Effects of Shu-Jing Tuina on TLR8/ERK Signaling Pathway and LncRNA-GAS5 in Rats with Neuropathic Pain and Its Mechanism: Postprint

Authors: Zongbo Wei, Long Bingcai, Wang Xiongjiang, Liang Yingye, Tang Hongliang, summer, Lu Dongming, Wang Xiongjiang

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Abstract

Background: In recent years, Shu-Jing Tuina manipulation has demonstrated favorable therapeutic efficacy in the treatment of neuropathic pain, but its specific mechanism of action has not been fully elucidated.

Objective: Using a rat model of neuropathic pain established by L5 spinal nerve ligation as the observation subject, this study observed the analgesic effect of Shu-Jing Tuina manipulation in rats based on various research indicators, and investigated whether it achieves analgesic effects by affecting LncRNA-GAS5 and thereby regulating spinal dorsal horn neuronal apoptosis.

Methods: The experiment was conducted from January to June 2021 at Guangxi University of Chinese Medicine and the Animal Medicine Experimental Center of Guangxi University. A total of 120 healthy female SD rats were selected and randomly divided into normal group, model group, sham operation group, sham Tuina group, and Shu-Jing Tuina group using a random number table method, with 24 rats in each group. The sham operation, model, sham Tuina, and Shu-Jing Tuina groups underwent L5 spinal nerve ligation to establish neuropathic pain rat models. Twenty-four hours after modeling, the sham operation group had the L5 spinal nerve exposed for several minutes without ligation, followed by layered wound closure; the sham Tuina group received gentle stroking of both hind limbs for 18 minutes; the Shu-Jing Tuina group received sequential stimulation of three acupoints (Huantiao, Yanglingquan, and Xuanzhong) on both sides of the Gallbladder Meridian of Foot-Shaoyang using a self-prepared massage device, with a stimulation force of 5 N and frequency of 2 Hz, applying 1 minute of intervention per acupoint and per technique, totaling 18 minutes for bilateral 6 acupoints and 3 techniques; the normal and model groups received normal feeding and observation without any intervention. Behavioral tests (mechanical withdrawal threshold and thermal withdrawal latency) were performed

before modeling and on days 1, 3, 7, and 14 after modeling. On days 7 and 14 of intervention, 12 rats were randomly selected for tissue sampling to detect expression of proteins mediating the TLR8/ERK signaling pathway in spinal cord tissue (protein expression levels of Bcl-2, Caspase-3, ERK, and TLR8, and gene expression levels of LncRNA-GAS5 and miR-21).

Results: (1) Behavioral aspects: Mechanical withdrawal thresholds in the model, sham Tuina, and Shu-Jing Tuina groups were lower than those in the normal group on days 1, 3, 7, and 14 after modeling ($P<0.05$). Mechanical withdrawal thresholds in the sham Tuina and Shu-Jing Tuina groups were higher than those in the model group on day 14 after modeling ($P<0.05$). The mechanical withdrawal threshold in the Shu-Jing Tuina group was higher than that in the sham Tuina group on days 7 and 14 after modeling ($P<0.05$). Thermal withdrawal latency in the sham operation and Shu-Jing Tuina groups was lower than that in the normal group on days 1, 3, and 7 after modeling ($P<0.05$). Thermal withdrawal latency in the sham Tuina and Shu-Jing Tuina groups was longer than that in the model group on days 7 and 14 after modeling ($P<0.05$). Thermal withdrawal latency in the Shu-Jing Tuina group was longer than that in the sham Tuina group on day 14 after modeling ($P<0.05$). (2) Signal pathway-related proteins and gene expression levels: On day 7 after modeling, Bcl-2 protein expression level in the normal group was lower than that in all other groups ($P<0.05$). Bcl-2 protein expression level in the Shu-Jing Tuina group was higher than that in the model group, while Caspase-3, ERK, and TLR8 protein expression levels were lower than those in the model group ($P<0.05$). Bcl-2, Caspase-3, ERK, and TLR8 protein expression levels in the Shu-Jing Tuina group were lower than those in the sham Tuina group ($P<0.05$). On day 14 after modeling, Bcl-2 protein expression level in the Shu-Jing Tuina group remained higher than that in the model group, Caspase-3 and TLR8 protein expression levels remained lower than those in the model group, while ERK protein expression level was higher than that in the model group ($P<0.05$). On day 7 after modeling, LncRNA-GAS5 gene expression levels in both the sham Tuina and Shu-Jing Tuina groups were higher than that in the model group, while miR-21 gene expression levels were lower than that in the model group ($P<0.05$). LncRNA-GAS5 gene expression level in the Shu-Jing Tuina group was higher than that in the sham Tuina group, while miR-21 gene expression level was lower than that in the sham Tuina group ($P<0.05$). On day 14 after modeling, lncRNA-GAS5 gene expression level in the model group was lower than that in the normal group, while lncRNA-GAS5 gene expression levels in both the Shu-Jing Tuina and sham Tuina groups were higher than that in the model group ($P<0.05$). miR-21 gene expression levels in both the Shu-Jing Tuina and sham Tuina groups were higher than that in the model group ($P<0.05$).

Conclusion: Shu-Jing Tuina manipulation exerted certain analgesic effects on rats with neuropathic pain. It is preliminarily speculated that the analgesic mechanism may involve upregulating LncRNA-GAS5 expression to adsorb miR-21, thereby mediating TLR8/ERK pathway-related proteins and inhibiting neuronal apoptosis. Although its specific mechanism has not been definitively con-

firmed, LncRNA-GAS5 is expected to become a novel therapeutic target for neuropathic pain in the future.

Full Text

Effect and Mechanism of Pivot Meridian Massage on TLR8/ERK Signaling Pathway and LncRNA-GAS5 in Rats with Neuropathic Pain

WEI Zongbo[1], LONG Bingcai[1], WANG Xiongjiang[1]*, LIANG Yingye[2], TANG Hongliang[3], XIA Tian[1], LU Dongming[2]

[1]Guangxi University of Chinese Medicine, Nanning 530041, China
[2]The First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine, Nanning 530023, China
[3]Fangchenggang Hospital Affiliated to Guangxi University of Traditional Chinese Medicine, Fangchenggang 538000, China
*Corresponding author: WANG Xiongjiang, Attending physician; E-mail: 958207546@qq.com

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Abstract

Background: Pivot meridian massage has demonstrated favorable efficacy in treating neuropathic pain in recent years, yet its specific mechanism of action remains incompletely elucidated.

Objective: Using a rat model of neuropathic pain induced by L5 spinal nerve ligation, this study observed the analgesic effects of pivot meridian massage and investigated whether its therapeutic effect is achieved by influencing LncRNA-GAS5 expression to regulate apoptosis of spinal dorsal horn neurons.

Methods: The experiment was conducted from January to June 2021 at the Experimental Center for Animal Medicine of Guangxi University of Chinese Medicine and Guangxi University. A total of 120 healthy female Sprague Dawley rats were randomly divided into five groups (n=24 each): normal, model, sham-operated, sham-manipulation, and pivot meridian massage groups. Neuropathic pain models were established in all groups except the normal group by ligating the L5 spinal nerve. Twenty-four hours post-modeling, interventions were applied as follows: the sham-operated group underwent exposure of the L5 spinal nerve without ligation; the sham-manipulation group received gentle

stroking of both hind limbs for 18 minutes; the pivot meridian massage group received sequential stimulation of three acupoints (Huan Tiao, Yang Ling Quan, and Xuan Zhong) on the bilateral Foot Shaoyang Gallbladder Meridian using a custom-designed massage device at 5 N force and 2 Hz frequency, with 1 minute per acupoint and technique (6 acupoints total, 3 techniques, 18 minutes total duration). The normal and model groups received no intervention. Behavioral tests (mechanical withdrawal threshold and thermal withdrawal latency) were performed before modeling and on days 1, 3, 7, and 14 post-modeling. On days 7 and 14 of intervention, 12 rats from each group were randomly selected for tissue sampling to detect expression of TLR8/ERK signaling pathway-related proteins (Bcl-2, Caspase-3, ERK, TLR8) and gene expression levels of LncRNA-GAS5 and miR-21 in spinal cord tissue.

Results: (1) Behaviorally, mechanical withdrawal thresholds in the model, sham-manipulation, and pivot meridian massage groups were significantly lower than the normal group on days 1, 3, 7, and 14 post-modeling ($P<0.05$). The sham-manipulation and pivot meridian massage groups showed higher mechanical withdrawal thresholds than the model group on day 14 ($P<0.05$), with the pivot meridian massage group exceeding the sham-manipulation group on days 7 and 14 ($P<0.05$). Thermal withdrawal latency in the sham-manipulation and pivot meridian massage groups was shorter than the normal group on days 1, 3, and 7 ($P<0.05$) but longer than the model group on days 7 and 14 ($P<0.05$), with the pivot meridian massage group showing superior performance compared to the sham-manipulation group on day 14 ($P<0.05$). (2) Regarding protein and gene expression, on day 7 post-modeling, Bcl-2 expression in the normal group was lower than all other groups ($P<0.05$). The pivot meridian massage group exhibited higher Bcl-2 but lower Caspase-3, ERK, and TLR8 protein levels compared to the model group ($P<0.05$), with all four protein levels also lower than the sham-manipulation group ($P<0.05$). On day 14, the pivot meridian massage group maintained higher Bcl-2 and lower Caspase-3 and TLR8 levels than the model group, while ERK expression became higher ($P<0.05$). At 7 days, both sham-manipulation and pivot meridian massage groups showed elevated LncRNA-GAS5 and reduced miR-21 expression compared to the model group ($P<0.05$), with the pivot meridian massage group showing more pronounced changes ($P<0.05$). By day 14, the model group's LncRNA-GAS5 expression remained lower than the normal group, while both intervention groups showed higher expression ($P<0.05$); miR-21 expression was also higher in both intervention groups compared to the model group ($P<0.05$).

Conclusion: Pivot meridian massage demonstrates analgesic effects in rats with neuropathic pain. The mechanism is hypothesized to involve upregulation of LncRNA-GAS5 expression, which adsorbs miR-21 to modulate TLR8/ERK pathway-related proteins and inhibit neuronal apoptosis. Although the specific mechanism requires further confirmation, LncRNA-GAS5 represents a promising novel therapeutic target for neuropathic pain.

Keywords: Pain; Neuropathic pain; Tuina therapy; Pivot meridian massage;

LncRNA-GAS5; Analgesia; Rats

Introduction

Neuropathic pain (NPP) refers to pain caused by damage to the somatosensory nervous system under central control, representing a refractory chronic pain syndrome characterized by spontaneous pain, allodynia, hyperalgesia, and sensory abnormalities. With a duration typically exceeding three months, NPP severely impacts sleep quality and induces anxiety, depression, and other mood disorders, significantly compromising patients' quality of life. NPP remains a major challenge in modern medicine, as its pathogenesis lacks comprehensive theoretical explanation and no curative treatment exists, necessitating urgent identification of safe and effective therapeutic approaches.

Traditional Chinese medicine has developed systematic understanding of pain etiology and pathogenesis, achieving notable analgesic efficacy in NPP treatment through acupuncture, tuina, and other external therapies. Compared to pharmacological or surgical interventions, these approaches offer advantages of economic viability, safety, superior efficacy, and fewer adverse effects, attracting considerable clinical attention. However, the lack of clearly defined analgesic mechanisms and inconsistent clinical research quality have hindered higher-level evidence-based recommendations for traditional Chinese medicine in pain management.

Pivot meridian massage is based on the pivot meridian theory, which emphasizes "pivot" as the core and "rotation" as the essence to govern the body's qi mechanism and regulate the circulation of qi, blood, and body fluids. As the 交接转枢 (pivot junction) of qi movement, pivot meridians coordinate internal-external yin-yang qi and regulate organ qi, thereby promoting qi flow, activating blood, and dredging meridians. Acupoints on the Shaoyang and Shaoyin meridians, when stimulated by tuina manipulation, can more effectively alleviate pain. Since NPP locations directly correlate with damaged areas, pivot meridian massage applied to affected regions may relieve pain.

Nerve injury alters microRNA expression, with miR-21 playing crucial roles in neuroprotection and repair. Extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) family, translocates to the nucleus upon activation, phosphorylates CREB via ribosomal S6 kinase, and regulates downstream factor transcription involved in synaptic plasticity, neuronal apoptosis inhibition, and neural repair. TLR8, a negative regulator of axonal growth and inducer of neuronal apoptosis, shows significantly increased expression in the spinal dorsal horn of NPP rats. Recent research indicates that TLR8, through miR-21 activation, induces ERK phosphorylation to mediate inflammatory mediator production and neuronal hyperexcitability in NPP. Therefore, inhibiting the miR-21-mediated TLR8/ERK pathway may affect spinal dorsal horn neuronal apoptosis and block pain transmission.

Long non-coding RNA GAS5 (LncRNA-GAS5) is closely associated with neuronal apoptosis. Our preliminary experiments found LncRNA-GAS5 to be downregulated in the spinal dorsal horn of NPP rats but upregulated after pivot meridian massage intervention. We therefore hypothesized that pivot meridian massage may participate in regulating NPP occurrence, development, and maintenance by influencing LncRNA-GAS5 expression. This study established an L5 spinal nerve ligation (SNL) pain model in rats to observe the effects of pivot meridian massage on LncRNA-GAS5, miR-21, ERK, TLR8, and other pathway-related factors in spinal dorsal horn tissue, aiming to elucidate the analgesic mechanisms of pivot meridian massage based on LncRNA-GAS5 and provide scientific evidence for clinical application.

Methods

1.1 Time and Location The experiment was conducted from January to June 2021 at Guangxi University of Chinese Medicine and the Experimental Center for Animal Medicine, Guangxi University.

1.2 Materials 1.2.1 Experimental Animals One hundred twenty healthy female Sprague Dawley rats weighing 140-160 g were purchased from Hunan Slack Company [License No. SYXK(Gui)2019-0004] and housed at the Experimental Center for Animal Medicine, Guangxi University. Housing conditions included temperature 20-25°C, humidity 35-45%, 12-hour natural light cycle, and ad libitum access to food and water. The study was approved by the Animal Experiment Ethics Committee of Guangxi University of Chinese Medicine (No. DW20220621-129).

1.2.2 Main Reagents Sodium penicillin for injection (Qiqihar Shuangfu Veterinary Medicine Co., Ltd., China), chloral hydrate (Chengdu Kelong Chemical Reagent Factory, China), DEPC-treated water (Shanghai Genechem Co., Ltd., China), BCA protein concentration assay kit (Beijing Solarbio Science & Technology Co., Ltd., China), rabbit anti-rat TLR8 antibody (Abcam, UK), and rabbit anti-rat ERK antibody (Abcam, UK).

1.2.3 Main Instruments Stereotaxic apparatus for rats (Narishiga, Japan), optical microscope (Olympus CX23, Qingdao, China), confocal microscope (Olympus BX43, Qingdao, China), microplate reader (OLYMPUS, Japan), low-temperature high-speed centrifuge (EPPENDORF, Germany), Von Frey filament pain tester (Stoelting, USA), and thermal pain tester (IITC, USA). The massage manipulation simulator was based on a Chinese invention patent (No. ZL200710187403.1).

1.3 Experimental Procedures 1.3.1 Animal Grouping After 7 days of acclimatization, rats were randomly divided into five groups (n=24 each): normal, model, sham-operated, sham-manipulation, and pivot meridian massage

groups. The sham-operated, model, sham-manipulation, and pivot meridian massage groups underwent neuropathic pain model establishment.

1.3.2 Model Preparation The SNL rat model was established according to the method of Kim et al. Rats were anesthetized via intraperitoneal injection of 7% chloral hydrate (4 mL/kg body weight). Following skin preparation and disinfection with 75% alcohol, a 1.8 cm incision was made along the longitudinal axis of the left paraspinal muscles at the level connecting the highest points of both posterior superior iliac spines. After blunt dissection of skin, superficial fascia, and deep muscle tissue to expose the L5 vertebral transverse process, the bony connections between L5 transverse process and L4/L6 vertebrae were removed with bone rongeurs to fully expose the L5 spinal nerve. The exposed L5 spinal nerve was tightly ligated in double layers with non-invasive nylon sutures, avoiding excessive traction. Successful modeling was confirmed by frequent licking of the affected limb, elevation of the operated hind paw at rest, protective suspension of the operated limb during activity, and absence of paralysis or dragging.

1.3.3 Intervention Methods Twenty-four hours post-modeling, interventions were administered as follows: the normal and model groups received no intervention; the sham-operated group underwent exposure of the L5 spinal nerve for several minutes without ligation; the sham-manipulation group received gentle stroking of both hind limbs for 18 minutes under cloth restraint; the pivot meridian massage group received sequential stimulation of three acupoints (Huan Tiao, Yang Ling Quan, and Xuan Zhong) on the bilateral Foot Shaoyang Gallbladder Meridian using a custom massage device simulating point, kneading, and plucking techniques. Stimulation parameters were 5 N force and 2 Hz frequency, with 1 minute per acupoint and technique (6 acupoints total, 3 techniques, 18 minutes total duration).

1.4 Observation Indicators 1.4.1 Mechanical Withdrawal Threshold Measurement Rats were placed in transparent cages with wire mesh floors (0.5 cm × 0.5 cm grid). After acclimatization, von Frey filaments of varying stiffness were applied to the central plantar surface of the left hind paw through the mesh floor. The threshold force eliciting paw withdrawal or shaking was recorded. Three consecutive measurements were averaged to determine the mechanical withdrawal threshold.

1.4.2 Thermal Withdrawal Latency Measurement With room temperature maintained at 25°C, rats were placed in a transparent organic glass cage containing a thermal pain tester set to 55°C. The timer was started upon placement, and stopped when frequent paw lifting or licking behaviors were observed. Three measurements were taken at 20-minute intervals and averaged.

1.4.3 Tissue Collection After 12-hour fasting, rats were anesthetized with 10% chloral hydrate (4 mL/kg) via intraperitoneal injection. Surgical instruments were soaked overnight in 0.1% DEPC-treated water to prevent enzyme

contamination. Following successful anesthesia (confirmed by absence of limb movement), rats were decapitated and spinal dorsal horn tissue from the lumbar enlargement (L2-L5) was rapidly dissected, weighed, snap-frozen in liquid nitrogen, and stored at -80°C for use within 3 months.

1.4.4 Protein Expression Detection Spinal cord tissue samples were analyzed by Western blot to determine protein levels of Bcl-2, Caspase-3, ERK, and TLR8. Protein concentrations were measured, samples were prepared, separated on 10% gels, transferred to membranes based on molecular weight, and subjected to immunochemical luminescence detection. Band densities were quantified using Bio-Rad software.

1.4.5 Gene Expression Detection Total RNA was extracted from spinal cord tissue using Trizol reagent and reverse-transcribed to cDNA using the Takara PrimeScript™ RT Master Mix kit (DRR036A). Real-time fluorescent PCR amplification was performed using 2× Taq PCR MasterMix according to the manufacturer's instructions to quantify LncRNA-GAS5 and miR-21 expression levels.

1.4.6 Immunofluorescence Staining Tissue sections underwent dewaxing in graded xylene (100%, 90%, 80%) and rehydration through graded ethanol (100%, 90%, 80%, 70%). After PBS washing, sections were incubated with 3% H_2O_2 for 15 minutes at room temperature, blocked with goat serum for 1 hour, and incubated overnight at 4°C with primary antibody NeuN (1:400). Following PBS washes, FITC-conjugated secondary antibody was applied for 1 hour at room temperature in the dark. After DAPI counterstaining for 25 minutes, fluorescent images were captured using a confocal microscope and ImageJ software.

1.4.7 Apoptosis Detection Sections were dewaxed and rehydrated as described above. After air-drying, 100 L of 1× Proteinase K was applied to each sample and incubated at 37°C for 20 minutes. Following PBS washes, samples were incubated with TdT Equilibration Buffer for 10-30 minutes, then with 50 L labeling working solution for 1 hour in a dark humidified chamber. After PBS washes and DAPI counterstaining, sections were mounted with anti-fluorescence quenching medium and imaged.

1.5 Statistical Analysis Data were analyzed using SPSS 25.0 software. Measurement data are expressed as mean ± standard deviation ($\bar{x}\pm s$). Inter-group comparisons were performed using one-way ANOVA, with pairwise comparisons conducted via LSD-t test. Statistical significance was set at $P<0.05$.

Results

2.1 Behavioral Observations 2.1.1 Mechanical Withdrawal Threshold Before modeling, no significant differences in mechanical withdrawal thresh-

old were observed among groups (P>0.05). Post-modeling days 1, 3, 7, and 14 showed significantly lower thresholds in the model, sham-manipulation, and pivot meridian massage groups compared to the normal group (P<0.05). The sham-operated group showed no difference from the normal group on days 7 and 14 (P>0.05). Both the sham-manipulation and pivot meridian massage groups exhibited higher thresholds than the model group on day 14 (P<0.05), with the pivot meridian massage group showing superior results compared to the sham-manipulation group on days 7 and 14 (P<0.05) .

2.1.2 Thermal Withdrawal Latency Pre-modeling thermal withdrawal latency showed no inter-group differences (P>0.05). The sham-operated and pivot meridian massage groups exhibited shorter latency than the normal group on days 1, 3, and 7 post-modeling (P<0.05), but no difference on day 14 (P>0.05). Both intervention groups showed longer latency than the model group on days 7 and 14 (P<0.05), with the pivot meridian massage group demonstrating superior performance on day 14 (P<0.05) .

2.2 Protein and Gene Expression 2.2.1 Spinal Cord Protein Expression On day 7 post-modeling, Bcl-2 expression in the normal group was lower than all other groups (P<0.05). The pivot meridian massage group showed higher Bcl-2 but lower Caspase-3, ERK, and TLR8 levels compared to the model group (P<0.05), with all four proteins also lower than the sham-manipulation group (P<0.05) , [Figure 1: see original paper].

On day 14, the pivot meridian massage group maintained higher Bcl-2 and lower Caspase-3 and TLR8 levels than the model group, while ERK expression increased above model group levels (P<0.05) , [Figure 2: see original paper].

2.2.2 Spinal Cord Gene Expression At 7 days post-modeling, both sham-manipulation and pivot meridian massage groups showed elevated LncRNA-GAS5 and reduced miR-21 expression compared to the model group (P<0.05), with the pivot meridian massage group exhibiting more pronounced changes (P<0.05) . By day 14, the model group's LncRNA-GAS5 expression remained lower than the normal group, while both intervention groups showed higher expression (P<0.05). MiR-21 expression was also higher in both intervention groups compared to the model group (P<0.05) .

2.3 Neuronal Apoptosis Immunofluorescence staining with TUNEL/DAPI and NeuN/DAPI revealed increased apoptotic cells (TUNEL-positive) and decreased neurons (NeuN-positive) in the model, sham-manipulation, sham-operated, and pivot meridian massage groups compared to the normal group on day 7 post-modeling. The pivot meridian massage group showed reduced apoptosis and preserved neuronal populations compared to the model and sham-manipulation groups [Figure 3: see original paper], [Figure 4: see original paper].

Discussion

Neuropathic pain is characterized by its intractable and refractory nature, with complex mechanisms underlying its initiation, development, and maintenance. Current theories include neural sensitization, plasticity of muscle nociceptive neurons, and gate control theory. The key pathological factors are nerve injury and inflammation, which trigger apoptosis of spinal dorsal horn neurons through pain signal transmission and pro-inflammatory factor release, ultimately increasing pain sensitivity. Therefore, blocking or attenuating neuronal apoptosis represents an effective strategy for NPP relief.

Pivot meridian massage, guided by the pivot meridian theory, selects acupoints along the Foot Shaoyang Gallbladder Meridian for point, plucking, and kneading manipulations. Our results demonstrate that both pivot meridian massage and sham manipulation significantly improved pain threshold indicators (mechanical withdrawal threshold and thermal withdrawal latency) compared to the model group, with pivot meridian massage showing superior efficacy. This confirms that tuina achieves analgesic effects by elevating pain thresholds, with pivot meridian theory-guided interventions demonstrating enhanced effectiveness.

Long non-coding RNAs (LncRNAs) regulate numerous diseases through epigenetic mechanisms and apoptosis control. LncRNA-GAS5, located on chromosome 1q25 and consisting of 12 exons and 11 introns, is associated with neuronal apoptosis, vascular remodeling, and inflammatory responses. Studies have shown that inhibiting LncRNA-GAS5 reduces apoptosis by decreasing Bax and increasing Bcl-2 expression in cerebral infarction models, while overexpression exacerbates neuronal apoptosis through enhanced glycolysis. Additionally, LncRNA-GAS5 functions as a “molecular sponge” for miRNAs. MiR-21 plays critical regulatory roles in neuronal protection and repair, with its expression significantly increased in SNL rat spinal cord and dorsal root ganglia. Intrathecal injection of miR-21 inhibitors alleviates mechanical allodynia and thermal hyperalgesia, suggesting miR-21 as a potential downstream target for NPP treatment.

Our findings revealed that model rats exhibited decreased LncRNA-GAS5 and increased miR-21 expression in spinal cord tissue. Pivot meridian massage significantly reversed these changes, suggesting that stimulation of peripheral somatic tissues receiving pain signals may partially block transmission to the superficial spinal dorsal horn while upregulating LncRNA-GAS5 expression. The elevated LncRNA-GAS5 likely “adsorbs” miR-21, reducing its expression and indirectly inhibiting neuronal apoptosis. Notably, miR-21 expression patterns changed between days 7 and 14, with model group levels decreasing (possibly due to natural repair processes) while pivot meridian massage group levels increased, suggesting potential involvement of negative feedback mechanisms requiring further investigation.

TLR8, a Toll-like receptor family member expressed primarily in small-to-medium-sized neurons, plays crucial roles in pathogen recognition and innate

immune activation. Research confirms TLR8 as a negative regulator of axonal growth and inducer of neuronal apoptosis. In SNL rats, TLR8 expression increases significantly in the spinal dorsal horn, where it activates ERK phosphorylation through miR-21 to mediate inflammatory mediator production and neuronal hyperexcitability. Our results showed that pivot meridian massage downregulated the apoptosis inducer TLR8 and miR-21-mediated ERK protein while upregulating the anti-apoptotic protein Bcl-2, which inhibits cytochrome c release from mitochondria and reduces Caspase-3 expression, ultimately decreasing neuronal apoptosis and alleviating pain.

In summary, pivot meridian massage guided by pivot meridian theory alleviates inflammatory responses at pain sites and regulates spinal dorsal horn neuronal apoptosis to relieve NPP. The mechanism likely involves upregulating LncRNA-GAS5 expression to downregulate miR-21 and modulate TLR8/ERK pathway components. This study provides experimental evidence for clinical application of pivot meridian massage in NPP and suggests LncRNA-GAS5 as a promising therapeutic target. Future studies should employ miR-21 inhibitors or gene knockout approaches to further elucidate the relationships among LncRNA-GAS5, miR-21, TLR8, and ERK, and to more thoroughly clarify the analgesic mechanisms of pivot meridian massage.

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