

Original Article

The effect of electrostimulation on apoptosis of spermatogenic cells of testes of the rats after spinal cord injury

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Abstract: Objective: To investigate the effect of electrostimulation on apoptosis of spermatogenic cells of the testes of the rats after spinal cord injury (SCI). Methods: The animal model of SCI was established by Allen's percussion. The apoptosis condition of spermatogenic cells in rat testes was detected by TUNEL 2 weeks, 4 weeks and 6 weeks after SCI and the expressions of Fas, p-ERK, p-JNK and p-p38 MAPK were detected by immunohistochemistry method. Results: The positive scores of TUNEL staining on the 2 weeks group, 4 weeks group and 6 weeks group were significantly decreased compared with those of the control group ($P < 0.01$). The positive scores of Fas, p-ERK, p-JNK and p-p38 MAPK on 2 weeks group, 4 weeks group and 6 weeks group were significantly decreased compared with those of the control group ($P < 0.01$). Conclusion: Electrostimulation can result in apoptosis of spermatogenic cells of the testes of the rats after SCI and the effect has timeliness. The mechanism may be related to the regulation of the expressions of Fas, p-ERK, p-JNK and p-p38 MAPK in spermatogenic cells of testes of the rats after SCI.

Keywords: Electrostimulation, spinal cord injury, apoptosis

Introduction

Spinal cord injury (SCI) is a serious threat to human health. It often causes paraplegia and quadriplegia, and leads to a series of serious complications and sequelae, such as urinary tract infection, respiratory tract infection, renal function impairment, and bedsores [1, 2]. SCI can substantially affect quality of life and family life situations, and make it an urgent, worldwide problem to be addressed in the field of medicine.

In recent years, there have been an increasing number of studies focused on the treatment, rehabilitation, and mechanisms of acute SCI. It is currently believed that mechanical injury to the spinal cord itself destroys its continuity and integrity. Following primary injury, the body initiates secondary injury to the spinal cord, leads to changes in the microenvironment, such as ischemia and hypoxia, inflammation, intracellu-

lar and extracellular calcium imbalance, excessive production of free radicals, excitatory amino acid changes, cytotoxic substances, and apoptosis, which are counterproductive to spinal cord functional recovery.

Apoptosis is an important biological process, which involves a series of gene-regulated, initiative, cell death processes. Previous studies have documented the role of neuronal apoptosis and apoptotic gene expression in SCI [3]. In recent years, many scholars at home and abroad found that electrical stimulation on nervi sacrales after spinal cord injury can increase bladder contraction, relaxation and contraction of urethral sphincter to improve urinary function [4]. Our previous studies showed that electrical stimulation on the sacral 3 nerve can reduce the level of spermatogenic cell of seminiferous tubule in the rats after spinal cord injury the reduction of sperm cells and sperm [5]. Although domestic scholars have reported

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that the relevant acupuncture points can inhibit the apoptosis of nerve cells after spinal cord injury, and reduce [6], but there is no report on whether electric stimulation of the sacral nerve after spinal cord injury at home and abroad is reported to be affected by Gao Wansheng.

The aim of this study was to establish a model of functional impairment of reproductive system in male rats after spinal cord injury, and to investigate the effects of electric stimulation on apoptosis condition of testicular spermatogenic cells by detecting the expressions of Fas, p-ERK, p-JNK and p-p38 MAPK on the different time points.

Material and methods

Animals and groups

72 SD rats were randomly divided into the three groups: sham operation group (SO), spinal Cord Injury Group (SCI), and spinal Cord Injury with electrostimulation (SCIE) group ($n = 24$). The rats were fasting for 12 hours before surgery, and were weighed. 1% sodium pentobarbital (40 mg/kg) was used for intraperitoneal anesthesia, the rats on a prone position. The surgical exposure was sterilized by 1% Iodophor wild, T9-T11 spinous process, lamina and T10 spinal cord.

Sham operation (SO) group: The hemostatic forceps bite the T10 spine and the lamina of the rats, the gelatin sponge was place to stop bleeding, and then the wound of the rats were sutured layer by layer.

Spinal cord injury (SCI) group: A circular area, which is about 4 mm in diameter, was exposed in the spinal cord of the T10. The plastic gasket (3 mm × 2 mm × 1 mm) soaked with iodophen was carefully placed in T10 spinal epidural after pre bending in accordance with the rat thoracic spinal. The homemade modified Allens device was installed, the spinal cord posterior median vascular was centered, and the restructured sleeve mouth was vertically and gently on the plastic gasket. 10 g blow stick from 5 cm height was freely fallen to impact sleeve mouth; the severe contusion injury was caused in T10 spinal cord by impacting plastic gasket. The tail and lower limbs of the rats were twisted violently, suggesting that the animal model is successful. Then, gelatin sponge was placed to stop bleeding; the wound was sutured layer by layer.

Spinal cord injury with electrostimulation (SCIE) group: On the basis of ALLEN's injury, the sacral canals of the rats were opened, and the third sacral nerve was dissected out, and the electrodes were placed in the 200 us 3 V (20 Hz 10 mA).

The animal model of spinal cord injury was established by Allen's percussion. The apoptosis of germ cells in rat testes was detected by TUNEL 2 weeks, 4 weeks and 6 weeks after spinal cord injury and the expressions of Fas, p-ERK, p-JNK and p-p38 MAPK were detected by immunohistochemistry method.

Samples collection

AM 8, 2 weeks, 4 weeks and 6 weeks after operation, 8 rats were chosen on each group, 1% pentobarbital sodium (40 mg/Kg) were injected into the abdominal cavity. The samples were collected at second weeks, 4 weeks, 6 weeks, 8 rats in each group, 1% sodium 400 ml, and the open chest exposed to the heart, and 30 minutes after the infusion of 4% minutes, 4% minutes after the removal of the skin and subcutaneous tissue. And then the dehydration, paraffin embedding, continuous in the middle of the testes to slice, slice thickness is 10 um.

TUNEL staining and immunohistochemistry

TUNEL staining was performed on paraffin sections using an in situ cell death detection kit (Rochev, Germany) according to the manufacturer's instructions. Sections were counterstained with hematoxylin. A negative control was similarly performed except for omitting TUNEL reaction mixture. Only cells showing nuclear condensation/fragmentation and apoptotic bodies in the absence of cytoplasmic TUNEL reactivity were considered apoptotic. For immunohistochemistry, sections, blocked using 2% normal goat serum in PBS, were incubated for overnight at 4°C with mouse monoclonal antibody against Fas, p-ERK, p-JNK and p-p38 MAPK (Maxim Biotech Inc, China) followed by followed by a biotinylated sheep anti-mouse antibody and avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA.) for 2 h. The slices were colorized with DAB/H₂O₂ solution, and then cell nucleuses were counterstained with hematoxylin. Each procedure was followed by several rinses in PBS. Blank staining was carried out in the same way as the

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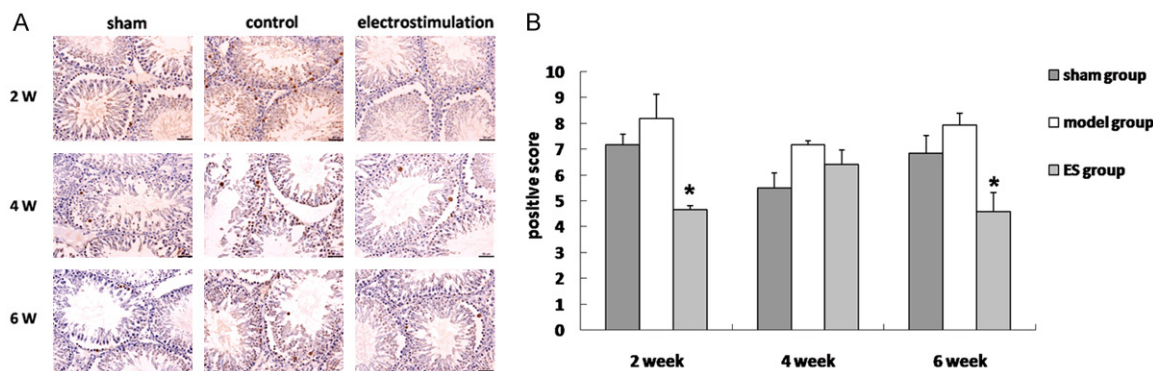


Figure 1. The effect of electrostimulation on apoptosis of the testes of the rats 2 weeks, 4 weeks and 6 weeks after SCI. A. TUNEL staining in situ end labeling. B. The statistical analysis of the comparison of the positive scores of apoptosis of spermatogenic cells of the rats. * $P < 0.05$, compared to model group, Scale bars = 200 μm .

above, except for eliminating the primary antibodies. Brown color of nuclei was taken as the positive staining of apoptotic neuronal cells and Brown color of cytoplasm was taken as the positive staining. For quantitative analysis, 10 microscopic fields were taken, and all neurons, including neurons with TUNEL staining were counted. The mean values of the percentage of neurons with TUNEL positive staining were taken for further processing.

Detection index

The detection of apoptosis in situ end labeling (TUNEL): 2 sections were randomly chosen in each testis for TUNEL staining, the two sections of each testis was randomly selected for TUNEL staining, the four visual fields were selected to record the number of TUNEL positive cells in high magnification to detect testicular students germ cell apoptosis. p-ERK, Bcl-2, p-JNK and MAPK Fas expression were detected by immunohistochemical method. The expression of p-p38, p-ERK, p-p38, p-JNK and MAPK Fas were detected by immunohistochemical method, respectively. The positive cells were counted and the average value was positive.

Statistical analysis

Mean values and standard deviations (mean \pm S.D.) were calculated for each variable studied. All statistical procedures were performed using SPSS 19.0. Normally distributed data were analyzed using one-way ANOVA with a Bonferroni post hoc test to evaluate the statistical significance of intergroup differences in all the tested variables. In all cases, statistical significance was $P < 0.05$.

Results

The effect of electrostimulation on apoptosis of the testes of the rats after spinal injury

To study the effect of electrostimulation on apoptosis of the testes of the rats after SCI, we adopted TUNEL method to detect apoptosis in situ end labeling. 2 weeks, 4 weeks and 6 weeks after electro-stimulation, the positive scores of apoptosis of spermatogenic cells of the rats on the SCIE group were significantly decreased compared with those of the rats on the control group ($P < 0.05$, $P < 0.01$) (Figure 1).

The effect of electrostimulation on expressions of Fas and p-38 of the testes of the rats after spinal injury

To study the effect of electrostimulation on expression of Fas and p-38 of the testes of the rats after spinal injury, we adopted immunohistochemical method to detect it. 2 weeks, 4 weeks and 6 weeks after electro-stimulation, the positive scores of the expressions of Fas and p-38 of spermatogenic cells of the rats on the SCIE group were significantly decreased compared with those of the rats on the control group ($P < 0.05$) (Figures 2, 3).

The effect of electrostimulation on expressions of p-ERK and p-JNK of the testes of the rats after spinal injury

To study the effect of electrostimulation on expression of p-ERK and p-JNK of the testes of the rats after spinal injury, we adopted immunohistochemical method to detect it. 2

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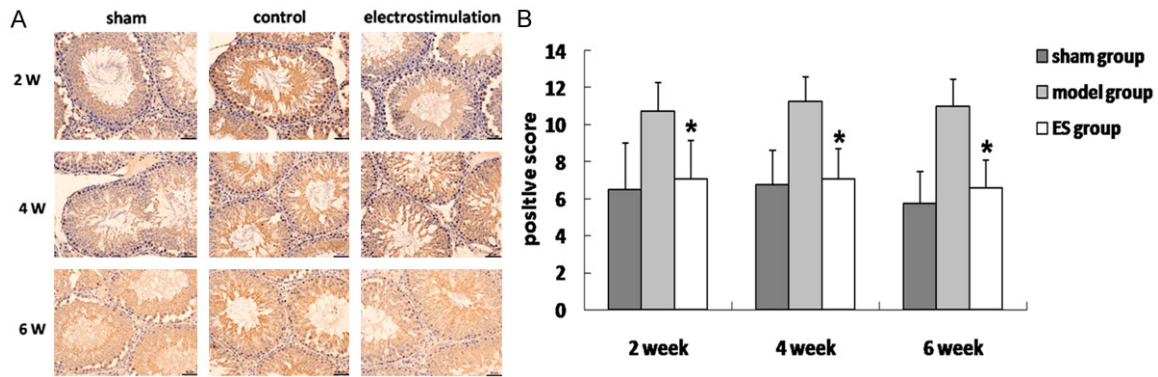


Figure 2. The effect of electrostimulation on the expressions of Fas of the testes of the rats 2 weeks, 4 weeks and 6 weeks after SCI. A. Immunohistochemical staining. B. The statistical analysis of the comparison of the positive scores of Fas of spermatogenic cells of the rats. * $P < 0.05$, compared to model group, Scale bars = 200 μm .

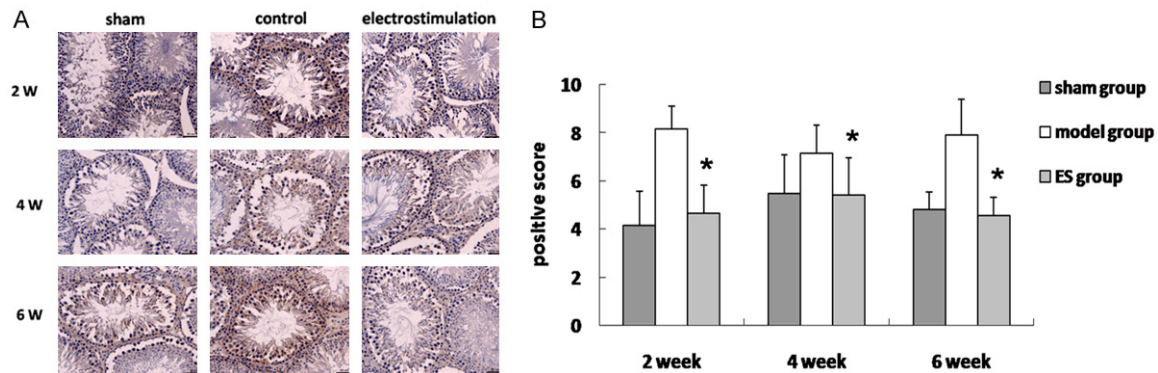


Figure 3. The effect of electrostimulation on the expressions of p-38 of the testes of the rats 2 weeks, 4 weeks and 6 weeks after SCI. A. Immunohistochemical staining. B. The statistical analysis of the comparison of the positive scores of p-38 of spermatogenic cells of the rats. * $P < 0.05$, compared to model group, Scale bars = 200 μm .

weeks, 4 weeks and 6 weeks after electro-stimulation, the positive scores of the expressions of p-ERK and p-JNK of spermatogenic cells of the rats on the SCIE group were significantly decreased compared with those of the rats on the control group ($P < 0.05$) (Figures 4, 5).

Discussion

In this study, we investigated the effect of electrostimulation on apoptosis of spermatogenic cells of the testes of the rats after spinal cord injury (SCI) by detecting the expressions of Fas, p-ERK, p-JNK and p38 on the different time points. We found electrostimulation can result in apoptosis of spermatogenic cells of the testes of the rats after SCI and the effect has time-liness. The mechanism may be related to the regulation of the expressions of Fas, ERK, JNK and p38 in spermatogenic cells of testes of the rats after SCI.

Spinal cord ischemia/reperfusion (I/R) injury may present immediate or delayed paraplegia that occurs 4% to 33% of patients undergoing surgery on the thoracic aorta [7]. Presently, SCI is characterized by high mortality, high disability rate, difficult rehabilitation, longer course, and high cost of treatment. SCI prevention, treatment, and rehabilitation have attracted increasing attention in the field of medicine and there is a great need for a simple and efficient means of treatment for this disease. The role of apoptotic mechanisms has been recently proposed in neuronal cell death following spinal cord I/R injury [8]. Several studies have suggested that apoptotic mechanisms were initiated at the molecular level in I/R neural cells [9, 10]. In this study, we adopted TUNEL method to detect apoptosis in situ end labeling. 2 weeks, 4 weeks and 6 weeks after electro-stimulation, the results showed that the positive scores of

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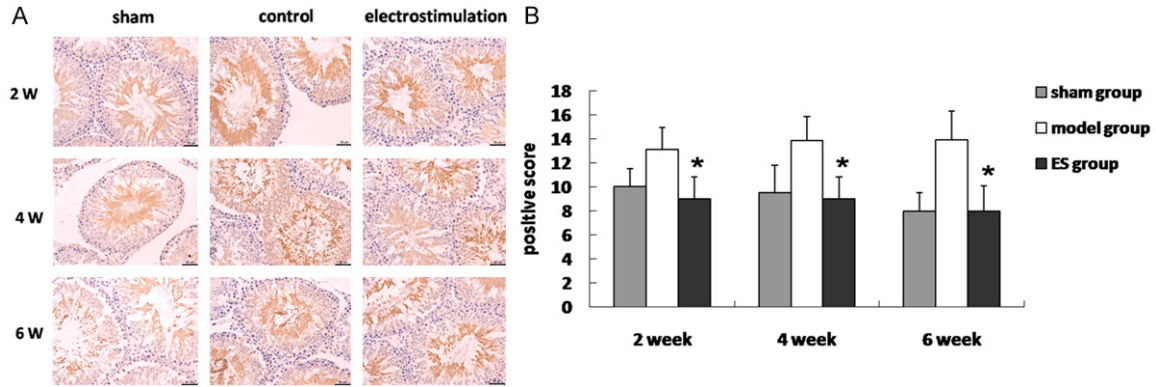


Figure 4. The effect of electrostimulation on the expressions of p-JNK of the testes of the rats 2 weeks, 4 weeks and 6 weeks after SCI. A. Immunohistochemical staining. B. The statistical analysis of the comparison of the positive scores of p-JNK of spermatogenic cells of the rats. * $P < 0.05$, compared to model group, Scale bars = 200 μm .

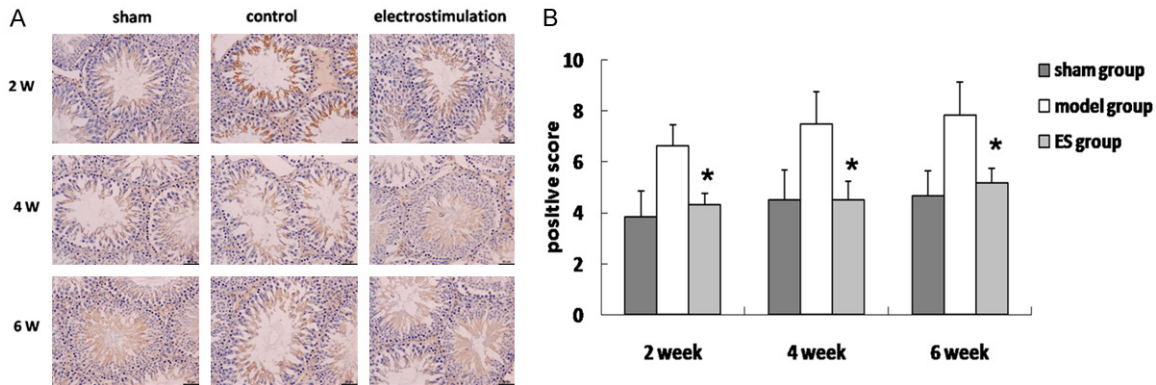


Figure 5. The effect of electrostimulation on the expressions of p-ERK of the testes of the rats 2 weeks, 4 weeks and 6 weeks after SCI. A. Immunohistochemical staining. B. The statistical analysis of the comparison of the positive scores of p-ERK of spermatogenic cells of the rats. * $P < 0.05$, compared to model group, Scale bars = 200 μm .

apoptosis of spermatogenic cells of the rats on the SCIE group were significantly decreased compared with those of the rats on the control group. It suggests that electrostimulation can result in apoptosis of spermatogenic cells of the testes of the rats after SCI.

Gong Yongguang et al first detected a large number of apoptotic cells in the testicular tissue after removal of the spermatic cord [11], and the number of apoptotic cells is increased with prolonged nerve time, which indicated that cord nerve had an important effect on spermatogenic cells. Fas is a member of the tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) family, which belongs to the type I transmembrane glycoprotein, which can initiate apoptosis by binding to Fas ligand (FasL). Mitogen activated protein kinase is an important enzyme in the signal transduction

pathways, including extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (c-Jun N-terminal kinase, JNK) and p38 etc. ERK is mainly expressed in nucleus of spermatogonium, or primary spermatocyte. JNK was found in between sertoli cells, between sertoli cells and spermatogenous cells, p38 was distributed in the cytoplasm of seminiferous tubule and leydig cells [12, 13], and it was proved that different members of MAPK family were activated by phosphorylation to regulate cell proliferation, differentiation and apoptosis. In this study, the data showed that 2 weeks, 4 weeks and 6 weeks after electro-stimulation, the positive scores of the expressions of Fas, p-ERK, p-38 and p-JNK of spermatogenic cells of the rats on the SCIE group were significantly decreased compared with those of the rats on the control group ($P < 0.05$). It indicates that the mechanism, which electrostimulation

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results in apoptosis of spermatogenic cells, may be related to the regulation of the expressions of Fas, p-ERK, p-JNK and p-p38 in spermatogenic cells of testes of the rats after SCI.

In conclusion, electrostimulation can result in apoptosis of spermatogenic cells of the testes of the rats after SCI and the effect has timeliness. The mechanism may be related to the regulation of the expressions of Fas, p-ERK, p-JNK and p-p38 in spermatogenic cells of testes of the rats after SCI.

Disclosure of conflict of interest

None.

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