Chapter-7

Summary & Conclusions

7.1 Summary

Chronic pain is one of the most prevalent clinical disorders which occurs due to persistent activation of neural pain pathways. Every person experiences pain at some point in their life span but the number of patients seeking medical care due to chronic pain is about 30% across the globe. Kinesins are the motor-proteins which are involved in the anterograde (from the cytosol to synapse) transport of cargo. Targeting the kinesins responsible for the delivery of receptors involved in chronic pain pathophysiology could provide a novel strategy in the understanding and treatment of neuropathic pain. NMDA receptor activation is known to induce the central sensitization and excitotoxicity which can be recognized by the glial cells followed by the release of cytokine storm at spinal and supraspinal level leading to chronic pain. NR2B if one of the important subunits of NMDA receptors which is trafficked across the neurons by KIF17 kinesin motor protein. Many regulatory proteins govern the transit of receptors by activating kinesin, and aurora kinase are one of them.

First, we study the protein-ligand architectural interplay using *in-silico* molecular dynamics simulation to delineate the dynamic interaction of aurora kinase with its pharmacological inhibitor, tozasertib. The findings demonstrate that tozasertib-aurora kinase complex is stabilized through hydrogen bonding, polar interactions, and water bridges. Next, we investigated the effect of tozasertib, a pan-Aurora kinase inhibitor, on nerve injury-induced evoked and chronic ongoing pain in rats and the involvement

of kinesin family member 17 (KIF17) and NMDA receptor subtype 2B (NR2B) crosstalk in the same. Rats with chronic constriction injury showed a significantly decreased pain threshold in a battery of pain behavioral assays. We found that tozasertib treatment showed significant and dose-dependent inhibition of evoked pain in nerve-injured rats. Tozasertib treatment significantly inhibits spontaneous ongoing pain in nerve-injured rats but did not produce any place preference behavior in healthy naïve rats pointing towards their non-addictive analgesic potential. Moreover, tozasertib treatment did not alter the normal pain threshold in healthy naïve rats and didn't produce central nervous system-associated side effects as well. Western blotting and reverse transcription-polymerase chain reaction studies suggested enhanced expressions of NR2B and KIF-17 along with increased nuclear factor-kappa β (NFkβ), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) levels in dorsal root ganglion (DRG) and spinal cord of nerve-injured rats which was significantly attenuated on treatment with different doses of tozasertib. Our findings suggest that inhibition of pan-Aurora kinase led to KIF-17-NR2B mediated inhibition of evoked and chronic ongoing pain in nerve-injured rats.

Chronic pain may consist of both neuropathic and inflammatory components, involving complex mechanisms such as excitatory synaptic transmission, microglial and macrophage activation, an altered action potential in nociceptive fibers, and central as well as peripheral sensitization. Thus, we have investigated the role of aurora kinase in the regulation of KIF17 and NR2B in the animal model of chronic inflammatory pain. Tozasertib, a pan aurora kinase inhibitor significantly attenuates acute inflammatory pain and suppresses enhanced pain hypersensitivity to heat, cold, and mechanical stimuli in CFA injected rats. Molecular investigations suggest enhanced

expression of KIF17/mLin10/NR2B in L4-L5 dorsal root ganglion (DRG) and spinal cord of CFA injected rats which was significantly attenuated on treatment with tozasertib. Moreover, tozasertib treatment significantly attenuated CFA-induced oxido-nitrosative stress and macrophage activation in DRG and microglia activation in the spinal cord of rats. Findings from the current study suggest that tozasertib mediates anti-nociceptive activity by inhibiting aurora kinase-mediated KIF17/mLin10/NR2B signaling.

To test the toxicity profile of tozasertib at highest dose used against chronic pain models we have performed acute toxicity study. We have observed that tozasertib 40mg/kg *i.p.* did not alter the behavioral responsiveness in rats including gait, skin color, lacrimation, sleepiness, writhing, convulsion, tremor, diarrhea, salivation as compared to the saline-treated rats. Next, we have found that tozasertib did not affect body weight, food-water consumption in rats as examined at different time points. Further, the hematological and biochemical studies have revealed that tozasertib did not produce any significant changes in the blood profile of rats as compared to the saline-treated rats. Finally, the histopathological analysis of the liver and kidney was performed in both the groups and our findings demonstrated that tozasertib treatment did not show any sign of necrosis or inflammation in rat organs. Our preliminary study suggests that single-dose administration of tozasertib at 40mg/kg *i.p.* is safe in rats and could be utilized as an effective tool to modulate the aurora kinase activity during chronic pain condition.

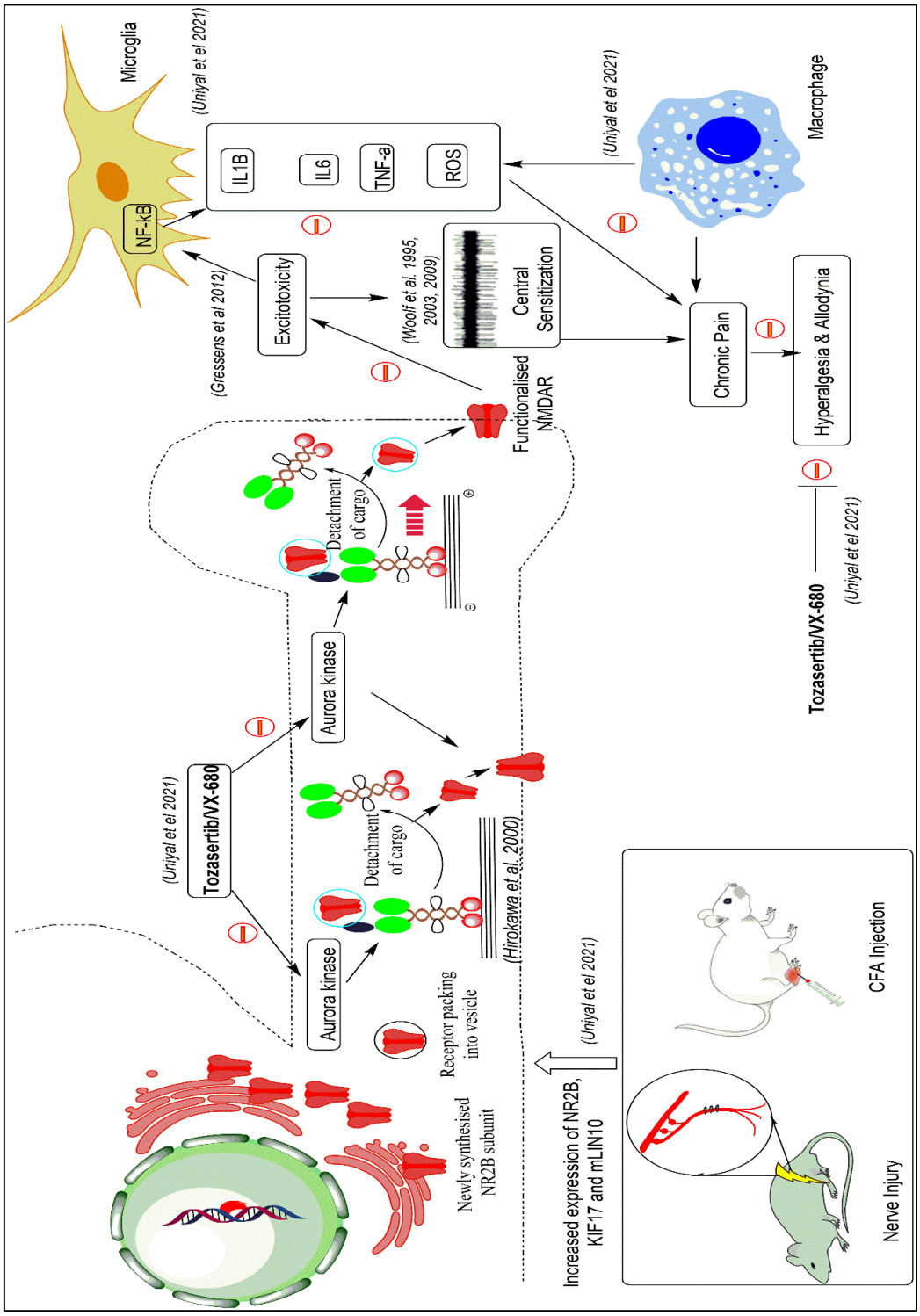

Figure 7.1 Summary of the thesis work

7.2 Conclusion

Pan-Aurora kinase inhibition produces evoked and spontaneous ongoing pain inhibition by regulating the KIF17-NMDA expression and neuroinflammation in the spinal cord and dorsal root ganglion of rats with chronic pain. Importantly, tozasertib does not produce CNS associated side effects and addiction at single dose administration. Hence, the present study suggests a novel line of action for the development of therapeutic strategies that interfere with aurora kinase and kinesin-mediated nociception. At present most of the therapies for the management of neuropathic pain are having limitations due to undesirable side effects. In this relevance, the kinesin targeted transport of receptors could provide a novel class of drugs with higher efficacy and broader safety margin.

7.3 Limitations and outlook for future work

The present study provides an important preliminary data and critical insights that can lead to new directions for the future studies involving the development of novel therapeutics against chronic pain. Finding from the current study will lay down the basis for future studies exploring new kinesins and enzymes involved in pain progression. This knowledge will be important for the development of novel pharmacotherapies for the treatment of chronic pain without producing any major side effects. We believe that our research scheme can be used to develop this class of pharmaceuticals and tozasertib can be investigated for various pathophysiological modalities at a low dose to target aurora kinase mediated regulation of kinesin motors. In present work we did not observe any acute toxicity, CNS toxicity and addictive potential of tozasertib single dose administration. However, future studies are required to draw the complete safety

profile of this compound with repeated dosing approach in preclinical settings prior to drug development. One major limitation observed in clinical setup with the use of aurora kinase inhibitors is potential side effects. Thus, future studies are required to unravel the detailed interaction of this compound with cellular system or an alternate approach can be utilized by screening novel compounds against the pan aurora kinase enzymes. To minimize the toxicity the specific inhibitors of aurora kinase isoforms could be used or adjunctive agents such as granulocyte stimulating factors might be added to the treatment. On the basis of our experiments, we could envisage that tozasertib can be investigated for various pathophysiological modalities at low doses to target aurora kinases.