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Chiral analysis of bambuterol, its intermediate and active drug in human plasma by liquid chromatography–tandem mass spectrometry: Application to a pharmacokinetic study

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ABSTRACT

A sensitive liquid chromatography-tandem mass spectrometry (LC–MS/MS) method has been developed for simultaneous chiral analysis of an antiasthma drug bambuterol, its key intermediate monocarbamate bambuterol and its active drug terbutaline in human plasma. All samples were extracted with ethyl acetate and separated on an Astec Chirobiotic T column under isocratic elution with a mobile phase consisting of methanol and water with the addition of 20 mm ammonium acetate and 0.005% (v/v) formic acid at 0.6 mL/min. The analytes were detected by a Xevo TQ-S tandem mass spectrometer with positive electrospray ionization in multiple reaction monitoring mode. The established method has high sensitivity with the lower limit of quantifications of 25.00 pg/mL for bambuterol enantiomers, and 50.00 pg/mL for monocarbamate bambuterol and terbutaline enantiomers, respectively. The calibration curves for bambuterol enantiomers were linear in the range of 25.00–2500 pg/mL, and for monocarbamate bambuterol and terbutaline enantiomers were linear in the range of 50.00–5000 pg/mL. The intra- and inter-day precisions were <12.4%. All the analytes were separated in 18.0 min. For the first time, the validated method was successfully applied to an enantioselective pharmacokinetic study of rac-bambuterol in 8 healthy volunteers. According to the results, this chiral LC-MS/MS assay provides a suitable and robust method for the enantioselectivity and interaction study of the prodrug bambuterol, the key intermediate monocarbamate bambuterol and its active drug terbutaline in human.

1. Introduction

Bambuterol, a bis-dimethylcarbamate prodrug of terbutaline, is a selective β_2-adrenoceptor agonist. It has been widely used for the treatment of asthma and chronic obstructive pulmonary disease in clinic for more than 20 years [1]. After administration, bambuterol is hydrolyzed into its active drug terbutaline with an intermediate metabolite monocarbamate bambuterol (shown in Fig. 1), which is catalyzed by butyrylcholinesterase (BChE) [2]. Both bambuterol and monocarbamate bambuterol are BChE inhibitors, inhibiting their own hydrolysis [3–4]. Our previous studies showed that monocarbamate bambuterol contributed significantly to the inhibition of BChE in humans after the administration of normal doses of bambuterol, which resulted in the rate-limiting step during the conversion of bambuterol into its active drug terbutaline [5–6]. Therefore, monocarbamate bambuterol concentrations should always be monitored during the pharmacokinetic studies of bambuterol [6].

As a chiral drug, bambuterol has two enantiomeric forms. Optically pure individual enantiomers of bambuterol have been successfully produced by asymmetric synthesis [7–8]. Previous study has demonstrated that S-bambuterol (distomer) was less active and caused adverse cardiac toxic effects, while R-bambuterol (eutomer) displayed high anti-bronchospasm effects [9–10]. It is generally known that the enantiomers of a chiral drug usually display pharmacological and pharmacokinetic processes in an enantioselective manner [11–12]. Therefore, both the US Food and Drug Administration (FDA) and China State Food and Drug Administration (SFDA) have defined more strict requirements for the pharmacological and toxicological evaluation of individual enantiomer of a developing chiral drug [13]. Thus, there is a high need to develop a sensitive and rapid analytical method

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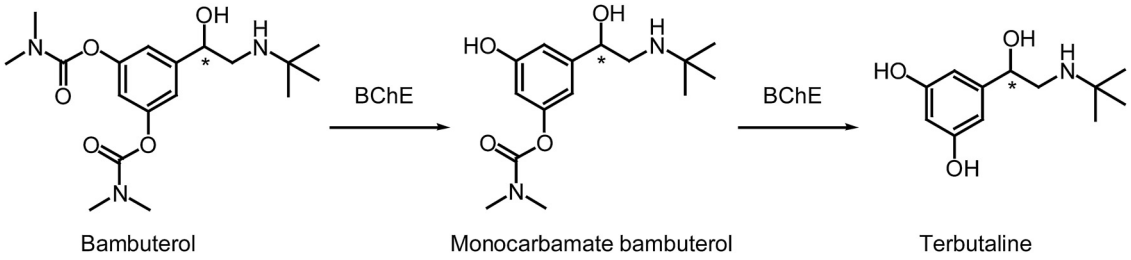

Fig. 1. The reaction of bambuterol to monocarbamate bambuterol and terbutaline catalyzed by BChE. *Marks the chiral center.

for chiral determination of individual enantiomers in biological samples.

Since the intermediate monocarbamate bambuterol plays a key role in the bioconversion of bambuterol into its active drug terbutaline, it is necessary to develop a chiral analytical method to simultaneously analyze bambuterol, monocarbamate bambuterol and terbutaline to study their interaction and clinical pharmacokinetic profiles. However, none of the studies have been reported yet and only one paper about simultaneous chiral analysis of bambuterol and terbutaline was currently available [14]. While for the enantioselective analysis of bambuterol, a few methods have been reported, including chiral liquid chromatography with UV [15–16] or MS detector [14]. And a bunch of assays have been reported for the determination of terbutaline enantiomers, including liquid chromatography (LC) with chiral solid phase column [14,17] or derivatization reagents [18], coupled achiral–chiral LC [19–20], electrospray high-field asymmetric waveform ion mobility spectrometry coupled to mass spectrometry [21], capillary electrophoresis (CE) using cyclodextrins or their derivatives as chiral selectors [22–23], CE with ionic liquids [24], and so on.

In this study, an enantioselective bioanalytical method using chiral LC–MS/MS was developed for the first time to simultaneously determine all the enantiomers of bambuterol, monocarbamate bambuterol and terbutaline in human plasma. This method was fully validated in terms of sensitivity, linearity, accuracy, precision and stability and was further applied to a stereoselective pharmacokinetics study of bambuterol in 8 healthy volunteers.

2. Experimental

2.1. Materials and reagents

R-bambuterol hydrochloride, R-terbutaline sulphate and R-salbutamol sulphate (IS) were obtained from Dongguan Key-Pharma Biomedical Co., Ltd. (Guangdong, China). Rac-bambuterol hydrochloride and rac-terbutaline sulphate were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). R-monocarbamate bambuterol hydrochloride and S-monocarbamate bambuterol hydrochloride (purity ≥ 98.9%, determined by HPLC analysis) were prepared in our laboratory according to a procedure reported elsewhere [6]. The test drugs were bambuterol tablets containing 10 mg of bambuterol hydrochloride, which were provided by Dongguan Key-Pharma Biomedical Co., Ltd. (Guangdong, China). Methanol, acetonitrile and ethyl acetate were of HPLC grade (Merck Company, Germany). Ammonium acetate, sodium carbonate and formic acid were of HPLC grade and purchased from Sigma–Aldrich (St Louis, MO, USA). Neostigmine methyl sulfate was purchased from Sigma–Aldrich (St Louis, MO, USA). Water used throughout the study was prepared with a Milli-Q Gradient A-10 water system (Millipore, Bedford, MA, USA).

2.2. LC-MS/MS condition

The LC–MS/MS analysis was carried out on a Waters Xevo TQ tandem quadrupole mass spectrometer (Waters Micromass MS Technologies, Manchester, UK) coupled with a Waters ACQUITY UPLC system (Waters, Milford, MA, USA), equipped with a binary solvent delivery system and an auto-sampler. The whole system was controlled by a computer employing the MassLynx^{TM} data acquisition software (Version 4.1) supplied by Waters.

All analytes were determined on an Astec Chirobiotic T column (250mm × 4.6 mm i.d., 5 μm, Advanced Separation Technologies Inc., USA) equipped with an ACQUITY UPLC In-Line Filter Kit (0.2 μm, Waters, USA). The mobile phase consisted of A (water; 20 mM ammonium acetate, 0.005% formic acid) and B (methanol; 20 mM ammonium acetate, 0.005% formic acid) used under isocratic elution. The flow-rate was 0.6 mL/min and the column temperature was set at 40 °C. The mass spectrometer with electrospray ionization (ESI) source was operated in positive mode. Mass spectrometric conditions were optimized to obtain maximum sensitivity. The optimized desolvation temperature was 600 °C. The capillary voltage was set at 3000 V. The nebulizer gas was set at 7.0 Bar. The cone gas flow and the desolvation gas flow were set at 150 and 1000 L/h, respectively. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 368.1 → 294.2 for bambuterol, m/z 297.1 → 223.1 for monocarbamate bambuterol, m/z 226.1 → 152.1 for terbutalin and m/z 240.1 → 148.0 for IS with a Dwell time of 0.317 s per transition. The optimized collision voltages chosen for bambuterol, monocarbamate bambuterol, terbutalin and IS were 18, 18, 16 and 18 V, respectively.

2.3. Preparation of stock and working solutions

The stock solution with a target concentration of 5.000 μg/mL for bambuterol enantiomers and 10.00 μg/mL for monocarbamate bambuterol and terbutaline enantiomers separately was prepared in methanol and stored at −20 °C. The stock solution was further diluted with methanol to yield working solutions at 0.2500, 0.5000, 1.250, 2.500, 5.000, 12.50, 17.50, and 25.00 ng/mL for bambuterol enantiomers, and 0.5000, 1.000, 2.500, 5.000, 10.00, 25.00, 35.00, and 50.00 ng/mL for monocarbamate bambuterol and terbutaline enantiomers, respectively. A solution containing IS of 2.000 ng/mL was obtained by further dilution of IS stock solution (500.0 μg/mL in methanol) with 1 M sodium carbonate aqueous solution (pH 11.4). The IS solution was stored at approximately 4 °C.

2.4. Preparation of calibration standards and quality control samples

Calibration standards were prepared by spiking appropriate amounts of the working solutions in 100 μL blank plasma obtained from healthy volunteers. Standard curves were prepared at concentrations of 25.00, 50.00, 125.0, 250.0, 500.0, 1250, 1750, 2500 pg/mL