Extended Abstract

Neuropathic pain is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage," according to the International Association for the Study of Pain. It is a defense mechanism that our body employs to protect from an injury that might cause damage to tissues, and as such, it is crucial for the survival and well-being of organisms. However, when pain becomes chronic it develops into a devastating medical condition imposing a huge burden on society and healthcare costs. Despite the progress made in unraveling the pathophysiology behind chronic pain, the existing therapies exhibit limited effectiveness and elicit various adverse effects, which eventually lead to treatment withdrawal and poor quality of life. The first line of treatment for neuropathic pain includes the use of calcium channel blockers like pregabalin and gabapentin. Apart from that, antidepressants such as tricyclic antidepressants (TCAs) like Amitriptyline and serotonin-noradrenaline reuptake inhibitors (SNRIs) like duloxetine are also often prescribed. These clinical pharmacotherapies provide only the symptomatic pretreatment and there are no disease-modifying agents available for neuropathic pain treatment. Gabapentinoids that are used clinically are associated with several non-specific clinical drawbacks. Among them, the major and frequent side effects of Gabapentin are associated with the increased risk of atrial fibrillation (Afib) along with somnolence, dizziness, and peripheral edema (10% to 15%). An anecdotal case report says > 10 % of the elderly above 65 years or older and ~ 1 % of the younger population (< 49 years) developed Afib within 3 months of initiation of gabapentin. Similarly, pregabalin-associated heart failure was also observed approximately 10 days after initiation and the withdrawal rate for pregabalin is 19%. Hence, there is a need for newer therapeutic strategies that would work on calcium channels.

Therefore, we have selected an inorganic compound i.e., Barium-doped bioactive glass (BaBG). Barium being endogenously present in the body may not pose any undesired effects. Barium is present in the exoplanets so we have evolved containing barium and all living beings are exposed to barium as it is present in rock, soil, and water. Unlike organic calcium channel blockers in the market, inorganic BaBG is biocompatible as our body has mechanisms to utilize barium and eliminate it. Apart from that, the resting membrane potential of the nerve is different from other tissues. So, the calcium channel blockers that are designed to act on other systems mostly the heart when repurposed to act on the nervous system may not act efficiently.

We synthesized BaBG using the sol-gel process that holds promise in biomedical research due to its unique properties, offering potential therapeutic applications in regenerative medicine and pain management. The synthesized batch of BaBG was characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). These techniques were utilized to analyze the crystal structure, morphology, and chemical composition of BaBG. These also provided crucial insights into the material's physicochemical properties, guiding its optimization for biomedical use. BaBG, a variant of 45S5, exhibited biocompatibility as its hemolysis index was less than 5 %. It also has the ability to form hydroxyapatite along with the leaching of physiologically active dopants from its framework in a time-dependent manner, making it suitable for various pharmacological uses. Incorporating barium into the glass matrix enhanced its biological activity, paving the way for innovative biomedical applications. Further, the *in vitro* studies played a vital role in assessing the regenerative potential of BaBG using the scratch assay which is an *in vitro* model of neurotrauma. We have also seen that BaBG has anti-inflammatory properties as evident from a decrease in pro-inflammatory cytokines i.e., IL-6 and TNF-α with

an increase in anti-inflammatory mediator, IL-10 which may be important for the repair mechanisms of post-traumatic injuries.

One intriguing aspect of BaBG is its potential pharmacological effects on calcium channels. Calcium ions play a crucial role in neuronal signaling and excitability, and dysregulation of calcium homeostasis has been implicated in various neurological disorders, including neuropathic pain. Barium ions released from BaBG selectively blocked the calcium currents measured from the dorsal root ganglion neurons of the rats as confirmed by the patch-clamp technique, thereby aiding in modulating the neuronal excitability and neurotransmitter release. Moreover, in the *ex vivo* electrophysiological setup, BaBG prolonged the repolarization phase of the action potential. Therefore, BaBG reduced the prorogation of action potential and in neuropathic pain conditions; there is hyper-excitability of nociceptors. Thus, understanding these mechanisms of BaBG on calcium channels helped in elucidating its potential therapeutic utility in neuropathic pain management.

Moving from *in vitro* studies to *in vivo* applications requires comprehensive preclinical pharmacokinetic evaluations. Quantitative analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) are used to measure the concentration of barium, calcium, and silicon ions in vital organs, providing insights into its pharmacokinetic profile and systemic biodistribution following oral administration. Post-administration of BaBG, there was a statistically significant level of dopants in the vital organs in a dose-dependent manner. Barium that was leached from BaBG was found in the peripheral as well as in the brain within the physiological limit. Moreover, BaBG was safer and its LD_{50} was more than 2000 mg/kg b.w. as per the OECD guidelines.

Neuropathic pain represents a significant clinical challenge, and understanding its pathophysiology is crucial for developing effective treatments. In the chronic constriction injury (CCI) models in rats, we observed that there was a significant increase in intracellular calcium levels, S100b and TNF-α in the sciatic nerve (SN) and spinal cord (SC). Mostly these effects were seen acutely 1h after the injury. This implies the involvement of calcium and S100b in the regulation of inflammation in the CCI model in a time-dependent manner. CCI-induced allodynia and hyperalgesia started from day 7 and was maximum on day 14. However, treatment with Pentamidine, a specific S100b inhibitor reversed these neuropathic pain phenotypes. There was also glial cell activation in this model which was mitigated by a specific S100 b inhibitor. Therefore, this implicates the involvement of S100b in the progression of neuropathic pain.

Pharmacological interventions targeting aberrant calcium signaling, neuroinflammation, and neuronal hyperexcitability hold promise for alleviating neuropathic pain symptoms. Being a calcium channel blocker, BaBG reversed all neuropathic pain symptoms in a dose-dependent manner. It also prevented the activation of S100b and reduced inflammation post-CCI of SN. Treatment with BaBG causes axonal repair and remodeling of the dendrites; hence has the potential to be used for neuropathic pain treatment.

In conclusion, the objectives outlined collectively aim to advance our understanding of BaBG synthesis, characterization, pharmacology, and therapeutic potential in the context of neuropathic pain management. By employing a multidisciplinary approach encompassing materials science, pharmacology, and toxicology, we strived to harness the unique properties of BaBG for the development of innovative therapies targeting neuropathic pain.