Study of the effects of psilocybin in three rat preclinical models of migraine: insights from the trigeminovascular system

Transpharmation
Science that translates into results

Owein Guillemot-Legris[1], Isabelle Shand[1], Joshua Featherstone[1], Monika Baryla[3], Joseph Araujo[2], Mark Duxon[1], K.R. Walker[2], Colleen Taylor[1], Guy A. Higgins[2]

[1]Transpharmation Ltd. UK. [2]Transpharmation Canada. [3]Transpharmation Poland

INTRODUCTION

Migraine is a complex disorder the aetiology of which is still not fully understood. Its pathophysiology has been found to involve complex processes implicating neurological and vascular mechanisms[1]. One structure thought to be central in these processes is the trigeminovascular system[1-2].

Recently, psychedelics have shown some very interesting effects in several models of neuropathic pain and in the fields of anxiety and depression[3-4]. In the clinics, psilocybin has been shown to be effective in migraineurs as it decreased migraine frequency and severity in participants[5]. Interestingly, however, psilocybin administration in healthy volunteers was shown to induce transient headaches[6].

As this compound could represent an interesting avenue to explore in terms of treatment, we wanted to assess, from a more mechanistic standpoint, the effects of psilocybin in three preclinical rat models of migraine while focusing on the trigeminovascular system.

OBJECTIVES

- Find a psilocybin dose able to reverse mechanical allodynia in the classical glyceryl trinitrate (**GTN**) migraine model.
- Investigate psilocybin efficacy in two other migraine models relying on very distinct pathophysiological pathways: the calcitonin gene-related peptide (**CGRP**) and the pituitary adenylate cyclase-activating polypeptide 38 (**PACAP-38**) migraine models.
- Measure inflammatory mediator levels in the trigeminovascular system: trigeminal ganglia (**TGG**), brainstem (**BST**), and plasma

METHODS

We used three different models of migraine in Sprague Dawley male rats with the same experimental timeline. The migraine inducer (**GTN, CGRP, and PACAP-38**) was administered at t=0. Psilocybin or gold standards (sumatriptan or PA-8) were dosed at t=30 minutes. Mechanical allodynia was assessed at t=90min with von Frey hairs in the periorbital region.

Finally, we assessed the effects of psilocybin on the levels of several inflammatory mediators (cytokines and chemokines) in the plasma, brainstem, and trigeminal ganglia of the **CGRP and PACAP-38** animals using Meso Scale Discovery (MSD) multiplex immunoassays.

RESULTS: Psilocybin reverses GTN-induced mechanical allodynia

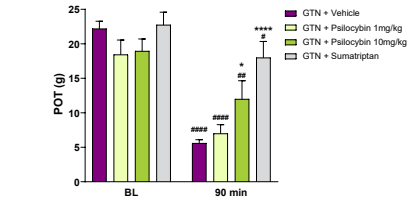

Figure 1. Effects of Psilocybin on periorbital mechanical allodynia in GTN-induced migraine in rat.
GTN (10mg/kg, i.p.) was administered at t=0. Vehicle, Psilocybin (1mg/kg and 10mg/kg), and Sumatriptan (1mg/kg) were administered at t=30min. Mechanical allodynia was assessed at t=90min with von Frey hairs in the periorbital region and is displayed as periorbital threshold (POT) in g. Data is mean ± sem, N=10 per group. Two-way repeated measure ANOVA with Dunnett's multiple comparisons post-hoc test. #p<0.05, ## p<0.01, and #### p< 0.0001 when compared to the respective baseline. *p<0.05, and ****p<0.0001 when compared to GTN + Vehicle at each timepoint.

GTN significantly induced mechanical allodynia in the periorbital region at 90 minutes when compared to baselines (**Fig.1**). Sumatriptan (1mg/kg) increased the periorbital threshold (POT) 90 minutes. We assessed the effects of two doses of psilocybin (1mg/kg and 10mg/kg). While the lowest dose did not increase the POT, psilocybin 10mg/kg was able to significantly increase the POT when compared to vehicle-treated animals at 90 minutes.

RESULTS: Psilocybin reverses CGRP-induced mechanical allodynia

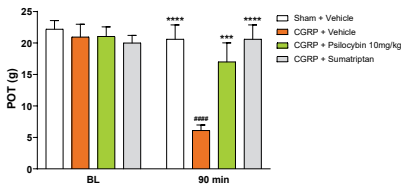

Figure 2. Effects of Psilocybin on periorbital mechanical allodynia in CGRP-induced migraine in rat.
CGRP (0.1mg/kg s.c.) was administered a t=0. Vehicle, Psilocybin (10mg/kg), and Sumatriptan (1mg/kg) were administered at t=30min. Mechanical allodynia was assessed at t=90min with von Frey hairs in the periorbital region and is displayed as periorbital threshold (POT) in g. Data is mean ± sem, N=10 per group. Two-way repeated measure ANOVA with Šídák's multiple comparisons post-hoc test. #### p< 0.0001 when compared to baseline and ***p<0.001, and ****p<0.0001 when compared to CGRP + Vehicle group.

CGRP significantly induced mechanical allodynia in the periorbital region at 90 minutes when compared to baseline (**Fig.2**). Sumatriptan (1mg/kg) increased the periorbital threshold (POT) at 90 minutes. Psilocybin (10mg/kg) was able to significantly increase the POT when compared to vehicle-treated animals at 90 minutes.

RESULTS: Psilocybin alters inflammatory marker levels in the trigeminovascular system during CGRP-induced migraine

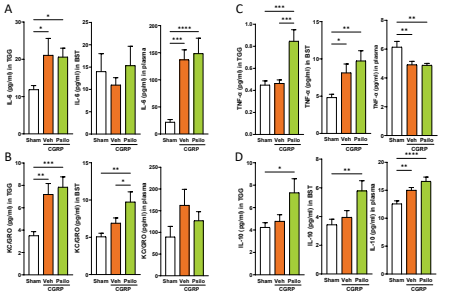

Figure 3. Inflammatory mediator levels measured in trigeminal ganglia (TGG), brainstem (BST), and plasma.
CGRP (0.1mg/kg s.c.) was administered a t=0. Vehicle (Veh) and Psilocybin 10mg/kg (Psilo) were administered at t=30min. Inflammatory mediator levels were measured at t=95min. Data is mean ± sem, N=6-10 per group. One-way ANOVA with Fisher's LSD post-hoc test or Kruskal-Wallis test with Dunn's post-hoc test. * p<0.05, **p<0.01 ***p<0.001, and ****p<0.0001.

CGRP increased the levels of IL-6 in both TGG and plasma while no change was measured in the BST (**Fig.3A**). KC/GRO levels were increased in the TGG and in the BST following CGRP dosing (**Fig.3B**). While IL-10 levels were increased in CGRP-treated animals in plasma, psilocybin 10mg/kg led to a selective increase in both the TGG and the BST (**Fig.3D**).

RESULTS: Psilocybin reverses PACAP-38-induced mechanical allodynia

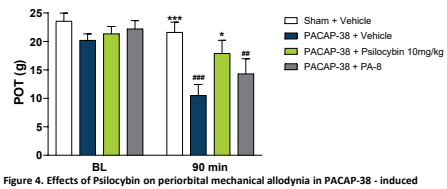

Figure 4. Effects of Psilocybin on periorbital mechanical allodynia in PACAP-38 - induced migraine in rat.
PACAP-38 (0.003mg/kg s.c) was administered a t=0. Vehicle, Psilocybin (10mg/kg), and Sumatriptan (1mg/kg) were administered at t=30min. Mechanical allodynia was assessed at t=90min with von Frey hairs in the periorbital region and is displayed as periorbital threshold (POT) in g. Data is mean ± sem, N=10 per group. Two-way repeated measure ANOVA with Šídák's multiple comparisons post-hoc test. ## p< 0.01 and ### p< 0.001 when compared to respective baselines and *p<0.05, and ***p<0.001 when compared PACAP-38 + Vehicle group.

PACAP-38 significantly induced mechanical allodynia in the periorbital region at 90 minutes when compared to baseline. Psilocybin (10mg/kg) was able to significantly increase the POT when compared to vehicle-treated animals at 90 minutes.

RESULTS: Psilocybin alters inflammatory marker levels in the trigeminovascular system during PACAP-38-induced migraine

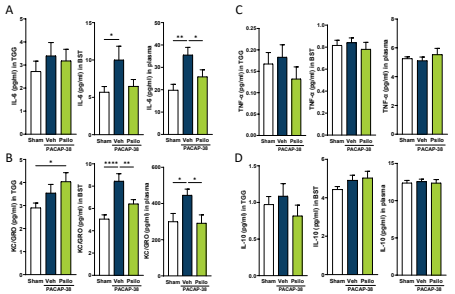

Figure 5. Inflammatory mediator levels measured in trigeminal ganglia (TGG), brainstem (BST), and plasma.
PACAP-38 (0.003mg/kg s.c) was administered a t=0. Vehicle (Veh) and Psilocybin 10mg/kg (Psilo) were administered at t=30min. Inflammatory mediator levels were measured at=95min. Data is mean ± sem, N=6-10 per group. One-way ANOVA with Fisher's LSD post-hoc test or Kruskal-Wallis test with Dunn's post-hoc test. * p<0.05, **p<0.01, and ****p<0.0001.

PACAP-38 increased IL-6 levels in the BST and in plasma while psilocybin 10mg/kg was able to decrease IL-6 in the plasma (**Fig.5A**). KC/GRO levels were increased by PACAP-38 (Veh) and Psilocybin 10mg/kg (Psilo) was able to decrease KC/GRO in the BST and in the plasma.

CONCLUSIONS

- Psilocybin was able to reverse mechanical allodynia in three distinct models of migraine in rats.
- The inflammatory mediator analysis in the trigeminovascular system showed that depending on the model considered, the changes observed were inducer-specific (CGRP vs PACAP-38).
- The effects of psilocybin were found to be model-dependent (plasma IL-6 in CGRP vs PACAP-38) as well as model-specific (IL-10 in the trigeminovascular system in CGRP vs PACAP-38).

REFERENCES

1. Hoffmann J. et al, *J. Cereb. Blood Flow Metab.* (**2019**) **2**. Akerman S. et al, *Nat. Rev. Neurosci.* (**2011**) **3**. McClure-Begley TD & Roth BL, *Nat. Rev. Drug Discov.* (**2022**) **4**. Hammo A., Wisser S. & Cichon J, *Nat. Neurosci.* (**2025**) **5**. Schindler E. et al, *Neurotherapeutics.* (**2021**) **6**. Johnson MW, Sewell RA & Griffiths RR, *Drug Alcohol Depend* (**2012**)