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Note

Measurement of xamoterol in plasma and urine by high-performance liquid chromatography

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Xamoterol, 1-(4-hydroxyphenoxy)-3-[2-(4-morpholinecarboxamino)-ethylamino]-2-propanol, hemifumarate, ICI 118,587, Corwin (Fig. 1), is one of a structurally related series of new, orally active drugs being investigated for use in the management of heart failure in man. Preliminary human and animal studies have shown that xamoterol is a highly selective, partial beta-adrenoceptor agonist [1—3].

We have recently studied the intravenous pharmacokinetics of xamoterol, the time course of its cardiovascular effects and their relationship to dose [4]. For this study, it was necessary to determine plasma and urinary concentrations of xamoterol. The present report describes the analytical procedure developed to measure xamoterol in plasma and urine.

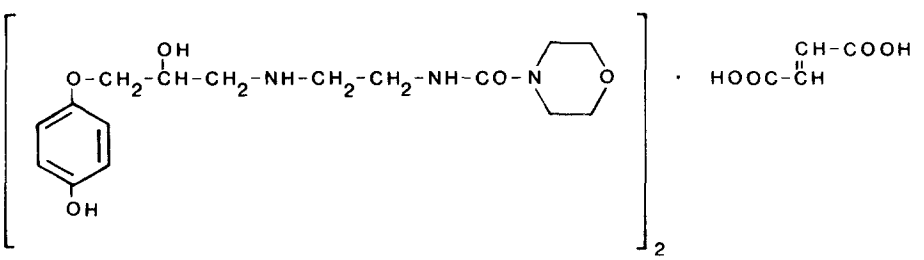

Fig. 1. Structural formula of xamoterol hemifumarate.

METHODS

Reagents

Xamoterol hemifumarate was generously supplied by Imperial Chemical

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Industries, U.K. Prenalterol hydrochloride (*S*-(−)-(4-hydroxyphenoxy)-3-isopropylamino-2-propanol hydrochloride) was generously supplied by Ciba-Geigy (Basle, Switzerland). Stock solutions (1 mg/ml) of both compounds were prepared in 0.1 *M* hydrochloric acid and stored at 4°C. Working solutions were prepared in 0.01 *M* hydrochloric acid in the range 800—12.5 ng/ml. Acetonitrile, 190-nm HPLC grade, was obtained from Waters Assoc. (Milford, MA, U.S.A.). Water for HPLC was redistilled from alkaline potassium permanganate. All other reagents were of analytical grade.

Bio-Rex 70 (50—100 mesh), Na^+, cation-exchange resin (Bio-Rad Labs., Richmond, CA, U.S.A.) was packed into polypropylene columns (4 × 1 cm, Bio-Rad Labs.) and washed sequentially with 3 *M* hydrochloric acid, 3 *M* sodium hydroxide, 3 *M* acetic acid, 1 *M* ammonium acetate, pH 6.5 and distilled water.

Chromatography

High-performance liquid chromatography (HPLC) was carried out using a Model 5000 liquid chromatograph fitted with a 100-μl universal loop injector (Varian Assoc., Palo Alto, CA, U.S.A.). A Spherisorb ODS 5-μm column (25 cm × 4.6 mm I.D.) (Laboratory Data Control, Riviera Beach, FL, U.S.A.) was used. The mobile phase was acetonitrile—0.01 *M* perchloric acid (15:85) at a flow-rate of 2 ml/min. Detection was by fluorescence using an FS-970 fluorimeter (Schoeffel, Westwood, NJ, U.S.A.) fitted with a deuterium arc source. Wavelength of excitation was 190 nm; wavelength of emission was selected by a Corning 7-60 glass filter with bandpass of 320—400 nm.

Biological samples

Blood was collected from normal subjects 0—8 h following a rapid intravenous infusion of xamoterol (100 μg/kg) into heparinised tubes on ice and the plasma separated and frozen at −20°C until assay. Drug-free blood was similarly collected from healthy human subjects on no medication. Plasma from patients on medication with potential for interference in the assay was also tested.

Urine was collected at 0—2, 2—4, 4—8 and 8—24 h from the xamoterol study subjects above. Sodium metabisulphite (0.1%) or 5 *M* hydrochloric acid were used as preservative and aliquots of the urine were stored at −20°C until assay.

Experimental procedure

To a 1-ml aliquot of plasma or 0.1-ml aliquot of urine were added 40 ng prenalterol as internal standard, 5 ml 0.1% disodium EDTA and 0.1 ml of 1 *M* ammonium acetate, pH 6.5. The solution was passed through a washed Bio-Rex 70 column and the resin washed twice with 10 ml distilled water; all effluents being discarded. Xamoterol and prenalterol were eluted from the column with 3 ml of 1 *M* ammonium hydroxide. The eluate was dried under vacuum at 50°C using a vortex evaporator (Buchler Instruments, Fort Lee, NJ, U.S.A.). The dried eluate was reconstituted in 0.25 ml of 0.1 *M* perchloric acid on ice. The sample was transferred to a 1.5-ml tapered tube and centrifuged at 10,000 *g* for 10 min at 4°C (Zentrifuge 5412, Eppendorf, Hamburg, F.G.R.). A 100-μl aliquot of the supernatant was taken for HPLC analysis.

The calibration curve was prepared by processing 1-ml aliquots of subject control plasma or drug-free plasma or 0.1-ml aliquots of subject control urine to which 0—800 ng xamoterol was added through the experimental procedure.

RESULTS AND DISCUSSION

A relatively simple procedure has been developed for the measurement of xamoterol in plasma and urine. Initial purification of xamoterol was obtained by using a weak cation-exchange resin, a technique successfully applied for other ethanolamine derivatives such as urinary metanephrines [5, 6]. Use of the resin was preferential to solvent extraction because xamoterol is not readily extracted by non-polar solvents such as diethyl ether and the use of more polar solvents would be likely to pose chromatographic problems. Xamoterol, like prenalterol [7], used as the internal standard, exhibits good fluorescence on low-wavelength excitation, thereby enabling sensitive and selective detection with HPLC.

The chromatographic system developed provided good resolution of xamoterol and prenalterol from endogenous plasma contaminants. Retention times were 6.9 min for xamoterol and 5.8 min for prenalterol. Sample chromatograms for standards and plasma and urinary extracts from subjects, before and following administration of xamoterol, are shown in Fig. 2.

Recoveries from plasma and urine were similar, averaging 82 ± 2% (mean ± S.E.) for xamoterol and 81 ± 2% for prenalterol. Linear and reproducible standard curves over the range 0—800 ng/ml were obtained with a mean equation, $y = (0.013 \pm 0.001)x - (0.020 \pm 0.010)$, $r^2 = 0.999$, $n = 21$, where y represents the peak height ratio of xamoterol/prenalterol and x represents the concentration of xamoterol hemifumarate in ng/ml.

The limits of sensitivity of the assay are 1 ng/ml for plasma and 10 ng/ml for urine, the difference being due to the sample size (1 ml for plasma versus 0.1 ml for urine). Accuracy and precision of the assay were determined by replicate analysis of plasma to which 1000, 100, 2.5 and 0 ng xamoterol were added. The results are given in Table I. The slope of the regression line for nanogram amounts xamoterol added versus nanogram amounts xamoterol obtained was 1.00 and the intercept was —0.094.

Analysis of patient control plasma or urine gave non-detectable assay blanks and no interference was seen from drugs such as diazepam, nitrazepam and quinidine or from those used in the clinical study, prazosin, clonidine and atropine [4]. The method was also successfully used to measure compounds structurally similar to xamoterol i.e. salbutamol and fenoterol (unpublished observations).

Following a 5-min intravenous infusion of xamoterol (100 μg/kg) into normal subjects, peak plasma concentrations of the drug ranging from 300 to 700 ng/ml were obtained on cessation of the infusion, then declined with an apparent half-time of 2—3 h (Fig. 3, upper panel). Xamoterol was excreted in urine predominantly as the unchanged drug. An average of 60% of the administered dose was excreted in the first 2-h collection period and a total of 82% for the 24-h collection period (Fig. 3, lower panel).

In summary, the method developed permits the sensitive and selective assay

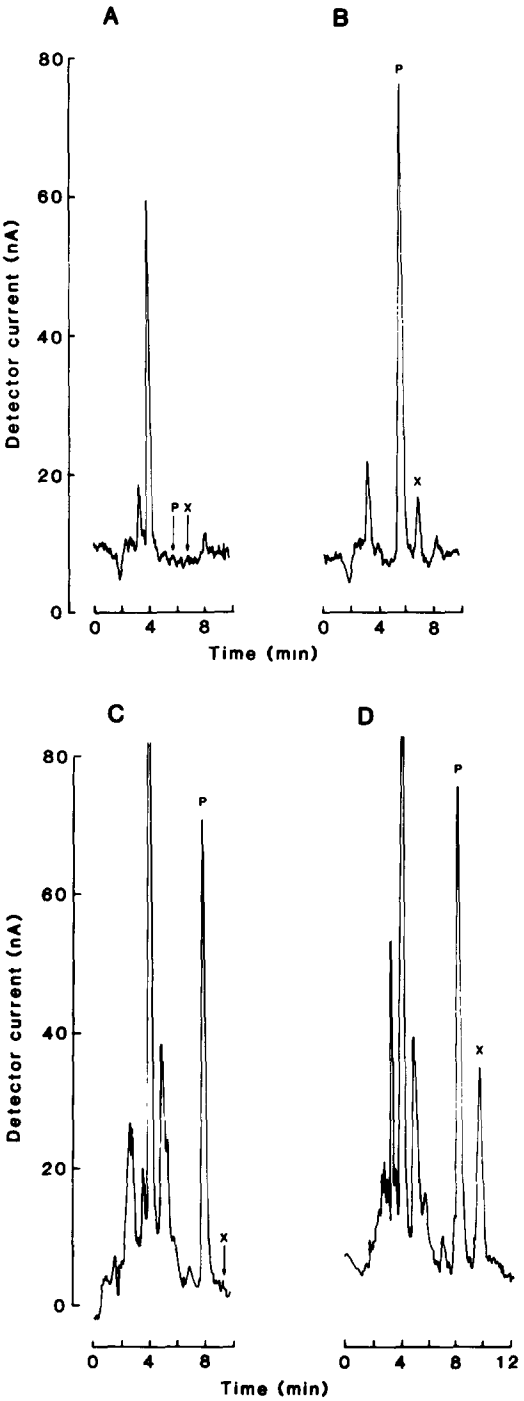

Fig. 2. Chromatographic traces of (A) extract of subject control plasma; (B) extract of subject plasma following a dose of xamoterol (100 μg/kg, intravenously) containing 5.3 ng/ml xamoterol; (C) extract of subject control urine with internal standard added; (D) extract of subject urine following a dose of xamoterol as in (B) containing 353 ng/ml xamoterol. Peaks: X = xamoterol; P = prenalterol, internal standard.

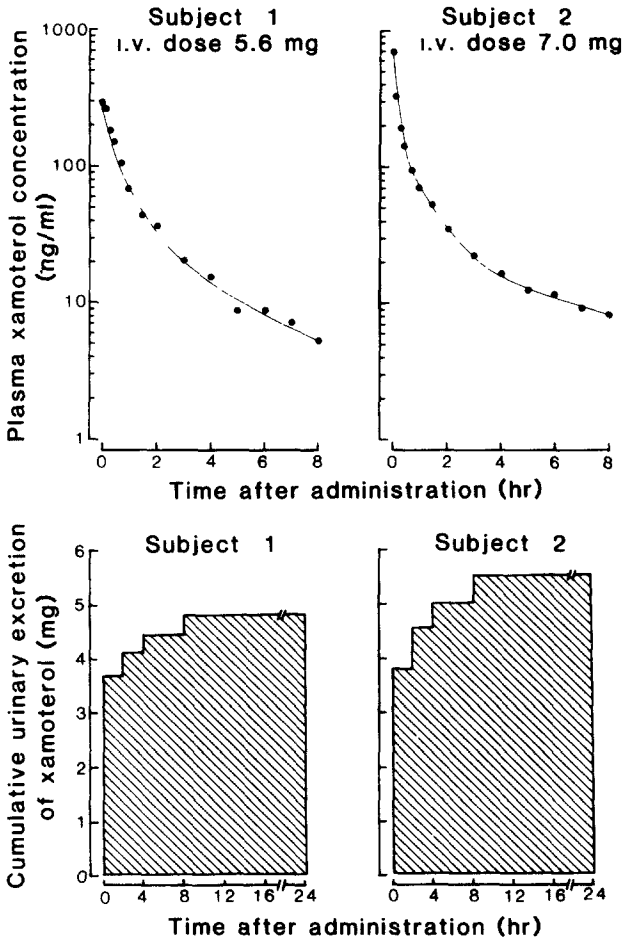

Fig. 3. Upper panel: the decline in plasma xamoterol concentrations immediately following cessation of an infusion of xamoterol (100 μg/kg, intravenously) in two typical subjects. Lower panel: cumulative urinary excretion of xamoterol in the same subjects following the xamoterol infusion.

TABLE I

ACCURACY AND PRECISION OF THE ASSAY (n = 6)

Xamoterol added (ng/ml)	Xamoterol obtained (ng/ml)	
	Mean ± S.D.	C.V. (%)
1000	1000 ± 28.7	2.9
100	98.7 ± 4.25	4.3
10	10.6 ± 0.64	6.1
2.5	2.71 ± 0.14	5.0
0	0 ± 0	—

of xamoterol in biological samples. The ion-exchange procedure shows a wider applicability than solvent extraction, increasing the potential use of the method for the analysis of similar drugs.

ACKNOWLEDGEMENT

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