Rolipram delivered by PgP nanocarrier via intrathecal injection enhances motor function and reduces neuropathic pain in a rat moderate contusion SCI model

Zhen Liao | Advisor: Dr. Jeoung Soo Lee
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Background: Traumatic spinal cord injury (SCI) is a major source of morbidity and mortality worldwide. SCI disrupts axonal pathways, leading to permanent motor, sensory, and autonomic dysfunction, as well as chronic pain, respiratory impairment, and loss of bowel or bladder control. Several complex pathophysiological mechanisms such as the reduction of cyclic adenosine monophosphate (cAMP), activation of myelin-associated inhibitors and formation of astrogliosis limit spontaneous recovery following SCI. Our long-term goal is to develop a combinatorial therapy, using a cationic amphiphilic polymeric nanocarrier, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP, US patent 10,232,050B1) for co-delivery of rolipram (Rm) and RhoA siRNA (siRhoA) modulating the cAMP and RhoA signaling pathways, respectively, with L1 neural cell adhesion molecule as a targeting moiety. In previous studies, we demonstrated that a single intraspinal injection of Rm-PgP nanoparticles restored the cAMP level and increased neuronal cell survival and reduced inflammatory response in a rat severe compression SCI model [1]. In this study, we evaluated the effect of Rm-PgP single and repeat injection via intrathecal catheter on neuroprotection and inflammatory response; motor function recovery; and neuropathic pain in a rat moderate contusion SCI model at short-term (1 week post injury: Acute response) and long-term (6 weeks post-injury: Chronic response) time points.

Methods: PgP was synthesized and characterized as previously reported [2]. Rm was loaded into PgP using the solvent evaporation method and the loading efficiency was measured by HPLC. Sprague Dawley rats were used to generate a moderate contusion injury model by impacting spinal cord and catheterization was performed. Rats were divided into 4 groups: sham, untreated SCI (saline, 40 µl), Rm-PgP (20 µg Rm, 40 µl) single injection (Rm-PgP-S) at immediately after injury, Rm-PgP (20 µg Rm, 40 µl) repeat injection at 0, 2, and 4 DPI (Rm-PgP-R). cAMP levels of spinal cords were measured by ELISA using a Mouse/Rat cAMP Parameter Assay Kit (R&D Systems). Motor functional recovery were evaluated using Basso Bettie and Brenahan (BBB) scoring system. Neuropathic pain were evaluated using von Frey test. Nissl staining was performed to measure cavity size and IHC conducted with antibodies specific for ED1 and Arg1 to identify M1 and M2 macrophages, NeuN and GFAP for neuronal nuclei and reactive astrocytes, respectively. The stained sections were imaged and analyzed using ImageJ software for lesion area and positive cell counting.

Results: We observed that cAMP levels in both Rm-PgP treated groups were significantly higher than that in untreated group at 7 DPI. Both Rm-PgP treated groups significantly reduced the lesion volume compared to untreated SCI. We also observed that both Rm-PgP treatment groups significantly reduced the number of $ED1^+$macrophages and increased number of $Arg1^+$ cells compared to untreated SCI group. The number of $NeuN^+$ cells was significantly increased and the GFAP fluorescence intensity was reduced by Rm-PgP treatment. For the motor function recovery, we observed that BBB scores of Rm-PgP injected animals were significantly higher than that of untreated SCI animals beginning at Day 5 and continuing throughout all subsequent time points. For neuropathic pain, we observed that pain level in both Rm-PgP treated groups were significantly lower than that in untreated SCI group.

Conclusion: These results suggest that Rm-PgP injection via intrathecal catheter, which is a less invasive and more clinically relevant administration route than direct intraspinal injection, significantly improve motor functional recovery and reduce neuropathic pain development.

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