

Determining the dose-efficacy relationship of intralesional injection of bone marrow mesenchymal stem cell-derived exosomes (BM-MSCs) for SCI

Introduction

Exosomes are small extracellular vesicles that carry proteins, lipids, and RNAs that modulate immune responses, allowing us to manipulate the body to aid in generation and recovery for a variety of injuries. While stem cell therapies are currently a large topic of interest for tissue regeneration, alternatively, stem-cell derived exosomes may provide similar clinical benefits without biosafety concerns that are coupled with the transplantation of living cells (Zhang & Cheng, 2023). Additionally, because exosomes aren't technically living cells, they don't undergo cell duplication and therefore have no risk of cancer development. For spinal cord injuries (SCI), this can be extremely impactful in helping regenerate damaged nerves and tissues.

Most exosome therapies are in their early clinical stages, however, have displayed promising results. NurExone's ExoPTEN, an allogeneic off-the-shelf exosome therapy, has been tested on rats with transection and compression SCI injuries. The treatment was administered via intranasal and intrathecal routes, and led to significant improvement in motor and sensory functions in SCI rats, as well as structural recovery (Avni et al., 2025). Exosome therapies administered via injections have shown to be effective as well. Researchers from Mackay Medical College in Taiwan found that injection of an exosome-loaded Gelfoam onto the T9 spinal cord improved SCI-induced motor dysfunction and neuropathic pain (Poongodi et al., 2024). The current research of exosome treatments has been very promising in terms of effectiveness, however, there has been little research on the optimal dosage of exosomes in treatments, with none of the current studies investigating dose-efficacy on SCI pathologies. The

limited studies that have looked at exosome dosage have found a significant difference in its effects. Researchers of the Key Laboratory of Brain Science Research at Hainan Medical University conducted a study comparing the same exosome type at different doses in treating the sciatic nerve of a rat's hind leg. They found that the "middle region" (EXO-D3, 0.9×10^{10} particles/ml) proved most effective by number of regenerated myelinated nerve fibers and largest fiber diameters (Zhao et al., 2020). Given the impact of dosage on the effectiveness of exosome therapies, it's important to establish an optimal range for exosome dosages in SCI pathologies to advance exosome therapies as a viable treatment for SCI.

Specific Aim

To determine the dose-efficacy relationship of Company X exosome treatment via intralesional injection in SCI.

Rationale

Many studies have looked into the effectiveness of exosomes in treating in vivo in a fixed dose in SCI, however, have not done a study comparing multiple exosome doses within the same study. Meta-analyses have tried to compare dose-effect relationships, however, these statistics are inconsistent with reported units.

Methods

This double-blind study will investigate exosome treatment dose-effect relationships from twenty adult participants ($n = 20$). All participants will be randomly assigned to a group. Five participants will be administered a placebo exosome injection, representing the control

group. The other fifteen participants will be divided into three experimental groups. Each group will be administered three different doses of the exosome treatment. The three groups will receive dosages of 100ug, 150ug, and 200ug respectively.

Exosomes will be sourced from bone marrow mesenchymal stem cells (BM-MSCs), which are very commonly used to source exosomes in exosome therapy studies for SCI. These exosomes will be isolated by differential ultracentrifugation, a BM-MSCs isolation method borrowed from a study led by Ming Deng, professor of the Department of Radiology at Wuhan University (Deng et al., 2025). They were cultured for 48 hours at 37 °C in a CO₂-regulated incubator in a 10% FBS medium to deplete EVs. To remove cellular debris, centrifugation was carried out at 500 g for 15 minutes at 4 °C after the supernatant was collected. After that, cell fragments or apoptotic bodies were removed by centrifugation at 2000 g for 15 minutes at 4 °C, and big vesicles were removed by centrifugation at 10,000 g for 20 minutes at 4 °C. After passing through a 0.22 µm filter, the centrifuged samples were centrifuged again at 110,000 g for 70 minutes at 4 °C. The samples were then resuspended in PBS and centrifuged at high speeds under the same circumstances again.

A lesion will be made at the participant's SCI injury site. The exosome treatment will then be administered to the participant via the lesion site. Effectiveness of the dosages will be measured using BBB scores, Von Frey, and Gait Analysis.

Pitfalls and Alternative Approaches

One caveat is a lack of homogeneity in spinal pathology of recruited patients. In other words, subjects with SCI participating in study don't all have the same injury, which can lead to varied results. However, the fact that they are all experiencing SCI means that we can point to similar

markers of effects such as BBB score, Von Frey, and Gait Analysis. Another pitfall is that with human participants, there could be a lack of them (we only have access to 10-20 subjects), have different SCI pathologies (different sites of injury, different genetic profiles and predispositions to recovery, inflammatory responses, reactivity to exosome treatment).

References

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