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TNFAIP3 Mediated by Novel Nano Composite Adsorbent on Tumor Necrosis Factor-α in Rats with Lumbar Disc Herniation by Inhibiting NF − κB Pathway

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ABSTRACT

Mobile phones and computers have been widely used in the work of people. The incidence rate of lumbar disc herniation(LDH) has gradually increased and the trend toward younger age has been increasing. According to the epidemiological survey, about half of people will experience lumbar pain in their life and the resulting huge social and economic burden. It has important clinical significance for the treatment of lumbar disc herniation of TNFaIP3 mediated by a new nanocomposite adsorbent on tumor necrosis factor(TNF)-α in rats with LDH by inhibiting the NF − κB pathway. This paper mainly studies the mechanism and efficacy of TNFaIP3 mediated by a new nanocomposite adsorbent on TNF-α in rats with LDH by inhibiting the NF − κB pathway. Eight groups of human nucleus pulposus cells were randomly divided into four groups: high inhibition group, medium inhibition group, low inhibition group and no inhibition group. After interfering with human nucleus pulposus cells by inhibiting the NF − κB pathway, the cells were allowed to stand for 24 hours to extract and detect TNF-α, p-p65, P50, IKB−α and IKK−β in the NF − κB signaling pathway to explore the mechanism of inhibiting NF − κB pathway on TNF-α in rats with LDH. The experimental results showed that after 24 hours of intervention, compared with the non-inhibition group, the expression of TNF in the low inhibition group, medium inhibition group and high inhibition group decreased relatively, and with the increase of inhibition degree, the expression of TNF in each group decreased more obviously, such as the expression of TNF −αin non-inhibition group was 1.48, the expression of TNF in low inhibition group was 1.31, the expression of TNF in medium inhibition group was 0.74, and the expression of TNF in high inhibition group was 0.58. The expression of P50 was 1.86 in non-inhibition, 1.47 in low inhibition, 1.32 in medium inhibition and 1.13 in high inhibition.

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Introduction

With the progress of society, the rhythm and working mode of people have changed dramatically. The pressure on survival in society has increased dramatically, and the incidence rate of various diseases has also increased (1-2). Among them, LDH has become one of the most common diseases and has a clinical incidence rate of about 20%, which accounts for 1/5 of outpatients with leg pain (3-4). Most of them are young adults between 20 and 50 years old, and the main patients are sedentary workers and heavy manual workers (5-6). The pathogenesis of LDH is still under constant exploration. At present, studies have found that the main causes include intervertebral disc degeneration, imbalance of intervertebral disc stress caused by vertebral instability, aggravation of acute and chronic injury, and even long-term compression compensation caused by weight, pregnancy, occupation, bone development and other factors, resulting in persistent injury (7-8); the main pathological mechanisms are mechanical compression, blood supply disorders, inflammatory stimulation, autoimmune response theory (9-10). The disease is considered as a difficult case by some doctors. The course of the disease is lingering and does not heal for a long time. It accompanies the patients for a long time, and can gradually develop to the extent that it affects the activities of both lower limbs and even the excretion of urine and feces (11-12). It brings great inconvenience to the study, work and life of the patients. Due to a large number of patients, a lot of medical resources are consumed every year, resulting in a waste of resources, therefore, in the context of current medical conditions, it is necessary to explore the treatment scheme with less cost and the best curative effect (13-14).

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In the study of LDH, many scholars at home and abroad have studied it and achieved certain results (15-16). Researchers found that the shear force on the intervertebral disc was greater and the damage to the intervertebral disc was more serious when the lumbar spine was flexing forward, so the porters, drivers and computer clerks were more prone to the disease. In addition, some people with abnormal bone development will also cause changes in lumbar biomechanics, which will affect the pressure on the intervertebral disc, causing damage and accelerating degeneration (17-18). Wang J and Hui believed that the herniated intervertebral disc tissue was recognized by the body as an exogenous antigen, and induced the body's autoimmune reaction to produce an inflammatory reaction. They confirmed the existence of antigen-antibody complex in the nucleus pulposus tissue by immunohistochemical staining, and the infiltration of T cells and macrophages in the intervertebral disc tissue (19-20).

This paper mainly studies the mechanism and efficacy of TNFaIP3 mediated by a novel nanocomposite adsorbent on TNF−α in rats with LDH through the NF − κB inhibitory pathway. In this paper, we studied the NF − κB signaling pathway, NF − κBand LDH, and inhibition of the NF − κB signaling pathway in nucleus pulposus to study the mechanism and efficacy of inhibition ofNF − κB signaling pathway on TNF−α in rats with LDH. In order to explore the mechanism of inhibiting the NF − κB pathway on TNF−α in rats with LDH, 8 groups of human nucleus pulposus cells were selected to test the expression of p-p65, P50, IKB−α, IKK−β and other key proteins in TNF−α and NF − κB signaling pathway.

The Mechanism and Effect of Inhibiting NF − κB Pathway on TNF−α in Rats with LDH

NF − κB Signaling Pathway

NF − κB is a kind of nuclear transcription factor, which is ubiquitous in the cytoplasm. It belongs to the Rel family because it can interact with other genes κBsequence is named after specific binding, which plays the role of signaling and inducing gene expression when cells are stimulated. Because it can regulate the gene expression of chemokines, adhesion molecules, growth factors and a variety of enzymes, and participate in the body's inflammatory response and immune regulation, it plays a key role in cell proliferation, differentiation and apoptosis. There are five members in NF − κB / Rel family: NF − κB1(p105), NF − κB2 (P100), RelA (p65), Rel B and C-Rel. They form NF − κB active molecules with each other, mainly in the form of homologous or heterodimers. P50 / p65 heterodimer is the main form of the NF − κB/ Rel family. The amino terminal of the NF − κB/ Rel family contains a Rel homologous domain (RHR), which is composed of about 300 amino acid residues. It contains the dimer domain, nuclear localization sequence and DNA binding domain, which is the functional domain of the NF − κB family (21-23).

NF − κB and LDH

As a ubiquitous transcription factor, NF − κB participates in and mediates a variety of immune responses, inflammatory reactions, cell proliferation and apoptosis. Therefore, the abnormal activation of the NF − κB signal transduction pathway enhanced transcriptional activity, and caused the cascade amplification effect of intercellular signal, leading to a series of diseases. Therefore, in recent years, the relationship between NF − κB and intervertebral disc degenerative diseases have become a hot topic in academic research. At present, it has been proved that NF − κB positive expression is found in nucleus pulposus cells and chondrocytes of animal intervertebral disc degeneration model, while NF − κB positive expression is not found or weakly expressed in nucleus pulposus cells and chondrocytes of self-negative control intervertebral disc. Activation of the NF − κB signaling pathway can enhance IL-1 β, IL-6, TNF−α, the expression of IL-8, MMPs, adhesion molecules and cyclooxygenase were detected. In addition, many inflammatory factors are also activators of the NF − κB signaling pathway, such as TNF−α The increased expression of IL-1B and IL-1B can further activate the NF − κB signaling pathway. Finally, the degeneration and necrosis of intervertebral disc cells, intervertebral disc degeneration, resulting in a series of clinical symptoms (24,25).

Inhibit the Activation of NF－κB Signaling Pathway in Nucleus Pulposus

The appearance of NF－κB in the degenerative intervertebral disc has been reported in a large number. At the same time, the NF－κB signaling pathway is activated in intervertebral disc tissue, and its products, such as TNF－α, IL-8 and IL-1B can accelerate the local inflammatory reaction and play a catalytic role in the degeneration of the intervertebral disc. At the same time, these products are activators of the NF－κB signaling pathway, which further increase the activation of the NF－κB signaling pathway, thus producing more inflammatory products, aggravating the local inflammatory reaction, and finally accelerating the process of intervertebral disc degeneration (26).

The Understanding of Modern Medicine on the Pathogenic Factors of LDH

LDH is a common and high-incidence disease in today's medical industry. It is caused by degeneration or injury of the nucleus pulposus, which becomes fragmented or scar-like connective tissue. In addition, the binding force of the annulus fibrosus around the nucleus pulposus decreases, resulting in a gap or fracture. The nucleus pulposus protrudes from the gap or fracture of the annulus fibrosus, stimulating and compressing the spinal nerve in the spinal canal, causing lumbar pain and unilateral or bilateral lower limb radiation pain, Sellar area sensory disturbance and even abnormal excretion of urine and urine is a disease (27). Modern medical research has found that it is mainly related to the following reasons:

Disc degeneration

Disc degeneration is a degenerative disease characterized by the degradation of the extracellular matrix and loss of cytoplasm. The changes in disc morphology and biomechanical properties are a gradual process. However, the degeneration can begin at the earliest from 20 years old. Relevant research finds that the degeneration of the disc and the changes in various collagen fiber content, insufficient nutrition supply, and the degradation of the disc are the main factors affecting the development of the disc degeneration apoptosis and local inflammatory response are related to these factors, which can cause the changes of nucleus pulposus dehydration, fiber ring relaxation and tear, and the decrease of the function of the nucleus pulposus constrained by the fiber ring, and finally the prolapse of the nucleus pulposus (28).

Lumbar instability

The lumbar spine is the most stressed part of the whole spine. It is usually composed of five lumbar vertebrae, corresponding spinous process, transverse process, superior and inferior articular process, vertebral arch plate, pedicle and other bone structures, as well as intervertebral disc, anterior and posterior longitudinal ligament, ligamentum flavum, interspinous ligament, supraspinous ligament and other soft tissues to stabilize the mechanical balance of the lumbar spine, in which the intervertebral disc plays a role in buffering external force Stable lumbar shape, support weight and another important role. When the lumbar facet joint disorder, lumbar muscle spasm, ligament relaxation, and intervertebral disc degeneration will cause lumbar mechanical imbalance, and then affect the function of the intervertebral disc, damage the structure of the intervertebral disc, leading to nucleus pulposus protrusion (29).

Acute and chronic injury factors

The injury of the intervertebral disc is mainly caused by two aspects: on the one hand, chronic strain, long-term overload of lumbar movement, repeated lumbar rotation and flexion, incorrect daily life and working posture and other reasons are easy to damage the function and structure of the intervertebral disc and cause premature degeneration; on the other hand, it is external violence. When the intervertebral disc is compressed, the annulus fibrosus expands to all sides. When the pressure on the intervertebral disc exceeds its bearing range, the weak part of the posterior annulus fibrosus ruptures. Both of these can cause nucleus pulposus protrusion, but the chronic strain is an important factor causing lumbar disc protrusion (30).

Other factors

In addition to the above reasons, LDH is also related to weight, pregnancy, heredity, occupation, skeletal dysplasia and other factors. Studies have shown that changes in hormone levels during

pregnancy make the ligaments and muscles at the lumbar spine and sacroiliac joints relax, resulting in instability of the vertebral body. With the increase in pregnant women's weight and abdominal pressure, the pelvis tilts forward, the lumbar lordosis, the center of gravity moves forward, and the pressure on the intervertebral disc increases, accelerating the degeneration of the intervertebral disc.

The Pathogenesis of LDH by Modern Medicine

It has been proved that sciatica is caused by LDH. After continuous exploration and research, researchers found that the pathogenesis of low back and leg pain caused by LDH includes mechanical compression, blood supply disorder, inflammatory chemical stimulation and immune response (31).

(i) Mechanical compression

Many researchers say mechanical compression of the spinal nerve is the main cause of low back pain and lower limb radiation pain. There are two kinds of situations in the mechanism of mechanical compression: pulling and compression, while the nerve root lacks the sheath of connective tissue protecting itself, so it is sensitive to mechanical compression. The nerve root will damage its structure when it is compressed by nucleus pulposus, which leads to the local blood supply and nutritional disorders, and causes the internal cells and surface tissues of DRG of dorsal root ganglion to apoptosis (32).

(ii) Blood supply disorder

In addition to the direct effect caused by mechanical compression, there are also indirect factors of blood supply disorder caused by compression. Studies have shown that more than half of the blood supply in the spinal canal comes from the artery that accompanies the nerve root to enter the spinal canal. When the nucleus pulposus compresses the nerve root, it will directly squeeze the blood vessel, resulting in hypoxia of the nerve root, obstruction of blood circulation, local swelling, and subsequently increased compression on the nerve root and blood vessel, aggravated local hypoxia, and apoptosis of nerve cells form a chain reaction. Mechanical compression leads to blood supply disorder, which aggravates the damage to nerve roots. Varicose veins in the spinal canal can also be seen during operation. When the protrusion is removed, the varicose veins disappear. It can be concluded that mechanical compression is closely related to blood supply disorder (33).

(iii) Inflammatory chemical stimulation

In the clinic, it was found that some patients did not have herniation of nucleus pulposus compressing nerve after auxiliary examination, and some patients with LDH had symptoms of low back and leg pain after the operation, so it can be seen that mechanical compression is not a necessary factor for LDH. Some scholars have proposed that the inflammatory reaction after the compression of the nerve root by the nucleus pulposus is the main factor of pain. The experimental results showed that there was an inflammatory reaction around the local tissue after injection of autologous nucleus pulposus, so it was found that autologous nucleus pulposus had an inflammatory reaction on nerve roots. In recent years, more studies have confirmed that LDH-induced lumbocrural pain is caused by an inflammatory reaction. Aseptic inflammation of the connective tissue around the nerve root stimulates the nerve endings and produces the symptoms of body pain, while a single mechanical compression can only cause numbness in the innervated area. Many studies have found IL-1 β, IL-6, and TNF- α, Although IL-8 and other inflammatory factors have no direct pain-inducing ability, they can promote the accumulation of inflammatory cells and the synthesis of direct pain-inducing factors, and also affect the local microcirculation, thus stimulating the spinal nerve and dorsal root ganglion to produce symptoms of low back and leg pain (34).

(iv) Immune response

The intervertebral disc is composed of a nucleus pulposus in the center, an annulus fibrosus around the disc and a closed cartilage plate. Nucleus pulposus is derived from the degeneration of mesoderm chorda. After the formation of the nucleus pulposus, it is surrounded by annulus fibrosus and cartilage plate to form a closed tissue without blood vessels and lymphatic circulation. The nucleus pulposus has never been exposed to the body's immune system, so it has antigenicity. When the nucleus pulposus first contacts the internal immune system of the body, the body

recognizes it as "alien" and promotes the immune response. Cartilage endplate matrix, glycoprotein and type I and type II collagen have antigenicity, and the content of IgG and IgM in intervertebral disc tissue and the content of IgG, IgM and IgA in serum in LDH patients were significantly increased. Stimulation of TNF by T cells in early LDH immune response- α Nerve root is injured by secretion, and nerve root pain appears. The content of IgG and IgM in serum was increased in the sciatic neuralgia model by placing autogenous nucleus pulposus beside the sciatic nerve in rats. The content of IgG and IgM was not increased in the control group without autogenous nucleus pulposus. LDH is a disease with complex pathogenesis. Although the above mechanisms are described separately, they do not appear alone in the development of the disease. These mechanisms are interrelated and influence each other in pathogenesis. When the disc herniation oppresses the spinal nerve, it also oppresses the blood vessels to block the blood supply; Poor blood circulation, varicose veins, further aggravate the compression of the spinal nerve, but also in the local aseptic inflammation; when the nucleus pulposus protrusion produces an immune response, it will also promote the secretion of inflammatory factors, so in the clinical treatment of LDH, we should first determine the dominant pathogenic mechanism, and then select the optimal targeted treatment (35).

TNF- α Pain and Hyperalgesia

TNF- α It is a member of the huge TNF family, TNF- α It is mainly secreted by macrophages, and its biological activity accounts for 70-95% of the total activity of TNF. It can participate in the regulation of cell function, inflammation and immune response. When the body is healthy, TNF- α After the mechanical injury, ischemia or inflammatory stimulation, the expression level of TNF in injured nerve tissue is lower- α The expression level increased rapidly. TNF - α in DRG- α The increase of the content will cause a pain-sensitive effect. Studies have found that TNF- α can increase the expression of p35, which can cause the phosphorylation of capsaicin receptor in the Cdk5 pathway, and then increase the influx of calcium ions into nociceptive neurons to produce pain. The expression of TNF - α in spinal dorsal horn or dorsal root ganglion- α. After binding with its receptor, it can activate nuclear factor kappaB ($NF-\kappa B$), and then affect the production of other inflammatory factors, resulting in chain reaction and pain. Many experiments confirm that TNF- α can regulate neuropathic pain and provide a target for the diagnosis and treatment of neuropathic pain (36).

Image Segmentation Algorithm for LDH

In this paper, the standard fuzzy c-means clustering segmentation algorithm is improved for the image segmentation of LDH. Considering the uncertainty and fuzziness of the image, the spatial information of the image is integrated into the standard FCM algorithm, and the gray statistics of the image are used to cluster instead of the pixels of the image, so as to further modify the membership function. The basic idea is: the pixel set X to be classified, n is the number of elements in the pixel set, P is the p-dimensional feature space, and C is the number of pixels to be classified. If the pixel set X is divided into C categories, then n pixels belong to the fuzzy membership function matrix of C categories respectively, the expression formula is as follows:

$$U = \{\mu_{ij}\} \in R^{cn} \quad [1]$$

Where $\mu_{ij}(1 \le i \le c,\ 1 \le j \le n)$ denotes the membership degree of the j-th pixel $x_j$belonging to the i-th category, which μ_{ij}should meet the following constraints:

$$\sum_{i=1}^{c} \mu_{ij}=1, 1 \le j \le n \quad [2]$$

$$0 \le \mu_{ij} \le 1, 1 \le i \le c, 1 \le j \le n, 2 \le c \le n \quad [3]$$

$$0 < \sum_{j=1}^{n} \mu_{ij} < n, 1 \le i \le c \quad [4]$$

Standard fuzzy C-means clustering is realized by minimizing the objective functions J_M(U, V) of membership matrix U and clustering center matrix V., the formula is as follows:

$$\min J_M(U,V) = \sum_{i=1}^{c} \sum_{j=1}^{n} \mu_{ij}^{m} d_{ij}^{2}(x_j, v_i) \quad [5]$$

Among them, $\mu_{ij}$is the membership of the j-th pixel to the i-th class, $U = \{\mu_{ij}\}$ is the membership matrix, V is the c cluster center set, and m is the fuzzy weighted index, which controls the fuzzy degree of the data partition process. When m=1, the fuzzy clustering degenerates to the hard C-means clustering $J_M(U,V)$ represents the sum of squares of clustering from pixels to clustering centers, and its objective function value reflects the degree of compactness and consistency

within a class under a certain definition of difference.J_M (U,V) the smaller the clustering is, the more compact the clustering is and the higher the quality of image segmentation is. Using the Lagrange multiplier method, two optimization iterative formulas can be derived.

$$\mu_{ij}=\frac{(d_{ij}^2)^{-1/(m-1)}}{\sum_{k=1}^{c}(d_{ij}^2)^{-1/(m-1)}},j=1,2,...,n \quad [6]$$

$$v_i=\frac{\sum_{k=1}^{n}\mu_{ik}^m x_k}{\sum_{k=1}^{n}\mu_{ik}^m},i=1,2,...,c \quad [7]$$

Suppose that the image adopts matrix M × N means that the gray value of a pixel in the image is represented by f (m, n) ∈ {0, 1,..., L - 1}, where L is the gray level of the image, which is generally 256 levels in medical images. The one-dimensional gray statistical function of the image is H (l), the formula is:

$$H(l)=\sum_{m=0}^{M-1}\sum_{n=0}^{N-1}\varphi[f(m,n)-l] \quad [8]$$

The data items in the standard FCM algorithm are replaced by histogram function H (l), and the new clustering objective function formula is obtained.

$$\min J_M(U,V:L)=\sum_{i=1}^{c}\sum_{l=0}^{L-1}\mu_{il}^m H(l)\|l-v_i\|_A^2 \quad [9]$$

By iteratively optimizing the objective function J_M (U,V:L) to achieve medical image segmentation, a new fast FCM membership matrix U and clustering center matrix V are obtained, the formula is as follows:

$$\mu_{ij}=\sum_{j=1}^{c}\frac{\|l-v_i\|_A^{2/(m-1)-1}}{\|l-v_j\|_A},j=1,2,\ldots,n \quad [10]$$

$$v_i=\frac{\sum_{l=0}^{L-1}\mu_{ij}^m H(l)l}{\sum_{l=0}^{L-1}\mu_{ij}^m H(l)},i=1,2,...,c \quad [11]$$

Materials and methods

Subjects

The subjects of this study were 8 groups of human nucleus pulposus cells from patients with LDH confirmed by clinical manifestations and imaging. Eight groups of human nucleus pulposus cells were randomly divided into four groups: high inhibition group, medium inhibition group, low inhibition group and no inhibition group. After interfering with human nucleus pulposus cells by inhibiting the NF − κB pathway, the expression of p-p65, P50, IKB−α, IKK−β and other key proteins in the TNF−α and NF − κB signaling pathway were extracted and detected for 24 hours, To explore the mechanism of inhibiting NF − κB pathway on TNF−α in rats with LDH.

Experimental Process Steps

The human nucleus pulposus cell tissue was taken out and put into the culture medium. It was taken to the laboratory within 30 minutes, washed twice with 1% penicillin, put into the culture dish containing the culture medium, and cut into the size of 1 mm * 1 mm * 1 mm; Move it into the centrifuge tube at 1000R / min and centrifuge for 5min; The supernatant was removed, 0.2% trypsin (1:3) was added and digested for 30 minutes; 1000 R / min, centrifugation for 5 minutes, discard the supernatant; after most of the tissues were digested and the culture medium was slightly turbid, the 150 mesh filter was used to collect the filtrate, and the serum-containing culture medium was added to stop digestion; transfer to 15ml centrifuge tube, 1000 / min, 5min, and remove the supernatant; Add 15% FBS medium, blow evenly, transfer into culture bottle, 37 degrees, 5% CO2; the cells were observed under the inverted microscope every day, and the medium was changed every 2-3 days. After 80% fusion, the cells were subcultured.

Human nucleus pulposus cells were taken to inhibit NF − κBpathway, after 24 hours of intervention, the expression levels of TNF−α, P50, IKB−α and IKK−β in nucleus pulposus cells of each group were extracted, and the cell proteins of each group were extracted. WB was used to detect the expression changes of p-p65, P50, IKB −αand IKK−β in the NF − κB signaling pathway, and the mechanism of inhibiting NF − κBpathway on TNF−α in rats with LDH was observed.

Results and discussion

The Mechanism of Inhibiting NF − κB Pathway on TNF-α in Rats with LDH

Inhibition of NF − κB Pathway on the Expression of TNF−α, P50, IKB−α, IKK−β in Human Nucleus Pulposus Cells

In order to understand the mechanism of inhibiting the NF − κB pathway on TNF−α in rats with LDH, four groups of control experiments were used to study the inhibition of the NF − κB pathway. The expression of TNF−α, P50, IKB−α and IKK−β in human nucleus pulposus cells was extracted from all

four groups, and the data were sorted out. The results are shown in Figure 1.

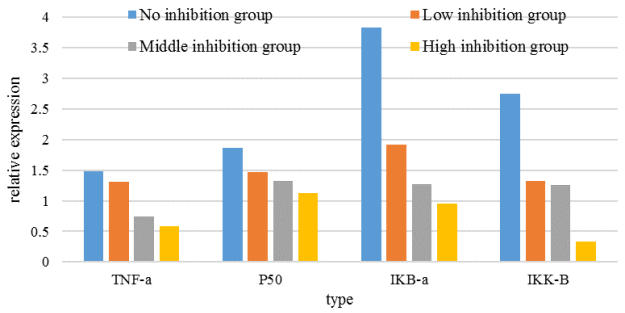

Figure 1. Comparison of TNF−α, P50, IKB−α and IKK−β expression in human nucleus pulposus cells

As can be seen from Figure 1, after 24 hours of intervention, compared with the non-inhibition group, the expression of TNF−α, P50, IKB−α, and IKK−β in the low inhibition group, medium inhibition group and high inhibition group decreased relatively, and with the increase of inhibition degree, the more obvious the decrease was, the expression of TNF−α in the non-inhibition group was 1.48, the expression of TNF−α in the low inhibition group was 1.31, the expression of TNF−α in the medium inhibition group was 0.74, and the expression of TNF−αin the high inhibition group was 0.58. The expression of P50 in the non-inhibition group was 1.86, the expression of P50 in the low inhibition group was 1.47, the expression of P50 in the medium inhibition group was 1.32, and the expression of P50in the high inhibition group was 1.13. The expression of IKB −α in the non-inhibition group was 3.83, the expression of IKB −α in the low inhibition group was 1.92, the expression of IKB −αin the medium inhibition group was 1.27, and the expression of IKB −αin the high inhibition group was 0.96. The expression of IKK−β in the non-inhibition group was 2.75, the expression of IKK−β in the low inhibition group was 1.33, the expression of IKK−βin the medium inhibition group was 1.26, and the expression of IKK−βin the high inhibition group was 0.34.

Effect of Inhibiting NF − κB Pathway on TNF−α in Rats with LDH

In order to understand the mechanism of inhibiting the NF − κB pathway on TNF−α in rats with LDH, four groups of control experiments were used to study the inhibition of the NF − κB pathway. The changes of TNF−α in rats with LDH before and after inhibition of NF − κBpathway were compared, and the data were sorted out. The results are shown in Figure 2.

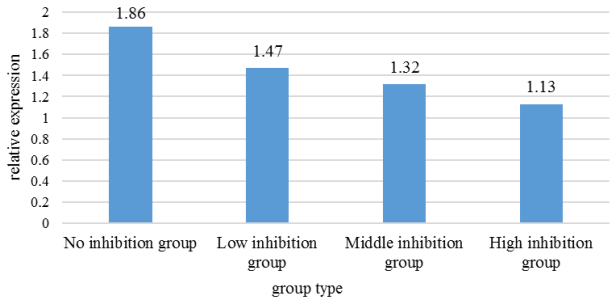

Figure 2. The relative expression of P50 in each group

As can be seen from Figure 2, after 24 hours of intervention, the TNF−α of LDH rats changed before and after inhibition of the NF − κB pathway in four groups. In the non-inhibition group, the change of TNF−α in LDH rats was the largest. The higher the degree of inhibition of the NF − κB pathway, the lower the quantitative change. The change of the non-inhibition group was 16.35, the low inhibition group was 8.44, the medium inhibition group was 6.16, high inhibition group was 3.61.

Effect of Inhibiting NF − κB Pathway on IKB−α in LDH

In order to understand the influence mechanism of inhibiting the NF − κB pathway on IKB−α in lumbar disc herniation, four groups of control experiments were used to study the inhibition of the NF − κB pathway. The changes of IKB−α in lumbar disc herniation before and after the inhibition of the NF − κB pathway in four groups were compared. The data results are shown in Figure 3.

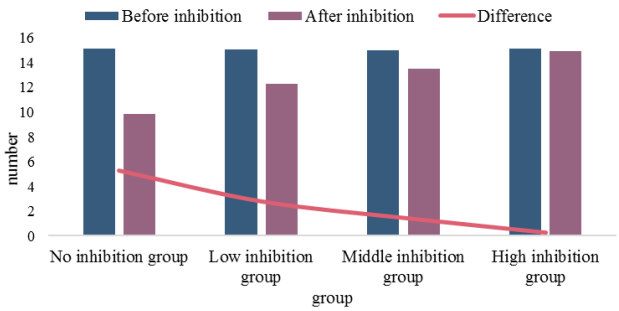

Figure 3. Comparison of changes of IKB−α

It can be seen from Figure 3 that after 24 hours of intervention, IKB−α changes in lumbar disc herniation before and after inhibition of the NF − κB pathway in four groups, andIKB−α changes in lumbar disc herniation in the non-inhibition group are the largest and the higher the degree of inhibition of the NF − κB pathway and the lower the quantitative changes have been shown. The change in the non-inhibition group was 5.28, the low inhibition group was 2.78, the medium inhibition group was 1.44, high inhibition group was 0.24.

Effect of Inhibiting NF − κB Pathway on IKK−β in LDH

In order to understand the influence mechanism of inhibiting the NF − κB pathway on IKK−β in lumbar disc herniation, four groups of control experiments were used to study the inhibition of the NF − κB pathway. The changes of IKK−β in lumbar disc herniation before and after the inhibition of the NF − κB pathway in four groups were compared. The data results are shown in Figure 4.

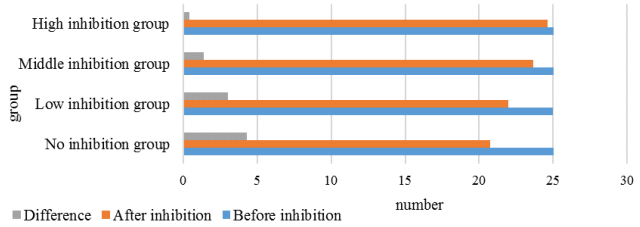

Figure 4. Comparison of changes of IKK−β

The change of the non-inhibition group was 4.29, the low inhibition group was 3.03, the medium inhibition group was 1.39, high inhibition group was 0.42.

Comparative Analysis of Relative Expression in Each Group

In order to understand the mechanism of inhibiting the NF − κB pathway on TNF −αin rats with LDH, four groups of control group experiments were used to study the inhibitory of the NF − κB pathway. The expression levels of TNF−α, P50, IKB−α and IKK−β in human nucleus pulposus cells were extracted from the four groups, and the data were sorted out as shown in Table 1.

Table 1. Comparison of relative expression levels in each group

Type	TNF−α	P50	IKB−α	IKK−β
No inhibition group	1.50±0.09	1.89±0.33	3.79±0.57	2.69±0.48
Low inhibition group	1.17±0.14	1.46±0.010	1.84±0.36	1.22±0.31
Middle inhibition group	0.66±0.06	1.17±0.20	0.90±0.10	1.15±0.32
High inhibition group	0.53±0.05	0.94±0.06	0.74±0.13	0.13±0.07

It can be seen from Table 1 that after 24 hours of intervention, compared with the non-inhibition group, the expression levels of the low inhibition group, medium inhibition group and high inhibition group were relatively decreased, and the more obvious the decrease was with the increase of inhibition degree.

Comparative Analysis of Protein Expression in Each Group

In order to understand the mechanism of inhibiting the NF − κB pathway on TNF −αin rats with LDH, four groups of control group experiments were used to study the inhibitory of the NF − κB pathway. The protein expression levels of TNF−α, IKB−α and IKK−β in human nucleus pulposus cells were extracted from four groups, and the data were sorted out as shown in Table 2.

Table 2. The protein expression of each group was compared

Type	TNF−α	IKB−α	IKK−β
No inhibition group	75.82±7.72	14.05±1.19	22.27±4.30
Low inhibition group	148.54±15.17	18.82±1.64	43.25±6.03
Middle inhibition group	135.60±14.47	17.96±1.77	40.32±5.53
High inhibition group	129.31±12.41	17.17±1.49	35.87±4.99

From Table 2, it can be seen that after 24 hours of intervention, the protein expression in the low inhibition group, medium inhibition group and high inhibition group were lower than that in the non-inhibition group, and the protein expression decreased significantly with the increase of inhibition degree.

Comparative Analysis of Protein Gray Value in Each Group

In order to understand the mechanism of inhibiting the NF − κB pathway on TNF −αin rats with LDH, four groups of control group experiments were used to study the inhibitory of the NF − κB pathway. In the four groups, p-p65, P50, IKB−α and IKK−β were extracted from human nucleus pulposus cells, and their protein gray values were studied. The results are shown in Table 3.

Table 3. Comparison of protein gray value in each group

Type	p-P65	P50	IKB−α	IKK−β
No inhibition group	0.156±0.005	0.282±0.008	0.048±0.001	0.538±0.022
Low inhibition group	0.143±0.004	0.216±0.007	0.034±0.001	0.497±0.008
Middle inhibition group	0.066±0.001	0.196±0.004	0.003±0.001	0.095±0.002
High inhibition group	0.049±0.004	0.094±0.008	0.002±0.001	0.070±0.005

It can be seen from Table 3 that after 24 hours of intervention, compared with the non-inhibition group, the gray value of protein in the low inhibition group, medium inhibition group and high inhibition group decreased relatively, and with the increase of inhibition degree, the gray value of protein in each group decreased more obviously.

Conclusions

Degenerative disc degeneration is affected by many factors and links. On the one hand, disc tissue damage is accumulating, and on the other hand, the repair function of biological tissue is also working at the same time. In order to explore the mechanism of inhibiting the NF－κB pathway on TNF−α in rats with LDH, 8 groups of experiments were conducted to study the effect of inhibiting the NF－κB pathway on the human nucleus pulposus cells. The experiment was left for 24 hours, and the expression levels of TNF−α, p-p65, P50, IKB−α, IKK−β and other key proteins in the NF－κB signaling pathway were detected, This study is of great significance for the clinical treatment of LDH.

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Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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