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Therapeutic Potential of Fungally Derived Psilocybin Extract in Morphine-Dependent Mice: A Research Protocol

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Abstract

Introduction: Psilocybin is a naturally occurring tryptamine derivative psychedelic compound potently produced by fungi members of the genus *Psilocybe*. Previous literature has highlighted psilocybin as a serotonin 2A receptor agonist with striking effects on neuralplasticity and cognition. Recent studies explore the usage of psilocybin in addressing addictive behaviours and substance abuse. Small psilocybin doses have shown promising anti-addictive and withdrawal-minimizing properties in alcohol-dependent mice. This study will further investigate this emerging field through a novel lens. We propose a novel study to elucidate psylobicin's effect on opioid addiction in mouse models.

Methods: Morphine-dependent mice will be administered either saline vehicle or differing doses of psilocybin during a morphine-available period to monitor consumption. Though mechanistically ambiguous, psilocybin's hypothesized entry into serotonin-dependent stress-conciliating pathways supports the hypothesis that mean morphine consumption will decrease in a dose-dependent manner to similar levels to popular opioid receptor agonist therapeutics, such as methadone. Furthermore, we will examine psilocybin's impact on withdrawal behaviour. After morphine deprival on dependent mice, sustained doses of psilocybin, methadone, and positive and negative controls will be administered.

Results: By analyzing stress-indicative behaviours in mice, the efficacy of psilocybin as a withdrawal assistance agent can be elucidated. Results from the morphine-dependent mice are expected to consume less morphine than controls and minimize withdrawal symptoms at a similar level to popular therapeutic options like methadone.

Discussion: If successful, psilocybin's anti-addictive potential will help provide a cheaper, more accessible therapeutic option in addressing the growing opioid crisis. Furthermore, controlled psilocybin dosages have been shown to have lesser dependence potential compared to modern opioid addiction therapeutics.

Conclusion: This study's novel approach will provide meaningful support in exploring the growing field of mycology and its potential to address other substance use disorders. Future research may explore outside the limitations of the mouse model, like the oral administration of the compounds rather than intraperitoneal injection.

Keywords: psilocybin; psychedelic; addiction; opioids; withdrawal; serotonin; dependence

Introduction

Known for its nearly-magical anti-anxiety and anti-depressant properties, psilocybin is a tryptamine derivative of the tryptophan amino acid found in members of the *Psilocybe* genus such as *P. cyanescen*, *P. azurescens*, and *P. semilanceata* fungi [1]. It is a psychedelic, serotonin 2A receptor agonist, and impacts intracellular signalling, neural plasticity, behaviour, and cognitive function [2].

Serotonin Binding

Psilocybin has a similar chemical structure to serotonin and can bind to 5-HT2A G protein-coupled serotonin receptors, which are responsible for conciliating anxiety and mood disorders. Psilocybin increases serotonin release upon binding to promote relaxation [3]. Not having enough serotonin can impact the onset and progression of mania, anxiety, depression, and other mental health concerns. Many antidepressants used to treat depression using selective serotonin reuptake inhibitors that work by blocking serotonin reuptake back into neurons, which ultimately increases the duration and activity of serotonin in the synaptic cleft between neurons [4]. Psilocybin has a similar chemical structure to serotonin and can bind to 5-HT2A G protein-coupled serotonin receptors, which are responsible for conciliating anxiety and mood disorders. Psilocybin increases serotonin release upon binding to promote relaxation [5]. Downstream signalling of psilocybin can increase serotonin release and decrease anxiety and mood disorders to relieve withdrawal symptoms and reduce addiction cravings [3].

Neuroplastic Properties

Neuroplasticity refers to the brain's capacity to adapt, encompassing changes in cell structure and synaptic transmission efficacy [6]. Disruptions in neuroplasticity are evident in addictions and mood disorders [4]. Unlike traditional antidepressants, even after a single administration, psychoplastogens can yield rapid and sustained beneficial behavioural effects [7]. It is suggested that atrophy of neurons in the prefrontal cortex plays a pivotal factor in the pathophysiology of addiction and related disorders [7]. Psilocybin is also a psychoplastogen as it increases neuralplasticity, aiding in neuropsychiatric disease treatment and addressing addiction [7]. Psilocybin's high affinity for serotonin receptors, particularly 5-HT2A receptors, enhances synaptic plasticity, leading to structural and functional changes in neurons; this mechanism is implicated in alleviating addictive symptoms in patients [6].

Opioid Addiction

Opioids directly caused over 75% of nearly 100,000 deaths by overdose in 2021 [9], and over 16 million people worldwide are currently addicted to opioids [7]. Opioid medicines like Fentanyl, Morphine, and Vicodin are severely addictive, as they activate reward centres in the central nervous system by producing artificial endorphins to reduce pain and stress. Endorphins increase dopamine release, which is associated with enhanced pleasure and, consequently, the onset of addiction [8]. Taking opioids for extended periods can increase the chance of developing drug tolerance or dependence, diminishing natural neural signalling. Current treatments for opioid addiction are primarily opioid receptor agonists like methadone. Although these treatments reduce the chances of an overdose, the reliance on opioids persists and the probability of a relapse exceeds 50% if the medication is stopped [10]. Comparatively, psilocybin acts independently of the opioid pathway, is cheaper, and has fewer long-term adverse side effects [11]. In a double-blind randomized experiment, Bogenschutz et al. studied psilocybin-assisted psychotherapy for use in decreasing heavy alcohol consumption. They identified an increase in blood pressure and psychedelic experiences believed to contribute to the observed reduction in drinking [2]. Psilocybin is accepted as an effective treatment for alcohol addiction, smoking addiction, binge eating, and depression [12], making it an attractive candidate for potential use in treating opioid addiction.

It is theorized that psilocybin promotes a heightened state, resulting in a feeling of enlightenment and causing a paradigm shift that can break the cycle of addiction [13]. Psilocybin has a low risk of addiction, is non-toxic, and acts on neurons which reduces long-term dependence on a medication by altering users' mental perspective [14]. Literature supports psilocybin treatment efficacy, but studies do not focus on opioid addiction. Therefore, there is a need for in-vivo testing to affirm the predicted results. We hypothesize that psilocybin is effective in treating opioid addiction, and it is predicted that lower levels of opioid consumption and addictive behaviours, including withdrawal, will be observed after psilocybin treatment when compared to controls.

Methods

Psilocybin Extraction

Psilocybin and psilocin will be extracted from *P. cyanescen* by grinding in chloroform. The extract will be derivatized with MSTFA, qualitatively analyzed by ion-mobility spectrometry, and quantitatively analyzed using gas chromatography-mass spectrometry.

Mouse Entrainment and Habituation

A mice model in vivo study will be done. Experiments will be conducted with adult male C57BL/6J mice following the treatment plan. Mice will be housed individually, entrained to a reverse light-dark cycle and habituated to bitterant quinine in their water for 7 days. Morphine addictive behaviour will be assessed via a two-bottle drinking model. Mice will be offered one bottle containing quinine and another containing morphine (0.15 mg/mL) solution at the beginning of the dark phase for 5 days, followed by a 2-day withdrawal period where morphine is replaced with quinine, repeated over 4 weeks. Each day, the morphine and quinine bottle positions will be alternated to ensure place preference is not acquired (Figure 1). Weight, behaviour and quinine and morphine intake will be monitored daily.

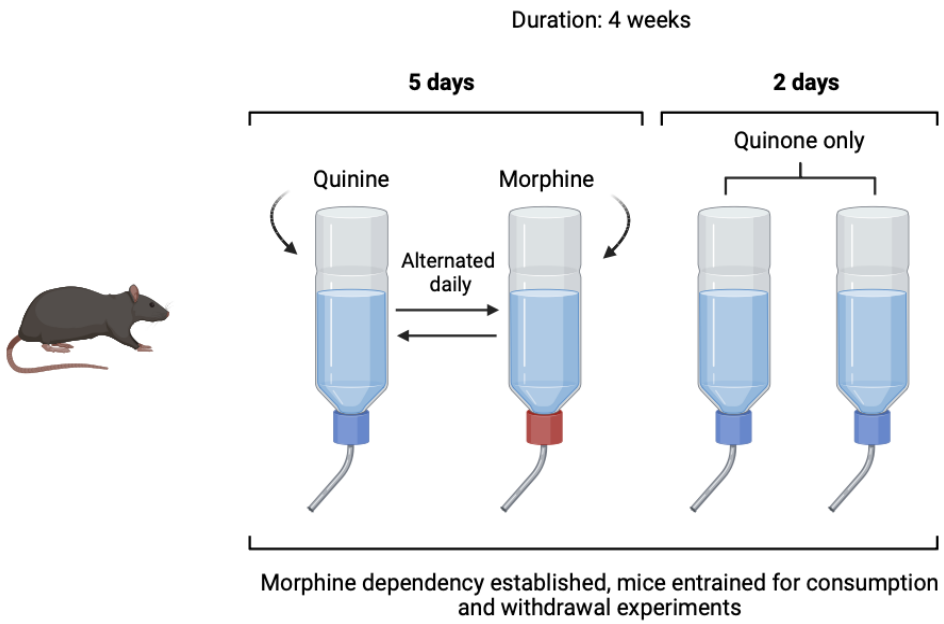

Figure 1. Morphine dependency establishment model. In this two-bottle paradigm model, place preference and taste aversion are minimized for mice to gain morphine dependence. This figure was created using BioRender (2023).

Experiment 1: Morphine Consumption in Response to Psilocybin Post-4-Week Dependence Establishment

Thirty minutes before the dark cycle of the third morphine-available day in the following week, mice will be divided into six groups (n=6 mice/group). The mice will be administered 0.9% saline vehicle, 20 mg/kg body weight methadone, or psilocybin at 0.3, 0.6, 1.2, and 2.4 mg/kg body weight intraperitoneally. These doses correspond to moderate psychoactive doses [15]. Quinine and morphine consumption will be measured daily at the end of the dark cycle for 7 days.

Experiment 2: Psilocybin Effect on Withdrawal Recovery

To measure withdrawal effects, mice will be deprived of morphine post-4-week entrainment for 14 days and administered 0.2 mg/kg naloxone subcutaneously. Mice will be divided into seven groups (n=8 mice/group). Mice will be administered 0.9% saline vehicle, 7 mg/kg body weight methadone, or psilocybin at 0.3, 0.6, 1.2, and 2.4 mg/kg body weight intraperitoneally daily thirty minutes before the beginning of the dark cycle. Positive control will be administered 0.9% saline and given access to a morphine bottle. Cage cameras will monitor mouse behaviour to quantify daily withdrawal symptoms including restlessness, sudden jumping, and paw tremors. Diarrhea and weight loss will also be assessed daily.

Statistical Analysis

The data will be presented as mean ± standard deviation. P-value ≤ 0.05 will be used as a threshold for statistical significance. To study the morphine consumption in the mice, we will use a repeated measures analysis of variance (ANOVA) test to compare the variables between the six studied groups of mice across the 7-days at the end of the dark cycle. Post-hoc comparisons, like Fischer's exact test, will determine if there are significant differences in quinine and morphine consumption between the treatment and control groups, with decreased morphine consumption in the former [16]. To study withdrawal effects, independent-samples t-tests will be used between psilocybin treated mice and control mice to determine any significance of mouse behavior.

Results

As this is a research protocol, the results presented are hypothetical and may differ if the experiments are actually performed. To reduce any confounding variables, mouse entrainment and habituation would be done to avoid place preference and taste aversion. From experiment 1, it may be observed that mice in the psilocybin treatment groups consume less morphine than controls, indicating a reduction in addictive behaviours. Morphine consumption will be recorded for each group throughout the 7-day period, estimating saline treated mice with higher levels of morphine consumption compared to psilocybin dose and methadone treated mice (Figure 2). Towards the end of the 7-day period, morphine consumption of psilocybin-treated mice is expected to be similar with methadone-treated mice. In addition, it is expected that psilocybin will minimize withdrawal symptoms at a similar level to methadone in experiment 2. Any significant changes in diarrhea and weight loss will be recorded to determine any adverse effects of the treatment groups.

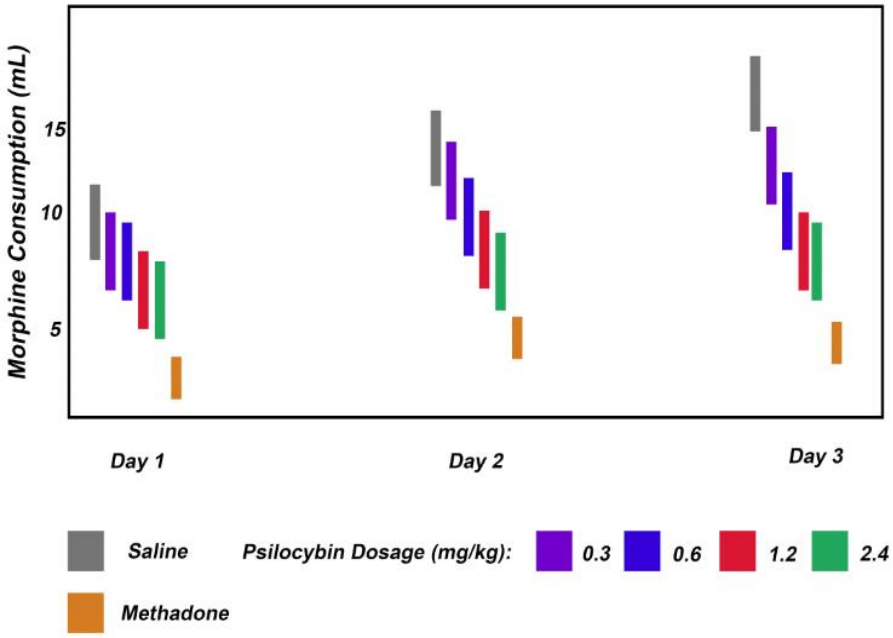

Figure 2. Experiment 1 expected results. Hypothesized morphine consumption (mL) over the first 3 days given saline, methadone or psilocybin treatment (0.3, 0.6, 1.2, 2.4 mg/kg body weight). This will be done daily during the 7-day period at the end of the dark cycle. This figure was created using GraphPad Prism version 10.0.0.

Discussion

The objective of this study is to examine the potential use of psilocybin as a withdrawal assistance agent in comparison to current treatments like opioids, where reliance and the probability of relapse persists. Within this study, we hypothesize the mice that were injected with differing doses of psilocybin would consume less morphine in comparison to the rats who were instead injected with saline. Furthermore, we hypothesize that the psilocybin treated mice would consume levels of morphine at a similar level to methadone treated mice, indicating increased tolerance and efficacy of treatment to current treatments. Minimized withdrawal symptoms of psilocybin-treated mice will also prove to its lesser dependence potential.

The therapeutic effects of psilocybin extract on morphine dependence likely involve multiple mechanisms. Psilocybin primarily interacts with serotonin receptors in the brain, particularly the serotonin 2A receptor (5-HT2A), which leads to changes in neural activity and connectivity [2]. This modulation of serotonin signaling pathways may contribute to reducing opioid withdrawal symptoms and restoring normal behavioral patterns [3]. Moreover, psilocybin has been found to enhance neuroplasticity and promote neuronal growth, potentially aiding in rewiring neural circuits disrupted by chronic morphine exposure [17].

These conclusions agree with known literature as psilocybin is expected to have anti-addictive properties [18]. In a study done that explored the use of psilocybin as a treatment for alcohol use disorder, alcohol consumption was reduced during the 3-day interval after psilocybin administration [16]. However, lower doses of 0.5 mg/kg body weight of psilocybin did not have a significant effect on alcohol consumption levels. Especially since patients react to medication differently at different doses, it is crucial to use psilocybin under medical attention. Another study investigated psilocybin-assisted therapy in major depressive disorder through a randomized double-blind clinical trial [15]. Symptom severity significantly decreased compared to baseline results in psilocybin treated patients compared to those in the placebo group. This alludes to the possible benefits of introducing psilocybin as a treatment in humans and its potential in aiding in withdrawal effects.

The experimental results may provide valuable information of clinical-wide applications and introduce psilocybin as a withdrawal assistance treatment towards opioid disorders. Limitations to this study are that the findings are based on animal models and may not directly translate to humans. Further research using human participants is required to validate the therapeutic efficacy of fungally derived psilocybin extract in the treatment of opioid addiction. Investigating other unwanted effects of psilocybin in addition to addiction or tolerance can determine other benefits psilocybin may provide in withdrawal assistance treatment. Moreover, the optimal dosage and treatment regimen of psilocybin extract need to be determined to maximize therapeutic benefits while minimizing potential adverse effects. This research protocol bridges the gaps in other studies by testing psilocybin treatment efficacy in opioid addiction.

Conclusions

This research protocol highlights the approach of using psilocybin as an alternative for opioid addiction therapeutics for its anti-addictive potential. These proposed findings indicate that psilocybin is as effective as current addiction therapies and should be considered as a superior alternative. Mycology applications in healthcare are an emerging field, and future studies should investigate oral administration of the compounds rather than intraperitoneal injection, a limitation of the mouse model. Researchers could also examine psilocybin's mechanism and determine if and how it chemically interacts with the opioid and reward pathway. Psilocybin is a cheaper, more accessible alternative to current opioid addiction therapies and, if approved for therapeutic consumption, could save the growing substance-dependent population.

Conflicts of Interest

The authors declare that they have no conflict of interests.

Ethics Approval and/or Participant Consent

This study did not require ethics approval and/or participant consent as the experiment was performed in mice hypothetically.

Authors' Contributions

LN: collected and analyzed literature and data, made contributions to the design of the study, drafted and revised the manuscript, and gave final approval of the version to be published.
SR: ideation, contributed to study design and planning, assisted with the collection and analysis of data, and gave final approval of the version to be published.
AG: conceptualization and ideation of the study design, the collection of data as well as interpretation and analysis of the data, and gave final approval of the version to be published.

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