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Potential role of soluble biglycan induces the production of ICAM-1 following spinal I/R injury through ERK1/2 pathway

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Abstract

Neurologic deficit (paraparesis and paraplegia) after repair of the thoracic and thoracoabdominal aorta remains a devastating complication. The elusive mechanism of spinal cord ischemia-reperfusion (I/R) injury has delayed the development of pharmacological adjuncts. The objective of this study is to investigate the neuroprotective role of biglycan in spinal injury following I/R after aortic occlusion via down-regulation of ICAM-1 involving ERK1/2 pathway. Adult male Albino rats were underwent aortic occlusion for 5 minutes, followed by 48 hours of reperfusion when spinal cords were removed for analysis. Rat treated with biglycan attenuated ICAM-1 expression with down-regulation of ERK1/2 pathway. Further, biglycan decreased cytokine production and had preserved functional outcomes and neuronal viability after thoracic aortic occlusion. In conclusion, biglycan attenuated neuronal injury after thoracic aortic occlusion in rat through decreased the activity of ERK1/2 pathway that significantly attenuated the production of proinflammatory cytokines.

Keywords: Biglycan; ICAM-1; ERK1/2; Spinal cord I/R

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Introduction

Neurologic deficit (paraparesis and paraplegia) after repair of the thoracic and thoracoabdominal aorta remains a devastating complication [1]. The rationale for this approach to aortic repair deserves some explanation. Increased cerebrospinal fluid (CSF) pressure during aortic cross-clamping has been implicated as a cause of

neurologic complications [2]. Some studies have shown that adjunctive CSF drainage can prevent these problems [3, 4, 5].

Biglycan consists of a protein core containing leucine-rich repeat regions and two glycosaminoglycan (GAG) chains consisting of either chondroitin sulfate (CS) or dermatan sulfate (DS), with DS being more abundant in most connective tissues. The CS/DS chains are attached at amino acids 5 and 10 in human biglycan [6]. The composition of the GAG chains has been reported as varying according to tissue of origin. Non-glycanated forms of biglycan (no GAG chains) increase with age in human articular cartilage [7].

Mice lacking biglycan also display pathologies typically associated with skeletal aging. Specifically, by three months of age, hallmark signs of osteoarthritis (OA) are evident in the mutant mice, including fissures, cell clustering and loss of the smooth articular cartilage surface on the joints. The OA is detected in all weight bearing joints as well as in the temporomandibular joint of the jaw [8]. The effects of biglycan loss are exacerbated by depletion of the related small leucine-rich proteoglycan fibromodulin (Bgn-/0; Fmod-/- DKO).

Molecular studies point to the abnormal sequestration of the potent growth factor TGF-beta in the combined absence of biglycan and fibromodulin causing it to be "unleashed" and subsequently overactive. The uncontrolled stimulation of TGF-beta in this context leads to hyper- proliferation, premature differentiation of cartilage derived cells, MMP induction and, ultimately, loss of the condyle tissue integrity [9]. The objective of this study is to investigate the neuro-protective role of biglycan in spinal injury following I/R after aortic occlusion via down-regulation of ICAM-1 involving ERK1/2 pathway.

Method and material

Animals

Albino rats (weighing 180 - 220 g) were purchased from Animal Resource Center, the approval that has been established by the Institutional Animal Care and Use Committee (IACUC) and submission the required applications. They were housed in the animal house (for one week) in a temperature-controlled (25°±1C) room (humidity was kept at

(60–65%) with alternating 12-h light/12-h dark cycles and were allowed to access freely regarding water and chow diet until the time of starting the experimental study. All rat were kept and bred in-house under pathogen-free condition. All animal procedures used in this study were conducted in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Kufa University for Research Animal Care Committee.

Spinal ischemic model

We constructed the rat model of thoracic artery occlusion and reperfusion described previously [10]. The rat were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (40 mg/kg), occlusion was achieved by placing vascular clamps on the aortic arch distal to the left common carotid artery and the subclavian artery for 5 minutes. After 5 minutes of ischemia, the ligature line was pulled to achieve reperfusion until 48 hours. We maintained the room temperature at about 25°C during and after surgery, and exposed the animals to incandescent lamp to keep their rectal temperature at (37°C±1°C).

Neurological deficits assessment

Neurological deficits were examined at 24h and 48h after spinal I/R using a five-point scale adapted and modified from Bederson scale and an adaptation of the 'sticky tape test [11] with a minor modification as follows: 0 – no observable neurological deficits (normal), 1 -failure to extend right forepaw (mild); 2 – circling to the contralateral side (moderate); 3 – loss of walking or righting reflex (severe). The 30-min time point was selected because behavioral changes are marked at this time after ischemia.

ELISA detection of the levels of cytokines

The levels of plasma cytokines IL-6, TNF-α, IL-1β, ICAM-1 and ERK1/2 were measured by an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions. The ELISA intra-assay and interassay coefficients of variation were <5% and <10%, respectively. All of the samples were measured in duplicate as described previously [12].

Statistics

Responses over time were compared using one-way analysis of variance. Data from different treatment groups were compared using the t-test or the Mann–Whitney U-test, and expressed as mean±standard deviation (S.D.) or median±S.D. as appropriate. Categorical data were evaluated using the $\chi 2$-test statistic. Correlations were performed using Spearman's rho. Significance was set at $P \leq 0.05$.

Results

Neurological deficits

Neurologic scores were higher (i.e. neurologic dysfunction greater) in animals without treated with biglycan after at 48 hours. Treated rat had mild functional deficit (paralysis) at 24 hours after aortic occlusion, which was not significantly different from sham rat (Fig. 1).

Effects of biglycan on expression of ICAM-1

ICAM-1 is the key inflammatory mediators in several kinds of central nervous system injury and play an important role in the inflammatory response to traumatic spinal cord injury. Suppressed expression of this cytokine have been demonstrated in rat treated with biglycan compared to untreated rat ($P<0.05$) 24h, 48h after spinal cord I/R (Fig. 2).

Effect on Pro-inflammatory cytokines (TNF-α IL-6 and IL-1β)

Results revealed a significant increase in proinflammatory cytokines (TNF-α IL-6 and IL-1β) levels in the untreated rat group as compared with the biglycan treated rat (Fig. 3).

Biglycan reduced the level of ERK1/ERK2

The expression of ERK1/ERK2 were measured to further investigate its effect on inflammatory response during spinal I/R. As shown in Figure 4, spinal I/R injury

induced markedly increases ERK1/ERK2 in comparison to biglycan treated rat group after injury (P <0 .05).

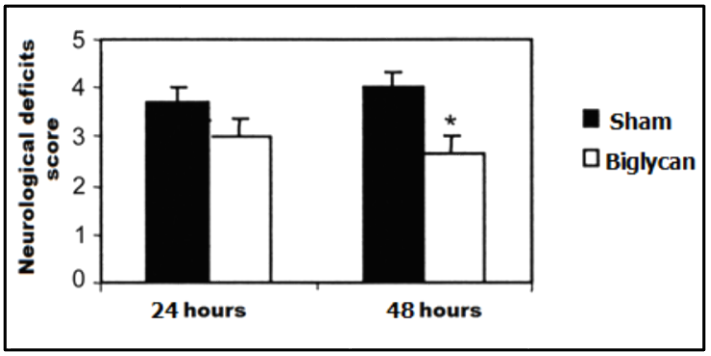

Figure 1.

Neurological deficits in sham and treated rat after spinal I/R

For transient aortic occlusion, untreated and biglycan treated rat were subjected to 4-min aortic occlusion followed by reperfusion, and neurological deficits were evaluated at 24 hours and 48 hours after reperfusion. Neurological deficits were measured using a five-point scale as described under "Experimental Procedures"; *P< 0.01 compared with the untreated animals.

Figure 2.

The levels of ICAM-1 is assay by ELISA. Biglycan treated rat tended to have decreased expression levels of ICAM-1 compared with the untreated rat. Statistically significant differences ($^{*}P<0.05$) were observed post spinal cord I/R.

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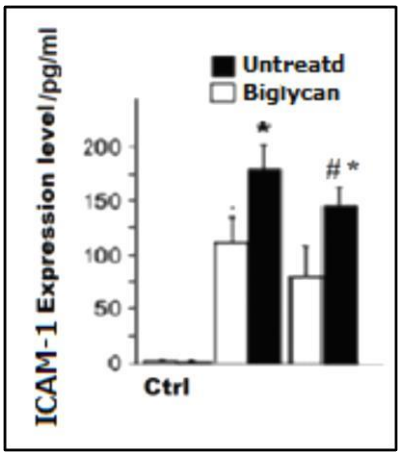

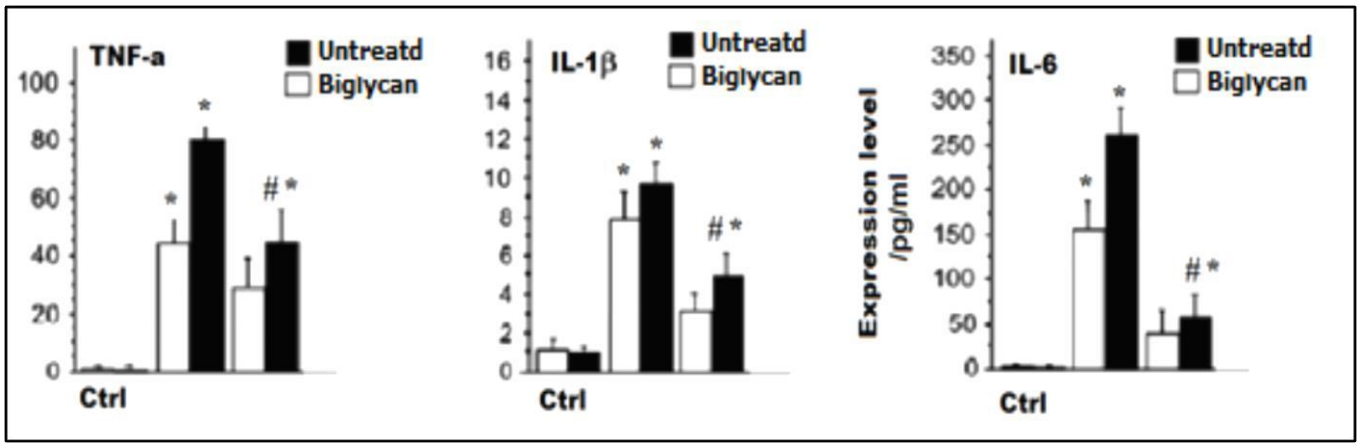

Figure 3.

The levels of pro-inflammatory cytokines (TNF□, IL-1□, and IL-6) are assay by ELISA. Biglycan treated rat tended to have decreased expression levels of cytokines compared with the untreated rat. Statistically significant differences ($^{*}P<0.05$) were observed post spinal I/R.

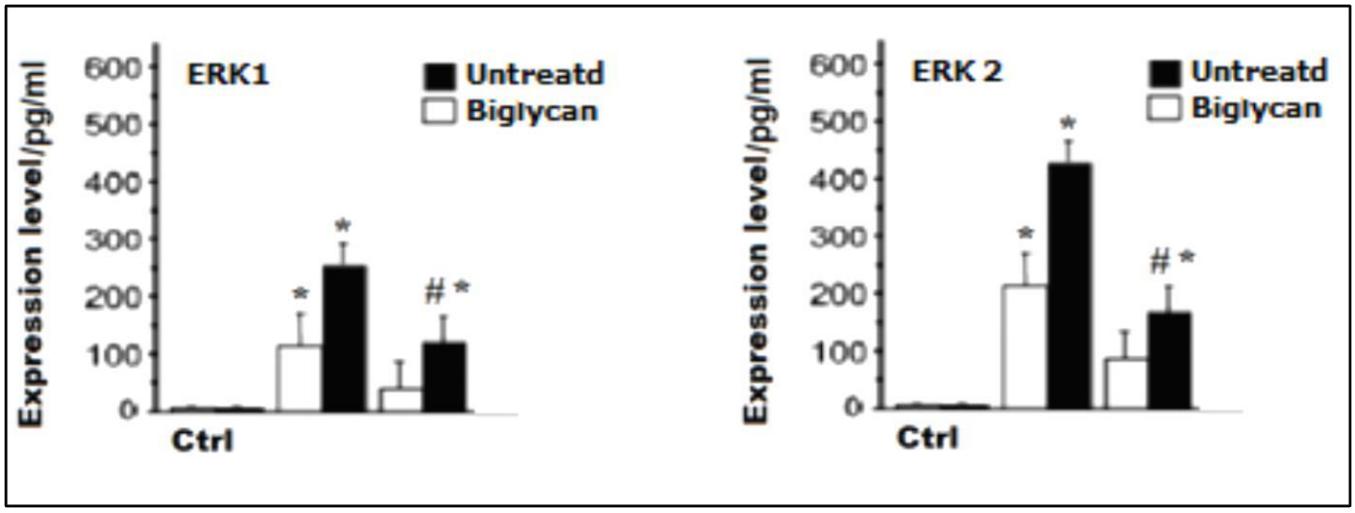

Figure 4.

The levels of ERK1/ERK2 are assay by ELISA. Biglycan treated rat tended to have decreased expression levels of ERK1/ERK2 compared with the untreated rat. Statistically significant differences ($^{*}P<0.05$) were observed post spinal I/R.

Discussion

This study is a pivotal demonstration of the anti-inflammatory effect for biglycan on a well-studied model of spinal cord injury. Paraplegia secondary to spinal cord ischemiareperfusion injury remains a devastating complication of thoracoabdominal aortic surgery [13, 14]. The complex interactions between the injured neurons and activated leukocytes have made investigation of neuron specific injury elusive. We hypothesize that the neuroprotective role of biglycan in spinal injury following I/R after aortic occlusion via down-regulation of ICAM-1 involving ERK1/2 pathway.

Spinal damage after ischemia and reperfusion is known to be modulated by soluble proinflammatory mediators such as cytokines, chemokines, adhesion molecules, and matrix metalloproteinases [15]. ERK1/2, appear to be of particular importance in regulating these injuries. After spinal cord IR, early chemokine production by local cellular populations may result in chemotaxis of more inflammatory cells that ultimately

lead to functional motor deficits [16]. Chemokine production by this second wave of inflammatory cells further amplifies the response and results in irreversible neurological injury. It is the accumulation of these molecular insults that reaches a threshold and thus results in clinically apparent neurological injury [17].

The I/R model used and the period observed were referred to previous studies, in which the evolution of inflammatory cytokine activation in the spinal cord peaks between 24 and 48 h after reperfusion [18]. The intact barrier can prevent vasogenic edema and pathological effects on CNS by restricting access of molecules and cells to the spinal cord under conditions of stroke and I/R [19]. It is generally believed that inflammatory factors play a critical role in leakage of BSCB as a result of aberrant vascular permeability due to dissociation of zonula occludens-1 (ZO-1) from the cytoskeletal complex and due to an increased level of matrix metalloproteinases (MMPs) and tumor necrosis factor α (TNF-α) [20]. As demonstrated in our previous study, BSCB permeability is altered in the course of spinal cord I/R injury, and these changes could be measured using extravasation of EB dye [21, 22, 23]. This damage may cause by inflammatory processes and it may further exacerbate inflammation. Recent evidence support the notion that microglia, components of the immune system are activated and play an important role in the initiation phase of I/R-induced neurodegenerative and inflammatory processes.

Furthermore, treatment with a murine anti-ICAM-1 antibody (enlimomab) has been investigated in patients with acute ischemic stroke in the Enlimomab Acute Stroke Trial (EAST). Unfortunately, the case fatality rate in this trial was significantly higher in the enlimomab patient group than in the placebo group [4].

In conclusion, biglycan attenuated neuronal injury after thoracic aortic occlusion in rat through decreased the activity of ERK1/2 pathway that significantly attenuated the production of proinflammatory cytokines.

Competing interests

The authors declare that there is no conflict of interest.

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