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Determination of Propranolol and Its Major Metabolite, Naphthoxylactic Acid, in Human Plasma by High Performance Liquid Chromatography

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High performance liquid chromatography (HPLC) with a fluorometric detector was applied to the determination of propranolol and its major metabolite, naphthoxylactic acid (NLA), in human plasma following a single oral low dose (20 mg) of propra olol HCl.

Propranolol and NLA were extracted successively from the sample aliquot. The carboxyl group of NLA was methylated with diazomethane in order to avaoid interference from blank plasma and a very strongly tailing peak on the HPLC chromatogram. The mean recoveries of propranolol and NLA subjected to the entire analytical protocol were better than 92%. The detection limits were 0.5 ng/ml for propranolol and 2 ng/ml for NLA. The calibration curves for propranolol HCl (20—100 ng/ml) and NLA (0.2—2 μg/ml), showed good linearity.

The plasma concentrations of propranolol and NLA following a single oral administration of 20 mg of propranolol HCl to three healthy male subjects were determined by this method.

Keywords——HPLC; propranolol; naphthoxylactic acid; human plasma; fluorometric detector; methylation; diazomethane

Propranolol, the widely used beta-adrenergic receptor blocking drug, is almost completely metabolized in man.[1] It has already been shown that 4-hydroxypropranolol and naphthoxylactic acid (NLA) are two major metabolites following oral administration of propranolol in man.[2] The former is equipotent with propranolol[3] as regards beta-blocking properties, and no information is available on the plasma concentration of the latter. Methods presently available to determine propranolol and its metabolites involve either spectrofluorometry,[4] thin-layer chromatography,[5] gas chromatography,[6] gas chromatography–mass spectrometry,[7] or high performance liquid chromatography (HPLC).[8,9] The HPLC method seems to be the most valuable because of its high sensitivity and procedural ease. Propranolol and six metabolites in human urine were determined by means of reversed-phase HPLC after extraction of both the acidic and basic metabolites of propranolol from the same urine aliquot.[8] However, some difficulties, especially interference by components of the plasma, low sensitivity and poor reproducibility due to the very strongly tailing peak of NLA, arose in our trials according to the procedures of Pritchard *et al.*[8]

This paper described the reverse-phase HPLC determination of propranolol and its metabolite, NLA, in human plasma following a single oral low dose (20 mg) of propranolol HCl. In this work, NLA was methylated to overcome the problems described above.

Experimental

Materials——Propranolol HCl and NLA were kindly supplied by ICI-Pharma Ltd. (Tokyo) and pindolol by Sankyo Company, Ltd. (Tokyo).

Apparatus——A high speed liquid chromatograph (Nihon Seimitsu, Tokyo) with a fluorometric detector (Hitachi 65-60) was used.

Chromatographic Condition——TSK-GEL LS 410 (5 μm; Toyo Soda Manufacturing Co., Ltd. Tokyo) was packed in a 250 × 4 mm i.d. stainless steel column. The column eluate was monitored with a fluorometer set at excitation and emission wavelengths of 295 and 337 nm, respectively. The column temperature was kept at 24°C.

Mobile Phase——Methanol: 5% ammonium acetate (70: 30) for propranolol determination, and acetonitrile: methanol: 0.2% ammonium acetate (30: 20: 70) for NLA determination were prepared. The flow

rate for both determinations was 1.0 ml/min.

Plasma——Normal healthy male volunteers were orally given 20 mg of propranolol HCl with 100 ml of water in the morning after overnight fasting. The blood samples were collected in heparinized tubes and immediately centrifuged at 3000 rpm for 5 min. The plasma samples were stored at −15°C until assayed.

Extraction of Samples——To 1.0 ml of plasma in a 10 ml glass-stoppered centrifuge tube, 1.0 ml of 0.5 M carbonate buffer (pH 10.2) and 6.0 ml of anhydrous ether were added. The tube was vigorously shaken for 10 minutes then centrifuged at 3000 rpm for 5 min. A 5.0 ml aliquot of the ether layer was transferred to a 10 ml glass-stoppered centrifuge tube and evaporated to dryness under a current of nitrogen at 25°C. This constituted the basic extract. The resulting residue was reconstituted in 100 μl of methanol containing quinidine H_2SO_4 (10 μg/ml) as the internal standard, and a 20 μl aliquot was injected into the HPLC column for the determination of propranolol. The remaining ether in the original extraction tube was carefully removed, and 0.5 ml of 10 N H_2SO_4 and 5.0 ml of anhydrous ether were added to the plasma aqueous phase. The tube was shaken for 10 min and centrifuged at 3000 rpm for 5 min. A 4.0 ml aliquot of the ether phase was transferred to a 10 ml glass-stoppered centrifuge tube, 0.5 ml of diazomethane ether solution was added, and the reaction mixture was allowed to stand for 10 min at room temperature and then evaporated to dryness under a current of nitrogen at 25°C. This constituted the methylated acidic extract. The resulting residue was reconstituted in 100 μl of methanol containing pindolol (100 μg/ml) as the internal standard and a 4—10 μl aliquot was injected into the HPLC column for the determination of NLA.

Results and Discussion

Both propranolol and NLA were extracted successively from the sample plasma aliquot and determined by HPLC. Chromatograms of extracts of blank plasma and plasma in which 8 ng/ml of propranolol HCl and 80 ng/ml of NLA had been spiked are shown in Fig. 1. Methy-

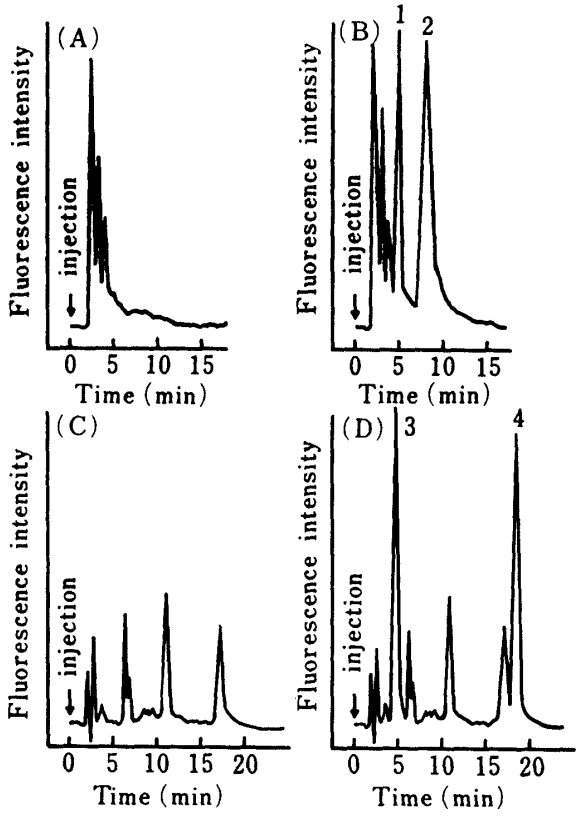

Fig. 1. High Performance Liquid Chromatograms

(A) Basic extract of blank plasma. (B) Basic extract of blank plasma spiked with 8 ng/ml of propranolol HCl. (C) Methylated acidic extract of blank plasma. (D) Methylated acidic extract of blank plasma spikep with 80 ng/ml of NLA. 1, propranolol; 2, quinine H_2SO_4; 3, pindolol; 4, methylated NLA.

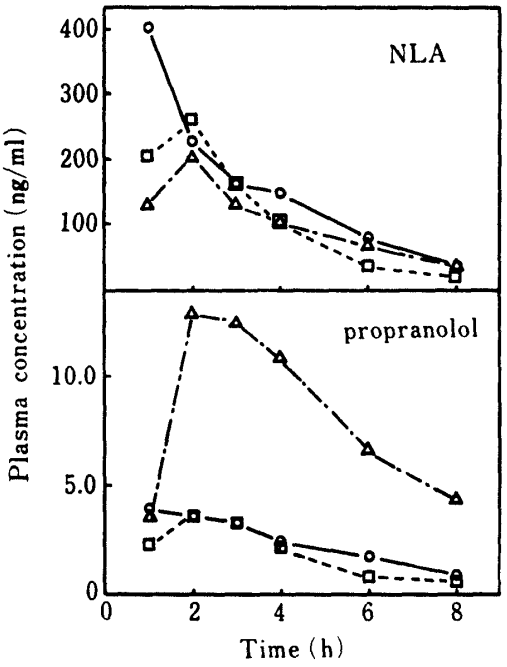

Fig. 2. Plasma Concentrations of Propranolol and NLA following a Single Oral Administration of Propranolol HCl (20 mg) to Volunteers A(○), B(□) and C(△)

Plasma concentration of propranolol is expressed as that of the HCl salt.

TABLE I. Recoveries of Propranolol and NLA from Human Plasma

Compound	Amount added (ng)	Amount found[a] (ng±SD[b])	Recovery (%±SD)
Propranolol HCl	3	2.95 ± 0.09[c]	98.4 ± 3.1
	8	7.51 ± 0.16	93.9 ± 2.0
NLA	30	27.6 ± 1.3	92.0 ± 4.4
	80	77.0 ± 2.6	96.3 ± 3.2

a) Each value is the mean of six determinations.
b) Standard deviation.
c) Calculated as the HCl salt.

lation of NLA gave the best separation of NLA from the plasma components and resulted in a sharp and symmetrical peak of NLA on the HPLC chromatogram as shown in Fig. 1. The calbration curves showed good linearity (r=0.999) for propranolol HCl (20—100 ng/ml) and NLA (0.2—2 μg/ml).

Table I shows the mean recoverises of propranolol HCl and NLA spiked in human plasma. The detection limits (2×baseline) were 0.5 ng/ml for propranolol and 2 ng/ml for NLA.

The plasma concentrations of propranolol and NLA following a single oral administration of propranolol HCl (20 mg) to three healthy volunteers (A, B and C) were determined by this method and the results are shown in Fig. 2. NLA concentrations were considerably higher than propranolol concentrations in every case. Walle *et al.*[10] reported that the maximum concentration of NLA in the plasma was ten times higher than that of propranolol following a single oral dose (20 mg), but Fig. 2 shows fifteen to one hundred times higher concentration of NLA than propranolol. The present method is being applied to pharmacokinetic studies, and the results will be reported later.

References and Notes

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