Stereospecific High-Performance Liquid Chromatographic Assay of Sotalol in Plasma

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A convenient high-performance liquid chromatographic (HPLC) assay was developed for determination of sotalol (STL) enantiomers in plasma. Following addition of the internal standard (IS; racemic atenolol), enantiomers of STL and IS were extracted using ethyl acetate. After evaporation of the organic layer, samples were derivatized with a solution of *S*-(+)-1-(1-naphthyl)ethyl isocyanate (NEIC). The resulting diastereomers were chromatographed with normal-phase HPLC with chloroform:hexane:methanol [65:33:2 (v/v)] as the mobile phase at a flow rate of 2 ml/min. The fluorescence detection wavelength was set at 220 nm for excitation with no emission filter. The suitability of the assay for pharmacokinetic studies was determined by measuring STL enantiomers in the plasma of a healthy subject after administration of a single 160-mg oral, racemic dose of STL.

KEY WORDS: sotalol; high-performance liquid chromatography (HPLC); enantiomers; β-adrenergic blocking agents (β-blockers); stereoselective pharmacokinetics.

INTRODUCTION

Sotalol (STL) is a β-adrenergic blocking (β-blocking) agent devoid of intrinsic sympathomimetic activity, membrane stabilizing actions, and cardioselectivity (1). STL is unique in comparison with other β-blockers by way of its ability to lengthen repolarization and the effective refractory period in all cardiac tissues (2). As a consequence of these electrophysiologic properties, STL is now also considered as an important class III antiarrhythmic agent (2,3).

As with other β-blocking drugs, STL is chiral and is marketed as the racemate. It is known that the two enantiomers have substantially different pharmacologic properties, whereby virtually all of the β-blockade activity has been attributed to l-STL (3–5). Both the d- and the l-enantiomers, however, contribute equally to the class III antiarrhythmic activity. Despite the fact that the enantiomers of STL have differing activities, reported methods for analyzing STL in biological samples have, to date, utilized nonstereospecific techniques (6–13). Thus, these nonstereospecific assays cannot be used to delineate the pharmacokinetics and pharmacodynamics of the enantiomers after administration of the racemate. In this report, we describe a convenient and sensitive HPLC method for the determination of STL enantiomers in human plasma.

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MATERIALS AND METHODS

Chemicals

Figure 1 depicts STL, which was obtained as the hydrochloride salt. The pure enantiomers of *d*- and *l*-STL, as well as the racemate, were obtained as gifts from Bristol-Myers (Ottawa, Ontario, Canada). Racemic atenolol hydrochloride was used as the internal standard (IS) and was obtained from ICI Pharma (Mississauga, Ontario. Canada). The chiral derivatizing reagent, *S*-(+)-1-(1-naphthyl)ethyl isocyanate (NEIC; Fig. 1), was obtained from Aldrich (Milwaukee, WI). Analytical-grade sodium hydroxide, methanol, glacial acetic acid, and chloroform were obtained from BDH chemicals (Toronto, Ontario, Canada), while analytical-grade ethyl acetate and hexane and HPLC-grade water were obtained from Mallinckrodt (Paris, KT). Analytical-grade triethylamine was obtained from Fisher Scientific (Fair Lawn, NJ).

Apparatus and Chromatography

Both normal-phase and reversed-phase conditions were used for determination of enantiomer concentration and derivatization, respectively. In both cases, the HPLC system consisted of a Model 590 pump, Model 712 Wisp autosampler, and Model 745B integrator (Waters, Mississauga, Ontario, Canada).

The normal-phase chromatography utilized a 25-cm stainless-steel silica column (Whatman Partisil 5, Clifton, NJ). Fluorescence detection (Applied Biosystems Model 980, Technical Marketing Associates, Edmonton, Alberta, Canada) was set at 220 nm for excitation; no emission filter was used. The mobile phase was chloroform:hexane:methanol (65:33:2, v/v) pumped at a flow rate of 2 ml/min.

The reversed-phase consisted of a Nova-Pak C_{18} 8-mm cartridge which was housed in an 8 × 10-cm radial compression module (Waters, Mississauga, Ontario, Canada). Fluorescence detection excitation was set at 235 nm; no emission filter was used. The mobile phase was water:methanol:acetic acid (64:35:1) pumped at 2 ml/min.

All samples were vortexed using a Vortex Genie 2 mixer (Fisher Scientific, Edmonton, Alberta, Canada) and centrifuged with a Dynac II centrifuge (Becton Dickinson, Parsippany, NJ). Solvents were evaporated using a Savant Speed Vac concentrator-evaporator (Emerston Instruments, Scarborough, Ontario, Canada).

Standard Solutions

A 100 μg/ml stock solution of racemic STL hydrochloride (as the base) was prepared in HPLC-grade water (solution 1). The IS solution consisted of 10 μg/ml (as the base) of racemic IS in HPLC-grade water (solution 2). Another stock solution of STL (used to determine extraction and derivatization yields) was prepared as 0.00375% triethylamine in methanol (v/v) to give a final concentration of 100 μg/ml of the base (solution 3). These solutions were stored at 5°C. The NEIC solution was prepared in chloroform (0.05%, v/v) and was stored at −20°C until just prior to use.

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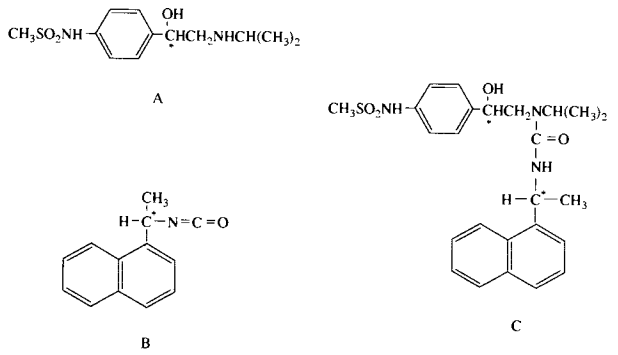

Fig. 1. Structures of (A) STL, (B) NEIC, and (C) derivatized STL.

Sample Preparation

Drug-free human plasma samples (0.5 ml each) were spiked with STL (solution 1) to give final concentrations of 50, 100, 250, 500, 1000, 2500, and 5000 ng/ml of each enantiomer. To this was added 5 μg of each enantiomer of IS (solution 2) and 30 μl of 1 *M* sodium hydroxide. The plasma was vortexed for 30 sec and centrifuged at 1800*g* (5 min) with two consecutive 4-ml volumes of ethyl acetate. The two ethyl acetate extracts obtained from each sample were combined and evaporated to dryness using the Savant Speed Vac concentrator-evaporator. Samples were then derivatized at room temperature with 0.2 ml of the NEIC solution, which was added to the residue. After addition of NEIC, tubes were vortexed for 30 sec and aliquots ranging from 75 to 150 μl were injected into the HPLC.

Extraction Yield

Solutions of either 100, 500, or 2500 ng/ml STL enantiomers (solution 3, $n = 3$) were added to clean, dry glass tubes and evaporated to dryness. After addition of 0.5 ml plasma to each tube, samples were extracted after addition of 30 μl 1 *M* sodium hydroxide and ethyl acetate (2 vol of 4 ml each). The tubes were then vortexed for 30 sec and centrifuged (1800*g*, 5 min) and the two extracts of each sample were combined in clean tubes, evaporated to dryness, derivatized, and chromatographed. To compare these samples with those that were not extracted, another set of tubes containing the above concentrations was prepared without the addition of plasma and subsequent extraction procedure. Peak areas of extracted STL versus unextracted equivalent STL concentrations were compared under identical chromatographic conditions.

Derivatization Yield

Using solution 3, concentrations of either 250 or 1000 ng of STL enantiomer ($n = 6$ for each concentration) were evaporated to dryness. To three samples of each concentration was added 0.2 ml of NEIC solution. These derivatized samples were compared to another three samples that were not derivatized after injection of aliquots ranging from 25 to 50 μl into the HPLC.

Applicability to Pharmacokinetic Studies

To test the utility of the stereospecific assay for pharmacokinetic studies, a single 160-mg racemic dose of STL was administered orally to a healthy 25-year-old male subject giving informed consent. Blood samples were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hr via an indwelling catheter inserted in a forearm vein. Plasma was collected by centrifugation and samples were stored at −20°C until the next day for analysis.

Treatment of Data

The peak area ratio of STL/IS was used to determine the concentration of each enantiomer. The first-eluting IS peak was used in these ratio calculations. Results are reported as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Separation, identification, and quantification of racemic

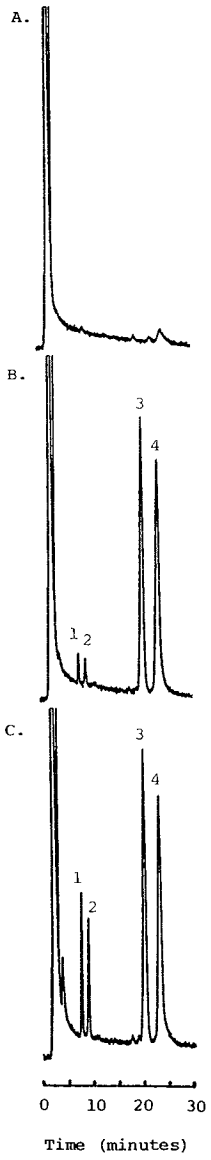

Fig. 2. Chromatograms of (A) blank plasma, (B) plasma spiked with 50 ng/ml of each STL enantiomer, and (C) plasma sample taken 12 hr after a single oral 160-mg dose of racemic STL. Peak identification: 1, *d*-STL; 2, *l*-STL; 3, *R*-IS; 4, *S*-IS.

Table I. Accuracy and Precision of the Method[a]

Enantiomer concentration (ng/ml)						
	Measured[b]		Accuracy, error %		Precision, CV %	
Added	d	l	d	l	d	l
50	49.0 ± 5.90	49.2 ± 6.20	11.3	11.8	12.0	12.6
100	99.5 ± 5.23	99.9 ± 5.53	5.50	4.87	5.26	5.54
250	245 ± 5.10	244 ± 4.89	2.28	2.22	2.08	2.00
500	495 ± 12.9	495 ± 12.9	2.16	2.13	2.61	2.61
1000	970 ± 43.4	971 ± 44.1	4.01	4.18	4.54	3.38
2500	2510 ± 84.7	2510 ± 84.4	2.71	2.71	3.38	3.36
5000	5030 ± 111	5030 ± 111	1.75	1.74	2.22	2.22

[a] $n = 9$ (3 sets for 3 days).
[b] Reported as mean ± SD.

compounds have received widespread attention (14,15). To date, separation of drug enantiomers has been accomplished using either chiral stationary phases (16) or homochiral reagents (17). Recently, our laboratory reported the sterospecific analysis of acebutolol and its metabolite diacetolol, as well as tocainide, using NEIC as the chiral derivatizing reagent (19–21). This analytical technique has since been applied to study the pharmacokinetics of acebutolol and diacetolol in healthy subjects (22).

In this report, separation of STL enantiomers was achieved using optically pure NEIC, thus forming diastereomers, which were chromatographed by normal-phase HPLC. Using this method, individual enantiomers of STL were measured in human plasma. To our knowledge, there has not been any assay reporting the analysis of STL enantiomers in plasma.

The reaction of NEIC with STL enantiomers resulted in baseline resolution of both STL and IS diastereomers ($R >$ 1.5, Fig. 2). Formation of these diastereomers seemed to occur virtually immediately, as incubation of samples using various concentrations of NEIC at various times and temperatures did not enhance derivatization. Peaks corresponding to the STL enantiomers eluted at approximately 7.5 and 8.7 min. The first- and second-eluting STL peaks corresponded to *d*- and *l*-STL, respectively, as confirmed by chromatography of the pure enantiomers. The *R*- and *S*-IS eluted at approximately 20 and 23 min, respectively. Consequently, the total run time for the assay was 25 min, which allowed for convenient processing of numerous clinical samples.

The assay was accurate, precise, and reproducible as summarized in Table I. Chromatograms were free from interfering peaks (despite the absence of an emission filter), and calibration curves for *d*- and *l*-STL were typically described by $y = -0.0141 + 0.000910(x)$ and $y = -0.0126 + 0.000910(x)$, respectively. These equations were described, where y is the peak area ratio and x is the enantiomer concentration (x and y were not weighted). Excellent linearity was observed for all calibration curves ($r^2 > 0.999$), and accuracy (%error) and precision (%CV) exceeded 10% only for the lowest concentration studied. Although the reported sensitivity of this assay was 50 ng/ml, greater sensitivity, of the order of 20 ng/ml, was obtained using a signal:noise ratio of 4:1.

Derivatization of structurally similar compounds with NEIC has been reported to be virtually complete (18). When using isocyanates to derivatize β-blocking drugs, previous studies have confirmed the formation of a urea, and not a carbamate, derivative (18,19). Further, the reaction between the β-blocker and the isocyanate was on a 1:1 molar basis (19). To determine the efficiency of the derivatization with STL, we tried to detect underivatized drug either under the stated normal-phase conditions or with changes to the mobile phase composition. Despite our efforts, however, underivatized STL was not detected. Consequently, a modification of a previously reported nonstereospecific reverse-phase HPLC method was utilized (10) to detect underivatized STL. Using this method, underivatized samples containing either 250 or 1000 ng of each STL enantiomer, resulted in a single peak at 3.38 min, which corresponded to racemic STL. Once samples were derivatized, the 3.38-min peak was absent, even at STL enantiomer concentrations of up to 1000 ng/ml. These data suggest that derivatization of STL under the stated conditions was complete (>99%). Furthermore, the derivatization reaction was not stereoselective, as the addition of NEIC to STL always resulted in virtually equal peak areas corresponding to the enantiomers. Finally, the diastereomers of both STL and IS appeared to be stable for at least 24 hr, as no changes were observed with the chromatograms upon repeated injection of the same samples at ambient temperature.

The extraction yield of STL from plasma was ≥75% over the concentration range studied. Although the extraction was not 100%, it was, nevertheless, sufficient to allow

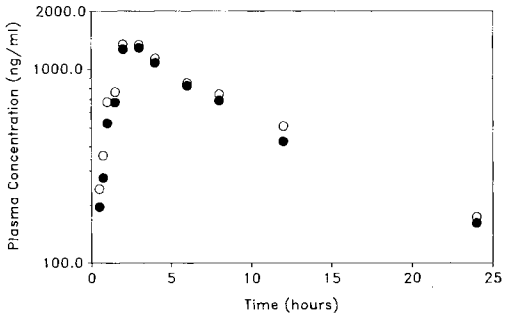

Fig. 3. Plasma concentration versus time profile of *d*-STL (open circles) and *l*-STL (filled circles) in a healthy male volunteer following a single oral 160-mg dose of racemic STL.

for the requisite sensitivity after administration of commonly used STL doses.

The plasma concentration versus time profile of *d*- and *l*-STL after oral administration of 160 mg racemic STL to a healthy 25-year-old male volunteer is depicted in Fig. 3. Although concentrations of *d*-STL were generally greater than those of *l*-STL, little stereoselectivity was observed.

In conclusion, the described assay is sensitive and convenient, allowing for numerous samples to be processed in a relatively short span of time. Furthermore, the assay is applicable to pharmacokinetic studies of STL in humans.

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