Moxibustion attenuates neurogenic detrusor overactivity in spinal cord injury rats by inhibiting M2/ATP/P2X3 pathway

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Research

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Abstract

Activation of muscarinic receptors located in bladder sensory pathways is generally considered to be the primary contributor for driving the pathogenesis of neurogenic detrusor overactivity following spinal cord injury. The present study is undertaken to examine whether moxibustion improves neurogenic detrusor overactivity via modulating the abnormal muscarinic receptor pathway.

Background

Spinal cord injury (SCI) is a serious traumatic event to the central nervous system, which can result in sensory and motor deficits, mental illnesses, and urinary disorders [1]. One of the most important consequences of SCI is loss of voluntary control over bladder function [2]. The initial phase following acute SCI is that of spinal shock, during which the bladder is hypocontractile and urinary retention occurs. Spinal shock is slowly replaced by an involuntary spinal micturition reflex pathway located at the lumbosacral spinal cord that is characterized by detrusor-sphincter dyssynergia [3]. Of note, urinary incontinence, due to development of neurogenic detrusor overactivity (NDO), has been reported in most

Methods

Female Sprague-Dawley rats were subjected to spinal cord injury with T9-10 spinal cord transection. Fourteen days later, animals were received moxibustion treatment for one week. Urodynamic parameters and pelvic afferents discharge were measured. Acetylcholine and adenosine triphosphate content in the voided cystometry uid were determined. Expressions of M2, M3 and P2X3 receptor in the bladder mucosa were evaluated.

Results

Moxibustion treatment prevented the development of detrusor overactivity in SCI rats, with an increase in the intercontraction interval and micturition pressure threshold and a decrease in afferent activity during filling. The expression of M2, but not M3, was markedly suppressed by moxibustion, accompanied by a reduction in the level of ATP and P2X3. M2 receptor antagonist methoctramine hemihydrate had similar effects to moxibustion on bladder function and afferent activity, while the M2-preferential agonist oxotremorine methiodide abolished the beneficial effects of moxibustion.

Conclusions

Moxibustion is a potential candidate for treatment of neurogenic bladder overactivity in a rat model of spinal cord injury possibly through inhibiting the M2/ATP/P2X3 pathway.
SCI patients, which is the main reason for the decline in quality of life [4]. In fact, after improving motor and sensory function, restoring bladder control is the highest priority for patients with SCI [5, 6], as it has a significant impact on social participation and daily work performance.

Experimental studies have shown that bladder C-fibers play an essential role in the pathogenesis of NDO [7–9]. NDO occurrence could be eliminated by the administration of systemic capsaicin in SCI rats [10], suggesting that the synaptic reorganization responsible for the new abnormal spinal micturition reflex is mediated by capsaicin-sensitive C-fiber afferents [11]. There is now compelling evidence supporting a role for the muscarinic receptors and their mediated bladder sensory pathways in the regulation of the excitation of C-fiber afferents [12, 13]. Under pathological condition, a number of neurogenic and non-neurogenic acetylcholine (Ach) released from urothelial cells, act on muscarinic receptors (mainly M2 and M3 muscarinic receptor subtypes) on bladder mucosa [14, 15]. Activation of muscarinic receptors stimulates the release of adenosine triphosphate (ATP) and the expression of purinergic P2X3 receptor, which increases the excitability of C-afferent nerves and elicits sensations of bladder fullness [16–19].

Traditionally, anti-muscarinic drugs are considered to be the mainstay for medical treatment of detrusor overactivity. However, their use is limited by the serious adverse effects such as dry mouth, constipation, blurred vision, and dyspepsia, so searching for new treatments is warranted [20]. Moxibustion, as a part of traditional Chinese medicine, is frequently used to treat lower urinary disease including urination disorders post stroke [21], female stress urinary incontinence [22], and dysuria after operation for lower limb fracture [23]. Clinical studies have shown that moxibustion remarkably improved bladder compliance and bladder function in patients with neurogenic bladder after SCI [24–26]. Our previous animal experiments observed that moxibustion inhibited the NDO in SCI rats and its effect is possibly relevant with reducing the expression of detrusor M2 receptors [27]. Nonetheless, the specific mechanisms responsible for the effects of moxibustion on NDO are still unclear, restricting its wide application. In view of the fact that muscarinic receptor mediated sensory pathways influence afferent nerve activity, the purpose of this study is to verify the hypothesis that moxibustion ameliorates NDO symptoms is related, in part, to regulation the M2/M3 receptors pathway.

**Methods**

**Animals**

Adult female Sprague-Dawley rats (female, 7-week old, Vital River Laboratory Animal Technology Co. Ltd, Beijing, China) weighing 180–250 g were housed at a constant temperature (23 ± 2 °C) and humidity (50%-60%) under a regular 12-hour light/dark schedule. Tap water and standard rat chow were freely available. All animal experiments were done in accordance with international guidelines for the ethical use of experimental animals and were examined and approved by the Institutional Animal Care and Use Committee of China Academy of Chinese Medical Sciences (Beijing, China).

**Experimental protocol**
Experiment I

To observe the effect of moxibustion on urodynamic parameters and afferent activity, we used a random number table method to randomly divide rats into 4 groups: Sham-operated (Sham), SCI, SCI + moxibustion (Mox) and SCI + capsaicin (Caps) groups (n = 8 per group). The rats in the Sham and SCI groups received the same operation as SCI + Mox and SCI + Caps groups without moxibustion treatment. The latter two groups received treatment 2 weeks after surgery. After 7 days of treatment, the rats were subjected to cystometric evaluation and bladder afferent nerve recording experiment.

Experiment II

To investigate whether moxibustion-induced improvement in bladder function was associated with bladder muscarinic receptor pathway, we randomly divide rats into 3 groups: Sham, SCI, and SCI + Mox groups (n = 8 per group). The SCI + Mox group received treatment 2 weeks after spinal cord transection surgery. After 7 days of treatment, we determined the level of ATP in urinary fluid by luciferine-luciferase assay, and detected the expressions of M2, M3, and P2X3 in the bladder mucosa by Western Blot and Real-time PCR.

Experiment III

To determine whether the improvement of bladder function in moxibustion-treated rats was on account of inhibiting the activation of muscarinic receptor, we used the oxotremorine methiodide (Oxo-M) to activate muscarinic receptor and the methoctramine hemihydrate (Metho) to block M2 receptor subtype. Rats were randomly divided into 7 groups: Sham, SCI, SCI + Mox, SCI + Oxo-M, SCI + Mox + Oxo-M, SCI + Saline, and SCI + Metho (n = 8 for each group). The urodynamic parameters and bladder afferent activity were detected after 7 days of treatment.

Spinal cord transection

The model of chronic SCI induced by a complete spinal cord transection, as previously described [28]. In brief, rats were anesthetized with 2% isoflurane and a dorsal midline incision was made over the thoracic spinal cord. Complete transection of the T9-10 spinal cord was performed with fine scissors and a sterile Gelfoam® sponge (Ferrosan, Soeborg, Denmark) was placed between the cut ends of the spinal cord. The overlying muscle and skin were then sutured separately to close the wound. Rats were treated postoperatively with ampicillin (100 mg/kg, intramuscularly) for 5 days. During the period characterized by bladder areflexia, bladders were manually emptied by abdominal compression twice a day until reflex voiding recovered. This period lasted for 10 days to 2 weeks. In the Sham group, animals were subject to the same operation except for spinal cord transection and underwent abdominal compression at the same time as the SCI rats.

Moxibustion treatment

Two weeks after the operation, rats in the SCI + Mox group received ginger-salt-indirect moxibustion treatment. The rats were gently fixed to a specific fixator in a supine position, with their navels exposed
(Fig. 1). After the skin was cleaned with alcohol, a certain amount of salt (about 1g) was put on the navel to cover the “Shenque” (CV8) acupoint and it was then covered by a fresh slice of ginger (30 mm in diameter and 4–5 mm in thickness). Then a moxa cone (pure worm wood fiber material, 15 mm in diameter and 30 mm in length; Tongrentang Inc, China) was placed on the fresh ginger slice and lit by the operator. Rats in the SCI + Mox group received three units of moxa cone (about 15 minutes) per day for 1 week [27]. The operator was required to observe the rat carefully and quickly remove the burning ash to avoid injury. Sham group and SCI group had the same treatment as the SCI + Mox group except that the moxa sticks were not ignited. All rats had been placed on the fixture every day for 15 minutes of adaptive training in the same posture a week before treatment.

**Drugs**

To induce C-fiber desensitization, capsaicin (125 mg/kg, dissolved in 10% ethanol, 10% Tween 80 and 80% physiological saline) was injected subcutaneously into rats. As described in previous studies [29–31], capsaicin was administered in divided doses on 2 consecutive days: 25 and 50 mg/kg at a 12-h interval on the 1st day and 50 mg/kg on the 2nd day, and the experiments were performed 4 days after the last injection. Oxo-M (Sigma; dissolved in saline; intraperitoneal injection of 0.2 mg/kg), a preferential M2 agonist [32, 33], was administrated at 15 minutes before each moxibustion treatment. Metho, a selective M2 receptor antagonist (Sigma; dissolved in saline; intraperitoneal injection of 0.3 mg/kg) [34], was used in our experiments.

**In vivo cystometric evaluation and bladder afferent nerve recording**

Rats were anesthetized with intraperitoneally urethane (1.0-1.2 g/kg) that is recommended as an anesthetic for electrophysiological experiments on the lower urinary tract in rats [35]. Core body temperature was kept between 36 and 37°C with the help of a heating pad controlled by a thermosensor connected to a rectal probe. After exposing the urinary bladder through a medial abdominal incision, a three-barrel catheter was inserted through its dome. One barrel was connected to an automated perfusion pump for saline infusion; a second barrel was attached to a pressure transducer for continuous monitoring of intravesical pressure. The left side postganglionic bladder nerve close to the bladder (branches of the pelvic nerve) was carefully dissected and thin non-absorbable, sterile sutures were tied around the identified nerve. A custom-made bipolar electrode, which consisting of two thin (diameter 0.1 mm) platinum-iridium hook-shaped wires was separated by a distance of 0.5-1 mm, was mounted on a micromanipulator and the identified nerve was carefully placed on the wires. The isolated pelvic nerve branch was crushed between the major pelvic ganglion and the electrode, to eliminate efferent nerve signals and afferent activity only was recorded. Warm saline (0.9 % NaCl) was infused into the abdomen to prevent drying out of the organs during surgery. After the surgical procedures, the saline was removed and the pelvic cavity was kept moist by warm paraffin oil throughout the electrophysiological measurements.
After surgical preparation, the micturition reflex was initiated by keeping the intravesical infusion of saline at a constant flow rate of 0.1 mL/min for 1h, which was within the range to obtain stable micturition cycles during continuous a cystometrogram in anaesthetized rats. Voiding contractions were assumed as large-amplitude rhythmic bladder contractions accompanied by urine draining through the urethra when bladder pressure reached a certain threshold. Intravesical pressure was monitored using a pressure transducer (MLT0380, AD Instruments, Colorado Springs, CO, USA) connected to a bridge amplifier (ML221, AD Instruments, Colorado Springs, CO, USA). Afferent nerve activity and pressure signals were continuously monitored on a computer screen with a PowerLab/8SP data acquisition system and LabChart 8 software (AD Instruments, Colorado Springs, CO, USA). The electrical signal of the pelvic nerve was correspond to the urination waveform of bladder pressure. During cystometry, intercontraction interval (ICI) and micturition pressure threshold (MPT) were measured. ICI and MPT are cystometry parameters that are required to initiate the voiding reflex and are normally associated with the sensitive component of the micturition reflex during the filling phase. The pressure transducer was calibrated using a column of water before the start of each experiment. The nerve signal was calibrated using a 1-V (500 Hz) sinusoidal test signal. The rats were euthanized at the end of the experiment with an overdose of urethane.

**Western Blot**

The entire bladder mucosa was dissected from the detrusor muscle under a microscope. The total, nuclear and cytoplasm proteins were extracted from mucosa and the protein concentrations were determined by the Pierce BCA Protein Assay kit (Thermo Scientific). Samples were separated by a 10% SDS-PAGE (Applygen) gel and electrically transferred onto nitrocellulose membranes (Protran). The membranes were blocked with 5% non-fat dried milk and incubated overnight at 4°C with anti-M2 antibody (1:100, Santa Cruz Biotechnology), anti-M3 antibody (1:200, Santa Cruz Biotechnology), anti-P2X3 antibody (1:1000, Abcam), and GAPDH (1:1000, Bioss). The membranes were incubated with secondary antibodies for 1 h at room temperature and visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences, Nebraska USA).

**Quantitative realtime-polymerase chain reaction (qPCR)**

Total RNA was extracted from the bladder mucosa tissues with a Trizol Reagent (*Introgen, Carlsbad, CA*). Two micrograms of total mRNA were used for first-strand cDNA synthesis with AMV reverse transcriptase (Promega, Madison, WI). The mRNA levels of genes of interest were analyzed by Real-time PCR, which was performed with 2x SYBR master mix (Takara, Otsu, Shiga, Japan), using a BIORAD iCycler iQ5 (Bio-Rad, Hercules, CA). Gene levels were normalized to that of β-actin. Relative mRNA expressions were calculated by the $2^{-\Delta\Delta Ct}$ method.

**Measurement of urinary ATP**

For measuring urinary ATP content, samples were collected from the draining barrel of the catheter inserted in the bladder during in vivo fluid cystometry experiments. Sterile samples were immediately
freeze-dried in liquid nitrogen and stored at -80°C until determination. ATP in the samples was measured by the ATP bioluminescent assay kit (Sigma-Aldrich, St Louis, MO, USA).

**Statistical Analysis**

All data are expressed as the mean ± SD. Results were analyzed using one-way ANOVA with the Bonferroni multiple comparison post hoc test. *P*-value < 0.05 was considered to be statistically significant.

**Results**

**Moxibustion improves urodynamic parameters and decreases bladder afferent C-fiber activity in SCI rats**

To evaluate the effect of moxibustion on voiding function, we measured the continuous infusion urodynamic parameters in SCI rats. Spinal cord transection induced bladder hyperactivity as evidenced by decreasing the ICI and MPT compared with the Sham group. Moxibustion considerably increased the ICI and MPT compared to the SCI group (Fig. 2A and B). As showed in Fig. 2C, a notable increase of afferent nerve activity was observed in the SCI group, which was weakened by moxibustion treatment. Although moxibustion ameliorated the urodynamic parameters and afferent activity, it was still worse than the Sham group, suggesting that moxibustion could improve bladder function but was unable to bring it back to normal. To further identify whether moxibustion influenced the activity of bladder afferent C fibers, we applied capsaicin to desensitize C-afferent nerve fibers. Capsaicin pretreatment blocked the SCI-induced enhancement of afferent firing as well as reduction of ICI and MPT (Fig. 2). Additionally, there was no significant difference between SCI + Mox group and SCI + Caps group in afferent nerve discharge, ICI and MPT.

**Moxibustion inhibits the expression of M2, but not M3 muscarinic receptor in the bladder mucosa of SCI rats**

To further assess whether the improvement of bladder function by moxibustion was associated with M2 and M3 muscarinic receptor subtypes in mucosa, we detected the expressions of M2 and M3 protein and mRNA. Interestingly, we found that the protein and mRNA levels of M2, but not M3, were substantially up-regulated in the SCI group in comparison with the Sham group, but obviously down-regulated after moxibustion treatment (Fig. 3).

**The M2 receptor antagonist simulates the improvement of bladder function induced by moxibustion, while the M2-preferential agonist reverses the beneficial effect of moxibustion**

Considering that the improvement in bladder function was mediated by M2 receptor, we reasoned that M2 antagonist Metho may simulate moxibustion on ameliorating continuous infusion urodynamic parameters and M2-preferential agonist Oxo-M may mitigate the beneficial effects of moxibustion.
Compared with SCI group, an evident improvement of ICI and MPT was observed in SCI + Metho group. There was no difference between SCI + Metho group and SCI + Mox group. However, application of Oxo-M before moxibustion treatment prevented the moxibustion-induced therapeutic effect in SCI rats. Moreover, compared to the SCI + Mox group, a notably decrease of ICI and MPT in the SCI + Oxo-M group and SCI + saline group (Fig. 4A and B).

The M2 receptor antagonist mimics the restoration of afferent activity induced by moxibustion, but the M2-preferential agonist abolishes the conductive effect of moxibustion

To confirm whether the M2 receptor is implicated in the moxibustion-mediated restoring effect on bladder afferent activity, we detected afferent nerve discharges after administration of Metho or Oxo-M in rats. Similar to the results of urodynamic parameters, there was no significant difference between SCI + Metho group and SCI + Mox group. Injection of Oxo-M in moxibustion-treated rats abolished the beneficial effect of moxibustion. Besides, afferent activity was enhanced in the SCI + Oxo-M and SCI + saline groups compared with the SCI + Mox group (Fig. 4C).

Moxibustion decreases the levels of ATP, and P2X3 in SCI rats

To explore whether the downstream pathway of M2 was also regulated by moxibustion, we next examined the expressions of ATP and P2X3. The level of ATP was significantly increased in the SCI group when compared to the Sham group, and moxibustion treatment decreased the content of ATP induced by SCI (Fig. 5A). In comparison with the Sham group, the protein and mRNA expressions of P2X3 were enhanced in the SCI group, but reduced after moxibustion treatment (Fig. 5B and C).

Discussion

In this study, we found that moxibustion prevented the development of detrusor overactivity in SCI rats. Additionally, moxibustion suppressed the expression of M2, but not M3 receptor, companied by a decrease in the release of ATP and the expression of P2X3 receptor in the bladder sensory pathway. Metho, a M2 receptor antagonist, mimicked the therapeutic effects of moxibustion, while Oxo-M, a preferential M2 agonist, notably abolished the effects. Thus, it is reasonable to conclude that the beneficial effects of moxibustion on bladder function seem to be correlated with the inhibition of M2/ATP/P2X3 pathway.

Moxibustion involves the application of ignited mugwort directly or indirectly at acupoints or other specific parts of the body to cure diseases [36]. This therapy is to warm the body and enhance the energy of patients to prevent and treat a variety of chronic deficiency diseases [37], including urinary incontinence [21]. It was manifested that moxibustion reduced residual urine volume and increased volume of bladder in patients with urinary retention after SCI [24]. A profound improvement in bladder function receiving moxibustion compared to intermittent clean urethral catheterization controls in patients of neurogenic vesical dysfunction [26]. Importantly, moxibustion ameliorated the mean time of
urination and urgent urinary incontinence each day for the patients [38]. In conformity to these results, our study demonstrated that SCI impaired bladder voiding function as evidenced by a reduction of ICI and MPT, while moxibustion reversed these changes.

Bladder afferent nerves originate in lumbosacral dorsal root ganglia and carry information concerning bladder fullness and pain. Composed of myelinated A-fibers and unmyelinated C-fibers, they terminate in the urothelium, suburothelial space and the muscle layers of the bladder and are activated by mechanical or chemical stimulation [39]. Normal urination depends on the activation of Aδ-fiber bladder afferents, whereas C-fiber afferent activation is generally considered to be associated with lower urinary tract dysfunction [40]. The neurotoxicity of capsaicin could prevent the occurrence of NDO in SCI cats, but had no effect on the micturition reflex of normal animals, further suggesting that the C-fibers is not the main afferent fiber in the normal micturition, but participates in the abnormal voiding contraction [10, 41, 42]. In this study, we also observed that a significant enhancement in the excitability of pelvic afferent nerve in rats with SCI and an obviously decrease following moxibustion treatment. Capsaicin application attenuated the abnormal firing of pelvic afferent nerves as well as improved bladder function, which demonstrates that NDO after SCI in rats is primarily mediated by the aberrant activity of C afferent fibers. It is noteworthy that there is no difference between moxibustion and capsaicin groups, implying that the therapeutic effect of moxibustion may be somewhat similar to that of capsaicin, which can ameliorate the bladder function of SCI rats via inhibiting the activity of bladder C-fiber afferents.

Accumulating evidence suggests that the initiation of NDO after SCI is attributed to the activation of muscarinic receptors in bladder sensory pathways [17]. Of note, M2 and M3 receptor subtypes are the predominant muscarinic receptors in bladder both rats and humans [43]. The number of M2 receptor subtype is more than that of M3 receptor subtype, but M3 receptor subtype is generally thought to be the main contributor of detrusor contraction [44, 45]. Intriguingly, in our study, we found M2, but not M3 receptor subtypes, expression in bladder mucosa was considerably increased by SCI, and was obviously reversed by moxibustion treatment. Similar to our results, matsumoto et al. [46] evaluated the role of M2 and M3 receptor subtypes in activity of bladder afferent pathways. They suggested that the M2 rather than M3 receptor subtype contributes to bladder overactivity in rats. Pontari et al. [47] verified that muscarinic receptor subtype mediating bladder contraction shifted from M3 to M2 in individuals with neurogenic bladder dysfunction caused by SCI. What's more, we observed that Oxo-M as a preferential M2 agonist significantly blocked the improvement of bladder function and afferent activity induced by moxibustion, while Metho as a M2-selective antagonist had the similar effect as the moxibustion, manifesting that moxibustion exerts beneficial effects on bladder function through inhibiting the activation of M2 receptor subtype.

The mechanism by which moxibustion treatment decreases M2 muscarinic receptor levels to alleviate bladder overactivity in SCI rats is still unclear. One possible mechanism might be related with ATP release and P2X3 receptor activation [48]. It has been well-established that during bladder filling, activation of M2 muscarinic receptors induced by Ach, might lead to increased release of ATP from the urothelium [49]. ATP, in turn, can activate P2X3 receptors located on the urothelial cells [50], myofibroblasts [51, 52] and
afferent C-fibers [53, 54] to trigger the micturition reflex. Previous reports have confirmed that the application of M2-preferential agonist Oxo-M caused substantial ATP release and mucosal contractions, which was abolished by the M2-specific antagonist Metho [55]. Intravesical administration of high dose Oxo-M increased voiding frequency via mechanisms involving ATP and NO release from the urothelium, while these effects were absent in rats pretreated with capsaicin to desensitize C-fiber afferent nerves [48]. Furthermore, high levels of intravesical ATP in a rat model of SCI positively modulated P2X3 and P2X2/3 receptors to intensify sensory signals and generate NDO [53]. In our research, moxibustion inhibited the expressions of ATP and P2X3 in SCI rat, which indicates that moxibustion improves bladder function possibly through modulation of M2/ATP/P2X3 pathway.

There are several limitations in this experiment. Firstly, our research only focused on the effects of moxibustion on ICI and MPT. More urodynamic parameters, such as urine output and residual urine, should be used to systematically evaluate the effect of moxibustion on bladder function in future studies. In addition, observation of the therapeutic effect of moxibustion was limited to 7 days after treatment. How long moxibustion takes effect and how long the effect can last still remain to be further verified. Importantly, moxibustion has similar therapeutic effects as antimuscarinic agents, but there is a lack of comparison and evaluation of their side effects. A number of experiments are necessary to be carried out to assess the safety of moxibustion in the treatment of NDO, so as to provide reliable evidence for clinical translation.

Conclusions

In summary, we demonstrated that moxibustion treatment ameliorated bladder function in SCI rats, which was probably associated with the inhibition of M2/ATP/P2X3 pathway (Fig. 6). In particular, moxibustion may be a promising M2 receptor inhibitor for treating NDO after SCI. The current research provides new insights into the protective effect of moxibustion and implicates moxibustion as a potential complement to pharmacological agents for neurogenic bladder overactivity.

Abbreviations

SCI: spinal cord injury; NDO: neurogenic detrusor overactivity; Ach: acetylcholine; ATP: adenosine triphosphate; Oxo-M: oxotremorine methiodide; Metho: methoctramine hemihydrate; ICI: intercontraction interval; MPT: micturition pressure threshold.

Declarations

Acknowledgments

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Authors’ contributions
LW, YBF, YL and CZL conceived and designed the work; YFB and GXS helped to coordinate support and funding; LW, YL, NNY, SMM, XRW and JH performed the experiments; LW analyzed the data and wrote the original draft; YBF, GXS, JWY and CZL reviewed and revised the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

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

**Ethics approval and consent to participate**

The protocol was approved by the Institutional Animal Care and Use Committee of China Academy of Chinese Medical Sciences, and conformed to the Guide for the Care and Use of Laboratory Animals.

**Consent for publication**

Not applicable.

**Competing interests**

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

**References**


**Figures**

![Figure 1](image)

**Figure 1**

The location of CV8 acupoint on the rat and the schematic representation of ginger-salt-indirect moxibustion treatment.
Moxibustion improves urodynamic parameters and inhibits bladder afferent nerve firing in SCI rats. (A) Intercontracion interval. (B) Micturition pressure threshold. (C) Nerve discharge frequency. Values are mean±SEM of 8 animals per group. **p<0.01, ***p<0.001, compared with the Sham group; ##p<0.01, ###p<0.001, compared with the SCI group.
Figure 3

Moxibustion reverses SCI-induced over-expression of M2, not M3 muscarinic receptor subtype in the bladder mucosa of SCI rats. (A-B) The protein levels of M2 and M3 and its corresponding GAPDH bands were determined using Western blot analysis. (C-D) The expressions of M2 and M3 receptor mRNA level were analyzed by qPCR. Values are mean±SEM of 8 animals per group. **p<0.01, ***p<0.001, compared with the Sham group; ##p<0.01, ###p<0.001, compared with the SCI group.
Figure 4

The M2 receptor antagonist mimics moxibustion-induced improvement of urodynamic parameters and afferent nerve activity, and the muscarinic receptor agonist reverses the beneficial effect of moxibustion. (A) Intercontracion interval. (B) Micturition pressure threshold. (C) Nerve discharge frequency. Values are mean±SEM of 8 animals per group. ***p<0.001, compared with the Sham group; ##p<0.01, ###p<0.001, compared with the SCI group; &p<0.05, &&&p<0.001, compared with the SCI+Mox group.
Figure 5

Moxibustion decreased the content of ATP in urinary fluid and the expression of P2X3 in the bladder mucosa of SCI rats. (A) The ATP level was quantified by the luciferin-luciferase bioluminescence assay. (B) The protein level of P2X3 and its corresponding GAPDH bands was determined using Western blot. (C) The expression of P2X3 receptor mRNA level analyzed by qPCR. Values are mean±SEM of 8 animals per group. **p<0.01, ***p<0.001, compared with the Sham group; #p<0.05, ###p<0.001, compared with the SCI group.
Figure 6

Proposed scheme showing how moxibustion inhibits SCI-induced neurogenic detrusor overactivity via the M2/ATP/P2X3 pathway. Moxibustion treatment alleviates neurogenic detrusor overactivity in SCI rats. These effects involve suppression of activation of the M2/ATP/P2X3 pathway. M2 antagonist Metho mimics the effects of moxibustion, while M2 agonist Oxo-M reverses the beneficial effects of moxibustion.