TARGETING PERIPHERAL G-PROTEIN COUPLED RECEPTORS FOR THE TREATMENT OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

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By

GADEPALLI ANAGHA

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI – 221005
INDIA

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CHAPTER 7: Summary & Conclusions

Chapter-7

Summary & Conclusions

7.1 Summary

Chemotherapy-induced neuropathic pain has emerged as a significant healthcare challenge in cancer patients undergoing chemotherapy, exerting a substantial impact on their overall well-being and quality of life. Unfortunately, a substantial proportion, approximately 70%, of patients do not get effective pain relief from currently prescribed medications. Moreover, most of the analgesic drugs primarily work by targeting the higher pain centers, present in the central nervous system 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, 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 nociceptors 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 the development of safer therapeutics devoid of central side effects and toxicities is of paramount importance. GPCRs present a range of therapeutic opportunities given their role in modulating pain signals by facilitating the transduction and neural activity in primary sensory neurons (PSNs). Notably, mu-opioid receptors (MOR) and Cannabinoid receptors (CBR) stand out as prominently targeted GPCRs in the realm of pain therapies, showcasing their significance in addressing pain-related challenges. Previous literature strongly supports the potential of peripheral MOR targeting in the

alleviation of various pain modalities including neuropathic pain, inflammatory pain and bone cancer pain However, whether activating peripheral MOR would attenuate chemotherapy-induced neuropathy was not reported previously. Thus, we designed our first study with an aim to investigate the therapeutic potential of peripherally restricted MOR agonist, loperamide, in animal model of paclitaxel-induced neuropathic pain. Rats with paclitaxel induced neuropathic pain showed a significantly decreased pain threshold in a battery of pain behavioral assays. We found that loperamide treatment showed significant and dose-dependent inhibition of evoked pain in paclitaxel induced neuropathic rats. Loperamide treatment significantly inhibits spontaneous ongoing pain in neuropathic rats but did not produce any place preference behavior in healthy naïve rats pointing towards its non-addictive analgesic potential. Moreover, loperamide treatment did not produce central nervous system-associated side effects as well. Further, molecular investigations revealed increased expression of ion channels such as TRPA1, TRPM8; voltage-gated sodium channels (VGSCs) and neuroinflammatory markers in the dorsal root ganglion (DRG) and lumbar (L4-L5) spinal cord of neuropathic rats, which was significantly downregulated upon loperamide treatment. These findings collectively suggest that activation of peripheral MORs contributes to the amelioration of both evoked and spontaneous pain in CINP rats by downregulating TRP channels and VGSCs along with suppression of oxido-nitrosative and neuroinflammatory cascade.

To further validate our findings, we evaluated the effect of a preferential peripheral MOR agonist Dermorphin [D-Arg2, Lys4] (1-4) amide (DALDA), on CINP. The primary objective was to assess the analgesic properties of DALDA and elucidate the underlying mechanisms governing its therapeutic activity. Our findings revealed that

DALDA treatment significantly ameliorated paclitaxel-induced evoked and spontaneous ongoing pain in rats without causing drug addiction and other central side effects. Molecular analyses further unveiled that DALDA treatment significantly downregulated ion channels (TRPs, VGSCs) and NR2B expressions, concomitant with the inhibition of microglial activation, resulting in the suppression of oxido-nitrosative stress and neuroinflammatory cascade induced by paclitaxel. Findings from these studies suggest that peripheral MORs may offer a potential target for the treatment of CINP, offering new avenues for improved pain relief while minimizing central side effects.

Building on the noteworthy success of peripherally restricted MOR agonists, like loperamide and DALDA, in mitigating chemotherapy-induced neuropathic pain, our investigation extended to explore the efficacy of Cannabinoid receptor (CBR) agonists. In this next study, we aimed to explore the impact of CB13, a peripherally restricted dual agonist of CB1/CB2 receptors, on alleviating CINP in rats. The local administration of CB13 exhibited a modality-specific inhibition of evoked pain in paclitaxel-induced neuropathic rats. Specifically, it showcased the alleviation of allodynia-like behavior in paclitaxel-induced neuropathic rats while leaving hyperalgesia unaffected, and notably, without inducing any central nervous system (CNS) toxicities. Molecular investigations revealed that CB13 treatment significantly attenuated the upregulation of nociceptors, including TRPA1, TRPM8, and voltage-gated calcium channels (VGCCs) such as Cav2.2 and Cav3.2, in the L4-L5 dorsal root ganglion (DRG) of paclitaxel-injected rats. Simultaneously, it exhibited a noteworthy attenuation of neuro-inflammatory signaling in both DRG and spinal cord tissues of the rats. These findings offer valuable insights that serve as a foundation for future

investigations into peripherally restricted therapeutics for CINP management devoid of CNS toxicities.

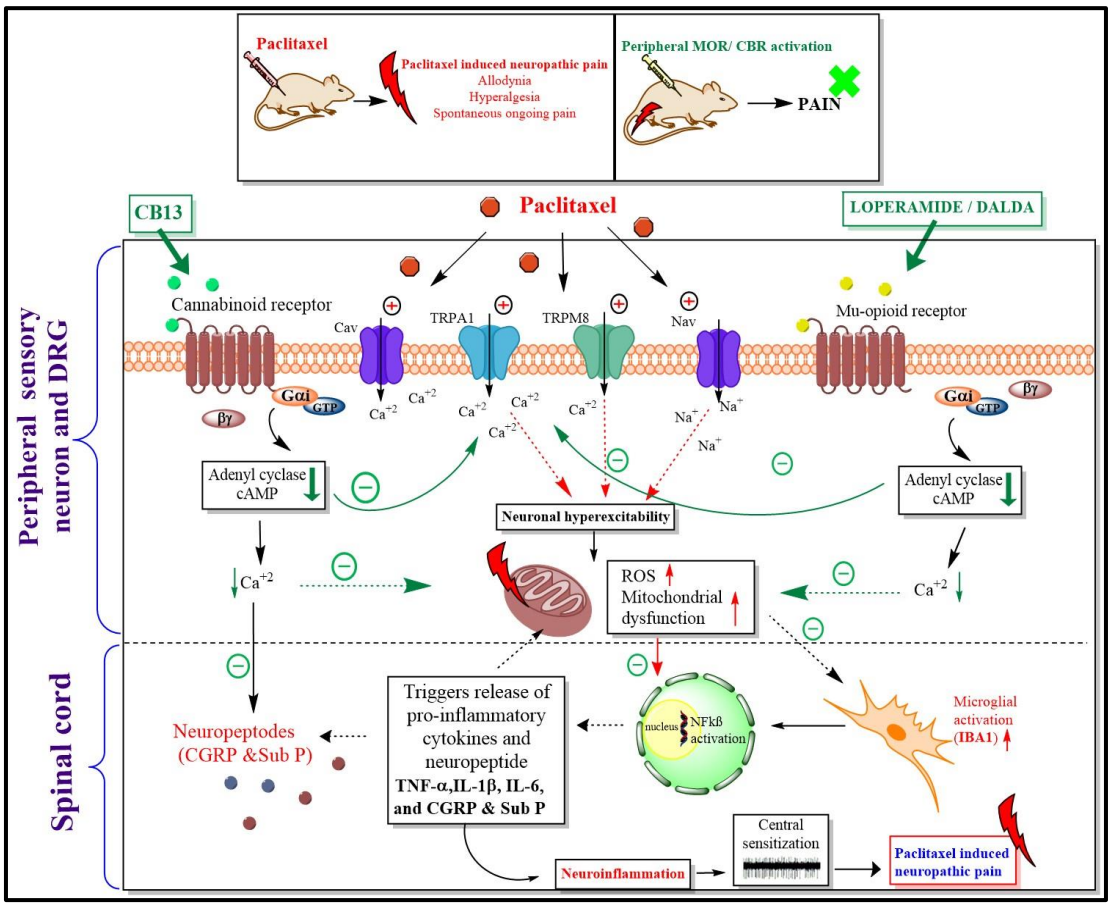

Figure 7.1 Summary of the thesis

7.2 Conclusion

The activation of peripheral G protein-coupled receptors (GPCRs) using agonists has been found to diminish both evoked and spontaneous ongoing pain in rats with paclitaxel-induced neuropathic pain. This effect is achieved by regulating the expression of TRP channels, VGSCs, VGCCs, and reducing neuroinflammation in the spinal cord and dorsal root ganglion. Importantly, the peripherally restricted drugs used in this study exhibit a noteworthy absence of central nervous system (CNS) associated

side effects and addiction potential. Consequently, our findings propose a novel avenue for developing therapeutic strategies targeting peripheral GPCRs, specifically MORs and CBRs. This is particularly significant given the existing limitations of many current neuropathic pain management therapies with central side effects. In the context of these limitations, strategies focused on peripheral restriction could pave the way for a new class of drugs, offering heightened efficacy and a broader safety margin in the treatment of chemotherapy induced neuropathic pain.

7.3 Limitations and outlook for future work

This study serves as a foundational step, providing valuable proof of concept for the potential efficacy of peripherally restricted MOR and CBR agonists in alleviating CINP. The highlighted findings with loperamide, DALDA, and CB13 offer promising leads, showcasing the feasibility of targeting the peripheral nervous system for enhanced pain relief in CINP condition, with minimized central side effects. However, as with any initial exploration, certain limitations necessitate consideration. Long-term effects, potential development of tolerance, and extended safety assessments represent critical aspects requiring further exploration. While the specific agonists presented show potential, diversification of the pharmacological toolbox is crucial for a comprehensive understanding. Future research should encompass a broader spectrum of candidates, considering varying pharmacokinetics and mechanisms of action. Additionally, an exploration of combinatorial strategies, investigating synergistic effects and understanding the interplay between different receptors and signaling pathways within the peripheral nervous system, is vital for a more nuanced understanding of CINP modulation.

Furthermore, addressing the heterogeneity of chemotherapy agents and their distinct neurotoxic mechanisms is crucial to developing a versatile therapeutic approach. In conclusion, this study marks a promising beginning, laying the groundwork for a more nuanced understanding of CINP management. As we move forward, refining current findings and expanding the scope of research will be essential for developing safe, effective, and clinically relevant therapeutics for individuals grappling with the debilitating impact of CINP.