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Note

Gas—liquid chromatographic determination of procetofenic acid in human plasma and urine

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Procetofenic acid or 2[4'-(*p*-chlorobenzoyl)phenoxy]-2-methylpropionic acid (LF 153) is the active metabolite of a new hypolipidemic drug, procetofene (LF 178—isopropyl ester of procetofenic acid). This compound is chemically related to the well known hypolipidemic drug, clofibrate or ethyl (*p*-chlorophenoxy)-2-methylpropionate, in which the *para*-chlorine atom is spaced by a benzoyl group. In the body, clofibrate is also completely transformed in an active metabolite, clofibric acid [1].

No methods suitable for the determination of this new compound have been reported as yet. An enzymic hydrolysis of the conjugates was performed prior to the urine analysis. The method described in this paper was developed for the determination of the active circulating metabolite, procetofenic acid, in human plasma and hydrolyzed urine down to the level of 100 ng/ml in 0.5-ml samples. The compound was determined as its methyl ester and the parent drug, procetofene, was used as the internal standard.

EXPERIMENTAL AND RESULTS

Reagents

The solvents used were of analytical grade. Ethereal diazomethane was prepared with Diazald (Aldrich, Milwaukee, Wisc., U.S.A.) in the Diazald-kit instrumentation set and stored at —20°. Procetofene and procetofenic acid were of analytical purity. β-Glucuronidase—aryl sulphatase preparations in water (B grade) were obtained from Calbiochem (San Diego, Calif., U.S.A.).

Quantitative analysis of procetofenic acid in human plasma and urine

Gas—liquid chromatography. A Varian Model 1445 gas chromatograph

equipped with an electron-capture detector (^{3}H Sc) was used. The column was a 5 ft × 2 mm I.D. coiled glass tube packed with 4% SE-30 on Gas-Chrom Q, 80—100 mesh (Applied Science Labs, State College, Pa., U.S.A.) and was conditioned at 270° for 48 h (nitrogen flow-rate 30 ml/min). The instrument settings were: column temperature, 230°; injection port temperature, 250°; detector temperature, 270°; ^{3}H Sc foil temperature, 240°; and carrier gas (ultra-pure nitrogen) flow-rate, 45 ml/min. Under these conditions, the retention time was 4.25 min for the methyl ester of procetofenic acid and 5.25 min for the internal standard, procetofene.

Enzymic hydrolysis of conjugates in urine. A 0.5-ml volume of urine and 4.5 ml of 0.2 *M* sodium acetate buffer pH 5 were incubated with the β-glucuronidase—aryl sulphatase preparation (10.000 units) at 37° for 24 h.

Extraction procedure. To a 60-ml stoppered tube were added successively 0.5 ml of plasma or hydrolyzed urine, 1 ml of distilled water, 2 ml of 3 *M* hydrochloric acid, 10 ml of diethyl ether and 1 ml of the internal standard solution (procetofene, 5 μg/ml in heptane). After continuous manual extraction for 30 sec and separation of the two layers, 8 ml of the upper layer were transferred to another tube. To this extract, 2 ml of ethereal diazomethane were added and the mixture was allowed to stand for 15 min. Evaporation on a Rotavapor at 40° afforded the residue for the gas chromatographic analysis. Before injection, solubilization was effected with 1 ml of *n*-hexane and 2 μl were injected into the gas chromatograph (duplicate injections).

Quantitation. One calibration graph for plasma and one calibration graph for urine were obtained by assaying respectively drug-free plasma or urine containing known amounts of procetofenic acid and plotting the ratio of peak heights (methylated procetofenic acid to internal standard) against the amount of procetofenic acid added. The graph covered the range 100 ng/ml—6 μg/ml of procetofenic acid in plasma with a slope of 1.32. For urine the slope was quite similar, 1.35.

The peak height ratio of procetofenic acid to internal standard was calculated for each sample and the amount of procetofenic acid present was determined by reference to each calibration graph. The reproducibility and accuracy were found to be 4 ± 1%. The sensitivity was about 100 ng/ml and the recovery was 96.5 ± 1% for both plasma and urine.

Specificity. In all of the plasma samples investigated, a small peak was found at the retention time of the internal standard (Fig. 1). This constant interference was small in contrast with the height of the internal standard peak. The urine chromatograms were free from interfering peaks.

Application. The above method was performed on plasma and urine samples from one healthy volunteer who was given a single oral dose of 300 mg of procetofene (Lipanthyl®, Laboratoires Fournier, Dijon, France) (Fig. 2 and Table I).

DISCUSSION

The method described for the quantitative analysis of procetofenic acid is based on the use of an internal standard with a similar chemical structure for human plasma and urine samples. Both dosages proved to be accurate and

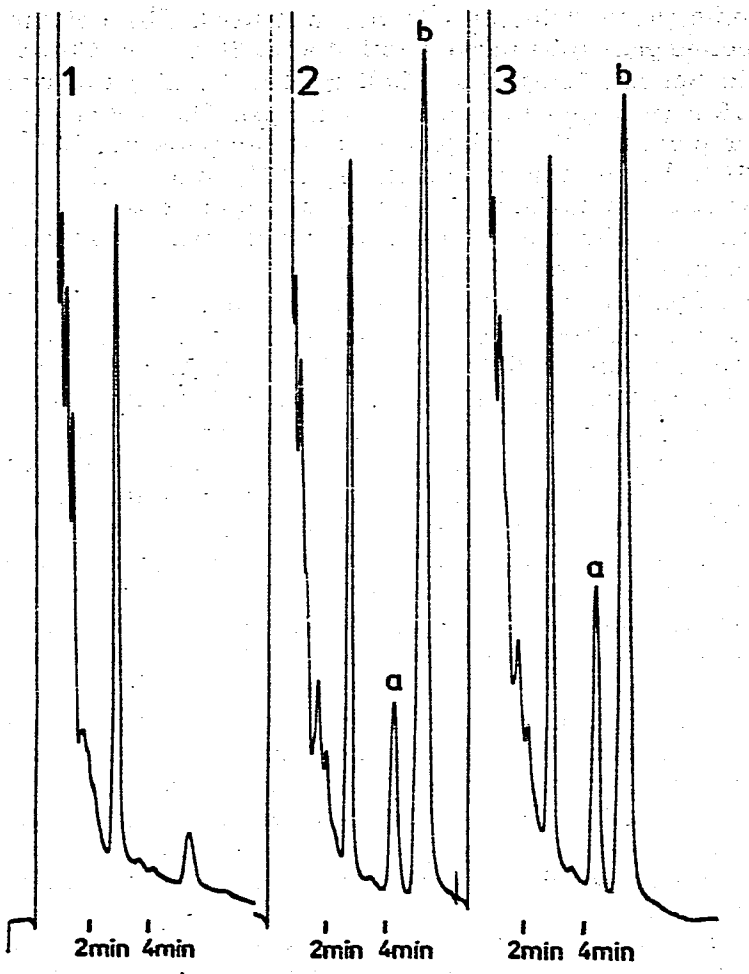

Fig.1. Chromatograms of a drug-free plasma extract (1), a plasma extract containing added procetofenic acid at a level of 2 μg/ml (2), and a plasma extract from a volunteer receiving procetofene (a= active metabolite; b= standard— (3).

easy to perform. A small but constant interfering peak in the plasma samples did not impair the results. To remain in the linear range, drug-free human plasma or hydrolyzed urine were used to dilute the samples in which a concentration above 6 μg/ml was found. No traces of procetofenic acid could be detected in drug-free samples (plasma and hydrolyzed urine) containing the internal standard. The stability of procetofene was so ascertained in our experimental conditions. Moreover, the parent drug was not found in the plasma and hydrolyzed urine samples as reported elsewhere [2].

The free fraction of procetofenic acid in human urine was estimated to be about 1 or 2%. The enzymic hydrolysis of the conjugates with a β-glucuronidase—aryl sulphatase preparation was prefered to the acid hydrolysis with hydrochloric acid. Between 25 and 35% of procetofenic acid was destroyed

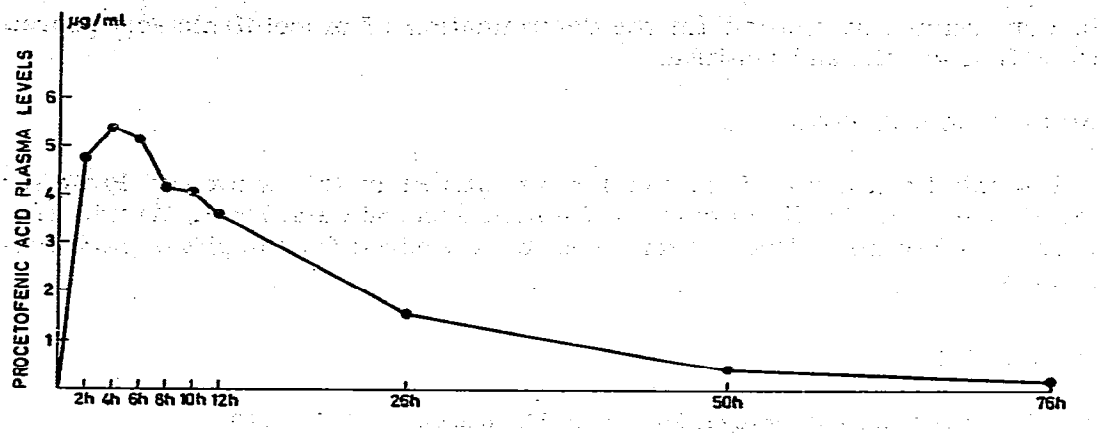

Fig.2. Graph illustrating the absorption and elimination from plasma of 300 mg of procetofene (Lipanthyl®) administered to one volunteer as a single oral dose.

TABLE I

LEVELS OF PROCETOFENIC ACID IN HYDROLYZED URINE FROM ONE HEALTHY VOLUNTEER RECEIVING 300 mg OF PROCETOFENE AS A SINGLE ORAL DOSE

Period of collection (h)	Procetofenic acid excreted (mg)
0—24	49.13
24—48	8.32
48—72	3.29
72—96	1.27

in the urine samples when they were hydrolyzed for 15 min at 100° with 3 *M* hydrochloric acid.

The sensitivity of the method made it possible to study the pharmacokinetic parameters of this new drug. If needed, it is possible to detect 10 ng/ml in the biological fluids.

The first results on a human volunteer showed that the drug is slowly cleared from the plasma, following a biexponential curve on a semi-logarithmic scale. The elimination half-life was about 24 h and 20.67% of the administered dose were recovered from the 96-h hydrolyzed urine collection. In a previous study [3] conducted with a radiochemical tracer in two healthy volunteers, a half-life of about 7 h was found and the existence of another metabolite of procetofene was suggested. However, the presence of this metabolite could not be confirmed [2]. In this study, the specificity of the method was checked using a coupled gas—liquid chromatographic—mass spectrometric system (LKB 9000; column, 3% OV-1 on Chromosorb W, 60—80 mesh; temperature 230°; helium flow-rate 30 ml/min; ionization potential, 70 eV). Only the mass spectra of the methyl ester of procetofenic acid was recorded during the gas—liquid chromatographic—mass spectrometric analysis of our samples.

In conclusion this method for the determination of procetofenic acid proved to be fast, specific and sensitive.

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