Effects of a single dose of psilocybin on cytokines, chemokines and leptin in rat serum

GEOFFREY M. BOVE* and DAVID J. MOKLER

Bove Consulting, USA

Received: August 20, 2022 ● Revised manuscript received: October 30, 2022 ● Accepted: November 2, 2022

ABSTRACT

Background and Aims: The hallucinogenic drug psilocybin is being widely tested in humans for the treatment of psychiatric disorders. Psilocybin and other psychedelics are proposed to work through serotonin 2a (5-HT2a) receptors, which are tightly linked to immune function. The purpose of the present study was to assess the effects of a single dose of psilocybin on a panel of cytokines, chemokines, and peptides in the short term (24 h) and long term (seven days) in female rats. Methods: Female rats were given a dose of psilocybin (20 mg kg⁻¹, i.p.) or a dose of synthetic interstitial fluid. At 24 h, the control group and one group of rats were anesthetized, and blood was withdrawn by intracardiac puncture. In a third group of rats, blood was withdrawn after seven days. Serum was analyzed by a separate lab (Eve Laboratories, Calgary, Canada) for 27 immunomodulators. Results: Serum levels of IL-1β, TNF-α, MCP-1, IP-10, G-CSF, IFN-γ, IL-10, IL-13, and leptin were significantly increased compared to controls after 24 h and were increased further after 7 days. Most of the other assays showed this same pattern of increase, although not statistically significant. Conclusions: Psilocybin induces the release of multiple immune factors, consistent with a generalized activation of the immune system, which can persist for at least seven days after a single dose. These findings may relate to the mechanism of action. The implications of these findings require additional research to determine how these findings relate to the clinical effects of psilocybin.

KEYWORDS

psilocybin, psilocin, hallucinogen, psychedelic, immune

INTRODUCTION

Psilocybin is currently being used in numerous clinical studies for its benefits in the treatment of psychiatric disorders including depression (Davis et al., 2021), anxiety (Agin-Liebes et al., 2020), and substance use disorders (Bogenschutz et al., 2022). The mechanism(s) of action of these effects are not well understood. Psychedelic drugs are proposed to work through 5-HT2a receptors, which have been linked to the effects of serotonin on the immune system (Flanagan & Nichols, 2022). Effects of psilocybin on the immune system including cytokines, chemokines, and other intracellular messengers, may lead to better understanding of how these drugs produce their effects.

The immune system is a very complex interaction of mediators that are released from a variety of tissues to mediate inflammation induced by any means (Stow & Murray, 2013). These mediators include cytokines, chemokines, and proteins. They are involved not only in the inflammatory response but are also involved in many homeostatic pathways.

Our understanding of the actions of psychedelic drugs on the immune system is very limited. Uthaug et al. (2020) examined changes in cortisol, IL-6 and IL-1β in human saliva immediately after treatment with 5-methoxy-dimethyltryptamine (5MeO-DMT). Cortisol was increased, IL-6 was decreased, and there was no change in IL-1β. Other studies have been done using in vitro techniques. House, Thomas, and Bhargava (1994) found that in vitro exposure to LSD suppressed t-lymphocytes, the production of IL-2, IL-4 and IL-6. Szabo, Kovacs, Frecska, and Rajnavolgyi (2014) examined the effects of 5-MeO-DMT and
N,N-dimethyl-tryptamine (N,N-DMT) on monocyte derived dendritic cells in vitro: significant changes were seen in cytokines and chemokines. Given the significant increase in our understanding of immune system complexity, the current investigation was designed as an initial probe into the effect of a single psychoactive dose of psilocybin on the blood concentrations of a panel of 27 immunomodulators.

METHODS

All procedures were approved by the Bove Consulting IACUC (OLAW Assurance D20-010188, protocol BL-2020-01) and conformed to the NIH Guide for the Care and Use of Laboratory Animals. We used 30 female Sprague-Dawley rats (175–200 g) obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in groups, with food and water available ad libitum. Synthetic psilocybin was obtained from the National Institute on Drug Abuse Drug Supply Program (Research Triangle Park, NC), and mixed with sterile synthetic interstitial fluid (Bretag, 1969) to make a stock solution of 8 mg mL⁻¹. 20 mg kg⁻¹ psilocybin was used since it has been reported to be a significant psychedelic dose (Halberstadt, Chatha, Klein, Wallach, & Brandt, 2020).

Ten animals were assigned to a vehicle group and given an intraperitoneal (ip) injection of synthetic interstitial fluid, and blood was drawn 24 h later. Ten animals were assigned to a group given 20 mg kg⁻¹ psilocybin ip and blood drawn at 24 h. Ten animals were assigned to a group given 20 mg kg⁻¹ psilocybin ip and blood drawn at 7 days. For blood collection, animals were deeply anesthetized with 5% isoflurane and blood drawn directly from the heart. Animals were then euthanized. Blood was allowed to sit for 1 h at room temperature. Samples were centrifuged at 1,500 rcf at 4 °C for 10 min. Serum was pipetted into 0.65 mL tubes and immediately placed into a –30 °C freezer until shipped.

Samples were shipped overnight on dry ice to Eve Technologies (https://www.evetechologies.com/, Calgary, Canada). The Rat Cytokine/Chemokine 27-Plex Discovery Assay® Array (RD27) was used on all samples. This assay measures the following biomarkers in rat serum: Eotaxin, EGF, Fractalkine, IFNy, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IP-10, GRO/KC, TNFα, G-CSF, GM-CSF, MCP-1, Leptin, LIX, MIP-1α, MIP-2, RANTES, VEGF-A. Samples were run in duplicate and the results averaged. Statistical analyses were done using analysis of variance, with $P \leq 0.05$ considered statistically significant. Data were tested for normality and equal of variance using SigmaPlot (San Jose CA). If normality tests failed, a non-parametric test was used. Post-hoc analyses were used to determine differences between groups, with $P \leq 0.05$ considered statistically significant.

RESULTS

Within 30 mins of receiving an i.p. dose of psilocybin, animals showed a characteristic behavior of remaining motionless but not sedated. A sudden noise would cause them to become alert. One of the authors (DJM) in previous work has seen this behavior in rats receiving high doses of LSD and other psychedelics (Mokler, Commissaris, Warner, & Rech, 1983). This behavior was not seen 24 h or 7 days after the injection. All animals were included in the analysis of serum levels at 24 h and 7 days. The results of 24 of the 27 assays are shown in Fig. 1 (levels of GM-CSF, GRO/KC, and MIP-2 were below reliable ranges and are not reported).

Significant increases were noted in several pro-inflammatory and anti-inflammatory cytokines as well as several other key inflammatory mediators, including IL-1β, IL-10, IL-13, IFN-γ, IP-10, G-CSF, MCP-1, TNFα, and leptin. As can be appreciated in the figure, most of the changes were similar in that the amount at 24 h was higher than vehicle, and the amount at 7 days was higher than at 24 h. Of note, most of the cytokines (IL-1α, IL-2, IL-4, IL-5, IL-6, IL-12/ p70, IL-17a, IL-18) as well as chemokines (EGF, eotaxin, fractalkine, MIP-1α) and VEGF showed a similar pattern of progressive increase, although the differences were not statistically significant.

DISCUSSION

The results of this experiment show that the rat immune system is affected by a single dose of psilocybin, and that the effect increases over a period of 7 days. This occurred even though psilocybin and psilocin are cleared from the bloodstream after a few hours (although there is some evidence that metabolites may still be excreted up to seven days in the rat (Dinis-Oliveira, 2017)). Limitations of our findings include low statistical power for many of the analyses due to high variability in the results, which is not surprising given the exploratory nature of this experiment. It is also unknown if the observed differences are functionally meaningful.

It is striking that most of the assays showed a similar pattern of increase over the 7 days of the experiment. Because the affected chemokines and cytokines have diverse and apparently opposite general functions, such as pro-inflammatory versus anti-inflammatory (Table 1), we suggest that this is not a specific or coordinated effect. Rather, the effect seems consistent with a generalized immune cell activation, inducing a general increase in post-Golgi trafficking and release (Stalder & Gershlick, 2020; Wang, Stanford, & Kundu, 2020). Psychedelic drugs such as psilocybin have been investigated for their anti-inflammatory activity, purported to be tied to their common mechanisms of action as 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, and 5-HT2C receptor agonists (Flanagan & Nichols, 2018; Inserra, De Gregorio, & Gobbi, 2021; Szabo, 2015). However, our data support a possible pro-inflammatory effect as well. An intriguing finding is the long-term increases in G-CSF and IFN-γ. Both of these chemokines have been shown to induce neurogenesis in the brain (Pereira, Medina, Baena, Planas, & Pozas, 2015; Schneider, Kuhn, & Schäbitz, 2005), a link that warrants additional investigation. It is beyond the scope of this article to comprehensively discuss
the details of the various mechanisms by which psilocybin might influence immune system function: a recent review goes into significant detail on this (Szabo, 2015). Much further research is necessary to investigate how psilocybin might affect immune-related chemicals and function and how the changes may impact psychiatric disorders.
ACKNOWLEDGEMENTS

This study was funded by HAVNLife, Vancouver, BC, Canada. The company had no input into the design of the study or in the interpretation of the results. DJM was a paid scientific consultant to HAVNLife from September 2020 to June 2022. There are no other potential conflicts of interest.

REFERENCES


Dinis-Oliveira, R. J. (2017). Metabolism of psilocybin and psilocin: Clinical and forensic toxicological relevance. Drug Metabolism
Mizuno, T., Kawanokuchi, J., Numata, K., & Suzumura, A. (2003). Open Access. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes – if any – are indicated.


