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Note

Improved determination of betaxolol in biological samples by capillary column gas chromatography

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Betaxolol (SL 75 212) is a new cardioselective β-adrenoceptor blocking agent with a long half-life of about 16 h and high bioavailability of about 80% [1—4].

During the early pharmacokinetic studies, the determination of the drug in blood and urine was carried out by a gas chromatographic method with a classical packed column using propranolol as the internal standard [5]. This method was quite satisfactory for studies in volunteers and in patients on monotherapy [1, 2, 4, 6, 7].

However, during long-term studies, when occasional polytherapy occurred, we could observe, in a few cases, interferences with both the betaxolol and its internal standard. It was then necessary to improve the efficiency of the original chromatographic method by the use of capillary columns. In addition, we thought it preferable to replace propranolol as internal standard by an analogue of betaxolol with a longer retention time, allowing higher analysis temperature and less risk of interference.

This paper describes the improved method for the accurate quantitation of betaxolol in blood, urine and tissues. The method has proved useful both for pharmacokinetic studies and for therapeutic drug monitoring.

EXPERIMENTAL

Standard and reagents

Betaxolol [4-(2-cyclopropylmethoxyethyl)-1-phenoxy-3-isopropylamino-propan-2-ol] and SL 76 020 [4-(2-cyclobutylmethoxyethyl)-1-phenoxy-3-iso-propylaminopropan-2-ol] were synthesized by Dr. Manoury, Chemistry Depart-

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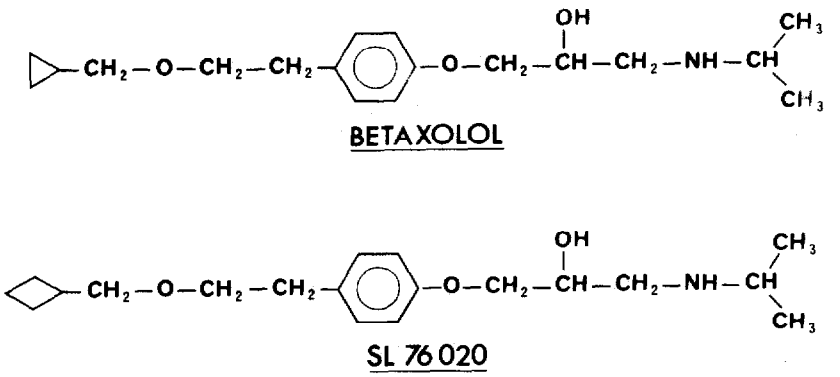

Fig. 1. Structural formulae of betaxolol and its internal standard, SL 76020.

ment LERS-Synthélabo (France). Their structural formulae are given in Fig. 1.

Analytical grade diethyl ether, ethyl acetate, sodium hydroxide, hydrochloric acid and *n*-hexane were obtained from Merck (Darmstadt, G.F.R.) and heptafluorobutyric anhydride (HFBA) was from Pierce (Rotterdam, The Netherlands).

Gas—liquid chromatographic conditions

Analyses were performed under isothermal conditions on a Model 5880 A Hewlett-Packard or a Model Sigma 4 Perkin-Elmer gas chromatograph, both equipped with a ^{63}Ni linear electron-capture detector.

A 0.02-mm OV-101 phase thickness quartz capillary column (25 m × 0.2 mm I.D.) was pretreated and tested by the manufacturer (Spiral, Dijon, France)

The operating conditions were: column temperature 225°C; injector temperature 300°C; split ratio 1:10; carrier gas argon—methane (95:5, v/v) 1 ml/min, make-up gas (argon—methane) 40 ml/min; detector temperature 300°C.

Samples of 1 µl were injected with the automatic injector HP 7672 A coupled with the HP 5880 gas chromatograph.

Calibration graph and quantitation

Standard solutions of betaxolol (1 µg/ml) and SL 76 020 (1 µg/ml), prepared in methanol, were stable for at least two months when kept at −20°C in the dark.

The calibration curves were prepared by adding 1, 5, 15, 30, and 50 ng betaxolol and 50 ng of SL 76020 to 1 ml of blank blood. The samples were extracted according to the method described below, and the extract was derivatized by heating with 200 µl of a solution of HFBA (10%, v/v) in ethyl acetate at 50°C for 10 min. In order to remove the excess reagent, this solution was then evaporated to dryness under a gentle stream of nitrogen at 60°C. To the dry residue 100 µl of hexane were added and 1 µl of this solution was injected onto the column. The curves were prepared by plotting the ratios of the peak height of betaxolol to the internal standard, against the known amounts of betaxolol. This curve was used to calculate the amount of betaxolol in unknown samples.

Extraction procedure for blood, urine and tissues

SL 76020 (50 ng) as internal standard, 100 μl of 2 *N* sodium hydroxide, and 7 ml of freshly distilled diethyl ether were added to 0.5–2 ml of blood in a 10-ml glass stoppered test-tube. The tubes were gently shaken on a rotating mixer for 15 min and then centrifuged at 4°C for 5 min at 800 *g*. The ether phase was transferred to another series of test-tubes containing 2.5 ml of 0.2 *N* hydrochloric acid, mixed on a Vortex mixer for 15 sec and then centrifuged for 2 min at 800 *g*. The upper organic phase was discarded. A further 5 ml of diethyl ether were added to the aqueous phase and the agitation and centrifugation repeated. After discarding the ether, 300 μl of 2 *N* sodium hydroxide solution were added to the aqueous phase together with 7 ml of diethyl ether. After extraction on a Vortex for 15 sec and centrifugation, 6.5 ml of the ether phase were transferred to another series of test-tubes evaporated to dryness under nitrogen in a water bath at 40°C, and then derivatized with HFBA as described above. An internal calibration curve with various amounts of betaxolol added to blank blood was carried through the procedure with the unknown samples.

An identical procedure was used for the analysis of urine and tissues.

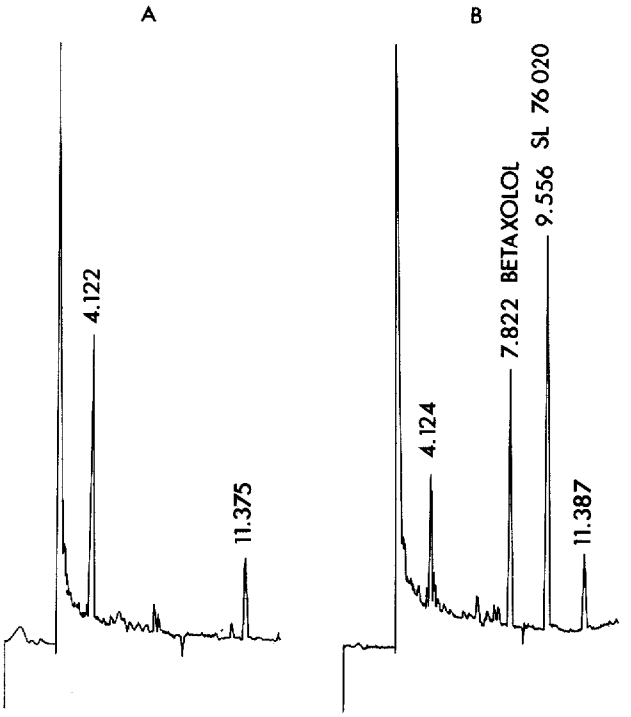

Fig. 2. Gas chromatograms, obtained with the capillary column, of a patient blood extract. (A) Blood collected before the beginning of the administration of betaxolol (t = 0); (B) blood collected 12 h after the oral administration of a 20-mg dose. No interfering peaks are present in the blood collected at t = 0.

RESULTS AND DISCUSSION

The chromatograms of a blood sample collected from a patient before the administration of betaxolol (time = 0) and a blood sample drawn 12 h after dosing with 20 mg of betaxolol are shown in Fig. 2. The retention times of betaxolol and SL 76 020 were 7.8 and 9.5 min, respectively. The peaks of betaxolol and its internal standard are well separated and no interfering peaks from endogenous substances or xenobiotics are present.

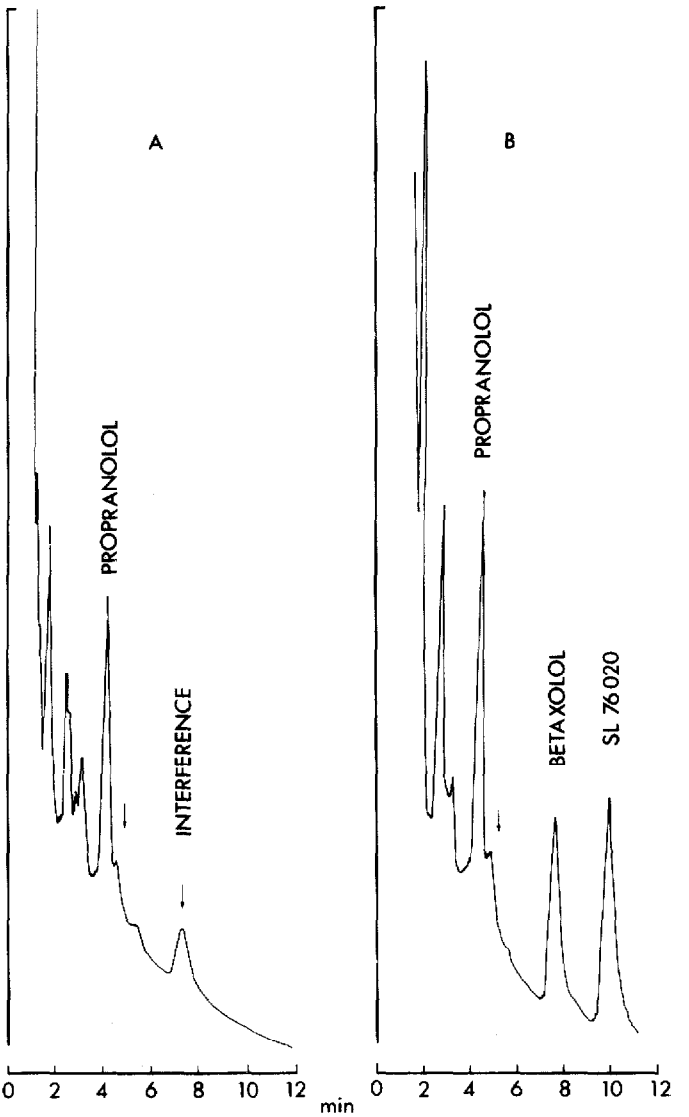

Fig. 3. Gas chromatograms, obtained with the packed column, of the same blood extract shown in Fig. 2. Propranolol was also added in this experiment. (A) Blood collected at $t = 0$: arrows indicate two peaks interfering with propranolol and betaxolol. (B) Blood collected 12 h after betaxolol dosing: the peak interfering with propranolol is still evident, whilst the other one is completely masked by the peak of betaxolol, which is thus overestimated.

The same samples, spiked also with propranolol, were analysed by the previously published method in which the separation is carried out on a packed column. As can be seen in Fig. 3, two interferences were present in the chromatogram. Fig. 3A shows the chromatogram obtained at time 0; an interfering peak at the end of the peak of propranolol and a second one with a retention time almost identical to that of betaxolol are present. Fig. 3B shows a chromatogram obtained from the sample collected 12 h after dosing and in which one can observe again the interference with propranolol, but not the interference with betaxolol, probably because betaxolol masks completely the interfering peak. Substituting propranolol by SL 76 020 should eliminate the interference with the internal marker, as shown in Fig. 3A, but would not avoid the possible interfering peak with betaxolol. The higher resolution of the capillary column overcomes this problem, as shown in Fig. 2.

The possible interference between betaxolol, SL 76 020 and a certain number of cardiovascular and diuretic drugs which could be associated with beta-blocker therapy, has been investigated. Guanethidine, clonidine, chlortalidone, furosemide, lidocaine, quinidine, diazepam, diltiazem, amiodarone and nitroglycerine have been added to samples containing betaxolol and SL 76 020, at concentrations known to be present during chronic treatment. No interfering peaks any of these compounds were observed. Moreover, many other beta-blockers such as alprenolol, oxprenolol, atenolol, acebutolol and metoprolol have a retention time shorter than that of betaxolol in our chromatographic conditions and cannot interfere with betaxolol.

The reproducibility of the method is shown in Table I. The coefficient of variation ranges from about 10% at lower concentration to 4% at higher concentration. For 1 ng, the coefficient of variation is 8.6%. It must be emphasized that these values were not obtained during a single experiment, but from 31 calibration curves prepared for routine analysis over a period of two months. Compared with the previously published procedure, the limit of sensitivity was lowered from 1 to 0.5 ng/ml of blood or urine with the present capillary column method. As already observed for betaxolol and propranolol,

TABLE I

CONCENTRATION OF BETAXOLOL FOUND IN PLASMA FOR KNOWN AMOUNTS OF THE DRUG ADDED

Amount added to plasma (ng)	No. of determinations	Amount recovered (ng/ml, mean ± S.D.)	Coefficient of variation* (%)
1	13	1.04 ± 0.09	8.6
5	10	4.6 ± 0.4	8.8
15	41	15.3 ± 1.6	10.4
30	31	30.1 ± 1.7	5.6
50	31	48.3 ± 2.4	4.9

*Coefficient of variation = $\frac{\text{S.D.} \times 100}{\text{mean}}$.

the ratio of the peak heights of betaxolol and its new internal standard (SL 76 020) is not modified over a one-week period, confirming the excellent stability of the HFBA derivatives. The absolute recovery of the method calculated independently for betaxolol and SL 76 020, of about 90%, confirmed the values previously obtained [5].

More than 1500 samples have been analysed with the capillary column method and no interferences have been detected or observed up to now.

CONCLUSIONS

The use of a quartz capillary column increased the sensitivity and especially the specificity of the previous packed column method. This two-fold increase in sensitivity obtained with the capillary column cannot be very relevant for clinical drug monitoring in chronically treated patients whose blood level at steady-state is about 40 ng/ml, but may improve the accuracy of the determination in the terminal phase of pharmacokinetic studies. The main advantage of the described method is the increase of selectivity due to the improved separation power of the capillary column. The possibility of interferences due to the concomitant intake of other xenobiotics cannot be excluded but it remarkably reduced, and samples which could not be analysed with the previous method give well-separated peaks necessary for quantitation. This method must be preferred to the previous one for routine monitoring of betaxolol in patients undergoing polytherapy.

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