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Original Article

Effects of botulinum toxin A on endometriosis-associated pain and its related mechanism

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Abstract: Endometriosis (EMS) is a common disease in women of childbearing age, and pain is the main clinical symptom. The primary clinical treatments are surgical excision and drug therapy targeting the ectopic lesions, but these have not been very effective. Botulinum neurotoxin serotype A (BTX-A) has been reported to be useful in the treatment of pain in a variety of diseases. Based on this, the aim of the present study was to explore the therapeutic effect and mechanism of BTX-A on EMS. We constructed a model of nerve injury induced by oxygen glucose deprivation (OGD) in PC12 cells and EMS mice. Model cells and mice were treated with different concentrations of BTX-A to observe the changes in pain behavior, to detect cell viability and the secretion of Norepinephrine (NE) and methionine enkephalin (M-EK) in cells and the spinal cord, and to evaluate the expression of apoptosis-related molecules in spinal cord nerves. The results revealed that BTX-A significantly reduced the amount of writhing in model mice, enhanced the activity of PC12 OGD cells, increased the secretion of NE & M-EK in model cells and the spinal cord of mice, and decreased the apoptosis of neural cells in the spinal cord of the model mice. Therefore, we believe that BTX-A may alleviate the pain induced by EMS by increasing the secretion of analgesic substances and promoting the repair of nerve injury. The present study provides a theoretical basis for the treatment of pain induced by EMS.

Keywords: Botulinum toxin A, endometriosis, analgesia, norepinephrine, methionine encephalin

Introduction

Endometriosis (EMS) is a common clinical chronic gynecological disease. It is caused by ectopic activation of endometrial cells and implantation outside of the uterine cavity. The incidence of EMS in women of reproductive-age is ~10% and increases annually. At present, the main treatment methods for the disease are surgical resection of the ectopic endometrium and drug treatment. However, it is prone to relapse and drug addiction or tolerance, therefore, there is no effective treatment as of yet. Pain is the main clinical symptom of EMS, but its mechanism is not clear [1]. EMS-induced pain may be caused by local inflammatory response stimulated by periodic hemorrhage in ectopic lesions, chronic inflammation in the abdominal cavity and the abnormal growth of lesions, which may result in peripheral and central nervous sensitization [2]. Prolonged exposure to pathogens or systemic inflammation can lead to chronic neuroinflammation, which in turn leads to functional and structural changes until the nerve cells die [3].

Botulinum neurotoxin serotype A (BTX-A) is a neuromuscular toxin secreted by *Clostridium botulinum* with high toxicity and pathogenicity [4]. It is widely used in the treatment of some diseases related to muscle hyperresponsiveness, such as squint, blink, torticollis, hemifacial spasm and cerebral palsy [5, 6]. At present, BTX-A has been used in the treatment of clinical pathological pain, such as migraine, musculoskeletal pain and refractory trigeminal neuralgia, and has achieved satisfactory efficacy [7, 8]. In addition, further research has demonstrated that BTX-A can also inhibit the activation of microglia and play a long-term role in

pain relief in animal models of inflammation and neuropathic pain [9]. Some studies have also shown that BTX-A can be used to repair nerve injury and promote the regeneration and growth of neurons [10].

To the best of our knowledge, no previous studies on BTX-A in the application of EMS-associated pain have been reported. Therefore, the present study constructed a model of nerve injury induced by oxygen glucose deprivation (OGD) in PC12 cells *in vitro* and in mice with EMS, in order to investigate whether BTX-A alleviates the pain induced by EMS in mice. These models were then used to study the effects of EMS and OGD on nerve injury repair and neuronal apoptosis as well as its molecular mechanism in order to provide an experimental basis for the clinical treatment of EMS.

Materials and methods

Cell culture

PC12 cells were purchased from the Cell Bank of Chinese Academy of Sciences. The cells were seeded in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) with 10% (v/v) fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 units/ml penicillin and 100 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.) with the appropriate cell density. Cells were maintained at 37°C in a humidified incubator containing 5% CO_2. Cell culture medium was replaced every two days.

PC12 OGD model

The PC12 cell suspension was seeded in 96-well culture plates (2×10^4 cells/well) and incubated at 37°C in a 5% CO_2 incubator. After 12 h of normal culture, the cells were cultured in DMEM without glucose in a hypoxic chamber (1% O_2, 94% N_2, and 5% CO_2) for 12 h. After OGD, the culture was washed with PBS three times and then cultured in normal DMEM under normoxic conditions for 24 h. BTX-A (100 U/box; Lanzhou Hengli; 10 U/ml, BL; 50 U/ml, BH) was separately added during the OGD and reoxygenation culture. Control cells without OGD were maintained under normal conditions. Finally, the culture medium was discarded, cell samples were collected and cytological tests were conducted.

Determination of cell viability

The PC12 cells were collected, and viability was detected by a Cell Counting Kit (CCK)-8 assay with CCK-8 (Dojindo Molecular Technologies, Inc.; cat. no. CK04). According to the kit's protocol, each well was mixed with 10 μl CCK-8, and incubated at 37°C for 1 h. The absorptivity of OD450 nm was measured using an enzyme-labelled meter (Thermo Fisher Scientific, Inc.; MuLTiSKAN MK3), which was used to determine the cell viability. Each experiment was performed in 6 parallel wells and repeated 3 times.

Immunocytochemistry

The PC12 cells, after BTX-A treatment, were fixed in 4% paraformaldehyde and permeabilized in PBS containing 0.5% Triton-X 100 for 15 min at 4°C. Coverslips were incubated in 4% bovine serum albumin (Thermo Fisher Scientific, Inc.; cat. no. 37525) for 1 h to block non-specific antibody-binding sites, and then incubated overnight with primary antibodies against β3-tubulin (Abcam; cat. no. ab52623; 1:500 dilution), followed by incubation with Alexa Fluor 594 goat anti-rabbit antibody (Thermo Fisher Scientific, Inc.; cat. no. R37117; 1:2,000 dilution) for 1 h. Then cells were stained with DAPI for 10 min, and examined using a Zeiss LSM710 fluorescence microscope.

Enzyme-linked immunosorbent assay (ELISA)

The levels of NE and M-EK in the PC12 cell supernatant and mouse spinal cord homogenate were detected using ELISA methods, and the operation was performed according to the instructions of the ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd.). The absorbance was measured at 450 nm by enzyme-labelled meter (Thermo Fisher Scientific, Inc.; MuLTiSKAN MK3). Each sample was made in triplicate.

Mouse EMS model

The present study constructed a mouse model of intraperitoneal EMS. The mice were purchased from Shanghai Sippr-BK laboratory animal Co. Ltd. To establish this model, 6-week-old (18-20 g) female BALB/c mice were fed adaptively for one week with a standard diet and water *ad libitum*, and the weight of the mice

was ~20-22 g. The mouse EMS model was established as described previously [11]. Briefly, estradiol benzoate at 0.5 mg/kg was administrated to donor animals by gavage for 2 days to induce the estrus period. Vaginal smears showed a large number of seedless keratinocytes in mice as donors. After a week, the uterus of the donor mice were collected, and minced using scissors in sterile normal saline. Then the uterus tissue debris was injected into the recipient mouse intraperitoneally and half of the preparation was injected into the peritoneum of each of two recipient mice with a syringe. A week later, the model mice were injected intraperitoneally with oxytocin at 20 IU kg^{-1}; observing evident pain behavior was defined as successful model mouse establishment [12]. Following the removal of unsuccessful model mice, the EMS mice were randomly divided into the control (normal mice with saline, ip), SHAM (model mice with saline, ip), botulinum toxin high dose (30 U/kg, ip; BH) and botulinum toxin low dose groups (10 U/kg, ip; BL). Oxytocin 20 IU kg^{-1} was injected intraperitoneally at every time point to observe the writhing reaction within 30 min on days 0, 1, 3, 5, 7, 14, 21 and 28 after BTX-A treatment. Among them, oxytocin was injected 1 hour after BTX-A treatment, and the time point was defined as day 0. The model mice didn't die during the behavioral observation period, and there were also no significant weight changes or behavioral variations. After 28 days of animal behavior observation, the mice were sacrificed by cervical dislocation, and spinal cord tissue was collected. The experiments and procedures using mice were approved by the Animal Ethics Committee of Obstetrics & Gynecology Hospital of Fudan University Shanghai.

Writhing test

Immediately after the oxytocin injection, visceral pain was measured by counting the number of writhing reflexes for 30 min in the experimental groups. The writhing reflexes were characterized by the presence of abdominal contraction, body distortion, hind limb extension and hip elevation. The investigator was blind to the drug treatment administered. The analgesic response was quantified as the percentage of the number of writhing decreases after treatment with oxytocin, which was calculated as the analgesic frequency: = [(no. of writhes in sham-no. of writhes in determined dose or control)/no. of writhes in sham] × 100% [13].

Western blot analysis

Total protein was extracted from tissues using radioimmunoprecipitation assay lysis buffer. Proteins were separated by SDS-PAGE. After SDS-PAGE, proteins were transferred to polyvinylidene fluoride membranes. Membranes were then incubated with primary antibodies and then with a secondary antibody. The primary antibodies included C-X3-C motif chemokine receptor 1 (CX3CR1; Abcam; cat. no. ab8021; 1:1,000 dilution), purinoceptor P2X 7 (P2X7; Santa Cruz Biotechnology, Inc.; cat. no. sc-514962; 1:200 dilution), Phospho-p38 mitogen-activated protein kinase (MAPK; CST Biological Reagents Co., Ltd.; cat. no. 4511; 1:1,000 dilution), Bax (Abcam; cat. no. ab32503; 1:3,000 dilution), Bcl2 (CST Biological Reagents Co., Ltd.; cat. no. 15071; 1:1,000 dilution) and β-tubulin (Santa Cruz Biotechnology, Inc.; cat. no. sc-101527; 1:500 dilution), which was used as the internal control. Then the blocked membranes were incubated with mouse secondary antibody (Jackson ImmunoResearch Laboratories, Inc.; cat. no. 315-035-003; 1:3,000) and rabbit secondary antibody (Jackson ImmunoResearch Laboratories, Inc.; cat. no. 111-035-008; 1:3,000). Bands were detected with a chemiluminescence reagent (Thermo Fisher Scientific, Inc.; cat. no. 34577).

Statistical analysis

All experiments were repeated at least three times. The data were presented as the mean ± standard deviation of the mean. Statistical analysis was performed using paired Student's t-test or one-way analysis of variance followed by Newman-Keuls' multiple comparison test with GraphPad Prism 5 software (GraphPad Software, Inc.). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

BTX-A treatment in vitro can reverse cell damage induced by an OGD model

In this study, the PC12 OGD cell damage model and β3-tubulin specific neuron markers were used to evaluate the effects of BTX-A on the

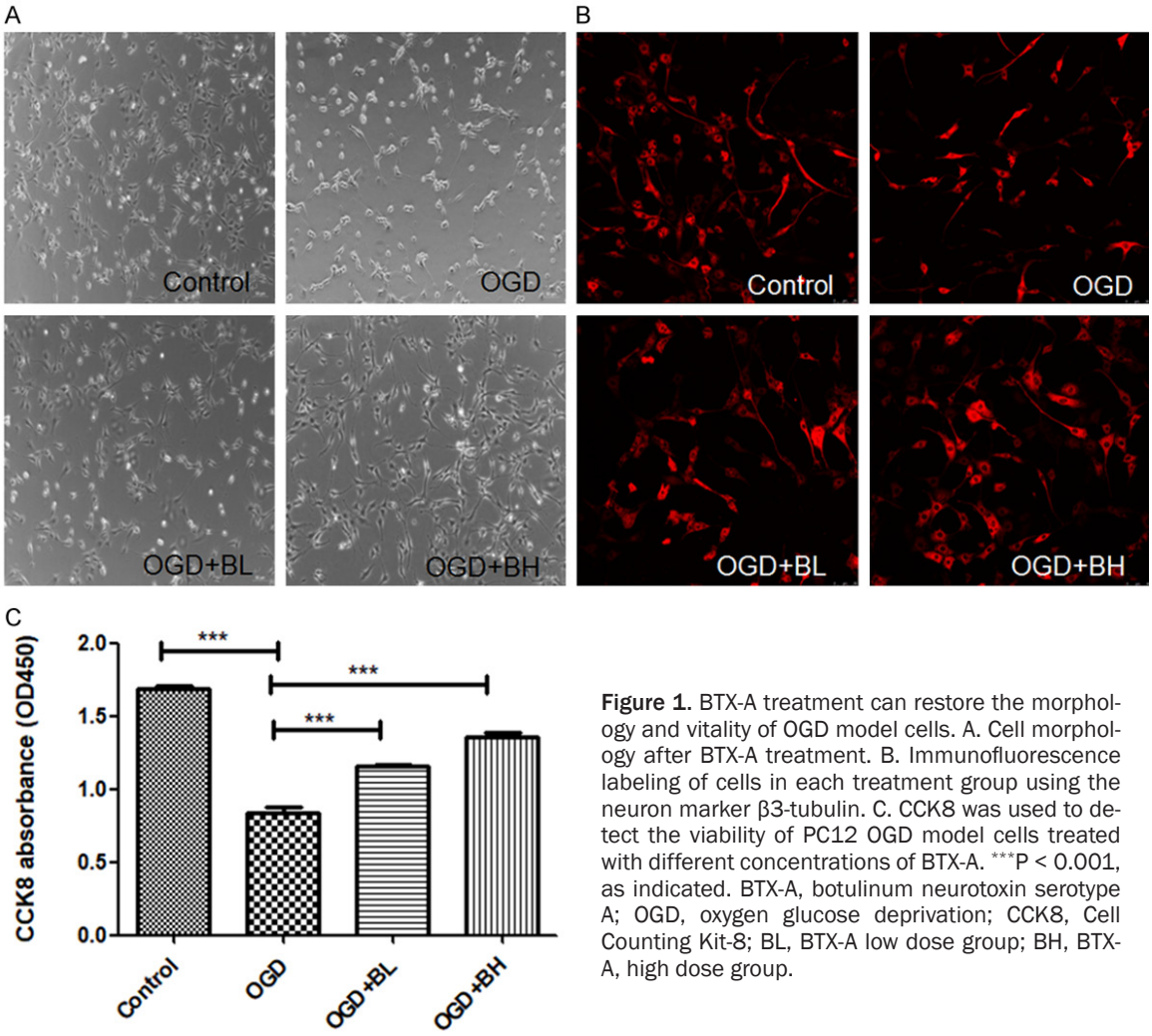

Figure 1. BTX-A treatment can restore the morphology and vitality of OGD model cells. A. Cell morphology after BTX-A treatment. B. Immunofluorescence labeling of cells in each treatment group using the neuron marker β3-tubulin. C. CCK8 was used to detect the viability of PC12 OGD model cells treated with different concentrations of BTX-A. ***P < 0.001, as indicated. BTX-A, botulinum neurotoxin serotype A; OGD, oxygen glucose deprivation; CCK8, Cell Counting Kit-8; BL, BTX-A low dose group; BH, BTX-A, high dose group.

repair of neuronal cell damage [14-16]. By observing the morphology of the cells, the normal PC12 cells were reported to be spindle or polygonal in shape. The cells had longer axons interlaced with each other. After OGD treatment, the cells became round, the axons became shorter, and the cells floated to form clusters. Cells treated with different concentrations of BTX-A gradually reversed the phenotype observed in OGD cells, and the higher the concentration, the better the effect (**Figure 1A** and **1B**). In addition, CCK8 was used to detect the effect of BTX-A on the viability of PC12 cells in the OGD model. The results revealed that compared with the normal group, the cell viability of the OGD treated group was significantly decreased ($P < 0.001$) to approximately half of that in the control group, while BTX-A could increase the OGD cell viability in a concentration-dependent manner (OGD-BL vs. OGD, $P < 0.001$; OGD-BH vs. OGD, $P < 0.001$; **Figure 1C**).

Activation of BTX-A on neurotransmitter secretion in PC12 cells under OGD

An increasing number of studies have reported that norepinephrine (NE) and methionine enkephalin (M-EK) have analgesic effects. Injection of NE and/or opioid agonists into the spinal dorsal horn can achieve analgesic effects [17, 18]. Microencapsulated PC12 cells can be transplanted into the subarachnoid space of rats, and as a source of microcellular pump, can continuously produce NE and M-EK and may have a good analgesic effect in a chronic neurogenic animal model [19, 20]. NE is extremely important for inhibiting neuropathic pain in the spinal cord, mainly through α2-adrenergic receptors, and it also improves

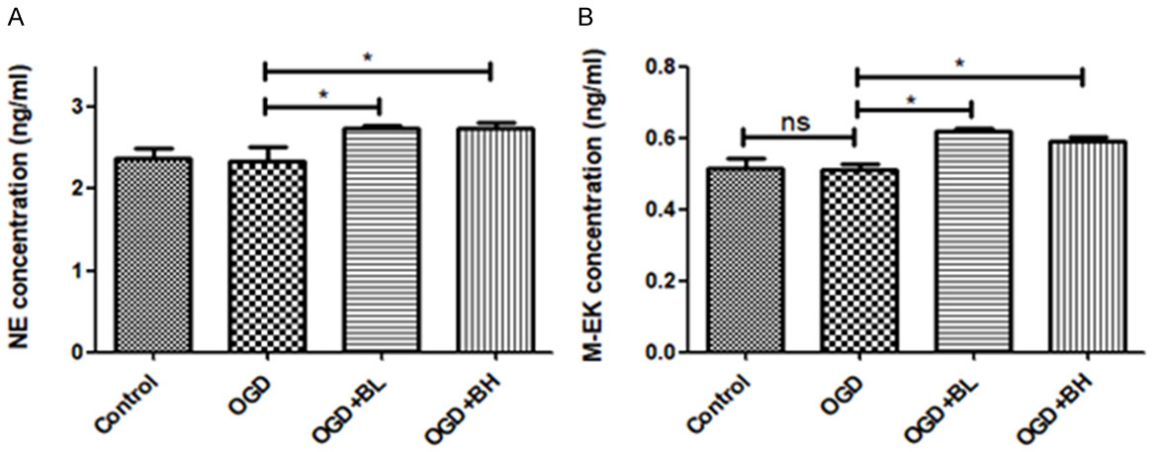

Figure 2. BTX-A increases neurotransmitter secretion in OGD model cells. (A) NE and (B) M-EK. Data are presented as the mean ± standard deviation of three independent biological replicates. *P < 0.05, as indicated. BTX-A, botulinum neurotoxin serotype A; OGD, oxygen glucose deprivation; NE, norepinephrine; M-EK, methionine enkephalin; ns, not significant; BL, BTX-A low dose group; BH, BTX-A, high dose group.

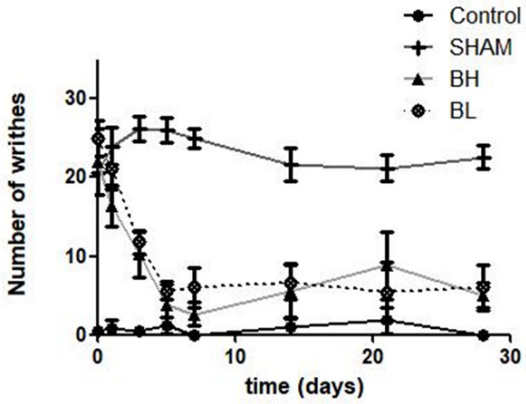

Figure 3. Effects of BTX-A on pain relief induced by EMS in mouse models. The control group consisted of normally fed mice, the Sham group was treated with saline after the uterine heterotopia model establishment, and the BTX-A group was treated with BTX-A at the corresponding concentration after uterine heterotopic model establishment. Data are presented as the mean ± standard deviation. BTX-A, botulinum neurotoxin serotype A; BL, BTX-A low dose group; BH, BTX-A, high dose group.

the function of the descending noradrenergic inhibitory system [21, 22].

In order to study the possible mechanism of pain relief involved in the repair of nerve cell injury caused by BTX-A, the present study used ELISA to detect the level of neurotransmitters in the cell supernatant. The results showed that BTX-A could significantly increase the secretion of NE and M-EK in PC12 cells (**Figure 2A** and **2B**). Therefore, the analgesic effect of BTX-A is probably achieved by increasing the analgesic factors secreted by cells.

Effect of BTX-A on pain induced by EMS

To explore the effect of BTX-A on the pain caused by EMS, we established EMS model mice. With this approach, the current success rate of this model in our laboratory was about 74%. We next observed the behavioral response of BTX-A to the pain induced by EMS. BTX-A was injected subcutaneously one week after model establishment. After that, oxytocin was intraperitoneally injected at 0, 1, 3, 5, 7, 14, 21 and 28 days, respectively. And the number of twisting was recorded within 30 min. Meanwhile, the mice were sacrificed at 28 days to confirm the presence of allograft. The results revealed that the amount of writhing in the control group was almost none within one month. The number of writhing events in the Sham and BTX-A treated groups were significantly higher than those in control group (21 ± 1, 22 ± 4 and 26 ± 2 on day 0, respectively). The writhing frequency of the control group and Sham group remained relatively stable at the different time periods, while the writhing frequency of the BTX-A group decreased sharply with increasing time, and the trend remained stable after the fifth day, the results were slightly higher than those observed in the control group. The trend in writhing times in the high and low BTX-A dose groups were basically the same within the one month period, but there was no significant difference between the two groups (**Figure 3** and

Table 1. Statistical analysis of number of writhes at 0, 1, 3, 5, 7, 14, 21, 28 days after surgery

day	Groups	Samples	Writhing frequency	Analgesic rate	day	Samples	Writhing frequency	Analgesic rate
0	Control	5	1 ± 0.57	96.8%	7	5	0 ± 0	100%
	Saline	5	21 ± 1	-		5	25 ± 2	-
	10 U/kg BTX-A	5	22 ± 4	-5.6%		5	3 ± 2	89.7%
	30 U/kg BTX-A	5	26 ± 2	-24.8%		5	6 ± 3.21	77.6%
1	Control	5	1 ± 1.15	97.1%	14	5	0 ± 0.57	98.5%
	Saline	5	23 ± 3	-		5	22 ± 3	-
	10 U/kg BTX-A	5	16 ± 3	29.7%		5	6 ± 3	74.9%
	30 U/kg BTX-A	5	21 ± 3.6	10%		5	6 ± 2.88	71.6%
3	Control	5	0 ± 0	100%	21	5	2 ± 2.08	92%
	Saline	5	27 ± 2	-		5	21 ± 2	-
	10 U/kg BTX-A	5	10 ± 3	61.75%		5	9 ± 4	58%
	30 U/kg BTX-A	5	12 ± 2	55%		5	5 ± 5.13	74.6%
5	Control	5	1 ± 1	94%	28	5	0 ± 0	100%
	Saline	5	26 ± 2	-		5	23 ± 2	-
	10 U/kg BTX-A	5	4 ± 3	85.3%		5	5 ± 2	77.9%
	30 U/kg BTX-A	5	5 ± 1	80.7%		5	6 ± 4.04	72%

Table 1). In general, BTX-A could relieve the pain induced by EMS.

Protective capability of BTX-A on spinal cord neurons in mice

As EMS induces inflammatory infiltration around the visceral tissue of the ectopic site, inflammatory factors constantly stimulate nearby neurons. Long-term stimulation will cause neuronal damage, and nerve injury may lead to long-term pain [3]. Next, we investigated whether the pain relief induced by BTX-A in EMS was achieved by repairing spinal cord neuron injury. The results showed that the level of β3-tubulin was significantly lower in the EMS model group treated with normal saline compared with the control group, but it increased to a certain extent in the BTX-A treatment group (**Figure 4**). It was suggested that BTX-A may repair spinal cord neuron injury to some extent *in vivo.*

Effect of BTX-A on neurotransmitter secretion in the spinal cord neurons of mice

The expression of NE and M-EK in the spinal cord homogenate of each treatment group was analyzed. The results showed that there was no significant change in NE and M-EK expression in the saline group compared with the control group, but the expression in BTX-A treatment group increased to different degrees, especially in the 30 U/kg BTX-A group. There was a significant difference.

(**Figure 5A** and **5B**). From the above results, it could be concluded that BTX-A could increase the release of NE and M-EK in the spinal cord of mice with EMS. The pain relief induced by endometriosis was probably due to an increase in neurotransmitter secretion.

Effect of BTX-A on the apoptosis of spinal cord neurons in mice

Previous studies have shown that the MAPK family member p38MAPK signaling pathway is closely related to nerve injury and regulates many physiological processes, such as cell differentiation, cell growth and apoptosis. Among them, Bcl2/Bax is an important component and an important index to determine apoptosis [23]. Therefore, the present study further investigated the effect of BTX-A on neuronal sensitization and apoptosis. Western blotting results showed that the microglia receptor CX3XR1 and P2X7 of chemokines fractalkine increased in the EMS model group compared with the control group. It was suggested that BTX-A may induce neuronal sensitization and injury by stimulating the activation of microglia around the spinal neurons and releasing a large number of inflammatory factors, or it may induce self-sensitization via the activation of P2X7 on

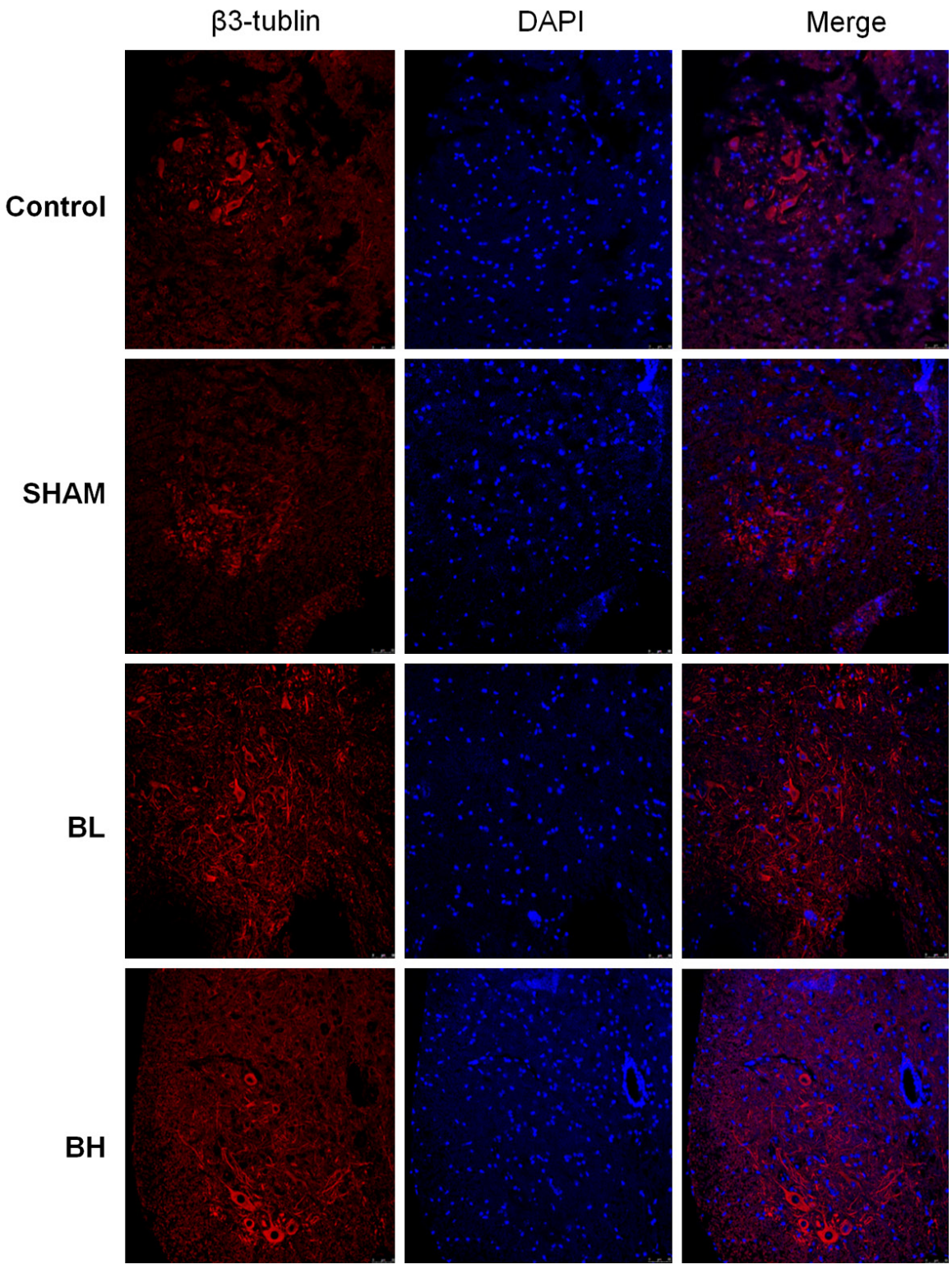

Figure 4. Effect of BTX-A on spinal cord neurons in mice. Spinal cord tissues were labeled with β3-tubulin; there were 5 rats in each treatment group, and at least three sections were labeled with antibodies in each group. BTX-A, botulinum neurotoxin serotype A; BL, BTX-A low dose group; BH, BTX-A, high dose group.

the surface of spinal neurons. Further research was required for confirmation. Similarly, the levels of phosphorylated p38MAPK and Bax increased, while the expression of Bcl2 de-

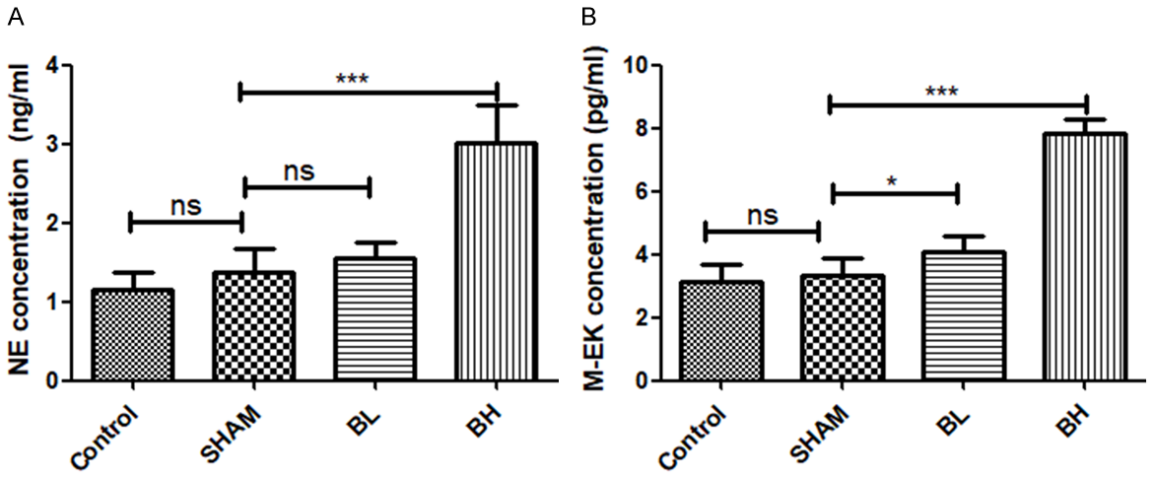

Figure 5. BTX-A can increase the secretion of spinal cord analgesic factors (A) NE and (B) M-EK in endometriosis model mice (n = 5 rats/group). $^{*}P < 0.05$ and $^{***}P < 0.001$, as indicated. BTX-A, botulinum neurotoxin serotype A; NE, norepinephrine; M-EK, methionine enkephalin; ns, not significant; BL, BTX-A low dose group; BH, BTX-A, high dose group; ns, not significant.

creased in the EMS model, but the levels were close to those of the control after BTX-A treatment (**Figure 6A** and **6B**).

Discussion

PC12 is a rat adrenal pheochromocytoma cell line with some characteristics of adrenal medullary chromaffin cells [14]. As adrenomedullary cells originate from the embryonic nerve spine, PC12 cells have neuronal cellular properties, and catecholamines (dopamine, epinephrine and norepinephrine) and enkephalin neurotransmitters, which are good analgesic substances, are secreted in conventional culture [20, 24]. Therefore, the OGD model was used in the present study to produce neuronal damage to investigate the molecular mechanism underlying the effect of BTX-A on neuronal injury and pain *in vitro*. The results revealed that BTX-A treatment could significantly increase the survival rate of PC12 OGD model cells and restore the morphology of the cells close to that of the control group in a concentration-dependent manner. This result was similar to a previous research, which showed that BTX-A can induce nerve outgrowth [10] and enhance Schwann cell proliferation [25]. At the same time, the repair of injured neurons was accompanied by an increase in the secretion of the analgesic factors NE and M-EK. It has been reported that these two substances have analgesic effects. Among them, NE can inhibit the release of excitatory substances in presynaptic neurons, promote the hyperpolarization of postsynaptic neurons, and decrease their excitability, thereby improving the inhibitory function of NE on the descending neural pathway [21]. M-EK, through its receptor, regulates the ion channels of neurons and inhibits the release of excitatory transmitters [26]. According to the available data *in vitro*, BTX-A can repair the PC12 cell damage induced by OGD to some extent, and may improve the analgesic ability of the cell.

Then, BTX-A was tested *in vivo* to reduce EMS-induced pain and spinal cord nerve injury. After subcutaneous injection of BTX-A, the writhing response of the mice continued to decrease within 30 min, and was close to the control group on day 5, while the high and low dose groups were significantly lower than the control group. The results demonstrated that BTX-A could significantly inhibit the pain response in EMS model mice. However, there was no significant difference between high and low dose groups, indicating that there might be a certain threshold for BTX-A analgesia *in vivo*. In addition, it was verified that BTX-A could repair the spinal cord injury induced by EMS. As expected, it did have a certain degree of injury repair by reducing apoptosis or increasing the outgrowth of spinal cord nerve cells. This conclusion was consistent with the previous research which reported that BTX-A could repair the pain caused by inflammation and neuropathology [27] and increase the growth of spinal cord axons [28]. Then the possible mechanism of spinal cord nerve injury repair underlying the analgesic effect was discussed. By detecting

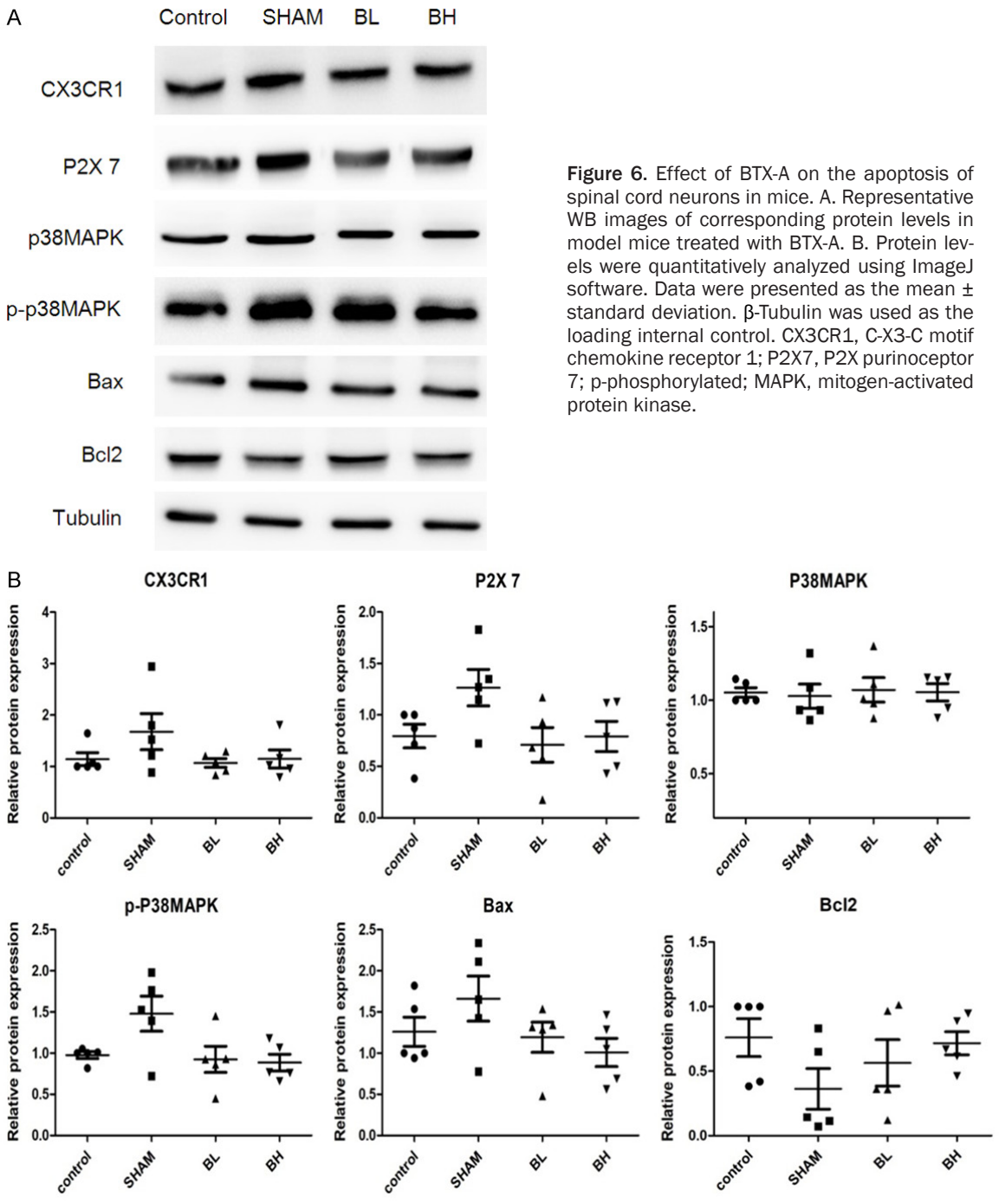

Figure 6. Effect of BTX-A on the apoptosis of spinal cord neurons in mice. A. Representative WB images of corresponding protein levels in model mice treated with BTX-A. B. Protein levels were quantitatively analyzed using ImageJ software. Data were presented as the mean ± standard deviation. β-Tubulin was used as the loading internal control. CX3CR1, C-X3-C motif chemokine receptor 1; P2X7, P2X purinoceptor 7; p-phosphorylated; MAPK, mitogen-activated protein kinase.

the level of NE and M-EK in spinal cord tissues, it was revealed that BTX-A could increase the levels of these neurotransmitters. In conclusion, the analgesic effect of BTX-A on EMS is likely to be induced by protecting spinal cord nerve cells from injury and promoting the secretion of analgesic factors by nerve cells.

Further study on the mechanism of BTX-A underlying the repair of spinal cord nerve cell injury at the molecular level was required. The MAPK family includes extracellular signal-regulated kinases, terminal kinases (such as JNK) and p38MAPK, which are widely expressed in neurons, astrocytes and microglia, and play different roles in different types of pain [29]. Among them, p38MAPK mediates the signal transduction of apoptosis when cells are stimulated by stress. Once activated, it can rapidly enter the nucleus from the cytoplasm and acti-

vate transcription factor p53, which leads to mitochondrial dysfunction by inhibiting members of the Bcl2 family and promoting the expression of Bax, which results in the release of apoptotic factors into the cytoplasm to activate caspase cascades leading to apoptosis [30-33]. From the results of the present study, the phosphorylation level of p38MAPK in the spinal cord of the EMS model was increased, while the expression of Bax increased and Bcl2 decreased. However, after BTX-A treatment, the expression levels of these three proteins were close to those of the control group. These results suggest that EMS can activate p38MAPK and induce spinal cord apoptosis through the Bax/Bcl2 signaling pathway. BTX-A, on the other hand, may inhibit the apoptosis of spinal cord nerve cells by inhibiting the activation of p38MAPK.

In conclusion, the most notable finding of the present study was that BTX-A could relieve EMS-induced pain and repair the apoptosis of spinal cord nerve injury *in vivo*. This repair pathway was probably associated with regulating p38MAPK and Bax/Bcl2, and the levels of NE and M-EK. As pain production is a very complex process, the relationship between the size of heterotopic plants and the degree of pain response needs further study. The exact mechanism by which BTX-A relieves pain needs to be verified in the future. It is expected to provide feasible plans and theoretical basis for the treatment of endometriosis and other related diseases.

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Disclosure of conflict of interest

None.

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