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SHORT COMMUNICATIONS

Stereoselective metabolism of 3-isopropyl-5-(1-naphthoxymethyl)oxazolidine, a prodrug of propranolol

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It is well established that all β-blocking drugs stereoselectively antagonize the binding of norepinephrine at the β-adrenergic receptor [1, 2]. Propranolol (Fig. 1) which is marketed as a racemic mixture of (*R*)- and (*S*)-enantiomers is being used in the treatment of hypertension, angina pectoris and cardiac arrhythmias. As proven by *in vitro* experiments, (*S*)-propranolol (Fig. 1) is about one hundred times more potent as a β-blocking agent than the (*R*)-isomer [3–5]. There are various reports exhibiting the stereochemical differences in the disposition of this drug [6, 7].

Further, propranolol has been shown to undergo extensive presystemic metabolism (first pass effect in liver) after oral administration leading to a reduced bioavailability [8, 9]. Previous studies indicate that the prodrugs of propranolol are more effective for the oral administration as they minimize the first pass effect in liver [10]. Particularly, the ester type of prodrugs of propranolol have been shown to be more effective by increased bioavailability [11]. A recent report describes the stereochemical difference in the hydrolysis of *O*-acetyl propranolol [12], while not much has been explored in terms of the stereochemical aspects of other derivatives.

In view of our interest in the synthesis of prodrugs [13], the present investigation describes the production of propranolol stereoselectively by incubation of 3-isopropyl-5-(1-naphthoxymethyl)oxazolidine (a cyclic derivative of propranolol, Fig. 1) [14] with post mitochondrial supernatant from rat liver.

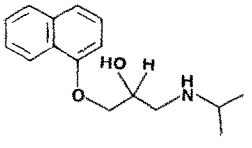
Materials and Methods

Chemicals. Racemic-propranolol was prepared by a standard procedure from 1-(α-naphthyloxy)-2,3-epoxypropane and isopropylamine. (*R*)- and (*S*)-Propranolol hydrochlorides were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). 3-Isopropyl-5-(1-naphthoxymethyl)oxazolidine was synthesized by a previously published procedure from racemic propranolol [14]. The 2-oxazolidone derivatives of (*R*)- or (*S*)-propranolol were prepared by the reported procedure [15]. All reagents and biochemicals used were commercially available. Solvents were HPLC or spectroscopy grade. The characterization and purity of the synthesized materials was established by i.r., TLC and HPLC.

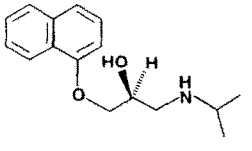
Isolation of propranolol and its identification was by comparison of i.r., NMR and MS properties with the standard sample.

High pressure liquid chromatography. The high performance liquid chromatographic (HPLC) method [15] was employed for the determination of the enantiomers of propranolol after derivatization to 2-oxazolidone derivatives (Fig. 1) [16].

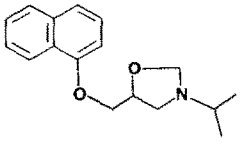
HPLC instrumentation consisted of a Shimadzu liquid chromatograph LC-6A equipped with a SCL-6A system controller, SPD-6A Fixed Wavelength UV Monitor as detector, FCV-100B Fraction Collector and a Chromatopac C-R4A Data Processor as recording integrator. The column was 4.6 × 250 mm Ultropac TSK ODS-120A, 5 μm (LKB) for hydrolysis studies of the prodrug and for semi-preparative work the column employed was 20 × 250 mm Shimpack PREP-ODS, 15 μm (Shimadzu). The eluents were water-methanol (70:30) with 1% acetic acid (v/v). Flow rates were 0.7 mL/min (analytical) and 7,8 mL/min (semi-preparative), monitored at 240 nm wavelength. For the enantiomeric excess determination an Enantiopac Cartridge column (4.0 × 100 mm, 10 ± 2 μm, LKB, Sweden) was used. This column was protected by a Ultropac Precolumn (Lichrosorb RP18, 4.0 × 30 mm, 7 μm, LKB). To assay the 2-oxazolidone derivative of propranolol, a mobile



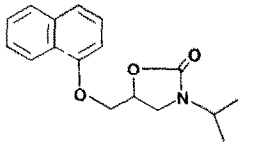
Racemic (R,S) Propranolol



(2S)-Propranolol



3-Isopropyl-5-(1-naphthoxymethyl)-oxazolidine



Oxazolidone Derivative

Fig. 1.

phase of 20% 2-propranol in 8 mM sodium dihydrogen phosphate/sodium hydrogen phosphate with 0.1 M sodium chloride, pH 6.9 was used. The flow rate was 0.25 mL/min and the column effluents were monitored at 230 nm wavelength.

UV and i.r. spectrometry. UV spectra were obtained with an LKB Ultrospec K spectrophotometer using methanol as solvent. i.r. spectra were recorded on a Perkin–Elmer 283B spectrophotometer and the samples were taken in KBr pellet form.

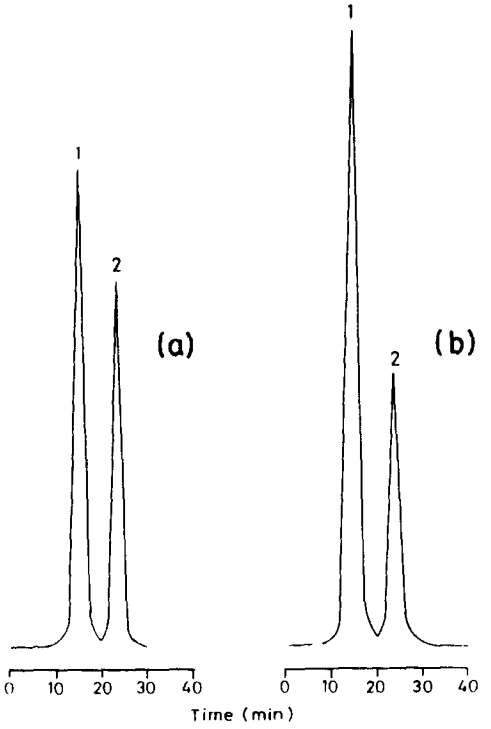


Fig. 2. Chromatograms of the oxazolidinone derivative of propranolol: (a) racemic oxazolidinone derivative of propranolol; (b) propranolol after its isolation from incubation of racemic 3-isopropyl-5-(1-naphthoxymethyl)oxazolidine with post-mitochondrial supernatant at 37° for 60 min (as an oxazolidinone derivative). Peak assignment: peak 1, (*R*)-form of the oxazolidinone derivative of propranolol; peak 2, (*S*)-form of the oxazolidinone derivative of propranolol.

NMR and mass spectrometry. ^{1}H-NMR spectra were taken in $CDCl_3$ on a Jeol FX90Q FT NMR spectrometer. TMS was used as an internal reference. EI-MS (electron energy 70 eV, trap current 200 μA) at source temperature 200° were obtained on a VG Micro-mass 7070H spectrometer equipped with a VG2015 data system.

In vitro *metabolism*. Hepatic post-mitochondrial supernatants were prepared from eight male Wistar rats (weighing 200–250 g). The livers were homogenized in four volumes of ice-cold 50 mM Tris–HCl buffer, pH 7.5, containing 5 mM $MgCl_2$ and 0.25 M sucrose. The homogenate was centrifuged at 4° at 15,000 *g* for 20 min. The supernatant was collected and the protein concentration was determined by the method of Lowry *et al.* [17] and was found as 18.9 mg/mL employing bovine serum albumin as a standard. 3-Isopropyl-5-(1-naphthoxymethyl)oxazolidine (60 μM) was added to the post-mitochondrial suspension and incubated at 37° in 0.01 M phosphate buffer (pH 7.4) at a protein concentration of 1.8 mg/mL. Samples, 100 μL, were collected at predetermined intervals. Reactions were stopped by the addition of 200 μL of acetonitrile. After removal of protein precipitate by centrifugation, the samples were assayed. After 60 min of incubation the propranolol formed as a metabolite was separated on a semi-preparative column employing a fraction collector (manual mode programme) and freeze-dried. Its enantiomeric composition was determined after derivatization to the 2-oxazolidone employing the reported procedure [15]. Pseudo-first-order rate constants for the hydrolysis of the oxazolidine were determined from the slopes of linear plots of the logarithm of propranolol obtained against time.

Another set of control incubations was carried out in boiled post-mitochondrial supernatant.

Results and Discussion

The hydrolysis rate of the enantiomers of racemic (*R*)- and (*S*)-3-isopropyl-5-(1-naphthoxymethyl)oxazolidine was determined in phosphate buffer (pH 7.4) and in hepatic post-mitochondrial supernatant at 37°. The reaction exhibited first-order kinetics under the experimental conditions employed. The hydrolysis of (*R*)-oxazolidine was about four times faster than that of (*S*)-oxazolidine in hepatic post-mitochondrial supernatant. This stereoselective difference in conversion of the (*R*)- and (*S*)-oxazolidine derivatives to propranolol is illustrated in Table 1 and Fig. 2. Similar rate constants were observed by employing the pure isomeric forms of the oxazolidine prodrug upon hydrolysis in hepatic post-mitochondrial supernatant. The hydrolysis of the oxazolidine was not observed in preheated post-mitochondrial supernatant. The *in vivo* metabolism studies of this prodrug in different species will be published later.

Table 1. The first-order hydrolysis rate constants of racemic 3-isopropyl-5-(1-naphthoxymethyl)oxazolidine in hepatic post-mitochondrial supernatant and phosphate buffer

	Hydrolysis rate constant ($min^{-1} \times 10^2$)	
	Post-mitochondrial supernatant	Boiled post-mitochondrial supernatant
R-Enantiomer	4.57 ± 0.07	0.03 ± 0.4
S-Enantiomer	1.27 ± 0.07	0.03 ± 0.4

Each value is the mean ± SD (N = 5).

In summary, this investigation suggests that the oxazolidine derivative of propranolol is a prodrug which is hydrolysed stereoselectively to propranolol by hepatic post-mitochondrial supernatant. The (*S*)-form of the prodrug is more stable in the biological system than its (*R*)-form.

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The fate of intravenously administered biotin-labelled hyaluronidase in the rat

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Traditionally, mammalian testicular hyaluronidases (hyaluronate 4-glycanohydrolase, EC 3.2.1.35) have been indicated in the treatment of a variety of clinical conditions [1] and are often used to enhance the intramuscular or subcutaneous administration of a variety of drugs. More recently, the efficacy of the enzyme has been evaluated for the treatment of myocardial infarction [2] and other conditions [3]. Efficacy remains to be established although there have been clinical improvements in some of the patients with myocardial infarction and a reduction in cumulative mortality [4–6]; however results from other studies are less clear [7, 8].

A major problem with the intravenous administration of hyaluronidase is the rapid removal of the enzyme from the bloodstream; the serum half-life in humans is approximately 3.2 min [9]. Similar values for half-life have been measured in rats using ^{125}I-labelled hyaluronidase where the bulk of the enzyme has accumulated in the liver within 10 min of administration [10]. Reducing the accumulation of enzyme by liver with a resultant increase in serum half-life should improve efficacy. The presence of three high-mannose type oligosaccharide subunits per polypeptide chain of hyaluronidase [11] is probably the reason for rapid uptake by liver. Previous studies with ^{125}I-labelled enzyme have shown that pre-administration of mannans significantly increased the serum half-life [10].

Preliminary studies have indicated that it is possible to label hyaluronidase with biotin as an alternative to ^{125}iodine [12]. This would allow the distribution and uptake of the enzyme to be monitored using an independent method based on detection with streptavidin-conjugates. In this paper we report on the use of biotin-labelled hyaluronidase