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Effect of Blade Needle Stimulation on the Expression of MyoD and HGF Proteins in Rats with Lumbar Multifidus Muscle Injury

Senming Xu[1*], Xiaolan Li[2*], Xingle Qin[2], Minli Jiang[3#]

[1]Department of Rehabilitation Medicine, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China
[2]Department of Rehabilitation Medicine, Liuzhou People's Hospital, Liuzhou, China
[3]College of Basic Medicine, Youjiang Medical University for Nationalities, Baise, China
Email: #00831@ymun.edu.cn

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Abstract

Objective: This paper aims to observe the effects of blade needle stimulation (BNS) therapy on the expression levels of myogenic differentiation antigen (MyoD) and hepatocyte growth factor (HGF) in the multifidus muscle of rats with lumbar multifidus muscle injury, and to explore the potential molecular mechanisms by which BNS therapy promotes skeletal muscle injury repair. **Methods:** Forty male SD rats were randomly divided into a normal group, a control group, a model group, an acusector group, and a BNS group, with 8 rats in each group. Except for the normal group, the lumbar multifidus muscle injury model was established by injecting 0.5% bupivacaine into the multifidus muscle, while the control group received an equal volume of normal saline. After modeling, the normal group, control group, and model group were only subjected to equal handling without any intervention. The acusector group received acusector treatment at the injured site starting from day 1 after modeling, once daily for 30 minutes each session. The BNS group received BNS at the same site on days 1 and 4 after modeling. The protein levels of MyoD and HGF in the multifidus muscle of rats in each group were measured by ELISA on days 4 and 8 after modeling, respectively. **Results:** On day 8 after modeling, there were statistically significant differences in the expression levels of MyoD and HGF among all groups ($P < 0.0001$). Compared with the normal group, the expression levels of MyoD and HGF in the model group were significantly decreased ($P < 0.0001$). The expression levels of MyoD in both the acusector group and the BNS group were significantly higher than those in the model group ($P < 0.0001$), with no statistically significant difference between the two

*Senming Xu and Xiaolan Li are co-first authors.
#Corresponding author.

treatment groups ($P > 0.05$). The expression level of HGF in the BNS group was significantly higher than those in the model group and the acusector group ($P < 0.05$, $P < 0.01$), while there was no statistically significant difference between the acusector group and the model group ($P > 0.05$). **Conclusion:** Both acusector and BNS therapy can effectively inhibit the downregulation of MyoD expression following lumbar multifidus muscle injury. Additionally, BNS significantly elevates HGF expression levels, demonstrating a distinct molecular regulatory advantage over acusector.

Keywords

Multifidus Muscle Injury, Myogenic Differentiation Protein, Hepatocyte Growth Factor, Acusector, Blade Needle Stimulation

1. Introduction

The multifidus muscle plays a critical role in maintaining spinal stability and regulating spinal movement, and its injury, atrophy, and functional disorders are closely associated with the development of various acute and chronic low back pain conditions [1] [2]. The repair process following multifidus muscle injury primarily depends on the proliferation and differentiation of muscle satellite cells (MSCs) [3] [4]; both myogenic determination factor (MyoD) and hepatocyte growth factor (HGF) are closely implicated in this proliferative process [3] [5].

In this study, we established a rat model of lumbar multifidus muscle injury and applied blade needle stimulation (BNS) technique to the lumbar multifidus muscle of the model rats. On days 4 and 8 after modeling, the expression levels of MyoD and HGF in the multifidus muscle of rats in each group were measured using Enzyme linked immunosorbent assay (ELISA) kits, in order to evaluate the effects of BNS on the repair process following multifidus muscle injury. Furthermore, we aimed to explore its potential application value in promoting the repair of lumbar multifidus muscle injury, thereby providing experimental evidence for the clinical promotion of this therapy.

2. Materials and Methods

2.1. Experimental Animals

A total of 40 specific pathogen-free (SPF) male Sprague-Dawley (SD) rats, aged 8 weeks and weighing 220 ± 20 g, were obtained from Changsha Tianqin Biotechnology Co., Ltd. (Changsha, China; license No.: SCXK (Xiang) 2019-0014). The rats were randomly and equally divided into 10 cages for rearing, with free access to food and water, a 12-hour light/dark cycle, an ambient temperature of 24˚C, and a humidity of 40% - 50%. They were acclimatized for 7 days prior to the experiment. All animal experiments were conducted in accordance with the guidelines of the Chinese Institute of Animal Research and were approved by the Ethics

Committee of Youjiang Medical University for Nationalities.

2.2. Main Reagents

Bupivacaine hydrochloride (Beijing Solarbio Science & Technology Co., Ltd., YZ-101034); Sodium pentobarbital (Shanghai Fude Chemical Co., Ltd.); Rat HGF ELISA Kit, 96T (Wuhan Huamei Biotech Co., Ltd.); Rat MyoD ELISA Kit, 96T (Shanghai Enzyme-linked Biotechnology Co., Ltd.).

2.3. Main Instruments

Standard microplate reader (Thermo Fisher Scientific, Multiskan MK3); Electrothermal constant-temperature incubator (Huangshi Hengfeng Medical Instrument Co., Ltd., SKP-02.600); Centrifuge (Sigma, USA, 3K18). Xinsheng brand acusector apparatus G6805-I.

2.4. Grouping and Model Establishment

The 40 rats were randomly divided into five groups using a computer-generated random number sequence: normal group, model group, control group, electroacupuncture group, and BNS group. Group allocation was concealed by an independent coordinator using a coded labeling system. Rats in the model group, acusector group, and BNS group were anesthetized with 1% sodium pentobarbital (50 mg/kg) [6], and the model was established according to the following method [7]: Six points on both sides of the spine at the L3, L4, and L5 levels were selected. A disposable 4-gauge needle syringe was used to draw 0.5% bupivacaine solution. The needle was inserted into the muscle close to the spinous processes until it contacted the bony surface of the articular processes and mammillary processes. The cannula was then retracted 1 mm to confirm no blood return, indicating that the needle had reached the multifidus muscle. Subsequently, 600 μL of bupivacaine solution (100 μL × 6) was injected, with each intramuscular injection lasting no less than 3 seconds to facilitate drug absorption. A single injection completed the model establishment. The control group received an equal volume of normal saline using the same method. All procedures were performed under sterile conditions, and the model was successfully established.

2.5. Intervention Methods

Interventions were initiated 24 hours after modeling in each group. The rats were fixed on an operating table with their backs and hind limbs exposed, referencing the rat anatomical atlas.

Normal group: All rats were handled, fixed, and locally disinfected simultaneously with the other groups, but received no other treatment. Samples were collected on days 4 and 8 after modeling.

Model group: No special treatment was given after modeling. Four rats were randomly selected for sampling on day 4 after modeling, and the remaining four rats were handled, fixed, and locally disinfected simultaneously with the other

groups, but received no other treatment, with final sampling on day 8 after modeling.

Control group: No special treatment was given after modeling. Four rats were randomly selected for sampling on day 4 after modeling, and the remaining four rats were handled, fixed, and locally disinfected simultaneously with the other groups, but received no other treatment, with final sampling on day 8 after modeling.

Acusector group: Intervention began on day 1 after modeling. The rats were first fixed on a specially designed holder with their backs exposed, and the L3, L4, and L5 spinal levels were locally disinfected with 75% alcohol. Huatuo brand disposable sterile acupuncture needles (0.35 × 50 mm) were then inserted into the muscle close to the spinous processes at the L3, L4, and L5 levels until the bony surface of the articular and mammillary processes was reached. After needling, the needles were connected to a Xinsheng brand acusector apparatus G6805-I, with sparse-dense waves at 10 - 26 cycles/min, a current intensity of 1 mA, for 30 minutes, once daily, for 7 consecutive days. Four rats were randomly selected for sampling on day 4 after modeling, and the remaining four rats continued the intervention, with final sampling on day 8 after modeling.

BNS group: Intervention began on day 1 after modeling. The rats were first fixed on a specially designed holder with their backs exposed, and the L3, L4, and L5 spinal levels were locally disinfected with 75% alcohol. Lejiu brand disposable blade needles (0.35 × 50 mm) were then inserted into the muscle close to the spinous processes at the L3, L4, and L5 levels until the bony surface of the articular and mammillary processes was reached, followed by rapid lifting and thrusting twice with an amplitude of 0.5 - 1.0 cm. Four rats were randomly selected for sampling on day 4 after modeling, and the remaining four rats received one additional intervention on day 4 after modeling, with final sampling on day 8 after modeling.

2.6. Specimen Collection and Processing

Rats were anesthetized intraperitoneally with 1% sodium pentobarbital (50 mg/kg). After the rats were deeply anesthetized, they were fixed on a board with their backs fully exposed. The dorsal fur was shaved, and the skin on the back was incised. The lumbar fascia, longissimus muscle, and iliocostalis muscle were dissected using tissue scissors and forceps. The lumbar multifidus muscles on both sides of the L3 - L5 spinous processes were harvested, and stored at −80˚C for later use. Tissue specimens (approximately 50 mg) were dissected from both the left and right sides of the target tissue. Each specimen was homogenized in ice-cold RIPA lysis buffer (containing protease inhibitor cocktail) at a ratio of 1:9 (weight/volume) using a tissue homogenizer. The resulting homogenates were incubated on ice for 30 minutes with intermittent vortexing/mixing, followed by centrifugation at 10,000 × g for 5 minutes at 4˚C. The clear supernatants were carefully collected for ELISA. To minimize intra-animal variability, the mean

value obtained from the bilateral samples was calculated and treated as a single independent data point for each animal in the subsequent statistical analyses. The investigators performing the experiments were blinded to the sample group assignments throughout the assay procedures.

2.7. Detection Indicators and Methods

A double-antibody sandwich ELISA method was used to detect the levels of MyoD and HGF. The specific procedures were performed in accordance with the instructions provided with the kits.

2.8. Statistical Methods

SPSS 26.0 statistical software was used for data analysis. For data that followed a normal distribution and had homogeneity of variance, data from each group were expressed as mean ± standard deviation ($\overline{x} \pm s$). Differences among groups were analyzed using one-way analysis of variance (ANOVA), with post hoc comparisons performed using Tukey's test. Intra-group comparisons were analyzed using independent samples t-test. Data that did not follow a normal distribution or had unequal variances were analyzed using non-parametric tests. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of BNS Therapy on MyoD Expression Levels Following Multifidus Muscle Injury

As shown in Figure 1(a), on day 4 after modeling, there was no statistically significant difference in MyoD expression levels among the groups (F = 2.938, P = 0.056). On day 8 after modeling, there was a statistically significant difference in MyoD expression levels among the groups (F = 15.758, P < 0.0001). Further post hoc analysis using Tukey's test revealed that, compared with the normal group, the control group showed no significant change in MyoD expression level (P > 0.05), while the model group showed a significant decrease in MyoD expression level (P < 0.0001). Compared with the model group, both the acusector group and the BNS group exhibited significantly elevated MyoD expression levels, with statistically significant differences (P < 0.0001). There was no statistically significant difference between the BNS group and the acusector group (P > 0.05).

As shown in Figure 1(b), when comparing different time points within the same group, the normal group, control group, acusector group, and BNS group all showed no significant changes in MyoD expression levels (P > 0.05). In the model group, the MyoD expression level on day 8 was significantly lower than that on day 4, with a statistically significant difference (P < 0.0001). These results indicate that following multifidus muscle injury, MyoD expression levels tend to decline. Both acusector therapy and BNS therapy can significantly inhibit the downregulation of MyoD expression after multifidus muscle injury. The specific MyoD expression levels for each group are presented in Table 1.

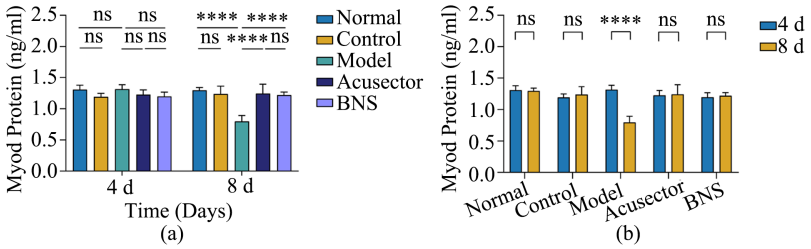

Figure 1. MyoD expression levels in rats of each group. (a) Comparison of MyoD expression levels among different groups at the same time point; (b) Comparison of MyoD expression levels within the same group at different time points. ns indicates no statistically significant difference. **** indicates P < 0.0001.

Table 1. MyoD expression levels of each group at different time points ($\overline{x} \pm s$, ng/ml).

Group	Detection time	
	Day 4	Day 8
Normal	1.31 ± 0.07	1.30 ± 0.05
Control	1.19 ± 0.05	1.24 ± 0.13
Model	1.32 ± 0.07	0.80 ± 0.10
Acusector	1.23 ± 0.08	1.24 ± 0.15
BNS	1.20 ± 0.07	1.22 ± 0.05

3.2. Effect of BNS Therapy on HGF Expression Levels Following Multifidus Muscle Injury

As shown in **Figure 2(a)**, on day 4 after modeling, there was no statistically significant difference in HGF expression levels among the groups (F = 2.916, P = 0.057). On day 8 after modeling, there was a statistically significant difference in HGF expression levels among the groups (F = 18.044, P < 0.0001). Further post hoc analysis using Tukey's test revealed that, compared with the normal group, the control group showed no significant difference in HGF expression level (P > 0.05), while the model group showed a significant decrease in HGF expression level (P < 0.0001). Compared with the model group, the acusector group showed no statistically significant difference (P > 0.05); however, the HGF level in the BNS group was significantly higher than that in both the model group and the acusector group, with statistically significant differences (P < 0.05, P < 0.01).

As shown in **Figure 2(b)**, when comparing different time points within the same group, the normal group, control group, and BNS group all showed no significant changes in HGF expression levels (P > 0.05). In both the model group and the acusector group, the HGF expression levels on day 8 after modeling were significantly lower than those on day 4, with statistically significant differences (P < 0.01). These results indicate that following multifidus muscle injury, HGF expression levels tend to decline. BNS therapy can significantly inhibit the downregulation of HGF expression, whereas acusector therapy did not demonstrate an inhibitory effect on the decline of HGF levels. The specific HGF expression levels for each group are presented in **Table 2**.

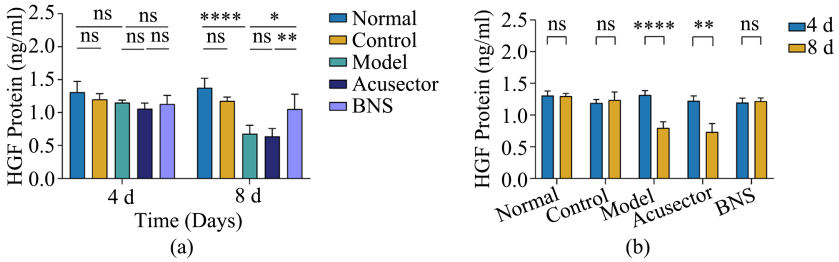

Figure 2. HGF expression levels in rats of each group. (a) Comparison of HGF expression levels among different groups at the same time point; (b) Comparison of HGF expression levels within the same group at different time points. ns indicates no statistically significant difference; * indicates $P < 0.05$; ** indicates $P < 0.01$; **** indicates $P < 0.0001$.

Table 2. HGF expression levels in rats of each group at different time points ($\overline{x} \pm s$, ng/ml).

Group	Detection time	
	Day 4	Day 8
Normal	1.31 ± 0.16	1.38 ± 0.14
Control	1.20 ± 0.09	1.17 ± 0.06
Model	1.15 ± 0.04	0.68 ± 0.13
Acusector	1.06 ± 0.08	0.64 ± 0.12
BNS	1.13 ± 0.13	1.06 ± 0.22

4. Discussion

Injury, atrophy, and functional disorders of the multifidus muscle are closely associated with the development of various acute and chronic low back pain conditions. Effective repair following multifidus muscle injury depends on the activation, proliferation, and differentiation of MSCs, a process precisely regulated by multiple growth factors and transcription factors. MyoD, a core member of the myogenic regulatory factor (MRF) family, serves as a critical transcription factor that initiates the expression of skeletal muscle-specific genes. It plays an irreplaceable role as a "molecular switch" in the transition of satellite cells into myoblasts [8], and is therefore recognized as a core biomarker reflecting both the proliferative activity of muscle satellite cells and the regenerative capacity of skeletal muscle [9]. HGF, on the other hand, is regarded as the most important activating signal for MSCs, mediating the activation, migration, and proliferation of quiescent MSCs, and is a key regulatory factor in the early stages of muscle regeneration [10]. In this study, by establishing a lumbar multifidus muscle injury model, we observed the effects of BNS therapy on the expression of these two key proteins, aiming to provide experimental evidence for the clinical application of BNS therapy.

Our study found that on day 8 after modeling, the expression levels of both MyoD and HGF in the multifidus muscle of the model group were significantly lower than those in the normal group, and within the same model group, the ex-

pression levels on day 8 were significantly lower than those on day 4. This result suggests that following multifidus muscle injury, endogenous MyoD and HGF expression is insufficient to drive effective muscle regeneration. As a deep postural muscle of the spine, the multifidus muscle is predominantly composed of slow-twitch fibers, and the proliferative capacity of its MSCs is relatively limited, which may represent the myobiological basis for the persistently low levels of regeneration-related factors in the model group.

In terms of therapeutic effects, both acusector and BNS therapy significantly inhibited the downregulation of MyoD expression, with comparable efficacy between the two. Upregulation of MyoD expression indicates that more MSCs are recruited into the myogenic lineage, which is a critical step for successful skeletal muscle regeneration. The effect of acusector in promoting MyoD expression may be related to the activation of signaling pathways such as MAPK/ERK and PI3K/Akt [11] [12]. BNS, on the other hand, may indirectly protect the myogenic capacity of MSCs by reducing excessive inflammation and oxidative stress in the injured area. Notably, no significant effect on MyoD expression was observed in the control group, implying that direct intervention at the site of injury exerts a significant modulatory effect on MyoD expression.

Regarding the regulation of HGF expression, BNS demonstrated a unique advantage—the HGF level in the BNS group was significantly higher than that in both the model group and the acusector group. This finding provides direct molecular evidence for the specificity of BNS therapy. There was no statistically significant difference between the acusector group and the model group, which may be related to the insufficient number of acusector treatment days. Previous studies have found that HGF is sensitive to mechanical stretching stimulation from acupuncture, which can induce the proliferation of MSCs and promote repair following skeletal muscle injury [13]. Therefore, BNS may induce HGF production through stronger mechanical stimulation, thereby activating skeletal MSCs. HGF can also inhibit macrophage polarization toward the M1 phenotype while promoting polarization toward the M2 phenotype, thereby facilitating skeletal muscle regeneration and repair [14] [15]. In addition, the stimulation and release effect of blade needle on deep fascia and adhesive tissues can rapidly improve local microcirculation, providing a favorable microenvironment for HGF expression.

Combining the MyoD and HGF results reveals that BNS treatment can simultaneously upregulate both HGF and MyoD, whereas acusector significantly affects only MyoD. This suggests that the two intervention modalities may act on different nodes of the muscle regeneration regulatory network—BNS acts at the more upstream level of HGF release, triggering a cascade reaction to achieve MyoD upregulation; acusector, on the other hand, may directly affect the initiation of the myogenic program through an HGF-independent pathway. The mechanistic differences between the two provide a theoretical basis for their complementary clinical advantages.

This study has certain limitations: the limited number of time points prevented

the depiction of a complete temporal course; ELISA could not definitively identify the cellular sources of the proteins; and changes at the gene level and downstream signaling pathways were not investigated.

5. Conclusion

In conclusion, both acusector and BNS can effectively inhibit the downregulation of MyoD expression following multifidus muscle injury, while BNS additionally significantly elevates HGF expression, demonstrating a distinct molecular regulatory advantage. This study provides key experimental evidence for elucidating the molecular mechanisms by which BNS promotes skeletal muscle regeneration, and also offers theoretical support for the use of BNS therapy in treating lumbar muscle injuries. Future studies may further explore the upstream signaling mechanisms through which BNS promotes HGF expression and its association with muscle functional recovery, providing a more comprehensive theoretical basis for therapy optimization.

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Author Contributions

Conceptualization, X.S. and L.X.; methodology, Q.X.; software, X.S.; validation, L.X., and J.M.; formal analysis, X.S.; investigation, J.M.; resources, Q.X.; data curation, X.S.; writing—original draft preparation, X.S.; writing—review and editing, Q.X.; visualization, X.S.; supervision, J.M.; project administration, X.S.; funding acquisition, X.S. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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