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Note

High-performance liquid chromatographic procedure for the quantitation of propafenone in serum and tissues

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Propafenone is a new antiarrhythmic agent effective in controlling ventricular and atrial arrhythmias [1] but little is known about its metabolism or pharmacokinetics. In a clinical study dealing with the relationship between pharmacological effects of propafenone and its serum concentrations, Keller et al. [2] found a good correlation between serum levels and atrioventricular conduction times. Serum levels of propafenone were determined by high-performance liquid chromatography (HPLC) by the above authors [2]. In the present communication, we describe a simple HPLC procedure for the quantitation of propafenone in serum and tissue extracts. The method is appropriate for use in the study of clinical and experimental pharmacokinetics of propafenone.

EXPERIMENTAL

Propafenone in injectable form (Rytmonorm® I.V.) was obtained from Knoll (Ludwigshafen, G.F.R.) and was used without further dilution. The internal standard Li 6115, 2′(2-hydroxy-3-ethylaminopropoxy)-3-phenyl-propiophenone hydrochloride, was a gift from Dr. E.B. Kirsten, Knoll Pharmaceuticals (Whippany, NJ, U.S.A.). Diethyl ether and methanol were HPLC grade (J.T. Baker, Phillipsburg, NJ, U.S.A.) and were filtered and degassed before use. All other solvents and chemicals were of reagent grade.

A Waters HLC/GLC Model equipped with a Waters M 6000A pump, a U6K injector, a Model 450 variable-wavelength detector and a Hewlett-Packard HP 3380 A integrator was used. Separations were achieved in an Altex (Beckman, Irvine, CA, U.S.A.) 150 mm × 4.6 mm I.D. column with silica gel (particle size

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5 μm) and a mobile phase consisting of methanol—diethyl ether (30:70, v/v) and 0.02% perchloric acid at a flow-rate of 1.0 ml/min [3]. Propafenone and the internal standard were detected at 254 nm.

The extraction procedure for propafenone from rabbit, dog and human sera was the same as that described by Keller et al. [2]. Rabbit hearts (300—500 mg) were homogenized in 5 parts distilled water and aliquots of supernatant in duplicate equivalent to 120—150 mg wet tissue were taken for analysis. Standard curves were prepared for propafenone and Li 6115 internal standard either together or individually in methanol, serum and myocardial tissue extracts. Interassay and intra-assay standardizations were carried out with rabbit sera.

RESULTS AND DISCUSSION

Fig. 1 shows the separation and quantitation of propafenone (peak I) using internal standard Li 6115 (peak II) in rabbit serum. The retention times of propafenone and internal standard were 4.9 min and 5.8 min, respectively. Fig. 1A shows the chromatogram of blank rabbit serum before propafenone was injected. Fig. 1B and C show the quantitation of propafenone from serum and myocardial tissue 3 h after intravenous (i.v.) injection of propafenone (2 mg/kg) to the same rabbit.

Standard curves were prepared by adding known amounts of propafenone and internal standard to sera from control rabbits, dogs and to normal human serum, and analyzing the samples and determining the peak ratios of propa-

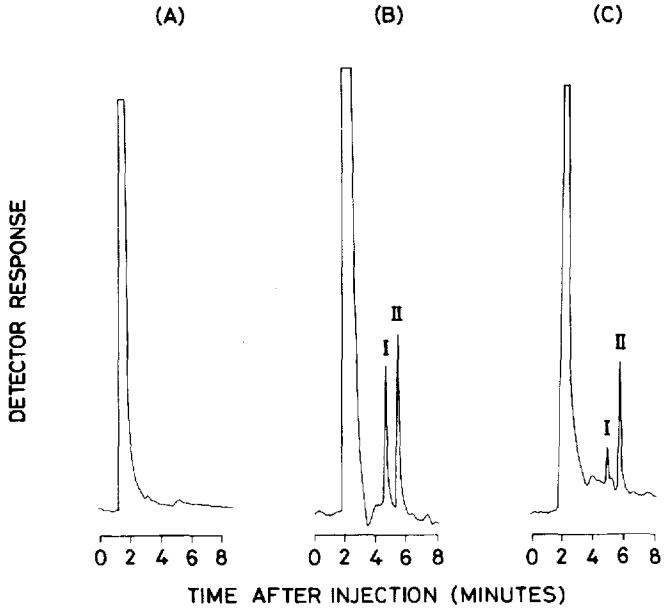

Fig. 1. Quantitation of propafenone from rabbit serum and myocardium. Chromatograms: (A) extracted blank serum sample; (B) and (C) are extracted serum and myocardium respectively from the same rabbit 3 h after i.v. injection of propafenone (2 mg/kg body weight). Peaks: I = propafenone and II = internal standard, Li 6115.

fenone to internal standard. The curves were linear from 0.025—2 μg/ml. Fig. 2 shows a calibration curve obtained using rabbit serum spiked with 0.1—2 μg/ml propafenone and 0.5 μg of Li 6115. The data points for each concentration represent mean ± S.E. from six determinations except for 0.1 and 0.25 μg (n = 4). A good correlation was found between propafenone added to rabbit serum and propafenone found. A linear curve passing through the origin was obtained for the given concentrations. A similar curve (not shown) was obtained for spiked serum samples from control dogs.

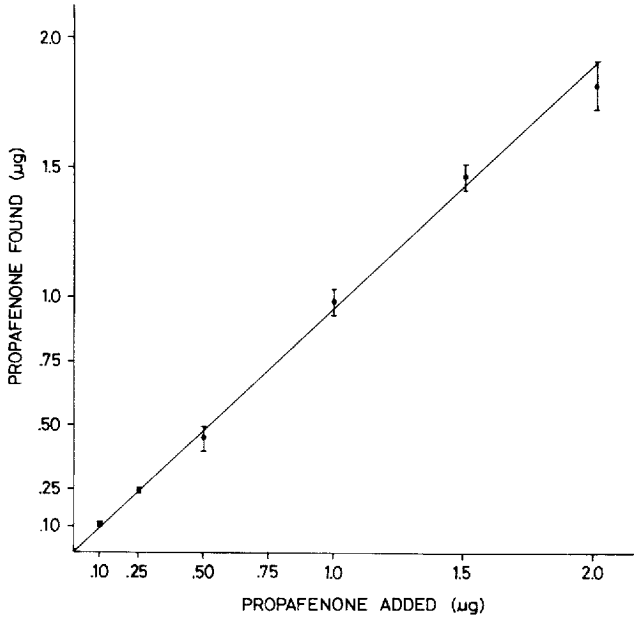

Fig. 2. Calibration curve for propafenone in rabbit serum. The data points represent mean ± S.E. from 4—6 determinations. Quantitation was done by measuring peak height ratios of propafenone to internal standard. Abscissa shows the amount of propafenone added to 1 ml rabbit serum and ordinate, amounts found by HPLC analysis.

The present method could quantitate up to 50 ng/ml propafenone with reasonable accuracy and the detection limit was 20 ng/ml. The intra- and inter-assay variations determined using spiked rabbit sera were 6.1% (n = 12) and 5.5% (n = 8), respectively.

The present method offers certain advantages over the recently published one of Brode et al. [4]. We have found that the Altex 5-μm normal-phase silica column gave better separations than the column used by previous authors [2, 4]. Separations were achieved using a methanol—ether mobile phase which is simple to prepare and which gives a steady baseline. In addition, this method has been successfully adapted for the analysis of tissue extracts such as from myocardial preparations. Experimental and clinical pharmacokinetics of propafenone are currently under study in our laboratory with the aid of this HPLC procedure for the quantitation of propafenone.

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