A Regenerative Approach to Spinal Cord Injury

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Injury to spinal cord is devastating also because of the inability of central neurons to regenerate correct axonal or dendritic connections. The consequences of injury are not just a break in communication between healthy neurons, but a cascade of events that can lead to neuronal degeneration and death. As reported early in the past century by Tello, central neurons CNS neurons can regrow processes if a favourable environment is provided. Transplantation of neural stem cells might represent such a desired tool. In a recent study we have shown that therapy with adult NSCs via i.v. route or intraspinal application may result as a useful treatment for spinal cord injury, and the effect is likely due to the early enhancement of local expression at site of injury of neurotrophic factors. Unfortunately NSCs are destroyed by macrophages between days 10 and 20 after injury and the recovery process is then stopped. Also embryonic stem cells die quickly at site of injury (Bottai et al. 2008, 2010). More recently, we have isolated from the SVZ and grown in vitro SNCs collected several hours after donor death (DR-NSC). These cells differentiate mostly in neurons (over 50%), present the activation of HIF-1α gene, and autosecrete erythropoietin. The blockade of erythropoietin or its receptor by means of specific antibodies inhibits DR-NSC differentiation into neurons. Results suggest that DRNSCs overcome macrophage action and accumulate at site of lesioning creating a bridge and promoting restoration of function.

Goal 1) ***Transplantation and evaluation of migration and homing to the lesion site, and in vivo differentiation of DR-NSCs.*** **1** million DRNSCs are injected i.v.. DR-NSCs differentiation is assessed by antibodies to neuronal and glial antigens, and neurotransmitter enzymes such as ChAT. Also the attenuation of apoptosis is a primary target and we shall use the TUNEL technique. ***Goal 2) Evaluation of recovery from disability.*** Recovery from hind limb disability was evaluated by means of behavioural tests, performed 24 hours, 4, 7, and every 4 days thereafter following SCI. The 21 points Basso, Beattie e Bresnahan (BBB) was used. ***Goal 3***). **Preservation of myelin at lesion site.** Since the preservation of discending ventral and segmental motor pathways contribute substantially to the motor recovery after spinal cord injury in mice, we evaluated preservation by means of fluoromyelin technique. **Goal 4) Evaluation of the reconstruction of the lesioned site and of the neuronal regeneration.** Quantitative morphometric techniques were used to quantify the protective action of DR-NSCs on the extent of the tissue damage and myelin preservation. Immunocytochemistry and quantitative techniques determined regeneration of 5-HT- and TH-positive pathways across the lesion site. ***Goal 5) Site of injury, cellular infiltration, and inflammatory cytokines.*** The effects of the transplants upon glia scar formation and macrophage infiltration were evaluated with GFAP and ED-1 antibodies respectively.