NEURAL REGENERATION RESEARCH
www.nrronline.org

Nucleoside modified mRNA-lipid nanoparticles as a new delivery platform for the repair of the injured spinal cord

Krisztián Pajer, Tamás Bellák, Antal Nógrádi*

Spinal cord injury and treatment opportunities: The adult mammalian spinal cord has a very limited capacity for spontaneous regeneration due to various intrinsic molecular and cellular factors. Although the spinal cord neurons have the capacity to regenerate their axons, the expression of growth inhibitory factors, lack or suppression of proper guidance cues, and profound inflammatory responses do not permit successful regeneration (Khyeam et al., 2021). Injury to the spinal cord affects both the long and short ascending and descending pathways thus separating the lower spinal cord segments from the higher motor and sensory centers. The primary physical injury is followed by a cascade of events, called secondary injury. During this phase, inflammation, apoptosis of neuronal and glial cells, glutamate excitotoxicity, disruption of the blood–brain barrier, demyelination of axons, and reactive astrogliosis occur (Silva et al., 2014). Therefore, safe and effective treatments need to be developed to preserve and if possible, regenerate the injured propriospinal and supraspinal tracts and induce favorable changes in the microenvironment of the cord around the lesion. Current therapeutic strategies using stem or progenitor cells, growth factors, or gene therapy via various methods have been attractive approaches to promote neuroprotection and neural regeneration following spinal cord injury (SCI) (Teng, 2019). Out of these many therapeutic ways, stem cells prove to be effective, especially a few stem cell lines, which are able to adapt to the specific needs of the injured cord in order to facilitate neuroprotection and regeneration. Recent evidence suggests that these effective undifferentiated stem cells produce a so-called "lesion-induced secretome" within the cord following transplantation, and they do not need to integrate permanently into the cord to achieve their beneficial effects (**Figure 1A**; Pajer et al., 2019). However, in cases of other stem cell types, integration into the injured cord is necessary to achieve a therapeutic effect.

Previous studies have determined the composition of the lesion-induced secretome produced by various undifferentiated stem cells (e.g. NE-4C neuroectodermal stem cells) or mouse and human induced pluripotent stem cells (Pajer et al., 2019; Bellák et al., 2020). As these biomolecules are very sensitive and have a very short half-life, we have sought opportunities to find alternative methods for administering molecules that (a) become expressed after delivery into the cord, (b) maintain their expression levels for the critical period necessary to successfully initiate neuroprotective and regenerative processes, and (c) are produced in therapeutically sufficient quantities.

The emergence of nucleoside-modified mRNA as a new therapeutic tool promises new potential perspectives in the treatment of spinal cord injuries. Nucleoside-modified mRNA is more stable and translatable than natural mRNA. The lipid nanoparticle (LNP) applied for coating the mRNA has proven to be an effective carrier for introducing mRNAs into tissues (Pardi et al., 2015). Based on these advantageous features, the use of mRNA-LNP appears to be a potential treatment to support neuroprotective and regenerative processes after SCI.

mRNA–LNP-based therapy as a new approach to repair the injured spinal cord: In our recently published work we used a human (h) IL-10-encoding mRNA-LNP construct for treating the injured rat spinal cord in the subacute phase of thoracic contusion injury (Gál et al., 2023). The aim of this research was to investigate the potential of intraspinally administered nucleoside-modified hIL-10 mRNA-LNP, whether it was able to initiate transient translation within the injured spinal cord and thus elicit substantial neuroprotection and subsequent improvement in locomotor function (**Figure 1B**; Gál et al., 2023).

To test the hypothesis that mRNS-LNP constructs are able to induce morphological and functional recovery after SCI, IL-10 was chosen, because it was a prominent component of the lesion-induced secretome in SCI and is known as an overall and powerful anti-inflammatory cytokine (Pajer et al., 2019). A study has provided evidence that the administration or induced expression of IL-10 protein following experimental SCI enhances neuronal survival and facilitates a certain degree of functional recovery via multiple mechanisms (Saraiva et al., 2020).

As the first step, we investigated the expression kinetics of mRNA translation in both intact and injured spinal cords. Following a single injection of a low dose of enhanced green fluorescent protein (eGFP) mRNA-LNP, astrocytes showed a continuous expression of eGFP for 3 weeks. In contrast, eGFP expression in neurons of injured cords was detectable for 14 days, while intact spinal cord neurons showed limited eGFP expression for 5 days. Interestingly, microglia/macrophages exhibited eGFP positivity only for a short period (Gál et al., 2023). These results allow us to conclude that certain characteristics of various cell types in the spinal cord and their current physiological state greatly influence the time course of protein expression.

Next, we used mRNA-LNP encoding hIL-10 intraspinally 1 week after the thoracic contusion injury. Remarkably, we observed sustained production of hIL-10 protein in neurons and astrocytes for at least 5 days following intralesional delivery, whereas microglia/macrophages expressed hIL-10 only for 2 days (Gál et al., 2023).

High or increased levels of IL-10 may be associated with functional recovery, so we used the Basso, Beattie, Bresnahan test and our video-based gait analysis system for testing the locomotor improvement. hIL-10 mRNA-LNP-treated animals demonstrated significant improvement through the use of both functional approaches compared with the control animals. Morphologically, the administration of hIL-10 mRNA-LNP resulted in a significantly smaller lesion area at the epicenter of the injury accompanied by significantly enhanced tissue sparing, and induced sparing and/or regeneration of proprio- and supraspinal connections of the injured spinal cord (Gál et al., 2023).

We were also interested in what cellular and molecular changes were induced by the hIL-10 mRNA-LNP treatment in the injured rat spinal cord. The ability of IL-10 to inhibit the expression of inflammatory cytokines and the activation of inflammatory macrophages in the injured spinal cord has been widely described. Our findings align with these results, as we observed a decrease in tissue densities of $CD68^+$ macrophages and $Iba\text{-}1^+$ microglia cells following treatment with hIL-10 mRNA-LNP. These results indicate a potent anti-inflammatory effect of hIL-10, exerting a favorable modulation of microglia/macrophage activities in the subacute phase of the injury. Interestingly, a notable, but temporary increase in hIL-10 protein expression was found in the serum on the first day following intraspinal administration of hIL-10 mRNA-LNP. This finding highlights the minimally invasive nature of intraspinal mRNA delivery as a route of administration in experimental animals. The Proteome Profiler Array analysis was used to assess the expression of 29 cytokines in the affected segments and blood serum. On day 5 following hIL-10 mRNA-LNP treatment, a significantly elevated chemiluminescence signal was detected for metalloproteinase inhibitor tissue inhibitor of metalloproteinase-1 and the neurotrophic factor ciliary neurotrophic factor in the affected segment. In contrast, there were no substantial alterations observed in cytokine levels within the blood serum of the hIL-10 mRNA-treated animals, except for a slight decrease in serum RANTES chemokine levels by day 5 after treatment. PCR-based quantification of pro-inflammatory IL-6 mRNA in the spinal cord revealed significantly increased levels, whereas tumor necrosis factor alpha, a central cytokine in inflammatory reactions, and the inflammatory protein C-C Motif Chemokine Ligand 3, known as macrophage inflammatory protein-1α, mRNAs showed significant decreases at days 1 and 2. Additionally, the pro-inflammatory cytokine IL-1b mRNA levels were significantly decreased. Collectively, these factors likely contributed to the active downregulation of microglia/macrophage activity and the preservation of spinal cord integrity following SCI (Gál et al., 2023). Overall, it can be stated that although the therapeutic protein was expressed only for 5 days, it caused a secondary turnover effect, likely contributing to the favorable transformation of the injury microenvironment. These processes are likely in close relationship with the activated neuronal IL-10 receptor, which provides trophic support and beneficial effects on neurons.

Our findings clearly demonstrate that mRNA-LNP serves as a promising and conceptually innovative non-viral delivery platform for various neurotrophic or anti-inflammatory factors to promote neuronal regeneration and regulate immune reactions after SCI.

Future perspective and challenges of the use of mRNA-LNP in SCI: The most important question regarding any therapeutic approach is always safety, and mRNA therapy is no exception to this. Although the prophylactic COVID-19 mRNA vaccine has been shown to be safe, similar safety measures will be required for any other mRNA-based therapy.

It is important to highlight that some LNPs may trigger inflammation (Vlatkovic, 2021), so their application in an inflammatory environment may become critical and generate further undesired, protracted inflammatory processes. Since there is a significant degree of inflammation in the acute and subacute phases of SCI, it is a central issue to use lipid-based transport molecules that do not induce an inflammatory response. Unregulated or chronic inflammation can inhibit neuronal regeneration and lead to greater tissue destruction than if no intervention had been used (Jin et al., 2023). It is important to emphasize that patients with SCI are often polytraumatized, thus administration of any neuroprotective or anti-inflammatory drug requires great consideration. Therefore, it is also important to modify the therapeutic mRNA molecules making them less immunogenic which can help reduce the inflammatory response triggered by the mRNA. The delivery routes of mRNA-LNPs (intraspinal or intravenous, etc.) and the dosage used can also influence the inflammatory responses. Optimizing these factors is critical to minimize inflammatory

processes while still achieving the desired therapeutic effect.

Another important aspect is that the experimental intraspinal application of mRNA-LNP is considered minimally invasive, but in humans, this type of therapeutic application can raise numerous complications such as re-opening the injured area with consequent destabilization of the vertebral column, possible leakage of the mRNA into the subarachnoidal space, etc. The alternative administration route would be the intravenous one (**Figure 1C**), but one should be aware of the fact that a significant amount of intravenously administered mRNA-LNPs is accumulated in the liver and spleen (Pardi et al., 2015). As a result, it may not be possible to achieve the required therapeutic concentration in the injured spinal cord, that would be able to result in significant morphological and functional recovery. To overcome the problem of mRNA-LNP "misrouting" to the liver and spleen, certain cell types (e.g. subtypes of leukocytes) can be theoretically tagged with or forced to take up mRNA-LNP (not known to have been performed yet) so that they may reach the site of injury as a target organ, exert the therapeutic effect and minimize drug exposure to non-target organs (**Figure 1**).

Our published work has also provided evidence that after the intraspinal administration of mRNA-LNP, IL-10 can be expressed for different periods (Gál et al., 2023). In light of this finding, it is important to determine the period until the given therapeutic protein will be expressed. Earlier studies have shown that short-term expression of some therapeutic proteins cannot achieve the desired neuroprotective effect, so repeated administration may be necessary, which should be considered in the case of an injured spinal cord. For this reason, a stable, long-acting mRNA that can be used to produce a long-lasting therapeutic protein is desirable for neuroprotection and proper neural regeneration, as well as for modulating the damaged environment. To extend the expression of mRNA-LNPs, several strategies can be employed, including mRNA modifications and special design of lipid nanoparticles (Hajiaghapour Asr et al., 2023). Interaction with cognate receptors expressed in the target organ or surface conjugation of targeting ligand would help us in the specific targeting of mRNA-LNPs. Coating the mRNA-LNPs with biocompatible polymers can help protect the nanoparticles and mRNA from degradation and rapid clearance by the immune system (Hajiaghapour Asr et al., 2023). These methods can enhance the specificity of mRNA-LNPs to reach their intended target cells or organs, which can be crucial for the effectiveness of therapeutic applications.

Looking ahead, the use of mRNA-LNP opens new paths in the field of neural regeneration and can even be used as a therapeutic tool in the treatment of certain neurodegenerative diseases or disorders, too. mRNA-LNPs can be utilized to deliver therapeutic mRNA molecules that encode specific proteins or factors involved in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, or in neurodevelopmental diseases. LNPs could be also used to deliver mRNA encoding factors that promote neuroprotection, neural regeneration, or angiogenesis following acute ischemic stroke (Ayala and Nguyen, 2021). The mRNA-LNP technique can play a significant role in the treatment of pain in the central nervous system due to the localized and specific expression of analgesic factors. For these applications, it is essential to modify the mRNA-LNP to cross the blood-brain barrier reaching the target area.

It is important to note that while the potential use of mRNA-LNPs for injuries and disorders in the central nervous system is promising, further research and (pre)clinical trials are necessary to explore their safety, efficacy, and feasibility for these specific applications.

Conclusion: In the present review, we have discussed the potential use of mRNA-LNP as a treatment strategy for the injured spinal cord. mRNA-LNP proved to be an effective gene delivery platform for the injured microenvironment resulting in a short term, but effective release of therapeutic protein. However, further understanding of the molecular mechanism, and how LNP modulates the transport, lifetime, and translation of mRNA to functional therapeutic protein will greatly contribute to the development of therapies targeting degenerative changes within the central nervous system.

We are grateful to Dénes Török (University of Szeged, Hungary) for his help with preparing Figure 1.

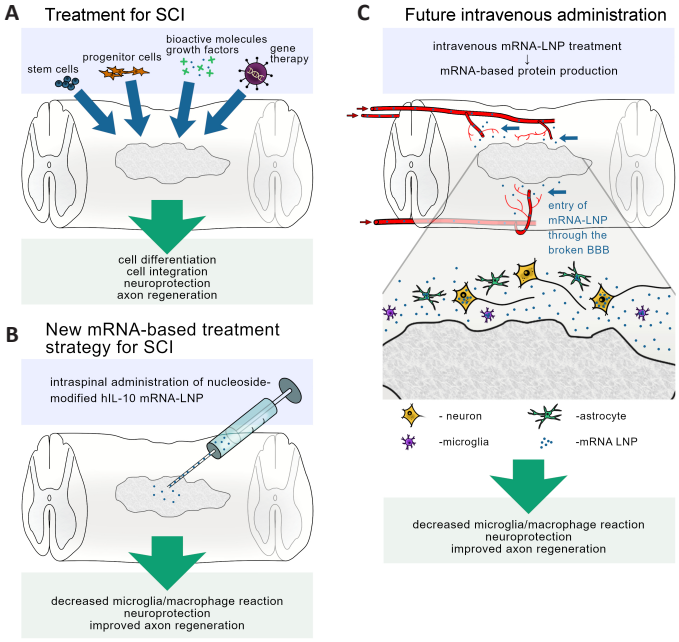

Figure 1 | **Various approaches to treat SCI.**
(A) A range of treatment options for spinal cord injuries; (B) a novel method for decreasing the microglia/macrophage reaction and paving the way for motor function recovery; (C) intravenous delivery of mRNA to neurons, astrocytes, and microglia/macrophages by LNP. Created with the graphical software GIMP (version 2.10.28). BBB: Basso, Beattie, Bresnahan; hIL: human interleukin; LNP: lipid nanoparticle; SCI: spinal cord injury.

Krisztián Pajer, Tamás Bellák, Antal Nógrádi*
Department of Anatomy, Histology and Embryology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary
***Correspondence to:** Antal Nógrádi, MD, PhD, DSc, nogradi.antal@med.u-szeged.hu.
https://orcid.org/0000-0002-0520-5350 (Antal Nógrádi)
Date of submission: July 22, 2023
Date of decision: April 20, 2024
Date of acceptance: May 13, 2024
Date of web publication: September 6, 2024

https://doi.org/10.4103/NRR.NRR-D-23-01231
How to cite this article: *Pajer K, Bellák T, Nógrádi A (2025) Nucleoside modified mRNA-lipid nanoparticles as a new delivery platform for the repair of the injured spinal cord. Neural Regen Res 20(8):2311-2312.*

References

Ayala YM, Nguyen AD (2021) RNA-based therapies for neurodegenerative diseases. Mo Med 118:340-345.
Bellák T, Fekécs Z, Török D, Táncos Z, Nemes C, Tézsla Z, Gál L, Polgári S, Kobolák J, Dinnyés A, Nógrádi A, Pajer K (2020) Grafted human induced pluripotent stem cells improve the outcome of spinal cord injury: modulation of the lesion microenvironment. Sci Rep 10:22414.
Gál L, Bellák T, Marton A, Fekécs Z, Weissman D, Török D, Biju R, Vizler C, Kristóf R, Beattie MB, Lin PJC, Pardi N, Nógrádi A, Pajer K (2023) Restoration of motor function through delayed intraspinal delivery of human IL-10-encoding nucleoside-modified mrna after spinal cord injury. Research (Wash D C) 6:0056.
Hajiaghapour Asr M, Dayani F, Saedi Segherloo F, Kamedi A, Neill AO, MacLoughlin R, Doroudian M (2023) Lipid nanoparticles as promising carriers for mRNA vaccines for viral lung infections. Pharmaceutics 15:1127.
Jin Y, Song Y, Lin J, Liu T, Li G, Lai B, Gu Y, Chen G, Xing L (2023) Role of inflammation in neurological damage and regeneration following spinal cord injury and its therapeutic implications. Burns Trauma 11:tkac054.
Khyeam S, Lee S, Huang GN (2021) Genetic, epigenetic, and post-transcriptional basis of divergent tissue regenerative capacities among vertebrates. Adv Genet (Hoboken) 2:e10042.
Pajer K, Bellák T, Redl H, Nógrádi A (2019) Neuroectodermal stem cells grafted into the injured spinal cord induce both axonal regeneration and morphological restoration via multiple mechanisms. J Neurotrauma 36:2977-2990.
Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, Madden TD, Hope MJ, Weissman D (2015) Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. J Control Release 217:345-351.
Saraiva M, Vieira P, O'Garra A (2020) Biology and therapeutic potential of interleukin-10. J Exp Med 217:e20190418.
Silva NA, Sousa N, Reis RL, Salgado AJ (2014) From basics to clinical: a comprehensive review on spinal cord injury. Prog Neurobiol 114:25-57.
Teng YD (2019) Functional multipotency of stem cells and recovery neurobiology of injured spinal cords. Cell Transplant 28:451-459.
Vlatkovic I (2021) Non-immunotherapy application of LNP-mRNA: maximizing efficacy and safety. Biomedicines 9:530.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y