NEURAL REGENERATION RESEARCH
January 2014, Volume 9, Issue 2
www.nrronline.org

Spinal root avulsion: an excellent model for studying motoneuron degeneration and regeneration after severe axonal injury

Carolin Ruven[1], Tak-Kwong Chan[1], Wutian Wu[1, 2]

1 Department of Anatomy, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China; 2 GHM Institute of CNS regeneration, Jinan University and The University of Hong Kong, Guangzhou, Guangdong Province, China

Spinal root avulsion is an excellent model for studying the response of motoneurons to severe injury to their axons (Koliatsos et al., 1994). In this model ('Avulsion Model'), spinal roots are torn off from spinal cord without removing the vertebra at different levels of spinal segments, usually at cervical and lumbar segments. Step-by-step procedures are described in detail elsewhere (Chu and Wu, 2009). The Avulsion Model resembles very well brachial plexus injuries in human beings. Around 70% of severe brachial plexus injuries in human involved avulsion of one or more roots (Narakas, 1985) and the main causes of traumatic brachial plexus injuries were motor vehicle accidents, sport injuries and difficult deliveries (Terzis et al., 2001). The Avulsion Model involves injury to both central nervous system (CNS) and peripheral nervous system (PNS) while nerve axotomy, transection and crush injuries only involve PNS.

The avulsion model provides an opportunity to study the phenomena of neuronal death and survival after spinal cord injuries. After avulsion injuies in rat, massive death of motoneurons occurs (Wu, 1993; Wu and Li, 1993). Motoneurons in the injured spinal cord segment begin to die one week after injury and 70% of the motoneurons degenerate by 3 weeks. By 6 weeks, almost all motoneurons degenerate (Li et al., 1995). In contrast, distal axotomy of spinal nerves does not cause any motoneuron death due to the presence of the remaining part of peripheral nerves. Studies have shown that at least 4 mm of remaining peripheral nerve is needed to avoid degeneration of a motoneuron (Gu et al., 1997). In the Avulsion Model, spinal roots are pulled away from spinal cord so that nerve roots are totally removed and cell bodies in the spinal cord do not get any external support from peripheral nerve (Wu, 1993; Koliatsos et al., 1994). It has been shown that glial cell derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF) from Schwann cells in peripheral nerves are the factors that keep the motoneurons in the spinal cord from dying (Li et al., 1995; Novikov et al., 1995; Chai et al., 1999; Wu et al., 2003). It is known that the changes within a cell have similar characteristics in both necrotic and apoptotic deaths (Li et al., 1998), but the exact mechanisms of how the motoneurons die are still unknown. Avulsion injury also triggers the inflammation response that brings macrophages and microglia to the lesion site (Koliatsos et al., 1994; Yuan et al., 2004). During the process of degeneration, motoneurons undergo many biochemical and structural changes (Li et al., 1998; Yang et al., 2006). Expression of nitricoxide synthase (NOS) is highly upregulated after an injury suggesting the important role of NOS in the degeneration of motoneurons (Wu, 1993). Expression of NOS can be inhibited by neurotrophic factors GDNF and BDNF that are produced by Schwann cells in peripheral nerve and that have shown the ability to prevent the degeneration of motoneurons (Novikov et al., 1995; Wu et al., 1995; Wu et al., 2003). These findings help us to understand and learn the mechanisms of cell deaths in CNS.

The rapid loss of motoneurons in the Avulsion Model also gives us an opportunity to test different methods of enhancing the survival and regeneration of motoneurons. As spinal roots are pulled off only from one side of spinal cord leaving the other side intact, the number of motoneurons in the contralateral side of spinal cord can serve as a control (Wu, 1993). Re-implantation of the avulsed ventral root was shown to be most effective in preventing motoneuronal deaths but the delay of surgery causes makes implantation technically very difficult due to retraction of the nerve stum (Carlstedt et al., 1993; Hallin et al., 1999; Carlstedt et al., 2000; Blits et al., 2004; Eggers et al., 2010; Carlstedt and Hayton, 2012; Su et al., 2013). An alternative method is to implant a peripheral nerve graft that could enhance the survival of motoneurons, axonal regeneration and functional recovery (Wu et al., 1994; Wu, 1996; Su et al., 2013). Survival and regeneration of motoneurons in the injury site is promoted by GDNF and BDNF produced by Schwann cells in the nerve graft (Frostick et al., 1998). These neurotrophic factors can be used alone as therapeutic agents because they can be easily applied on the surface of spinal cord close to the ventral root of the avulsion site. GDNF and BDNF are suggested to prevent motoneuronal death by inhibiting the expression of NOS in injured motoneurons (Novikov et al., 1995; Wu et al., 1995; Wu et al., 2003).

Besides studying neuronal death and survival, the Avulsion model is also good for studying axonal regeneration. Since spinal roots are torn off from spinal cord, the cell bodies of motoneurons are no longer connected with their axons. Target muscles lose innervation from motoneurons when injury is left unattended. To avoid this, we have to find possible ways to promote axonal regeneration. For functional recovery, regenerated axons have to myelinate and reach the target muscles. Axonal regeneration can be promoted by re-implantating the avulsed ventral root, or transplanting peripheral nerve graft or conduit (Carlstedt et al., 1993; Wu et al., 1994; Wu, 1996; Gu et al., 2004; Gu et al., 2005; Eggers et al., 2010; Su et al., 2013; Zhan et al., 2013). Brachial plexus avulsion model in rats is convenient for functional recovery studies because of the short distance that axons have to regrow. Therefore, axonal regeneration under different conditions can be tested with relatively short observation time.

In rats, brachial plexus root avulsion results in progressive atrophy of forelimb muscles due to the loss of innervation from motoneurons. In the Avulsion Model, both forelimb and hind limb muscles can be observed for atrophy when avulsion is made in the cervical spinal segment, while atrophy only occurs in hind limb muscles when avulsion is made in the lumbar segment. Similarly, traumatic injuries to human spinal cord often results in the paralysis of muscles innervated by the nerve roots at or below the lesion site. So, this model gives us an insight into processes occurring in traumatic muscle atrophy and diseases like spinal muscular atrophy (SMA). Briefly, muscle atrophy involves the reduction of muscle fiber size and muscle weight, increased amount of connective and fat tissue and overall weakness of muscles (Grimby et al., 1976; Scelsi et al., 1982; Castro

et al., 1999; Round et al., 2003). So far, transplantation of neural progenitor cells or other pluripotent cells into peripheral nerve seems to be a promising strategy to reduce muscle atrophy (Erb et al., 1993; Thomas et al., 2000; Su et al., 2012; Su et al., 2013).

In summary, the Avulsion Model will allow us to discover the mechanisms of motoneuronal death and survival that may in turn enhance our understanding about the pathological processes occurring in neurodegenerative diseases such as Alzheimer, Parkinson, and Huntington's disease.

Corresponding author: Wutian Wu, M.D., Professor, Department of Anatomy, LKS Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Hong Kong Special Administrative Region, China, wtwu@hku.hk.
doi:10.4103/1673-5374.125338 http://www.nrronline.org/
Accepted: 2014-01-02

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Copyedited by Zheng BH, Snow DM, Li CH, Song LP, Liu WJ, Zhao M