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Stereoselective hydrolysis of *O*-acetyl propranolol as prodrug in human serum

KOICHI TAKAHASHI, JUN HAGINAKA, SATOKO TAMAGAWA, TOSHIAKI NISHIHATA*, HIROYUKI YASUDA, TOYOSHI KATAGI, *Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11–68 Koshien Kyuban-cho, Nishinomiya, Hyogo 663, Japan and *Pharmaceutical Chemistry Department, University of Kansas, 2065 Constant Avenue, Lawrence, KS 66046, USA*

Abstract—A direct high-performance liquid chromatographic method was developed for the assays of the enantiomers of *O*-acetyl propranolol. Using this procedure, the stereochemical characteristics on hydrolysis of racemic *O*-acetyl propranolol as a prodrug have been studied in phosphate buffer (pH 7·4) and in 90% human serum. In the phosphate buffer, no difference in the hydrolysis rate between the esters of (*R*)- and (*S*)-propranolol was observed. In 90% human serum, the hydrolysis of the esters was accelerated, and the hydrolysis rate of the ester of (*R*)-isomer was about three times faster than that of the ester of (*S*)-isomer. The interconversion between (*R*)- and (*S*)-isomer was not observed during the hydrolysis of prodrug in buffer and in human serum. These results indicated that hydrolysis of *O*-acetyl propranolol occurs stereoselectively in human serum.

Propranolol is a nonselective β-adrenoceptor blocking agent used in the treatment of angina pectoris, hypertension, and cardiac arrhythmia. It has been shown to undergo extensive presystemic metabolism (first pass effect in liver) after oral administration leading to a reduced bioavailability (Lo et al 1982; Iwamoto & Watanabe 1985). Garceau et al (1978) and Anderson et al (1988) have shown that the hemisuccinate or acetate ester of propranolol, after dosing orally to beagle dogs or rats, yielded higher plasma propranolol concentrations than after an equivalent dose of propranolol hydrochloride; i.e. the prodrugs of propranolol are more effective forms for oral administration as they reduce the first pass effect in liver.

Propranolol has two optical isomers, dextro (*R*)- and laevo (*S*)-propranolol. The (*S*)-isomer is about 100 times more potent as a β-adrenoceptor blocker than the (*R*)-isomer (Barrett & Cullum 1968). A number of studies have demonstrated the stereochemical differences in the disposition of this drug (George et al 1972; Bai et al 1983; Olanoff et al 1984; Walle et al 1984). In spite of the reports on the effectiveness of the ester type of prodrug of propranolol in increasing bioavailability (Garceau et al 1978; Anderson et al 1988), there is no report on the stereochemical differences in their hydrolysis.

In the present study, a direct high performance liquid chromatographic (HPLC) method for the simultaneous determination of the enantiomers of *O*-acetyl propranolol has been developed and the stereoselective hydrolysis of *O*-acetyl propranolol as a prodrug demonstrated using a developed assay procedure.

Materials and methods

Materials. Racemic-propranolol hydrochloride was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). (*R*)- and (*S*)- propranolol hydrochlorides were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). All other reagents used were of analytical grade. The racemic mixture of *O*-acetyl propranolol hydrochloride was prepared by a previously published procedure from racemic propranolol (Nelson & Walker 1978). *O*-Acetyl (*R*)- or (*S*)-propranolol was prepared by the same procedure. Following extractive purification and recrystallization, the identity and purity of the synthesized materials was established by IR, TLC and HPLC.

Hydrolysis of O-acetyl propranolol. A 100 μL amount of *O*-acetyl propranolol solution (200 μg mL^{-1}) was added to 900 μL of human serum and incubated at 37°C. Samples, 100 μL, were collected at predetermined intervals and acetonitrile (200 μL) for quenching the hydrolysis was added. The agitated mixture was centrifuged at 10 000 *g* for 10 min, and the supernatant (100 μL) was mixed with 100 μL of mobile phase for HPLC and stored in an ice-water bath until the assay. A five μL aliquot of the sample was loaded onto the column. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual prodrug against time. The study on the hydrolysis of *O*-acetyl propranolol in phosphate buffer (pH 7·4, $\mu = 0{\cdot}5$) was also performed according to the procedures described above, without centrifugation.

Chromatography. The system consisted of a Jasco pump (TRI ROTAR-V, Tokyo, Japan), a Rheodyne 20 μL loop (Model 7125, Cotati, CA, USA), and Jasco fluorescence detector set (FP-210, Tokyo) at excitation and emission wavelengths of 285 nm and 340 nm, respectively. An ovomucoid-conjugated column (Ultron ES-OVM, 4·6 mm i.d. × 15 cm, Shinwa Kako Co. Ltd, Kyoto, Japan) was used. The analytical column was protected by a guard column (4·6 mm i.d. × 1 cm) packed with the same materials. To assay the propranolol prodrug, the mobile phase of 10% v/v acetonitrile in 20 mM sodium dihydrogen phosphate was used. The flow rate was 1·0 mL min^{-1} and the separation was performed at ambient temperature.

Results and discussion

Previously reported HPLC methods for the simultaneous determination of propranolol enantiomers in biological fluids have usually involved procedures such as extraction and derivatization (Silber & Riegelman 1980; Sedman & Gal 1983; Wilson & Walle 1984). Recently, Takahashi et al (1988) proposed an analytical method for propranolol enantiomers using chiral stationary-phase liquid chromatography. This method also involves the extraction step for propranolol. The ovomucoid-conjugated column having chiral recognition properties has been used for the chromatographic resolution of racemic compounds (Miwa et al 1987). To analyse the enantiomers of the propranolol prodrug, we developed a direct HPLC method requiring only one step of deproteinization followed by HPLC separation on this column. Under the conditions of HPLC, we observed no interfering peak for *O*-acetyl propranolol enantiomers, when blank samples of serum and buffer were analysed. The retention times were 12·7 min for *O*-acetyl (*R*)-propranolol and 15·8 min for the (*S*)-isomer (Fig. 1). Calibration curves used

Correspondence to: K. Takahashi, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11–68 Koshien Kyuban-cho, Nishinomiya, Hyogo 663, Japan.

Table 1. The first-order hydrolysis rate constants of racemic *O*-acetyl propranolol in phosphate buffer and human serum.

	Hydrolysis rate constant ($min^{-1} \times 10^2$)	
	Phosphate buffer	Serum
R-	0·47 ± 0·02	3·48 ± 0·09
S-	0·47 ± 0·02	1·12 ± 0·09

Each value represents mean ± s.d. ($n = 3$).

for quantification of *O*-acetyl propranolol in buffer and human serum exhibited excellent linearity at a concentration range of 0·4 to 15 μM for each isomer. With the mobile phase used to determine the prodrug, propranolol was observed as a single peak (Fig. 1, peak 1). But the enantiomers of propranolol were separately determined with the 10% v/v ethanol in 20 mM sodium dihydrogen phosphate (chromatogram not shown).

Using the racemic mixture, the hydrolysis rate of each enantiomer of *O*-acetyl propranolol was determined in the phosphate buffer (pH 7·4, $\mu = 0{\cdot}5$) and in 90% human serum at 37°C. Under the experimental conditions used, the reaction displayed strict first-order kinetics. There was no difference between *O*-acetyl (*R*)- and (*S*)-propranolol hydrolysis rate in the phosphate buffer. However, a stereoselective hydrolysis was observed in 90% human serum; i.e. the hydrolysis of *O*-acetyl (*R*)-propranolol was three times faster than that of *O*-acetyl (*S*)-isomer (Table 1). Using each pure isomer of prodrug, the same rate constants were obtained, and the interconversion between (*R*)- and (*S*)-isomer was not observed during the hydrolysis study in buffer and human serum (data not shown). These results suggest that hydrolysis of *O*-acetyl propranolol as a prodrug occurs stereoselectively in human blood. As mentioned in the introduction, *O*-acetyl propranolol is more effective than propranolol by oral administration because of the reduction of the first pass effect (Anderson et al 1988). Furthermore, the prodrug (*S*)-isomer is biologically stable compared with the prodrug (*R*)-isomer. From these facts, it may be possible that the action (or plasma concentration) of the (*S*)-isomer is sustained. The assay method developed seems to be a useful tool for investigating the stereochemical disposition of *O*-acetyl propranolol after its oral administration.

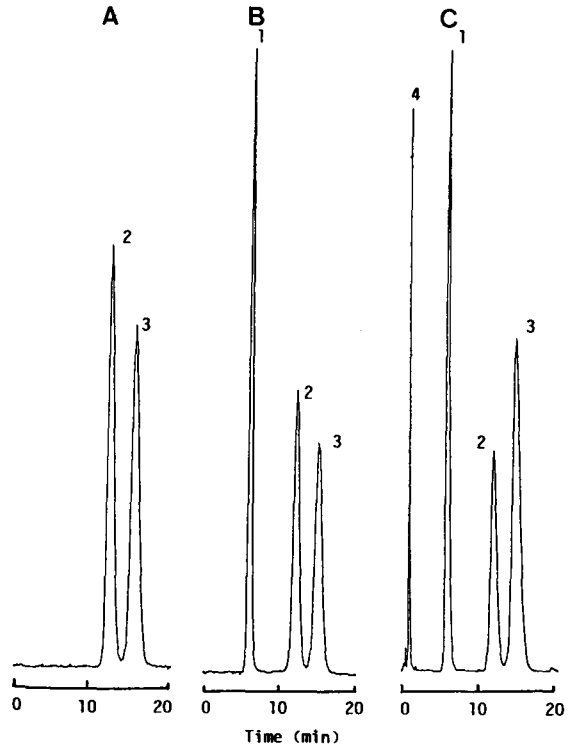

FIG. 1. Elution profiles of *O*-acetyl propranolol hydrolysed in serum or phosphate buffer. Part A, racemic *O*-acetyl propranolol before hydrolysis; B, racemic *O*-acetyl propranolol hydrolysed in 0·02 M phosphate buffer (pH 7·4, $\mu = 0{\cdot}5$) at 37°C for 90 min; C, racemic *O*-acetyl propranolol hydrolysed in 90% human serum at 37°C for 20 min.
Peak assignment: peak 1, propranolol; peak 2, *O*-acetyl (*R*)-propranolol; peak 3, *O*-acetyl (*S*)-propranolol; peak 4, serum blank.

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