ABSTRACT

Chemotherapy-induced neuropathic pain (CINP) is one of the most severe side effects associated with the administration of anticancer drugs. Prevalence of neuropathic pain in patients undergoing chemotherapy ranges from 18% to approximately 88% depending upon the treatment regimen. Despite extensive efforts in the last few decades, effective therapeutics for CINP treatment remains limited largely due to the multifaceted nature of the disease and the lack of well-defined targets. Currently, there are no US-FDA-approved therapeutics for the treatment of CINP in patients. The pathophysiology of CINP involves activation of nociceptors at peripheral nerve terminals leading to peripheral sensitization followed by neuronal hyperexcitability at spinal and suprspinal levels causing persistent awareness of neuropathic pain. Damage to sensory nerves caused by chemotherapy initiates cascades of free radical generation, ion channel dysregulation, neuroinflammation, and mitochondrial dysfunction contributing to the development of painful neuropathy. Activation of glial cells and the infiltration of mononuclear leukocytes into the dorsal root ganglions (DRG) exacerbate inflammatory signaling and promote peripheral sensitization. Transient receptor potential (TRP) channels, including transient receptor potential ankyrin 1 (TRPA1), transient receptor potential vanilloid 1 (TRPV1), and transient receptor potential melastatin 8 (TRPM8), plays a significant role in development of hyperalgesia and allodynia in patients suffering from CINP. Activation of TRP channels leads to increase in release of calcitonin gene-related peptide (CGRP) and substance P eventually leading to inflammation and neuronal hyperexcitability. Increasing evidence suggests the involvement of N-methyl-D-aspartate receptors (NMDARs), especially those containing the NR2B subunit, in the development and maintenance of CINP by facilitating central sensitization.

Despite numerous attempts in drug development for chemotherapy-induced neuropathic pain, the rate of successfully translating these drugs into clinical settings remains notably low, mainly due to several underlying factors. One such factor is the lack of reliable animal model which can clinically mimic the CINP and correlate the outcome to bridge the translational gap. For several decades the mono-chemotherapy model of CINP is being used by researchers for investigating pathophysiology and screening novel therapeutics against CINP. However, in the clinic, antineoplastic agents are mostly prescribed in combination with two or three drugs for the treatment of cancer. Paclitaxel/platinum/vincristine combinations delivered significant outcomes in phase III clinical trials and are now considered standard therapy for advanced

carcinomas including breast cancer and gastrointestinal cancers. Here, we hypothesized to develop a new animal model of CINP utilizing a combination of three commonly used chemotherapeutic drugs (paclitaxel, cisplatin, and vincristine) referred to as the combined chemotherapy model and compared it with conventional paclitaxel monotherapy model in rats. This alternative model may offer a promising avenue for the pharmacological screening of new analgesics and an in-depth exploration of intricate pathophysiology associated with CINP, thereby facilitating the seamless translation of novel therapeutics from the laboratory to clinical practice. In the present study, we have used behavioral and pharmacological approaches to establish and validate a novel combination-based chemotherapeutic model of neuropathic pain. Male Sprague Dawley rats were subjected to chemotherapy administration, followed by assessment of pain behavior at different time points post-chemotherapy. Paclitaxel-treated animals displayed enhanced thermal and mechanical hypersensitivity from day four onwards, which persisted until day thirty-five post the last paclitaxel injection. Notably, rats subjected to combined chemotherapy displayed prolonged hypersensitivity that emerged on day four and persisted until day fifty-six. Molecular analysis revealed a significant upregulation in DRG and spinal mRNA expressions of TRP channels (TRPA1, TRPV1, & TRPM8), pro-inflammatory cytokines (TNF-α & IL-1β), and neuropeptides, Substance P and CGRP in both pain models. Interestingly, the combined chemotherapy model demonstrated a significant increase in DRG and spinal NR2B expressions compared to rats solely treated with paclitaxel. Pharmacological investigations revealed that gabapentin treatment substantially mitigated pain hypersensitivity in both the combined chemotherapy and paclitaxel-administered groups, with the simultaneous reversal of cellular and molecular changes observed in the lumbar DRG and spinal cord of rats. This model not only recapitulates clinical biomarkers of neuropathy but also presents a potential alternative platform for screening analgesic drugs targeted at CINP. Further, we have screened the natural phytochemical, bergenin in the newly developed model of combined chemotherapy-induced neuropathic pain followed by dissecting the cellular and molecular mechanisms associated with its anti-nociceptive activity. Behavioral responsiveness assays were conducted in rats before and after CINP induction and at different time points post-bergenin treatment. Alterations in tight junction proteins, pro-inflammatory cytokines, transient receptor potential (TRP) channels (TRPV1, TRPA1, and TRPM8), and N- methyl-D-aspartate receptor subtype 2 (NR2B) in the lumbar dorsal root ganglion (DRG) and spinal cord of neuropathic rats was measured. Bergenin treatment led to a significant and dose-dependent reduction in pain-like behavior in neuropathic rats without causing central nervous system toxicity. Moreover, bergenin (25, 50, and 100mg/kg i.p.) and gabapentin (60 mg/kg

i.p.) treatment did not alter the acute pain response in healthy naïve rats. Combined chemotherapy administration resulted in the significant activation of TRP channels, more specifically TRPA1 and TRPV1, coupled with the disruption of spinal cord tight junction proteins, infiltration of pro- inflammatory cytokines, and NR2B activation. Bergenin treatment significantly restored all these alterations, except TRPM8, in the DRG and spinal cord of neuropathic rats.

The clinical significance of TRPA1 in pain patients is well recognized and interestingly, the findings from our study also confirmed the heightened levels of TRPA1 after combined chemotherapy administration in rats. Recently, RNA interference (RNAi) emerges as a powerful tool to silence the gene expression by neutralizing targeted mRNA molecules and it has become more popular in the application of targeted interference for pain regulation. The delivery of siRNA is very challenging as there are chances of degradation and generation of inappropriate immunogenic response which can lead to diminished efficacy of siRNA. Liposomes are synthetic carriers composed of a lipid bilayer resembling cellular membranes, enabling efficient delivery of encapsulated therapeutics while safeguarding them from premature degradation. Therefore, here we proposed to prepare the TRPA1-siRNA loaded nano-formulation for the treatment of CINP. TRPA1-siRNA based liposomal nanoforumulation was characterized using advanced techniques. Protein and mRNA expression studies convincingly confirmed the successful validation of TRPA1 knockdown post-intrathecal administration. The developed TRPA1-siRNA loaded liposomal formulation exhibited favorable characteristics through comprehensive characterization. Intrathecal and intravenous administration of the formulation significantly mitigated the attenuation of mechanical and cold hypersensitivity in neuropathic rats. Notably, sustained effects indicated controlled release, with plain siRNA showing no significant effects after day 3. Mechanistically, the silencing of the TRPA1 gene led to downregulated microglia activation and ICAM1, subsequently reducing the neuroinflammatory cascade in the spinal cord. Furthermore, the developed formulation significantly downregulated TRPA1 and IL6 mRNA expressions in the DRG of intravenously administered neuropathic rats compared to intrathecally administered rats. Findings from the current study will be helpful in the development of novel therapeutics for the treatment of chemotherapy-induced neuropathic pain without producing severe side effects and toxicities. This knowledge will lay down the basis for future studies investigating TRP channels along with siRNA-nanomedicine for the treatment of chronic pain and other neurodegenerative disorders.