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ELECTRON-CAPTURE GAS—LIQUID CHROMATOGRAPHIC DETERMINATION OF TOCAINIDE IN BIOLOGICAL FLUIDS USING FUSED SILICA CAPILLARY COLUMNS

G.K. PILLAI, J.E. AXELSON and K.M. McERLANE*

Faculty of Pharmaceutical Sciences, 2146 East Mall, University of British Columbia, Vancouver, B.C. V6T 1W5 (Canada)

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SUMMARY

Gas—liquid chromatography using capillary columns and electron-capture detection has been employed for the determination of a new antiarrhythmic drug, tocainide, in rat plasma and urine. The drug is extracted from a basified solution along with an internal standard, monoethylglycine xylidide, and subsequently reacted with heptafluorobutyric anhydride. The 50 m × 0.2 mm fused silica capillary column was coated in the laboratory with Carbowax 20M. Linearity of detector response was established in the range of 50—1000 ng of tocainide hydrochloride per 100 μl of plasma or urine. This represented 14—270 pg of the free base at the detector, using a split ratio of 1:25 and an injection volume of 2 μl. The derivatization method and chromatographic assay are well suited for monitoring of plasma or urine samples. The applicability of the method is demonstrated by the analysis of rat plasma collected over a period of 7 h after intravenous administration of 20 mg/kg of tocainide hydrochloride.

INTRODUCTION

Tocainide, 2-amino-2′,6′-propionoxylidide (I), is a primary amine analogue of lidocaine and an effective antiarrhythmic agent with complete oral bioavailability. For many years lidocaine has been the agent of choice for the treatment of ventricular arrhythmias, especially those occurring early after an acute infarction [1]. However, lidocaine cannot be administered orally due to extensive first-pass metabolism in the liver [2]. Tocainide, on the other hand, has been shown in clinical trials to be effective in the treatment of ventricular tachyarrhythmias by both the oral and intravenous routes in man [3—6]. Convenient oral dosage regimens are also possible with tocainide due to its long plasma half-life of approximately 11—14 h [4, 7]. Like many other antiarrhythmic drugs, tocainide exhibits steep plasma concentration—anti-

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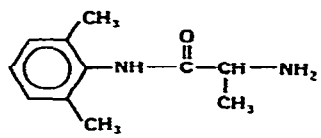
arrhythmic response curves [8] and careful dosage adjustment is necessary for effective treatment.

Analytical methods for the determination of tocainide in biological fluids have included high-performance liquid chromatography (HPLC) [9, 10] and gas–liquid chromatography (GLC) with flame ionization [4] or electron-capture detection [11, 12]. Many of these methods suffer from drawbacks such as tedious sample preparation [9], poor sensitivity [10] or the potential for interference from endogenous substances [11]. Since many of the anti-arrhythmic agents are used in combination with other antiarrhythmics, blood concentration monitoring may become more difficult with conventional packed-column analyses. The limitations of these methods prompted the development of a capillary-column GLC method that combined simple sample preparation, high selectivity, and sufficient sensitivity to measure the levels of tocainide in small volumes of rat plasma.

The analytical methodology described herein employs a fused silica capillary column, wall-coated with Carbowax 20M. Tocainide and the internal standard, monoethylglycine xylidide (II), a metabolite of lidocaine, are extracted and converted to their monoheptafluorobutyrate derivatives for chromatographic assay.



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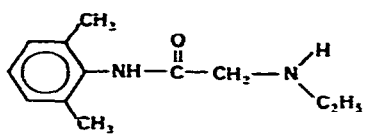

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EXPERIMENTAL

Materials

Tocainide hydrochloride (Astra Pharmaceuticals, Mississauga, Canada), monoethylglycine xylidide (Astra Pharmaceutical Products, Worcester, MA, U.S.A.), α-bromonaphthalene (ICN Pharmaceuticals, Plainview, NY, U.S.A.), Carbowax 20M (Alltech Assoc., Arlington Heights, IL, U.S.A.) and heptafluorobutyric anhydride (Pierce, Rockford, IL, U.S.A.) were used without further purification. Water and *n*-hexane were of HPLC grade (Fisher Scientific, Vancouver, Canada) and dichloromethane and benzene were distilled in glass (Caledon, Georgetown, Canada).

All analyses were carried out on a Model 5830 reporting gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with split/splitless

njection modes and a ^{63}Ni electron-capture detector operated in the pulsed node (150 μsec). Helium carrier gas flow-rate was maintained through the capillary column at 1 ml/min with 25 ml/min passing to the split vent. Argon—nethane (95:5) was used as the make-up gas for the detector at a flow-rate of 30 ml/min. The column inlet pressure was 1.84 bar (27 p.s.i.). The injection port and detector temperatures were 240°C and 350°C, respectively and the oven was operated isothermally at 180°C. Samples were introduced into the 50 m × 0.2 mm capillary column (Applied Science Labs., State College, PA, U.S.A.) using the split injection mode.

Preparation of fused silica column

The fused silica capillary column was coated by the mercury plug dynamic method [13]. A 2% solution of Carbowax 20M in dichloromethane was passed through the column under pressure from a coating reservoir (Alltech Assoc.) until the solution completely filled the column. A 10-cm plug of mercury was then forced into the column from the reservoir and allowed to pass through the column along with the stationary phase solution. After the last drop of mercury had left the column, the nitrogen flow-rate was increased and maintained for 3 h to remove the solvent. The coated column was transferred to the oven of the gas chromatograph and with a helium carrier gas flow-rate of 1 ml/min, the oven was heated at a rate of 1°C/min from 40°C to 220°C and maintained at this temperature for 24 h.

Stock solutions

Tocainide hydrochloride (1 μg/ml) and monoethylglycine xylidide hydrochloride (10 μg/ml) were prepared in HPLC-grade water. α-Bromonaphthalene (1 μg/ml) was prepared in *n*-hexane. An aqueous solution of tocainide hydrochloride, equivalent to 25 mg of the base, was rendered alkaline with 1 *N* sodium hydroxide and extracted with five 15-ml portions of benzene. The combined extracts were brought to a final volume of 100 ml and an aliquot was subsequently diluted with benzene to yield a final solution equivalent to 1 μg/ml of tocainide base.

Determination of optimum derivatization conditions

To 1 μg of tocainide base, evaporated from a 1-ml aliquot of the benzene extract, were added 100 μl of *n*-hexane and 30 μl of heptafluorobutyric anhydride. Six such samples were prepared and were heated for 15, 30, 45, 60, 75 or 90 min at 55°C in an aluminum block (Dri-Bath, Thermolyne, Dubuque, IA, U.S.A.). The excess reagent was removed under a gentle stream of clean, dry nitrogen and the residue was dissolved in 200 μl of *n*-hexane containing 0.2 μg of the internal standard, α-bromonaphthalene. A 1-μl aliquot was used for analysis.

Determination of linearity and response in plasma and urine extracts

To five 100-μl aliquots of plasma or urine obtained from untreated male Wistar rats were added 50, 100, 200, 500 or 1000 ng of tocainide hydrochloride from an aqueous solution (1 μg/ml). An aliquot of the internal standard solution, equivalent to 1 μg of monoethylglycine xylidide hydrochloride

(10 μg/ml), was added to each tube. Triplicate samples of each concentration were prepared. An aliquot of 0.5 ml of 1 *N* sodium hydroxide was added to each tube and the total volume of the aqueous phase was brought to 2.0 ml with water. Dichloromethane (5 ml) was added to each tube and the tubes were shaken for 15 min on a rotary shaker (Roto-Rak, Fisher Scientific). After centrifugation at 740 *g* for 10 min, 4 ml of the organic phase were transferred to a 15-ml PTFE-lined screw-capped centrifuge tube (Canlab, Vancouver, Canada) and the contents of the tube were evaporated to dryness in a water bath at 40°C under a gentle stream of clean, dry nitrogen. To the residue were added 100 μl of *n*-hexane and 30 μl of heptafluorobutyric anhydride. The tubes were tightly capped and heated at 55°C for 45 min in an aluminum block. The excess reagent was evaporated under a stream of nitrogen and the residue was reconstituted in 200 μl of *n*-hexane. A 1—2 μl aliquot was used for analysis.

In-vivo study

A solution of tocainide hydrochloride was prepared in normal saline (10 mg/ml) and a dose of 20 mg/kg was administered intravenously to a male Wistar rat (250 g). A jugular vein cannula, inserted one day prior to drug administration was used for dosing and blood collection. The cannula was flushed with an isotonic heparin solution (20 units) after dosing and after each blood collection. Blood samples (200 μl) were collected in Caraway capillary tubes (Sherwood Medical Industries, St. Louis, MO, U.S.A.) and centrifuged at 1000 *g* for 10 min. The plasma was separated and stored at −20°C until required. Tocainide and the internal standard were extracted from a basified solution of plasma (20—100 μl, depending on the volume of plasma available) and were converted to their monoheptafluorobutyrate derivatives as described above.

RESULTS AND DISCUSSION

The utilization of capillary column gas chromatography for the analysis of samples of biological origin has become more prevalent in the recent literature. The technique has been applied for steroid [14, 15] and fatty acid [16] profiling in normal and pathological conditions, and has recently been used for the analysis of a series of ten structurally related equine estrogens in a pharmaceutical product [17]. Many of the problems associated with glass capillary columns, such as fragility and the need for careful surface modification and deactivation, have been overcome with the introduction of fused silica columns in 1979. Such columns are readily coated with the lower polarity stationary phases and, due to the inert surface, facilitate the analysis of many functional groups such as alcohols, phenols, amines and carboxylic acids without derivatization [18]. The method described in the present paper utilizes a fused silica column which was readily coated in the laboratory using the mercury plug dynamic method [13]. Unlike the usual technique of employing a concentrated solution of the liquid phase, a dilute 2% solution of Carbowax 20M was used to deposit a thin film of liquid phase on the column surface.

To determine the optimum conditions required for derivatization of tocainide (I) a series of samples were heated at 55°C for periods varying from 15 to 90 min. The internal standard, α-bromonaphthalene, was chosen as a compound that would not react with heptafluorobutyric anhydride, thereby providing a basis on which to measure the increase in peak area of the tocainide derivative with time. The area ratio of tocainide to the internal standard was maximal and unchanging from 15 to 60 min but was reduced to 65% and less at 75 and 90 min.

For purposes of biological assays, an N-de-ethylated metabolite of lidocaine, monoethylglycine xylidide (II) exhibited a longer retention time than α-bromonaphthalene, and thus would not be subject to potential interference from early eluting peaks in biological samples. A study revealed that the area ratios of the monoheptaflucrobutyrate derivatives of tocainide and monoethylglycine xylidide were unchanged over a 7-day period when the derivatives were stored at 4° C.

Detector linearity and assay precision were determined from the calibration curves. Accordingly, triplicate samples of each of five concentrations of tocainide hydrochloride were injected into the gas chromatograph. The data given in Table I show the mean slopes for the calibration curves for plasma and urine. The mean coefficients of variation for plasma and urine were 8.3% and 6.2%, respectively. The differences in the two slope values are due to slight variations in extraction efficiency between plasma and urine. The extraction efficiency for the recovery of tocainide from plasma using similar methodology has been previously reported [11]. Since the present procedure utilizes an internal standard of similar structure to that of tocainide, and quantitation is based on identical handling of standards and samples, it was not considered necessary to determine absolute recovery values again.

The application of the method developed with the fused silica capillary column is illustrated in Figs. 1 and 2. The chromatograms depicted in Fig. 1

TABLE I

CALIBRATION CURVE DATA FOR PLASMA AND URINE

Tocainide* (ng)	Plasma area ratio (± S.D.)**	Urine area ratio (± S.D.)**
50	0.0592 ± 0.0083	0.0612 ± 0.0043
100	0.1100 ± 0.0144	0.1373 ± 0.0074
200	0.2149 ± 0.0020	0.2127 ± 0.0110
500	0.6693 ± 0.0324	0.6376 ± 0.0388
1000	1.2781 ± 0.1157	1.3358 ± 0.1013
Mean slope	1.3058	1.3456
Y-Intercept	—0.0168	—0.0209
Correlation coefficient	0.9989	0.9989

*Each sample contains the same amount of internal standard (1 μg).
**Area ratio determined for drug/internal standard. Standard deviations calculated for three aliquots prepared for each weight of tocainide HCl.

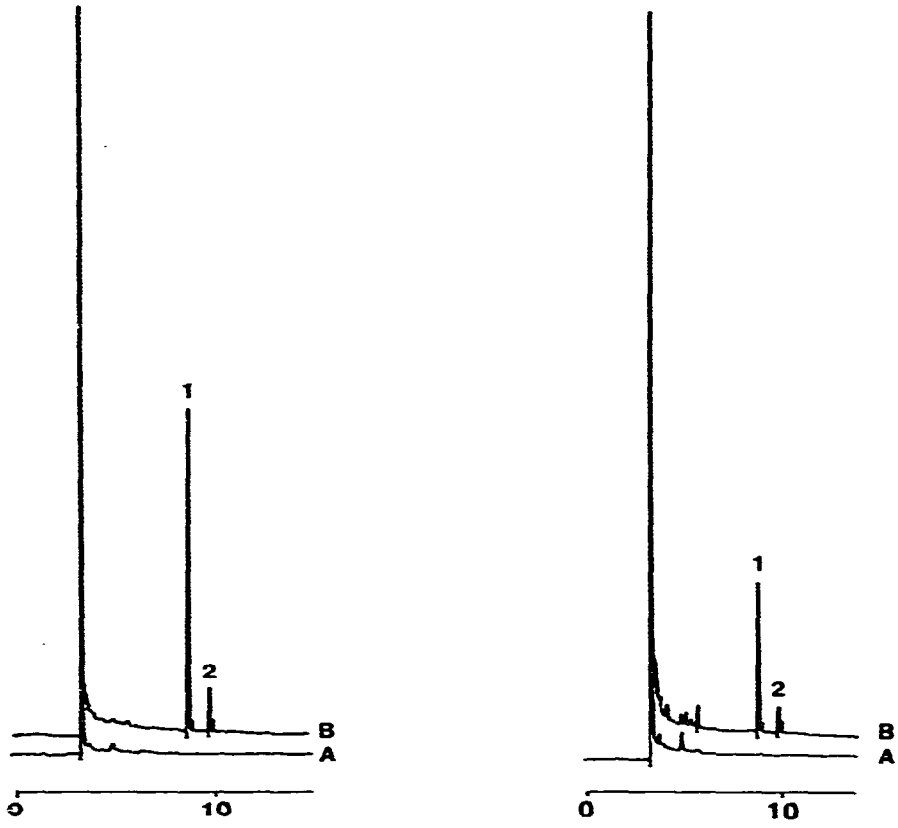

Fig. 1. Chromatograms of (A) rat plasma blank and (B) rat plasma containing 1 μg/ml of tocainide (2) and 10 μg/ml of internal standard, monoethylglycine xylidide (1).

Fig. 2. Chromatograms of (A) rat urine blank and (B) rat urine containing 1 μg/ml of tocainide (2) and 10 μg/ml of internal standard, monoethylglycine xylidide (1).

represent an extraction of blank rat plasma and plasma that contained 1 μg/ml of tocainide hydrochloride. Similarly, Fig. 2 is representative of blank rat urine and urine containing 1 μg/ml of the drug. In both extracts there is no interference from endogenous substances with the peaks due to tocainide or the internal standard. The sensitivity of the method is sufficient to detect as little as 2 pg of tocainide at the detector without appreciable baseline noise. Hence the method allows detection of 1/10 to 1/20 of the amount required for packed-column gas chromatographic methods operated at optimal conditions [11].

The potential for pharmacokinetic investigations in experimental animals is demonstrated by the assay of the drug in rat plasma collected at intervals over a period of 7 h after intravenous administration of a dose of 20 mg/kg to a 250-g male Wistar rat (Fig. 3). The data best fit a two-compartment model, which agrees with previous reports [19], however, further pharmacokinetic parameters were not calculated due to the limited amount of data on a single animal.

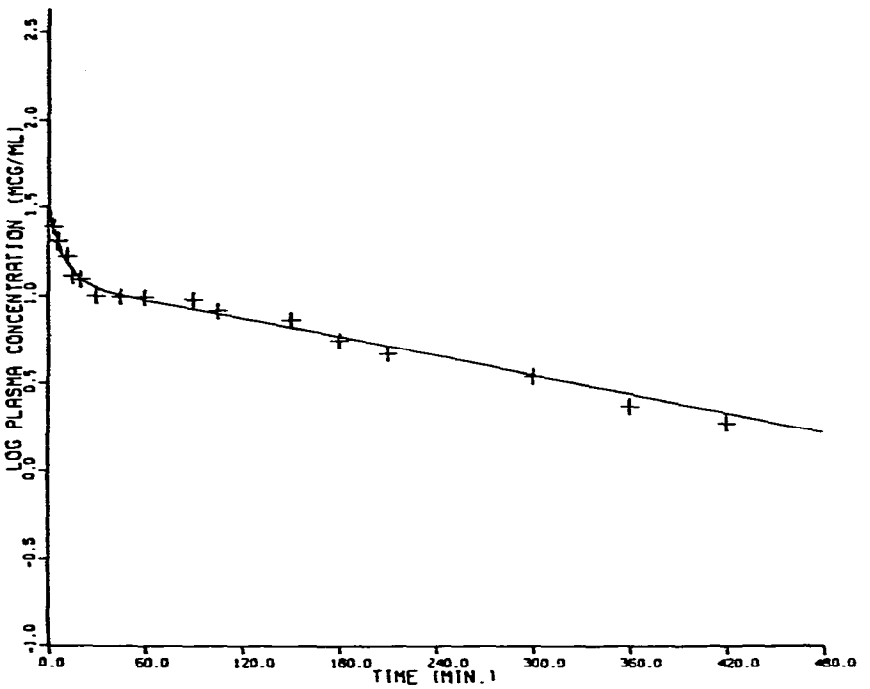

Fig. 3. Plasma tocainide levels in a rat receiving 20 mg/kg of tocainide hydrochloride intravenously.

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