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Mechanism of Qianghuo in Alleviating Knee Osteoarthritis Induced by Meniscal Injury in Rats

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Abstract. Background and Aim: Knee osteoarthritis (KOA) is a degenerative joint disease marked by cartilage degradation, inflammation, and pain, leading to reduced mobility. This study investigates how Qianghuo alleviates KOA induced by meniscal injury in rats.Experimental Procedure: KOA was induced in rats via right hind limb meniscectomy. Rats were divided into Sham, KOA, and Qianghuo treatment groups (1 g/mL, 0.5 mL/d for 21 days). In vitro, IL-1 β -induced chondrocyte models were used with Qianghuo (100 mol/L for 48 hours). Behavioral observations, CCK-8 assays, RT-qPCR, and Western blot analyses were performed.Results: KOA rats showed increased VAS scores, quadriceps atrophy, and decreased joint mobility. IL-1 β reduced chondrocyte proliferation and increased apoptosis, inflammation, oxidative stress, and matrix degradation. Qianghuo treatment reversed these effects.Conclusion: Qianghuo alleviates KOA by promoting chondrocyte proliferation, inhibiting apoptosis, reducing inflammation and oxidative stress, and preventing matrix degradation, highlighting its potential in KOA treatment.

Keywords: Qianghuo, knee osteoarthritis, chondrocytes, apoptosis, inflammation, oxidative stress, extracellular matrix.

1. Introduction

Knee osteoarthritis (KOA) is a prevalent musculoskeletal disease worldwide, also known as degenerative arthritis or proliferative arthritis. The incidence of KOA reaches up to 10% in individuals over 60 years old globally and is positively correlated with age. With improved living standards and increased awareness of exercise health, the risk of knee injuries from sports has also risen. Knee injuries occur in various sports, particularly in football, with injury rates ranging from 25% to 77%. This phenomenon is closely related to the unique anatomical structure of the knee joint and its altered biomechanics during movement.

The annular tension of the meniscus plays a crucial role in buffering shocks and distributing stress, thus protecting the joint cartilage. In football, players frequently perform rapid starts, stops, and changes in speed and direction, along with jumping and collisions, making knee meniscus injuries, especially medial meniscus (MM) injuries, more likely, leading to KOA. The root of the meniscus is directly connected to the bone, which is vital for maintaining its annular tension. Football players are particularly prone to medial meniscus root tears (MMPRT), which refer to bony or soft tissue avulsions or radial tears within 1 cm of the meniscal root attachment, accounting for about 29% of medial meniscus injuries(Figure1). Clinically, KOA induced by MMPRT accounts for more than 85% of traumatic arthritis cases. Therefore, this study constructs an animal model of KOA induced by partial meniscectomy to investigate related mechanisms.

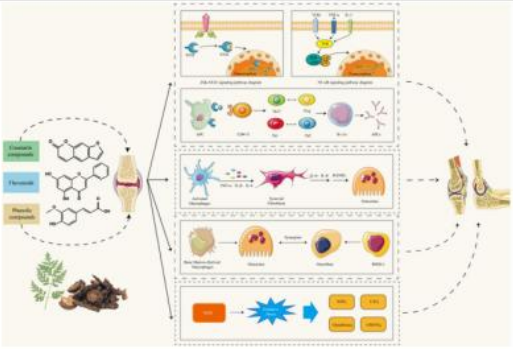

Figure 1.Schematic Diagram of the Special Anatomical Structure of the Knee Joint and Its Relationship with KOA Injury.

Figure 2.Surgical Procedure for Modeling.A. Disinfection and draping of the right knee surgical area.B. Transection of the lateral meniscus. C. Suturing of the joint capsule. D. Suturing of the skin.

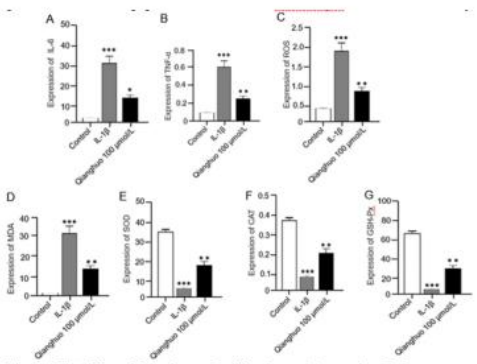

Figure 4.The Effect of Qianghuo on Proliferation and Apoptosis of Inflammatory Chondrocytes.A. CCK-8 Assay for Chondrocyte Viability. CCK-8 assay results showing the effect of different concentrations of Qianghuo on chondrocyte viability. B. Flow Cytometry Results Showing Chondrocyte Proliferation Activity.C. C. RT-qPCR Results Showing mRNA Expression of Bax, Caspase-3, and Bcl-2.D. D-G.Western Blot Results Showing Protein Expression of Bax, Caspase-3, and Bcl-2. Note: Compared with Control group: *P < 0.05, P < 0.01, *P < 0.001, ns: no significance.

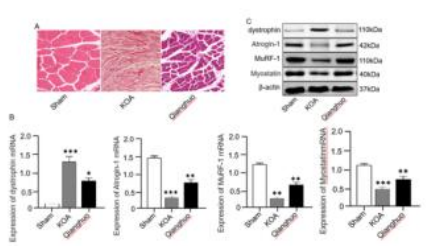

Figure 3. The Effect of Qianghuo on Muscle Atrophy in KOA Rats.A. HE Staining.B. mRNA Expression Analysis.C. Protein Expression Analysis. Note: Compared with Control group: *P < 0.05, P < 0.01, *P < 0.001, ns: no significance.

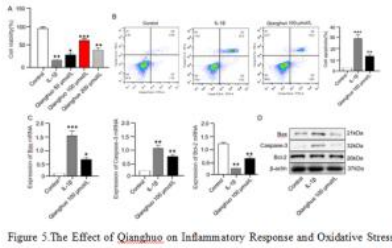

Figure 5.The Effect of Qianghuo on Inflammatory Response and Oxidative Stress in Inflammatory Chondrocytes.A, B: RT-qPCR results showing mRNA expression levels of IL-6 and TNF-α.C, D: RT-qPCR results showing mRNA expression levels of ROS and MDA.E, F, G: RT-qPCR results showing mRNA expression levels of SOD, CAT, and GSH-Px.Note: Compared with Control group: *P < 0.05, P < 0.01, *P < 0.001, ns: no significance.

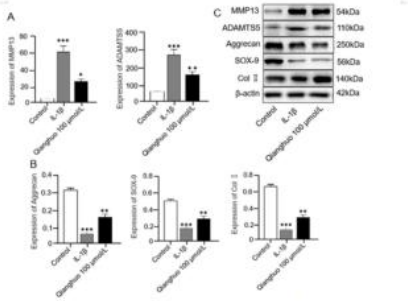

Figure6.The Effect of Qianghuo on Extracellular Matrix Degradation in IL-1β-Induced Inflammatory Chondrocytes.A. RT-qPCR results showing mRNA expression levels of MMP13 and ADAMTS5.B. RT-qPCR results showing mRNA expression levels of Aggrecan, SOX-9, and Col II.C. Western blot results showing protein expression levels of MMP13 and ADAMTS5.D. Western blot results showing protein expression levels of Aggrecan, SOX-9, and Col IINote: Compared with Control group: *P < 0.05, P < 0.01, *P < 0.001, ns: no significance.

2. Materials and methods

2.1 Experimental Materials and Reagents

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Qianghuo: Sourced from Bozhou Hongbaotang Trading Co., Ltd., Sichuan, China.

Preparation of Qianghuo Extract: Weigh 500 g of Qianghuo and soak it in 10 times the volume of 75% ethanol for 30 minutes. Extract twice using reflux at 90°C for 1 hour each time. Combine the filtrates, concentrate under reduced pressure to remove ethanol, and reconstitute with ultrapure water to a concentration of 1 g/mL of crude drug.

Reagents and Consumables: ATP content assay kit (Nanjing Jiancheng Bioengineering Institute, batch number 20230106), reverse transcription kit (TransGen Biotech, AT311), 2× Easy Taq PCR Super Mix (TransGen Biotech, AS111), nucleic acid dye (TransGen Biotech, GS101), anhydrous ethanol, chloroform, and isopropanol (analytical grade, domestic), PCR primers (Shanghai Bioengineering Co., Ltd.).

2.1.1.2 Animal Model Preparation and Drug Treatment

Experimental Animals: 30 male SPF-grade SD rats, 2 months old, weighing 225±25 g, purchased from the Animal Experiment Center of Xi'an Jiaotong University (Certificate No.: Shaanxi Medical Experimental Animal License No.17). The experiment was conducted in the Laboratory Center of the College of Physical Education, Yan'an University. Rats had free access to food and water with a 12-hour light/dark cycle. The experiment was conducted in strict accordance with the Shaanxi Provincial Experimental Animal Management Regulations.

Reagents and Consumables: Anti-MMP-3 and anti-ADAMTS-5 antibodies (Abcam, UK), β-actin antibody (Biosharp, China), HRP-conjugated goat anti-mouse/rabbit secondary antibodies (ZSGB-Bio, Beijing, China), surgical instruments (First Affiliated Hospital of Yan'an University), microscope (Olympus, Japan).

2.2 Animal Model Preparation and Drug Treatment

2.2.1 Experimental Animals

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Regulations.

2.2.2 Surgical Procedure[14,15]:

Grouping and Treatment: Successfully modeled rats were randomly divided into the model group (n=10) and the Qianghuo treatment group (n=10, Qianghuo solution 1 g/mL, 0.5 mL/d by gavage). The Sham and model groups received an equal volume of saline (0.5 mL) daily for 21 days.

2.2.3 Behavioral Observations of KOA Rats

Pain Behavior:The pain behavior of the operated limb was measured 1 day before surgery and at 1, 2, and 3 weeks post-surgery.

Pain Scoring (VAS Method):The visual analog scale (VAS) pain scores were recorded at rest and during joint movement at each observation point. Severe pain was rated as 10 points, and no pain was rated as 0 points.

Joint Swelling Evaluation:The swelling was scored based on the percentage difference between the pre- and post-injection values. Swelling score: <5% = 0 points, 515% = 1 point, 16-30% = 2 points, 31-60% = 3 points, > 60%=4points.Quadriceps

Lysholm Score[9]、Western Blot[10]、 Flow Cytometry[11] (FCM)、Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) .

Table 1.PCR Primer Sequences

Primer	Forward Sequence (5' - 3')	Reverse Sequence (5' - 3')
IL-1β	CAGTGGCCTGGTCACTGGCGA	TGGCGTTCTGGGTATTAAGATCGG
IL-6	GAGGCATCATGGGTATCCAGATCAAG	GAGCGGGGTGGAAATGTAACTTTAG
TNF-α	ATGTGGCTGACTTCGTGAG	AGGTATCTAAGGCATTGGTTCTG
MMP-13	CGG CAA TAG CTC TGT AT	CCT TGA AAC TTT GCC TCA
ADAMTS5	CAC AAG TCC GGA GAG GAG AC	CAG AAT TGC CAT TGC ACA AC
Aggrecan	AAAAGTGCTTACAGTGCAGGTAG	GGAAAAGTGCTTACAGTGCAGGT
SOX-9	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT
Col II	CCCAGGGAGGAGCAATACAG	GGGAGGACGCCATAACAACT
GAPDH	ACGGCAAGTTCAACGGCACAG	GAAGACGCCAGTAGACTCCACGAC

All experiments were conducted strictly according to the instructions provided in the reagent kits to ensure the reliability and reproducibility of the results.

Table 2.Effect of Qianghuo on VAS Scores in KOA Rats ($\bar{x}$±s, points, n=10)

Group	1 Day Before Surgery	1 Week After Surgery	2 Weeks After Surgery	3 Weeks After Surgery
Sham	0	0	0	0
KOA	1.24±0.97***	9.68±1.43***	9.60±1.19**	8.55±0.97**
Qianghuo	1.11±1.08**	6.71±0.97**	5.16±0.60**	3.12±0.44*

Note: Data are expressed as mean ± standard deviation. Statistical significance was determined by comparing the P-values between groups. Compared with Sham group: *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance.

Table 3.Effect of Qianghuo on Body Weight, Arthritis Index, and Knee Swelling in KOA Rats ($\bar{x}$±s,g, points, mm,n=10)

Group	Time	Body Weight	Arthritis Index	Knee Swelling
Sham	1 Day Before Surgery	253.31±9.25	0	0
KOA	1 Day Before Surgery	252.22±9.11	0	0
Qianghuo	1 Day Before Surgery	253.27±9.17	0	0
Sham	1 Week After Surgery	250.28±9.02	0	0
KOA	1 Week After Surgery	213.43±7.46**	3.72±0.69**	1.14±0.04**
Qianghuo	1 Week After Surgery	233.77±8.90**	2.51±0.36*	1.05±0.02*
Sham	2Week After Surgery	251.21±9.11	0	0
KOA	2Week After Surgery	222.57±8.61*	3.20±0.61**	0.99±0.04**
Qianghuo	2 Week After Surgery	238.69±8.88**	2.09±0.23**	0.86±0.03**
Sham	3 Week After Surgery	252.81±9.21	0	0
KOA	3 Week After Surgery	229.69±8.44**	3.12±0.58**	0.53±0.02**
Qianghuo	3 Week After Surgery	242.06±9.03**	1.44±0.55**	0.43±0.01**
F		*7.143*	*7.311*	*6.011*
P		*0.000*	*0.002*	*0.006*

Note: Data are expressed as mean ± standard deviation. Statistical significance was determined by comparing the P-values between groups. Compared with Sham group: *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance.

Table 4.Effect of Qianghuo on Muscle Atrophy in KOA Rats ($\bar{x}$±s,mm, n=10)

Group	1 Day Before Surgery	1 Week After Surgery	2 Weeks After Surgery	3 Weeks After Surgery
Sham	0	0	0	0
KOA	0.54±0.67^{ns}	4.70±1.26***	4.64±1.13***	4.50±0.90***
Qianghuo	0.50±0.28^{ns}	3.99±0.52***	2.02±0.47***	0.71±0.07**

Note: Data are expressed as mean ± standard deviation. Statistical significance was determined by comparing the P-values between groups. Compared with Sham group: *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance.

Table 5.Effect of Qianghuo on Knee Joint Mobility in KOA Rats ($\bar{x}$±s, points, n=10)

Group	1 Day Before Surgery	1 Week After Surgery	2 Weeks After Surgery	3 Weeks After Surgery
Sham	111.31±7.17	111.33±7.20	111.35±7.28	111.30±7.24
KOA	110.90±7.08^{ns}	84.08±6.90*	85.93±6.87**	86.34±0.90***
Qianghuo	109.99±7.33^{ns}	84.34±7.18*	92.37±7.40**	108.83±7.71***

Note: Data are expressed as mean ± standard deviation. Statistical significance was determined by comparing the P-values between groups. Compared with Sham group: *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance.

Table 6.Effect of Qianghuo on Lysholm Scores of Knee Joints in KOA Rats ($\bar{x}$±s, points, n=10)

Group	1 Day Before Surgery	1 Week After Surgery	2 Weeks After Surgery	3 Weeks After Surgery
Sham	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
KOA	100.00±0.00^{ns}	99.98.15±3.34^{ns}	29.11±3.54*	31.37±3.66**
Qianghuo	100.00±0.00^{ns}	100.11±3.94^{ns}	79.83±3.40**	87.05±4.00***

Note: Data are expressed as mean ± standard deviation. Statistical significance was determined by comparing the P-values between groups. Compared with Sham group: *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance.

2.3 Establishment and Grouping of Chondrocyte Inflammation Model

2.3.1 Isolation and Culture of Chondrocytes

Cartilage tissues obtained from the aforementioned experiments were cut into 1 mm³ pieces. These pieces were pre-digested with trypsin at 37°C for 30 minutes, followed by digestion with 0.2% collagenase for 2 hours. After filtration through a 200-mesh sieve, the suspension was centrifuged at 1,000 rpm for 5 minutes, and the supernatant was discarded. The resulting cells were washed twice with DMEM and then resuspended in DMEM containing 10% fetal bovine serum and 100 μg/L gentamicin. Cells were seeded at a density of 1×10^6 cells/mL into 50 cm² culture flasks and cultured at 37°C in a 5% CO2 incubator for primary cell culture. The medium was changed every 3 days. When cells reached 80-90% confluence, the medium was replaced with fresh DMEM, and cells were further cultured for 6 hours.

2.3.2 Establishment of Chondrocyte Inflammation Model

To establish the IL-1 β -induced chondrocyte inflammation model, different concentrations of simvastatin were added to the culture medium. After 48 hours, IL-1 β (10 ng/mL) was added, and the cells were cultured for an additional 24 hours[18].

2.3.3 Experimental Grouping

Using high-throughput drug screening (HTS), the optimal anti-inflammatory concentration of Qianghuo was determined to be 100 μmol/L for chondrocyte culture. The constructed chondrocyte inflammation model was divided into the following three groups:

2.3.4 Control Group: No drug intervention.

IL-1 β Group: IL-1 β (10 ng/mL) was added.

Qianghuo Group: 100 μmol/L Qianghuo was added, and after 48 hours, IL-1 β (10 ng/mL) was added and cultured for an additional 24 hours.

2.4 Statistical Analysis

Statistical analysis was performed using SPSS 23.0 software. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$). Comparisons of proportions between groups were conducted using the χ^2 test. A P-value of less than 0.05 was considered statistically significant.

3. Result

3.1 Effects of Qianghuo on Knee Joint Function in KOA Rats

3.1.1 Effects of Qianghuo on VAS Scores in KOA Rats

The Visual Analogue Scale (VAS) scores of rats in the KOA group showed a continuous downward trend with treatment time. One day before surgery, there was no statistically significant difference in the data between the groups ($P > 0.05$). One week after surgery, the differences between the groups were still not statistically significant ($P > 0.05$), indicating that the improvement in knee joint pain with Qianghuo was related to the treatment duration. Two weeks after surgery, the differences between the groups became statistically significant ($P < 0.05$), demonstrating that by the second week post-surgery, the therapeutic effect of the Qianghuo group was superior to that of the control and model groups ($P < 0.01$). Three weeks after surgery, the differences between the groups were even more significant ($P < 0.001$). These results suggest that the effect of Qianghuo on improving knee joint pain increases significantly over time (Table 2).

3.1.2 Qianghuo Significantly Improves Body Weight, Arthritis Index, and Knee Swelling in KOA Rats

Compared to the Sham group, the body weight of rats in the KOA group was lower at 1 week, 2 weeks, and 3 weeks post-surgery. In contrast, the body weight of rats in the Qianghuo group showed an increasing trend at 1 week, 2 weeks, and 3 weeks post-surgery compared to the KOA group. Additionally, the arthritis index and knee swelling in the KOA group were significantly higher than those in the Sham group at 1 week, 2 weeks, and 3 weeks post-surgery. However, the Qianghuo group exhibited a decreasing trend in both the arthritis index and knee swelling at 1 week, 2 weeks, and 3 weeks post-surgery compared to the KOA group (Table 3). These results indicate that Qianghuo can significantly improve body weight, arthritis index, and knee swelling in KOA rats, with these improvements becoming more pronounced over time (Table 3).

3.1.3 Qianghuo Significantly Improves Muscle Atrophy in KOA Rats

The degree of muscle atrophy in KOA rats exhibited a continuous downward trend over the treatment period, showing a negative correlation with the duration of treatment. One day before surgery, there were no statistically significant differences in the data between the groups ($P > 0.05$). One week after surgery, the differences between the groups were still not statistically significant ($P > 0.05$), indicating that the improvement in muscle atrophy with Qianghuo is related to the treatment duration. Two weeks after surgery, the differences between the groups became statistically significant ($P < 0.01$), demonstrating that by the second week post-surgery, the therapeutic effect of the Qianghuo group was superior to that of the Sham and KOA groups ($P < 0.01$). Three weeks after surgery, the differences between the groups became even more significant ($P < 0.01$). These results suggest that the effect of Qianghuo on improving muscle atrophy increases significantly over time (Table 4).

HE staining showed that the muscle fibers in KOA rats were markedly disordered, with a significant reduction in muscle fiber cross-sectional area. However, after Qianghuo intervention, the muscle fiber cross-sectional area significantly increased (Figure 3A). Western blot results indicated that the expression of dystrophin was reduced in KOA rats, while the expression of Atrogin-1, MuRF-1, and Myostatin proteins significantly increased ($P < 0.05$, Figure 3B). RT-qPCR results demonstrated that the mRNA expression of dystrophin was reduced in KOA rats, while the mRNA expression of Atrogin-1, MuRF-1, and Myostatin significantly increased ($P < 0.05$, Figure 1C).

These findings suggest that Qianghuo can significantly alleviate muscle atrophy induced by meniscectomy in rats.

3.1.4 Effect of Qianghuo on Knee Joint Mobility in KOA Rats

The knee joint mobility of KOA rats exhibited a continuous upward trend over the treatment period, showing a positive correlation with the duration of treatment. One day before surgery, there were no statistically significant differences in the data between the groups ($P > 0.05$). One week after surgery, the differences between the groups remained statistically insignificant ($P > 0.05$), indicating that the improvement in knee joint mobility with Qianghuo is related to the treatment duration. Two weeks after surgery, the differences between the groups became statistically significant ($P < 0.05$), demonstrating that by the second week post-surgery, the therapeutic effect of the Qianghuo group was superior to that of the Sham and KOA groups ($P < 0.01$). Three weeks after surgery, the differences between the groups became even more significant ($P < 0.01$). These results suggest that the effect of Qianghuo on improving knee joint mobility significantly increases over time (Table 5).

The knee joint mobility of KOA rats showed a continuous upward trend over the treatment period, with a positive correlation to treatment duration. One day before surgery, there were no statistically significant differences in the data between the groups ($P > 0.05$). One week after surgery, the differences between the groups remained statistically insignificant ($P > 0.05$), indicating that the improvement in knee joint mobility with Qianghuo is related to the treatment duration. Two weeks after surgery, the differences between the groups became statistically significant ($P < 0.05$), demonstrating that by the second week post-surgery, the therapeutic effect of the Qianghuo group on knee joint mobility was superior to that of the Sham and KOA groups ($P < 0.01$). Three weeks after surgery, the differences between the groups became even more significant ($P < 0.01$). These results suggest that the effect of Qianghuo on improving knee joint mobility significantly increases over time (Table 5).

3.1.5 Effect of Qianghuo on Lysholm Scores in KOA Rats

Following three consecutive weeks of Qianghuo administration, the Lysholm scores of KOA rats showed a continuous upward trend, positively correlated with the treatment duration. One week before surgery, there were no statistically significant differences in the data between the groups ($P > 0.05$). One week after surgery, the differences between the groups remained statistically insignificant ($P > 0.05$), indicating that the improvement in Lysholm scores with Qianghuo is related to the treatment duration. Two weeks after surgery, the differences between the groups became statistically significant ($P < 0.01$), demonstrating that by the second week post-surgery, the Lysholm scores of the Qianghuo group were superior to those of the Sham and KOA groups ($P < 0.05$). Three weeks after surgery, the differences between the groups became even more significant ($P < 0.001$). These results suggest that the effect of Qianghuo on improving Lysholm scores significantly increases over time (Table 6).

3.1.6 Effect of Qianghuo on IL-1β-Induced Proliferation and Apoptosis of Chondrocytes in Inflammatory Cartilage

CCK-8 Assay Results:The CCK-8 assay results showed that compared to the Control group, chondrocytes treated with 50 μmol/L, 100 μmol/L, and 200 μmol/L concentrations of Qianghuo in the presence of IL-1 β (10 ng/mL) exhibited varying levels of viability. Among these, the 100 μmol/L Qianghuo group showed the highest chondrocyte viability. Therefore, 100 μmol/L Qianghuo was selected for subsequent experiments ($P < 0.01$, Figure 4A).

Flow Cytometry Results:Flow cytometry results indicated that compared to the Control group, the proliferation activity of chondrocytes induced by IL-1 β was significantly reduced ($P < 0.01$, Figure 4A), while apoptosis was significantly increased ($P < 0.01$, Figure 4B).

RT-qPCR and Western Blot Results:RT-qPCR and Western blot results showed that in IL-1 β -induced inflammatory chondrocytes, the expression of Bax and Caspase-3 proteins and mRNA was

significantly elevated ($P < 0.01$), whereas the expression of Bcl-2 was significantly reduced ($P < 0.01$, Figures 4C and 4D). Treatment with Qianghuo significantly reversed these gene and protein expression changes.These results suggest that Qianghuo can promote chondrocyte proliferation, inhibit apoptosis, and ameliorate chondrocyte inflammation, thereby alleviating KOA.

3.1.7 Effect of Qianghuo on Inflammatory Response and Oxidative Stress in IL-1β-Induced Inflammatory Chondrocytes

RT-qPCR Results:RT-qPCR results showed that compared to the Control group, the expression of inflammation-related factors IL-6 and TNF-α was significantly increased in IL-1β (10 ng/mL)-induced inflammatory chondrocytes ($P < 0.001$, Figures 5A and 5B). Simultaneously, the expression of oxidative stress-related factors ROS and MDA was significantly elevated ($P < 0.001$, Figures 5C and 5D), while the expression of antioxidant enzymes SOD, CAT, and GSH-Px was significantly reduced ($P < 0.001$, Figures 5E, 5F, and 5G). These effects were significantly reversed by Qianghuo treatment.

These results suggest that Qianghuo can inhibit the inflammatory response and oxidative stress induced by IL-1β in inflammatory chondrocytes, thereby alleviating the inflammatory response and oxidative stress damage in chondrocytes and mitigating the pathological changes of KOA.

3.1.8 Effect of Qianghuo on Extracellular Matrix Degradation in IL-1β-Induced Inflammatory Chondrocytes

Compared to the Control group, IL-1β (10 ng/mL)-induced inflammatory chondrocytes exhibited significantly higher expression levels of extracellular matrix degradation enzymes MMP13 mRNA and ADAMTS5 mRNA ($P < 0.01$, Figure 6A), while the expression levels of Aggrecan mRNA, SOX-9 mRNA, and type II collagen (Col -II mRNA) were significantly lower ($P < 0.01$, Figure 6B). Additionally, the protein levels of MMP13 and ADAMTS5 were significantly increased in IL-1β -induced inflammatory chondrocytes ($P < 0.01$, Figure 6C), whereas the protein levels of Aggrecan, SOX-9, and Col -II were significantly decreased ($P < 0.01$, Figure 6D). These changes were significantly reversed by Qianghuo treatment.

These results suggest that Qianghuo can inhibit the expression of extracellular matrix degradation enzymes in IL-1β -induced inflammatory chondrocytes, reduce cellular degradation, and promote the synthesis of cartilage matrix components, thereby mitigating the pathological changes of KOA.

4. Discussion

Osteoarthritis (OA) is one of the most common clinical diseases with complex and diverse etiologies. Trauma is a common cause of knee joint injury and KOA in soccer players [18]. With improvements in living standards and changes in lifestyle, people are increasingly engaging in exercise to shape their bodies and alleviate chronic diseases. However, the incidence of sports injuries is also on the rise. Clinically, KOA manifests as joint swelling, pain, deformity, and impaired mobility, characterized by high incidence and high disability rates.

Chondrocytes are the only cells in articular cartilage, responsible for the synthesis and degradation of the cartilage matrix, maintaining the dynamic balance of cartilage. Matrix metalloproteinases (MMPs) play a crucial role in cartilage metabolism and development [19,20]. Studies have shown that the traditional Chinese medicine Qianghuo can upregulate cartilage-related genes, promote cartilage formation, inhibit cartilage degeneration, and maintain the dynamic balance of cartilage matrix degradation and synthesis in the knee joint[21].

Numerous studies have demonstrated that the occurrence, development, and prognosis of KOA are closely related to abnormal proliferation and apoptosis of chondrocytes [22]. Therefore, interventions that promote chondrocyte proliferation and inhibit chondrocyte apoptosis represent new targets and strategies for treating KOA. Qianghuo, a perennial herb, is affordable and abundant.

Research has found that Qianghuo can treat ankylosing spondylitis of the wind-cold-damp type, improve synovitis in rats by inhibiting the expression of inflammatory factors and promoting chondrocyte proliferation, and has anti-inflammatory effects and can prevent neuropathic pain [23]. Additionally, Qianghuo inhibits tumor cell proliferation, migration, and invasion, and induces tumor cell apoptosis.

However, there are few reports on Qianghuo's ability to alleviate KOA by inhibiting inflammation, oxidative stress, and extracellular matrix degradation. Our study results show that Qianghuo treatment significantly increases chondrocyte proliferation, with Bcl-2 levels increasing over time, while the proteins and mRNA of Bax, Caspase-3, and Caspase9 are significantly upregulated. This indicates that Qianghuo can promote chondrocyte proliferation and inhibit apoptosis, suggesting that Qianghuo may be a potential drug for treating KOA.

KOA is a degenerative joint disease characterized by cartilage degeneration, destruction, and bone proliferation due to chronic inflammation. The significant high expression of inflammatory factors IL-1 β , IL-6, and TNF- α in cartilage leads to cartilage degeneration [24]. Studies have confirmed that elevated levels of inflammatory factors such as TNF- α and IL-1 β play an important role in the occurrence and development of KOA [25]. From the neuroanatomical perspective of the knee joint, IL-1 β -induces the expression of other inflammatory factors, aggravates the inflammatory response, and stimulates peripheral nerves around the knee joint, lowering the pain threshold and causing pain [26]. Interleukin-6 (IL-6) is an important member of the interleukin family, involved in inflammatory response and anti-infective functions. Reports indicate that elevated serum IL-6 levels in patients with severe infections can be used as a reference for early diagnosis and efficacy evaluation [27]. TNF- α is a key factor in activating the cytokine system and plays a crucial role in the inflammatory response. When tissue damage occurs, TNF- α is rapidly highly expressed in the body, triggering a cascade effect and leading to an excessive inflammatory response [28]. Our study results show that Qianghuo intervention significantly reduces the expression of IL-1 β 、 IL-6, and TNF- α , suggesting that Qianghuo can alleviate KOA. Future studies will track the therapeutic basis of Qianghuo for KOA using transgenic mice and network pharmacology databases, identifying novel traditional Chinese medicines and active components with clear targets for KOA treatment, providing experimental evidence for the clinical application of Qianghuo extracts in treating KOA, and offering effective treatment methods for sports coaches and soccer players.

KOA is a complex autoimmune disease with oxidative stress and inflammatory response as two major injury mechanisms. Free radicals produced by oxidative stress act as both oxidants and inflammatory mediators in the pathological process of KOA [29]. Oxidative damage can induce the production of inflammatory factors such as IL-6, TNF- α , and IL-1 β in KOA patients, while the increased free radicals lead to an imbalance between oxidation and antioxidation, weakening the body's antioxidant capacity and further exacerbating the inflammatory response. Important antioxidant enzymes in the body that remove oxygen free radicals include SOD, CAT, and GSHPx, which protect chondrocytes from damage by eliminating oxygen free radicals [30,31]. MDA, a peroxidation product formed by the attack of oxygen free radicals on unsaturated fatty acids in biological membranes, reflects the degree of lipid peroxidation and cell damage in the body [32]. The study results show that Qianghuo can significantly reduce the expression of ROS and MDA while increasing the expression of SOD, CAT, and GSH-Px, indicating that Qianghuo can inhibit oxidative stress in chondrocytes and protect chondrocytes.

Matrix metalloproteinases (MMPs) and aggrecanases are enzymes that catalyze the hydrolysis of polypeptides or proteins, degrading the cartilage matrix, promoting cartilage degeneration, reducing joint stability, and inducing KOA [33]. MMP-13, which degrades cartilage matrix, is highly expressed in the cartilage, synovium, synovial fluid, and peripheral blood of KOA patients [34]. The ADAMTS family, which contains a thrombospondin type 1 motif, also plays an important role in the pathogenesis of KOA. Aggrecan, SOX-9, and type II collagen (Col-II) are major components

of the cartilage matrix, involved in the synthesis and degradation of the cartilage matrix, maintaining the cellular framework, and reflecting the pathological changes and metabolic state of joint cartilage tissue [35,36]. Our study results show that Qianghuo can significantly reduce the expression of MMP-13 and ADAMTS5 while increasing the expression of Aggrecan, SOX-9, and Col -II, suggesting that Qianghuo can maintain the normal physical and physiological functions of joints.

In conclusion, this study suggests that Qianghuo can protect cartilage and alleviate KOA by mitigating the inflammatory response, inhibiting oxidative stress, and reducing the expression of extracellular matrix degradation enzymes. Future research will utilize transgenic mice and network pharmacology databases to trace the therapeutic basis of Qianghuo for KOA, ultimately identifying innovative traditional Chinese medicines and active components with clear targets for KOA treatment, providing experimental evidence for the clinical application of Qianghuo extracts in treating KOA, and offering effective treatment methods for sports coaches and soccer players.

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