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Note

Fluorimetric determination of atenolol in plasma and urine by direct evaluation of thin-layer chromatograms

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For the quantitative determination of atenolol [4-(2-hydroxy-3-isopropylaminopropoxy)-phenylacetamide], a cardioselective beta-blocking drug, in plasma and urine, one fluorimetric and two gas chromatographic methods have been described. In the fluorimetric method described by Kaye[1], atenolol is extracted from alkaline solution with ethyl acetate and the fluorescence determined after re-extraction in a solution containing sodium dihydrogen orthophosphate in the cuvette. The disadvantage of this technically simple method is the relatively high detection limit of 50 ng/ml. The detection limits of the two gas chromatographic methods[2,3] are about 10–20 ng/ml.

It has been possible only with these methods to measure plasma levels of atenolol over a sufficiently long period after administration of therapeutic doses (50–100 mg). However, both methods require conversion of the atenolol with heptafluorobutyric anhydride, because for the detection of such small amounts an electron-capture detector is necessary.

We have now succeeded in decreasing the detection limit applying direct quantitative measurement of fluorescence after separation of the plasma extract and of the untreated urine by thin-layer chromatography. A further advantage of this procedure is that interference by plasma components extracted at the same time is excluded.

EXPERIMENTAL

Apparatus

A KM 3 chromatogram spectrophotometer (Carl Zeiss, Oberkochen/Württemberg, G.F.R.) with a Servogor Sb compensation recorder was used.

Materials

Atenolol hydrochloride (Tenormin) was kindly donated by ICI-Pharma (Plankstadt, G.F.R.). The chemicals and thin-layer plates (Kieselgel 60, 20 × 20 cm, without fluorescence indicator) were obtained from E. Merck (Darmstadt, G.F.R.).

Method for analysis of plasma

Extraction. To 1.00 ml of plasma in a stoppered test-tube 0.5 ml of 1 *N* sodium

hydroxide solution is added and the mixture is shaken for 15 min with 7 ml of butanol-1–cyclohexane (1:1). After centrifuging for 5 min, the organic phase is separated and placed in a 10-ml glass beaker. The sample is evaporated to dryness by heating while nitrogen is bubbled through.

Chromatography. The residue is dissolved in 2 drops of ethyl acetate by shaking vigorously and the solution is taken up with a 100-μl Hamilton syringe. This procedure is repeated twice. The whole of the solution contained in the syringe is applied with a Linomat III (Camag, Muttenz, Switzerland) on to a thin-layer plate (width of strip 8 mm). Then a known amount of a solution of atenolol hydrochloride in methanol is applied to three starting lines per plate as calibration spots. The amount of atenolol per spot is determined according to the expected content of the plasma.

Development. The plate is developed in a Desaga trough in solvent I (chloroform–methanol, 88:12, in an atmosphere of ammonia) under standard conditions. The R_F value of atenolol is 0.23.

Measurement. After drying, the plate is sprayed with a solution of 10 g of citric acid in 90 g of water–ethylene glycol (1:1). The fluorescence is determined on the moist plate, because the fluorescence decreases rapidly as the plate dries; the ethylene glycol keeps it moist.

The following conditions were used for measurement: monochromator probe method (M-Pr); excitation with mercury 265-nm line of an St 41 mercury medium-pressure lamp; measurement of radiation emission with an M 313 monochromatic filter; slit, 1.0 × 12 mm; high tension (550 V); intensification, 1–50 times; stage speed, 50 mm/min.

Evaluation. The areas under the peaks, calculated as peak height × width at half-height, are directly proportional to the amount of atenolol in the plasma sample in the range 0–2 μg. To determine the content of unknown samples it is sufficient to determine only one point on the calibration graph (mean of three determinations) and to connect this point to zero. The content of the sample can then be determined graphically or by calculation.

Method for analysis of urine

A 10-μl volume of urine is applied to a thin-layer plate as a strip (width 8 mm) with a Linomat III. Again, three starting lines with known amounts of standard solution are applied to each plate. The plate is developed in solvent II (toluene–ethanol–ethyl acetate–water, 60:40:20:6, in an atmosphere of ammonia[4]). The R_F value of atenolol is 0.29. Measurement and evaluation are carried out as for plasma.

RESULTS

In the thin-layer chromatographic separation of the plasma extracts or the sample of urine applied without previous treatment, all interfering substances are completely separated (Figs. 1 and 2). The detection limit is about 5 ng/ml of atenolol in plasma and about 0.5 μg/ml in urine.

To determine the recovery and the standard deviation of the method, eight samples of three different concentrations of atenolol hydrochloride in plasma and urine were investigated with the procedures described above. The plasma and urine

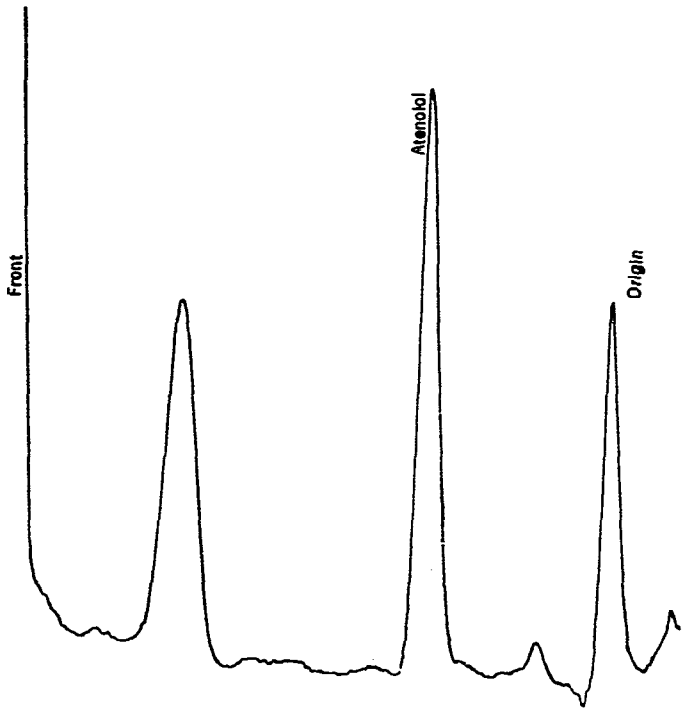

Fig. 1. Fluorescence intensity curve for an extract of 1 ml plasma with 172 ng atenolol [volunteer No. 16, 12 h after an oral dose of Tenormin (tablet containing 100 mg of atenolol hydrochloride)]. Sorbent: silica gel 60; solvent: chloroform–methanol (88:12) in an atmosphere of ammonia.

levels of atenolol after administration of therapeutic doses were taken as a basis for the selection of the concentrations.

The results are given in Table I and show that the mean recovery of atenolol in plasma was 64.6%, with a relative standard deviation of about 5% at high and medium concentrations. Atenolol was recovered quantitatively from the urine at

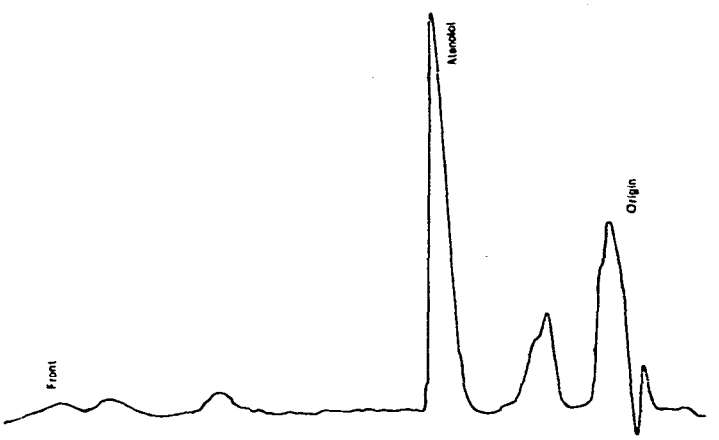

Fig. 2. Fluorescence intensity curve for 1.26 μg of atenolol in 10 μl of urine (volunteer No. 16, 0–6 h after an oral dose of Tenormin, 100 mg). Solvent: toluene–ethanol–ethyl acetate–water (60:40:20:6) in an atmosphere of ammonia.

high and medium concentrations, also with a relative standard deviation of about 5%. The recovery for low concentrations of atenolol in the urine was lower, presumably because this substance is photosensitive.

TABLE I

MEANS OF RECOVERIES AND RELATIVE STANDARD DEVIATIONS FOR ATENOLOL DETERMINATIONS IN PLASMA AND URINE

Eight samples of each of three different concentrations were investigated.

Sample	*Concentration of atenolol*	*Recovery (%)*	*Relative standard deviation (%)*
Plasma	500 ng/ml	65.5	3.78
	100 ng/ml	62.9	5.98
	20 ng/ml	66.0	12.06
Urine	100 μg/ml	102.4	4.77
	20 μg/ml	96.7	1.56
	2 μg/ml	85.4	5.43

Interference by hydroxyatenolol

About 10% of the atenolol excreted in urine is in the form of hydroxyatenolol[5]. Therefore, it was necessary to test whether the metabolite interfered in the determination of atenolol by the proposed method. As this metabolite is unobtainable commercially, about 100 μl of urine from each of four patients were applied as a strip to thin-layer plates (width of strip 5 cm). In the same way, urine from two healthy subjects who had received no atenolol was examined after addition of 100 μg of atenolol hydrochloride. In addition, 1 μg of atenolol hydrochloride (dissolved in methanol) was applied to each plate. The urines were chromatographed in solvent II. The areas of Kieselgel corresponding to the R_F value of atenolol were scraped off and shaken with 1 ml of methanol and the liquid was centrifuged from the Kieselgel. In each instance 99 μl of the clear supernatant solution was applied to the thin-layer plate as a strip 8 mm wide. The thin-layer plates were chromatographed in the following five solvents:

I: see above;
III: chloroform–methanol (90:30) in an atmosphere of ammonia;
IV: ethyl acetate–methanol–ammonia (60:30:10);
V: benzene–ethyl acetate–methanol (20:20:10) in an atmosphere of ammonia;
VI: butanol-1–cyclohexane (50:50) in an atmosphere of ammonia.

When the fluorescence intensity curves were plotted, no additional spots appeared after the second chromatography. Consequently, interference by hydroxyatenolol in the determination of atenolol seems improbable.

DISCUSSION

The detection limit for atenolol in plasma is about 5 ng/ml by direct quantitative measurement of fluorescence on thin-layer chromatograms. It is therefore about ten times lower than that obtained with the measurement of fluorescence in a cuvette and also lower than obtained with the two previous gas chromatographic methods.

The low detection limit is due particularly to the fact that with the chromatographic separation of plasma constituents extracted with the drug material it is possible to concentrate the entire atenolol content of the extract on to a very small area. Also, the intensity of the fluorescence is greater on excitation with a mercury medium pressure lamp at 265 nm than on excitation with a deuterium lamp at the maximum excitation wavelength of 280 nm. Further, a great increase in intensity of fluorescence is obtained by spraying the plate with citric acid solution.

A further advantage of the method is that no interference is caused by plasma constituents extracted with the drug, as they are completely separated by the change of solvent (extraction with butanol-1–cyclohexane, then taking up the extract in ethyl acetate after evaporating to dryness) and by the thin-layer chromatography. Fluctuating blank readings, such as are generally found in fluorescence measurements in a cuvette, were not found here. Interference by hydróxyatenolol could be excluded.

REFERENCES

1 C. M. Kaye, *Brit. J. Clin. Pharmacol.*, 1 (1974) 84.
2 B. Scales and P. B. Copsey, *J. Pharm. Pharmacol.*, 27 (1975) 430.
3 J. O. Malbica and K. R. Monson, *J. Pharm. Sci.*, 64 (1975) 1992.
4 S. Hiemstra, ICI-Pharma, personal communication.
5 J. McAinsh, *Postgrad. Med. J.*, 53, Suppl. 3 (1977) 74.