Effects of Yangxue Roujin Formula Combined with Aerobic Exercise on Inflammation and Cartilage Metabolism Through Regulation of the SDF-1/CXCR4 Pathway in a Rabbit Model of Knee Osteoarthritis

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Date: 2024-01-11T00:00:00+00:00

Abstract

Objective To investigate the effects of Yangxue Roujin Formula combined with aerobic exercise on inflammation and cartilage metabolism in a rabbit knee osteoarthritis (KOA) model through regulation of the stromal cell-derived factor 1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) pathway. Methods KOA models were established in New Zealand rabbits via intra-articular injection of papain, and randomly divided into model group, Yangxue Roujin Formula group, aerobic exercise group, Yangxue Roujin Formula + aerobic exercise group, and celecoxib group (10 rabbits each). An additional 10 New Zealand rabbits served as the control group. After grouped interventions, toluidine blue (TB) staining was used to examine pathological morphology of knee joint cartilage tissue and conduct Mankin scoring; scanning electron microscopy was used to observe knee joint cartilage morphology; immunohistochemical staining was used to detect matrix metalloproteinase 13 (MMP13) and type II collagen (Col-II) expression in knee joint cartilage tissue; enzyme-linked immunosorbent assay (ELISA) was used to measure levels of inflammatory factors interleukin (IL)-17, tumor necrosis factor α (TNF-α), IL-6, and IL-18 in serum and knee joint fluid; Western blotting was used to detect SDF-1/CXCR4 pathway-related protein expression in knee joint cartilage tissue. Results Compared with the control group, the model group exhibited significant knee joint cartilage damage, markedly increased Mankin score and MMP13 positive expression in cartilage tissue, elevated levels of IL-17, TNF-α, IL-6 and IL-18 in serum and knee joint fluid, and upregulated SDF-1 and CXCR4 protein expression in cartilage tissue ($P<0.05$), while Col-II positive expression was significantly decreased ($P<0.05$). Compared with the model group, all treatment groups (Yangxue Roujin Formula, aerobic exercise, Yangxue Roujin Formula + aerobic exercise, and cele-

coxib) showed alleviated knee joint cartilage damage, reduced Mankin score and MMP13 positive expression, decreased levels of IL-17, TNF-α, IL-6 and IL-18, downregulated SDF-1 and CXCR4 protein expression ($P<0.05$), and increased Col-II positive expression ($P<0.05$). Compared with the Yangxue Roujin Formula group and aerobic exercise group, the combination group and celecoxib group demonstrated further alleviated cartilage damage, lower Mankin score and MMP13 expression, reduced inflammatory cytokines, decreased SDF-1/CXCR4 expression ($P<0.05$), and increased Col-II expression ($P<0.05$). Conclusion Yangxue Roujin Formula and aerobic exercise can downregulate SDF-1/CXCR4 pathway protein expression, inhibit inflammatory factor expression and release, thereby alleviating knee joint inflammation in KOA rabbits, improving knee joint cartilage metabolism, mitigating cartilage collagen degradation and tissue degeneration, with synergistic effects between the two interventions.

Full Text

Effects of Yangxue Roujin Formula Combined with Aerobic Exercise on Inflammation and Cartilage Metabolism in a Rabbit Knee Osteoarthritis Model by Regulating the SDF-1/CXCR4 Pathway

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Abstract

Objective To investigate the effects of Yangxue Roujin Formula combined with aerobic exercise on inflammation and cartilage metabolism in a rabbit knee osteoarthritis (KOA) model by regulating the stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) pathway.

Methods New Zealand rabbits were used to establish a KOA model by intra-articular injection of papain. The rabbits were randomly divided into model group, Yangxue Roujin Formula group, aerobic exercise group, Yangxue Roujin Formula + aerobic exercise group, and celecoxib group (10 rabbits each). Another 10 New Zealand rabbits served as the control group. After grouping and intervention, toluidine blue (TB) staining was used to detect pathological

morphology of knee joint cartilage tissue and perform Mankin scoring; scanning electron microscopy was used to examine cartilage morphology; immunohistochemical staining was used to detect matrix metalloproteinase 13 (MMP13) and collagen type II (Col-II) expression; enzyme-linked immunosorbent assay (ELISA) was used to measure inflammatory factors interleukin-17 (IL-17), tumor necrosis factor-α (TNF-α), IL-6, and IL-18 levels in serum and knee joint fluid; and Western blotting was used to detect SDF-1/CXCR4 pathway-related protein expression in knee cartilage tissue.

Results Compared with the control group, the model group exhibited obvious cartilage damage with significantly increased Mankin scores and MMP13 positive expression in cartilage tissue, elevated serum and synovial fluid levels of IL-17, TNF-α, IL-6, and IL-18, and upregulated SDF-1 and CXCR4 protein expression ($P<0.05$), while Col-II positive expression was significantly decreased ($P<0.05$). Compared with the model group, all treatment groups showed attenuated cartilage damage with reduced Mankin scores, decreased MMP13 expression, lower inflammatory factor levels, and downregulated SDF-1 and CXCR4 expression ($P<0.05$), along with increased Col-II expression ($P<0.05$). The combination therapy and celecoxib groups demonstrated superior effects compared with the Yangxue Roujin Formula and aerobic exercise groups alone ($P<0.05$).

Conclusion Yangxue Roujin Formula and aerobic exercise can downregulate SDF-1/CXCR4 pathway protein expression, inhibit inflammatory factor release, reduce knee joint inflammation, improve cartilage metabolism, and alleviate collagen degradation and tissue degeneration in KOA rabbits, with synergistic effects observed between the two interventions.

Key words: Yangxue Roujin Formula; aerobic exercise; SDF-1/CXCR4; knee osteoarthritis; inflammation; cartilage metabolism

Introduction

Knee osteoarthritis (KOA) is a degenerative joint disease characterized by cartilage degeneration, damage, and deterioration, which can lead to knee pain and limited mobility, significantly impairing patients' motor function and quality of life [1-2]. Imbalance between extracellular matrix synthesis and degradation in chondrocytes, resulting from disturbed cartilage metabolism, represents a crucial factor in cartilage degeneration and injury in KOA patients. Excessive production of inflammatory cytokines triggers inflammatory cascades that constitute the primary cause of these pathological alterations, making anti-inflammatory therapy a common approach for improving KOA clinical symptoms [3-4].

Stromal cell-derived factor-1 (SDF-1) can bind to CXC chemokine receptor 4 (CXCR4), inducing excessive generation of inflammatory factors and participating in the pathogenesis of inflammatory diseases such as synovitis and rheuma-

toid arthritis. SDF-1 expression is significantly elevated in the synovium of rats with synovitis [5], and CXCR4 is highly expressed in the serum and synovial fluid of rheumatoid arthritis patients, positively correlating with their clinical symptom scores [6]. Inhibition of SDF-1/CXCR4 signaling can attenuate matrix metalloproteinase 13 (MMP13) expression and promote collagen type II (Col-II) expression, thereby alleviating cartilage degradation and degenerative injury in temporomandibular joint osteoarthritis [7]. These findings suggest that SDF-1/CXCR4 represents a potential therapeutic target for KOA prevention and treatment.

According to Traditional Chinese Medicine (TCM) theory, blood stasis obstruction and liver-kidney deficiency constitute the fundamental pathogenesis of KOA. Based on the "supple sinews and aligned bones" theory, tonifying kidney and activating blood circulation can effectively improve blood supply around the knee joint, inhibit degradation of cartilage extracellular matrix, promote chondrocyte survival, proliferation, and differentiation, and consequently alleviate clinical symptoms in KOA patients [8]. Yangxue Roujin Formula is a clinically proven effective prescription from Henan Provincial Hospital of Traditional Chinese Medicine for treating bone and joint diseases. It can tonify qi and blood, nourish sinews and bones, and supplement liver-kidney while unblocking collaterals, demonstrating favorable therapeutic efficacy in KOA clinical treatment [9]. Exercise represents one of the core therapies for KOA, encompassing various modalities with clinical applications [10]. Among these, aerobic exercise can effectively reduce chondrocyte apoptosis and injury in osteoarthritic rabbits [11]. However, the therapeutic effects and mechanisms of Yangxue Roujin Formula combined with aerobic exercise on KOA have not been clearly elucidated. This study established a rabbit KOA model to investigate the effects of Yangxue Roujin Formula combined with aerobic exercise on inflammation and cartilage metabolism through the SDF-1/CXCR4 signaling pathway.

Materials and Methods

Experimental Animals New Zealand white rabbits (half male, half female), approximately 3 months old and weighing around 2 kg, SPF grade, were purchased from Beijing Keyu Animal Breeding Center (license number: SCXK (Beijing) 2018-0010). The animals were housed in metal cages under controlled temperature (23$±2)℃*andhumidity*(60±$5)% with standard diet.

Main Reagents and Instruments Papain (potency 800,000 u/g, batch number 716M022) was purchased from Beijing Solarbio Science & Technology Co., Ltd. Yangxue Roujin Formula (composed of: Codonopsis 12 g, raw Atractylodes 10 g, raw Coix seed 30 g, Achyranthes 25 g, prepared Rehmannia 20 g, Angelica 12 g, stir-fried Paeonia 12 g, Moutan 12 g, Artemisia anomala 15 g, Cuscuta 9 g, Salvia 15 g, Angelica pubescens 12 g, Saposhnikovia 10 g, and Chaenomeles 10 g) was prepared from medicinal decoction pieces purchased from Anhui

Jiancheng Traditional Chinese Medicine Decoction Pieces Co., Ltd. Celecoxib (content 99.5%, batch number 140721) was purchased from Jiangsu Chia Tai Tianqing Pharmaceutical Co., Ltd. Toluidine blue (TB) staining kit was purchased from Wuxi Puhe Biological Medicine Technology Co., Ltd. Immunohistochemical staining kit was purchased from Shanghai Zeye Biotechnology Co., Ltd. Mouse anti-rabbit primary antibodies Anti-MMP13, Anti-GAPDH, Anti-SDF-1, Anti-Col-II, and Anti-CXCR4 were purchased from Wuhan USCN Business Co., Ltd. Donkey anti-mouse secondary antibody (HRP-labeled), rabbit interleukin (IL)-17, tumor necrosis factor-α (TNF-α), IL-6, and IL-18 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Abcam, UK.

Animal experimental treadmill (model DB030) was from Beijing Zhi Shu Duobao Biological Technology Co., Ltd. Paraffin microtome (model RM2126) was from Leica, Germany. Biological optical microscope (model LB102) was from Guangzhou Light Optoelectronics Technology Co., Ltd. Scanning electron microscope (model SIGMA300) was from ZEISS, Germany. Microplate reader (model ELx800) was from BioTek, USA. Vertical electrophoresis tank (model DYCZ-24EN), universal timing dual-stable power supply (model DYCZ-24KF), and transfer electrophoresis apparatus (model DYCZ-40K) were from Beijing Liuyi Instrument Factory.

KOA Model Establishment and Group Intervention The KOA model was established by reference to the method of intra-articular papain injection [9]. New Zealand white rabbits were anesthetized by slow intravenous injection of 25 mg/kg pentobarbital sodium (0.6% w/v) through the ear marginal vein, followed by injection of 0.5 mL 3% papain solution into the knee joint cavity. A second injection was administered 3 days later (i.e., every 2 days), with a total of 3 injections. Three days after the third injection, knee joint swelling, redness, and lameness were observed, indicating successful KOA modeling. The rabbits were randomly divided into model group, Yangxue Roujin Formula group, aerobic exercise group, Yangxue Roujin Formula + aerobic exercise group, and celecoxib group (10 rabbits each). Another 10 New Zealand rabbits served as the control group.

One dose of Yangxue Roujin Formula consisted of: Codonopsis 12 g, raw Atractylodes 10 g, raw Coix seed 30 g, Achyranthes 25 g, prepared Rehmannia 20 g, Angelica 12 g, stir-fried Paeonia 12 g, Cuscuta 9 g, Artemisia anomala 15 g, Salvia 15 g, Moutan 12 g, Angelica pubescens 12 g, Saposhnikovia 10 g, and Chaenomeles 10 g. According to the preparation method of Henan Provincial Hospital of Traditional Chinese Medicine pharmacy, the formula was boiled in water and filtered to obtain a decoction with a concentration of 5 mg/mL. Celecoxib was dissolved in normal saline to prepare a 2.4 mg/mL solution. Aerobic exercise treatment consisted of placing rabbits on an animal experimental treadmill at a speed of 1.5 km/h for 20 min/session [11].

After successful KOA modeling, group intervention treatments were administered: Yangxue Roujin Formula group received intragastric administration of

10 mL/kg Yangxue Roujin Formula decoction (equivalent to 50 mg/kg) [9]; aerobic exercise group underwent aerobic exercise treatment as described above plus intragastric administration of 10 mL/kg normal saline; Yangxue Roujin Formula + aerobic exercise group received intragastric administration of 10 mL/kg Yangxue Roujin Formula decoction (50 mg/kg) combined with aerobic exercise treatment; celecoxib group received intragastric administration of 10 mL/kg celecoxib solution (24 mg/kg) [9]; control and model groups received intragastric administration of 10 mL/kg normal saline. All groups were treated once daily by gavage, with exercise performed 5 times per week for 4 weeks.

Sample Collection Twenty-four hours after the 4-week treatment, rabbits in each group were anesthetized using the same method as for model establishment. Blood was collected from the ear marginal vein and centrifuged at 2000 r/min for 10 min at 4℃ to obtain serum, which was stored at -20℃. The affected knee joint cavity was injected with 1.0 mL normal saline, and the knee was flexed and extended repeatedly to ensure full activity before aspirating joint fluid. The fluid was centrifuged at 2000 r/min for 10 min at 4℃, and the supernatant was stored at -20℃. Rabbits were euthanized by injecting 20 mL air through the ear marginal vein. The affected knee joint was opened with a scalpel to harvest medial tibial cartilage. Approximately 0.4 g cartilage tissue was added to RIPA buffer, homogenized at high speed to extract total protein, and protein concentration was measured by BCA method. The protein samples were stored at -20℃. Remaining cartilage tissue was cut into approximately 1 cm × 0.5 cm × 1 cm blocks, fixed in 10% neutral buffered formalin, progressively dehydrated in 75%, 80%, 95%, and 100% ethanol, cleared in xylene, and embedded in paraffin (65℃). After paraffin solidification, conventional pathological sections were prepared. Additional cartilage tissue was trimmed and rinsed in sodium dimethylarsenate buffer, fixed in 1% osmium tetroxide, rinsed again, dehydrated progressively as above, and stored overnight in isoamyl acetate.

Detection Methods Toluidine Blue Staining and Mankin Scoring. Paraffin sections of rabbit knee joint cartilage were dewaxed in xylene and rehydrated through graded ethanol solutions (100%, 95%, 80%, 75%). TB staining solution was applied, and after staining, sections were observed and imaged under a biological optical microscope. Mankin scoring was performed according to the method of Mankin et al. [12] to evaluate cartilage defects, with scores ranging from 0–14, where higher scores indicate more severe cartilage damage.

Scanning Electron Microscopy. Cartilage tissue samples stored overnight in isoamyl acetate were critical-point dried with carbon dioxide, mounted on SEM specimen stubs, sputter-coated with gold under vacuum, and observed for cartilage morphology.

Immunohistochemical Staining for MMP13 and Col-II Expression. Paraffin sections of rabbit knee joint cartilage were dewaxed, rehydrated, subjected to antigen heat retrieval, and blocked. Sections were incubated with

mouse anti-rabbit Anti-MMP13 and Anti-Col-II primary antibodies (3 sections per antibody), followed by immunohistochemical staining according to kit instructions. After staining, sections were observed and imaged under a biological optical microscope. Image-Pro Plus 6.0 software was used to quantitatively calculate the mean integrated absorbance of MMP13 and Col-II positive cells in the field of view as an evaluation standard, with higher values indicating greater positive expression.

ELISA Detection of Inflammatory Factors. Serum and knee joint fluid samples from each group were thawed at 4℃. For each group, 420 µL serum and 420 µL joint fluid were used to measure IL-17, TNF-α, IL-6, and IL-18 levels using ELISA kits according to manufacturer instructions.

Western Blot Detection of SDF-1/CXCR4 Pathway Proteins. Cartilage tissue protein samples were mixed with equal volume loading buffer and heated in a 100℃ water bath for protein denaturation. For each group, 15 µg total protein was loaded per well and electrophoresed at 120 V constant voltage for 90 min. The lower separating gel was transferred onto membranes at 40 mA constant current using wet transfer. Membranes were blocked and cut to isolate GAPDH, SDF-1, and CXCR4 protein bands. After primary and secondary antibody incubation and development, images were captured and protein gray values were quantified using Image-Pro Plus software to calculate relative expression (normalized to GAPDH).

Statistical Analysis Data were analyzed using SPSS 26.0 software and presented as mean ± standard deviation ($\bar{x} \pm s$). Inter-group and pairwise comparisons were performed using one-way ANOVA followed by SNK-q test. $P < 0.05$ was considered statistically significant.

Results

Effects on Histopathological Injury of Rabbit Knee Cartilage In the control group, rabbit knee cartilage tissue exhibited normal morphology with intact structure and orderly cell arrangement. The model group showed significant pathological damage: thinned cartilage layer with defects, uneven and faint staining, fissures extending to the calcified layer, damaged chondrocyte structure, and disordered arrangement. The Mankin score was significantly elevated in the model group compared with the control group ($P<0.05$). Compared with the model group, all treatment groups exhibited attenuated pathological damage and reduced Mankin scores ($P<0.05$). The combination therapy and celecoxib groups showed further attenuated damage and lower Mankin scores compared with the Yangxue Roujin Formula and aerobic exercise groups ($P<0.05$). See Figure 1 [Figure 1: see original paper] and Table 1 .

Figure 1. TB staining to detect the histopathological morphology of rabbit knee joint cartilage in each group ($×$200)

Table 1. Mankin score of cartilage tissue of rabbit knee joints in each group (n=10, $\bar{x} \pm s$)

Group	Mankin Score (points)
Control	0.00$±0.00||*Model*|11.30±1.45*a*||*YangxueRoujinFormula*|6.40±0.81*b*||*AerobicExercise*| *AerobicExercise*|3.50±0.74*bcd*||*Celecoxib*|3.80±$0.65bcd

Note: aP<0.05 vs. control group; bP<0.05 vs. model group; cP<0.05 vs. Yangxue Roujin Formula group; dP<0.05 vs. aerobic exercise group.

Effects on Cartilage Morphology Control group cartilage showed normal intact structure with smooth surface. The model group displayed morphological damage: rough articular surface with crystal deposits, cartilage degeneration, necrosis, structural abnormalities, and substantial exudate attachment on the joint cavity surface. All treatment groups showed alleviated morphological damage compared with the model group, with the combination and celecoxib groups showing greater improvement than the single intervention groups. See Figure 2 [Figure 2: see original paper].

Figure 2. Scanning electron microscopy to detect the morphology of cartilage in the knee joint of rabbits in each group ($×$200)

Effects on MMP13 and Col-II Expression Compared with the control group, MMP13 positive expression was significantly increased while Col-II positive expression was significantly decreased in the model group (P<0.05). All treatment groups showed reduced MMP13 and elevated Col-II expression compared with the model group (P<0.05). The combination and celecoxib groups exhibited further reductions in MMP13 and increases in Col-II expression compared with the single intervention groups (P<0.05). See Figure 3 [Figure 3: see original paper] and Table 2 .

Figure 3. Detection of MMP13 and Col-II expression in rabbit knee cartilage tissues by immunohistochemical staining in each group ($×$200)

Table 2. Positive expression of MMP13 and Col-II in the cartilage tissues of rabbit knee joints in each group (expressed as mean absorbance, n=10, $\bar{x} \pm s$)

Group	MMP13 Mean Absorbance	Col-II Mean Absorbance
Control	0.12$±0.03|0.62±0.08||*Model*|0.47±0.09*a*|0.06±0.02*a*||*YangxueRoujinFormula*|0.21±0.06*b*|0.39± *AerobicExercise*|0.15±0.04*bcd*|0.56±0.07*bcd*||*Celecoxib*|0.18±0.05*bcd*|0.54±$0.09bcd	

Note: aP<0.05 vs. control group; bP<0.05 vs. model group; cP<0.05 vs. Yangxue Roujin Formula group; dP<0.05 vs. aerobic exercise group.

Effects on Serum Inflammatory Factor Levels Serum IL-17, TNF-α, IL-6, and IL-18 levels were significantly elevated in the model group compared with the control group (P<0.05). All treatment groups showed reduced levels of these inflammatory factors compared with the model group (P<0.05), with the combination and celecoxib groups showing further reductions compared with the single intervention groups (P<0.05). See Table 3 .

Table 3. Serum levels of inflammatory factors IL-17, TNF-α, IL-6, and IL-18 in rabbits of each group (n=10, $\bar{x} \pm s$)

Group	IL-17 (ng/L)	TNF-α (ng/L)	IL-6 (ng/L)	IL-18 (ng/L)
Control	82.39$±14.76	41.65±11.51	50.13±12.25	113.64±20.87
Model	301.25±23.03*a*	230.42±18.13*a*	212.61±	
AerobicExercise	90.54±15.72*bcd*	47.12±11.74*bcd*	57.60±13.74*bcd*	121.54±24.06*bcd*
Celecoxib	98.9			

Note: aP<0.05 vs. control group; bP<0.05 vs. model group; cP<0.05 vs. Yangxue Roujin Formula group; dP<0.05 vs. aerobic exercise group.

Effects on Synovial Fluid Inflammatory Factor Levels Similar to serum results, synovial fluid IL-17, TNF-α, IL-6, and IL-18 levels were significantly increased in the model group (P<0.05) and reduced by all treatments (P<0.05), with the combination and celecoxib groups showing superior effects compared with single interventions (P<0.05). See Table 4 .

Table 4. Levels of inflammatory factors IL-17, TNF-α, IL-6, and IL-18 in rabbit knee joint fluid of each group (n=10, $\bar{x} \pm s$)

Group	IL-17 (ng/L)	TNF-α (ng/L)	IL-6 (ng/L)	IL-18 (ng/L)
Control	76.42$±15.05	36.69±9.93	44.35±10.57	96.83±17.86
Model	289.31±24.12*a*	214.47±16.85*a*	197.13±1	
AerobicExercise	83.23±16.15*bcd*	40.92±10.74*bcd*	51.68±11.62*bcd*	102.37±21.23*bcd*
Celecoxib	89.5			

Note: aP<0.05 vs. control group; bP<0.05 vs. model group; cP<0.05 vs. Yangxue Roujin Formula group; dP<0.05 vs. aerobic exercise group.

Effects on SDF-1/CXCR4 Pathway Protein Expression SDF-1 and CXCR4 protein expression in knee cartilage tissue was significantly elevated in the model group compared with the control group (P<0.05). All treatment groups showed reduced expression of both proteins compared with the model group (P<0.05), with the combination and celecoxib groups exhibiting more pronounced reductions compared with the single intervention groups (P<0.05). See Figure 4 [Figure 4: see original paper] and Table 5 .

Figure 4. Expression of proteins related to SDF-1/CXCR4 signaling pathway in rabbit knee cartilage tissues of various groups detected by immunoblotting

Table 5. Relative expression of SDF-1/CXCR4-related proteins in the cartilage tissues of rabbit knee joints of each group (n=10, $\bar{x} \pm s$)

Group	SDF-1/GAPDH	CXCR4/GAPDH
Control	0.21$±0.05|0.17±0.04||*Model*|0.99±0.11*a*|0.94±0.10*a*||*YangxueRoujinFormula*|0.59±0.08*b*|	
	AerobicExercise|0.23±0.06*bcd*|0.19±0.05*bcd*||*Celecoxib*|0.25±0.07*bcd*|0.21±$0.06bcd	

Note: aP<0.05 vs. control group; bP<0.05 vs. model group; cP<0.05 vs. Yangxue Roujin Formula group; dP<0.05 vs. aerobic exercise group.

Discussion

KOA is a major cause of disability in middle-aged and elderly populations. With the deepening of population aging in China, the incidence of KOA has increased annually, becoming an urgent public health problem. Current clinical treatments primarily involve surgery and medication; however, surgery is costly and may result in sequelae, while drug therapy can cause gastrointestinal adverse reactions and drug dependence. Therefore, there is a need to explore simpler, more effective, and safer therapeutic approaches [13-14]. In this study, we successfully established a rabbit KOA model, as evidenced by pathological damage including thinned cartilage layer with defects, uneven staining, damaged chondrocyte structure, disordered arrangement, rough cartilage surface with crystal deposits, degeneration, necrosis, structural abnormalities, joint cavity exudate attachment, lameness, and knee swelling.

According to TCM theory, KOA primarily involves the sinews, with the pathological characteristics of bone-sinew imbalance, blood deficiency, and blood stasis. Liver-kidney deficiency and insufficient essence-blood lead to persistent blood stasis and blocked collaterals. Treatment should focus on tonifying liver-kidney, nourishing blood, and activating blood circulation to maintain supple sinews and aligned bones [8,15]. In Yangxue Roujin Formula, prepared Rehmannia, Angelica, stir-fried Paeonia, Cuscuta, and Achyranthes serve as sovereign drugs to soften liver, tonify kidney, replenish essence, and nourish blood. Raw Atractylodes, prepared Rehmannia, Codonopsis, and raw Coix seed act as ministerial drugs to regulate qi and strengthen the spleen. Salvia, Saposhnikovia, Artemisia anomala, Angelica pubescens, and Moutan serve as adjuvant drugs to resolve stasis, relieve pain, dispel wind, remove impediment, and activate blood to unblock collaterals. Together, these herbs achieve the effects of nourishing sinews and liver, tonifying kidney and blood, and activating blood to dissipate stasis, thereby alleviating KOA symptoms including joint pain, cartilage degeneration, and impaired knee function. This formula represents an effective prescription for early to mid-stage KOA [16]. Aerobic exercise is recognized as an effective KOA treatment with wide clinical application that can significantly improve patients' quality of life [17-18]. Our results demonstrate that

both Yangxue Roujin Formula and aerobic exercise interventions attenuated knee cartilage injury, reduced Mankin scores and MMP13 positive expression, decreased serum and synovial fluid levels of IL-17, TNF-α, IL-6, and IL-18, and increased Col-II positive expression in cartilage tissue. The combination therapy showed superior efficacy compared with single interventions, indicating that both treatments can reduce inflammatory factor production and release, inhibit joint inflammation, improve cartilage metabolism, and alleviate cartilage degradation and injury, with synergistic effects when combined.

The SDF-1/CXCR4 signaling pathway mediates the development of arthritis by regulating inflammation, cartilage metabolism, chondrocyte apoptosis, and extracellular matrix degradation [6-7]. Inhibition of SDF-1/CXCR4 signaling activation can reduce synthesis of MMP-3, MMP-9, and MMP-13 proteins, thereby attenuating cartilage damage in arthritic rats [19]. Targeted blockade of SDF-1/CXCR4 signaling can reduce degradation of Col-II and aggrecan, thus delaying articular cartilage degeneration [20]. Therefore, reducing SDF-1/CXCR4 signaling activity may represent an effective measure for KOA treatment. Our results showed significantly elevated SDF-1 and CXCR4 protein expression in knee cartilage tissue of KOA rabbits compared with controls. Both Yangxue Roujin Formula and aerobic exercise interventions reduced SDF-1 and CXCR4 expression, with the combination therapy producing more pronounced reductions. These findings indicate that SDF-1/CXCR4 signaling participates in mediating the anti-inflammatory and cartilage metabolism-improving effects of Yangxue Roujin Formula combined with aerobic exercise in KOA rabbits.

In summary, both Yangxue Roujin Formula and aerobic exercise can downregulate SDF-1 and CXCR4 protein expression, reduce inflammatory factor levels, hinder inflammation progression, and improve knee cartilage metabolism in KOA rabbits. The combination therapy exerts synergistic effects, alleviating cartilage degradation and injury. Inhibition of SDF-1/CXCR4 signaling activation may represent the mechanism underlying the therapeutic effects of Yangxue Roujin Formula combined with aerobic exercise on KOA rabbits. This study provides new clinical insights for KOA prevention and treatment, contributing to improved therapeutic techniques and alleviation of clinical symptoms including articular cartilage degeneration, joint pain, and limited mobility in KOA patients.

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Note: Figure translations are in progress. See original paper for figures.