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Determination of a novel phosphodiesterase-4 inhibitor chlorbipram in mouse plasma and brain by UFLC–MS/MS: Application in pharmacokinetic studies after intravenous administration

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ABSTRACT

In this study, we evaluated a simple and sensitive method for determination of a novel phosphodiesterase-4 (PDE4) inhibitor, chlorbipram, in mouse plasma and brain using ultra-fast liquid chromatography-tandem mass spectrometry (UFLC–MS/MS). Separation was achieved using an Acquity UPLC BEH C18 column (50 mm × 2.1 mm, particle size 1.7 μm) with a gradient mobile phase consisting of water and methanol at a flow rate of 0.25 ml/min. Detection was performed in the multiple reaction monitoring (MRM) mode using electrospray ionization (ESI) in the positive ion mode. The liquid–liquid extraction method with ethyl acetate was used for both pretreatment of plasma and brain homogenates. The calibration curves of chlorbipram showed good linearity over the concentration range of 0.5–200 ng/ml ($R^2 > 0.994$) for mouse plasma and over the range of 0.25–100 ng/ml ($R^2 > 0.994$) for mouse brain homogenate. The extraction recovery was in the range of 78.3–84.8% for chlorbipram and the internal standard (IS) ZXI14 in two different biological matrices. The intra- and inter-day precision values were less than 13.0% and the accuracy ranged from 97.8% to 106.0% for quality control samples. No noteworthy matrix effects and instability were observed for chlorbipram. This validated method was successfully applied to a pharmacokinetic study of chlorbipram in mice after intravenous administration. The results show that this novel drug crosses the blood-brain barrier and provides the basis for further studies on chlorbipram.

1. Introduction

Depression is a common, but serious, psychiatric disorder that is characterized by a combination of symptoms, including persistent low mood, loss of pleasure, low energy, feelings of hopelessness, disturbed sleep or appetite and concentration difficulties. The clinical efficacy of current anti-depressant therapies is unsatisfactory, with many anti-depressant drugs showing low response rates and associated with the risk of adverse side-effects [1,2]. Thus, new strategies for the treatment of depression are urgently required.

Phosphodiesterase-4 (PDE4) has been studied as a potential target for the treatment of depression for more than 20 years. Previous studies using the first generation of selective PDE4 inhibitors (e.g. rolipram) demonstrated the association of PDE4 with depression [3]. Rolipram was shown to exert rapid anti-depressant effects by activating cyclic AMP (cAMP)/cAMP response element-binding protein (CREB) signaling pathways [4,5], thereby, regulating the level of brain-derived neurotrophic factor (BDNF), which has an important effect on depression [6].

PDE4 is encoded by four separate genes (PDE4A-PDE4D) that can give rise to multiple splice variants and are differentially expressed in the central nervous system (CNS). Among the different classes, the PDE4D subtype is likely to influence memory [7] and accumulating evidence suggests that PDE4D is a therapeutic target for Alzheimer's disease [8,9]. However, to date, no PDE4 inhibitors have been approved for use as anti-depressants in the clinic due to side-effects such as nausea and emesis that are unacceptable to patients [10,11].

Abbreviations: PDE4, phosphodiesterase-4; UFLC–MS/MS, ultra-fast liquid chromatography-tandem mass spectrometry; MRM, multiple reaction monitoring; ESI, electrospray ionization; IS, internal standard; cAMP, cyclic AMP; CREB, cAMP response element-binding protein; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; QC, quality control; LLOQ, lower limit of quantification; $T_{1/2}$, terminal elimination half-life; MRT, mean residence time; C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; AUC_{0-t}, area under the plasma concentration versus time curve; CL, clearance; LLE, liquid–liquid extraction; SPE, solid-phase extraction.

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The novel PDE4 inhibitor chlorbipram (2-((3′-chloro-6-methoxy-[1,1′-biphenyl]-3-yl) methyl)-6-((2-(2-methoxyphenoxy) ethyl) amino) pyridazin-3(2H)-one, Chinese invention patent application number: CN201210037980.3) was synthesized in our laboratory [12]. Chlorbipram was designed based on the theory of PDE4D allosteric modulators that do not completely inhibit enzymatic activity, and thus, have reduced potential to cause emesis. Our previous studies suggested that chlorbipram exerted anti-depressant-like effects under acute single administration (0.075–0.6 mg/kg) as well ascognition-enhancing effects in attenuating scopolamine-induced cognitive dysfunction in rodents after treatment with different doses in the range of (0.5–1.5 mg/kg). [12].

In the preliminary stages of designing and screening candidate drugs, pharmacokinetic studies are beneficial for optimization of the structure of compounds by providing information regarding the drug half-life and feasibility of transport to the site of action. Further clinical development of chlorbipram requires preliminary pharmacokinetic studies in rodents, which depend on the availability of a reliable, accurate, and sensitive bioanalytical assay for detection of this drug. Moreover, knowledge of the capacity of chlorbipram to penetrate the blood–brain barrier is critical in evaluating its potential as a CNS targeting agent. Compared to other methods, ultra-fast liquid chromatography with tandem mass spectrometry (UFLC–MS/MS) offers a powerful tool for quantification of drugs in biological matrices with high specificity, selectivity and sensitivity [13,14].

In this study, a rapid, sensitive and selective UFLC–MS/MS assay method was developed and validated for the detection of the novel PDE4 inhibitor chlorbipram in mouse plasma and brain. The present method was applied successfully to a pharmacokinetic study of intravenous administration of chlorbipram in mice.

2. Experimental

2.1. Chemicals and reagents

Chlorbipram (purity > 99%) and the internal standard ZXI14 (IS, purity > 99%) were synthesized by the Department of Pharmacology, School of Pharmaceutical Sciences, Southern Medical University (Guangzhou, China). Methanol (HPLC grade) was purchased from Dikma (Richmond Hill, NY, USA). Ethyl acetate was purchased from Yuwang (Chemical Reagent Plant, Shandong, China). All other chemicals were of analytical grade. Water was purified by redistillation and filtered through a 0.22 μm membrane filter before use.

2.2. LC–MS/MS instrument and conditions

2.2.1. Liquid chromatography

Chromatography was performed using the SHIMADZU LC-20A D_{XR} UFLC system with an autosampler and column oven enabling temperature control of the analytical column. A BEH C18 column (50 mm × 2.1 mm, particle size 1.7 μm; Waters, USA) was employed. The column temperature was maintained at 40 °C and

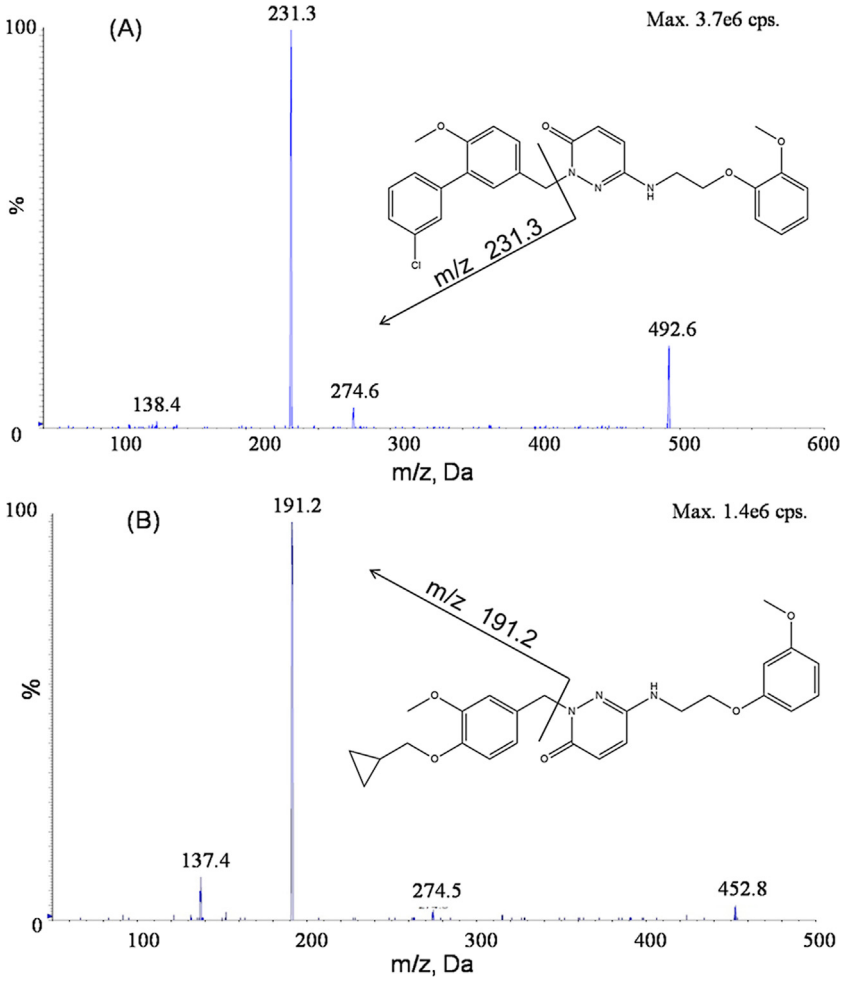

Fig. 1. Product ion spectra of chlorbipram (A) and the internal standard ZXI14 (B, IS).