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Note

Determination of metoprolol in human blood plasma using high-performance liquid chromatography

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Metoprolol is a cardioselective β-adrenoceptor antagonist which has been shown to be effective in the management of hypertension [1,2], angina pectoris [3], and cardiac arrhythmias [4]. Monitoring the concentration of the drug in blood plasma can be helpful in maintaining optimum dosage [5], and is also needed in pharmacokinetic and some pharmacodynamic studies. Published methods for the determination of metoprolol in plasma include procedures based on gas chromatography [6—8], radioisotopes [9,10], gas chromatography—mass spectrometry [11] and high-performance liquid chromatography (HPLC) [12—16]. The HPLC methods are all based on fluorescence detection and appear useful, but some have significant drawbacks, e.g., lack of use of an internal standard [13,16], interference from other drugs [15], unduly long (26 min) retention times [14], long (1 h) extraction time [14], low drug recovery [12,13], or elaborate derivatization schemes [16].

Some time ago we developed a simple HPLC method for the determination of metoprolol in human plasma, and used it in pharmacological studies [17] of the drug. In this communication, we describe the analytical procedure.

EXPERIMENTAL

Chemicals

Metoprolol tartrate was obtained from Ciba Pharmaceutical (Summit, NJ, U.S.A.), and alprenolol tartrate was purchased from Sigma (St. Louis, MO, U.S.A.). Sodium hydroxide, hydrochloric acid, 0.1 N, and sulfuric acid, 0.1 N, all Baker Analyzed reagent-grade, and phosphoric acid (85%, Fisher Scientific, Pittsburgh, PA, U.S.A.) were obtained from routine chemical suppliers.

Methanol, acetonitrile, and *n*-butyl chloride, all distilled-in-glass grade, were from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Human blood plasma was provided by the Belle Bonfils Memorial Blood Bank of the University of Colorado Health Sciences Center. Water used was twice distilled.

Standards

Stock solutions of metoprolol tartrate and alprenolol tartrate at 50 μg/ml free-base concentration were prepared in 0.01 N hydrochloric acid and were stored at 4—6°C. The working internal standard was prepared by diluting the stock alprenolol solution with 0.01 N hydrochloric acid to a concentration of 500 ng/ml. Metoprolol standards in plasma were obtained by appropriate dilutions of the stock solution with plasma to achieve free-base concentrations of 5, 10, 25, 50, 100, 250 and 500 ng/ml.

Chromatography

A Waters Assoc. (Milford, MA, U.S.A.) Model U6K injector and Model 6000A pump were used in combination with a Hitachi-Perkin-Elmer (Norwalk, CT, U.S.A.) Model 204-A fluorescence detector. The mobile phase, delivered at 2.0 ml/min, was prepared by mixing 150 ml acetonitrile, 300 ml methanol and 550 ml water, and adjusting the acidity of the mixture to pH 3.0 with phosphoric acid. The chromatographic column, 30 × 3.9 mm, contained octadecylsilane reversed-phase (μBondapak C_{18}, Waters) of 10-μm particle size. The detector was operated at 275 and 300 nm excitation and emission wavelengths, respectively.

Procedure

To a 1.0-ml aliquot of the sample (standard or patient plasma) in a 12-ml conical extraction tube were added 0.5 ml of the working internal standard solution, 0.1 ml of 2 N sodium hydroxide and 3.0 ml of *n*-butyl chloride. The mixture was shaken vigorously for 30 sec, followed by centrifugation at 500 g for 2 min. The organic (top) layer was then transferred to another conical tube, and 0.1 ml of 0.1 N sulfuric acid was added. The mixture was mixed on a vortex-type mixer, followed by centrifugation at 500 g for 1 min. Most of the organic (top) layer was removed by aspiration and discarded, and 20 μl of the aqueous layer was injected into the HPLC system.

Calculations

The standard curve was defined by linear-least-squares regression analysis of the data obtained from the spiked plasma samples. The concentration of metoprolol in the unknown samples was determined from the standard curve.

RESULTS

Fig. 1 shows the chromatogram of metoprolol and alprenolol in the HPLC system used. The retention times are: metoprolol, 2.8 min; alprenolol, 6.0 min. The overall recoveries of metoprolol and alprenolol were 74 ± 3% and 74 ± 4%, respectively (n = 5). The method was evaluated over the metoprolol concentration range of 5—500 ng/ml, and was found to be linear, with
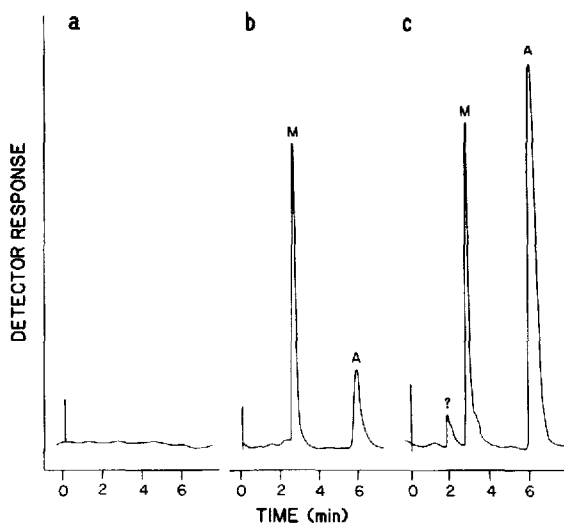
Fig. 1. (a) Chromatogram obtained upon analysis of blank plasma without addition of internal standard; (b) chromatogram of standard metoprolol (M) and alprenolol (A); (c) chromatogram obtained upon analysis of plasma of volunteer receiving metoprolol. Concentration of metoprolol: 79 ng/ml. The unknown peak may be due to α-hydroxy-metoprolol, see Discussion.

metoprolol:alprenolol peak height = 0.010 metoprolol concentration − 0.001 ($r = 0.998$) being a typical least-squares regression line. The minimum detectable amount (peak height = 3X baseline noise) was 2 ng/ml. The analysis of ten plasma samples containing metoprolol at 100 ng/ml concentration yielded an intra-assay (within-day) mean value of 99 ng/ml and a coefficient of variation (C.V.) of 4.1%. The corresponding values for inter-assay (day-to-day) were 98 ng/ml and a C.V. of 6.7%.

Interference from other drugs was studied including hydralazine, prazosin, furosemide, lidocaine, digoxin, quinidine, procainamide, N-acetylprocainamide, propranolol, salicylic acid, acetaminophen and diazepam. Plasma samples containing high therapeutic concentrations of the potentially interfering drug were analyzed by the procedure for metoprolol and were found to produce no interfering peaks.

DISCUSSION

Alprenolol was selected to serve as internal standard in the determination of metoprolol. The two compounds are very similar in chemical structure (Fig. 2), and the extraction, chromatographic, and fluorescence properties of alprenolol make it highly suitable for the role of internal standard. In addition, alprenolol is readily available from a commercial supplier at low cost (where internal standards are used in previously published HPLC procedures [12,14,15] the compounds are not routinely available from commercial suppliers, but must be obtained as gifts from pharmaceutical companies, a constraint we find somewhat inconvenient). While alprenolol is not available for routine clinical use in the United States, it is marketed in some countries. However, metoprolol

R
CH₂CH=CH₂

ALPRENOLOL

R
X

R = OCH₂CHCH₂NHCH(CH₃)₂
|
OH

X

METOPROLOL: CH₂CH₂OCH₃

O−DEMETHYLMETOPROLOL: CH₂CH₂OH
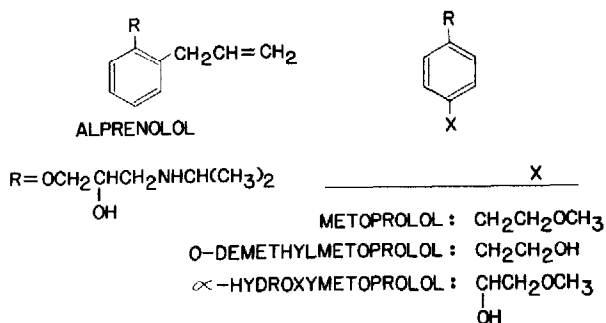
∝−HYDROXYMETOPROLOL: CHCH₂OCH₃
|
OH

Fig. 2. Chemical structures of the compounds studied.

and alprenolol are not normally administered together, and therefore the two drugs are generally not present simultaneously in patients. Nonetheless, it should be noted that this procedure cannot be used if alprenolol (other than that added as internal standard) is present in the sample (the presence of alprenolol in a sample can be readily determined by analyzing it without addition of internal standard).

Among the previously published HPLC procedures for metoprolol only one used monochromators for both excitation and emission, set at 280 and 300 nm, respectively [13]. In our HPLC system, slightly greater sensitivity was achieved by using 275 instead of 280 nm for excitation.

Among the metabolites of metoprolol [18] only the basic compounds α-hydroxymetoprolol (Fig. 2) and O-demethylmetoprolol (Fig. 2) would be extracted from alkalinized plasma. O-demethylmetoprolol is rapidly further oxidized in vivo and negligible amounts are found in plasma [19]. α-Hydroxy-metoprolol has been detected in human plasma at concentrations reaching ca. 50% of those of metoprolol [19,20]. We did not evaluate the chromatographic behavior of this metabolite, but in view of its chemical structure (Fig. 2) it is expected to have a shorter t_R value than that of metoprolol in reversed-phase HPLC, as has been found by others [15]. Indeed, a peak with a t_R of 1.7 min appeared in the chromatogram upon analysis of plasma samples from volunteers receiving metoprolol (Fig. 1). The peak was not present in blank plasma-derived chromatograms, and may well be due to α-hydroxy-metoprolol.

The procedure described is selective, simple, rapid, and displays good accuracy and precision. Furthermore, the same HPLC column and mobile phase can be used (with different fluorescence monochromator settings) for the determination of the concentration of propranolol [21] and salicylate [22] in plasma.

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