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Research Article

Endocan; a Novel Inflammatory Indicator for Spinal Trauma? an Experimental Study in Rats

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Abstract:

Aim: The prognosis of spinal cord injuries is determined through the use of various inflammatory markers and scoring systems. In this study, it was aimed to investigate the relationship between trauma severity and serum endothelial cell-specific molecule 1 (ESM-1; Endocan) levels, a novel inflammatory marker, in an experimental spinal cord trauma model in rats.

Materials and Methods: Sixteen adult male Sprague-Dawley rats were assigned to 2 groups: control (Group 1) and trauma (Group 2). The spinal cord injury was induced by a modified Allen's weight-drop technique. Tail blood was collected from the subjects in both groups at 0 minutes, 6, 24, and 48 hours, centrifuged at 360 rpm for 10 minutes and endocan levels were quantified. All rats were sacrificed after 48 hours. We evaluated all tissue samples obtained from the trauma area by light microscopy on H&E-stained slides following routine tissue examination in the pathology laboratory. Neuronal degeneration was scored semiquantitatively according to nuclear shrinkage, pyknosis, and hyperchromasia, with a score of 0 representing normal/almost normal histology, and mild, moderate, and severe degeneration as scores 2, 3, and 4, respectively. ANOVA and one sample T-tests were used to analyze the difference in endocan levels between the 2 groups and the results were confirmed by Friedman and Paired sample T-tests ($p<0.005$).

Results: A significant difference was found between both groups in endocan levels measured at 24 and 48 hours ($p=0.00$). There was a relatively small difference between the two groups in endocan levels measured at 0 min; however, this difference was not significant ($p=0.067$). The pathological examination of the tissues revealed a score of 1 for neuronal degeneration in Group 1, a score of 2 for two subjects, a score of 3 for four subjects, and a score of 4 for two subjects in Group 2. Serum endocan levels of rats were correlated with neuronal degeneration scores determined after the pathological examination.

Discussion: In this experimental study in rats, serum endocan levels increased significantly after spinal cord injury. Elevated serum endocan levels could be used as a prognostic marker to predict the extent of neuronal damage and to determine the need for clinical follow-up after spinal cord injury. Further studies are needed to elucidate the pathways of increase in serum endocan levels.

Keywords: esm-1; endocan; spinal cord injury; spinal trauma; neuronal damage

Introduction

Spinal cord injury (SCI) is one of the most important public health problems today since it is a devastating neurologic condition that leads to a number of serious physical, psychological, and economic complications, and there is currently no universally accepted treatment protocol [1, 2]. Its incidence varies between 20-40/1000.000 and it is known that the mean age of these patients is 16-30. [3, 4]. Motor vehicle accidents, falls, gunshot wounds, penetrating injuries from sharp objects, and sports-related injuries are the most common causes of SCI [5]. In light of the significant costs incurred by these patients for their treatment and care, as well as the loss of their labor force and income, as well as their social and psychological problems, this is a serious health issue that impacts not only the patient but also his or her family and the national economy [6].

It has been well-documented in experimental and clinical studies that primary and secondary damage occurs after SCI. Primary damage is the injury at the time of trauma. Meanwhile, secondary damage is a form of damage that develops after the primary damage and occurs with different physiopathological mechanisms including hemorrhage, edema, axonal or neuronal necrosis, demyelination, cyst formation, and ischemia in a longer process [7]. In the literature, some biochemical markers such as IL-6, MDA, and TNF-alpha, are used as indicators of spinal cord injury. However, none of these have yet become routine diagnostic and prognostic markers. Endothelial cell-specific molecule-1 (ESM-1), or endocan, is a soluble proteoglycan that can be measured freely in law and is usually released from vascular endothelium in inflammatory cells. Endocan is known to be a part of the chain of angiogenesis and endothelial cell cells required for inflammatory process cells [9]. Serum endocan levels are elevated alone or in combination with other biomarkers for different conditions, including some cancers (eg, brain, brain, growth, electrical and ventilation), systemic inflammation, and birth defects [9]. The operation performed shows tumor progression in cancer patients in serum endocan overgrowth and their spread in cases of endothelial cell structures or dysfunction in inflammatory diseases [10, 11]. Recent epidemiological study suggests that endocan is an inflammatory marker of endothelial dysfunction [12]. There is no data on SCI and endocan levels in the literature. The aim of this study was to determine the correlation between SCI, which is known to initiate an inflammatory process, and endocan levels and whether endocan levels have prognostic value in trauma severity.

Material and Methods

This experimental study was conducted in the laboratory after obtaining the approval of the ethic committee (No: 401). 16 male Sprague-Dawley rats (weighing between 250-300 g) were used for this study and randomly divided into 2 groups as; Group 1: Laminectomy (L) group (n: 8) (Control group)

and Group 2: Laminectomy+ Trauma (LT) group (n: 8).

Surgical Procedure

After general anesthesia (60 mg/kg of ketamine hydrochloride IP-Ketalar, Pfizer İstanbul, Turkey and 5 mg/kg of xylazin -Rompun, Bayer, İstanbul, Türkiye) the rats were stabled to the operating table by supporting the abdominal regions with a sponge in the prone position. After skin cleansing with povidone iodine scrub, a 3 cm midline incision was made over the spinous processes in the lumbar region. After dissecting the paravertebral muscles, a single-distance laminectomy was performed. The control group was only administered laminectomy. After laminectomy of the L4-5 vertebrae, the dura mater was exposed (Figure 1).

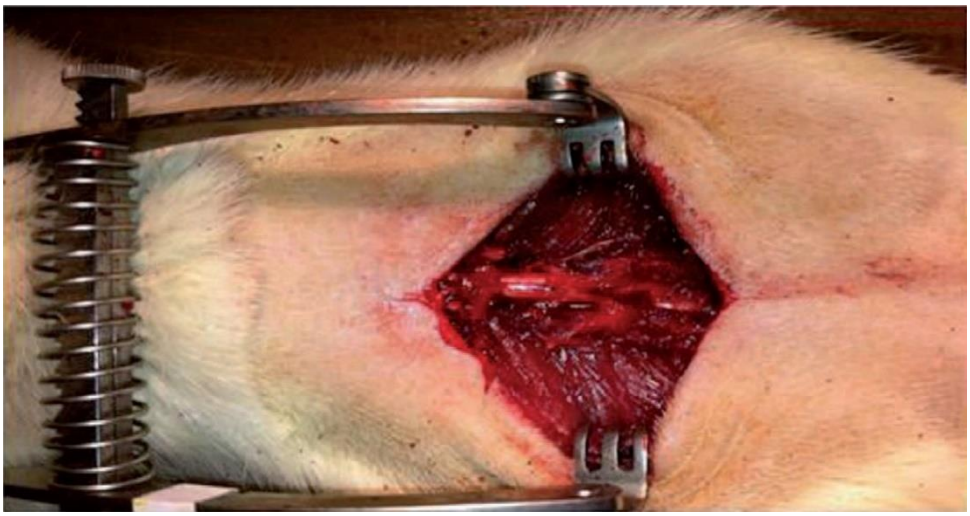

Figure 1: Dura mater exposed after laminectomy

Trauma procedure

Using the Modified Allen's weight-drop technique, a 5-mm-diameter cylindrical glass tube was positioned at a 90º angle on the surface of the exposed dura mater, and a 4g cylindrical constant weight was dropped from a 10-cm height through the tube onto the spinal cord [13].

Biochemical analysis

Tail blood was collected from both groups, a total of 4 times starting from the moment of trauma, at 0 minutes, 6, 24, and 48 hours. After centrifugation at 360 rpm for 10 minutes, the separated sera were stored at -80°C. Serum endocan concentration was measured by ELISA method via a manual endocan kit [Boster Biological Technology, USA).

Histopathological evaluation:

After routine tissue follow-up in pathology laboratory all tissue samples were evaluated in HE stained slides by light microscope. The extent of tissue damage was evaluated on the basis of neuronal degeneration and necrosis. The neuronal degenaration was scored semi-quantitatively according to the nuclear shirankage, pyknosis and the hyperchromasia. Score 1 represented normal-almost normal histology whereas mild, moderate and severe degeneration was scored as 2, 3 and 4 respectively.

Statistical analysis

Statistical analysis was conducted on a computer using the SPSS version 22.0 program. Normally distributed numerical data were expressed as mean ± standard deviation, while non-normally distributed data were expressed as median (minimum-maximum). Categorical variables were expressed as frequency (percentage). Group comparisons for variables with normal distribution were conducted by one sample t-test and ANOVA, and group comparisons for variables without normal distribution were conducted by the Shapiro-Wilk test. Correlation analysis between numerical variables was conducted using Paired sample t-test. P<0.05 was considered statistically significant.

Results

Biochemical results

Our study consisted of 16 subjects divided into two groups: Group 1: L (n:8) and Group 2: LT (n:8). According to results there is an increase in endocan levels each groups and this increase creates a significant difference between groups (p<0.05) (Table 1). When the control group and trauma groups is compared, there is a significant difference between control and trauma groups after 24h and 48h time periods (p<0.05). On the other hand, there is no significant difference after 6 hours, and there is a relatively small differene between the group 1 (Table 2).

	Mean Difference	Std. Error	Sig. (p)
Control	.36000	.05877	.001
0th min	.53125	.03336	.001
6th hour	.38125	.03260	.003
24h hour	.82875	.04605	.002
48th hour	.74375	.05364	.001

Table 1. Mean values of serum Endocan levels for each group

The differences in the mean values among the groups are greater than would be expected by chance; there is a statistically significant difference (p=<0,005).

	Mean	Std. Deviation	Sig (p)
Pair 1 Control- 0th min	-.17125	.22408	.067
Pair 2 Control- 6th hour	-.02125	.21663	.789
Pair 3 Control- 24h hour	-.46875	.18795	.000
Pair 4 Control- 48th hour	-.38375	.17254	.000

Table 2. Mean values of serum Endocan levels for control- each group and their p values

Histopathological results

In the light microscopic examinations of the L group, the gray and white matter neuroglial structuring of the spinal cord was normal/almost normal (Score 1) (Figure 2). The neuronal degeneration scores of the LT group were score 2 (n:2), score 3 (n:4), and score 4 (n:2) (Figure 3). Mean neuronal degeneration scores (NDS) were 0 and 1.125 (p=0.001) for Groups L and LT, respectively. Differences between neuronal degeneration scores were statistically significant among the two groups (p<0.05). Serum endocan levels of rats were correlated with neuronal degeneration scores determined after the histopathologic examination.

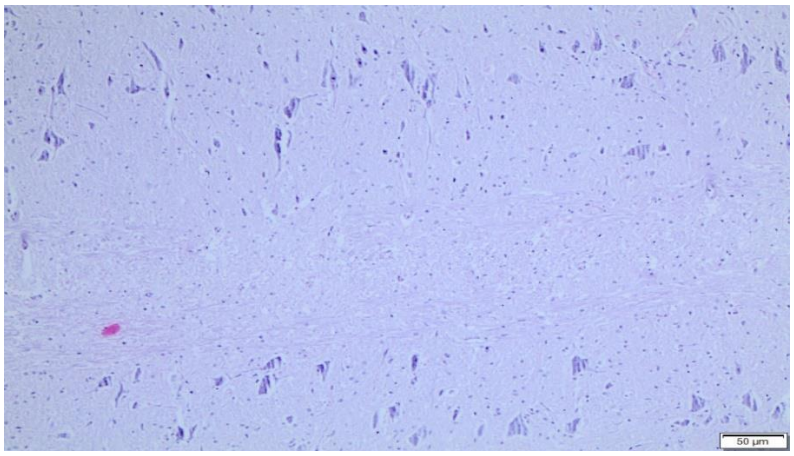

Figure 2. Non-degenerated normal neurons in normal spinal cord sections, score1 (H&EX100)

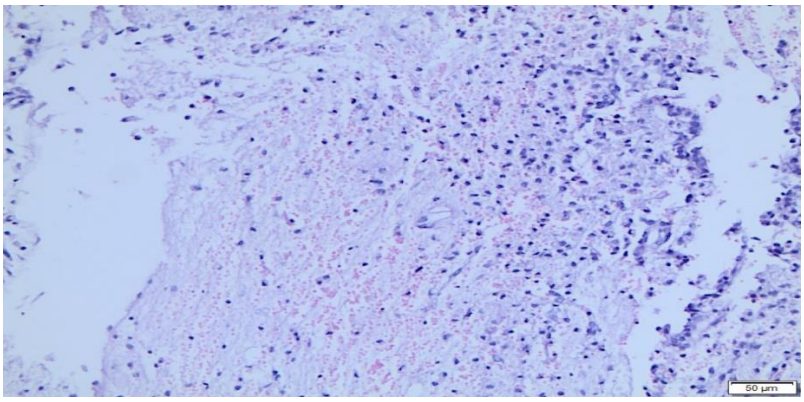

Figure 3. Diffuse hemorrage and congestion (H&EX100)

Discussion

We found that there was a statistically difference between control and trauma group regarding endocan levels. Furthermore, there was also positive correlation between the severity of SCI and endocan levels.

Primary injury is the initial injury caused by the mechanical trauma to the spinal cord at the time of injury. However, there is no treatment modality to reverse the effects of the primary injury [14]. Secondary spinal cord injury is a process that starts within minutes or hours and continues for weeks following the primary injury. In this period, many intertwined pathologic processes, especially ischemia due to impaired perfusion, are involved in the occurrence of secondary injury. These cascades have different sub-reactions of injury, mitochondrial damage, apoptosis, and cell death [7]. The aim of research on secondary spinal cord injuries is to find and use pharmacological agents and measures to protect neurons in the lesion area, which are still alive and connected with distal neurons after primary injury, to increase their resilience or to halt pathological processes that will damage them. Some of the secondary damage mechanisms include free radical theory, lipid peroxidation, and inflammatory changes [15]. A free radical is a chemical compound with an unpaired, free electron in its outer orbit. This electron is transferred to other biological molecules, leading to oxidation. Excessive increase in free radicals causes cell death [16,17]. A key step in ensuring the survival of cells is to prevent the formation of intense free radicals. Because potential oxygen-toxic metabolites are continuously formed in normal cellular respiratory processes. [15,18]. Even though neurological examinations, MRI evaluations, and electrophysiological examinations can provide insight into the severity of the disease in patients with spinal cord injury, all of them have limitations. For instance, neurological examinations in patients with multiple extremity fractures or uncooperative patients, and electrophysiological examinations due to loss of reflexes in patients with spinal shock may be inadequate in diagnosing and prognostic follow-up. Midden drop et al. emphasized the importance of the need to find potential biomarkers in patients with spinal cord injury due to these limitations [19]. Endocan is an indicator of angiogenesis and endothelial cell activation [8]. It was first described and reported in 1996 [20]. Endocan may be involved in molecular interactions required for the regulation of a wide range of biologically active modalities, such as cell adhesion, migration, and biological processes related to proliferation and

neovascularization. A group of specialized cells known as tip cells are more important for blood vessel growth and development than other cells known as stalk cells and mediate vascular growth by acting as sensors [9]. Furthermore, it is thought to be involved in the pathogenesis of vascular disorders, inflammation, and endothelial dysfunction [8]. Considering that endocan is released especially from inflamed endothelial cells, this suggests that its spread from vascular endothelial cells may also increase in inflammatory functions involved in the spinal cord injury process. The contents show that the serum shows a correlation with the pathology of endocan vessels [11]. In our study, the presence of serum endocan was correlated with the degree of neural degeneration performed histopathologically. This suggests that endocan is an inflammatory marker of endothelial dysfunction and may increase in color with the severity of the pathology [12]. In a study by Balta et al. showing the relationship between endocan in Behçet's Disease, a chronic inflammatory disease, plasma endocan levels in 33 Behçet's patients were found to be higher than in 35 healthy volunteers. The endocan level has shown a significant positive correlation with CRP, erythrocyte sedimentation rate, and disease activity [21]. Initially, Kose et al, comparing 53 acute coronary syndrome patients and 30 healthy controls, found that the expression levels of serum endocan in patients with acute coronary syndrome was significantly increased [22]. A study by Balta et al, including 18 newly diagnosed, untreated hypertensive patients, found that serum endocan levels were significantly higher in those hypertensive patients than in the control group [12]. Based on these findings, the researchers revealed that endocan levels were associated with disease severity and mortality and suggested that endocan may be a marker of endothelial dysfunction. On the other hand, our study had several limitations: the number of rats was insufficient, samples were not taken from CSF, and blood samples were taken only during sacrifice, rather than at different times after the trauma.

Conclusion

Serum endocan levels may be a novel biomarker of endothelial dysfunction in patients with spinal trauma and may be involved in the pathogenesis of spinal cord ischemia followed by contusion or compression injury. Therefore, endocan may be used to evaluate prognosis ischemia of spinal cord in patients with spinal trauma. Monitoring serum endocan levels can help identify high risk individuals, and early intervention, may reduce the prevalence of sekonder effects of spinal cord injury. However, additional prospective clicical studies are still needed to validate this conclusion.

Highlights

The primary injury resulting from trauma and the secondary injury, which is dominated by the inflammatory process and results in ischemia, are responsible for neurological damage following a spinal cord injury.

Endocan, secreted by vascular endothelial cells and is an indicator of angiogenesis and endothelial cell activation.

The serum endocan levels clearly increased especially 24-48 hours after spinal cord injury.

Increased endocan levels are observed in plasma of patients in the course of inflammatory processes like spinal cord injury suggesting that it might be a potential indicator of neuronal ischemia.

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