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Short communication

Validated LC–MS/MS method for the simultaneous determination of rotigotine and its prodrug in rat plasma and an application to pharmacokinetics and biological conversion in vitro

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ABSTRACT

Rotigotine behenate (RGTB), a long chain alkyl ester of the prodrug of rotigotine (RGT), has been synthesized for use in a sustained delivery system. The aim of the present report was to develop and validate a simple, sensitive and reliable LC–MS/MS method for the simultaneous determination of RGT and its prodrug RGTB in rat plasma samples. Detection was performed on a 1290 Infinity UPLC coupled Triple Quad 4500 mass spectrometer operated in positive MRM mode using an Eclipse XDB-CN chromatography column (2.1 mm × 100 mm, 3.5 μm) by isocratic elution using a 0.2% formic acid aqueous solution and acetonitrile, with stable isotope labeled RGT as an internal standard. The sample preparation method employed 50 μL of a plasma sample and liquid-liquid extraction with a mixture of diethyl ether–dichloromethane (3:2, v/v) as the extraction solvent.

The proposed method was fully validated by assessing its specificity, linearity, precision and accuracy, recovery, matrix effects and stability. Good linearity was found within the range of 0.1–10.0 ng/mL for both analytes ($r > 0.996$). This method was successfully applied to a pharmacokinetic study of a slow release RGTB formulation in rats following a single intramuscular injection and biological conversion in vitro.

1. Introduction

Rotigotine is the non-ergoline $D_3/D_2/D_1$ dopamine receptor agonist formulated for the market as the transdermal patches Neupro®, which provide continuous drug delivery over 24 h. Rotigotine has been approved for the treatment of Parkinson's disease (PD), both as a monotherapy in early stages and in association with levodopa as the disease advances, as well as for restless legs syndrome (RLS) [1]. Parkinson's disease (PD) is a progressive neurological disorder that affects nearly seven million people globally [2,3]. In China, the prevalence of PD is approximately 2% in people over 65 years old [4–6]. RLS is a motor disorder characterized by the urge to move the legs during periods of evening rest or inactivity and is accompanied by unpleasant sensations (paresthesia, itching, and pain) [7]. It affects up to 11% of the general population, with a two-fold preponderance among women [8]. Rotigotine has been widely used to treat RLS, and its biological and clinical effects are well documented, demonstrating that it is able to reduce motor complications compared with other currently available drugs [9].

Prodrugs are chemicals with little or no pharmacological activity, undergoing biotransformation to a therapeutically active metabolite. This prodrug strategy is especially promising for determining pharmaceutical and pharmacokinetic properties. The majority of prodrug approaches face the challenge of enhancing or prolonging the concentration of the active principle at the site of action [10]. The treatments of PD and RLS are proposed by a new therapeutic concept of continuous dopaminergic simulation (CDS). Therefore, we have developed rotigotine-loaded microspheres in our previous study which could supply a 7-day sustained drug release. As patients require long-term treatment, it is necessary to develop a much longer sustained release preparation of rotigotine to improve the compliance of the patients significantly. Rotigotine behenate (RGTB), the prodrug of rotigotine (RGT), is an investiga-

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tional rotigotine drug product that has been formulated to function in an extended-release microsuspension for intramuscular injection. It has been synthesized for use in a sustained delivery system to hopefully greatly reduce the frequency of administration and provide convenience for patients. The feasibility of these prototypes should subsequently be evaluated with appropriate in vivo pharmacokinetic experiments and in vitro conversion. Unfortunately, the literature has not characterized this compound. If the prodrug can be detected in the plasma and/or target tissue after administration of the prodrug, one should try to narrow or, preferentially, identify the enzyme(s) and potential transporters involved in prodrug transport and activation [11]. Therefore, we want to stress the fact that pharmacokinetic experiments and conversion in vitro have to be performed early.

In the present study, we developed a highly specific and sensitive liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) method to measure RGTB and RGT in rat plasma, and we applied this method to the evaluation of the slow release profile of the RGTB microsuspension after an intramuscular injection of 10 mg/kg per month. To the best of our knowledge, this is the first profile of the RGTB microsuspension for RGTB and RGT, and the method was also applied to the conversion in vitro for liver microsomes, blood and muscle to explain the profile in vivo for better.

2. Experimental methods

2.1. Reagents and chemicals

Rotigotine behenate (purity 99.4%) and rotigotine (purity 99.7%) were supplied by Shandong Luye Pharmaceutical Co., Ltd (Yantai Shandong P.R.China). Rotigotine-d3 hydrochloride salt used as internal standard (I.S.) was purchased from Toronto Research Chemicals, Canada. HPLC grade acetonitrile was purchased from Merck (Darmsadt, Germany). HPLC grade formic acid and acetic acid were supplied by Fluka (St.Louis.MO, USA). Millipore Milli-Q gradient purified water was used throughout the study (Millipore, Molsheim, France). Centrifuge tubes were supplied by Eppendorf (Hamburg, Germany). Liver microsomes were purchased from Research Institute for Liver Diseases of Shanghai. All other chemicals were of analytical grade and used without further purification.

2.2. LC–MS/MS conditions

The HPLC system (Agilent 1290 series) (Agilent, Santa Clara, US) consisted of a binary pump, autosampler, and column oven maintained at 35 °C.The analytes of interest were separated on an Eclipse XDB-CN chromatography column (2.1 mm × 100 mm, 3.5 μm, Agilent, US) with the mobile phase consisting of 0.2% formic acid in MilliQ water (solvent A) and 0.2% formic acid in acetonitrile (solvent B) with isocratic elution (12:88,v/v) at a flow rate of 0.5 mL/min.

Detection was performed on a Triple Quad 4500 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) with an ESI source operating in the positive ion mode. Multiple reaction monitoring (MRM) involved RGTB at m/z 638.7 → 147.2, m/z 316.2 → 147.2 for RGT and 319.4 → 147.2 for the internal standard. The optimized MS conditions were as follows: curtain gas, gas 1 and gas 2 were nitrogen set at 35, 55 and 55 units, respectively; the ion spray voltage was 5500 V; the source temperature was 550 °C; the declustering potentials were 80, 58 and 74 V for RGTB, RGT and IS, respectively; and the collision energies were 55, 38 and 34 eV, respectively.

2.3. Preparation of calibration standards and quality control (QC) samples

A stock solution of rotigotine behenate (1 mg/mL) in tetrahydrofuran and a stock solution of rotigotine (1 mg/mL) in acetonitrile were diluted with acetonitrile –water- formic acid (90:10:0.2, v/v/v) to give a series of standard solutions in the range of 0.10-10.0 ng/mL for both RGTB and RGT. A series of calibration standards were then prepared by spiking blank plasma samples (50 μL) with 50-μL aliquots of the standard solutions to give RGTB and RGT concentrations in plasma of 0.10, 0.25, 0.50, 1.00, 2.00, 5.00 and 10.0 ng/mL. Low, medium and high QC samples were prepared by spiking blank plasma samples with QC solutions (0.20, 1.0 and 8.0 ng/mL) independently prepared in the same way.

2.4. Sample preparation

The liquid-liquid extraction was a simple and suitable method to treat rotigotine and prodrug samples. All frozen rat plasma samples were thawed at room temperature prior to preparation. After vortexing, a 50 μL aliquot of the plasma sample was pipetted into an Eppendorf EP tube. Then, 50 μL of the IS working solution (rotigotine-d3, 5 ng/mL), 50 μL of acetonitrile –water- formic acid (90:10:0.2, v/v/v), and 200 μL of water were added. The mixture was then shaken with 3 mL of diethyl ether-dichloromethane (3:2, v/v) for 10 min and centrifuged at 3500g for 10 min. The organic layer was transferred to a glass tube and evaporated to dryness at 37 °C under nitrogen. The residue was reconstituted in 50 μL of acetonitrile (0.1% formic acid): 0.1% aqueous formic acid (90:10, v/v) and 5 μL were injected into the LC–MS/MS system.

2.5. Assay validation

Assay validation was performed according to FDA guidelines [12]. The fundamental parameters for validation of a bioanalytical method are accuracy, precision, selectivity, sensitivity (LLOQ), matrix effects, extraction recovery, and stability. Selectivity was evaluated by analyzing blank rat plasma from six different sources. LLOQ was defined as the lowest concentration that could be quantified with acceptable precision and accuracy. Linearity was assessed by a weighted ($1/x^2$) least-squares linear regression of the calibration curves based on the peak area ratios. Precision and accuracy were evaluated based on assays of six replicates of QC samples on three days.

The matrix effect was investigated by six replicate analyses of QC samples prepared from six lots of rat plasma. The matrix factor (MF) of each analyte or IS was calculated in each lot of matrix by calculating the ratio of the peak area from the post extraction spiked samples to the peak area from the pure solution. The IS normalized MF was calculated by dividing the MF value of the analyte by that of the IS. Extraction recovery was determined by comparing the peak areas of the analytes and internal standards obtained from QC samples with those from the post-extraction spiked samples.

The stability of the analytes in rat plasma was investigated by analyzing three replicates of low and high QC samples under a variety of storage and processing conditions: (a) bench-top storage (2 h at room temperature), (b) three freeze-thaw cycles, (c) post-preparation stability (24 h at room temperature), and (d) long-term freezer storage (3 months at −35 °C).

2.6. In vivo pharmacokinetics

Six male Sprague Dawley rats (weight 200 ± 20 g) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, PR China). All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council of USA, 2011). Approval for the animal experiments was granted by the Yantai University Ethical Committee for Animal Experimentation.

A single dose of 10 mg/kg (RGT base) was injected intramuscularly into the right biceps femoris (hind leg). All injections were