Stem cells for nerve repair and prevention of muscle atrophy

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt förvar i Sal KB3A9, KBC huset, tisdagen den 8 september, kl. 09:00. Avhandlingen kommer att förvaras på engelska.

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Abstract
Peripheral nerve injury (PNI) is common and despite modern microsurgical techniques of repair, functional restoration is always incomplete. This results in impaired sensation and reduced motor function alongside pain and cold intolerance. Traumatic PNI are often associated with loss of nerve tissue, creating a gap, and direct repair of the two damaged nerve stumps is not possible. These types of injuries are reconstructed using autologous nerve grafts but this is far from ideal since it necessitates the sacrifice of a functional nerve from elsewhere in the body. Chronic muscle atrophy because of the prolonged delay in nerve regeneration across gaps is a significant impediment to an optimal functional recovery.

Tissue engineering and regenerative medicine approaches to nerve repair might one day replace the need for autologous nerve grafts. This thesis investigates the effects of adipose derived stem cells (ASC) on nerve regeneration and muscle recovery by using the stem cells for intramuscular injection and combined with a biomaterial, poly-3-hydroxybutyrate (PHB), to create a bioengineered artificial nerve repair construct. The mechanisms of interaction between the stem cells and neuromuscular system cells were investigated and with a view to translating this work into clinical practice, an optimal source of cells was investigated from human donors.

It was hypothesized that injecting regenerative cells into muscle would reduce nerve injury induced muscle atrophy. A rat sciatic nerve lesion was performed and three different types of cells were injected into the denervated gastrocnemius muscle; either (1) undifferentiated ASC, (2) ASC induced to a ‘Schwann cell-like’ phenotype (dASC) or (3) primary Schwann cells. Nerves were either repaired by direct end-end suture or capped to prevent muscle reinnervation. One month later, functionality was measured using a walking track test, and muscle atrophy was assessed by examining muscle weight and histology. The Schwann cells and dASC groups showed significantly better scores on functional tests when compared with control injections of growth medium alone. Muscle weight and histology were also significantly improved in the cell groups in comparison with the control group.

PHB strips seeded with either primary Schwann cells or dASC suspended in a fibrin glue matrix were used to bridge a 10mm rat sciatic nerve gap. After 12 weeks, functional and morphological analysis (walking track test, electromyography, muscle weight and muscle histology) was performed. The results showed significantly better functional results for the PHB strips seeded with cells versus the control group with fibrin matrix only. This correlated with less muscle atrophy and greater distal axon myelination in the cell groups.

To further optimize the nerve regeneration and muscle recovery, the nerve gap lesion was repaired by treatment with the bioengineered constructs seeded with dASC or nerve autograft in combination with stem cell injection in the muscle. After 6 weeks, the best results were obtained in the nerve graft group combined with intramuscular dASC injection which showed significantly less atrophy than the other groups. The results also showed that using the stem cells in a matrix on a PHB strip in combination with intramuscular injections could significantly reduce muscle atrophy.

In vitro experiments showed that dASC expressed a wide range of neurotrophic and myogenic factors including BDNF, VEGF-A, IGF-1 and HGF. Stem cell conditioned medium enhanced the proliferation of myoblast cell lines and primary Schwann cells. Various signaling pathways (PKA, MAP kinase) were involved in these effects dependent on the cell type investigated. Furthermore, in direct co-culture with myoblast cells, a small population of the cells fused together to form myotube-like structures and expressed myogenic markers.

Human ASC were isolated from the deep and superficial layers of abdominal fat tissue obtained during abdominoplasty procedures. Cells from the superficial layer proliferated significantly faster than those from the deep layer. Superficial layer ASC induced significantly enhanced neurite outgrowth from neuronal cell lines when compared with the deep layer cells. However, RT-PCR and ELISA analysis showed that ASC isolated from both layers expressed similar levels of the neurotrophic factors NGF, BDNF and GDNF.

In summary, these results show that stem cell therapy at both levels (the nerve lesion site and in the target denervated muscle) offers a promising approach for clinical application for treatment of peripheral nerve lesions. The bioengineered artificial nerve construct, combining PHB strip with cells, also provides a beneficial environment for nerve regeneration. Many of the benefits of the ASC are likely to be mediated through their secretome, a rich source of neurotrophic and myogenic factors. Thus adipose tissue contains a pool of regenerative stem cells which have significant potential application to tissue engineering and regenerative medicine for nerve repair.

Keywords
adipose stem cells, biomaterial, muscle, nerve injury, neuromuscular junction, regeneration