Piperine Treating Sciatica Through Regulating Inflammation and MiR-520a/P65 Pathway

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[ABSTRACT]
Mongolian medicine, Naru-3, is composed of the radix aconiti agrestis, the fructus chebulae and the long pepper, which has the effects of reducing swelling, relieving pain and treating sciatica. Previous researchs have found that piperine, an intrinsic ingredient in the long pepper, may has analgesic and anti-inflammatory effects. To verify the regulatory relationship between miR-520a and p65, and to explore how miR-520a/p65 affects the levels of pro-inflammatory cytokines (IL-1β, TNF-α) and anti-inflammatory cytokines (IL-10, TGF-β1) under the action of piperine.

The rat model of sciatica was firstly made. The factors of sciatica inflammatory response were evaluated by ELISA. Gene chip analysis of the miRNAs was then performed to analyze the miRNA expressed differently in the model group and the drug-administered group, and to predict the downstream targeting of the miRNA. Real-time quantitative PCR was used to detect the expressions of miR-520a, P65, pro-inflammatory cytokines (IL-1β and TNF-α) and anti-inflammatory cytokines (IL-10 and TGF-β1) at the mRNA level. Western blot showed that P65 expression was positively correlated with pro-inflammatory factors and negatively correlated with anti-inflammatory factors at protein level. HE staining showed that the nerves in the sham group were closely arranged and orderly, in the model group, the nerve damage was extremely severe, with obvious vacuoles and severe deformation of nerve fibers. After drug administration, nerve fibers were repaired, vacuoles were significantly reduced, and the damage degree of nerve fibers was also improved. Immunohistochemical analysis showed that P65 was not expressed in the sham group, P65 was severely expressed in the model group, and the expression of P65 was decreased after administration. The dual luciferase reporter assay again confirmed that luciferase signal was significantly decreased when co-transfected with the recombinant plasmid of miR-520a mimics and P65 3'UTR compared with the other groups (P < 0.01). When miR-520a mimics was expressed at high or low, there was no significant decrease in luciferase signal after co-transfection with the mutated P65 3'UTR recombinant plasmid, suggesting that miR-520a had a specific target effect on P65.

[KEY WORDS] Piperine; sciatica; miR-520a; P65; inflammatory factor

[Background]
Sciatica is a kind of combined pain caused by stimulation and compression of various factors, resulting in stabbing, burning and dull pain along the route of the sciatic nerve and in the surrounding areas, which brings great physical and psychological pain to patients. The mechanism of sciatica is...
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extremely complicated and is often caused by the protrusion of the lumbar disc [4]. Lumbar disc herniation, which causes damage to the spinal nerve roots and autoimmune inflammatory response of the nucleus pulposus, is the mechanism of sciatica [5]. Nowadays, medical treatment methods are broadly divided into drug-based treatment and non-drug treatment. Drug-based treatments are mainly analgesics such as ibuprofen, celecoxib, pentazocine, and glucocorticoids, but these two types of drugs are prone to related adverse reactions [6–7].

Surgical therapy is also a common method, namely decompression spinal nerve roots, so that patients are no longer subject to compression caused by lumbar disc herniation. However, the operation can only delay the pain, and the treatment for those who have been sick for more than two years is not significant. And after surgery, it is also prone to complications such as infection and leakage of cerebrospinal fluid [8].

Mongolian medicine compound Naru-3, also called Naru Sumu Zhuer, whose prescription is from Supreme and Important Prescription, consists of such three medicines as the radix aconit agrestis, the fructus chebeulae and the long pepper. The theory of Mongolian medicine expresses that Naru-3 can eliminate “being glutinous” and “Xieri Wusu”, relieve pains, and fit for curing the rheumatism, the arthralgia, Waist pain, toothache and the diphtheritis. [9] Some research has indicated that Naru-3 has a curing function to rats’ AIA as anti-inflammatory and analgesia. [10] Through modern pharmacology, the research on Mongolian medicine Naru-3 found that Naru-3 have anti-inflammatory, analgesic and antibacterial effects [11]. Piperine in the long piper is the main active chemical substance in Naru-3. It belongs to the cinnamamine series, and is widely found in nature, especially in the pepper plant. [12] Current studies have found that piperine has a good anti-inflammatory, immune regulation and anti-tumor effects [13–15].

In recent years, it has been found that various miRNAs have anti-inflammatory or pro-inflammatory effects on pain [16–18]. P65 is a subtype unit of nf-kappab, and the expression of it is affected by central and peripheral nerve injury [19–23]. Although there is a research on mechanism of P65 in pain, there are few studies on the interaction between P65 and miRNAs. This paper will study the relationship between P65 and miRNAs based on the corresponding arguments.

This article is based on the theory of inducing lumbar disc herniation and causing root sciatica. This paper will study how piperine regulates miRNA, mediates the expression of P65, regulates inflammatory factors, and finally treats sciatica. It provides new ideas and methods for the treatment of sciatica in the future.

**Materials and methods**

**Laboratory animals and grouping**

Male SD rats, weighing 200-220 g (experimental animal center of Inner Mongolia medical university, Hohhot, China), at a room temperature 20 ± 1 °C, in humidity 40-50%, with 12 hours sun exposure, were raised by food and water for standard rodents. They were divided into four groups as sham group, model group, celecoxib group and piperine group, with 12 rats in each group.

**Rat model of non-compressed lumbar disc herniation and evaluation of pain-related behavior**

10% chlorohydrate was prepared, and the rats were anesthetized by intraperitoneal injection according to a dose of 3.5 ml/kg. With the L4-L6 spinous process as the center, the longitudinal incision of the midline back was performed to expose the bilateral L4 and L5 lamina. The two intervertebral discs of the larger tail were found along the lumbar vertebrae, removed the nucleus pulposus 5mg, and then gently placed the nucleus pulposus at the ruptured ganglion, and finally sutured. The sham group sutured directly after destroying the ganglion, and did not place the nucleus pulposus [24]. Mechanical withdrawal threshold(MWT) of four groups were measured respectively before the operation, at the first, third, fifth, seventh, tenth, fourteenth day after the operation and pain-related behavior evaluation was performed to determine whether the model was established successfully [25].

**Dosage and method of administration**

Sham group: no intragastric administration. Model group: gastric normal saline 0.75ml per day. Celecoxib group: celecoxib (Pfizer, California, USA, J20140072) was prepared into a suspension with a mass concentration of 42mg•ml⁻¹ with 0.9% normal saline, and 0.75ml was given by gavage every day. Piperine group: Piperine (purity > 97%, Sigma, Missouri, USA) was prepared into a suspension with a mass concentration of 4mg•ml⁻¹ with 0.9% normal saline, and 0.75ml was given by gavage every day. All the drugs were given for 15 consecutive days.

**Behavioral observation of rats**

The rats with non compression lumbar disc herniation were placed in the observation room for 30 minutes to observe the posture of hind limbs. The behavior of rats was observed three times in 15 minutes, each time for 300 seconds. The gait and autonomic behavior of rats were mainly observed to see if the affected limb landed lightly, the medial edge of the foot landed, limped, the foot slightly everted, the heel landed, the hind foot suspended, licked, swung and even bit the foot.

**Determination of the expression level of inflammatory cytokines in rats serum by ELISA**

The blood was cooled by centrifugation, the supernatant was taken, the standard was diluted, and the standard for each well was diluted to 50 μl. Samples were added and rabbit anti-p65 antibody (Maixin Biotechnology co., Ltd., Fujian, China) was used for incubate color. Finally, 50 μl of termina-
tion solution (Huijia Biotech, Xiamen, China) was added to each well to make the blue liquid turn yellow, that is, the reaction stopped. The blank wells are returned to zero after adding the termination solution, and the OD value of each well at a wavelength of 450 nm is measured. The final actual sample concentration is equal to the sample concentration calculated by multiplying the OD value by a dilution factor of 5 times.

The miRNA microarray technique screening miRNAs of different expression in rat sciatic nerve tissue and predicting downstream targeting

Tissues of rats in the model group were taken as a group, and tissues of rats in the celecoxib group and the Piperine group were taken as the other group. The total RNA of the two groups was extracted with Trizol reagent (Thermo Fisher Scientific, Massachusetts, USA). MiRNA microarray (Agilent, Inc., California, USA) was used to analyze miRNA expression profile of cells in the two groups. Using online budgeting software TargetScan (http://www.targetscan.org/), PicTar) and miRanda (http://www.microrna.org/) predicted the miRNA target genes bioinformatics. Downstream targets that have a targeted regulatory relationship with miRNA and are related to the regulation of inflammatory development were screened out.

Detection of mRNA expression level in sciatic nerve tissue by real-time fluorescent quantitative PCR

MRNA expressions of miR-520a, P65, IL-1β, TNF-α, IL-10 and TGF-β1 in the sciatic nerve were detected as follows. The following primers were obtained from Sangon Biotech, in which miR-520a used U6 as an internal reference, and the others used β-action as an internal reference(Table 1). Total RNA was extracted and reverse transcribed. The cDNA obtained by the reverse transcription reaction was used as a template to carry out PCR amplification. Finally, data analysis was performed using Sequence Detection System (SDS) 2.3 software. The expression level of mRNA of miR-520a, P65, and each inflammatory cytokines in the sciatic nerve was expressed by 2-ΔΔCt.

Western blot analysis of protein expression levels of inflammatory cytokines and P65 in rat sciatic nerve tissue

At the end of the administration, the rats sciatic nerve was collected, the lysate was added, and the supernatant was centrifuged, followed by protein extraction. The concentration of the sample protein was calculated. After that, glue preparation, glue filling, sample loading and rotary die were carried out. Rabbit anti-p65 primary antibody and Goat anti-rabbit antibody (Maixin Biototechnology co., LTD., fujian, China) were incubated in a shaker bed at 37 °C, and the prepared DAB solution was added to the front of the membrane to develop color at room temperature. The density ratio of P65 to GAPDH bands was analyzed by Image-J software, and the relative expression of protein was calculated.

HE staining experiment of rat sciatic nerve tissue

The tissue was first sliced, placed in a liquid, carried on a glass slide, and dried at 38 °C for 24 hours. The dried slices were dewaxed and hydrated, stained with hematoxylin for 5.5min, washed with tap water for 10s to return to blue, and finally stained with eosin for 8s. The stained sections were dehydrated, transparent, and finally sealed with a neutral gum and a cover slip.

Immunohistochemical analysis of rat sciatic nerve tissue

Tissue sections were first prepared, placed in a liquid, carried on a glass slide, and dried at 38 °C for 2 hours. The dried tissue sections were dewaxed and hydrated. The antigenic repair and closure of the tissue sections were performed by direct boiling of the citrate tissue antigen repair solution. The PBS solution was removed, rabbit anti-p65 primary antibody was added, and it was incubated overnight at room temperature. The enzyme-labeled goat anti-rabbit IgG polymer antibody was added, incubated at 37 °C for 15 minutes. DAB coloring solution was added and incubated for 5 minutes. After the counterstaining, the sections were dehydrated, transparent, and finally sealed with a neutral gum and a cover slip.

Dual luciferase reporter gene assay

The upstream and downstream primers of miR-520a were designed by PRIMER5.0, then amplified, and the amplified product was isolated. The target fragment of about 1600 bp was recovered and purified. The vector pmirGLO (Ambion, USA) was treated with restriction enzyme (Pmel and Xbal). Large fragments of the vector and the recovered PCR products were taken to transform the competent DH5 alpha cells and selected the positive clones. A small amount of recombinant plasmids were extracted by alkali lysis method, and then sequenced for identification. The recombinant plasmid pmirGLO-P65-3'UTR was constructed in the same man-

<table>
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<th>Table 1 RT-PCR Primer sequence</th>
<th>Forward 5'-CACACGCCATCATCACATTTGC-3'</th>
<th>Reverse 5'-GACCTACAGGCTGACGCCAG-3'</th>
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<tr>
<td>miR-520a</td>
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<tr>
<td></td>
<td>Forward 5'-CTGCTTCGACGACA-3'</td>
<td>Reverse 5'-AACGCTACAGGTTCGCT-3'</td>
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<td></td>
<td>P65</td>
<td></td>
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<tr>
<td></td>
<td>Forward 5'-CTGGTGGCTGGTCAAATCA-3'</td>
<td>Reverse 5'-GGTTGTCGTTTCCCTTAG-3'</td>
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<td></td>
<td>IL-1β</td>
<td></td>
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<tr>
<td></td>
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<td>Reverse 5'-CCTFACATCAAGACCCYCAA-3'</td>
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<td></td>
<td>TNF-α</td>
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<tr>
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<td>Forward 5'-AGGGTACGTCCCTGATA-3'</td>
<td>Reverse 5'-CTCTCCGGCTGGCTACAG-3'</td>
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<td>IL-10</td>
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<td>Reverse 5'-AGCTGCTGGCCAGTCCTCG-3'</td>
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<td>TGF-β</td>
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<tr>
<td></td>
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<td>Reverse 5'-ATCGTGGGAGCAGGAAGAT-3'</td>
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<tr>
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<td>Forward 5'-CTGTTAATGTCACGCA-3'</td>
<td>Reverse 5'-TTAATACTCTCATCACACC-3'</td>
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ner. Finally, a recombinant plasmid with a site mutation was constructed, namely pmirGLO-mutant-P65-3’UTR. The recombinant plasmid of miR-520a gene and the luciferase reporter plasmid containing P65 3’UTR fragment were cotransfected into HEK293 cells. The luciferase double reporter gene assay was used to identify the targeting relationship between miR-520a and P65.

Data analysis

All data are expressed as average numbers±standard deviations. SPSS 20.0 has been used for the statistical analysis. Differences between groups were determined by Student’s t test and analysis of variance of repeated measures. P < 0.05 was considered statistically significant and there was a difference. P < 0.01 means significant difference.

Results

Rat model of non-compressed lumbar disc herniation

By measuring the MWT value of the untreated rats in sham group, model group, celecoxib group and piperine group(Table 2), there was no significant difference in the preoperative MWT values between the four groups (P > 0.05). The MWT values of model group, celecoxib group and piperine group at different time were significantly different from the sham rats (P < 0.01). The MWT values of model group, celecoxib group and piperine group began to decrease. There were significant differences between the postoperative and the preoperative MWT values of each group (P < 0.01), indicating that the rats in model group, celecoxib group and piperine group have developed pain. When the MWT values of the rats in model group, celecoxib group and piperine group were compared with each other, it was found that there was a significant difference between the 3 days and 1 day, 5 days and 3 days after surgery (P < 0.01), and the celecoxib group had difference (P < 0.05). There was no significant difference in the piperine group (P > 0.05). When compared with 5 days and 7 days after surgery, the MWT values of the rats in model group, celecoxib group and piperine group showed significant differences (P < 0.01), indicating that the rats showed pain peak from the 7th day after surgery. The results of this experiment are consistent with the reports, indicating that the rat model of this study was successfully produced.

Behavioral observation of rats

All rats were in good mental state and food intake, no death and autophagy. From the 4th day after the operation, the rats in the model group and the administration group had pain gait. The toes of each foot could not be unfolded at will. The rats walked with limp gait. The hind leg of the sham group was relatively normal. The above situation was more obvious on the 7th day and reached the peak on the 14th day. No autotomy was observed, indicating that the model was successful produced.

After administration, the rats in each group were in good health. The rats in the sham group had normal activities, and the rats in the model group, the celecoxib group and the piperine group had different degrees of gait limping, licking, slight valgus, suspension, and weak walking of lower limbs. In the model group, the rats were limping, following the ground, not daring to touch the ground, licking their feet, and a few biting their feet. Compared with the model group, the above symptoms in the piperine group were alleviated on the 7th and 15th day after administration, most of which were spontaneous leg lifting reduction, foot licking less, and walking was more normal. Compared with the piperine group, the celecoxib group was more obvious, and the recovery of hind limb light landing or activity was faster, which had been relieved or without symptoms on the 15th day.

Determination of inflammatory factor expression levels in rat serum by ELISA

By measuring the levels of pro-inflammatory factors (IL-1β, TNF-α) and anti-inflammatory factors (IL-10, TGF-β1) in rat serum (Fig. 1), it was found that compared with the sham rats, the contents of TNF-α and IL-1β in model group were significantly increased (P < 0.01), while the levels of IL-10 and TGF-β1 were significantly decreased (P < 0.01). Compared with the model rats, the levels of IL-1β and TNF-α in the celecoxib group and the piperine group were significantly decreased (P < 0.01), and the levels of IL-10 and TGF-β1 were significantly increased (P < 0.01). It indicates that in-

<table>
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<th>Table 2</th>
<th>Results of preoperative and postoperative MWT values in four groups of rats(±SD)</th>
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<tr>
<td>Group</td>
<td>sham</td>
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<tr>
<td>Preoperative</td>
<td>35.14 ± 3.29</td>
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<tr>
<td>1 day after surgery</td>
<td>26.42 ± 3.62</td>
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<tr>
<td>3 days after surgery</td>
<td>24.64 ± 3.17</td>
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<tr>
<td>5 days after surgery</td>
<td>28.30 ± 2.64</td>
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<tr>
<td>7 days after surgery</td>
<td>29.49 ± 2.45</td>
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<tr>
<td>10 days after surgery</td>
<td>31.30 ± 2.97</td>
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<tr>
<td>14 days after surgery</td>
<td>33.63 ± 1.96</td>
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</table>

Note: The model group, celecoxib group and piperine group were compared with the sham group at each time point after surgery *P<0.05, **P<0.01
Inflammatory reaction is prominent in rats before administration, and the content of pro-inflammatory factors in serum is increased, while the content of anti-inflammatory factors is decreased. The inflammatory reaction was relieved after administration of celecoxib and piperine, so the levels of IL-1β and TNF-α were decreased, and the levels of IL-10 and TGF-β1 were elevated. The results of this study are consistent with the functions of inflammatory factors reported in the literatures [26-29]. It indicated that IL-1β, TNF-α, IL-10 and TGF-β1 can be used as an indicator for judging the inflammatory reaction in rats and suffering from persistent sciatica.

miRNAs with differential expression in rat sciatic nerve before and after administration and prediction of downstream targeting to P65

The miRNA microarray technique was used to detect the differential expression of miRNA in rat sciatic nerve before and after administration. The expression of miR-520a was found to be the most significant (FC) ≥ 2, P < 0.05 (Fig. 2-A). PCR experiment was used to confirm again that compared with the sham group, the expression of miR-520a in the model group was decreased (P < 0.01), and the expression of miR-520a was up-regulated after treatment (P < 0.01) (Fig. 2-B). This result is consistent with the results of microarray technology screening, indicating that piperine can up-regulate miR-520a. This study also used real-time fluorescent quantitative PCR to detect the expression of P65 at mRNA level (Fig. 2-C). It was found that compared with sham rats, the expression of p65 in model rats increased (P < 0.01); after administration, compared with model rats, the expression of p65 decreased (P < 0.01). Moreover, the expression of P65 was negatively correlated with the expression of miR-520a at the mRNA level, indicating that the increase of miR-520a expression at the mRNA level can regulate the downstream target P65 and reduce its expression when piperine was given.

Expression of inflammatory factors in sciatic nerve at mRNA and protein levels

As shown in Figure 3, B-E showed the expressions of IL-1β, TNF-α, IL-10 and TGF-β1 in the sciatic nerve of the four groups at the mRNA level, and found that the expressions of pro-inflammatory factors (IL-1β, TNF-α) were significantly increased in the model rats compared with the sham rats (P < 0.01). The expression of anti-inflammatory factor IL-10 was decreased (P < 0.05), and the expression of TGF-β1 was decreased significantly (P < 0.01). Compared with the model rats, the expressions of IL-1β and TNF-α in administration of celecoxib and piperine were significantly decreased (P < 0.01), and the expressions of IL-10 and TGF-β1 were significantly increased (P < 0.01). In the model group, the inflammatory reaction caused severe pain, while the administration of celecoxib and piperine showed that the expressions of pro-inflammatory cytokines were decreased, and the expressions of IL-10 and TGF-β1 were increased. This result indicated that piperine can relieve pain and is equivalent to celecoxib. The protein expression of IL-1β, TNF-α, IL-10 and TGF-β1 in the sciatic nerve was further determined.
shown in Fig.3-A, the protein expressions of IL-1β and TNF-α in the model rats were significantly higher than that in the sham rats (P < 0.01). The expressions of IL-10 and TGF-β1 were significantly decreased (P < 0.01). The expressions of IL-1β and TNF-α were significantly decreased after administration (P < 0.01), and the expression of IL-10 was increased in piperine group (P < 0.05). The expressions of IL-10 in the celecoxib group and TGF-β1 in the two administration groups were significantly increased (P < 0.01). This result indicated that piperine has a certain effect on various inflammatory factors at the protein level.

**Protein expression and pathological analysis of P65 in sciatic nerve tissue**

Western blot was used to analyze the expression of P65 in the sciatic nerve. The banding (Fig.4-A) and relative density ratio (Fig.4-B) showed that compared with the sham group, the expression of P65 in the model group was significantly increased (P<0.01). After administration, the expressions of P65 protein in celecoxib group and piperine group were decreased (P<0.01). Therefore, at the protein level, the expression of P65 is positively correlated with pro-inflammatory factors and negatively correlated with anti-inflammatory factors. HE staining analysis of the sciatic nerve tissue morphology of rats before and after administration (Fig.4-C) showed that the nerves were arranged closely and orderly in the sham group. Compared with the sham rats, the nerve damage in the model group was extremely severe, with obvious vacuolar, severe deformation of nerve fibers and light staining. After administration, compared with the model group, the degree of vacuolization in the celecoxib group was reduced, the nerve fibers were repaired, and the intact nerve fibers were clearly seen; the vacuolar in the piperine group showed that the nerves were arranged closely and orderly in the sham group. Compared with the sham rats, the nerve damage in the model group was extremely severe, with obvious vacuolar, severe deformation of nerve fibers and light staining. After administration, compared with the model group, the degree of vacuolization in the celecoxib group was reduced, the nerve fibers were repaired, and the intact nerve fibers were clearly seen; the vacuolar in the piperine group showed that the nerves were arranged closely and orderly in the sham group. Compared with the sham rats, the nerve damage in the model group was extremely severe, with obvious vacuolar, severe deformation of nerve fibers and light staining. After administration, compared with the model group, the degree of vacuolization in the celecoxib group was reduced, the nerve fibers were repaired, and the intact nerve fibers were clearly seen; the vacuolar in the piperine group showed that the nerves were arranged closely and orderly in the sham group.

**Fig. 2**  A: The differential miRNA clustering analysis between the model group and the drug-administered group. The left side represents the model group and the right side represents the drug-administered group. Blue is low expression and red is high expression. The expression difference of miR-520a was the most significant (FC) ≥ 2, P < 0.05. B: The real-time quantitative PCR result of miR-520a. The expression of miR-520a at the mRNA level is consistent with the results of the miRNA microarray technology. ##P < 0.01 Compared with sham rats, **P < 0.01 compared with model rats. C: The result of detection of P65 expression at the mRNA level. The expression of P65 at the mRNA level was inversely correlated with the expression of miR-520a at the mRNA level. #P < 0.01Compared with sham rats, **P < 0.01 compared with model rats.
plasm of the model group showed brown color, that is, P65 was severely expressed; The cytoplasm of the celecoxib group was light brown, P65 was mildly expressed; the piperine group was brownish yellow, and p65 was moderately expressed. It is further illustrated that piperine works by inhibiting the expression of P65.

_Dual luciferase reports confirmed that P65 is a downstream target of miR-520a_

This study used TargetScan and online (http://www.targetscan.org/) to find that the miR-520a theoretical binding site is P65 (Fig. 5-B). In order to re-verify the targeting effect of P65 and miR-520a, we used the double luciferase reporter gene experiment. As shown in figure 5, when miR-520a mimics was co-transfected with the recombinant plasmid of P65 3 ’UTR, luciferase signal was significantly decreased (P < 0.01) compared with other groups. When miR-520a mimics was expressed at high or low, there was no significant decrease in luciferase signal after co-transfection with the mutated P65 3’UTR recombinant plasmid. Therefore, miR-520a can specifically and directly target P65.

**Discussion**

In the sciatica model, the most commonly used is the CCI model, which causes mechanical compression. However, when making the model, a gut containing chemical substances, namely chromium, is used. It has been found that the toxicity of chromium can also damage the nerves, thus affecting the accuracy of the pathogenesis of pain model. Clinical observation found that many patients with lumbar disc herniation leading to lumbocural pain did not have compression of nucleus pulposus nerve root through imaging examination. In recent years, some scholars have found that mechanical compression is not the main cause of pain. Lu Zhidong et al. showed that after transplantation of autologous nucleus pulposus into the epidural space of rats, there was obvious hyperalgesia without obvious mechanical compression. At the same time, Olmarker et al. showed an inflammatory response through observation of the nerve roots and light microscopy of the model rats. This study further confirmed that transplantation of autologous nucleus pulposus can cause induced pain and hyperalgesia compared with sham group. According to Yang weiqi and other researchers, the local immune response in the animal model makes the nerve root fiber demyelinate, which leads to more sensitive hyperalgesia. Therefore, the author believes that there are many reasons for the onset of sciatica, but mechanical compression is not the only cause of sciatica, and the inflammatory characteristics of the nucleus pulposus is an important mechanism of pain caused by nerve injury. Therefore, this study does not use the traditional CCI rat model. In this study, the rat model of non compression lumbar disc herniation was used, and the sham group was designed to eliminate the trauma factors of the sham group. This method is easy to operate and can maintain pain for up to 28 days, which can be used to support the whole experimental cycle.

The full name of miRNAs, MicroRNA, is made up of 21 to 25 nucleotides, a non-coding protein. MiRNAs transcriptional regulation of most genes in eukaryotic genome through RNA interference mechanism is an inherent gene regulatory function of eukaryotes. In recent years, it has been found that various miRNAs have expressed abnormally in various pain caused by inflammation. This suggests that various miRNAs have anti-inflammatory or pro-inflammatory effects in pain. In chronic inflammatory pain, the expres-
Expression of miR-125b and miR-28 decreased significantly. Further study confirmed that in clinical, the content of miR-28 in the blood of patients with chronic inflammatory pain decreased significantly. In addition, miR-28 was found to participate in Ca²⁺ signal pathway and inhibit inflammatory pain. This finding provides a basis for clinical examination of chronic pain. Therefore, miRNAs will become an important index to study the mechanism of pain and inflammation in the future.

MiR-520a is derived from the human miR-520 family, which is located on human chromosome 19, overexpressed in undifferentiated human embryonic stem cells, and plays an important role in the regulation of the polarity of embryonic stem cells. However, there are few studies on the function of miR-520a in inflammation. In this study, TargetScan (http://www.targetscan.org/) was used to screen the upstream miRNAs that P65 may bind for the first time, and then the expression of miR-520a at mRNA level was analyzed again. Finally, it is predicted that P65 may be targeted downstream of miR-520a.

NF-κB is a kind of protein that can regulate the expression of many genes. It is widely found in the cytoplasm of various animals and participates in cellular inflammation and immune response. P65, a subtype of NF-κB, is involved in the regulation of gene expression. In the process of inflammation and immunity, p65 has been shown to regulate the expression of key inflammatory mediators. It has been reported that p65 can affect the levels of TNF-α and IL-1β in AIA rats, so p65 can cause neuropathic pain. It has also been reported that the level of IL-1β and TNF-α can be downregulated when p65 and cyclooxygenase-2 are limited to activate together. This study verified by a series of experiments that P65 can be significantly activated in the model group. Through the detection of p65 and various inflammatory factors, we found that p65 can significantly increase the release of IL-1β, TNF-α, and induce pain. After piperine administration, the mechanically induced pain was alleviated.

Fig. 4 A: P65 protein expression bands of rat tissues in sham group, model group, celecoxib group and piperine group. B: Relative density ratio of P65 to internal reference GAPDH. #P < 0.01 Compared with sham rats, **P < 0.01 compared with model rats. C: HE staining results of sham group, model group, celecoxib group, and piperine group. D: Results of P65 immunohistochemistry in the tissues of sham group, model group, celecoxib group and piperine group. The positive expression of P65 appears in the cytoplasm.
the level of pro-inflammatory factors was down-regulated, and the level of anti-inflammatory factors was increased, indicating that it was associated with inhibition of P65. Therefore, p65 can promote or inhibit the pain of sciatic nerve by regulating the expression of inflammatory factors.

Some studies have shown that inflammatory factors, such as TNF-α, IL-1β and IL-6, can cause pain persistence: they can stimulate the increase of the content of pain-causing substances, and can also increase their own content through autocrine secretion. For example, when the nerve is not injured, the content of TNF-α is very low. When the nerve is damaged, the content of TNF-α can rapidly increase, accelerating the occurrence of inflammation. In addition, inflammatory factors can interact with sodium and calcium channels on the cell membrane, rapidly increase the excitability of neurons, and lead to a continuous increase in the conductivity of these channels, leading to pain. After pretreatment of rats with TNF antibody, chronic compression injury of sciatic nerve did not show pain and hypersensitivity, but after one week of compression injury, TNF antibody could not prevent the occurrence of pain sensitivity. Therefore, p65 can promote or inhibit the pain of sciatic nerve by regulating the expression of inflammatory factors.

Conclusions

In this study, we successfully transferred the nucleus pulposus to L5 dorsal root ganglion to cause sciatic nerve pain. The results of this study indicate that the administration of piperine can down-regulate the transient production of pro-inflammatory factors TNF-α and IL-1β in rat serum and up-regulate the levels of anti-inflammatory factors IL-10 and TGF-β1. At the same time, mechanical induced pain and pro-inflammatory factors decreased, which was related to the inhibition of miR-520a/p65.

Clinically, non-steroidal anti-inflammatory drugs for the treatment of sciatica caused by the protrusion of the lumbar disc are not ideal, because there will be side effects such as diarrhea, gastroenteritis, gastric ulcer, gastroesophageal reflux, etc. In the treatment of inflammation and inflammation-related pain, the sensitivity due to basal pain and the safety of endogenous mediators were not altered. However, the Mongolian medicine, such as Naru-3, is mild in taste and side effects. In addition, this study found that piperine, the main active ingredient in Naru-3, has a similar therapeutic effect to celecoxib, thus providing a new option for the treatment of inflammation and pain diseases.
on analgesia and inflammation. MiR-520a can directly target P65, promote the expression of miR-520a, and then inhibit the expression of P65. Under this mechanism, the pro-inflammatory factors IL-1β, TNF-α are down-regulated and anti-inflammatory factors IL-10 and TGF-β1 are up-regulated to treat sciatica. Therefore, these findings may provide a new theoretical basis for the treatment of sciatica.

Declarations

Availability of data and materials

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

Abbreviations

ELISA: enzyme-linked immunosorbent assay
SD: standard deviations
PBS: phosphate-buffered saline
PCR: Polymerase Chain Reaction
HE: hematoxylin-eosin
DAB: Diaminobenzidine
NF-KB: Nuclear factor kB
NF-AT: Nuclear factor of activated T cells
MWT: Mechanical withdrawal threshold

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Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

The authors declare no competing financial interests.

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