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Note

Determination of the beta-adrenoceptor blocking drug sotalol in plasma and tissues of the rat by high-performance liquid chromatography with ultraviolet detection

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Sotalol hydrochloride {4-[1-hydroxy-2-(isopropylamino)ethyl]methanesulphonanilide hydrochloride, Fig. 1} is a β-adrenoceptor blocking drug which differs in molecular structure and ionic character from the other β-blockers in use, e.g. propranolol, metoprolol and atenolol, the high-performance liquid chromatographic (HPLC) analysis of which we described earlier [1]. Sotalol has an arylethanolamine structure similar to that of the β-receptor agonist isoprenaline, differing only by the aryl substituent, i.e. sotalol has a methanesulphonamide group instead of two hydroxyl groups. Furthermore, the acidic nitrogen of the metanesulphonamide group (pK_a = 8.3) and the basic nitrogen of the amino group in the side-chain (pK_a = 9.8) yield the zwitterionic character of the sotalol molecule. This property requires modifications in the extraction procedure as described for the other β-blockers mentioned above [1].

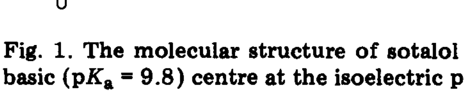

Fig. 1. The molecular structure of sotalol and the charges of the acidic (pK_a = 8.3) and the basic (pK_a = 9.8) centre at the isoelectric point (pH 9.0).

Different methods have been described for measuring sotalol concentrations in biological fluids. Garrett and Schnell [2] reported a fluorimetric assay, Walle [3] a gas—liquid chromatographic method, and Lefebvre and co-workers [4, 5] an HPLC method with fluorimetric detection. However, no method was described for the determination of sotalol levels in tissues. Since we are interested in the pharmacokinetic behaviour of different β-adrenoceptor blocking drugs in plasma and in tissues [6—8], which are target organs for their pharmacological effects, an HPLC method with ultraviolet (UV) detection for sotalol was developed in order to be able to quantify this hydrophilic compound in plasma as well as in organs.

EXPERIMENTAL

Standards and reagents

All reagents used were reagent grade and purchased from E. Merck (Darmstadt, F.R.G.). Only deionized glass-distilled water was used. The racemic mixture of sotalol · HCl was kindly supplied by Bristol-Myers (Bergisch-Gladbach, F.R.G.).

Standard solutions from 2 mg/ml to 10 ng/ml were obtained from an aqueous stock solution (2 mg/ml). This solution was stored at 4°C for up to one month.

HPLC instrumentation and conditions

The HPLC system consisted of a constant-flow pump (Gynkotek, 600/200), an autosampler (Waters, WISP 710 B), a reversed-phase column (Knauer, 10 cm × 4 mm; Shandon ODS 5 μm), a spectrophotometer with a deuterium lamp (Kratos, Spectroflow 773) and a computing integrator (Spectra Physics, SP 4100).

The mobile phase was methanol—water—acetonitrile (55:45:20) containing 1% acetic acid and 0.005 *M* of dodecyl sodium sulphate. The flow-rate was 1 ml/min and the monochromator of the spectrophotometer was set at 227 nm. The spectrophotometer was connected to the integrator; areas were measured. Chromatography was carried out at ambient temperature.

Sample preparation

Plasma and tissue samples of five organs (heart, muscle, lung, liver, kidney) from light—dark synchronized male Wistar rats of about 150—180 g body weight were used. The rats were sacrificed by decapitation, and blood was collected in 12-ml conical glass tubes containing 50 μl of heparin (250 I.U.). After centrifugation (900 *g*, 15 min), 1-ml plasma portions were pipetted into 12-ml screw-capped glass tubes and kept at —35°C. The organs were dissected out, rinsed in ice-cold 0.9% saline solution, blotted on filter paper, weighed, frozen in liquid nitrogen and finally stored at —35°C.

Plasma extraction

The extraction procedure is summarized in Fig. 2. The 1-ml plasma samples were thawed and 2 ml of acetonitrile were added. After vortexing, then centrifuging for 10 min at 800 *g*, 2.5 ml of each supernatant were transferred to 12-ml screw-capped glass tubes and evaporated to dryness under a stream of dry nitrogen. The residues were redissolved in 1 ml of buffer (0.5 *M* borate buffer, pH 9.0) by vortexing. Then 6 ml of extraction mixture (benzyl alcohol—chloroform, 60:40) were added, mixed by shaking for 10 min and separated by centrifugation for 10 min at 800 *g*. Aliquots of 5 ml of the organic layer

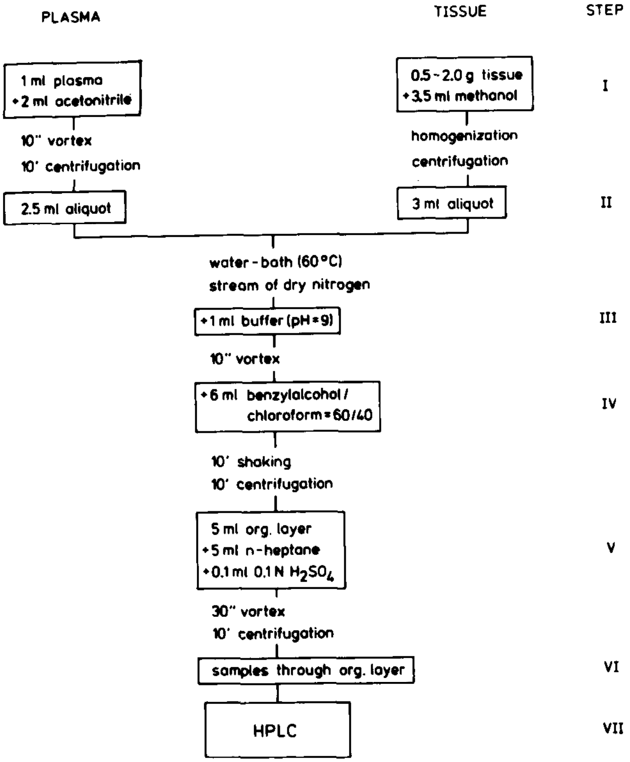

Fig. 2. Schematic outline of the extraction procedure.

were transferred to 12-ml screw-capped glass tubes; 5 ml of *n*-heptane and 0.1 ml of 0.05 *M* sulphuric acid were added, and the tubes were vortexed for 30 sec, then centrifuged for 10 min at 800 *g*. Finally, aliquots of the aqueous layer were injected into the HPLC system.

Tissue extraction

The extraction procedure is summarized in Fig. 2. To each sample of frozen tissue (0.5—2.0 g wet weight) 3.5 ml of methanol were added. The samples were homogenized with an Ultra-Turrax homogenizer in glass tubes at 4°C. After centrifugation at 7000 *g* for 10 min, 3 ml of each supernatant were transferred to 12-ml screw-capped glass tubes and evaporated to dryness under a stream of dry nitrogen. The further procedure was as described for the plasma samples (see Fig. 2).

Standard curves and recovery studies

Blank plasma and tissue samples from untreated rats were spiked with varying amounts of sotalol. These samples were treated as described above and standard curves of added concentrations versus peak area were calculated. From these data recovery in each organ was calculated over the whole concentration range. Sotalol plasma and tissue concentrations ex vivo were determined in the respective tissues after 60, 110, 160 and 210 min after intravenous injection of 6 μmol/kg sotalol · HCl in rats.

RESULTS AND DISCUSSION

The aim of the present investigation was to develop a method to be able to quantify sotalol concentrations in organs as well as in plasma, the determination of which is necessary for pharmacokinetic studies. The methods described by Garrett and Schnell [2] and Lefebvre and co-workers [4, 5] are similar as regards the extraction procedure. Using HPLC with fluorimetric detection, Lefebvre and co-workers [4, 5] succeeded in lowering the detection limit of sotalol to 20 ng/ml of plasma, whereas Garrett and Schnell [2] achieved a detection limit of 100 ng/ml ten years ago when using only a spectrofluorimetric method without HPLC. In preliminary experiments we were unable to reproduce a recovery of 60% (as calculated from refs. 4 and 5) from plasma with the extraction procedure described by Lefebvre et al. [4]; only a recovery of 38% for plasma and even less for tissue was obtained. Since, for the intended pharmacokinetic studies, extraction of sotalol from tissues is the most crucial point, a new HPLC method with UV detection for sotalol was developed by which low drug concentrations could be detected and reproducibly quantified in plasma as well as in various tissues.

In order to optimize the extraction procedure and to increase the recovery from tissues, various solvents were tested in preliminary studies. The extraction procedure finally adopted is depicted in Fig. 2. To precipitate the protein, methanol or acetonitrile was used (Step I), instead of perchloric acid as described for sotalol by Lefebvre et al. [4, 5] and Garrett and Schnell [2], since the samples had to be evaporated (Steps II and III). Methanol was a better solvent for homogenizing the tissue samples while acetonitrile yielded a better precipitate of the plasma proteins (Step I).

The mixture used for extraction of the redissolved sotalol from buffer (Step IV) was the result of a study on the extraction behaviour of 13 solvents and 48 binary mixtures of these solvents [9]. The best results concerning extraction (Steps IV and V) and back-extraction (Steps V and VI) into 0.05 *M* sulphuric acid were obtained by a mixture of 60 parts of benzyl alcohol and 40 parts of chloroform, with which about 69% of sotalol was extracted from the buffer solution (pH 9; Step III) into the aqueous layer (Step VI). The extraction ratio from buffer (pH 9) into the organic layer (Steps III and IV) was 74% and the ratio of the back-extraction from this layer into 0.05 *M* sulphuric acid

TABLE I

DATA FOR SOTALOL OBTAINED FROM SPIKED PLASMA AND TISSUE SAMPLES

Organ	No. of samples	Concentration* (ng/sample)	Correlation coefficient	Recovery (%) (mean ± S.E.M.)
Plasma	12	44— 880	0.999	58.4 ± 2.2
Heart	12	88—1760	0.999	41.5 ± 2.6
Muscle	12	88—1760	0.981	43.9 ± 1.7
Lung	12	88—1760	0.997	38.4 ± 1.7
Liver	12	88—1760	0.994	36.1 ± 0.8
Kidney	12	88—1760	0.999	50.5 ± 2.1

*Depending on the plasma and tissue concentrations expected in the pharmacokinetic studies.

(Steps V and VI) was 93%. Thus, the above-mentioned 69% of total recovery from buffer resulted. When plasma samples were spiked, an extraction ratio of about 60% was obtained (Table I). At Step V of the extraction procedure addition of an equivalent volume of *n*-heptane was useful to change the density of the organic layer; thus about 90% of the aqueous phase could be taken by a syringe through the organic layer (Step VI). Addition of *n*-heptane had no effect on the recovery. In accordance with Lefebvre et al. [5], sulphuric acid was superior to hydrochloric acid in the ratio of the back-extraction of sotalol.

The mobile phase used was a ternary mixture of methanol, water and acetonitrile containing acetic acid and counter-ion which proved to be the best of various mixtures studied. Addition of acetonitrile (20%) to the mobile phase decreased the retention time of sotalol.

The HPLC method described has been successfully used to study the kinetic behaviour of sotalol after a single application of the drug (6 μmol/kg, intravenously) in plasma and five organs of the light—dark synchronized rat. Fig. 3 shows chromatograms obtained from blank, spiked (44—440 ng/ml, depending on the plasma concentrations expected in the pharmacokinetic studies) and ex vivo plasma samples (60, 110, 160 and 210 min after application). As can be seen, the sotalol peak is well separated from the other peaks and at least 10 ng/ml could be detected. The correlation coefficients between drug concentrations and peak areas were greater than 0.99 for plasma and all organs except muscle for which a correlation coefficient of 0.98 was calculated (Table I). The recovery was 58% from plasma and 36—50% from the organs (Table I). Representative chromatograms of blank and ex vivo samples of all five organs are depicted in Fig. 4. In tissues sotalol concentrations of at least

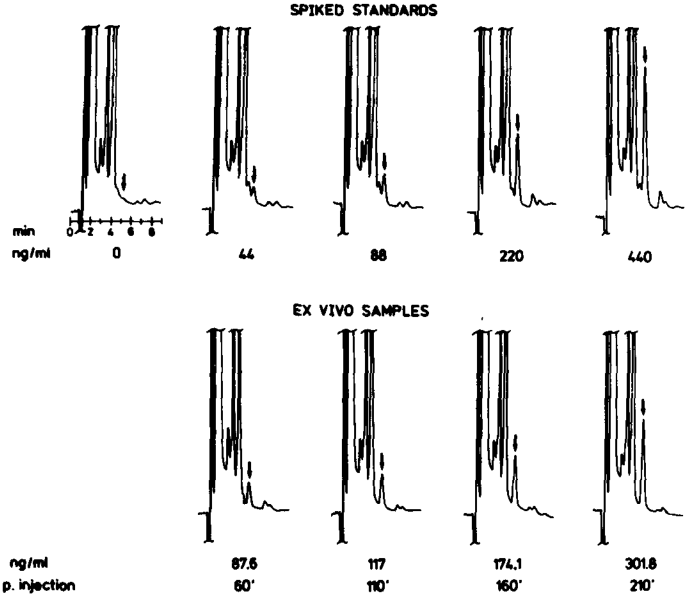

Fig. 3. Representative chromatograms of sotalol in plasma samples. Upper panel shows spiked plasma samples in which sotalol was added at 0—440 ng/ml. Lower panel shows chromatograms of ex vivo samples; sotalol was injected intravenously into rats (6 μmol/kg), and plasma samples were taken 60—210 min after drug application. The concentrations obtained are indicated.

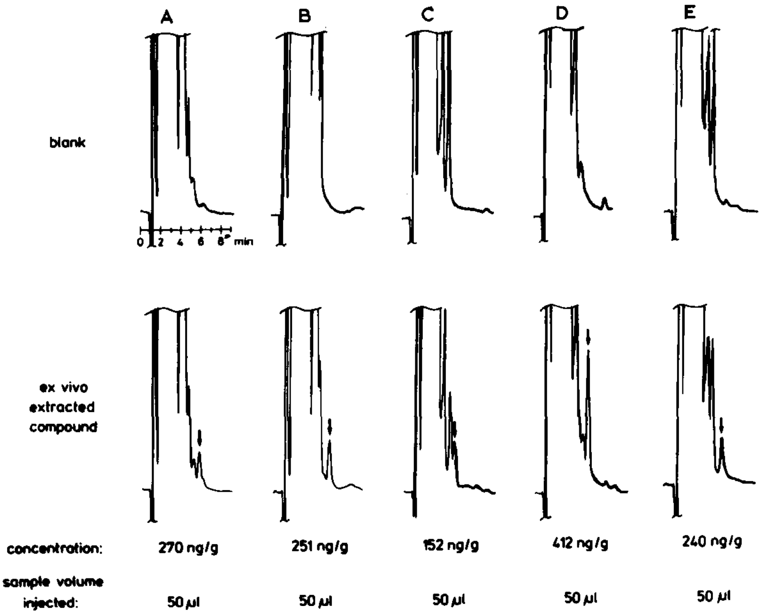

Fig. 4. Representative chromatograms of sotalol in heart (A), muscle (B), lung (C), liver (D) and kidney (E). Upper panel shows blank tissue samples of the respective organs from untreated rats. Lower panel shows the corresponding ex vivo samples (6 µmol/kg, intravenously) which were isolated 210 min after drug application. The concentrations obtained are indicated.

40 ng/g could be measured. As can be seen, there is no baseline separation of the sotalol peak in lung tissue, but the separation allowed sotalol concentrations to be determined in this organ even 210 min after drug application.

In conclusion, the HPLC method presented here provides the applicability of a liquid—liquid extraction of a zwitterionic compound and the short-wave UV detection of this compound within a pharmacokinetic study not only in plasma but also in such target organs as heart, muscle, lung, liver and kidney.

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