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Short communication

Analysis of enantiomers of sibutramine and its metabolites in rat plasma by liquid chromatography–mass spectrometry using a chiral stationary-phase column

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ABSTRACT

Sibutramine, a monoamine reuptake inhibitor, is used as a racemate, for the treatment of obesity. It is converted *in vivo* mainly to two desmethyl active metabolites, mono-desmethylsibutramine (MDS) and di-desmethylsibutramine (DDS). In the present study, we introduced a rapid and simple chromatographic method for separating the *R*(+)- and *S*(−)-isomers of sibutramine, MDS, and DDS, respectively. The stereoisomers of the three compounds were extracted from rat plasma using diethyl ether and n-hexane under alkaline conditions. After evaporating the organic layer, the residue was reconstituted in the mobile phase (10 mM ammonium acetate buffer adjusted to pH 4.03 with acetic acid:acetonitrile, 94:6, v/v). The enantiomers in the extract were separated on a Chiral-AGP stationary-phase column and were quantified in a tandem mass spectrometry. The accuracy and precision of the assay were in accordance with FDA regulations for the validation of bioanalytical methods. This method was used to measure the concentrations of the enantiomers of sibutramine, MDS, and DDS in plasma after a single oral dose of 10 mg/kg racemic sibutramine in rats.

1. Introduction

Sibutramine, a monoamine reuptake inhibitor, is currently used as a racemate, for the treatment of obesity. It is converted *in vivo* mainly to two desmethyl active metabolites, mono-desmethylsibutramine (MDS) and di-desmethylsibutramine (DDS) [1,2]. The enantioselective behaviors of sibutramine and its two major active metabolites have been of interest from a pharmacokinetic as well as a pharmacodynamic point of view. The selective effects of the enantiomers on pharmacological consequences have been well characterized, with the *R*(+)-enantiomer being 200-fold potent more than the *S*(−)-enantiomer [3]. However, their kinetic characteristics are still unclear.

To date, sibutramine and its metabolites in biological samples have been determined using gas or liquid chromatography/mass spectrometry (LC–MS) [4,5]. However, their chiral separation has been limited to pharmaceutical drug products [6,7]. Therefore, we developed a chiral chromatography method to determine the enantioselective pharmacokinetics of sibutramine, MDS, and DDS in rat plasma.

The chiral resolution of enantiomers has been carried out using a specific chiral stationary phase, a mobile phase with a chiral reagent, and a derivatization method [8,9]. In the present study, we introduce a rapid and simple chromatographic method for separating the *R*(+)- and *S*(−)-isomers of sibutramine as well as its two active metabolites. The method is based on the use of a chiral column and a tandem mass spectrometry. We successfully used the method to characterize the time course of changes in the plasma concentrations of the stereoisomers of sibutramine as well as those of its two active metabolites in rat plasma, following the oral administration of racemic sibutramine.

2. Experimental

2.1. Reagents and materials

Sibutramine, MDS, and DDS were kindly donated by Yuhan Pharmaceutical Co. (Seoul, Korea) and the purity of three compounds is more than 99.5%. Domperidone (internal standard, IS) was purchased from Sigma (Seoul, Korea), and n-hexane and diethyl ether

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were obtained from J.T. Baker (Seoul, Korea). All other chemicals and solvents were of the highest analytical grade available. *R*(+)- and *S*(−)-enantiomers were separated with dibenzoyl-D-tartaric acid at a medicinal chemistry laboratory in College of Pharmacy, Catholic University of Daegu, which was based on the literature previously published by Fang et al. [10].

2.2. Preparation of standards and quality controls

Sibutramine, MDS, DDS, and the IS were dissolved in methanol to obtain a concentration of 1.0 mg/ml. These solutions were diluted serially with the mobile phase (10 mM ammonium acetate buffer adjusted to pH 4.03 with acetic acid:acetonitrile, 94:6, v/v), and 5 μl of each solution was added to 85 μl of drug-free plasma, to obtain final concentrations of 1, 2, 5, 10, 20, and 50 ng/ml sibutramine; 1, 2, 5, 10, 50, and 100 ng/ml MDS; and 10, 50, 200, 1000, and 4000 ng/ml DDS. Using linear regression, six calibration graphs were derived from the ratio between the area under the peak of each compound and the IS.

Quality control samples were prepared in 85 μl of blank rat plasma by adding 5 μl of serially diluted solutions of each of the three racemates, to obtain low, intermediate, and high concentrations in control samples. These samples were used to evaluate the between days and within day precision and accuracy of the assay.

2.3. Characterization of the product ions using tandem mass spectrometry

In brief, 10 ng/ml each of the sibutramine, MDS, DDS, and IS solutions were separately infused into the mass spectrometer at a flow rate of 10 μl/min, to characterize the product ions of each solution. The precursor ions $[M+H]^+$ and the pattern of fragmentation were monitored using the positive ion mode. The major peaks in the MS/MS scan were used to quantify sibutramine, MDS, DDS, and the IS.

2.4. Analytical system

Plasma concentrations of sibutramine, MDS, and DDS were quantified using an API 4000 LC/MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization interface that was used in the positive ion mode ($[M+H]^+$).

The compounds were separated on a chiral stationary-phase column (Chiral-AGP, 100 mm × 2.0 mm internal diameter, 5-μm particle size; ChromTech Ltd., Congleton, Cheshire, UK) with a mobile phase that consisted of 10 mM ammonium acetate adjusted to pH 4.03 with acetic acid:acetonitrile (94:6, v/v). The column was heated to 22 °C, and the mobile phase was eluted at 0.2 ml/min using an HP 1100 series pump (Agilent, Wilmington, DE, USA). The Turboion spray interface was operated in the positive ion mode at 5500 V and 450 °C. Sibutramine, MDS, DDS, and domperidone (IS) produced mainly protonated molecules at *m*/*z* 280.2, 266.0, 252.1, and 427.2, respectively. The product ions were scanned in Q3 after collision with nitrogen in Q2 at *m*/*z* 125.2 for sibutramine, MDS, and DDS, and at *m*/*z* 175.1 for domperidone. Quantitation was performed by multiple reaction-monitoring (MRM) of the protonated precursor ions and the related product ions, using the ratio of the area under the peak for each solution and a weighting factor of $1/y^2$. The analytical data were processed with Analyst software (version 1.4.1, Applied Biosystems).

2.5. Sample preparation

One hundred microliters of the IS (100 ng/ml in mobile phase) and 0.01 ml of 10 N NaOH were added to 0.1 ml of rat plasma, followed by liquid–liquid extraction for 10 min with 1.5 ml of diethyl ether:n-hexane (4:1, v/v). The organic layer was separated and removed at 40 °C in a heated centrifugal evaporator (EYELA CVE-200D; Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The residue was reconstituted in 50 μl of the mobile phase by vortex-mixing for 15 s, and 5 μl of this solution was injected onto the column.

2.6. Validation procedure

The validation parameters were selectivity, extraction recovery, precision, and accuracy. Blank plasma samples obtained from five rats were screened to determine specificity. The extraction recoveries of sibutramine, MDS, and DDS were calculated by comparing the peak area ratios measured for the standard solution, considering condensation, with those obtained for the plasma extracts after

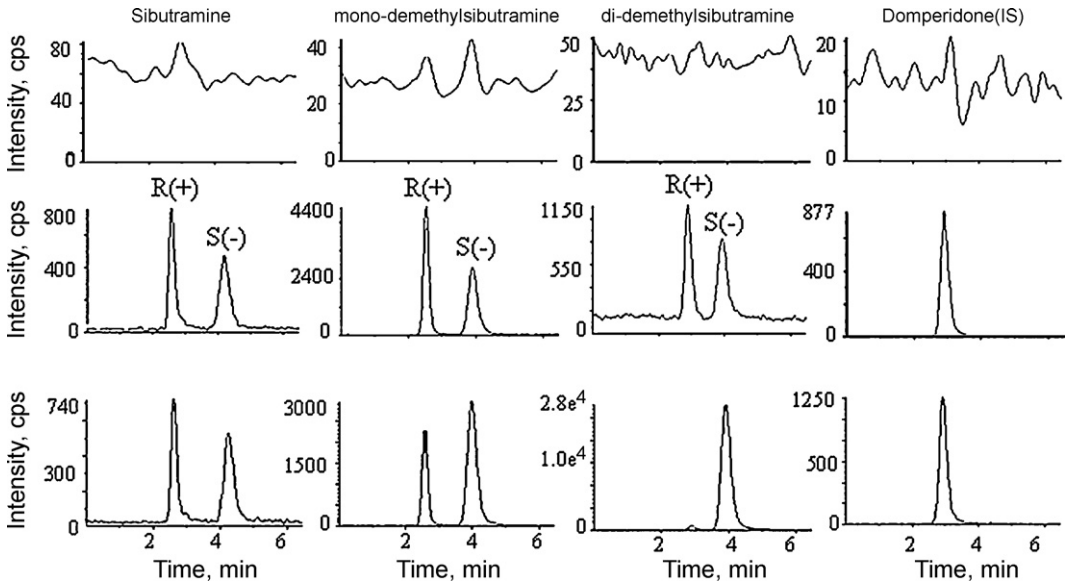

Fig. 1. Chromatograms of sibutramine, MDS, and DDS enantiomers and domperidone. Top, double-blank plasma; middle, plasma spiked with 5 ng/ml sibutramine, MDS, and DDS, and 100 ng/ml domperidone (IS); bottom, plasma sample of *S*(−)- and *R*(+)-isomers equivalent to 7.5 and 5.8 ng/ml for sibutramine, 9.5 and 3.8 ng/ml for MDS, and 308.0 and 6.9 ng/ml for DDS, respectively, in a sample obtained from a rat 30 min after oral administration of 10 mg/kg sibutramine.