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Research Article

Tanshinone IIA Regulate Inflammatory Response and Promote Functional Recovery in Rats with Spinal Cord Injury

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Abstract

Purpose: The treatment of spinal cord injury (SCI) is a clinical challenge. The study attempted to investigate the effects of Tanshinone IIA on SCI in rats. *Methods*: The SCI model of rat was established based on Allen's animal model. The rats were randomly divided into four groups as follows: Control, Sham, SCI model, SCI + Tanshinone IIA. Rats were administrated with Tanshinone IIA (30mg/kg) respectively daily within one week after establishment of SCI model. Scores of Basso, Beattie, Bresnahan (BBB) was evaluated on the 1st, 3rd, 5th and 7th day after operation. Rats were sacrificed seven days after SCI, and the pathological injury of spinal cord tissue was assessed by HE staining. The levels of inflammatory cytokines (IL-1β and TNF-α) were detected by ELISA. *Results*: On the 7th day after operation, the BBB score of SCI + Tanshinone IIA group was significantly better than that of SCI group ($P<0.01$). Compared with SCI group, the pathological changes, neuronal pyknosis, hemorrhage, inflammatory infiltration, and white matter cavity formation in SCI + Tanshinone IIA group were reduced. Compared with SCI group, the level of IL-1 β and TNF-α in SCI+ Tanshinone IIA group were significantly lower ($P<0.01$). *Conclusion*: Tanshinone IIA can significantly improve motor function inhibit inflammation and repair spinal cord function after SCI.

Keywords

Tanshinone IIA, Spinal Cord Injury, Inflammatory

1 Introduction

Trauma spinal cord injury (SCI) is a devastating neurological condition that can lead to permanent disability. It is a heavy burden on individuals, families and society as a whole because of the loss of workforce, long-term rehabilitation, diversion of significant medical resources and expensive medical costs [1, 2]. The annual incidence of SCI worldwide is estimated at 10.4 to 83.0 cases per 1 million people [3, 4]. The study showed that the incidence from 45 cases per million population in 2009 to 66 cases per million population in 2018 [5]. As a traumatic disease with high disability rate, SCI reduces the quality of life of pa-

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tients to a great extent [6]. How to treat effectively is one of the most important directions of clinical research.

Tanshinone IIA is extracted from Salvia miltiorrhiza and has the effects of anti-inflammation, anti-apoptosis, antioxidation and neuroprotection [7]. It is often used in the treatment of diseases such as cardiovascular and cerebrovascular syndrome [8, 9]. At present, more and more studies have confirmed that Tanshinone IIA can increase the blood flow in the injured area of the central nervous system, inhibit neuronal apoptosis, reduce the ischemia-reperfusion injury and a series of pathophysiological injuries of nerve cells, protect nerve tissue and promote the recovery of nerve function [10]. However, The effects of Tanshinone IIA on the injury processes after SCI is still largely unknown.

In order to investigate the effects of Tanshinone IIA on SCI in rats, a rat model of SCI was established in this study. Tanshinone IIA was used to interfere with rats to study their effects on limb motor function, inflammation and histopathology after acute SCI in rats, and to explore the effect of Tanshinone IIA on neurological recovery in rats after SCI.

2. Materials and Methods

2.1. Animal

24 SPF SD rats (male, weighing 180-220 g) were purchased from Zhejiang Center of Laboratory Animals [license number: SCXK (Zhejiang) 2019-0002]. All rats were cultured at 20-25˚C with 40-70% relative humidity at a 12-h light and dark cycle, with free access to food and water. One week after adaptation, the rats were prepared for use. The animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Zhejiang Center of Laboratory Animals, and all animal care and experiments were conducted in accordance with the following Chinese laboratory animal standards: Guideline for ethical review of animal welfare (GB/T 35892-2018) and General requirements for animal experiment (GB/T 35823-2018).

2.2. Establishment and Experimental Design of SCI Model in Rats

Establishment of SCI Model in Rats based on Allen's Animal Model [11]. To put it simply, all rats were anesthetized with intraperitoneal injection of pentobarbital (30mg/kg), and T10 spinous process as the center to cut open the skin of the T9-11 spinous process on the back, separate the muscles on both sides of the spinous process, expose the spinous process and vertebral arch plate, bite off the spinous process and lamina with rongeur, expose the spinal cord, and hit the T10 spinal cord vertically with an Allen's batter, hitting height 5 cm, weight 10 g, diameter 2 mm. The model was considered successful when there were contusions in the injured site, spasm of both lower limbs and continuous swinging of the tail [12]. The paraspinal muscles and skin of rats were sutured layer by layer. Within 1 h after SCI, rats in all groups were intraperitoneally injected with penicillin 200 U / 100g for 3 days. After operation, the bladder of the rats was squeezed to perform artificial urinary aid until the spontaneous urination of the rats was restored.

After one week of adaptation, the rats were randomly divided into 4 groups according to the experimental design: Control group, Sham group, SCI group, and SCI+ Tanshinone IIA group. The Control group received no surgical treatment or drug intervention and was fed normally every day. In the Sham group, the spinal cord was exposed but not injured according to the above methods and was fed normally every day. In SCI group, the SCI model was established by the above method, and normal saline was injected intraperitoneally every day. SCI+ Tanshinone IIA group was given intraperitoneal injection of Tan IIA 30 mg/kg every day after operation. The drug intervention in each group lasted for one week.

2.3. Behavioral Score

The functional scores of rats in each group were evaluated on the 1st, 3rd, 5th and 7th day after SCI. The evaluation criteria of hindlimb motor function are based on the 21-point Basso, Beattie, Bresnahan (BBB) locomotor rating scale [12, 13]. The evaluation criteria of hindlimb muscle tension, motor and sensory function and spinal cord reflex function are based on the 11-point Reuter scoring system [14, 15]. The behavioral score was performed by three colleagues who did not know the content of the experiment, and the average score represented the score of each mouse.

2.4. HE Staining of Spinal Cord Tissue

All rats were anesthetized by intraperitoneal injection of pentobarbital (200mg/kg) at the end of the behavioral score on the 7th day after SCI modeling. Enter according to the original surgical incision, take the injured segment as the center, the upper and lower 0.5cm to obtain the spinal cord tissue, and obtain the spinal cord tissue length about 1.2 cm.

The spinal cord tissue specimens were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, and sliced with a thickness of about 5 μm. After routine dewaxing and rehydration, 5 min was stained with hematoxylin solution, soaked in 1% acidic ethanol for 5 s, 10 min was rinsed in distilled water, and 5 min was stained with eosin solution. Then different concentrations of ethanol were used for fractionation dehydration, and the slides were sealed with neutral gum. The images were taken and collected under microscope (×200), and the morphological changes of SCI in each group were observed.

2.5. ELISA

IL-1β and TNF-α ELISA kits (Jianglaibio, Shanghai, China) were used to detect the level of inflammatory factors in rat spinal cord tissue. The kit balances the 60 min at room temperature,

configures the required reagents and tests according to the manufacturer's instructions. A blank hole with 50 μL of sample diluent, a standard hole of 50 μL and a sample hole of 50 μL were set up. 100 μL HRP-labeled antibodies were added to each hole and incubated at 37 ℃ for 60min. Remove the liquid from the hole, wash and pat dry the absorbent paper. Then each well was incubated with fresh chromogenic substrate at 37 ℃ for 15 min to avoid light. Immediately after the termination of the reaction, the OD value of each hole was measured by spectrophotometer at the 450nm wavelength.

2.6. Statistical Analysis

SPSS 24.0 software was used to analyze the data. The data were expressed as mean ± standard deviation. One-way ANOVA or two-way ANOVA and Turkey test were used for comparison between groups. The difference was statistically significant (P<0.05).

3. Results

3.1. Tanshinone IIA Can Improve Functional Recovery After SCI in Rats

After the establishment of the model, all the rats were paralyzed in both lower limbs, had no activity, decreased muscle tension below the injury plane, and had no obvious response to acupuncture. The rats had less activity, less food and drinking water, urinary retention was common, accompanied by hematuria, pyuria, pyuria, abdominal flatulence, defecation difficulty, a few appeared urinary incontinence, limb autophagy and so on. After SCI, the behavioral scores of rats in each group were shown in table 1. Compared with Sham group, BBB score was significantly lower (P<0.001). On the 7th day after operation, the BBB score of SCI+ Tanshinone IIA group was significantly better than that of SCI group (P<0.01).

Table 1. *BBB score.*

Group	D1	D3	D5	D7
Control	18.75 ±0.84	19.61 ±0.50	20.81 ±0.44	21.05 ±0.03
Sham	18.83 ±0.73	19.40 ±0.53	20.80 ±0.42	20.84 ±0.42
Model	0.32 ±0.81**	2.01 ±2.11**	6.27 ±1.57**	7.63 ±1.74**
Tanshinone IIA	0.73 ±1.12	3.52 ±1.52	6.30 ±1.21	9.64 ±1.02$^{\#}$

Basso, Beattie, Bresnahan (BBB) scores on the 1st, 3rd, 5th and 7th day after SCI, n = 3. All data were expressed as mean ± SD. Two-way ANOVA and Tukey's multiple comparison test were used to analyze the differences between groups. *P<0.05 vs. sham group, **P<0.01, $^{\#}$P<0.05 vs. SCI group.

3.2. Tanshinone IIA Improve the Histopathological Changes of Spinal Cord After SCI in Rats

The histopathological changes of spinal cord in each group were observed by HE staining on the 7th day after SCI, and the results were shown in figure 1. No obvious pathological changes were observed in spinal cord tissue in Control group and Sham group, the structure of white matter was closely arranged, and the structure of neurons in gray matter was clear. In SCI group, the pathological changes of spinal cord tissue were severe, the neuronal cells in gray matter area were severely pyknotic, small area hemorrhage, macrophage infiltration, white matter demyelination to cavity formation. Compared with SCI group, the pathological changes, neuronal pyknosis, hemorrhage, inflammatory infiltration and white matter cavity formation in SCI+Tanshinone IIA group were reduced.

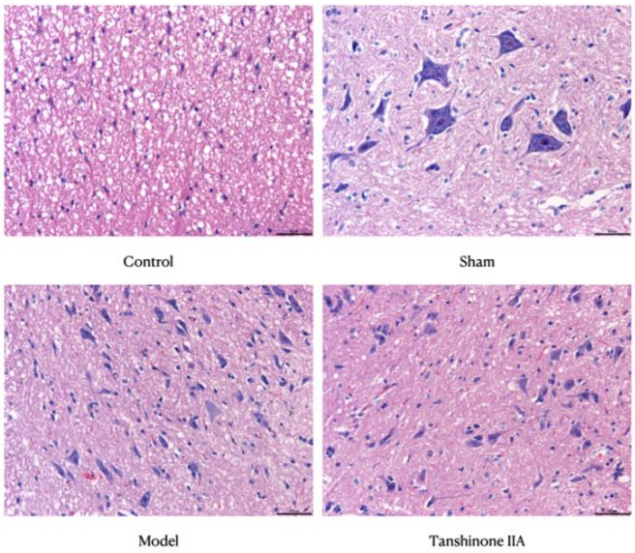

Figure 1. *Effects of Tan IIA on histological changes at 7 days after SCI. Scale bar = 50 μm.*

3.3. Tanshinone IIA Can Reduce Inflammation After SCI in Rats

ELISA was used to detect the level of inflammatory factors in rat spinal cord tissue.

As can be seen from figure 2, the level of IL-1 β in SCI group is significantly higher than that in Sham group (P<0.01). Compared with SCI group, the level of IL-1 β and TNF-α in SCI+Tanshinone IIA group was significantly lower (P <0.01).

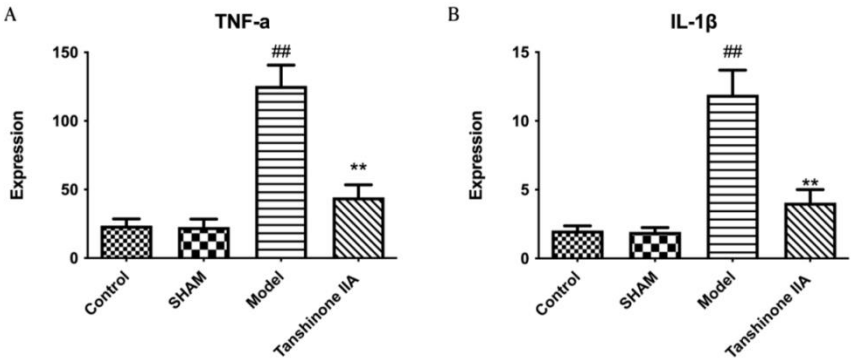

Figure 2. *Effects of Tan IIA on the level of inflammatory factors in spinal cord tissue 7 days after SCI.*

A-B Seven days after SCI, the levels of IL-1β and TNF-α were detected by ELISA. n = 3. All data were expressed as mean ± SD. One-way ANOVA and Tukey's multiple comparisons test were used to analyse differences among groups. **P<0.01 vs. sham group, $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. SCI group.

4. Discussion

In this study, Tanshinone IIA has protective effects on SCI in rats, showing a good recovery of motor function, improving histopathological changes and inhibiting the level of pro-inflammatory factors. In the study of animal model of SCI, it is often necessary to evaluate the motor function of hind limbs or lower limbs of experimental animals, among which BBB score [12, 13] and Rivlin inclined plate test [14, 15] are the most commonly used. The results of Zhang et al [16] showed that after the use of exogenous Shh, the functional recovery measured by the changes of BBB score was improved after SCI. The results of this study showed that Tanshinone IIA could significantly improve BBB score after SCI 7 days. Similarly, Yin et al [17] found that Tanshinone IIA promoted the recovery of motor function based on the BBB score 10 days after SCI. Several studies [16, 17] have confirmed the evidence of edema, hyperemia and incompact structure in SCI tissue. We also observed the same results in the rat model of SCI and found that Tanshinone IIA significantly improved SCI.

Inflammation plays an important role in the occurrence and development of SCI. TNF-α is a key initiating factor in inflammatory response, which plays a pivotal role in inflammatory response and can regulate the expression level of other cytokines [18]. IL-1β is the main regulatory factor in inflammatory response. In the early stage of SCI, the level of IL-1β in local spinal cord tissue increased rapidly, and the expression of IL-1β was earlier than inflammatory cell infiltration, which was one of the main causes of neuronal apoptosis [19]. Previous studies [17] have shown that Tanshinone IIA can reduce the expression of inflammatory factors such as TNF- α and IL-1β in SCI, reduce inflammatory response, significantly relieve spinal cord edema and promote the recovery of spinal cord motor function. The results showed that the expression of TNF-α and IL-1β in spinal cord tissue increased significantly after SCI. Tanshinone IIA could inhibit the inflammatory response after SCI and alleviate the further damage of spinal cord tissue structure, which was beneficial to the recovery of spinal cord function.

5. Conclusion

It can be seen that, Tanshinone IIA can significantly improve motor function after SCI, maintain the normal morphology of spinal cord tissue, inhibit inflammation, and repair spinal cord function. In the future, with the elucidation of the specific mechanism of Tanshinone IIA on SCI, it will provide a new idea for the research direction of our research group.

Abbreviations

SCI: Spinal Cord Injury
BBB: Scores of Basso, Beattie, Bresnahan
ELISA: Enzyme-Linked Immunosorbent Assay

Ethical Approval

The animal use protocol listed below has been reviewed and approved by the Institutional Animal Care and Use

Committee of Zhejiang Center of Laboratory Animals (approval no. ZJCLA-IACUC-20010449).

Patient Consent for Publication

Not applicable.

Author Contributions

Bin Lin performed the experiments and wrote the paper; Aini Lin and Weiting Chen collected and analyzed the data; Bin Lin gave guidance on experimental technology; Bin Lin, Aini Lin and Weiting Chen conceived the study. All authors read and approved the final manuscript.

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Data Availability Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Conflicts of Interests

The authors declare that they have no competing interests.

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