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Stereospecific high-performance liquid chromatographic determination of tocainide

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ABSTRACT

A sensitive high-performance liquid chromatographic assay was developed for the determination of tocainide enantiomers in plasma. Following extraction of tocainide from plasma, the enantiomers were derivatized with *S*-(+)-1-(1-naphthyl)ethylisocyanate. The resulting diastereomers were separated and quantified using normal-phase chromatography with fluorescence detection set at 220/345 nm (excitation/emission). The peaks, resolved with a resolution factor greater than 1.5, were free from interference. Linearity was established over the concentration range 0.25–10.0 mg/l for each enantiomer in plasma ($r^2 > 0.998$). The inter-assay variability was less than 10% at all concentrations examined. The method can be used to determine the pharmacokinetics of tocainide enantiomers in man.

INTRODUCTION

Tocainide [2-amino-N-(2,6-dimethylphenyl)propanamide, TOC, Fig. 1] is an antiarrhythmic agent indicated in the treatment of intractable ventricular arrhythmias [1]. Although structurally analogous to lidocaine, TOC is less suscep-

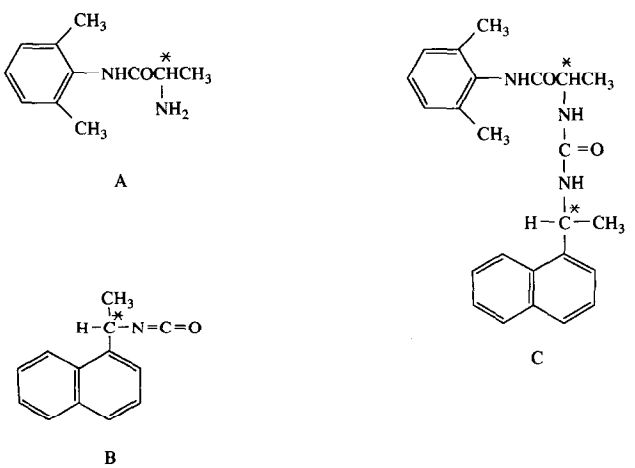

Fig. 1. Structures of (A) tocainide, (B) NEIC, and (C) derivatized tocainide.

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tible to hepatic metabolism [2], has a longer biological half-life, and is effective following oral dosing [3]. Also, unlike lidocaine, TOC possesses a chiral center and is administered to patients as the racemate. Antiarrhythmic activity, however, resides primarily with the *R* (−)-enantiomer [1,4,5].

As TOC has a narrow therapeutic index, therapeutic monitoring of plasma concentrations is often necessary [6]. However, analytical techniques must be able to measure individual enantiomer concentrations, as previous studies have reported stereoselective differences in TOC pharmacokinetics [7–11]. As such, the majority of reported assays which measure total (*R*- plus *S*-enantiomer) concentrations [3,6,12–15] have limited utility. Although either gas chromatographic (GC) [2,16,17] or high-performance liquid chromatographic (HPLC) [8] stereospecific methods have been reported, lengthy derivatization and/or incubation processes are generally required.

In this communication, we describe a convenient method for measuring plasma tocainide enantiomers using normal-phase HPLC and derivatization with *S*-(+)-1-(1-naphthyl)ethylisocyanate (NEIC, Fig. 1).

EXPERIMENTAL

Chemicals

(±)-TOC (Astra, U.K.) and (±)-acebutolol (internal standard, I.S., Rhône-Poulenc Pharma, Dagenham, U.K.) were obtained as gifts. The NEIC was obtained from Aldrich (Milwaukee, WI, U.S.A.). Analytical-grade sodium hydroxide was obtained from BDH (Toronto, Canada). Chloroform, hexane and methanol were obtained from Mallinckrodt (Paris, KY, U.S.A.) and were all analytical grade. Water was HPLC grade and triethylamine was analytical grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.).

Chromatography

All samples were vortex-mixed using a Vortex Genie 2 mixer (Fisher Scientific, Edmonton, Canada) and centrifuged with a Dynac II centrifuge (Becton Dickinson, Parsippany, NJ, U.S.A.). Solvents were evaporated using a Savant Speed Vac concentrator–evaporator (Emerston Instruments, Scarborough, Canada). For enantiomer separation, a 25 cm × 4.6 mm I.D. stainless-steel silica column (Whatman Partisil 5, Clifton, NJ, U.S.A.) was used. The HPLC apparatus consisted of a Model 590 pump, a Model 712 Wisp autosampler and a Model 745B integrator (Waters, Mississauga, Canada). Fluorescence detection (Applied Biosystems Model 980, Technical Marketing Assoc. Edmonton, Canada) was set at 220 and 345 nm for excitation and emission, respectively. The mobile phase was hexane–chloroform–methanol (60:38:2, v/v) pumped at a flow-rate of 2.0 ml/min. Preparation and analysis of samples was conducted at ambient temperature.

Confirmation of identity of the diastereomers was by high-resolution mass spectral analysis (AEI, MS9, Manchester, U.K.). The sample was introduced via

direct insertion utilizing fast atom bombardment (FAB). The medium was glycerol; positive-ion mode.

Derivatization yield was established using a PU8700 series UV–VIS spectrophotometer (Philips, U.K.) with the wavelength set at 192.3 nm.

Standard solutions

A 100 mg/l (TOC base) stock solution of racemic TOC hydrochloride was prepared in HPLC-grade water (solution 1). Another stock solution (used to determine extraction and derivatization yields) of TOC was prepared in 0.2% triethylamine in chloroform (v/v) to a concentration of 100 mg/l base (solution 2). For the I.S. solution, a 10 mg/l stock solution of (±)-acebutolol was prepared in HPLC-grade water (solution 3) as was another stock solution of I.S. containing 10 mg/l acebutolol in 0.2% (v/v) triethylamine in chloroform (solution 4). A solution of 0.05% (v/v) NEIC was prepared in chloroform.

Sample preparation

Drug-free human plasma samples were spiked with TOC (solution 1) to give final concentrations of 0, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/l of each enantiomer ($n = 9$ replicates). To 0.5 ml of plasma containing TOC was added 250 ng of I.S. (solution 3) and 0.20 ml of 1 *M* sodium hydroxide. After addition of 5 ml of chloroform, the mixture was vortex-mixed (30 s) and then centrifuged at 1800 *g* for 5 min. The aqueous layer was discarded and the organic layer was evaporated to dryness using the Savant Speed Vac concentrator–evaporator.

The residues remaining after evaporation were derivatized with the solution of NEIC (0.2 ml). The tubes were then vortex-mixed for 30 s and again evaporated using the Savant Speed Vac concentrator–evaporator. The residues were reconstituted with 0.2 ml of chloroform and aliquots ranging from 0.05 to 0.175 ml were injected into the HPLC system.

Extraction yield

Chloroform solutions of TOC (solution 2) were evaporated to dryness and then reconstituted with 0.5 ml plasma to give concentrations of 0.25, 2.5, or 10 mg/l (three sets of each). After addition of 0.2 ml of 1 *M* sodium hydroxide, chloroform (5.0 ml) was added and the tubes were vortex-mixed for 30 s and centrifuged (1800 *g*, 5 min). The aqueous layers were aspirated and discarded and exactly 2.5 ml of the remaining organic phase was placed in clean tubes. The I.S. (250 ng; solution 4) was added to each tube, evaporated, and derivatized. The peak areas of TOC extracted *versus* unextracted equivalent concentrations of drug were compared under identical chromatographic conditions.

Derivatization yield

Calibration curves ($n = 3$) of underivatized TOC (solution 1) were prepared over the range of concentrations of 0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 7.5 mg/l.

Three additional samples containing 5.0 mg/l racemic TOC (solution 2) were added to clean tubes and evaporated to dryness. To the remaining residue was added 0.2 ml of NEIC solution. After addition of 2 ml of HPLC-grade water, the mixture was vortex-mixed (30 s) and centrifuged at 1800 *g* for 5 min. A 1-ml volume of the aqueous layer was then transferred to a clean tube and the concentration of underivatized TOC remaining was calculated from the calibration curves by comparing the UV chromatograms.

Treatment of data

The peak-area ratio of TOC/I.S. was used to determine the concentration of each enantiomer. The first I.S. peak to elute was used in the ratio calculations. Results are reported as mean ± S.D.

RESULTS AND DISCUSSION

Type I antiarrhythmic drugs, including TOC, are thought to exert their pharmacologic action primarily via blockade of fast sodium channels. In addition, binding of these type I compounds to cardiac sodium channels is likely to be stereoselective [18]. Consequently, as noted by others, the electrophysiological effects associated with the individual enantiomers differ [1,4,19]. The complexity of the effects observed clinically is further complicated by the realization that the disposition of these agents may also be stereoselective [19]. Thus, it is necessary that investigations of TOC utilize stereospecific measures.

The presence of a primary amine functional group on TOC readily allows for precolumn derivatization with reagents including isocyanates and isothiocyanates. However, to this point, separation of TOC enantiomers has been achieved by utilizing either chiral stationary phases [16,17] or chiral derivatizing reagents such as *R*(−)-O-methylmandelic acid [8]. With the former technique, derivatization with heptafluorobutyric anhydride prior to enantiomer separation was required, while the latter technique could only be applied to urine samples. Recently, our laboratory reported the use of NEIC in the separation and quantitation of the chiral β-blocking adrenergic drug, acebutolol [20]. NEIC is commercially available, has high optical purity (99%), and fluoresces.

The application of NEIC to the separation of TOC enantiomers resulted in baseline separation of the two enantiomers ($R \geq 1.5$) (Fig. 2). Peaks corresponding to the enantiomers eluted at about 3.5 and 4.5 min. Although the order of elution of the two peaks could not be determined with absolute certainty, it was surmised that the identity of the peaks could be ascertained by knowledge of the enantiomeric disposition (*i.e.*, the area under the plasma concentration *versus* time curve for *S*-TOC being greater than that of *R*-TOC) [8,17]. Consequently, the first peak likely corresponded to *S*-TOC and the second peak to *R*-TOC. The *R*-I.S. and *S*-I.S. eluted at about 14.9 and 16.5 min, respectively.

Derivatization of the primary amine of TOC resulted in formation of a urea

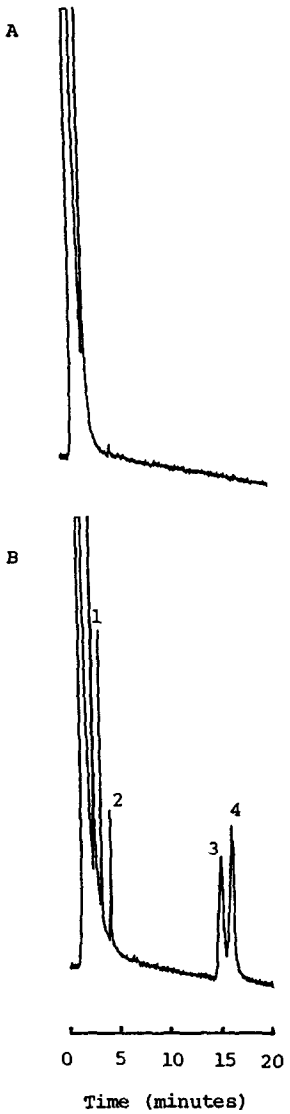

Fig. 2. Chromatograms of (A) blank plasma and (B) plasma spiked with 1 mg/l tocainide. Peaks: 1 = *S*-TOC; 2 = *R*-TOC; 3 = *R*-I.S.; 4 = *S*-I.S.

diastereomer (Fig. 1). As expected, the mass spectral analysis indicated that the derivative was mono-substituted. This observation was made based on the mass spectral data which indicated a prominent peak corresponding to the molecular ion (MH^+) at m/z 390. The formation of a urea from an amine derivatized with NEIC has previously been reported [20,21].

The derivatization yield was 46.5 ± 16.9%, as calculated by comparing the UV spectra of underivatized TOC with the spectra of underivatized TOC remaining after addition of NEIC. Although less than 50% of TOC was derivatized, the method has validity in that (1) the required sensitivity for clinical utility was obtained and (2) the derivatization yield did not change with time under our reaction conditions. Hence, the same sample analyzed repeatedly did not give different results. However, if after addition of NEIC the samples were not evaporated, it was noticed that the reaction continued to proceed over approximately 8 h. When samples were subjected to heat at temperatures from 80 to 100°C, the formation of diastereomers ceased to proceed beyond 1.5–2 h. Interestingly, samples that were evaporated immediately after addition of NEIC and subsequently reconstituted with chloroform yielded chromatograms where the diastereomer peaks did not change with time.

The extraction yields of TOC from plasma, as indicated by comparing extracted *versus* unextracted samples, were 84.2 ± 23.5, 86.4 ± 7.75, and 71.0 ± 15.7% for *S*-TOC at concentrations of 0.25, 2.5 and 10 mg/l, respectively. For *R*-TOC, the extraction yields were 81.0 ± 12.8, 89.7 ± 9.82, and 71.2 ± 14.8% for 0.25, 2.5, and 10 mg/l, respectively. Additionally, both TOC enantiomers extracted equally (*i.e.*, not stereoselective).

The assay is accurate, precise and reproducible as summarized in Table I. The chromatograms were free from interfering peaks, and calibration curves were typically described by $y = -0.015 + 0.16x$ and $y = -0.0093 + 0.15x$ over the entire examined concentration range for *R*- and *S*-TOC, respectively, where y was the peak-area ratio of either *R*- or *S*-TOC/I.S. and x was enantiomer concentration. However, despite the observed excellent linearity ($r^2 > 0.998$) of both curves, the accuracy (percentage error) and precision (coefficient of variation, C.V.) of the method for the less concentrated samples sometimes exceeded 10%.

TABLE I

ACCURACY AND PRECISION OF THE METHOD

$n = 9$ (three sets for three days).

Enantiomer concentration (mg/l)			Accuracy (error %)		Precision (C.V., %)	
Added	Measured (mean ± S.D.)					
	S	*R*	*S*	*R*	*S*	*R*
0.25	0.24 ± 0.032	0.23 ± 0.021	7.3	4.2	9.2	8.9
0.5	0.47 ± 0.059	0.48 ± 0.045	6.9	3.3	10	9.4
1.0	1.0 ± 0.0098	1.0 ± 0.0067	0.68	0.42	1.0	0.67
2.5	2.2 ± 0.19	2.2 ± 0.16	12	12	8.6	7.3
5.0	4.7 ± 0.42	4.7 ± 0.43	6.5	6.5	8.9	9.2
10.0	10 ± 0.10	10 ± 0.11	0.23	0.22	1.1	1.1

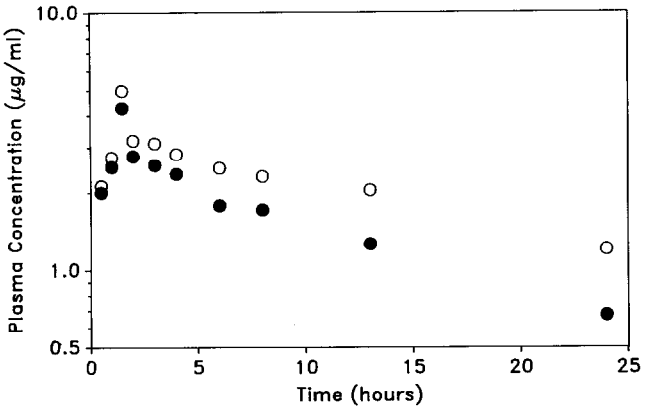

Fig. 3. Concentration *versus* time profile of *R*-TOC (●) and *S*-TOC (○) in a healthy male volunteer following a single oral 400-mg racemic dose of TOC.

Therefore, for the lower concentrations of 0, 0.25, 0.5, and 1.0 mg/l, a separate linear regression equation was used to estimate TOC concentrations. Hence, for concentrations of 1.0 mg/l or less, the calibration curves were described by $y = 0.00 + 0.12x$ and $y = 0.00 + 0.13x$ for *R*- and *S*-TOC, respectively ($r^2 > 0.998$). Using this procedure, the inter-assay C.V. was always less than 10% throughout the range of concentrations examined. The sensitivity of the assay was 0.25 mg/l, based on the concentration which resulted in an acceptable accuracy and precision. However, with a 4:1 signal-to-noise ratio, a greater sensitivity in the order of approximately 25 μg/l would ensue.

The plasma *versus* time profile of *R*- and *S*-TOC after oral administration of racemic TOC (400-mg tablet) to a healthy 32-year-old male is depicted in Fig. 3. Similar to previous reports [8,17], there was a stereoselective disposition of TOC throughout the examined time interval. Concentrations of *S*-TOC were consistently greater than those of the *R*-TOC. Furthermore, the sensitivity of the assay would allow samples to be collected over longer intervals, presumably for at least 24 h following a single 400-mg oral, racemic dose of TOC.

In conclusion, this stereospecific analysis of TOC is rapid and convenient, allowing for the processing of multiple samples in a limited amount of time. Use of the commerically available NEIC resulted in the formation of diastereomers which were separated by conventional normal-phase chromatography utilizing fluorescence detection. This method also appears to be useful for the separation of other structurally similar compounds, including mexiletine.

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