Dexmedetomidine relieves inflammatory response in rats with acute spinal cord injury through IKK/NF-κB signaling pathway

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Abstract

Purpose: To examine the effect of dexmedetomidine (DEX) on inflammatory response in spinal cord damage, and involvement of IKK/NF-κB signaling pathway in the process.

Methods: Sixty healthy male Wistar rats were selected and randomly divided into sham, control, IKK inhibition, and DEX groups. Rat hind limb motor function in each group at 1, 24 and 48 h after surgery was determined using Basso Beattie Bresnahan (BBB) method, while Western blot assay was used to evaluate the protein expression levels of IL-1β, IKK and NF-κB in spinal cord tissue.

Results: In DEX rat group, neuromotor function at 24 and 48 h, and protein expression level of IL-1β in spinal cord tissue significantly decreased, relative to IKK inhibition group (p < 0.05). There were higher protein expressions of IKK and NF-κB in IKK inhibition and DEX groups than in sham rats, but were lower than the corresponding values for model group (p < 0.05). However, these proteins were significantly downregulated in DEX group, relative to the IKK inhibition rats (p < 0.05).

Conclusion: DEX mitigates inflammatory response in rats with acute spinal cord injury via IKK/NF-κB signaling pathway. This provides some ideas for research and development of other drugs for the treatment of acute spinal cord injury.

Keywords: Dexmedetomidine, Acute spinal cord injury, Nuclear factor kappa B, Inflammatory response

INTRODUCTION

Acute spinal cord injury (ASCI), a neuronal traumatic disease, is a complication of SCI, which may lead to severe paralysis or even death [1]. Currently, surgery is generally used for the clinical treatment of ASCI, but this does not produce the desired clinical outcome, leading to severe physical and psychological pain in patients [2,3]. Apoptosis of spinal cord nerve cells is regulated by immune inflammatory response. It has been reported that ASCI induces secretion of large amounts of inflammatory cytokines and neurotoxins, which further lead to cellular damage in the central nervous system (CNS) [4]. Nuclear factor-Kappa B (NF-κB), a key factor in the expression of inflammatory genes, regulates inflammatory response in the CNS [5]. Studies have shown that IkK activates NF-κB, and that the IKK/NF-κB signaling pathway is
closely associated with the pathophysiological processes involved in ASCI-induced inflammation [6]. In clinical practice, DEX is used as a protective drug for neurons. However, there are limited studies on the neuroprotective effect of DEX in ASCI, and the mechanism involved [7]. In the present study, 60 healthy male Wistar rats were selected as experimental animals for investigation of the effect of DEX on a rat model of ASCI, and the involvement of the IKK/NF-κB signal route in this process.

EXPERIMENTAL

Animals

Sixty healthy male Wistar rats with mean age of 11.00 ± 1.00 weeks, and mean weight of 275 ± 25 g, were provided by Shanghai Regen Biotechnology Co. Ltd. All rats were adaptively fed for one week prior to commencement of the study. The animal studies were approved by the institutional animal ethical committee, and followed international guidelines for animal studies.

Establishment of model

All rats were fasted without water for 12 h before the operation for establishment of a rat model of acute spinal cord injury. The rats were fixed on a table and anesthetized with 10 % chloral hydrate. The back of each rat was skinned and thoroughly disinfected. The 9th thoracic vertebra (T9) was operated on. Both sides of the T10 - T12 lamina were exposed and allowed to make complete contact, so that the spinal cord was clearly exposed. The exposed spinal cord was hit with a Kirschner wire from a 3-cm free fall, resulting in acute spinal cord injury. The criteria for successful establishment of model were spinal cord edema and hemorrhage following spinal cord impingement, and tail wagging reflex in rats, with delayed paralysis of lower limbs after recovery from anesthesia. After surgery, all rats were injected intraperitoneally with 40,000 units of penicillin and 1 mL of glucose injection for 3 consecutive days, in order to prevent infection. Moreover, the rats were maintained on ordinary feed. Daily bladder massage was used to stimulate the establishment of spontaneous urination reflex in rats.

Grouping of rats

The 60 rats were divided into four groups: sham, model, IKK inhibition and DEX groups. Sham surgery animals (15 rats) had surgical laminectomy only, without acute SCI, while rats in model group (n = 15) were ASCI rats given only physiological saline. Rats in IKK inhibition group (n = 15) were ASCI rats subjected to intrathecal BMS-345541 (IKK inhibitor), while rats in DEX (n = 15) were ASCI rats treated with intrathecal DEX at a dose of 3 μg/kg.

Animal sacrifice, sample collection and analysis

All rats were euthanized by dislocating the spinal column from the skull, and the body surface of each rat was disinfected by soaking in 75 % ethanol for 5 min. Each rat was placed on ice, and the whole spinal column was put into a petri dish. The lamina of each rat in each group was cut so that the spinal cord could be completely removed and put into tissue buffer, and the spinal cord was preserved in a refrigerator at -80 °C.

Evaluation of neurological function

The BBB score was applied to determine hind limb motor functionality in rats in the four groups at 1, 24 and 48 h. The scores were within the range of 0 - 21, and they covered a total of 22 grades. The score for completely normal hind motor function was 21, while the score for immobility (paralysis) was 0.

Western blot assay

Total protein was extracted from spinal cord tissues of rats in each group by homogenizing the tissue in liquid nitrogen. After homogenization, the spinal cord tissue homogenates were placed on ice for 10 min, followed by ultrasonic lysis of cells for 1 min, and centrifugation at a speed of 1200 rpm at -4 °C for 15 min. The protein contents of the supernatants were determined using BCA method. Thereafter, equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis, followed by transfer to PVDF membranes which were subsequently incubated with 5 % bovine serum albumin solution for 2 h to block non-specific binding of the blot. Then, 12 h incubation with 1° antibodies was done at 4 °C, followed by incubation with fluorescence 2° antibodies at laboratory temperature. Then, the membranes were washed thrice with TBST, and the expression levels of IL-1β, IKK and NF-κB were obtained with image analysis software.

Histological and morphological examination of spinal cord tissues

Morphological changes in rat neurons were determined histologically using H & E staining method.
**Table 1:** Neuromotor function of the rats at different time periods \((n = 15)\)

<table>
<thead>
<tr>
<th>Group</th>
<th>1h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7.215±0.423</td>
<td>7.025±0.381</td>
<td>6.427±0.492</td>
</tr>
<tr>
<td>Model</td>
<td>7.267±0.482</td>
<td>23.481±1.437&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.746±1.637&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IKK inhibition</td>
<td>7.352±0.394</td>
<td>18.467±1.493&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.418±1.592&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DEX</td>
<td>7.294±0.372</td>
<td>12.592±1.516&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>14.282±1.053&lt;sup&gt;abc&lt;/sup&gt;</td>
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</table>

\(F = 0.28\)
\(P = 0.841\)

\(^aP < 0.05, \text{ vs sham operation rats}; ^bP < 0.05, \text{ vs model}, ^cP < 0.05, \text{ vs IKK inhibition rats}\)

**Table 2:** Protein expression levels of IL-1β in spinal cord tissues of the 4 groups at different time points \((n = 15)\)

<table>
<thead>
<tr>
<th>Group</th>
<th>1h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>183.17±9.24</td>
<td>185.93±7.93</td>
<td>187.56±12.42</td>
</tr>
<tr>
<td>Model</td>
<td>181.83±7.37</td>
<td>893.18±16.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1342.74±21.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IKK inhibition</td>
<td>182.49±8.43</td>
<td>534.56±12.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>783.41±14.84&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DEX</td>
<td>182.72±8.62</td>
<td>386.27±9.47&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>617.59±11.72&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

\(F = 0.06\)
\(P = 0.978\)

\(^a,b,cP < 0.05, ^a\text{ vs sham operation}; ^b\text{ vs model, } ^c\text{ vs IKK inhibition}\)

Spinal cord tissues were taken from the surgical area of rats and fixed in 4 % paraformaldehyde for 24 h. After dehydration, embedding, sectioning, H & E staining and slide sealing, pathological and morphological changes in spinal cord tissues were examined under a light microscope, and photographed.

**Statistical analysis**

All data in this study were analyzed with SPSS 21.0 software package. Measurement data are presented as mean ± standard deviation (SD). Two groups were compared with \(t\)-test. Enumeration results are presented \(n (\%)\], and two groups were compared with chi-square \((\chi^2)\) test. Statistical significance was assumed at \(p < 0.05\).

**RESULTS**

**Neuromotor function**

Neuromotor function was comparable among the four groups at 1 h \((p > 0.05)\). In contrast, 24-h and 48-h neuromotor function values in model group, IKK inhibition group and DEX group were markedly higher than sham values, but 24-h and 48-h neuromotor function values of IKK inhibition and DEX-treated rats were markedly reduced, relative to model rats \((p < 0.05)\). However, the 24-h and 48-h neuromotor function in DEX rats were markedly reduced, relative to IKK inhibition group (Table 1).

**Expression levels of IL-1β protein in spinal cord tissues**

At 1 h, protein expression levels of IL-1β in spinal cord of the four groups were unchanged. In contrast, IL-1β protein in spinal cord of model, IKK inhibitory and DEX animals were markedly increased, relative to the corresponding sham levels at 24 and 48 h, but IL-1β protein in spinal cord of IKK inhibition and DEX groups were down-regulated, relative to model at 24 and 48 h. In addition, there was markedly lower IL-1β protein levels in DEX group at 24 and 48 h than in IKK inhibition rats \((p < 0.05)\). These results are presented in Table 2.

**IKK and NF-κB proteins in spinal cord tissues**

There were significant up-regulations of protein expressions of IKK and NF-κB in IKK inhibition group and DEX group. However, IKK and NF-κB proteins in IKK inhibition group and DEX group were markedly decreased, relative to model \((p < 0.05)\). Moreover, compared with IKK inhibition group, IKK and NF-κB proteins in DEX group were significantly decreased \((p < 0.05; \text{ Table 3})\).

**Histopathological features of rat spinal cord**

Structures of spinal cord tissue and surrounding tissues of rats in sham operation group were intact and clear. In contrast, there were structural disorderliness and spinal cavity in model group,
indicating serious tissue damage. However, after treatment, there were decreased spinal cord injury, increased spinal cord neurons, and decreased syringomyelia. These results are shown in Figure 1.

Figure 1: Pathological changes in spinal cord tissues of rats in each group (H & E, ×200). A: Image of spinal cord tissue of rats in sham-operation rat; B: Image of spinal cord tissue model rat; C: Image of spinal cord tissue in IKK inhibition rat; D: Image of rat spinal cord in DEX group

DISCUSSION

The etiology of ASCI is complicated. Thus, development of an effective treatment strategy for ASCI has continued to pose a challenge to clinical researchers [8]. Currently, the medical community has made great progress in the study of the pathology of ASCI, although there has been no breakthrough in the study of clinical application of ASCI [9]. At present, clinical research on the treatment of ACSI may be divided into two aspects: one aspect is aimed at protecting neuromotor function so as to minimize secondary injury, while the second aspect deals with studies on development of methods for nerve regeneration [10].

The physiological and pathological processes of secondary ASCI injury are closely related to NF-κB, a key regulatory factor of the inflammatory genes. Indeed, the expressions of multiple cytokines in ASCI are closely associated with the regulation of inflammatory response [11,12]. It is known that IKK promotes the activation of NF-κB protein and regulates gene transcription. Therefore, it is of great clinical significance to find new and effective drugs for further studies on ASCI, with respect to the IKK/NF-κB pathway [13,14].

Dexmedetomidine (DEX) is a highly selective α-adrenergic receptor agonist which exerts analgesia, sedation, anti-sympathetic nerve activity, protective effect on spinal nerve, and significant inhibitory effect on inflammation in CNS [15]. The IL-1β protein levels in 24 and 48 h in spinal cord tissues of IKK inhibition group and DEX group were markedly higher than the sham operation values, but were low, relative to model values. Moreover, IL-1β protein expressions at 24 and 48 h were lower in DEX rats than in IKK inhibition rats. These results suggest that ASCI increased the release of inflammatory cytokines, while DEX down-regulated the expressions of these inflammatory cytokines.

Research has shown that NF-κB is crucial for activation pathway of IKK by regulating inflammatory response and cell proliferation in ASCI patients [16]. Previous studies have shown that DEX exerts a significant protective effect on neuronal function [17]. It reduces the excitability of glutamate and inhibits the release of tea aminophen in ASCI, which not only improves the blood supply to neurons, but also regulates neuronal apoptosis. In the present study, the protein expression levels of IKK and NF-κB were significantly increased in model group, IKK inhibition group and DEX group, relative to sham-operated rats. Moreover, the protein expression levels of IKK and NF-κB in IKK inhibition group and DEX group were markedly higher than the corresponding levels in sham group, but were lower than model group values. However, the protein expression levels of IKK and NF-κB were markedly down-regulated in DEX-treated rats, relative to IKK inhibition rats. Thus, DEX relieved inflammation in ASCI rats through suppression of IKK/NF-κB signal route.

The recovery of neuromotor function in ASCI patients can be used as an index for evaluation of the effectiveness of drug therapy (since the main purpose of ASCI treatment is the protection of nerve cells, and BBB score can be used as a standard for evaluating neural function in rats [18]). In this study, the 24 and 48-h neuromotor function levels of rats were markedly higher in IKK inhibition and DEX groups than in sham-operated rats, but were lower than the corresponding values in model group. The 24 and 48-h neuromotor function levels of rats in DEX group were significantly lower than those of IKK inhibition group. Results from H & E staining showed that the structures of spinal cord tissue and surrounding tissues of rats in sham operation group were intact and clear.

However, in the model group, there were blood cells, disordered structure, and spinal cavity in the spinal cord tissue, indicating that the tissues were severely damaged. In contrast, spinal cord injury was decreased, while the number of spinal cord neurons increased after treatment, and syringomyelia was reduced. These results suggest that DEX restored neurological function in rats with ASCI. There are currently no effective treatments for ASCI, although in recent years, continuous investigations by medical science researchers have led to some new ideas.
CONCLUSION

The findings of this study suggest that DEX suppresses inflammatory factors and alleviates inflammatory responses in ASCI rats via IKK/NF-κB signaling pathway. However, the drug concentration, timing and route of DEX application need to be further studied.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jinbao Wang designed the study, supervised the data collection, and analyzed the data. Haijuan Zhu interpreted the data and prepared the manuscript for publication. Haijuan Zhu, Jinbao Wang and Lihua Huang supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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