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14. ABSTRACT

This project is to rigorously evaluate if activating spinal GPR37 (G protein-coupled receptor 37) has a potential of treating and preventing pain chronification by erasing 'spinal pain memory' without posing a risk of abuse. As the first step, we determined if the putative GPR37 agonists TX14A and protectin D1 (PD1) produce any adverse effects on normal motor and sensory functions when given intrathecally. We found that TX14A at doses 10-50 ug and PD1 1 at 50-100 ng had no immediate (1 hour post intrathecal injection) or long-lasting (24 hour) effect on the grip strength and rotarod performance. In addition, at these doses, the two agonists did not affect normal mechanical and heat sensitivity longitudinally measured up to 24 hours post intrathecal injection. In the intraplantar capsaicin injection model, TX14A facilitated the resolution of mechanical pain hypersensitivity produced by the acute chemical injury. These results suggest that activating spinal GPR37 erases an acute injury-induced spinal pain memory without affecting normal motor and sensory functions.

15. SUBJECT TERMS
None listed.

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1. Introduction

This project is to rigorously evaluate if activating spinal GPR37 (G protein-coupled receptor 37) has a potential of treating and preventing pain chronification by erasing 'spinal pain memory' without posing a risk of abuse. Specifically in this reporting period, the purpose of experiments is to determine (1) if the GPR37 agonists TX14A and protectin D1 (PD1) produce any adverse effects on normal motor and sensory functions when given intrathecally, and (2) at the doses without any adverse effects, if a single intrathecal injection of the GPR37 agonists resolves long-lasting pain hypersensitivity in an acute injury model.

2. Keywords

G protein-coupled receptor 37, Spinal pain memory, Pain hypersensitivity, Pain chronification

3. Accomplishments

3.1. What were the major goals of the project?

The major goals and milestone of the project in this reporting period are:

(1) To examine adverse effects of intrathecal (i.th) GPR37 agonists (TX14A and PD1) on normal sensory and motor functions for determining doses of the agonists to be tested in pain models, and

(2) To determine effects of i.th. GPR37 agonists on pain hypersensitivity in acute injury models

3.2. What was accomplished under these goals?

(1) Major activities

During this reporting period, we conducted experiments proposed to fulfill the major goals of this project, focusing on the specific objectives described below.

(2) Specific objectives

The specific objectives in this reporting period are:

- To complete behavioral tests checking normal motor functions (grip strength test and rotarod test) and sensory functions (von Frey filament test and radiant heat test), and
- To test the GPR37 agonists, at the doses shown to be without an apparent adverse effect in the abovementioned tests, in the intraplantar capsaicin injection model that shows long-lasting mechanical and heat pain hypersensitivity.

(3) Significant results

To ensure robust and unbiased results, the experiments described below were performed by researchers blinded as to the experimental nature of animals except for sex. At least n=7 mice were used for each dose per drug. TX14A was dissolved in saline; PD1 was reconstituted in 10% dimethyl sulfoxide (DMSO) after evaporating the packaging solvent in a chamber filled with 100% N_2. These GPR37 agonists (as a 5 μL single bolus) were intrathecally injected by a lumbar puncture (between L5 and L6 vertebrae) method.

- <u>The effects of TX14A and PD1 on normal motor functions</u>
  We tested three doses (10, 25, and 50 μg) of TX14A and two doses (50 and 100 ng) of PD1 vs. their vehicles (saline for TX14A and 10% DMSO for PD1) in the grip strength test and the rotarod test. As our preliminary results in the intraplantar capsaicin model indicated both rapid onset (~ 1 hour) and long-lasting (~24 hours) effects of TX14A on pain hypersensitivity, we determined the motor functions of mice treated with each dose of TX14A or PD1 at these two time points. As shown in **Figure 1**, compared with vehicle, all three doses of TX14A showed no significant effect on grip strengths and motor performance on a rotarod in both sexes. Likewise, compared with vehicle, all two doses of PD1 had no significant effect on these motor functions in both sexes (**Figure 2**).

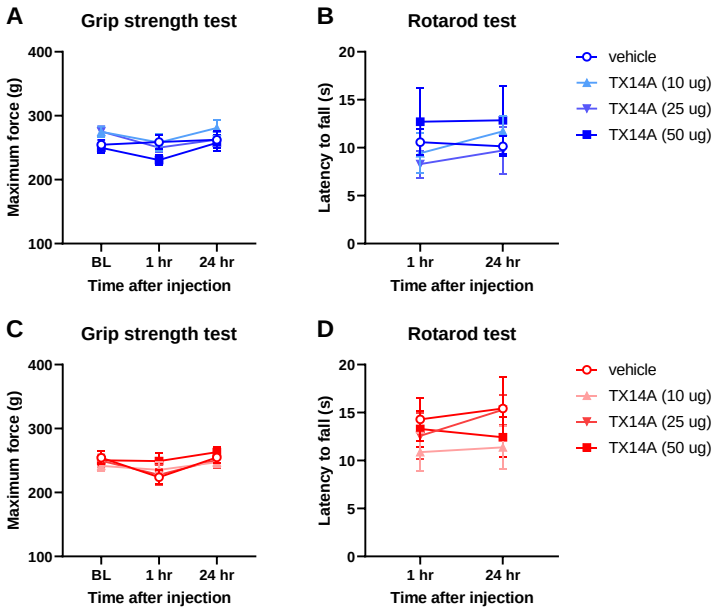

Figure 1. Intrathecally injected GPR37 agonist TX14A has no significant effect on normal motor functions in both (A & B) male and (C & D) female mice. TX14A was injected under anesthesia immediately after the baseline (BL) measurement in the grip strength test. Note that the baseline was not measured in the rotarod test before the injection for preventing the impact of exhaustion on the performance. Data are presented as mean±SEM. Statistical comparisons were made by 2-way repeated measures AVOVA followed by Dunnett's t-test (i.e., vs. vehicle at each time point) in each sex.

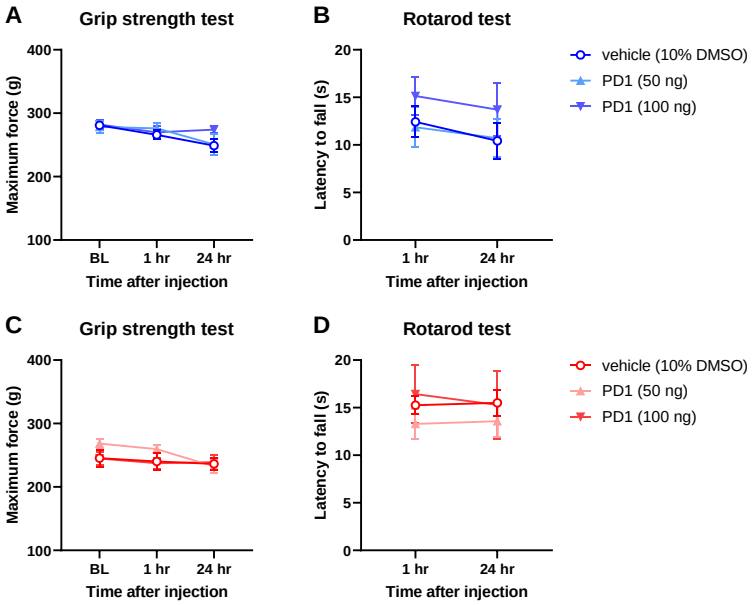

Figure 2. Intrathecally injected GPR37 agonist Protectin D1 (PD1) has no significant effect on normal motor functions in both (A & B) male and (C & D) female mice. PD1 was injected under anesthesia immediately after the baseline (BL) measurement in the grip strength test. Note that the baseline was not measured in the rotarod test before the injection for preventing the impact of exhaustion on the performance. Data are presented as mean±SEM. Statistical comparisons were made by 2-way repeated measures AVOVA followed by Dunnett's t-test (i.e., vs. vehicle at each time point) in each sex.

- <u>The effects of TX14A and PD1 on normal sensory functions</u>
  We tested three doses (10, 25, and 50 μg) of TX14A and two doses (50 and 100 ng) of PD1 vs. their vehicles (saline for TX14A and 10% DMSO for PD1) in the mechanical sensitivity (von Frey

filament test with 0.98 mN and 9.8 mN forces) and heat sensitivity (radiant heat test) assays. After a single bolus injection, we longitudinally measured the mechanical and heat sensitivity up to 24 hours. Compared with vehicle, all three doses of TX14A (**Figure 3**) and all two doses of PD1 (**Figure 4**) had no significant effect on either mechanical or heat sensitivity.

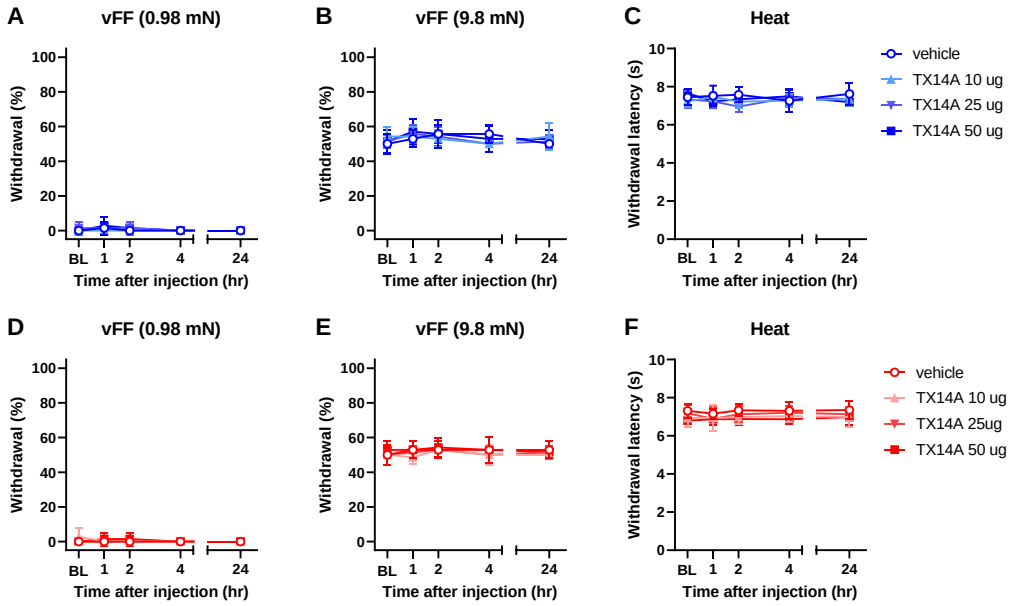

Figure 3. Intrathecally injected GPR37 agonist TX14A has no significant effect on normal (A, B, D, & E) mechanical sensitivity nor (C & F) heat sensitivity in both (A-C) male and (D-F) female mice. TX14A was injected under anesthesia immediately after the baseline (BL) measurement. Data are presented as mean±SD. Statistical comparisons were made by generalized linear mixed model followed by sequential Sidak test between groups in each sex.

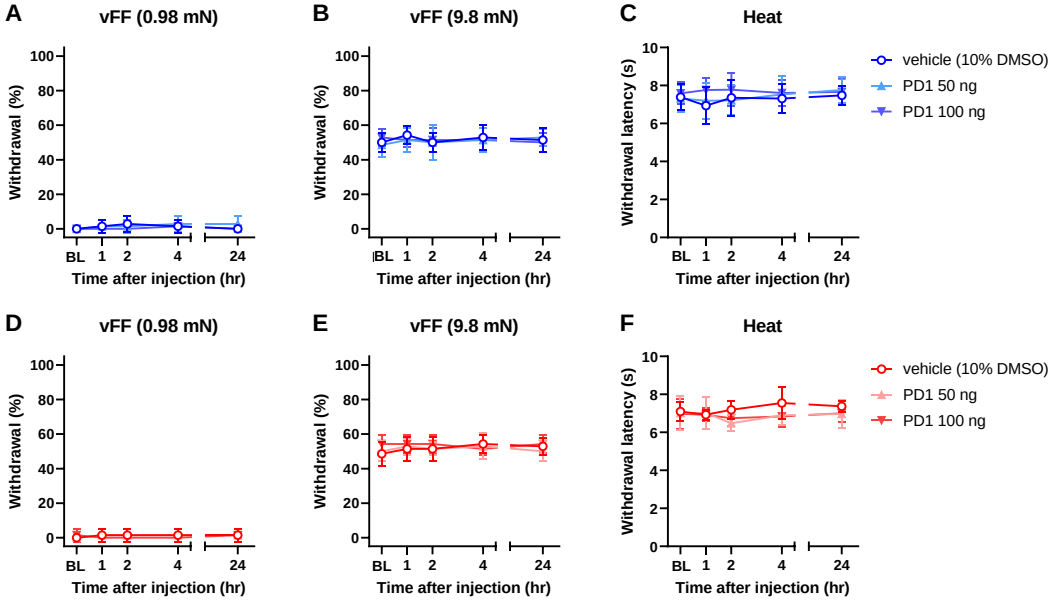

Figure 4. Intrathecally injected GPR37 agonist Protectin D1 (PD1) has no significant effect on normal (A, B, D, & E) mechanical sensitivity nor (C & F) heat sensitivity in both (A-C) male and (D-F) female mice. PD1 was injected under anesthesia immediately after the baseline (BL) measurement. Data are presented as mean±SD. Statistical comparisons were made by generalized linear mixed model followed by sequential Sidak test between groups in each sex.

- The effects of TX14A on pain hypersensitivity induced by an acute chemical injury (intraplantar capsaicin injection)

  Capsaicin activates nociceptors expressing Transient Receptor Potential channel V1 (TRPV1) and induces peripheral sensitization at the nociceptor level and central sensitization at the spinal level; the latter manifests as mechanical pain hypersensitivity outside the capsaicin injection area, termed secondary mechanical hypersensitivity. Two distinct forms of secondary mechanical pain hypersensitivity develop: allodynia and hyperalgesia. While the former is dependent on ongoing nerve activity at the capsaicin injection area and short-lasting, the latter is independent of such nerve activity and long-lasting because of long-terms changes in the spinal nociceptive circuits (i.e., pain memory). In animals, capsaicin-induced secondary mechanical allodynia and hyperalgesia can be detected by low (0.98 mN; normally not evoking withdrawals)- and high (9.8 mN; normally evoking ~50% withdrawals)-intensity von Frey filament stimulation of the area outside of the capsaicin injection site. In addition, capsaicin produces neurogenic inflammation, resulting in heat pain hypersensitivity.

  We intrathecally injected three doses (10, 25, and 50 μg) of TX14A or its vehicles (saline) in this intraplantar capsaicin injection model and determined their effects on mechanical and heat pain hypersensitivity. As shown in **Figure 5**, a single intrathecal injection of TX14A dose-dependently alleviated mechanical and heat pain hypersensitivity long-term in both male and female mice. However, we noticed that the effect of TX14A (at 50 μg) on hyperalgesia-like secondary mechanical hypersensitivity (i.e., to 9.8 mN force) was more pronounced in males than in females, virtually abolishing the hypersensitivity.

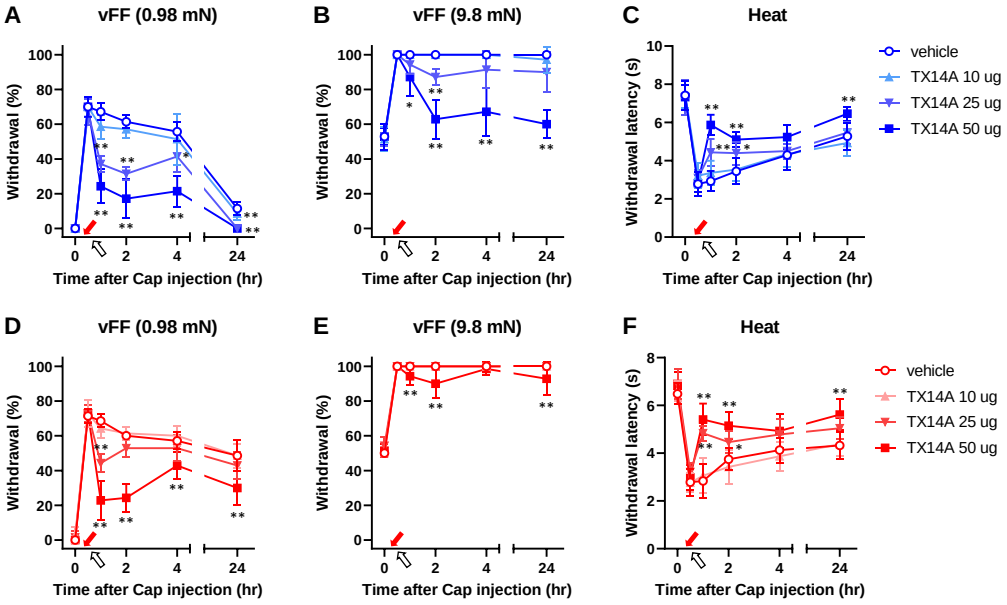

Figure 5. A single intrathecally injection of GPR37 agonist TX14A long-term alleviates mechanical and heat pain hypersensitivity induced by intraplantar capsaicin injection in both (A-C) male and (D-F) female mice. TX14A (white arrows) dose-dependently inhibited the pain hypersensitivity induced by capsaicin (red arrows). Note that TX14A (50 ug) almost abolished mechanical hypersensitivity to 9.8 mN stimulation at 24 hr post-capsaicin only in males. Data are presented as mean±SD. *p<0.05 and **p<0.01 vs. vehicle by generalized linear mixed model followed by sequential Sidak test between groups in each sex.

Similar to TX14A, a single intrathecal injection of PD1 (50 and 100 ng) dose-dependently alleviated mechanical and heat pain hypersensitivity in the intraplantar capsaicin injection model (**Figure 6**). Unlike TX14A, however, there was no apparent sex difference in the effect of PD1 (at 100 ng) on hyperalgesia-like secondary mechanical hypersensitivity.

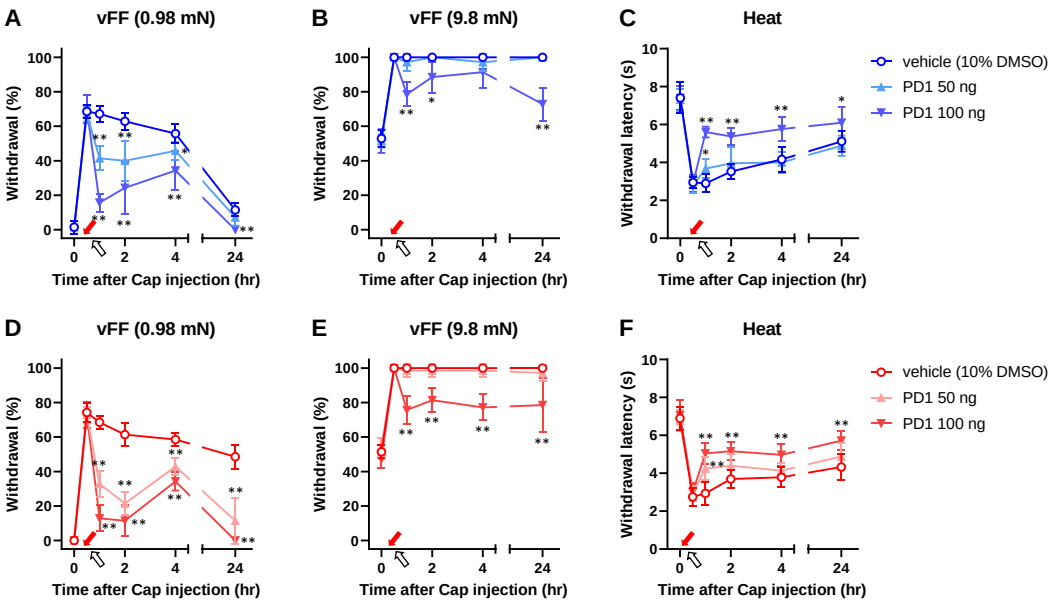

Figure 6. A single intrathecally injection of GPR37 agonist PD1 long-term alleviates mechanical and heat pain hypersensitivity induced by intraplantar capsaicin injection in both (A-C) male and (D-F) female mice. PD1 (white arrows) dose-dependently inhibited the pain hypersensitivity induced by capsaicin (red arrows). Note that unlike 50 ug TX14A, PD1 (100 ng) showed similar efficacy against mechanical hypersensitivity to 9.8 mN stimulation. Data are presented as mean±SD. *p<0.05 and **p<0.01 vs. vehicle by generalized linear mixed model followed by sequential Sidak test between groups in each sex.

(4) Conclusions

The results of experiments conducted in this reporting period demonstrate that the putative GPR37 agonists TX14A and PD1 do not produce adverse effects on normal motor and sensory functions while Tx14A can effectively erase an acute injury-induced spinal pain memory, facilitating the resolution of pain hypersensitivity resulted from the acute injury.

3.3. What opportunities for training and professional development has the project provided?

Nothing to report.

3.4. How were the results disseminated to communities of interest?

Nothing to report.

3.5. What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, we will complete the experiments testing the effects of the GPR37 agonists TX14A and PD1 on pain chronification in acute injury models (Major Task 2, hyperalgesic priming model). Next, based on these results, we will choose the most effective dose of each GPR37 agonist and test them in a chronic injury model (i.e., the L5 spinal nerve ligation model), determining their effects on both sensory-discriminative and affective-motivational domains of neuropathic pain (Major Task 3).

4. Impact

4.1. What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

4.2. What was the impact on other disciplines?

Nothing to report.

4.3. What was the impact on technology transfer?

Nothing to report.

4.4. What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/problems

Nothing to report.

6. Products

Nothing to report.

7. Participants & Other collaborating organizations

7.1. What individuals have worked on the project?

Name:	Jun-Ho La
Project Role:	PI
Researcher Identifier (e.g., ORCID ID):	ORCID 0000-0003-4306-0305
Nearest person month worked:	2
Contribution to Project:	Dr. La obtained an IACUC approval to initiate the study and supervised its performance and progress.

Name:	Jin Mo Chung
Project Role:	Co-I
Researcher Identifier (e.g., ORCID ID):	ORCID 0000-0003-2601-1720
Nearest person month worked:	1
Contribution to Project:	Dr. Chung supervised the progress of the project and provided an administrative support as a department Chair.

Name:	Ramesh Pariyar
Project Role:	Research Scientist
Researcher Identifier (e.g., ORCID ID):	
Nearest person month worked:	8
Contribution to Project:	Dr. Pariyar performed behavior experiments and mouse colony management together with Dr. Koo.

Name:	Ho Koo
Project Role:	Research Scientist
Researcher Identifier (e.g., ORCID ID):	
Nearest person month worked:	10
Contribution to Project:	Dr. Koo performed behavior experiments and mouse colony management together with Dr. Pariyar.

7.2. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Title: Developing GPR37 activators as non-opioid pain therapeutics
Goals: The goal of this project is to assemble a multi-disciplinary research team that can collect preliminary data supporting the feasibility of developing a drug targeting the spinal G protein-coupled receptor 37 (GPR37) as pain therapeutics.
Project Number: R61 NS127286
Name of PD/PIs: Jun-Ho La (contact); Jia Zhou; John Allen
Source of Support: NIH / NINDS
Primary Place of Performance: University of Texas Medical Branch
Project/Proposal Start and End Date: 04/2022 – 03/2024
Total Award Amount (including Indirect Costs):
Person Months (Calendar) per budget period.

Year	Jun-Ho La (PI)	Jin Mo Chung (co-I)
1. 2022-23	3 person month	1.2 person month
2. 2023-24	3 person month	1.2 person month

7.3. What other organizations were involved as partners?

Nothing to report.

8. Special reporting requirements

8.1. Quad Chart: an updated Quad Chart is attached to this report