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Short communication

Small blood volumes from children for quantitative sotalol determination using high-performance liquid chromatography

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Abstract

A sensitive high-performance liquid chromatographic method using fluorescence detection has been developed for sotalol determination in small plasma samples of children and newborns with limited blood volume. In sample sizes of 100 μl of plasma, sotalol was extracted using an internal standard and solid-phase extraction columns. Chromatographic separation was performed on a Spherisorb C_6 column of 150×4.6 mm I.D. and 5 μm particle size at ambient temperature. The mobile phase consisted of acetonitrile–15 m*M* potassium phosphate buffer (pH 3.0) (70:30, v/v). The excitation wavelength was set at 235 nm, emission at 300 nm. The flow-rate was 1 ml/min. Sotalol and the internal standard atenolol showed recoveries of 107±8.9 and 97±8.1%, respectively. The linearity range for sotalol was between 0.07 and 5.75 μg/ml, the limit of quantitation 0.09 μg/ml. Precision values expressed as percent relative standard deviation of intra-assay varied between 0.6 and 13.6%, that of inter-assay between 2.4 and 14.4%. Accuracy varied between 86.1 and 109.8% (intra-assay) and 95.4 and 103.3% (inter-assay). Other clinically used antiarrhythmic drugs did not interfere. As an application of the assay, sotalol plasma concentrations in a 6-year-old child with supraventricular tachycardia treated with oral sotalol (3.2 mg/kg per day) are reported.

Keywords: Sotalol

1. Introduction

Sotalol is a non-selective β-receptor blocker with additional class III properties which is used for the treatment of supraventricular and ventricular arrhythmias in adults and children [1,2]. Pediatric patients treated with sotalol are more likely to develop proarrhythmic effects (e.g., torsades de pointes) or bradycardia than adults probably due to inadequate dosing [3]. Dosing regimens for children are entirely based empirically and extrapolated from adult studies [2] because pharmacokinetic investigations in children or newborns have not been performed. Analytical methods which meet the special needs for quantitative determination of sotalol in small blood volumes are missing. Various methods for the determination of sotalol in human plasma have been reported in the literature using high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detection [4–10]. All of these methods are validated and adapted for pharmacokinetic investigations in adults using 300 to

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1000 μl of plasma. Given the increasing clinical importance and widespread use of sotalol in children, we developed a sensitive and quantitative HPLC method with high accuracy and precision to measure sotalol in small sample volumes. We used plasma samples of only 100 μl. This allows us to perform profiles of sotalol plasma concentrations to characterise absorption, distribution and elimination of sotalol in children, infants and newborns. As a result dosing regimens for these age groups will be recommendable in future.

2. Experimental

2.1. Materials

HPLC-grade acetonitrile and methanol was purchased from E. Merck (Darmstadt, Germany). Boric acid, *o*-phosphoric acid, potassium hydroxide, *R,S*-sotalol and atenolol were supplied by Sigma (St. Louis, MO, USA). All other chemicals were analytical-reagent grade.

2.2. Extraction procedure

For determination of sotalol, the internal standard atenolol (5.21 μg/ml) was added to 100 μl of plasma and alkalinised with 500 μl 0.05 *M* borate buffer, which was made out of 3.09 g boric acid dissolved in 1 l of double distilled water and adjusted to a pH of 9.0 with an aqueous solution of potassium hydroxide (3 *M*). Simultaneously, sotalol-free plasma (Department of Transfusion Medicine, Universitätsklinikum Hamburg–Eppendorf) was fortified with 100 μl of a known sotalol standard (5.75 μg/ml), 100 μl of atenolol standard (5.21 μg/ml) and 500 μl of borate buffer (pH 9.0). The samples were vortex-mixed and centrifuged at 4°C at 2700 *g* for 20 min. The supernatant was percolated slowly through Bond-Elut cartridges packed with C_8-bound silica particles of 40 μm (100 mg/ml of column volume; Analytichem International, Harbor City, CA, USA) under vacuum (Vac-Elut vacuum manifold; Chromatographie Service, Langerwehe, Germany). Columns were washed twice with 1 ml of double distilled water. Finally, sotalol and atenolol were eluted twice with 400 μl of methanol, evaporated to dryness under a stream of nitrogen at 40°C and reconstituted with 200 μl of acetonitrile–15 m*M* potassium dihydrogenphosphate buffer (pH 3.0) (70:30, v/v) which was made out of 1.47 g *o*-phosphoric acid dissolved in 1 l of double distilled water and adjusted to a pH of 3.0 with an aqueous solution of potassium hydroxide (3 *M*).

2.3. High-performance liquid chromatography

A HPLC system LC Workstation Class LC10 (Shimadzu, Kyoto, Japan) consisting of a SIL-10A autoinjector, an LC-10AT liquid chromatograph, an RF-10A spectrofluorometric detector, and software provided by the manufacturer, was used. Chromatographic separation was performed on a Spherisorb C_6 column (150×4.6 mm I.D., 5 μm particle size; Chromatographie Service) with a Spherisorb C_6 guard column (17×4 mm I.D., 5 μm particle size) at ambient temperature. The mobile phase consisted of acetonitrile–15 m*M* potassium phosphate buffer (pH 3.0) (70:30, v/v). The excitation wavelength was set at 235 nm, emission was measured at 300 nm. The flow-rate was 1 ml/min. Aliquots of the extracts (100 μl) were injected into the HPLC system. Sotalol was quantified by relating the peak height ratio of sotalol and the internal standard atenolol in the unknown sample to the peak height ratio of a known standard concentration.

2.4. Standards

Stock solutions of 5.75 μg/ml sotalol and 5.21 μg/ml atenolol were made up in double distilled water. Aliquots of the sotalol stock solution were diluted and added to drug-free plasma for obtaining standard curves over the range of 0.04 to 5.75 μg/ml and in the presence of 5.21 μg/ml internal standard. Other stock solutions were stored at −20°C until assay. They remained stable for at least 6 months.

2.5. Data analysis and statistics

Data are given as arithmetic means±standard deviation (SD). Intra- and inter-assay variation was determined by replicate analysis of samples at a sotalol concentration of 0.09, 0.13, 0.18, 0.36, 1.44 and 5.75 μg/ml. The precision was expressed as

relative standard deviation (RSD). The accuracy is expressed as percentage of the sotalol concentration measured in each sample relative to the known amount of sotalol added.

2.6. Application

Sotalol was determined in plasma samples taken from a 6-year-old patient treated with sotalol for supraventricular tachycardia. Eight blood samples were drawn before and 1, 2, 3, 4, 6, 8 and 12 h after oral administration of a single 30 mg sotalol dose. The 6-year-old patient weighed 22.1 kg and was treated with 70 mg of sotalol per day (3.2 mg/kg per day) divided into three daily doses of 30–20–20 mg sotalol. Treatment at this dosing regimen had been performed for a minimum of 3 days and could be assumed as steady state on the basis of pharmacokinetic parameters in adults.

This procedure had been approved by the local Ethics Committee (Ethik-Kommission der Ärztekammer Hamburg, No. 1448). The pharmacokinetic parameters of sotalol were determined by compartmental pharmacokinetic analysis. Non-linear regression modelling was used to fit the measured sotalol plasma concentrations to a one-compartment model with first-order input. Oral clearance, volume of distribution and terminal half-life were calculated form the model-dependent parameters, using the software package KINETICA v2.0 from Innaphase, Champs-sur-Marne, France.

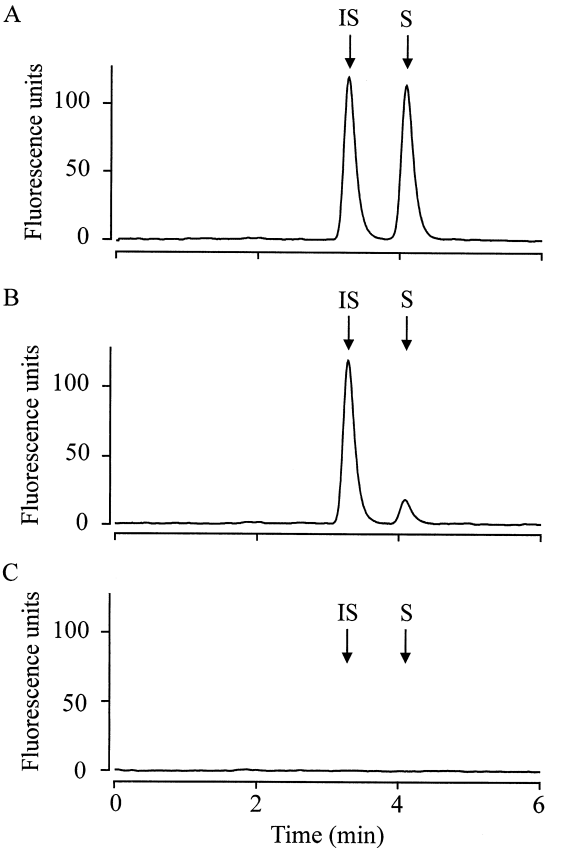

Fig. 1. Representative chromatograms of (A) plasma fortified with 5.21 μg/ml internal standard atenolol (I.S.) and 5.75 μg/ml sotalol (S), (B) a plasma sample of a 6-year-old patient 4 h after oral administration of 30 mg sotalol and a concentration of 0.86 μg/ml sotalol, (C) blank plasma without addition of sotalol or internal standard.

3. Results and discussion

3.1. Assay characteristics

Fig. 1A shows a representative chromatogram of blank plasma fortified with 5.75 μg/ml sotalol and 5.21 μg/ml atenolol. Fig. 1B represents a chromatogram of extracts from a 6-year-old patient with supraventricular tachycardia 4 h after administration of 30 mg sotalol at steady state (more than 3 days of sotalol treatment). Sotalol concentration was determined to 0.86 μg/ml. Fig. 1C shows a representative chromatogram of blank plasma from a healthy volunteer without sotalol medication and without addition of the internal standard. In all chromatograms, no interferences with other clinically used antiarrhythmic drugs like digoxin, flecainide or propafenone could be found.

3.2. Limits of quantitation, calibration curves and recoveries

The limit of quantitation (LOQ) of sotalol was determined at 0.09 μg/ml in this study and is similar to values previously reported at 0.08 μg/ml in 300 μl of human plasma [5]. In the present study we only used 100 μl of plasma which allows us to measure sotalol concentrations in small sample volumes from

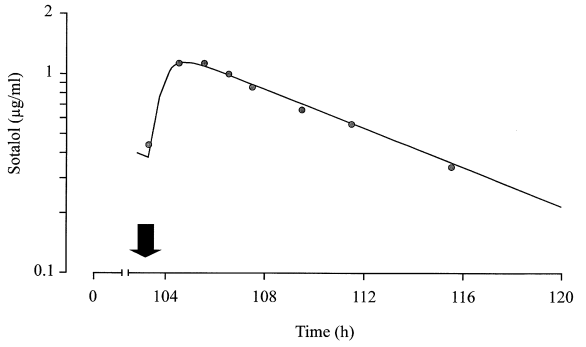

Fig. 2. Plasma concentrations of sotalol (·) in a 6-year-old patient after oral administration of 30 mg sotalol under steady state conditions (minimum 3 days of treatment) and a daily dose of 3.2 mg/kg per day. The solid line represents fitted plasma concentrations according to a one compartment model using KINETICA v2.0, Innaphase, Champs-sur-Marne, France. The application time point of oral sotalol is indicated by the arrow.

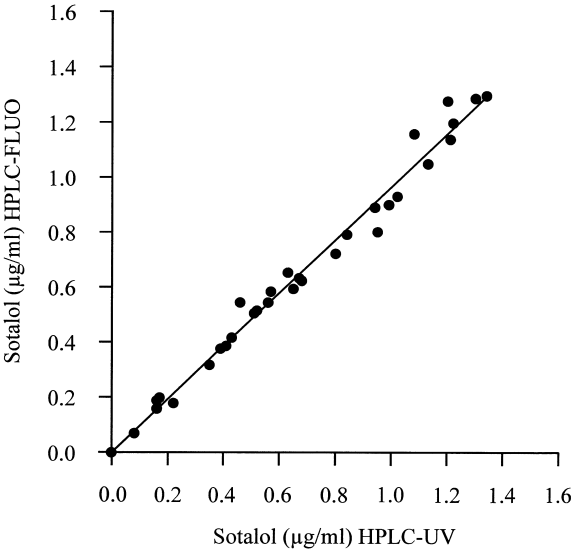

Fig. 3. Sotalol plasma concentrations from healthy volunteers after oral administration of 160 mg sotalol. Extraction of sotalol from 500 μl plasma and UV detection (HPLC–UV) [11] was correlated with extraction of sotalol from 100 μl plasma and fluorescence detection (HPLC–FL). Correlation coefficient was $r=0.993$, the slope $m=0.974$ and the intercept $b=0.0015$ in 35 plasma samples ($n=35$).

children treated with oral sotalol therapy (Fig. 1B and Fig. 2). We used fluorimetric detection which reduces the noise when compared to UV detection and in addition we cleaned up the plasma samples with solid-phase columns. Thus, the analytical procedure for sotalol established in this study can use only small amounts of biological specimens without loosing the sensitivity to detect sotalol concentrations needed to monitor patients. The cross-validation was performed with an established HPLC method using UV detection [4] and plasma samples from healthy volunteers after sotalol administration of 160 mg [7]. Sotalol plasma concentrations determined by HPLC–UV showed good correlation with concentrations determined by HPLC and fluorescence detection (FL). The correlation coefficient was $r=0.993$, the slope 0.974 and the intercept 0.0015 (Fig. 3).

The calibration curves were prepared by the addition of sotalol to blank plasma samples. The standard curve for sotalol was linear over the concentration range of 0.07 to 5.75 μg/ml ($r=0.999$). The limit of detection was 0.07 μg/ml based on a signal-to-noise ratio of 3:1. At LOQ, sotalol showed accuracy values between 86.1 and 109.8% and a precision expressed as RSD between 7.7 and 13.6% (Table 1). Precision throughout the whole working range was between 0.6 and 13.6%. The recoveries of sotalol were between 121 and 89.5% over the whole linear range. Precision and accuracy were adequate for sufficient pharmacokinetic analyses and were comparable to previously reported methods [4–10].

3.3. Application

This HPLC method was used to determine sotalol plasma concentrations in paediatric patients with tachycardia. Sotalol could be detected within 100 μl of plasma at 0, 1, 2, 3, 4, 6, 8, 12 h after oral administration of 30 mg sotalol to a 6-year-old child at concentrations of 0.44, 1.13, 1.13, 1.00, 0.86, 0.66, 0.56 and 0.34 μg/ml. The child was treated with a daily dose of 3.2 mg/kg per day typically used for the treatment of tachycardia. The maximal sotalol concentrations (C_{max}) were determined to 1.13 μg/ml and were reached 1 and 2 h (T_{max}) after sotalol administration. Pharmacokinetic analysis was performed and terminal half-life was calculated to 6.2 h, oral clearance of sotalol to 2.63 ml/(min kg) and the volume of distribution to 1.43 l/kg.

Table 1
Intra- and inter-assay accuracy and precision of sotalol in 100 μl of human plasma samples

	Nominal concentrations of sotalol in plasma (μg/ml)					
	0.09	0.13	0.18	0.36	1.44	5.75
Concentration found (arithm. mean value) (μg/ml)						
Day 1 (n=3)	0.08	0.14	0.17	0.34	1.43	5.80
Day 2 (n=3)	0.08	0.14	0.19	0.34	1.36	6.11
Day 3 (n=3)	0.10	0.13	0.18	0.36	1.40	5.91
Inter-assay (n=9)	0.09	0.14	0.18	0.34	1.39	5.94
Precision (arithm. mean value) (%)						
Day 1 (n=3)	7.7	9.2	1.8	4.4	1.6	1.3
Day 2 (n=3)	3.6	6.2	1.1	1.8	3.1	0.5
Day 3 (n=3)	13.6	4.9	1.0	2.9	0.6	1.2
Inter-assay (n=9)	14.4	6.9	4.3	3.9	2.8	2.4
Accuracy (arithm. mean value) (%)						
Day 1 (n=3)	86.1	105.8	95.2	94.5	99.1	100.9
Day 2 (n=3)	90.4	107.9	104.5	95.5	94.2	106.2
Day 3 (n=3)	109.8	100.5	98.7	100.2	97.3	102.8
Inter-assay (n=9)	95.4	105.2	99.5	96.7	96.9	103.3

4. Conclusion

In summary, the HPLC method reported here offers a technique to quantify sotalol concentrations in small blood volumes of pediatric patients with good accuracy and precision. This offers the possibility to take sufficient numbers of blood samples for pharmacokinetic data in children and newborns and to delineate dosing guidelines.

Acknowledgements

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