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Research Report

NDGA reduces secondary damage after spinal cord injury in rats via anti-inflammatory effects

Hui Xue[a], *Xiu-ying Zhang*[c], *Jia-mei Liu*[a,*], *Yu Song*[d,a], *Ting-ting Liu*[a], *Dong Chen*[b,**]

[a]Department of Histology & Embryology, Bethune School of Medical Science, Jilin University, Changchun 130021, China
[b]Department of Histology & Embryology, Guangdong Medical College, Dongguan 523808, China
[c]Department of Fundamental Nursing, School of Nursing, Jilin University, Changchun 130021, China
[d]Department of Anatomy, Changchun Medical College, Changchun 130021, China

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ABSTRACT

After spinal cord injury (SCI), a series of complex pathophysiological processes follows the initial injury. Because inflammation plays a key role in this secondary pathology damage, anti-inflammatory drug treatment may reduce secondary damage and protect neurons after SCI. Though nordihydroguaiaretic acid (NDGA) can inhibit inflammatory responses, its potential roles in neuroprotection and anti- inflammation in an SCI model have not been studied. In this study, we investigated the anti-inflammatory effects of NDGA in SCI. First, histopathological alterations were evaluated with hematoxylin/eosin (HE) and Nissl staining, showing an increased number of neurons after NDGA administration. Additionally, the extent of secondary damage was assessed by TUNEL assay and measurement of astrocyte proliferation. The data showed that the numbers of apoptotic cells and the proliferative extent of astrocytes were significantly decreased by the use of NDGA. The anti-inflammatory effect of NDGA was evaluated by measuring myeloperoxidase (MPO) levels as an indicator of neutrophil activity, macrophage/microglia numbers, and expression of inflammatory cytokines including IL-1β and TNF-α. NDGA treatment significantly decreased the MPO level and the number of macrophages/microglia. In addition, NDGA also suppressed the expression of IL-1β and TNF-α after SCI. These data suggest that anti-inflammatory action by NDGA can reduce secondary damage after SCI

1. Introduction

Spinal cord injury (SCI) is a traumatic event that has great physical, psychological, and social impacts on individuals, families, and society. After SCI, an inflammatory response is initiated, which plays an important role in the progression of secondary destructive phenomena (Fleming et al., 2006; Sinescu et al., 2010). During the immune response after SCI, inflammatory events in the first few days result in the most destructive activity (Leskovar et al., 2000; Sinescu et al., 2010). Those early inflammatory events also create a hostile microenvironment for various SCI treatments such as cell transplantation, thus creating obstacles for transplantation-based therapies (Coyne et al., 2006; Okano et al., 2003). The early

*Corresponding author.
**Corresponding author. Fax: +86 769 22896 395.
E-mail addresses: liujiamei100@yahoo.com.cn (J.-m. Liu), 527414465@qq.com (D. Chen).

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immune response after SCI is induced by neutrophilic signaling and magnified by neutrophil-activated macrophages and microglia (Hirose et al., 2000; Liu et al., 2011; Taoka et al., 1998). Thus, anti-inflammatory treatment that prevents neutrophil and macrophage inflow, activation of microglia, or phagocytic and secretory activity of macrophages can be administered to improve the microenvironment for cellular transplantation after SCI (Sinescu et al., 2010).

Nordihydroguaiaretic acid (NDGA) is a selective 5-LOX inhibitor from the creosote plant (Larrea tridentata) (Lu et al., 2010b). It has broad medicinal properties including inhibition of inflammation (Bhattacherjee et al., 1988; Salari et al., 1984), oxidation (Floriano-Sanchez et al., 2006; Lu et al., 2010a), virus activity (Craigo et al., 2000; Hwu et al., 2008), and tumor growth (Kubow et al., 2000; Nishimura et al., 2002; Park et al., 2004). Studies have shown that NDGA application in amyotrophic lateral sclerosis and chronic colitis can inhibit inflammation responses to promote tissue repair (Fitzpatrick et al., 1990; West et al., 2004). It has also been shown that NDGA can promote the survival of microencapsulated allogeneic islets by preventing the activation and chemotaxis of macrophages (Yang et al., 2005). In this study, we demonstrated that NDGA has a neuroprotective role through its anti-inflammatory effects after SCI.

2. Results

2.1. *Reduction of secondary damage and neuroprotection by NDGA after SCI*

HE staining illustrated that at 1 week after operation, the injured area of spinal cord in two groups was filled with connective tissue or formed a large cavity. Surrounding the lesion, significantly fewer neurons survived in the injury group compared with the NDGA group (Fig. 1). In line with the HE results, Nissl staining showed that the number of neurons in the NDGA group was significantly more than the injury group (Fig. 2). Neuron counting results showed that the difference between the two groups was statistically significant (Fig. 2), suggesting that NDGA promoted the survival of neurons after the injury. TUNEL staining showed that apoptotic cells were dramatically reduced in the NDGA group (Fig. 3), suggesting there was less neuronal apoptosis in the NDGA group. GFAP immunofluorescence staining 1 week post-operation showed that compared with the injury group, the percentage of GFAP-positive area in the NDGA group was significantly reduced (Fig. 4). This indicated that the application of NDGA reduced the proliferative extent of astrocytes after SCI, further supporting its role in limiting secondary damage.

2.2. *Anti-inflammation effects of NDGA in SCI*

The MPO value measured results of postoperative day 3 demonstrated that the MPO values of the control group were significantly higher than those of the NDGA group (Fig. 5), suggesting that NDGA reduces neutrophil infiltration after SCI. The ED-1 immunofluorescence staining showed that the number of ED-1 positive cells in the NDGA group was significantly decreased compared with the injury group (Fig. 6). This result indicated that the NDGA application reduced the infiltration of macrophages/microglia after SCI. The results of Western blots for IL-1β (30 kD) and TNF-α (17 kD) showed a significantly decreased level in the NDGA group (Fig. 7), indicating that NDGA can efficiently decrease inflammatory factors in damaged spinal cord tissues. Taken together, the above data suggest that NDGA reduced the inflammatory response by inhibiting the inflammatory cells and molecules in SCI.

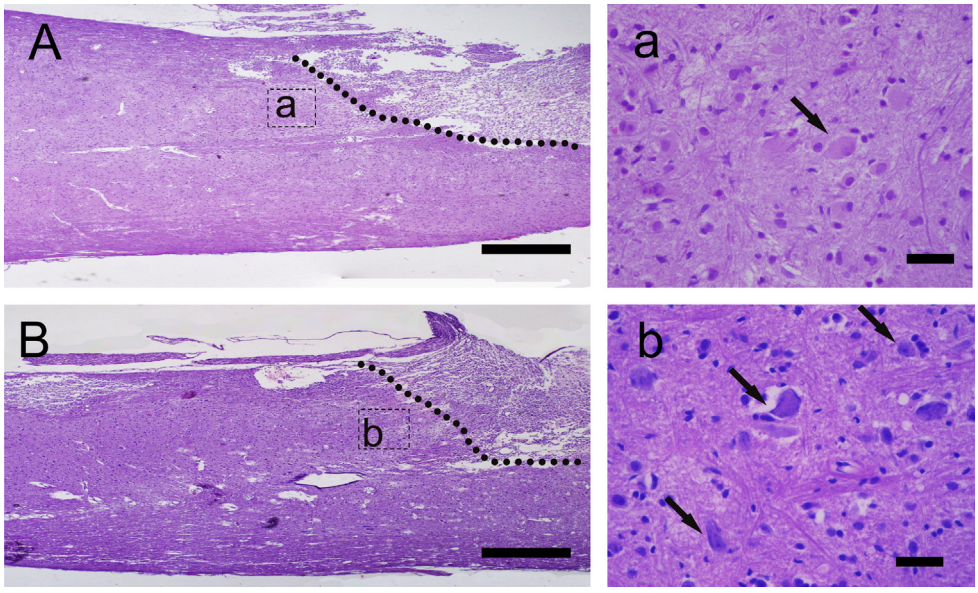

Fig. 1 – Coronal sections from each group at 1 week post-surgery stained with HE. Images of representative sections are from the injured control group ((A), (a)) and the NDGA-treated group ((B), (b)). Note that injured areas are filled with connective tissue. More neuronal survival is observed within the peripheral region of damage in the treated group than the injury group. Broken lines mark the lesion borders of injured spinal cords. Arrows indicate surviving neurons. Scale bars: (A) and (B), 625 μm; (a) and (b), 30 μm.