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Note

Determination of tulobuterol in human serum by electron-capture gas–liquid chromatography

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Tulobuterol–HCl { α -[(*tert.*-butylamino)methyl]-*o*-chlorobenzyl alcohol hydrochloride} (Fig. 1) is a new bronchodilator which has been shown, in animals and in early clinical studies, to possess an intensive and selective β_2 profile [1, 2].

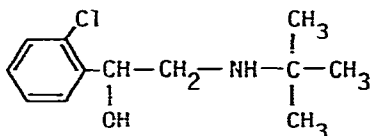


Fig. 1. Structure of tulobuterol.

Determination of the pharmacokinetic profile of a new therapeutic agent may be important for gaining a better understanding of its mechanism of action and for ensuring more efficient therapeutic application. Because of the low therapeutic dose of tulobuterol–HCl (1–3 mg/day), a sensitive analytical method is needed for its determination in human serum after oral administration.

In order to conduct a clinical pharmacokinetic study, we developed a selective and sensitive electron-capture gas chromatographic assay for the determination of tulobuterol in human serum.

EXPERIMENTAL

Reagents and materials

The tulobuterol-HCl used was synthesized [3] in our laboratory. 1,1-Bis-(4-fluorophenyl)-2,2-dichloroethane (internal standard) was obtained from Aldrich (Milwaukee, WI, U.S.A.) and trifluoroacetic anhydride (TFAA) was from Nakarai Chemical (Kyoto, Japan). Other reagents and solvents were of analytical grade and were used without further purification.

Drug administration and sample collection

The healthy volunteers received an oral dose of 2 mg of tulobuterol-HCl in tablet form (2 × 1-mg tablet). Blood samples of about 8 ml were withdrawn before the administration and at 1, 2, 3, 4, 5, 6, 8 and 10 h. After the samples had been left to stand for 30–60 min, the serum was separated by centrifugation (2000 g, 10 min) and stored frozen until analysis.

Gas chromatographic conditions

A Shimadzu Model 5A gas chromatograph, equipped with a ^{63}Ni electron-capture detector operated at a pulse interval of 8 μsec , was used. A glass column, 2 m and 3 mm I.D., packed with 2% OV-1 on Chromosorb W AW DMCS, 60–80 mesh (Gasukuro Kogyo, Tokyo, Japan), was operated at a temperature of 130°C. The injection port and detector temperatures were 230°C and 250°C, respectively, and the carrier gas (nitrogen) flow-rate was 60 ml/min.

Extraction procedure and derivatization

One millilitre of serum was placed in a 15-ml glass centrifuge tube and 0.5 ml of 1.0 N sodium hydroxide was added. The tube was stoppered and extracted with 9 ml of hexane by shaking for 10 min. After centrifugation (2000 g, 5 min), 8 ml of the hexane layer were transferred to a second tube containing 2 ml of 1 mM hydrochloric acid (adjusted to pH 3.0), and the tube was shaken and centrifuged. After complete removal of the hexane layer, 0.5 ml of 1.0 N sodium hydroxide was added to the aqueous phase and extracted with 6 ml of hexane. Five millilitres of the hexane layer were transferred to a third tube and evaporated to dryness under reduced pressure in a water-bath at 30–35°C.

The extract was dissolved in 4 ml of ethyl acetate containing TFAA (1%, v/v) and the mixture was heated at 70°C for 45 min. After evaporation of the reaction mixture, 5 ml of distilled water were added to the residue which was finally extracted with 1 ml of hexane containing internal standard (35 ng). The tube was shaken, centrifuged, and part of the hexane phase (6 μl) was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Fig. 2 shows a gas chromatogram of a sample extracted from human serum

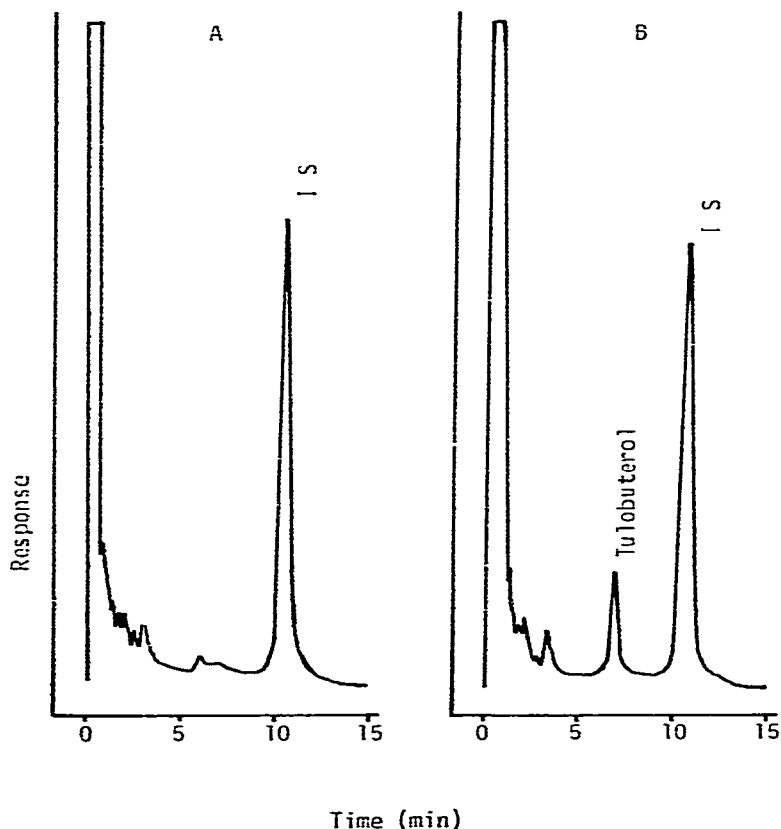


Fig. 2. Gas chromatograms of a serum extract. (A) Serum free from drug; (B) serum to which known amounts of tulobuterol-HCl (4 ng) and internal standard (IS) (35 ng) were added.

to which was added tulobuterol-HCl (4 ng/ml) and internal standard (35 ng/ml). The technique successfully separated the compound of interest from the endogenous materials in the serum.

The ratio of the peak height of tulobuterol to that of the internal standard varied linearly with the serum tulobuterol concentration in the range studied (up to 10 ng/ml). The statistical regression line can be presented as $Y = 0.130X + 0.006$ ($r = 0.9995$). The minimum detectable level of tulobuterol was 0.5 ng/ml.

Table I demonstrates the precision and accuracy of the present method. The recovery of our method was 95.5–102.1%. Over the therapeutic tulobuterol concentration range of below 10 ng/ml, the assay precision was satisfactory.

Electron-capture gas chromatographic analysis was employed for the determination of serum samples from an absorption study using ten subjects. Fig. 3 gives typical chromatograms for serum after oral administration of 2 mg of tulobuterol-HCl; no characteristic peaks other than that of unchanged tulobuterol were seen in the chromatogram.

Our previous study by mass fragmentography [4] quantitated the formation of some metabolites in human urine after a therapeutic dose of tulobuterol—

TABLE I

PRECISION IN THE DETERMINATION OF TULOButEROL IN SPIKED HUMAN SERUM SAMPLES

Concentration of tulobuterol-HCl (ng/ml)	Mean concentration found (ng/ml, \pm S.D.)	Coefficient of variation (%) ($n = 6$)
2.00	1.96 \pm 0.07	3.6
4.00	3.82 \pm 0.18	4.7
7.00	7.15 \pm 0.19	2.7

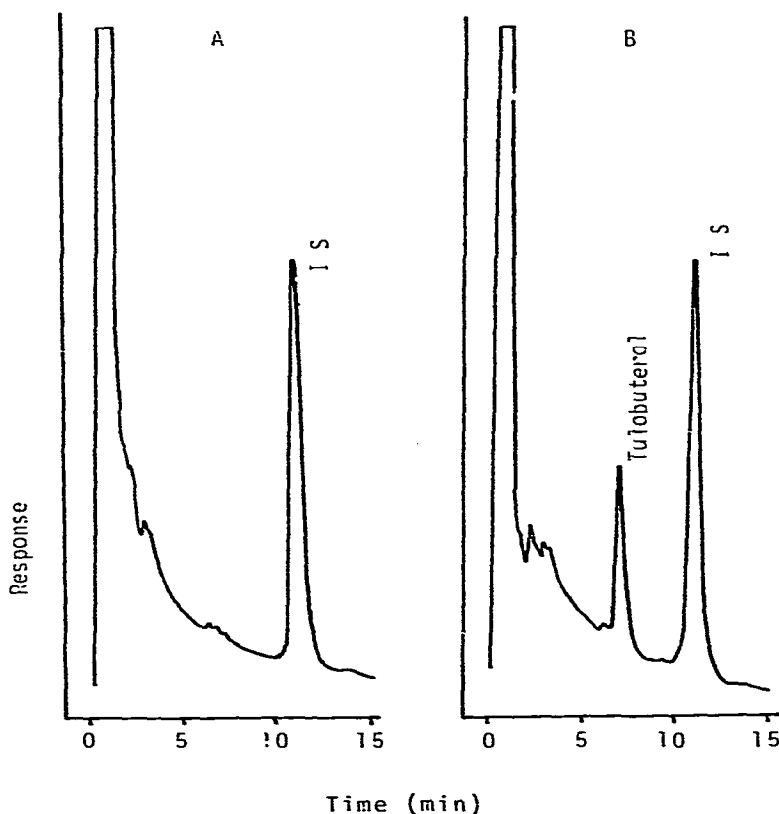


Fig. 3. Typical chromatograms of a serum extract (subject I). (A) Pre-dose; (B) 3 h after oral administration of 2 mg of tulobuterol-HCl.

HCl. So we also adapted this derivatization method with TFA to the ring-hydroxylated metabolites of tulobuterol, but did not succeed because of the instability of the resulting derivatives. However, bronchodilators which possess a hydroxy group on the benzene ring, such as salbutamol [5] and terbutaline [6, 7], have been determined as their silylated derivatives, but no report about TFA derivatization of these drugs has been presented. From these facts, it

seems that the hydroxy group on the benzene ring would probably be responsible for the instability of this type of derivative.

The mean serum concentration—time curve of tulobuterol is shown in Fig. 4. A peak serum tulobuterol concentration of 6 ng/ml was reached at 3 h after dosing. The mean values for the elimination half-life and area under the concentration—time curve were 3.19 h and 30.5 ng h ml⁻¹, respectively.

The sensitivity and selectivity of this method make it suitable for bio-availability and pharmacokinetic studies.

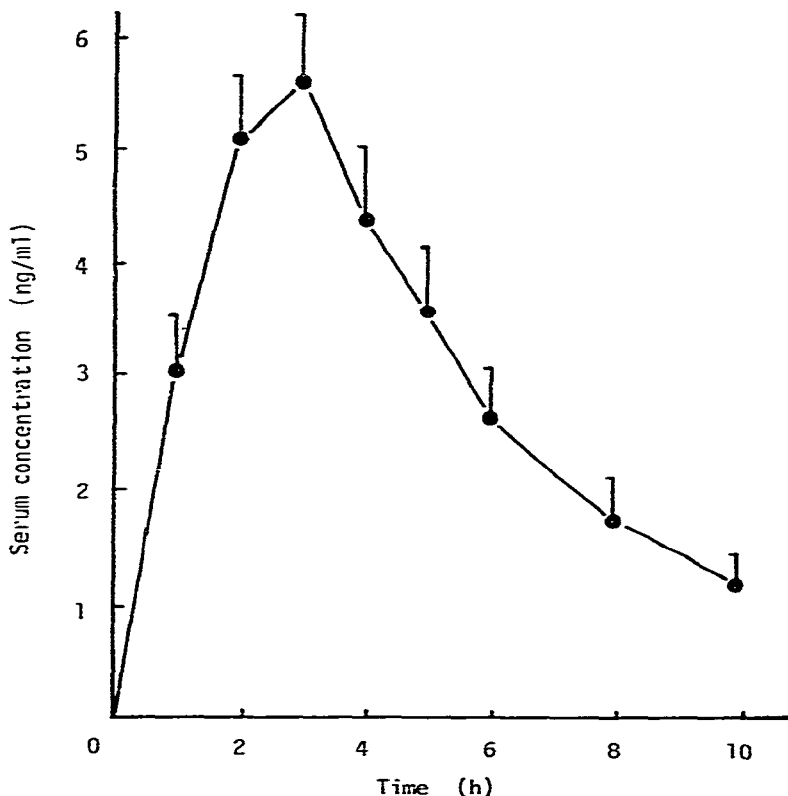


Fig. 4. Serum concentration versus time curve after oral administration of 2 mg of tulobuterol—HCl to human subjects ($n = 10$).

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