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An exploration of the mechanisms behind peripheral nerve injury

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Abstract
Despite surgical innovation, the sensory and motor outcome after peripheral nerve injury is incomplete. In this thesis, the biological pathways potentially responsible for the poor functional recoveries were investigated in both the distal nerve stump/target organ, spinal motoneurons and dorsal root ganglia (DRG). The effect of delayed nerve repair was determined in a rat sciatic nerve transection model. There was a dramatic decline in the number of regenerating motoneurons and myelinated axons found in the distal nerve stumps of animals undergoing nerve repair after a delay of 3 and 6 months. RT-PCR of the distal nerve stumps showed a decline in expression of Schwann cells (SC) markers, with a progressive increase in fibrotic and proteoglycan scar markers, with increased delayed repair time. Furthermore, the yield of SC which could be isolated from the distal nerve segments progressively fell with increased delay in repair time. Consistent with the impaired distal nerve stumps the target medial gastrocnemius (MG) muscles at 3- and 6-months delayed repair were atrophied with significant declines in wet weights (61% and 27% compared with contralateral sides). The role of myogenic transcription factors, muscle specific microRNAs and muscle-specific E3 ubiquitin ligases in the muscle atrophy was investigated in both gastrocnemius and soleus muscles following either crush or nerve transection injury. In the crush injury model, the soleus muscle showed significantly increased recovery in wet weight at days 14 and 28 (compared with day 7) which was not the case for the gastrocnemius muscle which continued to atrophy. There was a significantly more pronounced up-regulation of MyoD expression in the denervated soleus muscle compared with the gastrocnemius muscle. Conversely, myogenin was more markedly elevated in the gastrocnemius versus soleus muscles. The muscles also showed significantly contrasting transcriptional regulation of the microRNAs miR-1 and miR-206. MuRF1 and Atrogin-1 showed the highest levels of expression in the denervated gastrocnemius muscle. Morphological and molecular changes in spinal motoneurons were compared after L4-L5 ventral root avulsion (VRA) and distal peripheral nerve axotomy (PNA). Neuronal degeneration was indicated by decreased immunostaining for microtubule-associated protein-2 in dendrites and synaptophysin in pre-synaptic boutons after both VRA and PNA. Immunostaining for ED1-reactive microglia and GFAP-positive astrocytes was significantly elevated in all experimental groups. qRT-PCR analysis and Western blotting of the ventral horn from L4-L5 spinal cord segments revealed a significant up-regulation of apoptotic cell death mediators including caspases-3 and -8 and a range of related death receptors following VRA. In contrast, following PNA, only caspase-8 was moderately up-regulated. The mechanisms of primary sensory neuron degeneration were also investigated in the DRG following peripheral nerve axotomy, where several apoptotic pathways including those involving the endoplasmic reticulum were shown to be upregulated. In summary, these results show that the critical time point after which the outcome of regeneration becomes too poor appears to be 3-months. Both proximal and distal injury affect spinal motoneurons morphologically, but VRA induces motoneuron degeneration mediated through both intrinsic and extrinsic apoptotic pathways. Primary sensory neuron degeneration involves several different apoptotic pathways, including the endoplasmic reticulum. **Keywords:** Peripheral nerve injury, target organ, spinal motoneurons, primary sensory neurons, degeneration

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