogen or room air, in glass or plastic tubes (with or without silanization) or in the light or dark. However, mepivacaine is stable at room temperature for at least 48 h.

Pharmacokinetic results

Fig. 3 shows plasma concentrations of tocainide in the volunteer subject. A peak tocainide concentration of 3.95 μg/ml was reached at 2 h after dosage. Thereafter, plasma concentrations declined exponentially with an apparent half-life of 14 h. Assuming complete absorption of the entire dose of tocainide base, apparent volume of distribution and oral clearance were calculated. The values were: volume of distribution, 2.8 l/kg; total clearance, 2.3 ml/min kg. The pharmacokinetic values were similar to those reported by Graffner et al. [30] for healthy subjects.

CONCLUSION

The present report describes a rapid, sensitive, automated method for the quantitation of tocainide in plasma. The straightforward extraction procedure allows one person to prepare a large number of samples in a standard working day. With the automated sampler, 100 or more samples can be chromatographed in a 24-h period with no technical personnel in attendance. The method is sensitive enough for therapeutic monitoring and for most pharmacokinetic studies.

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