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BRAIN RESEARCH

Research Report

Modulation of extracellular matrix components by metalloproteinases and their tissue inhibitors during degeneration and regeneration of rat sural nerve

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ABSTRACT

The success of peripheral nervous system regeneration has been associated with changes on the microenvironment, particularly on the extracellular matrix (ECM) components. In the present study we analyzed by indirect immunohistochemistry, electron microscopy and Western blotting, the distribution of ECM components, metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), during Wallerian degeneration (WD) and nerve regeneration (2nd, 7th and 21st days after injury) on crushed rat sural nerves. Our results showed that laminin α_3-chain and α_2-chain are over expressed during the early stages of degeneration and regeneration respectively, whereas type IV collagen expression increased progressively after crush. On the other hand, the expression of chondroitin sulfate was down regulated during the early stages of degeneration, returning progressively to normal values during nerve regeneration. The expression of MMP-3 was almost normal immediately after lesion, and then reduced progressively achieving the smallest expression at 21 days after crush; on the contrary, the expression of MMP-7 increased significantly immediately after crush (2nd day) returning to normal values afterwards. TIMP-1 and TIMP-2 were over expressed at the beginning of WD, returning progressively to normal values after that. These results indicate that the modifications of ECM components, which are favorable for nerve regeneration, are correlated with changes on the balance between MMPs and TIMPs.

1. Introduction

Damage to peripheral nervous system (PNS) is followed by Wallerian degeneration (WD) in the nerve distal segment. WD can be induced by experimental nerve crush or section, which promotes a cascade of events described by Waller in 1850. The sequence of characteristic events of WD starts with Ca^{+2} influx (George et al., 1995; Martinez and Ribeiro, 1998) followed by axonal cytoskeleton disintegration and myelin sheath breakdown. After that, macrophages are recruited to the lesion area in order to phagocyte axon and myelin sheath debris (Stoll et al., 1989, 2002). All these changes provide differential signals to Schwann cells, which become dedifferentiated and then proliferate forming the bands of Büngner. The process of

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regeneration starts with axon sprouting at the nearest node of Ranvier in the nerve proximal stump (Stoll and Müller, 1999). These sprouts achieve the bands of Büngner and are guided to the target tissue by interacting with Schwann cells basal lamina components, and following different gradients of growth factors (Martini, 1994).

The extracellular matrix (ECM) molecules, present in the basal laminae, are produced by Schwann cells (Chernousov and Carey, 2000). Some ECM components such as laminin (LN) (Labrador et al., 1998), fibronectin (FN) (Vogelezang et al., 1999) and type IV collagen (CIV) (Tonge et al., 1997) are known to help axonal growth and elongation during either normal development or regenerative process. However, other ECM molecules such as chondroitin sulfate proteoglycans (CSPG) can inhibit this growth (Zuo et al., 1998b). The balance of positive and negative signals from ECM molecules to Schwann cells give rise to a unique response, which will or not allow axonal growth and elongation (Stevens and Jacobs, 2002).

ECM is a dynamic structure, which is in constant synthesis and degradation. This turnover can be modified during development, morphogenesis, and pathologic processes (Murphy and Gavrilovic, 1999; Jones et al., 2003). Metalloproteinases (MMPs) are a family of enzymes specialized in ECM components degradation. The main characteristic of MMPs is the presence of a Zn^{+2}-dependent domain. MMPs are secreted to extracellular space as pro-active enzymes, becoming activated when its Zn^{+2}-dependent domain is cleaved, normally by another MMP (Murphy et al., 1999; Misko et al., 2002). During WD, MMP-3 and MMP-7 have their expression modified in order to promote changes on the balance of ECM components (Ferguson and Muir, 2000; Hughes et al., 2002). After transcription and activation, MMPs can be regulated by its high affinity tissue inhibitors (TIMPs). These molecules are homologue proteins that can bind to MMPs in a proportion of 1:1 (Bode et al., 1999). Currently, there are four TIMPs described in the literature (1 to 4), and similar to MMPs, their expression modulates several processes during normal development and pathologic processes (Brew et al., 2000).

Since degradation and remodeling of ECM components are important events of peripheral nerve degeneration and regeneration, we analyzed in this study, the distribution and concentration of some basal membrane ECM components and also of MMPs and TIMPs after sural nerve crush. Understanding the role of ECM molecules on the degenerative and regenerative processes occurring in an injured nerve can elucidate our knowledge on the success of PNS regeneration and may help to find strategies that can optimize PNS regeneration. Recently, a broad-spectrum inhibitor of MMPs has been used after nerve crush, but the results did not point to a better nerve regeneration (Demestre et al., 2004). Therefore, further studies are necessary to elucidate the role of these molecules on nerve degeneration and regeneration. Most of all, these studies will help to find strategies that can induce CNS regeneration as already suggested by other authors (Ahmed et al., 2005).

2. Results

2.1. *Light microscopy of the sural nerve*

Fig. 1A illustrates a contralateral nerve showing a normal structural pattern. Nerves submitted to crush showed, on the 2nd day, evidence of degeneration characterized by axonal and myelin sheath disruption and increase of perineurium thickness (Fig. 1B). On the 7th day many degenerating fibers

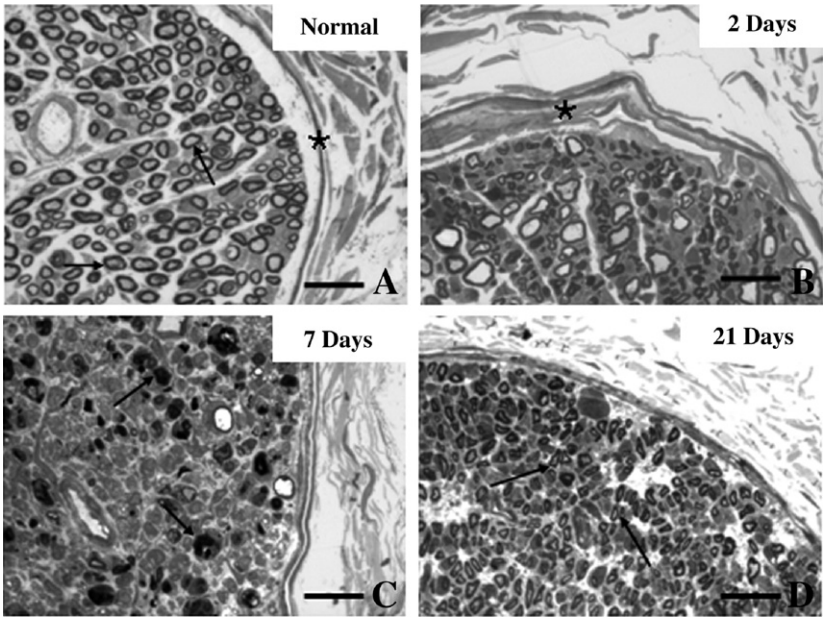

Fig. 1 – Transverse semithin sections of sural nerve stained with Toluidin Blue. (A) Control nerve showing characteristic myelinated fibers (→) and epineurium (*). (B) Nerve on the 2nd day after lesion showing degenerating nerve fibers and areas of epineurium thickening (*). (C) Nerve on the 7th day after crush, showing several degenerating fibers (→). (D) Nerve on the 21st day after lesion showing small myelinated fibers (→). Scale bars=40 μm.