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Development of a selective and sensitive high-performance liquid chromatography-tandem mass spectrometry assay to support pharmacokinetic studies of LY-487,379 in rat and marmoset

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ABSTRACT

Drugs modulating the metabotropic glutamate type 2 receptor ($mGluR_2$) activity may have therapeutic benefits in treating a large spectrum of neuro-psychiatric disorders, from schizophrenia to Parkinson's disease, both as a symptomatic therapy and potential disease-modifying paradigm. LY-487,379 is a highly selective $mGluR_2$ positive allosteric modulator that is widely used to study $mGluR_2$ function using experimental animal models. The common marmoset is a small primate that has long been used in neuroscience. However, given its small size and small circulating blood volume, conducting studies to determine the PK profile of LY-487,379 is challenging. We developed and validated a sensitive and selective analytical method that enables quantification of LY-487,379 using a limited volume of plasma (10 µL). The analytical method consists of protein precipitation followed by high-performance liquid chromatography with heat assisted electrospray ionization mass spectrometry (HPLC-HESI-MS/MS). The chromatographic separation was achieved using gradient elution with a mobile phase consisting of acetonitrile and 0.01% formic acid in water on a Thermo Scientific Aquasil C18 analytical column (100 × 2.1 mm I.D., 5 µm) operating at 40 °C and at a flow rate of 300 µL/min. The method displays a linear relationship ranging from 0.2 to 100 ng/mL. Intra- and inter-day relative standard deviations are < 1.4% and 7.9%, respectively and the relative error ranged from −6.9 to 9.7%. The method was used to quantify LY-487,379 in both rat and marmoset plasma, and PK parameters were determined after a single subcutaneous dose of 1.0 mg kg^{-1} in both species and significant differences in C_{max}, AUC and $T_{1/2}$ were observed.

1. Introduction

Metabotropic glutamate receptors (mGluRs) were identified as potential therapeutic targets for neurodegenerative diseases because of their distinctive function as modulator of signals via G-protein dependent pathways [1]. There are eight mGluRs subtypes divided into 3 groups based on their sequence homology, signal transduction pathways and ligand binding patterns [2]. Group I (i.e. $mGluR_1$, $mGluR_5$) are expressed postsynaptically and are implicated in the modulation of neuronal excitability through activation of phospholipase C (PLC) leading to an increase of intracellular calcium. Group II (i.e. $mGluR_2$ and $mGluR_3$) and group III (i.e. $mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$) are essentially localised presynaptically and their activation negatively modulates neuronal excitability [3]. Activation of mGluR group I can therefore increase postsynaptic excitability, worsening neuronal damage. In contrast, group II and III activation will reduce glutamatergic signalling and therefore reduce neuronal excitability, which may lead to neuroprotection [4]. Activation of mGluR group II and III leads to an inhibition of adenylyl cyclase, activation of potassium channels and inhibition of presynaptic voltage-gated calcium channels, thus reducing

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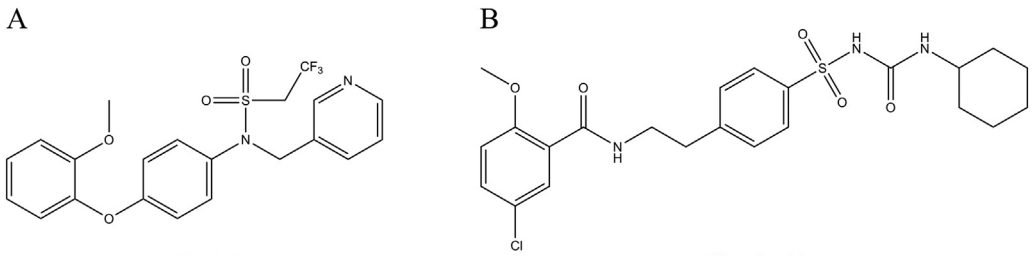

Fig. 1. Chemical structures of LY-487,379 and internal standard glibenclamide.

calcium entry into the cell and modulating neurotransmitter release in the synaptic space [5–7]. Also, mGluR group II and III are known to trigger other signalling pathways including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) pathways. This pairing appears to be central in the regulation of synaptic plasticity and processes of neuroprotection [8].

Drugs modulating $mGluR_2$ activity may have therapeutic benefits in treating a large spectrum of neuro-psychiatric disorders, from schizophrenia to Parkinson's disease, both as a symptomatic therapy and potential disease-modifying paradigm [9]. N-(4-(2-methoxyphenoxy)-phenyl-N-(2,2,2-triflu-oroethylsulfonyl)-pyrid-3-ylmethylamine) (LY-487,379) (Fig. 1A) is a highly-selective $mGluR_2$ positive allosteric modulator (PAM). LY-487,379 is regarded as the prototypical $mGluR_2$-PAM and has elicited anxiolytic and antipsychotic activity in animal models [10–14]. However, despite its use in research, the pharmacokinetic (PK) profile of LY-487,379 has not been published and, to our knowledge, there is no state-of-the-art analytical method or pharmacokinetic data on LY-487,379 described in the literature. Determination of LY-487,379 PK profile in animals is an important step to better understand how the drug exerts its effects and will facilitate the interpretation of potential up-coming behavioural studies with this highly-selective $mGluR_2$ PAM. The objectives of this study were to, first, develop and validate a HPLC-MS/MS assay to quantify LY-487,379 in rat and in common marmoset plasma at trace levels and second, provide a description of the PK properties of LY-487,379 in both species.

2. Material and methods

2.1. Chemicals

LY-487,379 hydrochloride was purchased from Tocris Bioscience through Cedarlane (Burlington, ON, Canada). Glibenclamide (Fig. 1B), formic acid, hydrochloric acid and dimethyl sulfoxide (DMSO) were purchased from MilliporeSigma (St. Louis, MO, USA). Drug-free rat and marmoset plasma containing K_3EDTA as anticoagulant was acquired from Bioreclamation (Westbury, NY, USA). Other chemicals, including, methanol, acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Stock and standard solutions

LY-487,379 standard stock solution was prepared in DMSO to obtain a 1.0 mg/mL free base solution. The internal standard stock solution was also prepared at 1.0 mg/mL of glibenclamide in methanol. Standard working solutions were obtained by dilution of the LY-487,379 standard stock solution with methanol. Due to limited matrix (marmoset plasma), calibration standards were solely prepared in blank rat plasma by spiking with the standard working solutions at 2% (v/v) to obtain an analytical range from 0.2 to 100 ng/mL. However, quality control samples were prepared in both rat and marmoset plasma. The internal standard working solution (ISWS) was prepared at a nominal concentration of 1.0 ng/mL of glibenclamide in methanol.

2.3. Sample preparation

LY-487,379 was extracted from rat or marmoset plasma after protein precipitation. One hundred and fifty microlitres of ISWS were added to an aliquot of 10 µL of sample. The sample was vortexed for approximately 5 s and let stand for a period of 10 min, then centrifuged at 16,000 × *g* for 10 min. The supernatant was transferred into a glass tube. Fifty microlitres of 0.5 N HCl in water were added to the tube. The sample was vortexed and evaporated to dryness at 50 °C and 10 psi of nitrogen. The dried extract was re-dissolved with 150 µL of methanol and transferred to an injection vial for analysis.

2.4. Chromatographic conditions

A gradient mobile phase was used with a Thermo Scientific Aquasil C18 analytical column (100 × 2.1 mm I.D., 5 µm) with a Phenomenex C18 security guard cartridge operating at 40 °C. The initial mobile phase condition consisted of acetonitrile and 0.01% of formic acid in water at a ratio of 30:70, respectively, and this ratio was maintained for 1 min. From 1 to 5 min, a linear gradient was applied up to a ratio of 80:20 and maintained for 1.5 min. At 6.6 min, the mobile phase composition was reverted to the original conditions and the column was allowed to equilibrate for 3.5 min for a total run time of 10 min. The flow rate was fixed at 300 µL/min and LY-487,379 and glibenclamide both eluted at 5.4 min. Five microlitres of the extracted sample were injected and the total run time was set to 10 min.

2.5. Mass spectrometry conditions

A Thermo Scientific TSQ Quantiva triple quadrupole mass spectrometer was interfaced with the Thermo Scientific Dionex 3000 UHPLC system using a pneumatic assisted heated electrospray ion source (HESI). MS detection was performed in positive ion mode, using selected reaction monitoring (SRM). The MS/MS parameters were optimised by infusing standard solutions of LY-487,379 and glibenclamide into the mass spectrometer. Following optimisation, the parameters employed were as follows: nitrogen, used for the sheath and auxiliary gases was set to 50 and 15 arbitrary units, the HESI electrode was set to 3500 V, the capillary temperature was set to 350 °C and the vaporiser temperature was set to 400 °C. Argon was used as collision gas at a pressure of 2.5 mTorr. The SRM transitions selected for LY-487,379 and glibenclamide were set to 453.1 → 181.1 and 494.2 → 169.0, respectively (Fig. 3). The collision energy (E_{lab}) for LY-487,379 and glibenclamide were set to 55 and 34 eV, respectively. Total cycle time was 0.25 s. The mass spectrometer was calibrated using the Thermo automated algorithm and peak widths of Q1 and Q3 were both set at 0.7 full width half mass (FWHM).

2.6. Animal studies

Experiments were conducted on Sprague-Dawley rats (n = 6) and common marmosets (*Callithrix jacchus*, n = 6). The method described