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Letter to the Editor

Determination of atenolol in plasma by high-performance liquid chromatography with application to single-dose pharmacokinetics

Sir,

Atenolol is a beta-receptor antagonist used in the treatment of hypertension, angina pectoris, and arrhythmias [1]. Atenolol may be detected in plasma by gas chromatography with derivatization [2], thin-layer chromatography [3] and high-performance liquid chromatography (HPLC) with ultraviolet or spectrofluorometric detection [4—10]. The HPLC methods reported in humans use solid-phase extractions or liquid extractions with clean-up steps, and are limited in sensitivity. We describe a method that is sensitive to 5 ng/ml and specific for atenolol. The method is simple, inexpensive, and rapid, due to the straightforward extraction procedure and short chromatographic retention times.

EXPERIMENTAL

Materials

Pure samples of atenolol and the internal standard, metoprolol, were provided by Stuart Pharmaceuticals and Ciba-Geigy, respectively. All other reagents, analytical-reagent grade or better, were purchased from commercial sources and used without further purification. Mobile phase components were filtered prior to and after mixing.

Apparatus and chromatographic conditions

The HPLC system consisted of a Waters solvent delivery system and a Perkin-Elmer 650-105 spectrofluorometer operated at excitation 280 nm and emission 300 nm (slit widths 10 and 13 nm, respectively). The separation system was a 25 cm × 3.9 mm stainless steel (10 μm particle size) μBondapak C_{18} reversed-phase column (Waters Assoc.). Samples were injected automatically with a WISP-710 injector (Waters Assoc.). Mobile phase (water—acetonitrile—glacial acetic acid, 50:50:1) was run at a flow-rate of 1.0 ml/min. All analyses were performed at room temperature.

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Preparation of samples

A 50-μl volume of stock solution (1 μg/ml) of metoprolol was added to each of a series of 15-ml glass round-bottom tubes with PTFE-lined screw-top caps. A 1.0 ml sample of unknown plasma was added to each tube. An aliquot of 100 μl 0.5 *M* sodium hydroxide was then added to each tube. Calibration standards for atenolol were prepared by adding 5, 10, 25, 50, 75, 100 and 200 ng of drug to consecutive tubes. Drug-free control plasma was added to each of the calibration tubes. One blank sample, taken from the subject prior to drug administration, was analysed with calibration standards and each set of unknown samples.

Extraction procedure

To each tube were added 2 ml of ethyl acetate. The tubes were agitated gently in the upright position on a vortex mixer for 30 s, then centrifuged at 20°C for 10 min at 400 *g*. The organic layer was transferred to a 15-ml glass conical centrifuge tube and evaporated to dryness at 40°C at moderately reduced pressure. The samples were reconstituted into 0.15 ml mobile phase. Aliquots of 15—50 μl were then injected into the sample loop.

Single-dose pharmacokinetic study

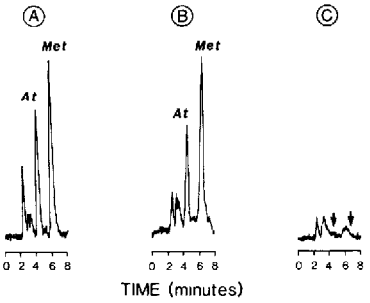
A healthy 40-year-old male volunteer participated. Atenolol (50 mg) was administered orally with water. Multiple venous blood samples were drawn into Venoject heparin-containing tubes over the following 24 h. Concentrations of atenolol were determined by the method described above. Pharmacokinetic analyses were performed by standard methods.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, atenolol and the internal standard, metoprolol gave symmetric well resolved chromatographic peaks (Fig. 1A—C) with retention times 4.2 min for atenolol and 6.2 min for metoprolol. The blank plasma after extraction consistently contains no interfering peaks. The relation between atenolol concentrations and peak height ratio (vs. internal standard) is linear from at least 5 to 300 ng/ml ($y = 107x$). Correlation coefficients are always greater than 0.98. The relative standard deviations, calculated by doing six replicate analyses of known standards (5, 10, 25, 50, 75, 100 and 200 ng/ml), were 3.6% at 5 ng/ml, 5.4% at 10 ng/ml, 6.0% at 25 ng/ml, 4.7% at 50 ng/ml, 3.8% at 75 ng/ml, 3.6% at 100 ng/ml and 5.0% at 200 ng/ml. Minimum detectable concentration was 5 ng/ml (signal-to-noise ratio 3:1). The between-day variability was assessed by inclusion of previously mixed quality-control samples of 50 or 75 ng of drug during extractions and analyses. The relative standard deviations for three analyses each were 4.7% and 3.2% respectively (50.5 ± 2.4 and 71.7 ± 2.3 ng). Extraction efficiency was greater than 90%, as determined by residue analysis.

Fig. 2 shows plasma atenolol concentrations after a single 50-mg oral dose. Kinetic variables were: peak plasma concentration, 123.2 ng/ml; time of peak 1.5 h; elimination half-life, 6.9 h; oral clearance, 79.5 ml/min.

This report describes a reliable, sensitive, and selective method for the



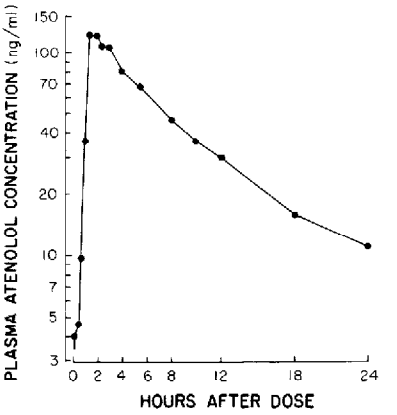


Fig. 1. (A) Chromatogram of an extract of a calibration standard containing atenolol 25 ng/ml and the internal standard, metoprolol 75 ng/ml. (B) Chromatogram of a single plasma sample from a subject taken 12 h after atenolol (50 mg) administration. (C) Chromatogram of a plasma sample taken from a subject prior to atenolol administration. Peaks At. = atenolol; Met. = internal standard, metoprolol

Fig. 2. Plasma atenolol concentrations in a subject following a single oral dose of 50 mg atenolol.

quantitation of atenolol in plasma using HPLC. The method has improved sensitivity and greater simplicity of sample preparation in comparison with other previously published techniques. These methods reliably detect 10—25 ng/ml in humans [4—10], and all require an initial liquid extraction followed by back extraction into a concentrated acid phase, or relatively expensive solid-phase extraction. In contrast, the present method employs a simple basic extraction and concentration into a small volume for auto-injection. This method produces blank plasma samples that are consistently free of contaminants in the areas corresponding to the retention times for atenolol and metoprolol. The method is sensitive to 5 ng/ml, which is sufficient for single-dose pharmacokinetics.

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