The serotonin receptor agonist psilocybin as a novel therapeutic approach for NAFLD: preclinical studies

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Background

Although the prevalence of liver steatosis is rising globally, in line with other metabolic disorders including obesity and type 2 diabetes, there are no drugs approved for its cure. The serotonin receptor (5-HT2AR) agonist psilocybin, an alkaloid contained in *Psilocybe* mushrooms, reduced obesity in animal models. This study assessed the effects of non-psychedelic doses of psilocybin in mice with high-fat-high-fructose diet (HFHFD)-induced liver steatosis. Mechanistic studies were also performed on HepG2 cells and 3T3L1-derived adipocytes.

Methods

The *in vitro* effect of psilocin was assessed on HepG2 cells treated with a mixture of palmitic and oleic acid (PA:OA mix, 1:2 ratio), in presence or absence of the 5-HT2AR antagonist ketanserin (Figure 1). Further evaluations were performed on 2D culture and spheroids of 3T3L1-derived adipocytes (Figure 2). Intracellular lipid accumulation was assessed using Bodipy staining. Psilocybin was tested *in vivo* on C57BL/6 male mice fed with HFHF (60% kcal from fat) for 17 weeks. One group (n=10) received daily 0.05 mg/Kg*bw psilocybin and the other (n=10) received vehicle by oral gavage (Figure 3). Standard diet-fed mice (n=10) were used as controls. Body weight and food intake were assessed weekly. T-maze and Light-Dark-box tests were used to assess anxiety-like behavior and memory. Oral glucose tolerance test was performed, and fasting glucose and triglycerides were measured before sacrifice. Liver histology was assessed by H&E and ORO staining, and plasma and hepatic triglycerides content was quantified after organic extraction. Blood immunophenotyping was performed by FACS and mRNA expression was evaluated by qPCR.

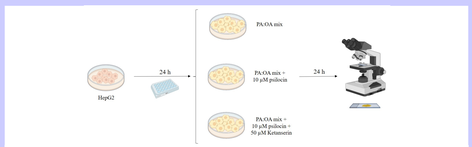

Figure 1. Scheme of treatment of HepG2 cell line. 24-hour treatment of HepG2 cell line with PA:OA mix, in presence or absence of 10 μM psilocin and/or 50 μM ketanserin.

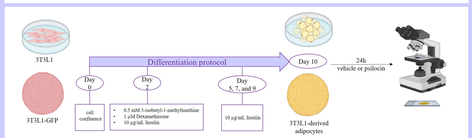

Figure 2. Protocol of differentiation of 3T3L1 cell line and scheme of treatment. Differentiation of 2D culture and spheroids of 3T3L1 and 3T3L1-GFP cell lines into pre-adipocytes, and 24-hour treatment w/ or w/o 10 μM psilocin.

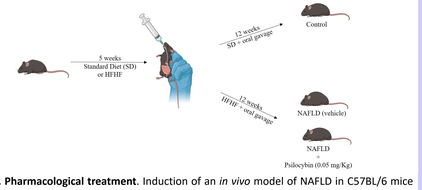

Figure 3. Pharmacological treatment. Induction of an *in vivo* model of NAFLD in C57BL/6 mice and relative pharmacological treatment with vehicle or 0.05 mg/Kg psilocybin

In vitro results

Psilocin decreased lipid accumulation in HepG2 cells (Figure 4), and this effect was reverted by the cotreatment with the 5HT2A antagonist ketanserin. A similar result was reported in 3T3L1-derived adipocytes, both in a 2D cell culture (Figure 5) and in spheroids, where the psilocin effect was particularly evident (Figure 6).

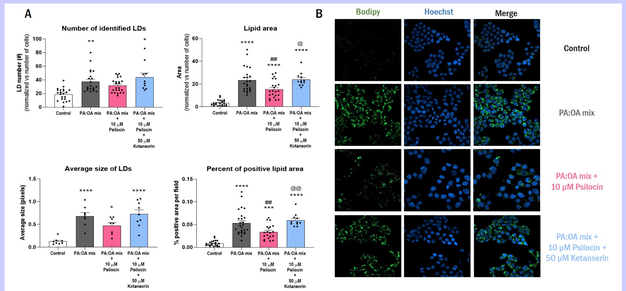

Figure 4. 5HT2A-dependent *in vitro* effect of psilocin on lipid droplets (LDs) formation in HepG2 cells treated with PA:OA mixture. The quantification of LDs is reported in (A), and the images of LDs stained green with Bodipy (nuclei stained blue with Hoechst) are reported in (B). Data are presented as mean ± S.E.M of nine independent experiments, each performed in triplicate. *p<0.05, **p<0.01, ***p<0.005, and ****p<0.0001 vs control; ##p<0.01 vs PA:OA mix; @p<0.05, and @@p<0.01 vs PA:OA mix + 10 .

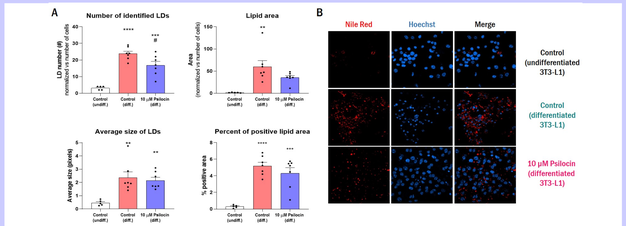

Figure 5. *In vitro* effect of psilocin on lipid accumulation in 3T3L1-derived adipocytes. Psilocin reduced number of the LDs stored inside adipocytes. The quantification of LDs is reported in (A), and the images of LDs stained red with Nile Red (nuclei stained blue with Hoechst) are reported in (B). Data are presented as mean ± S.E.M of two independent experiments, each performed in triplicate. **p<0.01, ***p<0.001, ****p<0.0001 vs undifferentiated 3T3-L1 cells; #p<0.05 vs differentiated 3T3L1 cells.

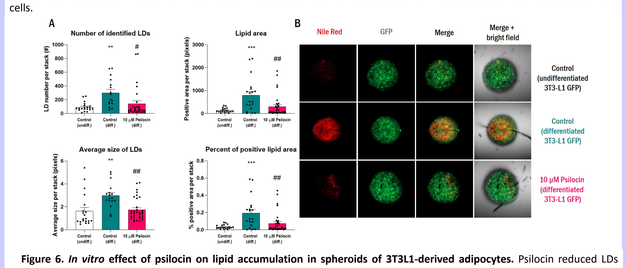

Figure 6. *In vitro* effect of psilocin on lipid accumulation in spheroids of 3T3L1-derived adipocytes. Psilocin reduced LDs number, lipid-positive area and dimensions (A). The confocal images show the LDs stained with Nile red and the green fluorescence of GFP (B). Data are presented as mean ± S.E.M of the stacks of spheroids. **p<0.01, ***p<0.001, ****p<0.0001 vs undifferentiated 3T3-L1 cells; #p<0.05 vs differentiated 3T3L1 cells.

In vivo results

Psilocybin, beside ameliorating liver histology (Figure 10), reduced body weight by 12% (Figure 7) and restored to normal level plasma and liver triglycerides in HFHFD-fed mice (Figure 12). A significant decrease of fasting glucose (p<0.001, Figure 8C) and AUC was observed in the OGTT (Figure 8B). Psilocybin reduced anxiety-like behavior (Figure 9B), and modulated hepatic genes involved in de novo lipogenesis, glycolysis, β-oxidation, i.e., SREBP1 (2-fold decreased, p<0.05, Figure 11A), PPARα (2-fold decreased, p<0.01, Figure 11B) ChREBP (2-fold increased, p<0.01 vs controls, Figure 11C) and CPT1α (3-fold increased, p<0.05, Figure 11D), and restored the phenotype of circulating NK cells (Figure 13B), by reverting the increase of PD-1 expressing exhausted NK cells of HFHFD-fed mice (p<0.01).

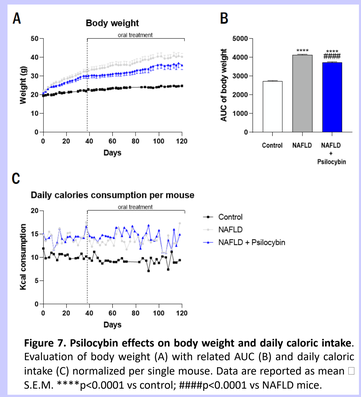

Figure 7. Psilocybin effects on body weight and daily caloric intake. Evaluation of body weight (A) with related AUC (B) and daily caloric intake (C) normalized per single mouse. Data are reported as mean ± S.E.M. ****p<0.0001 vs control; ####p<0.0001 vs NAFLD mice.

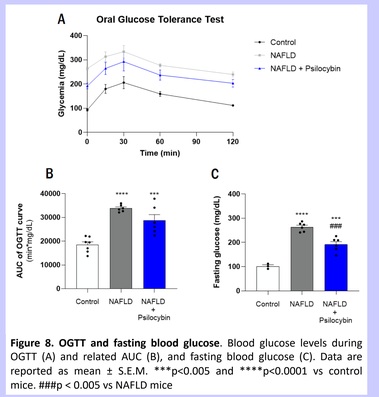

Figure 8. OGTT and fasting blood glucose. Blood glucose levels during OGTT (A) and related AUC (B), and fasting blood glucose (C). Data are reported as mean ± S.E.M. ***p<0.005 and ****p<0.0001 vs control mice. ###p < 0.005 vs NAFLD mice

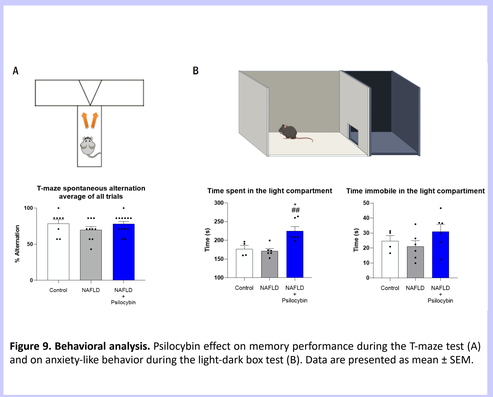

Figure 9. Behavioral analysis. Psilocybin effect on memory performance during the T-maze test (A) and on anxiety-like behavior during the light-dark box test (B). Data are presented as mean ± SEM.

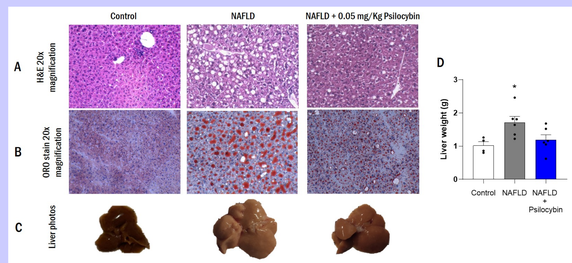

Figure 10. Liver Histology. Effect of psilocybin on steatosis. Histological images obtained with H&E (A) and ORO (B) stain, 20X magnification. Images of livers collected after animal sacrifice (C), and liver weight in the different groups of treatment (D). Data are reported as mean ± S.E.M *p<0.05 vs control mice.

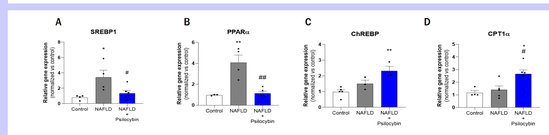

Figure 11. Effect of Psilocybin on mRNA expression of genes involved in hepatic lipid metabolism. Relative mRNA expression of transcription factors involved in *de novo* lipogenesis ((A), (B), and (C)), and β-oxidation (D). Data are presented as mean ± S.E.M. *p < 0.05, **p < 0.01 vs control; #p < 0.05, ##p < 0.01 vs NAFLD mice.

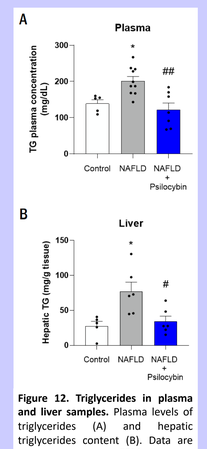

Figure 12. Triglycerides in plasma and liver samples. Plasma levels of triglycerides (A) and hepatic triglycerides content (B). Data are presented as mean ± SEM.
*p < 0.05 vs control mice; #p < 0.05, ##p < 0.01 vs NAFLD mice.

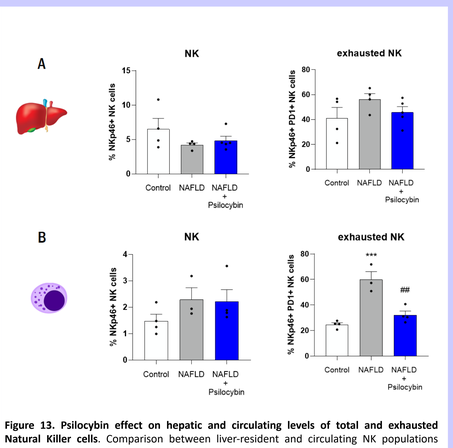

Figure 13. Psilocybin effect on hepatic and circulating levels of total and exhausted Natural Killer cells. Comparison between liver-resident and circulating NK populations (total and exhausted) after chronic treatment with microdoses of psilocybin. Data are reported as mean ± SEM.* p < 0.05, and *** p < 0.005 vs control; ## p < 0.01 vs HFHF

Conclusion

Low-dose psilocybin significantly reduces hepatic steatosis, blood glucose levels and body weight in HFHF-fed mice, without detrimental CNS effects, by a pleiotropic action on lipid and glucose metabolism. These in vivo results are supported by *in vitro* evaluations on different models. Psilocybin at low non-psychedelic doses may be a novel candidate therapy for liver steatosis and associated metabolic disorders, i.e., obesity and type 2 diabetes mellitus.

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