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A simple HPLC-fluorescence method for the measurement of R,S-sotalol in the plasma of patients with life-threatening cardiac arrhythmias

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Abstract

R,S-sotalol, a ß-blocker drug with class III antiarrhythmic properties, is prescribed to patients with ventricular, atrial and supraventricular arrhythmias. A simple and sensitive method based on HPLC-fluorescence is described for the quantification of R,S-sotalol racemate in 500 µl of plasma. R,S-sotalol and its internal standard (atenolol) were eluted after 5.9 and 8.5 min, respectively, from a 4-micron C_{18} reverse-phase column using a mobile phase consisting of 80 mM KH_2PO_4, pH 4.6, and acetonitrile (95:5, v/v) at a flow rate of 0.5 ml/min with detection at λ_{ex} = 235 nm and λ_{em} = 310 nm, respectively. This method, validated on the basis of R,S-sotalol measurements in spiked blank plasma, presented 20 ng/ml sensitivity, 20-10,000 ng/ml linearity, and 2.9 and 4.8% intra- and interassay precision, respectively. Plasma sotalol concentrations were determined by applying this method to investigate five high-risk patients with atrial fibrillation admitted to the Emergency Service of the Medical School Hospital, who received sotalol, 160 mg *po*, as loading dose. Blood samples were collected from a peripheral vein at zero, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after drug administration. A two-compartment open model was applied. Data obtained, expressed as mean, were: C_{MAX} = 1230 ng/ml, T_{MAX} = 1.8 h, AUC_T = 10645 ng h^{-1} ml^{-1}, K_{ab} = 1.23 h^{-1}, α = 0.95 h^{-1}, ß = 0.09 h^{-1}, t(1/2)ß = 7.8 h, Cl_T/F = 3.94 ml min^{-1} kg^{-1}, and Vd/F = 2.53 l/kg. A good systemic availability and a fast absorption were obtained. Drug distribution was reduced to the same extent in terms of total body clearance when patients and healthy volunteers were compared, and consequently elimination half-life remained unchanged. Thus, the method described in the present study is useful for therapeutic drug monitoring purposes, pharmacokinetic investigation and pharmacokinetic-pharmacodynamic sotalol studies in patients with tachyarrhythmias.

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- High-risk patients

R,S-sotalol is a ß-adrenergic blocker with class III antiarrhythmic properties. It is efficient for the treatment of supraventricular tachycardia and complex ventricular and refractory arrhythmias (1-5). This drug can be also useful as an alternative in the pharmacological reversal of atrial fibrillation or to control ventricular tachycardia when other drugs, e.g., quinidine, amiodarone, digoxin, verapamil and esmolol, or electrical cardioversion are not satisfactory (6,7). Several adverse effects, such as bradycardia (3%), *torsades de pointes* (2.5%), proarrhythmic effect (2.5%) and cardiac heart failure (1%), have been reported in patients with severe ventricular arrhythmia. During the first week of treatment, adverse effects arise more frequently as a consequence of the prolongation of QT interval (1-3).

As a weak base, R,S-sotalol hydrochloride is absorbed in the gastrointestinal tract after a *po* dose and is excreted primarily as the unmetabolized drug in a high extent by the kidney by glomerular filtration due to its hydrophilic nature that leads to low plasma protein drug binding (4,5). Consequently, its biological half-life (8-10 h) is increased in patients with renal failure and in elderly subjects (12-24 h) and decreased in patients on hemodialysis (5,8-10).

Several analytical methods have been described for the quantification of R,S-sotalol in biological fluids (11-14). Different techniques to determine sotalol racemate have been applied using fluorimetry (15), gas-liquid chromatography (16), and high-performance liquid chromatography, HPLC-UV (12-14) or HPLC-F (11,17), with HPLC-F being more sensitive. A serious problem of the chromatographic methods is the choice of internal standard, with many cardiovascular drug structural analog compounds such as atenolol, bisoprolol (ß-blockers) or others such as procainamide (antiarrhythmic drug) having been described (12,14,17). The large blood volume required for plasma analysis, as well as the low recovery of R,S-sotalol after sample clean-up, or time-consuming analytical methods indicate that an improved method should be developed for therapeutic drug monitoring purposes and pharmacokinetic-pharmacodynamic (PK-PD) studies.

Five high-risk patients (3 males and 2 females) with atrial fibrillation aged 54.8 ± 11.1 years (mean ± SD), mean weight, 66.6 ± 5.3 kg, mean height, 169 ± 5 cm, and mean BSA 1.76 ± 0.09 m^2 were investigated. Patients without renal, hepatic and endocrine dysfunction or heart failure were selected and included in the controlled study protocol. All of them gave informed written consent to participate in the study carried out in the Emergency Service of the Medical School Hospital and the protocol was approved by the Hospital Ethics Committee. The patients admitted with cardiac tachycardia or atrial fibrillation were selected on the basis of clinical evaluation and laboratory profile. R,S-sotalol (160 mg *po*, Sotacor™, Bristol-Myers Squibb, São Paulo, SP, Brazil) was administered as loading dose to reverse cardiac arrhythmias. Venous blood samples were collected at zero, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after drug administration.

Plasma R,S-sotalol concentration was determined by HPLC-F after the clean-up of biological samples consisting of a precipitation of plasma protein at 5°C by adding 100 µl 23% perchloric acid to a glass tube containing 500 µl of plasma plus its internal standard (atenolol). The mixture was vortexed for 20 s and kept in an ice bath; after 5 min, 4 M K_2HPO_4 (500 µl) was added to the mixture, followed by centrifugation at 3000 *g* for 15 min.

The supernatant solution was injected automatically using a Shimadzu SIL B Autosampler (Tokyo, Japan) into the HPLC apparatus, Shimadzu LC-6A, connected to a 4-µm reverse-phase NovaPak™ C18 column, 150 x 3.9 mm. The mobile phase was prepared as described previously by Boutagy and Shenfield (11), with some modifications

and adaptations, consisting of 80 mM KH_2PO_4, pH 4.6, and acetonitrile (95:5, v/v) pumped isocratically at a flow rate of 0.5 ml/min, at room temperature.

Calibration curves for R,S-sotalol in plasma were plotted after the clean-up of biological standard samples with spiked blank plasma ranging from 20 up to 20,000 ng/ml. The analytical method described here was validated for sotalol racemate and presented higher sensitivity (20 ng/ml), better linearity (20-10,000 ng/ml) and good precision (2.9 or 4.8% for intra- or interassay precision, respectively) compared to those reported previously (11).

R,S-sotalol and atenolol (internal standard) which were eluted at 5.9 and 8.5 min, respectively, were monitored with a fluorescence detector Shimadzu RF 535 with $\lambda_{excitation}$ = 235 nm and $\lambda_{emission}$ = 310 nm (Figure 1). Peak areas were integrated and the peak area ratio for R,S-sotalol/atenolol was estimated with a Shimadzu printer-plotter Chromatopac CR-6A. Consequently, only 10 min were necessary to complete each run, permitting 100 injections per 17 h.

For the kinetic modeling, a two-compartment open model was applied to obtain the plasma decay curve for R,S-sotalol, logC *vs* time (18). The pharmacokinetic parameters are listed in Table 1. The systemic availability:area under the curve (AUC_T), drug elimination:total body clearance (Cl_T), drug distribution:apparent volume of distribution (Vd_{AREA}), half-life and rate constants were estimated (18). The Statgraphics software was applied to the data obtained in the present study. The chi-square, Spearman, Wilcoxon, Mann-Whitney and rank sum tests for paired and unpaired data were used for statistical analysis. The data are reported as medians and the level of significance was set at $P<0.05$ (19).

The original fluorimetric nonchromatographic method to quantitate sotalol racemate in plasma was described by Garret and Schnelle (15), and has been used extensively. Then, a series of modifications in the clean-up of plasma samples were proposed to determine plasma sotalol levels by the selective HPLC technique using UV or fluorescence detectors (12-14,17). Extensive sample clean-up and time-consuming analysis are the main disadvantages for those methods, detailed as follows: large plasma volumes required (1 ml or greater) for sample clean-up by plasma protein precipitation, drug extraction using mixtures of organic solvents at appropriate pH, in general by one or two steps, followed by a back-extraction into aqueous acid solution before quantification by HPLC (12-14,17).

A solid-phase extraction of sotalol racemate from plasma samples has been described previously by Sallustio et al. (20). Large volumes of extracting solvents required

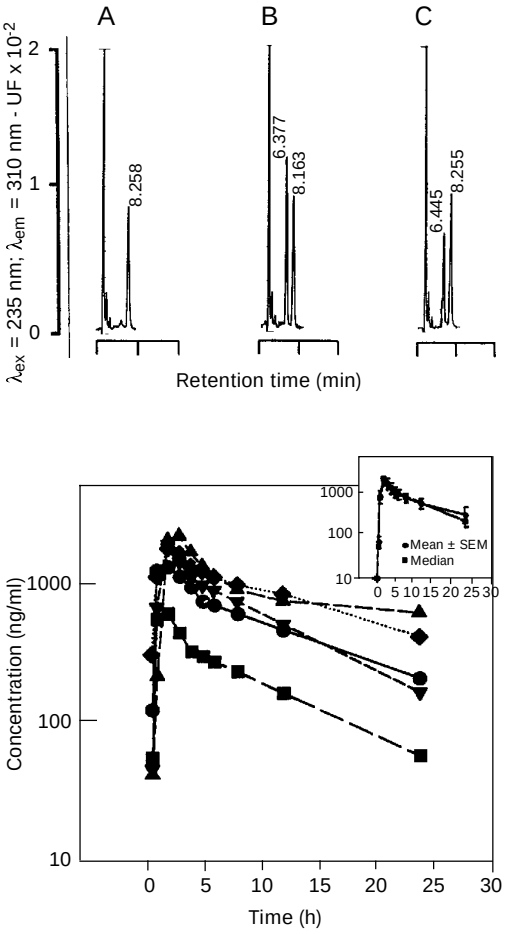

Figure 1 - Chromatographic profile of plasma sotalol and its internal standard (atenolol) (top). *A*, Blank of plasma control plus atenolol (IS). *B*, Control of plasma sample spiked with sotalol (500 ng/ml) plus IS. *C*, Plasma of a patient who received sotalol hydrochloride, Sotacor™, 160 mg *po* loading dose. Sotalol plasma decay curve (bottom). Life-threatened patients who received sotalol hydrochloride, Sotacor™, 160 mg *po* as loading dose in the emergency room. *Inset*, Means ± SEM, N = 5 (circles), and medians, N = 5 (squares). Individual data, log C versus T for five patients (No. 1-5) with atrial fibrillation. No. 1, Triangles; No. 2, circles; No. 3, squares; No. 4, lozenges, and No. 5, inverted triangles.

for the measurements in plasma are the main disadvantage of this procedure. Low recovery of sotalol extraction was obtained by Lemmer et al. (13) after the precipitation of plasma protein followed by a series of drug extractions, i.e., the mixture consisting of benzylalcohol and chloroform (60:40, v/v) was used for the main extraction. Reextraction with heptane followed by a back-extraction to 0.1 N sulfuric acid are the main reasons for the low recovery obtained (13).

Ion-pair liquid chromatography has been used and reagent modifiers such as alkylamines, e.g., n-octylsodium sulfate or 1-heptanesulfonic acid, have been added to the mobile phase (12-14,17,20). Good selectivity was obtained, but ternary or quaternary mobile phases at high flow rate were required. Large volumes of extracting solvents required for the measurements in plasma are the main disadvantage of these procedures. Recovery of sotalol extraction was improved by adding an alcohol derivative (isopropanol, 1-butanol or 1-pentanol) in a mixture with chloroform after the precipitation of plasma protein followed by a series of drug extractions, but extremes of pH are the main reason for shortened half-life of the analytical column (12). Reextraction with solvents followed by a back-extraction to 0.1 N hydrochloric acid is responsible for the low recoveries obtained (17). Additionally, the large number of drugs in the mixture and low precision are the main disadvantages of these methods.

More recently, a superior reverse phase chromatographic system was described by Boutagy and Shenfield (11) using a binary system instead of ternary or quaternary systems as mobile phase and detection by fluorescence. If the simplification of the number of components in the mobile phase is an advantage, as is the case for the latter method (11), the lower sensitivity reported can limit the application for this binary system, when their data (60 ng/ml) were compared to others (20 ng/ml) reported previously (11-14,17,20). However, the present study reports a quite specific and sensitive analytical HPLC-F method to measure plasma R,S-sotalol levels eluted from the reverse phase column with a binary system as mobile phase. Only 500 µl of biological samples was required for a rapid clean-up, and drug analysis was performed in a 10-min run; good linearity (20-10,000 ng/ml) and high sensitivity (20 ng/ml) were obtained, sufficient for R,S-sotalol plasma measurements and dose adjustment by therapeutic drug monitoring, since reference range based on trough or peak levels are 400 or 3000 ng/ml, respectively.

Therapeutic schemes for the reversal of atrial fibrillation with R,S-sotalol described

Table 1 - Kinetic modeling of plasma sotalol in life-threatened patients with atrial fibrillation after 160 mg *po* loading dose.

Data are reported as mean ± SD (median) for five patients with tachyarrhythmias who received a 160 mg sotalol hydrochloride *po* loading dose. Kinetic parameters: C_{MAX}: peak plasma concentration; T_{MAX}: time to reach C_{MAX}; AUC_T: area under the curve related to systemic availability of drug; α and ß: hybrid rate constants related to distribution and elimination, respectively; Vd_{AREA}: apparent volume of distribution; K_{ab}: absorption rate constant; t(1/2)ab: absorption half-life; t(1/2)α: distribution half-life; t(1/2)ß: biological half-life; Cl_T: plasma clearance. [a]Patients (atrial fibrillation), present study; [b]elderly hypertensive patients and healthy volunteers (9).

Kinetic parameters	Atrial fibrillation[a]	Healthy volunteers[b]	Arterial hypertension in elderly patients[b]
C_{MAX} (ng/ml)	1230 ± 292 (1325)	890 ± 318	1420 ± 317
T_{MAX} (h)	1.8 ± 0.7 (1.2)	2.5 ± 1.0	2.9 ± 1.5
AUC_T (ng h^{-1} ml^{-1})	10645 ± 5063 (8105)	10446 ± 4723	14583 ± 3863
K_{ab} (h^{-1})	1.23 ± 0.40 (1.35)	1.72 ± 1.14	1.36 ± 0.59
t(1/2)ab (h)	0.58 ± 0.28 (0.45)	0.40 ± 0.08	0.51 ± 1.14
α (h^{-1})	0.95 ± 0.46 (0.87)	1.24 ± 0.37	0.76 ± 0.30
t(1/2)α (h)	0.97 ± 0.64 (0.9)	0.56 ± 0.08	0.91 ± 2.31
Vd (l/kg)	2.53 ± 0.81 (2.54)	3.55 ± 1.77	2.22 ± 0.84
ß (h^{-1})	0.09 ± 0.02 (0.10)	0.07 ± 0.02	0.05 ± 0.03
t(1/2)ß (h)	7.8 ± 1.7 (7.1)	7.1 ± 3.1	11.4 ± 4.8
Cl_T (ml min^{-1} kg^{-1})	3.94 ± 1.61 (4.21)	5.93 ± 3.46	3.32 ± 0.69

in the literature are detailed as follows. Sotalol racemate (*po*) can be administered at 80-320 mg twice a day, 12/12 h (160 to 640 mg/daily) or 400-600 mg three times a day, 8/8 h (1200-1800 mg/daily), showing equivalent plasma drug concentrations related to the minimum and maximum (trough and peak) plasma levels expressed as 95%CI range (median): 440 to 1180 (750) ng/ml for the trough and 1222 to 2466 (1720) ng/ml for the peak plasma R,S-sotalol levels.

Data obtained and estimated parameters derived from the plasma decay curve by PK modeling expressed as mean (median) for our patients were peak plasma concentration (C_{MAX}): 1230 (1325) ng/ml, and time to reach it (T_{MAX}): 1.8 (1.2) h, AUC_T: 10645 (8105) ng h^{-1} ml^{-1}, K_{ab}: 1.23 (1.35) h^{-1}.

Concerning the systemic availability of R,S-sotalol, the data obtained in the present study showed a lower AUC_T than that reported for hypertensive subjects, probably due to the older age of the latter, although similar results were observed when our patients and healthy volunteers were compared (9). Rate of bioavailability, C_{MAX} and T_{MAX} also showed similar results when compared to data reported by the same authors (9). Consequently, arrhythmic patients showed a faster sotalol absorption than hypertensive elderly subjects (9). A good bioavailability was reached for all patients investigated after drug administration receiving 160 mg *po* as the loading dose for the reversal of atrial fibrillation (Table 1 and Figure 1).

Drug distribution was measured by alpha (α), a hybrid rate constant, its respective half-life and the apparent volume of distribution, Vd_{AREA}. Distribution was reduced by 30%, when patients with cardiac arrhythmias or arterial hypertension and healthy volunteers were compared (Table 1). Additionally, distribution half-life should be prolonged in the elderly (9).

The volume of distribution for our patients was similar to data obtained for hypertensive elderly patients (2.22 l/kg *vs* 2.53 l/kg), but reduced when compared to healthy volunteers (3.55 l/kg) (Table 1).

Drug elimination was evaluated by the following parameters: elimination rate constant (ß), biological half-life (t(1/2)ß) and total body clearance (Cl_T). The total body clearance showed a 34% reduction (3.94 ± 1.61 *vs* 5.93 ± 3.46 ml min^{-1} kg^{-1}), when data from our patients and healthy volunteers were compared (9). In fact, this difference is probably due to changes in hepatic blood flow in patients with atrial fibrillation.

The elimination half-life of R,S-sotalol as a function of its volume of distribution and plasma clearance remained unchanged for our patients because both parameters were reduced to a similar extent. Interindividual variation of the data as measured here by the 95%CI agrees with data reported previously for sotalol racemate PK modeling in hypertensive patients and in healthy volunteers (9).

These findings suggest that changes in hemodynamic parameters probably affecting blood perfusion could alter the kinetic disposition of sotalol, reducing the volume of distribution and plasma clearance with consequences of clinical relevance for these high-risk patients with atrial fibrillation (Table 1).

If drug accumulation increases, but cannot be predicted, dose adjustment is necessary to avoid adverse effects in these patients. Therefore, PK modeling, considered to be a powerful tool, is critical for the individualization and optimization of pharmacological therapy since drug accumulation increases as a consequence of plasma clearance and volume of distribution, both reduced in a same extension with relevant clinical effects in these patients.

In conclusion, the clinical pharmacokinetics of sotalol racemate in high-risk patients with ventricular arrhythmias was investigated by applying a simple and sensitive analytical method using HPLC-F as validated by the present controlled study proto-

col. Finally, we showed the clinical relevance of pharmacological R,S-sotalol therapy in the reversal of cardiac arrhythmias in life-threatened patients by applying PK modeling.

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