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Determination of serum atenolol using HPLC with fluorescence detection following isolation with activated charcoal*

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Abstract: In order to determine the bio-availability and pharmacokinetics of two oral 100 mg atenolol preparations, a simple analytical method was developed. Atenolol was determined in serum submitted to an extraction procedure consisting of: (a) adsorption of atenolol to activated charcoal at pH 11, (b) washing the charcoal with water to remove co-extracts and (c) elution of atenolol from the charcoal with organic solvent. The extracts were then analysed by high-performance liquid chromatography (HPLC) with fluorescence detection. The pharmacokinetic parameters obtained from eight healthy humans involved in a clinical bioavailability trial are also presented.

Keywords: *Atenolol; HPLC with fluorescence detection; adsorption to activated charcoal; bioavailability; pharmacokinetics.*

Introduction

Atenolol is a selective beta-adrenergic blocker with an oxypropylamine side chain, which gives rise to characteristic biological and physicochemical properties. Its absorption from the gastrointestinal tract is rather low [1], with the bioavailability ranging from 40 to 60% [2, 3]. HPLC has previously been used for atenolol determination [4, 5]. In drug analysis often multistep and time consuming extractions with organic solvents are used. Previous experience in these laboratories with adsorption of drugs to activated charcoal prompted the use of this adsorbant in the present analytical methodology. Due to the fluorescence properties of atenolol, luminescence spectrometry was used as the detection method.

This paper deals with the development of a new analytical method for atenolol determination in a biological matrix.

Experimental

In order to determine the pharmacokinetics and bioavailability of two oral atenolol

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preparations, an open cross-over randomized trial in human volunteers was designed. The trial protocol was approved and accepted by the Hospital Ethical Drug Committee.

Subjects

Eight healthy volunteers of both sexes, students aged 19–37 years ($\bar{x} \pm S_x$ = 28.8 ± 6.9 years), of body weight 59–77 kg ($\bar{x} \pm S_x$ = 64.8 ± 6.1 kg), were medically examined and informed in detail with regard to the study design and objectives of the trial. The subjects were not allowed to ingest any drugs or alcohol for at least 7 days prior to and during the trial.

Protocol

The interval between two doses was 7 days. On the day of the trial, an indwelling venous cannula was inserted in one of the veins of the forearm of each volunteer. At 8.00 a.m. they were administered a 100 mg dose of either atenolol preparation according to the randomization list. The tablets were taken with 100 ml of water. Blood samples were collected predose, and 1, 2, 3, 4, 6, 8, 10 and 12 h postdose. Sera was separated by centrifugation and stored at −20°C until determination.

Materials

All the chemicals and solvents used were of p.a. quality, obtained from Kemika, Zagreb. The activated charcoal was p.a., obtained from Merck, Darmstadt.

Analytical method

To 1 ml of serum in a test tube, the internal standard (procainamide, 5 μg) was added and the mixture alkalinized with 3 ml of pH 11 carbonate buffer. Then, 100 μl of suspended, activated charcoal (Merck, p.a., 500 mg per 100 ml water) was added and the test tubes vortexed several times for 10 min. After centrifugation (2000 *g* for 10 min), the supernatant was discarded. The charcoal was then washed with 3 ml of water. Atenolol and procainamide were extracted into an organic solvent by vortexing the charcoal with 3 ml of absolute ethanol for 10 min. After centrifugation, the supernatant was transferred to another test tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in the mobile phase (0.1 ml) and analysed by HPLC with fluorescence detection.

The chromatographic system consisted of a Perkin–Elmer LC-10 pump, a Perkin–Elmer LS-5 luminescence spectrometer, a Perkin–Elmer LCI-100 laboratory computing integrator and Rheodyne injector with a 20 μl loop. The column used was an RP-18 Perkin–Elmer analytical 10 μm column (25 cm × 4 mm), with a pre-column filled with the same sorbent. The mobile phase consisted of pH 6.3, 6.7 mmol phosphate buffer and acetonitrile (90:10, v/v). The flow rate was 1.5 ml min^{-1}, and the excitation and emission wavelengths were 230 and 360 nm, respectively. Under the above conditions, atenolol and procainamide capacity factors were 3.7 and 5.7, respectively (Fig. 1), and α = 1.54. The peak area ratio of atenolol to the internal standard was linearly related to the amount of atenolol added to blank plasma.

Calculations

Statistical comparisons were made using the ANOVA test. Pharmacokinetic parameters were calculated using a two-compartment model. The AUCs were calculated using the trapezoid method.

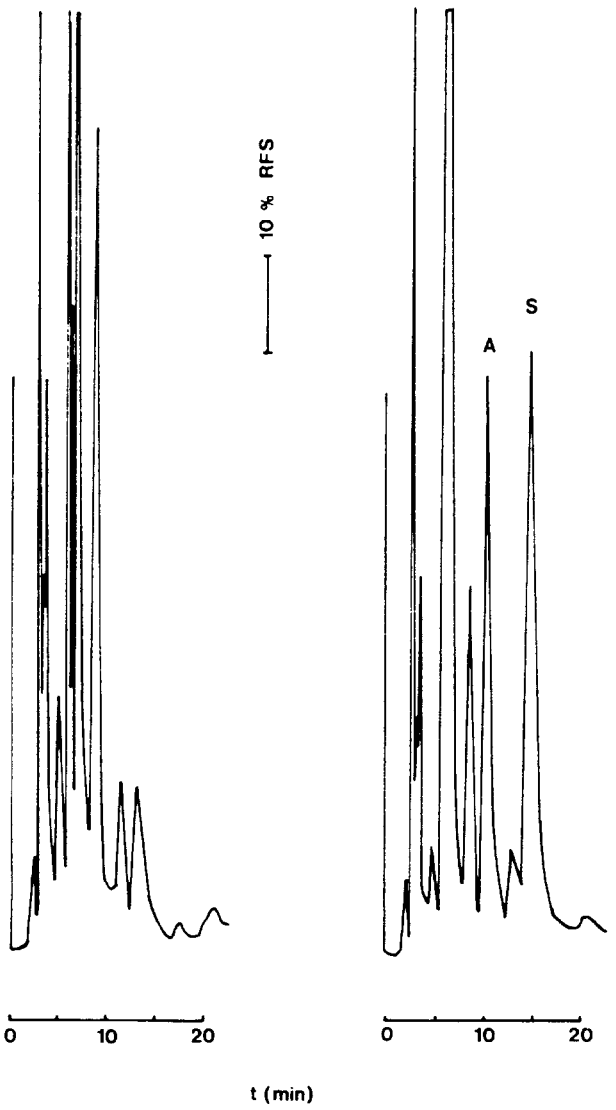

Figure 1

Results and Discussion

The analytical method presented here was based on previously published papers [4–6], with certain modifications which resulted in an overall simplification of the methodology for routine work. The primary advantage results from refinement of the extraction step. The approach chosen allows for the simultaneous processing of multiple samples (usually about 20), the adsorption and elution taking place in the same test tube. The throughput of the resulting extraction method was within the range of about 60 serum samples per 8 h. In comparison with liquid–liquid extraction several pipetting steps are avoided, thus minimizing the error of the analytical procedure. Also, extraction with organic solvents

often leads to the formation of (stable) emulsions or a lipid layer between the organic and the aqueous phases. These problems appear to be overcome by the present extraction method utilizing activated charcoal.

The assay detection limit was 0.020 mg l^{-1} in serum. The precision was 4% at 0.5 mg l^{-1} (n = 10) and the extraction efficiency averaged 83%. No interference was observed from either lipemic or haemolysed blood samples. Figure 1 shows the typical chromatograms for blank plasma and for the same plasma spiked with atenolol (0.5 μg ml^{-1}) and internal standard procainamide (5 μg ml^{-1}). This method was used to determine atenolol in sera of eight healthy volunteers. These volunteers participated in a bioequivalence study comparing 100 mg atenolol tablets from two manufacturers, ICI (Tenormin) and Pliva. The mean atenolol serum levels after the intake of 100 mg tablets (Tenormin or Pliva preparation) in the eight subjects are shown in Fig. 2, while the average pharmacokinetic parameters obtained from the concentration curves are depicted in Table 1.

Previous experience in these laboratories with extractions using activated charcoal [6] have shown the approach to be suitable for the extraction of primary and secondary

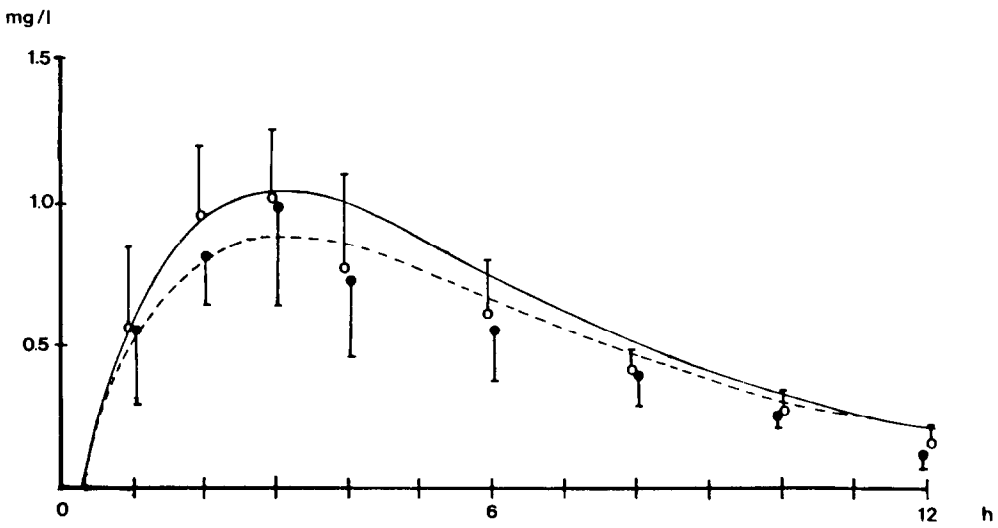

Figure 2

Table 1
Average pharmacokinetic parameters obtained from the concentration curves after administration of a single 100 mg atenolol tablet (n = 8)

Pharmacokinetic parameter		Atenolol preparation Temormin (ICI)		Pliva	
		$\bar{x}$	S_x	$\bar{x}$	S_x
t_{lag}	(h)	0.21	0.39	0.38	0.53
t_{max}	(h)	2.6	0.7	2.6	0.5
C_{max}	(mg l^{-1})	1.07	0.27	1.0	0.32
AUC_{0-12}	(mg h l^{-1})	6.1	1.1	5.8	1.3
beta	(h^{-1})	0.23	0.22	0.19	0.06

amines as well as carboxylic acids, providing that a wash step to remove co-extracts is possible prior to the elution step. However, this procedure is not suitable for amphoteric and non-polar drugs due to the presence of numerous co-extracts.

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