Nuclear Factor Kappa Beta (NF-κB) and Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) Expression in Spinal Cord Injury of Rats

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Abstract: Spinal cord injury is a life threatening condition. It is essential to evaluate spinal cord injury in the field of forensic medicine. Spinal cord injury affects the function of neurons and glia cells with inflammation and degenerative effects. Male Sprague Dawley rats (n:20) were divided as control, and trauma. Animals were applied a surgical procedure causing spinal cord injury and treated for 7 days then sacrificed under anesthesia. The spinal cords were dissected and fixed in 10% formaldehyde solution for immunohistochemical evaluations. In spinal cord injury increased cytokine activity was increased by increasing Nuclear factor kappa beta (NF-κB) expression in macrophage cells and apoptotic neurons and glial cells due to inflammation around blood vessels due to spinal cord injury. Platelet endothelial cell adhesion molecule-1 (PECAM-1) expression was positive in endothelial cells, PECAM-1 expression was significant in some hypertrophic funicular and radicular cells in substantia grisea. The most important factors in spinal cord injury are to increase the proteins that cause inflammation by disrupting the blood vessel structure and to show the activation of signaling pathways in inducing angiogenesis.

Keywords: Spinal cord injury, Nuclear factor kappa beta (NF-κB), PECAM-1, rat

1. Introduction

Spinal cord injury may be the main cause of disability or death after traumatic events. Therefore, the determination of spinal cord injury is very essential in forensic medical evaluation. In spinal cord injury (SCI), motor, sensory and autonomic dysfunction may be seen at all spinal cord levels (1). Rat models can be used to develop treatment modalities and to understand the pathophysiology of spinal cord injuries. Several studies have been conducted on spinal cord injury in rats in recent years.

It has been shown to induce neuron and glial cell loss parallel to the increase in inflammation, although it causes damage to the vascular wall structure (2,3).

Oxygen derived free radical formation originating from spinal cord neuronal lesion leads edema and inflammatory response. Loss of sensory, motor and vegetative sensory system abilities, chronic pain, muscle cramps, urinary system diseases can be seen after spinal cord injury (4).

It has been shown that vascular endothelial cells that cross endothelial cell-cell junction during inflammatory processes have an important role in vascular regulation. These proteins are key modulators of integrity of endothelial barrier and neuroinflammation caused by endothelial damage and have been reported to occur in response to traumatic injury (5).

Platelet endothelial cell adhesion molecule 1 (PECAM-1) has been reported to have a key role in the selective targeting of lymphocytes (6,7).

The main participant inflammation cells in SCI are endothelial cells, macrophages, microglia and astrocytes. Nuclear factor kappa beta (NF-κB) transcription factors in the spinal cord are prominent in glial cells and blood vessels that function primarily to mediate various mechanisms such as inflammation. The NF-κB signal transduction pathway has been shown to play role both in interfering the mechanism of SCI and in the repair of SCI (8).

The purpose of current study is to show the mechanisms of vascular endothelium and inflammation causing structures in spinal cord injury by immunohistochemical method.

2. Material and Method

All experimental protocol approvals were taken from local ethical committee. The study was carried out in guidance of the Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing 200–230 g were kept in a room with 12-h light: 12-h dark cycle, with the 22±2ºC temperature and 60±10% humidity. Twenty rats were divided as control group (group 1, n=10) and spinal cord injury group (group 2, n=10). Rats were anesthetized with anesthetics including combination of ketamine (50 mg/kg intraperitoneal) and chlorpromazine (1 mg/kg intraperitoneal). A rectal probe was used to monitor body temperature of rats positioned on a heating pad. Spinal processes and arcus lamina of T7-10 vertebrae were removed after T5-T12 midline incision and paravertebral muscle dissection under sterile conditions. The duramater was left intact. Weight-drop induced SCI model was
performed. The animals were forced to an impact of 100 g/cm to the dorsal side of the spinal cord. The rats were forced by a 3 mm diameter tip weighting 10 g stainless steel rod with rounded surface. The rod was left perpendicularly through a 10-cm guide hole to the center of the spinal cord (9). The incisions were sutured then. Following the procedure, the rats were taken to room to maintain normal body temperatures until they wake up completely. All rats were sacrificed under anesthesia 7 days after the surgical procedure.

Spinal cord tissues taken from T7-10 segments were extracted. Specimens were submitted in a 10% formalin solution, and embedded in paraffin blocks for histopathologic evaluation. 5µm sections taken from paraffin blocks were stained immunohistochemically for light microscopic evaluation.

Immunohistochemical Staining

Sections were put to distilled water and washed 5 minutes for 3 times in phosphate-buffered saline (PBS) (catalog no. 10010023, Thermo Fisher Scientific, Fremont, California). Antigen retrieval was performed using a 700 watt microwave (Bosch) for 2 minutes at 90°C. They were treated with a heating process in a microwave oven at 700 watts in a citrate buffer (pH 6.0) solution for proteolysis. Sections were washed 3×5 min in PBS and incubated with hydrogen peroxide (3 mL 30% H2O2+ 27 mL methanol) for 20 minutes. Sections were washed 5 minutes 2 times in PBS and blocked with Ultra V Block (Thermo Fisher) for 8 minutes. After draining, primary antibodies were directly applied to sections distinctly (Endothelin-1 mouse monoclonal antibody, 1:200, Nuclear factor kappa beta (NF-κB) monoclonal antibody, 1:200). Sections were incubated at 4°C during the night. Sections were washed 5 minutes for 3 times in PBS and then incubated with biotinylated secondary antibody (Thermo Fisher) for 14 minutes. After washing with PBS, streptavidin peroxidase (Thermo Fisher) was applied to sections for 15 minutes. Sections were washed 5 minutes for three times in PBS, and diaminobenzidine (DAB) (Thermo Fisher) was applied to sections for 10 minutes. Slides showing reaction in PBS were ceased (10). Counterstaining was performed with Harris hematoxylin for 45 seconds, dehydrated through ascending alcohol, and cleared in xylene. Slides were mounted with Entellan (Sigma-Aldrich) and examined under an Olympus BH-2 light microscopy.

3. Results

Immunohistochemical examination showed negative expression of NF-κB in funicular and radicular cells within the substantia grisea and in glia cells and in glial cells and basket cells in the substantia alba in control group sections (Figure 1a). In spinal cord injury group, macrophage cells around dilated blood vessels and degenerated neurons and glia cells increased NF-κB expression (Figure 1b). Negative PECAM-1 expression was found in endothelial cells in the blood vessel wall and in some glia cells in another control group section (Figure 1c). In the spinal cord injury group, PECAM-1 expression was positive in blood vessel endothelial cells showing significant dilatation and in some hypertrophic funicular and radicular cells in the substantia grisea (Figure 1d).

Figure 1: Explanations are given in the text.
4. Discussion

Spinal cord injury is one of the cornerstone of forensic medical examination. Spinal cord injury has been reported to be associated with high-energy trauma (11-12). There are many children with spinal cord injury who had traumatic myelopathy but without overt vertebral column damage shown with plain X-ray, computed tomography, myelography, and dynamic flexion/extension X-rays (13). Spinal cord injury may be associated with death in shaken baby syndrome even there is no evidence of trauma with physical examination and imaging which is an important issue in forensic medical evaluation (14). It is important to address whether the trauma threatens life or not in the forensic medicine evaluation in Turkish Criminal Code. Because, spinal traumas are life threatening injuries (15).

During the acute phase of SCI neutrophils, monocytes, lymphocytes, and microglias are collected and release inflammatory cytokines (16). At low concentrations, these pro-inflammatory cytokines can trigger inflammation to protect normal tissues in the spinal cord. If pro-inflammatory factors are overexpressed, they tend to activate transcription factors, such as NF-κB, which can lead to cell death, changing the positive to negative role of pro-inflammatory factors (17). NF-κB is very important among the transcription factors which are the negative result of inflammation.

Various pro-inflammatory molecules, such as TNF-α, IL-1, IL-6, and inducible NOS, have been reported to mediate the core NF-B complex, possibly causing inflammation in the spinal cord. NF-κB has been reported to play a role in apoptosis. In the early stage of SCI, TWEAK (TNF-like weak inducer of apoptosis) showed that its effect on NF-κB expression could combine proinflammatory factors to provoke cell apoptosis (18). Loss of oligodendrocytes in rats has been reported to cause axonal demyelination and peaks approximately 24 hours after injury. When demyelination occurs in SCI, microglia phagocytes remove myelin debris (19). In our study, increased cytokine activity was increased by increasing NF-κB expression in macrophage cells and apoptotic neurons and glial cells due to inflammation around blood vessels due to spinal cord injury.

PECAM-1 has been shown to regenerate angiogenic and antiapoptotic isofoms, as well as repair and remodel the damaged blood-brain barrier (20,21). PECAM-1 mediated activation of the phosphoinositide-3-kinase-protein kinase B (PI3K-Akt) pathway upregulates NF-κB-mediated transcription to facilitate angiogenesis, cell survival/growth, and the recovery of the endothelial cell barrier (22,23). Cetin et al. reported that an increase in VEGF expression levels, vascular permeability, and rapid interaction of VEGF receptors in endothelial cells lead to destruction of the vascular wall in the blood brain barrier and edema. It has been reported that the neuroprotective function of VEGF results from a combination of direct neuroprotective effects and stimulation of angiogenesis (24). Spinal cord injury caused dilatation of the blood vessel, PECAM-1 expression was positive in endothelial cells, PECAM-1 expression was significant in some hypertrophic funicular and radicular cells in substantia grisea. As a result, the most important factors in spinal cord injury is to increase the proteins that cause inflammation by disrupting the blood vessel structure and to show the activation of signaling pathways in inducing angiogenesis. Current findings from this study may guide the clinician in spinal cord injury in forensic medical examinations.

References


