Deciphering Psilocybin: Cytotoxicity, Anti-inflammatory Effects, and Mechanistic Insights

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Introduction

Psilocybin is the serotonergic psychoactive alkaloid found in the mushroom genus Psilocybe, commonly known as "magic mushrooms". Research into its properties was halted by its classification as a schedule I substance in the 1960s. However, in 2006, a groundbreaking study revealing psilocybin's ability to safely reduce fear of death in cancer patients reignited interest in psychedelic research and shifted public opinion. Since then, a substantial body of work uncovered psilocybin's ability to treat a range of neuropsychiatric symptoms with an unparalleled safety profile, earning it a breakthrough therapy designation by the FDA for treatment resistant depression (TRD) and major depressive disorder (MDD). Yet, its mechanism of action remains to be elucidated.

Objective

This study aims to understand the mechanism of action of psilocybin by investigating the cytotoxic and immunomodulatory effects of psilocybin and psilocin on both resting and LPS-activated RAW 264.7 murine macrophages.

Methods

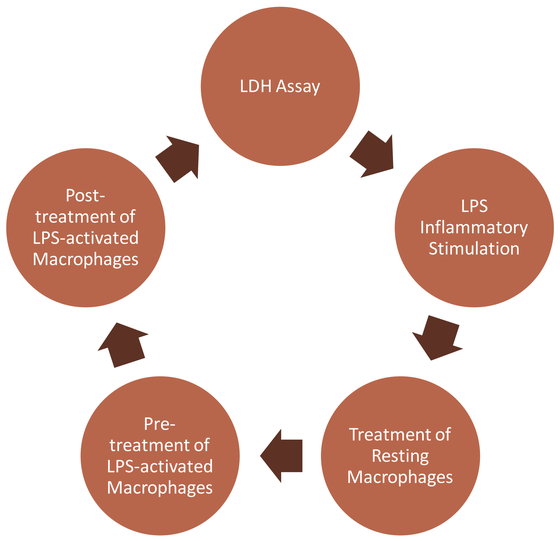


Figure1. Five separate experiments were conducted on RAW 264.7 cells to understand the cytotoxic and immunomodulating effects of psilocybin and psilocin. An LDH assay across various doses was used to establish cytotoxicity. Psilocybin and psilocin cytokine expression was also measured via ELISA on resting macrophages and in the presence of LPS. Different doses, including those above and below the LC50, were used in both pre-treatment and post-treatment approaches.

Results

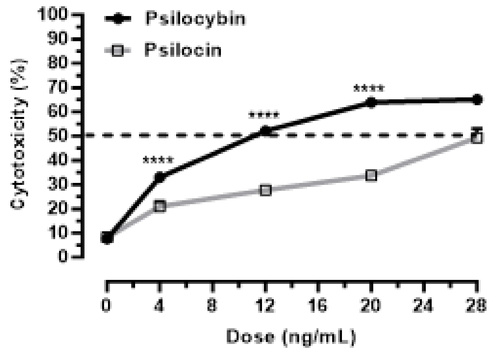
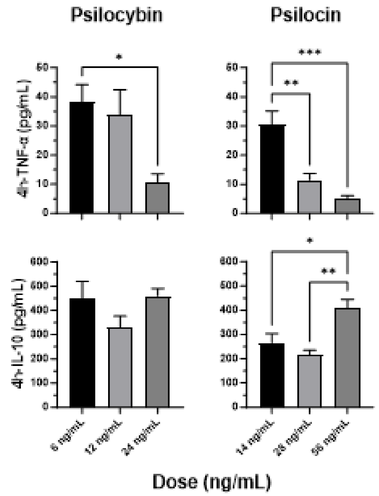


Figure 2. Psilocybin and psilocin dose response curves. Psilocybin is more cytotoxic than psilocin at all equivalent doses. LC_{50} doses for psilocybin and psilocin are noted at 12ng/ml and 28ng/ml respectively. The dash line represents 50% cytotoxicity

Figure 3. RAW264.7 macrophages primed with psilocybin or psilocin in the absence of LPS. Cells treated with media supplemented with Psilocybin (left) or psilocin (right) at various doses were incubated for 4 hours. Pro-inflammatory TNF-α and anti-inflammatory IL-10 were measured by ELISA. **Results for 24 h showed no significant changes.**



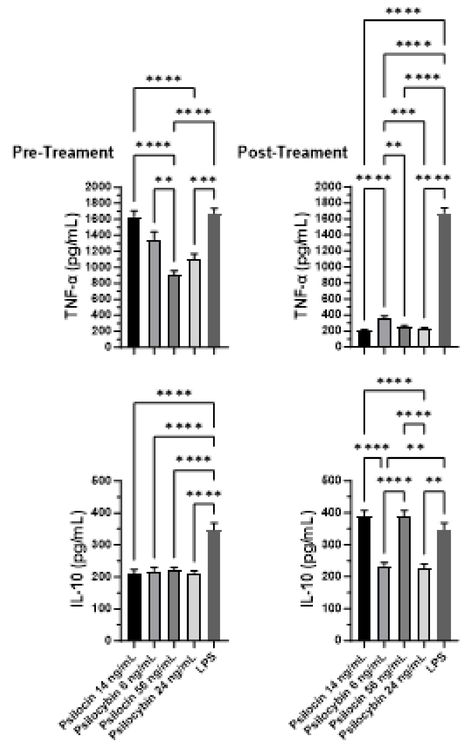


Figure 4. RAW264.7 macrophages pre-treated (left) or post-treated (right) with psilocybin or psilocin In pre-treatment conditions, cells were treated with either psilocybin or psilocin before LPS treatment. In post-treatment conditions, cells were treated with LPS, followed by psilocybin or psilocin. LPS (1 µg/mL) served as statistical comparison after a 4-hour incubation. TNF-α (top) and IL-10 (bottom) were measured in media by ELISA.

Results Continued

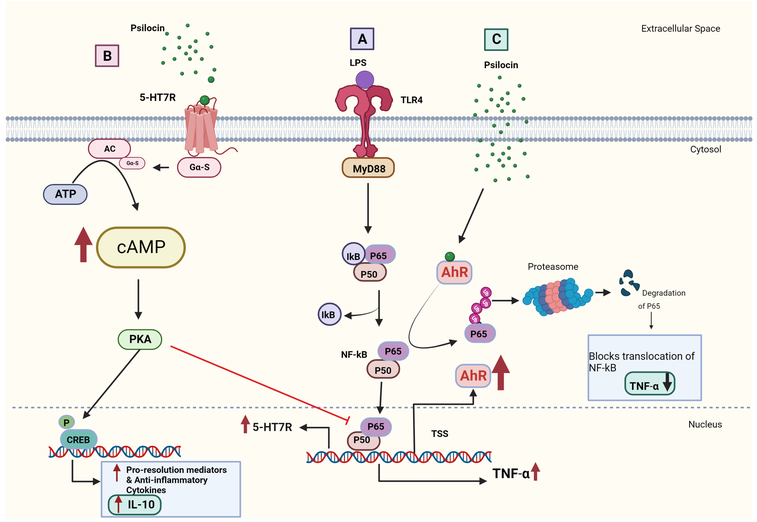


Figure 5. Proposed anti-inflammatory mechanisms of psilocin. (A) LPS binding to TLR4 leads to MyD88- mediated activation of NF-kB, which translocates into the nucleus to initiate transcription of pro-inflammatory cytokines, Aryl Hydrocarbon Receptor (AhR) and 5-HT7 serotonin receptor. **(B)** Psilocin binding to 5HT7 receptors on LPS-activated cells increases intracellular cAMP, leading to PKA-mediated inhibition TNF- α transcription and activation of CREB-mediated IL-10 transcription. **(C)** Psilocin crosses the plasma membrane and binds the LPS-upregulated intracellular AhR receptors, leading to the ubiquitination and proteasomal degradation of the p65 subunit, ultimately preventing the translocation of NF-kB into the nucleus.

Conclusions

We have identified two pathways that require pre-activation of macrophages:

- **Hypothesis 1:** Psilocin's binding to 5HT7 receptors on LPS-activated cells increases cAMP, which, through PKA activation, boosts IL-10 expression in the early phase of inflammation and inhibits the NF-kB-initiated TNF- α transcription (Figure 5B).

- **Hypothesis 2:** The lipophilic nature of psilocin allows cell entry and interaction with AhR, upregulated by the LPS-TLR4 signaling, leading to the degradation of the p65 subunit in a nongenomic manner. The nuclear translocation of NF-kB is prevented and transcription of TNF- α mRNA is reduced (Figure 5C).