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Transplantation of mesenchymal stem cells and injections of microRNA as therapeutics for nervous system repair

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Avhandlingen kommer att försvaras på engelska.

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Abstract
Traumatic injuries to the spinal cord (SCI) and peripheral nerve (PNI) affect several thousand people worldwide every year. At present, there is no effective treatment for SCI and despite continuous improvements in microsurgical reconstructive techniques for PNI, many patients are still left with permanent, devastating neurological dysfunction. This thesis investigates the effects of mesenchymal stem cells (MSC) derived from adipose (ASC) and dental (DSC) tissue and chitosan/microRNA-124 polyplex particles on regeneration after spinal cord and peripheral nerve injury in adult rats. Dental stem cells were obtained from apical papilla, dental pulp, and periodontal ligament. ASC and DSC expressed MSC surface markers (CD73, CD90, CD105 and CD146) and various neurotrophic molecules including BDNF, GDNF, NGF, VEGF-A and angiopoietin-1. Growth factor stimulation of the stem cells resulted in increased secretion of these proteins. Both ASC and DSC supported in vitro neurite outgrowth and in contrast to Schwann cells, ASC did not induce activation of astrocytes. Stimulated ASC also showed an enhanced ability to induce capillary-like tube formation in an in vitro angiogenesis assay. In a peripheral nerve injury model, ASC and DSC were seeded into a fibrin conduit, which was used to bridge a 10 mm rat sciatic nerve gap. After 2 weeks, both ASC and DSC promoted axonal regeneration in the conduit and reduced caspase-3 expression in the dorsal root ganglion (DRG). ASC also enhanced GAP-43 and ATF-3 expression in the spinal cord, reduced c-jun expression in the DRG and increased the vascularity of the implant. After transplantation into injured C3-C4 cervical spinal cord, ASC continued to express neurotrophic factors and laminin and stimulated extensive ingrowth of 5HT-positive raphaespinal axons into the trauma zone. In addition, ASC induced sprouting of raphaespinal terminals in C2 contralateral ventral horn and C6 ventral horn on both sides. Transplanted cells also changed the structure and the density of the astroglial scar. Although the transplanted cells had no effect on the density of capillaries around the lesion site, the reactivity of OX42-positive microglial cells was markedly reduced. However, ASC did not enhance recovery of forelimb function. In order to reduce activation of microglia/macrophages and the secondary tissue damage after SCI, we tested the role of microRNA-124. In vitro transfection of chitosan/microRNA-124 polyplex particles into rat microglia resulted in the reduction of reactive oxygen species and TNF-α levels and lowered expression of MHC-II. Upon microinjection into uninjured rat spinal cords, particles formed with Cy3-labeled control sequence RNA, were specifically internalized by OX42 positive macrophages and microglia. Alternatively, particles injected in the peritoneum were transported by macrophages to the site of spinal cord injury. Microinjections of chitosan/microRNA-124 particles significantly reduced the number of ED-1 positive macrophages after SCI. In summary, these results show that human MSC produce functional neurotrophic and angiogenic factors, creating a more desirable microenvironment for neural regeneration after spinal cord and peripheral nerve injury. The data also suggests that chitosan/microRNA-124 particles could be potential treatment technique to reduce neuroinflammation.

Keywords
Mesenchymal stem cells, spinal cord injury, peripheral nerve injury, microRNA-124, rat

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