CHAPTER 2: Rationale, Objectives and Plan of Work

Chapter-2

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2.1 Rationale

CINP is a noteworthy complication stemming from the administration of chemotherapeutic agents, affecting around 50-90% of individuals undergoing chemotherapy treatment. Despite its increasing recognition, CINP continues to pose a significant challenge, as it currently lacks viable curative approaches. Unfortunately, a substantial proportion, approximately 70%, of patients do not get effective pain relief from the currently prescribed medications including SNRIs such as duloxetine, anticonvulsants like gabapentin and pregabalin, TCAs and patches of lidocaine, capsaicin and both weak and strong opioids. Most of these drugs primarily works by targeting the higher pain centers, present in the CNS and are associated with several unwanted side effects such as hepatic impairment, renal insufficiency, fatigue and central toxicities like anxiety, dizziness, sedation, respiratory depression, cognitive dysfunction, addiction, and abuse potential.

Nonetheless, a growing body of clinical evidence suggests the involvement of the peripheral nervous system (PNS) in the progression and maintenance of chronic pain. Targeting peripheral GPCRs modulate pain signals at their source, thereby relieving the pain with higher specificity, and minimizing off-target CNS side effects. Thus, targeting the PNS for development of safer therapeutics devoid of central side effects and toxicities is of paramount importance.

Opioids and cannabinoids, historically recognized as potent analgesics and once considered first-line treatments, were later downgraded as the last-resort alternative due

to their CNS toxicities. Exploring drugs with similar mechanisms of action but confined to the peripheral nervous system presents a promising avenue for potential therapeutic advancements in CINP. We hypothesize that the activation of peripheral MOR or CBR may be effective in treating CINP while avoiding the central side effects commonly associated with other centrally acting analgesics.

2.2 Objectives

The objective of this study is to investigate the effectiveness of activating peripheral mu-opioid receptors (MOR) and cannabinoid receptors (CBR) using agonists as a potential therapeutic strategy for managing CINP devoid of central toxicities. The key objectives of this study are as follows:

Aim 1. To investigate the effect of Loperamide, a peripheral MOR agonist on Chemotherapy-induced Neuropathic Pain.

Aim1A. To study the effect of loperamide on evoked and spontaneous ongoing pain behaviors in CINP rats.

Aim1B. To investigate the possible CNS toxicities of loperamide.

Aim1C. To elucidate the downstream signaling through which loperamide is attenuating CINP in PTX treated rats.

Aim 2. To evaluate the therapeutic potential of DALDA, a preferential peripheral MOR agonist on Chemotherapy-induced Neuropathic Pain.

Aim2A. To study the effect of DALDA on evoked and spontaneous ongoing pain behaviors in CINP rats.

Aim 2B. To investigate the possible CNS toxicities of DALDA.

Aim2C. To investigate the downstream signaling through which DALDA is attenuating CINP in PTX treated rats.

Aim 3. To study the effect of peripheral cannabinoid receptor activation on Chemotherapy-induced Neuropathic Pain.

Aim. 3A. To study the effect of CB13 on evoked and spontaneous ongoing pain behaviors in CINP rats.

Aim 3B. To investigate the possible CNS toxicities of CB13.

Aim 3C. To dissect the downstream signaling through which CB13 is attenuating CINP in PTX treated rats.

2.3 Plan of work

2.3.1 Study I

The first study is designed with an aim to investigate the effectiveness of activating peripheral MORs using systemic loperamide for the management of paclitaxel induced evoked and ongoing pain. Our investigations further delved into the cellular and molecular mechanisms that contribute to the emergence of paclitaxel induced neuropathic pain, encompassing crosstalk between TRP channels, VGSCs, N-methyl D-aspartate receptor subtype 2B (NR2B), and neuroinflammatory signaling. Using molecular biology tools, we investigated the mechanism of action of loperamide responsible for attenuation of chemotherapy-induced evoked and ongoing pain in rats. CNS associated side effects are the major limitation of currently available analgesics in clinic therefore we examine the effect of loperamide on motor coordination and locomotion activity. The animal grouping was designed as below:

Table 2.1 Animal grouping to investigate the effect of loperamide on rat model of paclitaxel-induced neuropathic pain.

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	8
2.	Paclitaxel + Vehicle	8
3.	Paclitaxel + Loperamide 5mg/kg s.*c.*	8
4.	Paclitaxel + Loperamide 10mg/kg s.c.	8
5.	Paclitaxel + Loperamide 20mg/kg s.c.	8
6.	Paclitaxel + Gabapentin 60mg/kg s.*c.*	8

2.3.2 Study II

To further validate our findings on activating peripheral MORs, we utilized another preferential peripheral MOR agonist, Dermorphin [D-Arg2, Lys4] (1-4) amide (DALDA) and evaluated its effect on CINP, along with an in-depth exploration of associated neurobiological mechanisms. The grouping for the study was as follows:

Table 2.2 Animal grouping to investigate the effect of DALDA on rat model of paclitaxel-induced neuropathic pain:

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	8
2.	Paclitaxel + Vehicle	8
3.	Paclitaxel + DALDA 2.5mg/kg s.*c.*	8
4.	Paclitaxel + DALDA 5mg/kg s.c.	8
5.	Paclitaxel + DALDA 10mg/kg s.c.	8
6.	Paclitaxel + Gabapentin 60mg/kg s.*c.*	8

2.3.3 Study III

In this next study, our investigations extended to explore the efficacy of CBR agonists. We aimed to explore the impact of CB13, a peripherally restricted dual agonist of CB1/CB2 receptors, on alleviating CINP in rats. Simultaneously, we delved into understanding the neurobiological mechanisms associated with CB13 in mitigating CINP. The grouping for this set of study was as follows:

Table 2.3 Animal grouping to investigate the effect of CB13 on rat model of paclitaxel-induced neuropathic pain:

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	8
2.	Paclitaxel + Vehicle	8
3.	Paclitaxel + CB13 10µM/paw i.pl.	8
4.	Paclitaxel + CB13 20µM/paw i.pl.	8
5.	Paclitaxel + CB13 40µM/paw i.pl.	8
6.	Paclitaxel + Gabapentin 60mg/kg s.*c.*	8