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Research paper

The effect of sacral nerve root magnetic stimulation on bladder urodynamics and M3 receptor expression in rats with neurogenic bladder

Junhua Li, Chenhao Tang, Longfei Yang, Chen Song, Yanbin Wang *

Department of Urology, Hangzhou Third People's Hospital, Hangzhou 310009, China

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ABSTRACT

Objective: To investigate the effect of sacral nerve root magnetic stimulation(SMS) on bladder urodynamics and M3 receptor expression in rats with neurogenic bladder.

Methods: 30 adult female SD rats were randomly divided into Normal Group, Model Group, and Magnetic Stimulation Group, with 10 rats in each group. The Model Group and Magnetic Stimulation Group used the spinal cord transtion method to establish the neurogenic bladder animal model. After successful modeling, the Magnetic Stimulation Group received magnetic stimulation treatment once per day for 8 consutive weeks. After 8 weeks, bladder urodynamics were measured under anesthesia, rats were sacrificed, and bladder detrusor muscle tissue was collected for histological and ultrastructural observation, and M3 receptor expression levels were measured.

Results: The maximum bladder capacity and bladder compliance in the Magnetic Stimulation Group were higher than those in the Model Group (all $P < 0.05$), and the leak point pressure was lower than that in the Model Group ($P < 0.05$); there were no significant differences between the Magnetic Stimulation Group and the Normal Group in these three parameters (all $P > 0.05$). H&E staining of bladder detrusor muscle tissue in the Magnetic Stimulation Group revealed minimal neutrophil infiltration. Moreover, the morphology and arrangement of the mucosal epithelial cells were closer to those observed in the Normal Group when compared with the Model Group. Under transmission electron microscopy, detrusor muscle cells had a smooth surface, slightly widened intercellular spaces, relatively uniform arrangement, and relatively intact mitochondrial structure. The expression level of M3 receptors in the bladder detrusor muscle tissue of the Magnetic Stimulation Group was significantly higher than that in the Normal Group and the Model Group (all $P < 0.05$); there was no significant difference between the Model Group and the Normal Group ($P > 0.05$).

Conclusion: Sacral nerve root magnetic stimulation has a certain effect on improving bladder function in rats with neurogenic bladder, which may be related to the increased expression level of M3 receptors in the bladder detrusor muscle tissue.

Introduction

Bladder dysfunction caused by injury to the central or peripheral nervous system is called neurogenic bladder (Niu et al., 2018), clinically manifested as bladder hypertension, urinary incontinence, recurrent urinary tract infections, hydronephrosis, and uremia. It is a very difficult condition to treat and remains an unresolved medical challenge. In recent years, with the rapid development in the field of neuro-urology, sacral nerve root magnetic stimulation technology, as a new non-invasive and non-traumatic neural stimulation system, has shown promising application prospects (Niu et al., 2018). This experiment prepared a rat model of neurogenic bladder to observe the effects of sacral nerve root magnetic stimulation on bladder urodynamics and M3 receptor expression in bladder detrusor muscle tissue after spinal cord injury, exploring potential mechanisms to provide a reference for solving clinical challenges.

Abbreviations: SMS, Sacral nerve root magnetic stimulation; SD, Sprague dawley; MBC, Maximum bladder capacity; BLPP, Bladder leak point pressure; ANOVA, One-way analysis of variance.

* Correspondence to: 38, West Lake Avenue, Hangzhou, Zhejiang 310009, China

E-mail addresses: lijunhua7016@163.com (J. Li), 745460210@qq.com (C. Tang), 704409406@qq.com (L. Yang), 253472521@qq.com (C. Song), 15068741817@163.com (Y. Wang).

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Materials and methods

Experimental animals

30 adult female SD rats were purchased from Shanghai Slake [Certificate No.: SCXK (Shanghai, china): 2017–004].

Animal grouping and treatment

The 30 rats were randomly divided into 3 groups: Normal Group, Model Group, and Magnetic Stimulation Group, with 10 rats in each group. Except for the Normal Group (no treatment), the other two groups were used to establish a neurogenic bladder animal model by spinal cord transtion method (Lee et al., 2022):Rats were anesthetized by subcutaneous injection of 2 % sodium pentobarbital(0.3 ml/100 g, Shanghai Chemical Reagent Procurement and Supply Station Repackaging Plant) in the abdomen, their limbs were spread and fixed on an experimental board, shaved, and disinfected routinely. The skin and subcutaneous tissue of the rat's back were sequentially cut, the bilateral erector spinae muscles were split longitudinally along the spinous process, the spinal cord tissue was severed at the T9 segment, residual blood clots and nerve tissue were cleared from the injury cavity, and then the wound was sutured in layers and disinfected, completing the model construction. Each rat was housed individually in a cage and initially assisted with urination by abdominal compression. One day after successful modeling,rats in the Magnetic Stimulation Group received the first sacral nerve root magnetic stimulation treatment in the awake state: Using a magnetic stimulator produced by Wuhan Yirui Company, The circular coil of magnetic stimulation was fixed to the sacrum of the rat, with the midpoint of the central point of the upper sacrum and the upper edge of the coccyx as the central point of the coil (Fig. 1). The stimulator was connected to a power source, and the frequency was set to 0. 5 Hz, magnetic stimulation intensity was (13 ± 4)mT, adjusted to cause twitching of the rat's sacrum and/or tail without causing whole-body tremors. Treatment was given once per day, with 30 stimulation impulses per session, each impulse lasting for 2 min, for 8 consutive weeks. During the treatment period, the rats were all awake and fixed on the workbench without stress. The Model Group had the circular coil placed on the rat's sacrum, but it was not connected to a power source, and no magnetic stimulation was given. All three groups of rats were routinely fed. One rat in the model group and two rats in the magnetic stimulation group died of pulmonary hemorrhage and pulmonary edema, resulting in 10, 9, and 8 rats completing the experiment in the Normal, Model, and Magnetic Stimulation Groups, respectively. After the treatment ended, the rats were anesthetized for bladder urodynamics measurement, sacrificed, and their bladder detrusor muscle tissue was collected for H&E staining and ultrastructural observation. The M3 receptor expression level in the bladder detrusor muscle tissue was also measured.

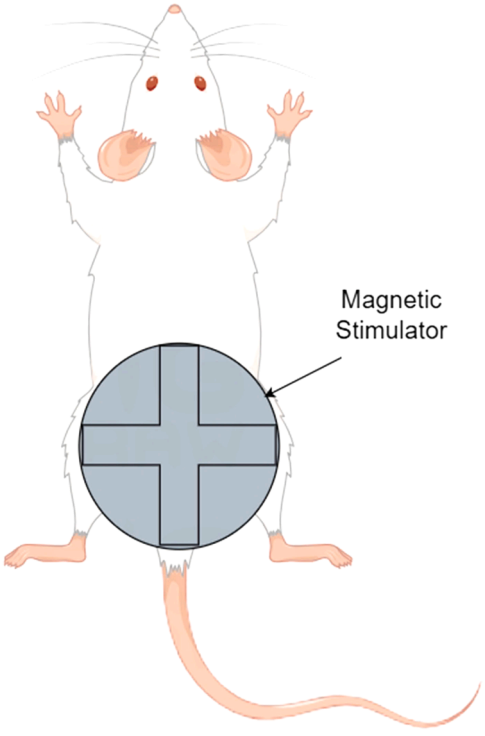

Fig. 1. The circular coil of magnetic stimulation was fixed to the sacrum of the rat, with the midpoint of the central point of the upper sacrum and the upper edge of the coccyx as the central point of the coil.

Bladder urodynamics

Before conducting urodynamic testing, gentle pressure was applied to the bladder area of the rats. In case of the presence of hematuria or turbidiuria in the rats, it was considered as a combined urinary tract infection, and levofloxacin(Harbin Pharmaceutical Group Pharmaceutical Factory, Harbin, China) at a dosage of 10 mg per 100 g body weight was administered via gavage until the symptoms subsided prior to urodynamic testing. Measurement Rats were anesthetized with ether, an F2 pressure catheter was inserted into the bladder, connected to the urodynamic pressure measuring tube, zeroed, and 0. 9 % sodium chloride solution was infused at a rate of 2 ml/h using a microinfusion pump (Abolhasanpour et al., 2020). Urodynamic parameters such as maximum bladder capacity(MBC), bladder leak point pressure(BLPP), and bladder compliance were recorded. Maximum bladder capacity was defined as the total infusion volume when continuous leakage occurred at the urethral opening. Bladder compliance was calculated as infusion volume/leak point pressure.

Bladder detrusor muscle histological observation

Using H&E staining, full-thickness bladder tissue was taken from the rats, fixed in 10 % formaldehyde, embedded in paraffin, and stioned. The stions were incubated in a 68 ℃ oven for 1 h, deparaffinized with xylene, dehydrated in a gradient ethanol series (from high to low concentration), rinsed with running water for several min, stained with hematoxylin for 0. 5 h, rinsed with running water to blue for several min, stained with eosin for 5 s, dehydrated in a gradient ethanol series (from low to high concentration), cleared with xylene, and sealed with neutral resin. Observations were made under a light microscope.

Ultrastructural observation of bladder detrusor muscle

Bladder detrusor muscle tissue from rats (approximately 1 mm×1 mm×1 mm) was fixed in 4 % glutaraldehyde solution at low temperature, embedded in paraffin, and pre-stioned (thickness 4–6 mm). After osmium staining for localization, ultrathin stions (thickness 0. 1 μm) were cut and observed under a transmission electron microscope.

The expression of M3 receptor in bladder tissue was determined by Western blot

Total protein was extracted from rat bladder tissue with RIPA lysis buffer (Thermo Scientific) containing protease inhibitor cocktail (Sigma). Total protein concentration was measured using the BCA assay (Pierce, Rockford, IL, USA). Total protein was separated by SDS-PAGE and transferred to a PVDF membrane (Merck Millipore). The membrane was incubated with anti-M3 Receptor and β-actin (Abcam, MA) primary antibodies. Next, goat anti-rabbit IgG and goat anti-mouse IgG (Biodragon, Beijing, China) sondary antibodies were incubated.

Immunoblotting was assessed using the Chemi Doc XRS + imaging system (Bio-Rad, Redmond WA, USA).

Statistical analysis

SAS 8. 0 statistical software was used for data analysis. Continuous data are expressed as mean ± standard deviation (Mean±SD). One-way analysis of variance (ANOVA) was used for comparisons among multiple groups, Bonferroni's test was used for post-hoc test. Pairwise comparisons were performed using the SNK-q test. A significance level of $P < 0.05$ was considered statistically significant.

Results

Comparison of bladder urodynamic parameters among the three groups

Before urodynamic testing, three rats in the model group and two rats in the magnetic stimulation group had hematuria symptoms, which disappeared within 3–5 days after treatment with gavage of levofloxacin. Comparison of maximum bladder capacity, bladder leak point pressure(BLPP), bladder compliance, and other urodynamic parameters among the three groups of rats showed statistically significant differences (all $P < 0.05$). Specifically, the maximum bladder capacity and bladder compliance in the Magnetic Stimulation Group were higher than those in the Model Group (all $P < 0.05$), and the bladder leak point pressure was lower than that in the Model Group ($P < 0.05$). There were no statistically significant differences in these three parameters between the Magnetic Stimulation Group and the Normal Group (all $P > 0.05$), as shown in Table 1.

Bladder detrusor muscle in three groups of rats

Observation of H&E staining results: In the Normal Group, the bladder detrusor muscle fibers were neatly arranged, cells did not show obvious enlargement (Fig. 2a). In the Model Group, the stroma has massive hyperemia, massive neutrophil infiltration, smooth muscle arrangement disorder (Fig. 2b). In the Magnetic Stimulation Group, the stroma was nearly normal, with a little neutrophil infiltration, hyperemia basically disappeared, and the smooth muscle arrangement was nearly normal(Fig. 2c).

Comparison of ultrastructure of bladder detrusor muscle in three groups of rats

In the Normal Group, the bladder detrusor muscle cells had normal structure with narrow extracellular space and intact organelle structures (Fig. 3a). In the Model Group, the bladder detrusor muscle cells were of varying sizes, with widened and disordered extracellular spaces. Numerous vacuolar structures were observed within the cells, and the normal organelle structures were indistinguishable (Fig. 3b). In the Magnetic Stimulation Group, the bladder detrusor muscle cells had smooth surfaces, slightly wider extracellular space, relatively uniform arrangement, the mitochondrial structure was basically intact. The number of vacuolar structures was significantly reduced, a small number of mitochondria were swollen but not ruptured, and the cytoplasmic features were generally well preserved (Fig. 3c).

Table 1
Comparison of bladder urodynamic parameters among three groups of rats (Mean±SD).

Groups	n	MBC (ml)	BLPP (cmH_2O)	Bladder Compliance (ml/cmH_2O)
Model Group	10	0. 56 ± 0. 32	33. 86 ± 1. 44	0. 016 ± 0. 007
Magnetic Stimulation Group	9	1. 29 ± 0. 52*	26. 58 ± 1. 53*	0. 043 ± 0. 011*
Normal Group	8	1. 32 ± 0. 12	22. 41 ± 1. 57	0. 057 ± 0. 012
P value		< 0. 05	< 0. 05	< 0. 05

Note: * $P < 0.05$ when compared with the Model Group

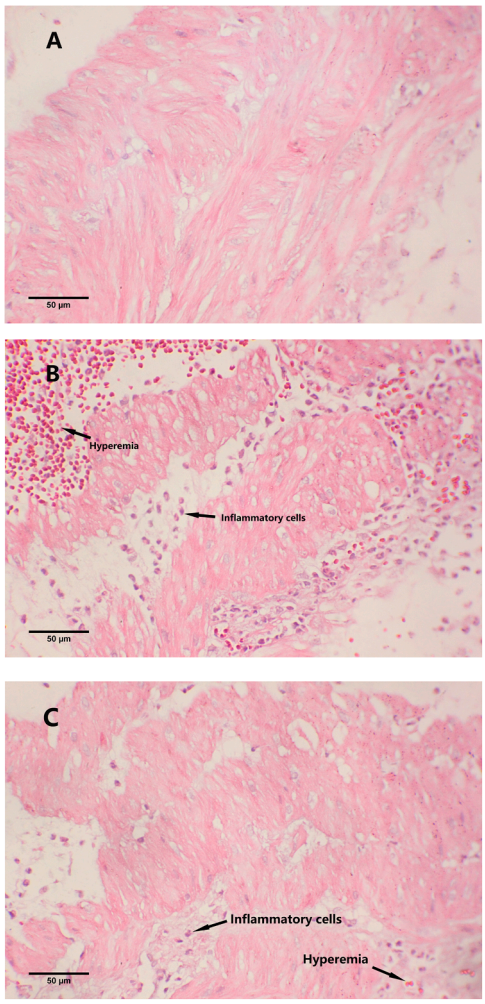

Fig. 2. Histological observation of bladder detrusor muscle tissues in three groups of rats (A: Normal Group; B: Model Group; C: Magnetic Stimulation Group; H&E staining, ×400 magnification).

Comparison of M3 receptor expression levels in bladder detrusor muscle tissue among three groups of rats

Comparison of M3 receptor expression levels in bladder detrusor muscle tissue among the three groups showed statistically significant differences ($P < 0.05$). Specifically, the Magnetic Stimulation Group exhibited significantly higher expression levels compared to both the Normal Group and the Model Group (all $P < 0.05$). There was no statistically significant difference between the Model Group and the Normal Group ($P > 0.05$), as shown in Fig. 4.

Discussion

Neurogenic bladder dysfunction caused by central or peripheral nervous system damage is termed neurogenic bladder (Niu et al., 2018). This condition is more common in patients with spinal cord injuries (Taweel and Seyam, 2007), presenting with symptoms such as frequent urination, difficulty urinating, urinary incontinence, and recurrent urinary tract infections. In severe cases, it can lead to hydronephrosis and renal failure. Therefore, early intervention and treatment are crucial.

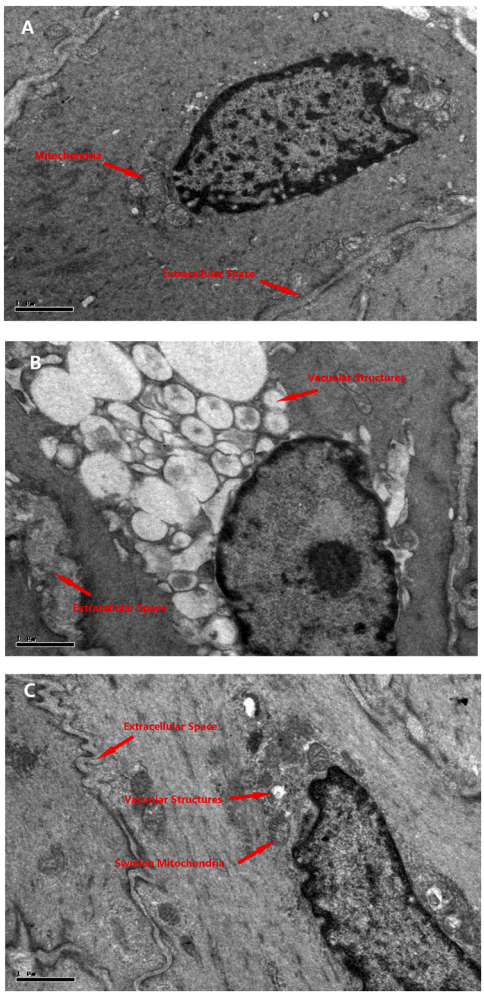

Fig. 3. Comparison of ultrastructure of bladder detrusor muscle among three groups of rats (A: Normal Group; B: Model Group; C: Magnetic Stimulation Group; ×8900 magnification).

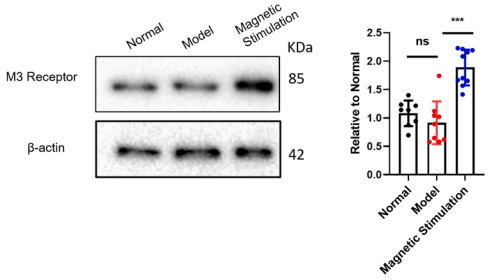

Fig. 4. Expression of M3 receptor in rat bladder tissue from three groups. Western blot showed that the expression level of M3 receptor protein in the Magnetic Stimulation Group was significantly higher than that in the Normal Group and the Model Group. There was no significant difference between the Model Group and the Normal Group. n = 8–10 rat in every group. ns $P > 0.05$, ***$P < 0.001$, versus the normal group.

Current clinical treatment methods include indwelling catheters, drug therapy, surgical treatment, and sacral nerve modulation (Jiang et al., 2024), each of which has its drawbacks. For example, indwelling catheters or clean intermittent catheterization require self-operation by patients, which if not done properly, can easily lead to lower urinary tract infections; drug therapy progresses slowly or may have unsatisfactory effects; post-surgical treatment requires long-term catheterization, causing significant inconvenience to patients. Sacral nerve modulation is a minimally invasive reversible treatment method (Chen and Li, 2023) that achieves certain therapeutic effects, but it also has issues such as invasive treatment, expensive equipment, susceptibility to infections, and disease recurrence, thereby limiting its widespread implementation.

Sacral nerve root magnetic stimulation is a form of electrotherapy. Evidence suggests that it has similar effects to sacral nerve electrical stimulation, generating a similar electric field, but with lower impedance and less attenuation (Takahashi and Kitamura, 2003; Neyroud et al., 2015). Other studies indicate that sacral nerve root magnetic stimulation can all Promote and inhibit the release of substances related to urination, and can improve bladder voiding function (Zhao et al., 2022; Xu et al., 2020). This experiment's results also found that in the Magnetic Stimulation Group, the maximum bladder capacity and compliance were higher than those in the Model Group, with lower bladder leak point pressures compared to the Model Group. The M3 cholinergic receptor are present on the membrane of detrusor smooth muscle cells, mediating detrusor contraction (Mansfield et al., 2009). Researchers found significantly increased levels of M3 receptor expression in a rat model of denervation injury, possibly related to reduced acetylcholine neurotransmitter levels and decreased detrusor contractility following nerve injury, suggesting compensatory upregulation of M3 receptors to maintain detrusor contraction (Wallis and Napier, 1999). In this experiment, H&E staining of the Magnetic Stimulation Group revealed moderate congestion and edema in submucosal tissues, mild infiltration of mononuclear macrophages, and slight proliferation of fibroblasts, while the morphology and arrangement of mucosal epithelial cells were closer to normal compared to the Model Group. Transmission electron microscopy showed smooth surfaces of detrusor muscle cells with slightly widened intercellular spaces, relatively uniform arrangement, and intact mitochondrial structure in the cytoplasm. Immunohistochemistry results indicated significantly elevated levels of M3 receptor expression in bladder detrusor muscle tissues compared to the normal and Model Groups.

Conclusion

In summary, sacral nerve root magnetic stimulation has a certain beneficial effect on bladder function in rats with neurogenic bladder, which may be related to increased expression of M3 receptors in bladder detrusor muscle tissue. The specific mechanisms of action require further investigation.

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Ethical Statement

All experimental procedures were approved by the Institutional Animal Care and Use Committee of of Hangzhou Third People's Hospital (NO. 2022KA016) and were in accordance with the guidelines of the International Association for the Study of Pain (IASP).

CRediT authorship contribution statement

Junhua Li: Writing – original draft, Formal analysis. **Chenhao Tang:** Software, Data curation. **Longfei Yang:** Software, Formal analysis, Data curation. **Chen Song:** Resources, Methodology. **Yanbin Wang:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

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Data availability

Data available on request from the authors.

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