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Preclinical pharmacokinetic and toxicological evaluation of MIF-1 peptidomimetic, PAOPA: Examining the pharmacology of a selective dopamine D2 receptor allosteric modulator for the treatment of schizophrenia

Mattea L. Tan[a,1], Dipannita Basu[a,1], Jacek M. Kwiecien[a,b], Rodney L. Johnson[c], Ram K. Mishra[a,*]

[a] Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, Ontario, Canada
[b] Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada
[c] Department of Medicinal Chemistry, University of Minnesota, Minneapolis, United States

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ABSTRACT

Schizophrenia is a mental illness characterized by a breakdown in cognition and emotion. Over the years, drug treatment for this disorder has mainly been compromised of orthosteric ligands that antagonize the active site of the dopamine D2 receptor. However, these drugs are limited in their use and often lead to the development of adverse movement and metabolic side effects. Allosteric modulators are an emerging class of therapeutics with significant advantages over orthosteric ligands, including an improved therapeutic and safety profile. This study investigates our newly developed allosteric modulator, PAOPA, which is a specific modulator of the dopamine D2 receptor. Previous studies have shown PAOPA to attenuate schizophrenia-like behavioral abnormalities in preclinical models. To advance this newly developed allosteric drug from the preclinical to clinical stage, this study examines the pharmacokinetic behavior and toxicological profile of PAOPA. Results from this study prove the effectiveness of PAOPA in reaching the implicated regions of the brain for therapeutic action, particularly the striatum. Pharmacokinetic parameters of PAOPA were found to be comparable to current market antipsychotic drugs. Necropsy and histopathological analyses showed no abnormalities in all examined organs. Acute and chronic treatment of PAOPA indicated no movement abnormalities commonly found with the use of current typical antipsychotic drugs. Moreover, acute and chronic PAOPA treatment revealed no hematological or metabolic abnormalities classically found with the use of atypical antipsychotic drugs. Findings from this study demonstrate a better safety profile of PAOPA, and necessitates the progression of this newly developed therapeutic for the treatment of schizophrenia.

1. Introduction

Recent studies in drug development have shown remarkable advances in the use of allosteric modulators (AMs) to alter receptor activity. In contrast to orthosteric ligands, AMs bind to a site on the receptor topographically distinct from the active site, to subtly modulate its activity. This mechanism of action allows AMs to have significant advantages over orthosteric ligands. Allosteric sites on receptors exhibit a much greater sequence divergence compared to active sites, thus allowing for the design of highly receptor subtype-specific ligands. Additionally, AMs are quiescent by themselves, and only work to enhance or attenuate endogenous receptor activity, thus subtly manipulating the natural physiological tone. Progress in the field of drug development has therefore identified AMs as promising molecules that can be researched and developed into better research tools and therapeutic drugs for the near future.

Melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1), also known as L-Pro-L-Leu-glycinamide (PLG), is

Abbreviations: 5-HT2, 5-hydroxytryptamine; 6-OHDA, 6-hydroxydopamine; AM, allosteric modulator; AUC, area under curve; AUC_{INF}, area under curve extrapolated to infinity; AUC_{last}, area under curve from dosing to observation; AUMC, area under first moment curve; CL, total body clearance; C_{max}, maximum plasma concentration; CNS, central nervous system; H&E, hematoxylin and eosin; HDL, high-density lipoprotein; HILIC, hydrophilic interaction chromatography; HOMA-IR, homeostatic model assessment; HPLC–MS, high performance liquid chromatography–mass spectrometry; i.p., intraperitoneal injection; i.v., intravenous; LDL, low-density lipoprotein; LLQ, limit of quantitation; MIF-1, melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1); PAOPA, 3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide; PLG, L-Pro-L-Leu-glycinamide; $T_{1/2}$, half-life; T_{max}, time to maximum plasma concentration; TSQ, triple stage quadrupole; p.o., *per oram* (oral); VCMs, vacuous chewing movements; V_{ss}, volume of distribution at steady state; V_z, volume of distribution.

* Corresponding author at: Department of Psychiatry and Behavioral Neurosciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada. Tel.: +1 905 525 9140x22396; fax: +1 905 522 8804.
E-mail address: mishrar@mcmaster.ca (R.K. Mishra).
[1] Both are considered first authors of this manuscript.

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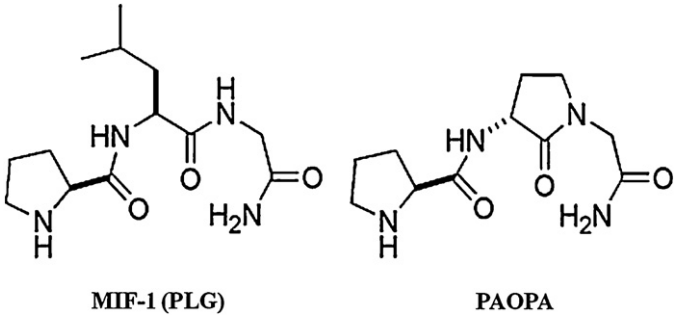

Fig. 1. Structure of melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1), also known as L-Pro-L-Leu-glycinamide (PLG), an endogenous brain peptide derived from oxytocin is indicated on the left. 3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (PAOPA) is a more potent conformationally restrained peptidomimetic of PLG, and is indicated on the right.

an endogenous brain peptide derived from oxytocin, and is involved in allosterically modulating dopaminergic transmission [10,11]. Past studies have shown PLG, and its potent peptidomimetic, 3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (PAOPA) (Fig. 1), to have strong therapeutic effects in a range of neurological and mental illnesses disorders which implicate the dopaminergic circuits. PLG has shown to be effective in the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease, suggesting its ability to enhance the hypodopaminergic state of this disease [5,14,20]. PLG also antagonizes the development of the antipsychotic drug (haloperidol)-induced vacuous chewing movements in rats, a well-accepted preclinical model of tardive dyskinesia [5]. The more potent peptidomimetic, PAOPA, on the other hand, has shown highly encouraging preclinical therapeutic effects in animal models of schizophrenia, including the amphetamine and the MK-801 drug-induced models [2,7]. Studies have shown PAOPA, a conformationally constrained peptidomimetic of PLG, to be 100–1000 times more potent than the parent compound, PLG, making it more clinically relevant for drug development. Thus, future investigations on advancing these small molecules into therapeutics will focus mainly on PAOPA.

PAOPA exerts its therapeutic effects in preclinical models of schizophrenia by specifically interacting with an allosteric site on the dopamine receptor, particularly the D2 subtype. As an AM, PAOPA modulates dopaminergic activity by increasing agonist (dopamine) binding to the D2 receptor at lower concentrations, and decreasing this binding at higher concentrations, leading to a unique biphasic pharmacological phenomenon [23,25]. Scatchard analysis on radioligand binding studies of agonist binding have shown PAOPA to significantly decrease the dissociation constant K_D while causing no significant changes in B_{max} [15], indicating PAOPA to have an effect by increasing the percentage of high affinity, GDP-bound, D2 receptors, but not the total number of receptors. The exact mechanism of action of PAOPA is currently under investigation, and is expected to present novel strategies to better understand and improve the treatment of mental disorders, particularly schizophrenia.

The current study helps progress the development of PAOPA beyond preclinical trials, toward possible human clinical trials. In this study, rodent models were used to extensively and methodically study the pharmacokinetic characteristics and toxicological effects of potential new drug, PAOPA. Results gathered from this study will provide a platform to predict key characteristics for this novel AM, PAOPA, for development it into an improved therapy for patients suffering from schizophrenia.

2. Materials and methods

2.1. Animals and drugs

Male Sprague Dawley rats (250 g) were obtained from Charles River Laboratories (Wilmington, MA) and allowed to acclimatize for 1 week. Animals were housed at the McMaster Central Animal Facility, and maintained under constant temperature and humidity, with a 12 h light-dark cycle, and ad libitum access to food and water. All animal studies were conducted in compliance with the guidelines set out by the Canadian Council on Animal Care, and all procedures were reviewed and approved by the McMaster Animal Research Ethics Board. PAOPA was synthesized by Dr. R. L. Johnson (University of Minnesota, MN), as previously described [27]. Haloperidol was obtained from Sigma (H1512) and olanzapine was obtained from Eli Lilly.

2.2. Drug brain distribution analysis

[^{3}H] PAOPA (35 mCi/mmol) was custom synthesized by PerkinElmer (Woodbridge, ON), and rats ($n = 3$) were injected with 100,000 DPMs of [^{3}H] PAOPA intraperitoneally. Two hours later, rats were sacrificed and the brain quickly dissected on ice. The striatum, frontal cortex, cerebellum, and hypothalamus were dissected and stored in −80 °C until further processing. The various brain regions were weighed, tissue was homogenized in Tris–EDTA (pH 7.4), and homogenate was placed in a scintillation tube in triplicates. Scintillation cocktail was added and tubes were counted in a Beckman LS5000TA scintillation counter. Total number of DPMs obtained/brain tissue weight reflected the total activity in each of the measured brain regions.

2.3. Pharmacokinetic assessment

Pharmacokinetic parameters of PAOPA were performed by using the custom in vivo research services of Cerep (Redmond, WA). Parameters were determined following oral and intravenous administration. The investigation of drug levels in the body over time and analysis of its pharmacokinetic parameters can offer critical information regarding the interaction of the target drug with organs in the body. Results will provide key information regarding the kinetics of drug exposure and thus bring understanding vis-à-vis the activity and pharmacological performance of the compound as a drug. The pharmacokinetic study and standard operating procedures to determine these parameters have been reviewed and approved by the Cerep Institutional Animal Care and Use Committee.

2.4. In vivo pharmacokinetics

For in vivo pharmacokinetic assessment, Sprague Dawley rats, weighing 180–250 g, were divided into 3 groups. Group 1 ($n = 3$) was administered with PAOPA (mol. wt.: 254.28 g/mol) via an intravenous (i.v) dosing at 1 mg/kg, Group 2 ($n = 3$) was administered with PAOPA via an oral (p.o) dosing at 5 mg/kg, and Group 3 ($n = 2$) was non-treated. PAOPA was dissolved in a phosphate-buffered saline at pH 7.4 and administered via either i.v. or p.o. For intravenous administration, rats were administered with PAOPA via a jugular catheter injection and blood was subsequently collected at various time points. For oral administration, rats were gastric gavaged with PAOPA and blood was collected at various time points following. Blood was only drawn once from control animals via cardiac puncture, and this was used to produce drug-free plasma for use in the linearity assay and calibration curve (not shown).

Blood was sampled at various time points (3, 10, 30, 60, 120, 240, 360, and 1440 min), and analyzed according to Moon