ROLE OF NOVEL RECEPTOR GPR171 IN CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

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INTRODUCTION

First-line chemotherapies against solid tumors are highly efficacious in reducing the tumor burden, but have many adverse side-effects including nerve damage, leading to chronic pain. Non-addictive, efficacious pain relievers are an area of active interest, and we propose a novel target to address this pressing issue. GPR171 is a G-Protein Coupled Receptor that was recently deorphanized and was identified to be expressed in the brain in regions that regulate reward, anxiety, and pain. Within the pain circuit, it was shown previously that systemic administration of the GPR171 agonist enhances morphine antinociception in acute pain tests. Preliminary data from our lab has shown that GPR171 activation can also alleviate persistent inflammatory pain. However, the role of this receptor has not been investigated in other chronic pain models. Given these findings in acute and inflammatory pain, we hypothesize that GPR171 can reduce neuropathic pain. To test this hypothesis, we investigate the role of GPR171 in chronic neuropathic pain. We tested the efficacy of a GPR171 agonist in a chemotherapy-induced neuropathy mouse model.

METHODS

Subjects

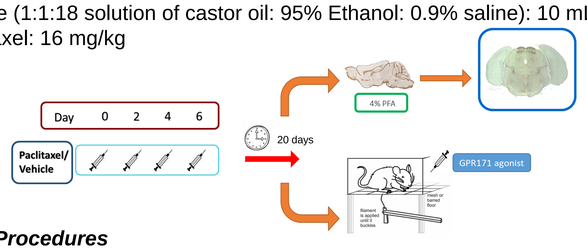
Male C57/BL6J mice (20-34 g) were used in this study. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Neuropathic pain

Intraperitoneal (i.p.) injections every other day:

Vehicle (1:1:18 solution of castor oil: 95% Ethanol: 0.9% saline): 10 mL/kg

Paclitaxel: 16 mg/kg



General Procedures

24 mice were treated on days 0, 2, 4, and 6 with vehicle or paclitaxel. We performed Von Frey mechanical nociception threshold behavioral testing on the mice to assess development of neuropathic pain. From the 15th day onwards, half of the mice were treated with MS15203 (10 mg/kg), a compound that activates the GPR171 receptor. 30 min following the injection, the mice were tested again on the von Frey to assess the analgesic effects of GPR171 activation. The remaining mice were transcardially perfused with 4% paraformaldehyde in PBS and their brains were removed.

Von Frey Assay

Mice were placed in transparent plexiglass chambers on a mesh platform. Fine plastic filaments with calibrated forces were applied to the plantar surface of the left and right hind paws. The lowest force at which the animal exhibited a pain response was recorded as their mechanical threshold.

Von Frey Setup

Von Frey Filaments

RESULTS

GPR171 agonist can relieve allodynia caused by neuropathic pain

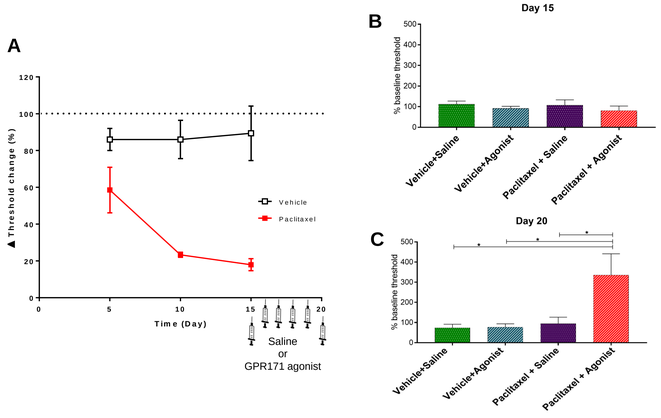


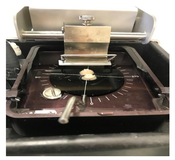
Figure 1: A) Changes in mechanical threshold of C57BL/6 mice upon instatement chemotherapy-induced peripheral neuropathy. Mechanical allodynia in Paclitaxel-treated mice develops by Day 7 and persists up to Day 15 of testing. The threshold is normalized to each animal's response on Day 0, prior to commencement of Paclitaxel administration. B) One dose of GPR171 agonist cannot relieve chronic pain. C) Repeated dosing of the agonist over 5 days can relieve chronic pain by increasing thresholds by 3x compared to baseline on day 15 (1-way ANOVA p-value <0.05).

METHODS

Immunofluorescence

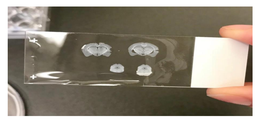
The perfused brains were mounted on a vibratome and 50 µm thick slices were prepared. Three slices of the PAG were selected from each treatment and proteins of interest were analyzed by fluorescent immunohistochemistry. These slices were labeled with GPR171.

A phosphate buffered saline solution was used to wash the slices. A 1% $NaBH_4$ solution is used to remove the paraformaldehyde layer to allow the antibodies to bond to the antigens within the brain. The slices were treated with a blocking serum which includes PBS, goat serum, and Triton X-100. The primary antibodies are delivered to the slices via a buffer solution. The secondary antibody is administered using the buffer solution the next day. DAPI, a blue-fluorescent DNA stain, is attached after a series of washes to the slices. The slices are run through another PBS wash. The slices are placed on a microscope slide and were imaged using a confocal microscope (Zeiss). Images were analyzed using ImageJ (NIH) and statistical analyses were performed using GraphPad Prism.



Brains were sliced using a vibratome

Brain slices were processed in a 24-well plate



Brain slices were stained and mounted for imaging

A Zeiss confocal microscope for taking fluorescence images

RESULTS

Paclitaxel treatment results in lower GPR171 levels in the PAG

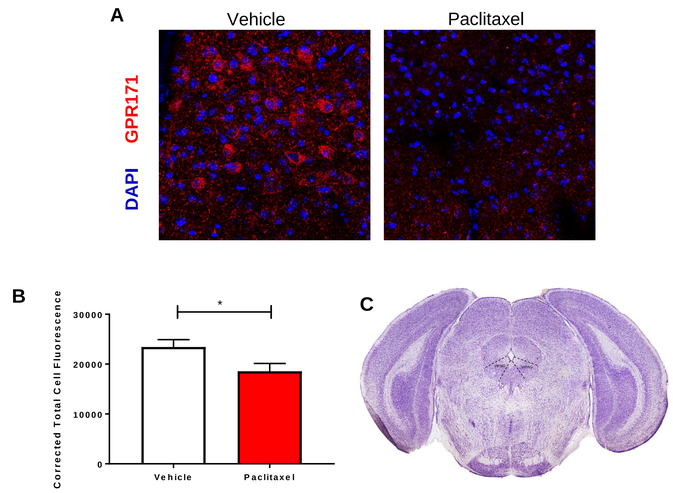


Figure 2: Paclitaxel treatment results in lower GPR171 levels in the PAG. A) 20x confocal image of GPR171 in vehicle and paclitaxel treated animals. B) Comparison of corrected total cell fluorescence (CTCF) of GPR171 signal intensity in PAG sections. C) Representative coronal diagram corresponding to the brain region selected for GPR171 analysis.

CONCLUSIONS

- Paclitaxel treatment makes mice more sensitive to mechanical stimuli.
- GPR171 agonist is a potential non-opioid therapeutic for chronic pain.
- Following 5 days of GPR171 agonist treatment, there was an increase in mechanical thresholds of animals in chronic pain.
- There is a decrease in GPR171 receptors in the PAG of mice that have chronic pain. The agonist can bind to the available receptors to produce pain relief.
- *Future directions*: Use molecular biology tools to evaluate changes in GPR171 levels after long-term agonist treatment.

Acknowledgements

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