Journal of Chromatography, 309 (1984) 193—197
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2126

Note

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

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(Received January 13th, 1984)

Bupranolol [1-chloro-2-(2-hydroxy-3-*tert.*-butylamino)propoxy-4-methylbenzene, Fig. 1] is the only β-adrenoceptor blocker with a chlorine substituent in the molecule. Until now, no methods have been available for the quantitative determination of bupranolol in plasma and tissues. On account of the chlorine atom, bupranolol does not fluoresce when activated at about 220 or 280 nm, as demonstrated for other β-blockers and which can easily be measured and quantified by spectrofluorimetric detection following high-performance liquid chromatographic (HPLC) separation (see, for example, refs. 1—3). Since we are interested in the structure—activity relationship of β-blockers of different polarities including their pharmacokinetic behaviour [4, 5] an HPLC method with ultraviolet (UV) detection at 200 nm for bupranolol was developed to be able to determine plasma and tissue levels of this lipophilic compound.

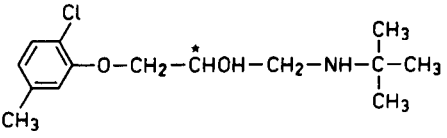

Fig. 1. The molecular structure of bupranolol.

EXPERIMENTAL

Standards and reagents

All reagents used were at least reagent grade and purchased from E. Merck (Darmstadt, F.R.G.). Only deionized glass-distilled water was used. The racemic mixture of bupranolol · HCl was kindly supplied by Sanol Schwarz (Monheim, 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.

0378-4347/84/$03.00

HPLC instrumentation and conditions

The HPLC system consisted of a constant-flow pump (Gynkotek, 600/200), an autosampler (Waters, WISP 710 B), a cyano column (Bischoff, 12.5 cm × 4.6 mm; Shandon CPS 5 μm), a spectrophotometer with a deuterium lamp (Kratos, Spectroflow 773) and a computing integrator (Spectra Physics, SP 4100).

The mobile phase was acetonitrile—water (70:30) containing 0.1% phosphoric acid. The flow-rate was 1.5 ml/min and the detection wavelength 200 nm. Chromatography was carried out at ambient temperature.

Sample preparation

Plasma and tissue samples of four organs (heart, muscle, brain, lung) 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

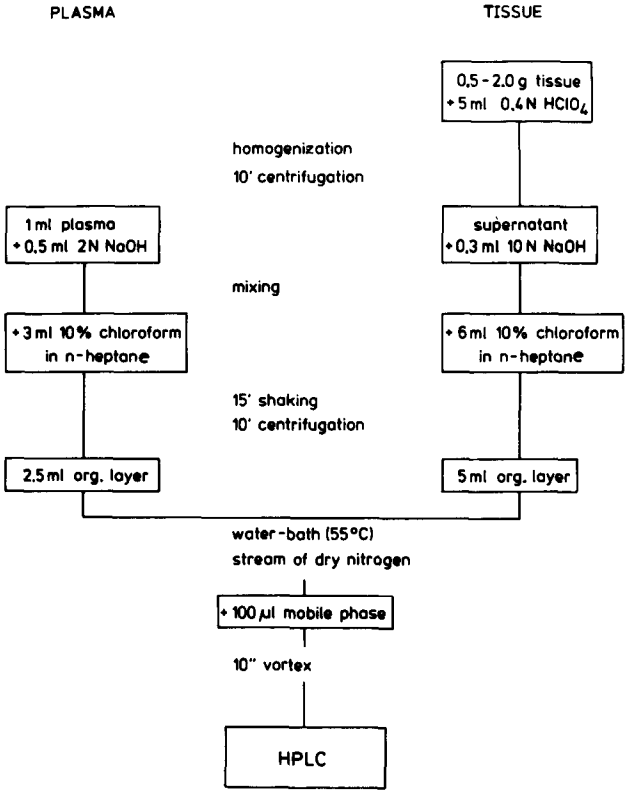

Fig. 2. A schematic outline of the extraction procedure.

were thawed and 0.5 ml of 2 *M* sodium hydroxide (saturated with sodium chloride) was added. Then 3 ml of a mixture of 10% chloroform in *n*-heptane were added; the tubes were shaken for 15 min and the layers separated by centrifugation. Aliquots of 2.5 ml of the organic layer were transferred to 12-ml screw-capped conical glass tubes and evaporated in a water bath at 55°C under a stream of dry nitrogen. After cooling the dry tubes in an ice bath, 100 μl of the mobile phase were added to redissolve the residue. The tubes were vortexed for 15 sec and aliquots (50—80 μl) were injected into the HPLC system.

Tissue extraction

The extraction procedure is summarized in Fig. 2. The frozen tissues (0.5—2.0 g wet weight) were homogenized in 5 ml of 0.4 *M* perchloric acid (saturated with sodium chloride) with an Ultra-Turrax homogenizer in glass tubes at 4°C. After centrifugation at 7000 *g* for 10 min, the supernatants were transferred to 12-ml screw-capped glass tubes containing 0.3 ml of 10 *M* sodium hydroxide and vortexed for 10 sec. Then 6 ml of the extraction mixture (10% chloroform in *n*-heptane) were added; the samples were shaken for 15 min and centrifuged for 10 min at 800 *g*. Aliquots of 5 ml of the organic layer were transferred to 12-ml screw-capped conical glass tubes. The further extraction was as described for the plasma samples (Fig. 2).

Standard curves and recovery studies

Blank plasma and tissue samples from untreated rats were spiked with varying amounts of bupranolol. 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.

Bupranolol plasma and tissue concentrations ex vivo were determined in the respective tissues 60, 90, 120 and 150 min after the intravenous injection of 6 μmol/kg bupranolol · HCl to rats.

RESULTS AND DISCUSSION

The HPLC system described provides for the first time a method of determining bupranolol concentrations not only in plasma but also in target organs such as heart, muscle, brain and lung, which are important when studying the kinetic behaviour of β-adrenoceptor blocking drugs [3—5].

Fig. 3 shows chromatograms of blank and spiked plasma samples from untreated rats and ex vivo plasma samples. As can be seen the extraction procedure employed yielded clean samples without interfering peaks in the chromatograms measured at 200 nm. Concentrations as low as 1 ng/ml plasma could be quantified. Chromatograms of blank and ex vivo samples of bupranolol in the four organs studied are depicted in Fig. 4. The chromatograms of the tissue samples also do not show any interfering peaks. The detection limit from tissue was about 10 ng/g.

The data obtained from spiked standard samples are shown in Table I. As can be seen, the correlation coefficients for plasma and all organs were greater

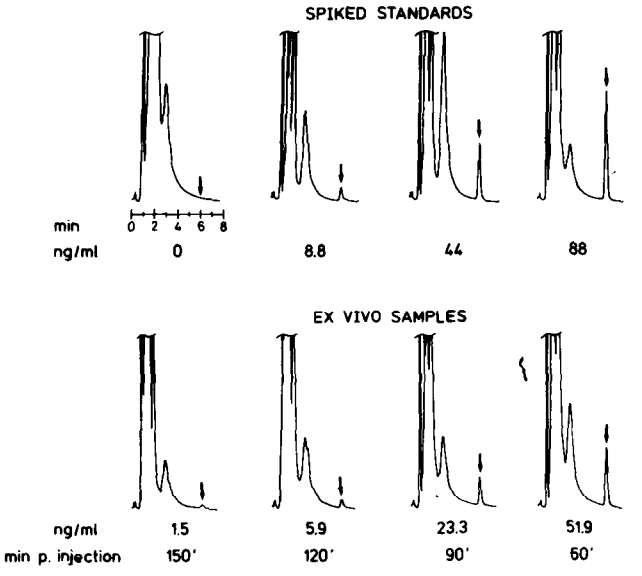

Fig. 3. Chromatograms of bupranolol plasma samples. Upper panel shows spiked plasma samples in which bupranolol was added at 0—88 ng/ml. Lower panel shows chromatograms of ex vivo samples; bupranolol was injected intravenously into rats (6 μmol/kg) and plasma samples were taken 60—150 min after drug application. The concentrations obtained are indicated.

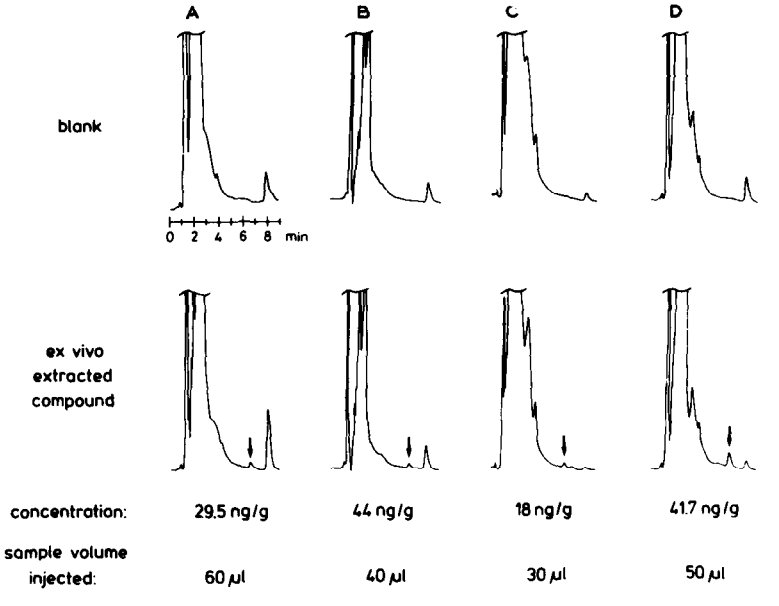

Fig. 4. Representative chromatograms of bupranolol in heart (A), muscle (B), brain (C) and lung (D). Upper panel shows blank tissue samples of the respective organ from untreated rats. Lower panel shows the corresponding ex vivo samples which were isolated 150 min after drug application (6 μmol/kg). The concentrations obtained are indicated.

TABLE I

DATA FOR BUPRANOLOL OBTAINED FROM SPIKED PLASMA AND TISSUE SAMPLES

Organ	No. of samples	Concentration* (ng/sample)	Correlation coefficient	Recovery (%) (mean ± S.E.M.)
Plasma	10	8.8—264.0	0.999	96.6 ± 0.8
Heart	10	17.6—52.8	0.988	26.8 ± 2.3
Muscle	10	8.8—264.0	0.992	21.5 ± 1.8
Lung	10	44.0—880.0	0.995	24.9 ± 1.7
Brain	10	88.0—1320.0	0.995	26.5 ± 2.4

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

than 0.98. The recoveries achieved were 97% from plasma and 22—27% from the organs. The difference in the recoveries from plasma compared to those from the tissues is similar to the difference we found when studying the kinetic behaviour of propranolol [1, 2], which is a β-blocker of nearly the same polarity as bupranolol. These lipophilic compounds have a strong affinity for tissue membranes which results in a lower recovery from tissues than from plasma. A dependency of the bupranolol recovery upon the amount of tissue extracted as described for propranolol [1, 2] could not be observed.

In conclusion, the HPLC system presented for the determination of bupranolol concentrations has shown its applicability within a study on the kinetic behaviour of this drug (6 μmol/kg, intravenously) in plasma and four organs of the rat [4]. It has been shown that it is possible to quantify substances in biological material by short-wavelength UV detection without difficulty.

ACKNOWLEDGEMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Le 318/8-2—318/8-4). The technical assistance of U. Schmidt is gratefully acknowledged.

REFERENCES

1 H. Winkler, W. Ried and B. Lemmer, J. Chromatogr., 228 (1982) 223.
2 H. Winkler, Thesis, Frankfurt, 1983.
3 B. Lemmer and K. Bathe, J. Cardiovasc. Pharmacol., 4 (1982) 635.
4 B. Lemmer, H. Winkler and G. Neumann, Naunyn-Schmiedeberg's Arch. Pharmacol., 321 (1982) R20.
5 B. Lemmer, K. Bathe, P.H. Lang, G. Neumann and H. Winkler, J. Amer. Coll. Toxicol., 2 (1983) 347.