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Chromatographic resolution and pharmacological investigation of ICI 118551, a new β_2-blocker

M.G. Quaglia*, N. Desideri and E. Bossù

Dipartimento Studi Farmaceutici, Università degli Studi "La Sapienza", P.le A. Moro 5, 00185 Rome (Italy)

A. Farina

Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome (Italy)

L.A. Morrone

Istituto di Farmacologia e Farmacognosia, Università degli Studi "La Sapienza", P.le A. Moro 5, 00185 Rome (Italy)

ABSTRACT

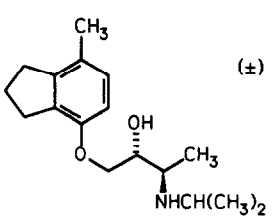
ICI 118551, a highly selective β_2-blocking compound, and its *threo* isomer, one of the possible impurities of drug, were resolved by high-performance liquid chromatography on a chiral stationary phase. The enantiomers of ICI 118551 were separately collected and the β_2-blocking activity of each isomer was tested on the guinea pig trachea.

INTRODUCTION

The β-adrenergic receptor antagonists (β-blockers) are drugs widely used in therapy for the treatment of hypertension and ischaemic heart disease. The β-receptors can be subdivided into β_1 (cardiac receptors) and β_2 (periferal receptors) subtypes. Generally the β_2-blockers have also a β_1-blocking activity.

Most of the β-blockers are arylpropanolamines or aryloxypropanolamines and are used as racemic mixtures, although the pharmacological activity is mainly due to the *S* isomer [1]. The introduction of a methyl substituent on C-3 of the chain leads to another centre of asymmetry with four possible stereoisomers, two *erythro* and two *threo* enantiomers. The 3-methyl substitution can confer a degree of β_2-selectivity [2].

ICI 118551, the racemic mixture of the *erythro* form of 1-[(2,3-dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol, is one of the first selective β_2-blockers to be synthesized [3], which will be applied in therapy for the treatment of vasomotor migraines and somatic problems related to anxiety states. No data have been reported on the activity of the individual enantiomers of ICI 118551.



(±) ICI 118551

In this paper, a chromatographic method to resolve the racemic mixture of ICI 118551 and the pharmacological activity of the separated

* Corresponding author.

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enantiomers is reported. This method can also be useful to reveal the presence of the less β_2-selective *threo* isomer, which could remain as an impurity in the bulk material, although a stereoselective synthesis of the *erythro* isomer has been developed [4].

The chiral discrimination of ICI 118551 and of its *threo* isomer was carried out using a cellulose tris(3,5-dimethylphenylcarbamate) adsorbed on macroporous silica gel as a chiral stationary phase (CSP). The good resolution of racemic ICI 118551 allows the separated enantiomers to be collected and their pharmacological activity to be tested.

EXPERIMENTAL

Chemicals

All solvents and chemicals were of HPLC or analytical-reagent grade (Merck, Darmstadt, Germany).

Pure standards of ICI 118551 and its *threo* form were kindly supplied by Italian Zeneca, Pharmaceutical Division (Milan, Italy).

Equipment

The HPLC discrimination of enantiomers was carried out with a Waters (Milford, MA, USA) Model 6000 A system equipped with an HP 1040 M linear photodiode-array detector controlled by an HP 9000 Model 310 computer (Hewlett-Packard, Palo Alto, CA, USA). Sample solutions were injected via a Rheodyne Model 7125 valve using a 6-μl sample loop. The photodiode-array detector conditions were 220 and 276 nm, acquisition rate of spectra 1.280 ms, bandwidth for each channel 4, sensitivity range 50, reference wavelength 450 nm and reference bandwidth 50.

Circular dichroism (CD) spectra were obtained with a Jasco 500-A dichrograph, kindly provided by Professor P. De Santis (Dipartimento di Chimica, Università "La Sapienza", Rome, Italy).

Chromatographic analysis

The chromatographic separation of enantiomers was carried out by using as chiral selector a commercial CSP Chiracel OD (10 μm) column (250 mm × 4.6 mm I.D. (Daicel Chemical Industries). The mobile phase was hexane–2-propanol (8:2, v/v) at a flow-rate of 0.8 ml/min.

The identity in the UV spectra of each enantiomeric pair was checked and the *erythro* isomers were separately collected from different runs. The optical purity of the resolved enantiomers was 98% enantiomeric excess (ee) for the first-eluted enantiomer and 93% ee for the most retained enantiomer. The hexane–2-propanol solutions were evaporated under vacuum and the residues were dissolved in ethanol for further tests (circular dichroism and pharmacological assay) and the solution concentrations were determined chromatographically.

Circular dichroism

CD spectra were recorded on ethanolic solutions (3.6 mM) using 0.1-mm cells.

Pharmacological assay

The β_2-blocking activities of the racemic compound and of the two separated *erythro* enantiomers were tested on the guinea-pig trachea. The trial was carried out on a tracheal zig-zag strip by measuring the ability of each enantiomer to counteract the tissue relaxation previously induced by salbutamol, a selective β_2 agonist [5–7].

Guinea-pig trachea was chosen because a remarkable predominance of β_2-adrenergic receptors on β_1-receptors is present in this tissue [8–11]. Generally, the β-adrenergic activity of drugs is tested on the tracheal chain [12]. In the pharmacological assay of ICI 118551 enantiomers, the zig-zag strip preparation was preferred because it gives quantitative responses similar to those obtained with a tracheal chain, but it can be prepared more quickly and easily and it provides two preparations with one trachea.

The experimental procedure was that of Emmerson and Mackay [13]. The isolated tracheal strip was set up under 0.5 g tension in a 10-ml organ bath containing Tyrode solution bubbled with a gas mixture of O_2–CO_2 (95:5) and maintained at 37°C. Changes in tension were recorded by means of an isotonic force transducer. The preparations were allowed to equilibrate for 50–60 min. They were then stimulated with a high concentration of acetylcholine (10^{-6} M) to

ascertain their suitability. After a further 15–20 min, salbutamol was added to the bath. Salbutamol (10^{-7} *M*) caused a relaxation of the tissue. The drugs to be tested were solubilized in ethanol–water (1:1) and were administered 1, 5 or 10 min before salbutamol. Only the data obtained when the drugs were administered 1 min before salbutamol are reported, as the effects of the drugs given 5 or 10 min before the agonist were very similar (Table I, Fig. 3).

The trials showed that the racemic compound counteracted in a dose-related way the salbutamol-induced relaxation of the tissue; only the second-eluted enantiomer elicited a similar dose-dependent inhibition; the first-eluted enantiomer did not affect the salbutamol-induced relaxation even at doses ten times higher than that of salbutamol. The results were expressed as IC_{50} (molar concentration producing 50% of the maximum inhibition response; Table I).

RESULTS AND DISCUSSION

The use of a Chiracel OD column allows the resolution of the *erythro* isomers and the detection of trace amounts of the *threo* isomers in the sample under investigation. Chromatograms with on-line resolution of *erythro* and *threo* racemic mixtures and the UV spectra of the peaks are reported in Fig. 1a and b, respectively. As the first-eluted enantiomer of each pair has the same retention time, the superimposition of the chromatograms (Fig. 2) shows three well separated peaks. Therefore, the proposed chromatographic method can be used for the detection of one isomer of the *threo* impurity in ICI 118551, whereas the ion-pair reversed-phase chromatographic method, used to analyse the other impurity, is unqualified [14].

The proposed method was successfully applied to the preparative separation of the *erythro* stereoisomers. These were therefore collected and characterized for their optical purity by using the same HPLC method.

The β_2-blocking activity of the single enantiomers was evaluated on the guinea-pig trachea. The biological results suggest that the antagonist action of the racemic compound on the β_2-receptors is due only to the second-eluted enantiomer (Table I and Fig. 3).

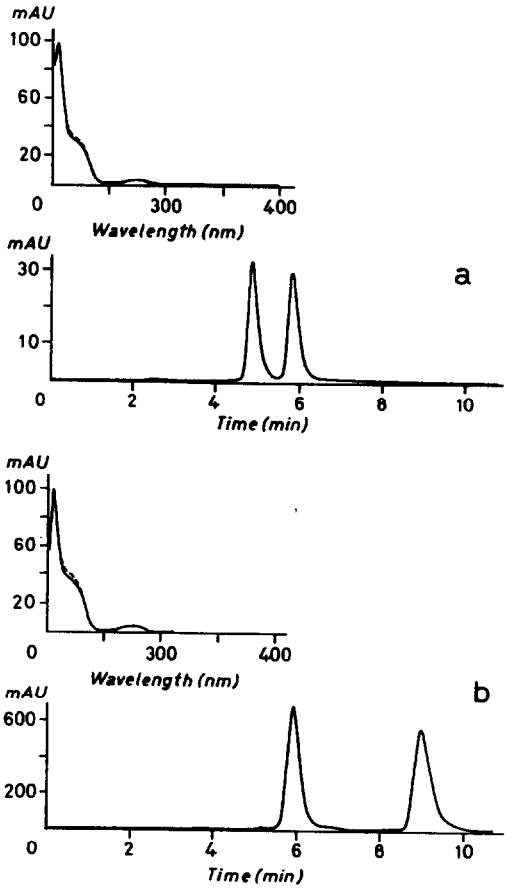

Fig. 1. Chiral discrimination and relative UV spectra of (a) *erythro* and (b) *threo* forms.

The mechanism affording the pharmacological activity of ICI 118551 is highly stereoselective

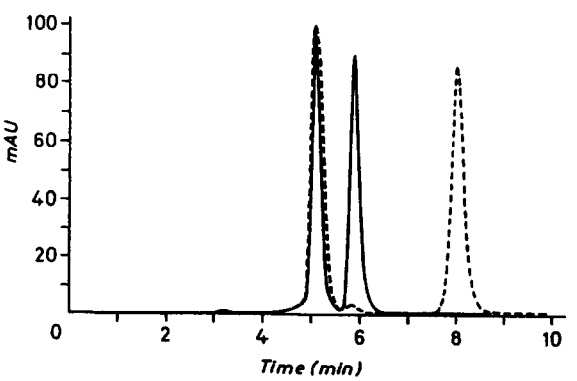

Fig. 2. Superimposition of chromatograms in Fig. 1a and b.

TABLE I

β_2-BLOCKING ACTIVITY OF ICI 118551 ENANTIOMERS AND RACEMATE

Drug	IC_{50} (M) in presence of salbutamol	
	$4.18 \cdot 10^{-7}$ M	$1.6 \cdot 10^{-8}$ M
Racemic	$3.00 \cdot 10^{-7}$ [a]	$2.20 \cdot 10^{-8}$ [b]
Peak 1	Not active[a]	Not active[a]
Peak 2	$3.00 \cdot 10^{-7}$ [a]	$2.10 \cdot 10^{-8}$ [a]

[a] Means of experiments on five tissues.
[b] Mean of experiments on four tissues.

and the stereochemical characterization of the drug, still under investigation, will be fundamental for understanding the chiral discrimination mechanism.

Structurally related chiral β-blockers with only the C-2 on the propanolamine chain as a stereogenic centre show pharmacological activity as mainly related to the *S* enantiomer. Moreover, several of these drugs were resolved using cellulose tris(3,5-dimethylphenylcarbamate) as a CSP. Hydrogen bonding between the hydroxy group of the β-blockers and the carbonyl group of the CSP seems to play the most important role in chiral recognition, and the *S* enantiomer is the more strongly retained in all instances [15,16]. On these bases, a 2*S*, 3*R* configuration could be tentatively suggested for the more retained enantiomer.

The CD spectra of resolved *erythro* isomers

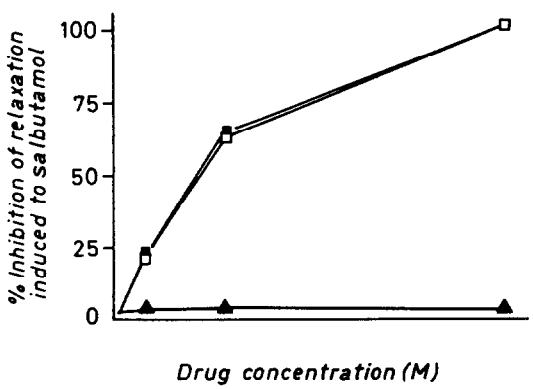

Fig. 3. Dose–effect diagram relative to (■) *rac*-ICI 118551, (▲) first-eluted enantiomer and (□) second-eluted enantiomer on relaxation induced by salbutamol ($4.2 \cdot 10^{-7}$ M).

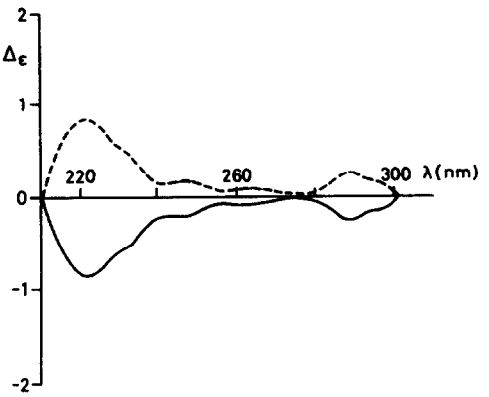

Fig. 4. Circular dichroism spectra of (solid line) first-eluted enantiomer and (broken line) second-eluted enantiomer of ICI 118551.

were recorded and are reported in Fig. 4. The first-eluted enantiomer exhibits some negative bands of low intensity between 300 and 210 nm and the second-eluted enantiomer similar positive bands. Unfortunately, we have not been able to compare our CD data with those of related systems owing to the lack of comparable literature data.

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