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Original Article

Effects of platelet-rich plasma combined with isometric quadriceps contraction on cartilage in a rat model of knee osteoarthritis

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ABSTRACT

Background: Intra-articular injection of platelet-rich plasma (PRP) or isometric contraction of quadriceps (ICQ) has shown positive effects in patients with knee osteoarthritis (KOA). However, the synergistic effect of combining PRP and ICQ intervention (joint intervention) on cartilage repair has not been validated. Thus, this study aimed to explore the reparative effects of joint intervention on cartilage in a KOA rat model.

Methods: Fifty-four 2-month-old female Sprague-Dawley rats were randomly divided into the control group (CG, n = 6) and model group (injected with sodium iodoacetate, n = 48). After 1 week, six rats from the model group were randomly selected for validation. The remaining 42 rats were further divided into seven groups: PRP group (PRPG), ICQ group (ICQG), joint intervention group (JIG), normal saline group (NSG), acupuncture group (AG), normal saline and acupuncture group (NSAG) and model blank group (MBG). The intervention lasted for 4 weeks, with PRPG and JIG receiving PRP injections (twice) and ICQG and JIG undergoing ICQ (five times per week, 15 min each session).

Results: Histological staining with haematoxylin and eosin as well as transmission electron microscopy revealed severe cartilage damage in MBG, AG, NSAG and NSG, followed by PRPG and ICQG. JIG exhibited a more intact cartilage structure. Compared with JIG, the Mankin scores increased remarkably in PRPG, ICQG, AG, NSAG and NSG ($P < 0.01$). Relative mRNA expression levels showed the upregulation of IL-1β in ICQG, NSAG and NSG compared with JIG ($P < 0.05$) and the upregulation of IL-6, IL-18 and MMP-13 in AG and NSAG ($P < 0.05$). Compared with PRPG, IL-1β and IL-6 were upregulated in ICQG, AG, NSAG and NSG ($P < 0.05$). In addition, IL-18 was upregulated in AG ($P < 0.01$), and IL-18, MMP-13 and TNF-α were upregulated in NSAG ($P < 0.05$). Compared with ICQG, IL-1β, IL-18, MMP-13 and TNF-α were upregulated in NSAG ($P < 0.05$), and IL-1β and IL-18 were upregulated in AG ($P < 0.05$).

Conclusion: The combination of PRP and ICQ can alleviate inflammatory responses in cartilage, promote chondrocyte regeneration and facilitate matrix tissue repair. Compared with single interventions, a synergistic effect is observed.

1. Introduction

Knee osteoarthritis (KOA) has a high incidence rate, and it is considered as the fourth leading cause of disability amongst the elderly and middle-aged population by the World Health Organization because of its high rate of deformity and disability in the advanced stages [1,2]. The incidence rate of KOA in China is approximately 8.1%, with about 120 million people suffering from the pain associated with KOA [3]. With the acceleration of China's aging population, the prevalence, number of patients and resulting disabilities from KOA are remarkably increasing. The diagnosis and treatment of KOA impose a substantial economic and medical burden on patients, families and society, making it an urgent social issue that requires research and resolution. Given the complex

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pathogenesis of KOA, effective treatment options are lacking, with clinical management primarily focusing on a comprehensive approach, including medication, physical therapy and exercise therapy [3]. Although early oral medication and physical therapy can alleviate clinical symptoms, they fail to prevent or delay the degeneration of articular cartilage or inhibit the chronic erosion of the knee joint caused by repeated inflammatory damage. Moreover, total knee arthroplasty still faces many issues, as it cannot truly simulate and replace the complex structure of the human joint and is associated with a range of complications and sequelae such as joint infection and joint stiffness, making it difficult for most patients to accept [4]. Therefore, exploring new methods and schemes to improve the clinical efficacy of KOA is a challenging and hot topic in the fields of sports medicine and orthopaedics.

Platelet-rich plasma (PRP) is a plasma product rich in platelets [5], which, once activated, releases 50 to 80 α-granules [6]. These granules further release various growth factors that participate in specific biomolecular functions during tissue repair [7]. A clinical study has confirmed the safety and efficacy of PRP injections [8], which promote the proliferation of chondrocytes and increase the lubricating effect of the produced regional proteins, thereby reducing cartilage friction and wear [9]. Evidence-based medicine has confirmed the positive effects of PRP injections on patients with KOA, reducing pain and WOMAC scores [10,11]. Exercise therapy can effectively alleviate the joint pain in patients with KOA and improve the function of the affected knee [12]. The guidelines issued by the American College of Rheumatology, the British Orthopaedic Association [13] and the Chinese Medical Association's Orthopedic Branch [14] all include exercise therapy as a first-line treatment strategy. However, whether the combination of the two methods has a better effect requires further verification.

Limited clinical studies have reported on the impact of PRP combined with exercise on patients with KOA, suggesting that the combination approach can achieve better clinical outcomes [15]. Compared with a single approach, PRP combined with exercise has been shown to be more effective in patients with moderate-to-severe KOA [16]. This result is related to the reduction in the expression level of interleukin-1β (IL-1β), tumour necrosis factor α (TNF-α), interleukin-18 (IL-18) and matrix metalloproteinases-13 (MMP-13) [17,18]. Inflammation plays an important role in the development of KOA, with factors such as IL-1β, TNF-α, IL-18 and MMP-13 being key mediators of chondrocyte apoptosis, leading to the degradation of the cartilage matrix [19]. Clinically, the expression level of the abovementioned factors is elevated in the cartilage of patients with KOA, inducing inflammatory reactions in chondrocytes and accelerating chondrocyte apoptosis [20]. In our previous studies on three research topics regarding PRP intervention in osteoarthritis, we found that PRP can promote chondrocyte proliferation and has reparative effects on cartilage in KOA animal models. Our previous clinical research found that isometric contraction of quadriceps femoris (ICQ) can markedly alleviate the symptoms of KOA, improve joint function and delay joint degeneration [21]. However, literature on whether the combined intervention of PRP and ICQ (joint intervention) has a better effect on KOA repair is limited.

Thus, this study starts from clinical problems, uses a KOA animal model to elucidate the reparative effects of joint intervention on KOA cartilage and provides a reference for subsequent clinical intervention in KOA. We hypothesise that joint intervention has a synergistic effect on KOA compared with single interventions.

2. Materials and methods

This study was approved by the Animal Experiment Ethics Committee of Chengdu Sport University (approval No. 202213). Female Sprague-Dawley (SD) rats, aged 2 months and weighing 180–220 g, were purchased from Chengdu Dashuo Laboratory Animal Co., Ltd. (certificate no. SCXK-2022-030, SPFSD), and were strictly raised in accordance with the standard breeding protocols. Experiments were conducted strictly in accordance with the requirements and were in compliance with animal welfare standards.

After 7-day acclimatisation, 54 rats were randomly assigned to the control group (CG, n = 6) and modelling group (n = 48) using a numerical allocation method. After 1 week, six rats from the modelling group were randomly selected for model validation [22,23]. The remaining 42 rats were further divided into seven groups using a numerical random allocation method (six cards each with numbers 1–7): platelet-rich plasma group (PRPG), isometric contraction of quadriceps group (ICQG), joint intervention group (JIG), normal saline group (NSG), acupuncture group (AG), normal saline and acupuncture group (NSAG) and model blank group (MBG, Fig. 1).

2.1. Induction and validation of knee arthritis model

Intra-articular injection of sodium iodoacetate can induce the degradation and loss of cartilage, as well as changes in the cartilage matrix, making it a commonly used animal model for studying KOA [24–26]. The rats were anesthetised by intraperitoneal injection of pentobarbital sodium (2% concentration, 0.2 mL/100 g). Under ultrasound guidance, with the rats' knees flexed, sodium iodoacetate (50 μL, 40 mg/mL; catalogue no. NY 14072, USA 1-716-774-6700; CAS no. 305-53-3) was injected into the knee joint cavity of both hind limbs, 1 mm lateral to the medial side of the patellar ligament [26]. The control group received an equal volume of physiological saline. After 1 week of modelling, six rats were randomly selected and euthanised (by intraperitoneal injection of pentobarbital sodium, 150–200 mg/kg). Then, knee joint cartilage was collected for haematoxylin–eosin staining (HE) to observe cartilage lesions, serving as the basis for successful modelling.

2.2. Intervention methods

After 1-week post-modelling acclimatisation, a 4-week intervention was conducted. PRPG and JIG were injected with PRP (two times, at baseline and week 2, 0.1 mL per knee joint) [27–29], whereas ICQG and JIG underwent electrical stimulation (acupuncture + current passage) to simulate ICQ (five times per week, 15 min per session). NSG and NSAG were injected with an equal volume and number of physiological saline injections, and AG and NSAG received acupuncture (without current passage). Furthermore, MBG and CG were not intervened.

2.3. Preparation of PRP

An additional 10 female SD rats aged 2 months were anesthetised by intraperitoneal injection of pentobarbital sodium (0.3%, 0.1 mL/100 g). Approximately 6 mL of blood was collected from the abdominal aorta. A platelet count was performed on 1 mL of whole blood, and the remaining blood was transferred into a tube containing ACD anticoagulant at a ratio of 1:10 and mixed well. PRP was prepared using a double-centrifugation method [30], with a centrifuge (Xiangyi, Hunan, China, Model: CHT 210R) performing two rounds of centrifugation (1st: 200 g, 10 min; 2nd: 200 g, 10 min), resulting in a platelet concentration in the PRP approximately six times the baseline concentration [31,32].

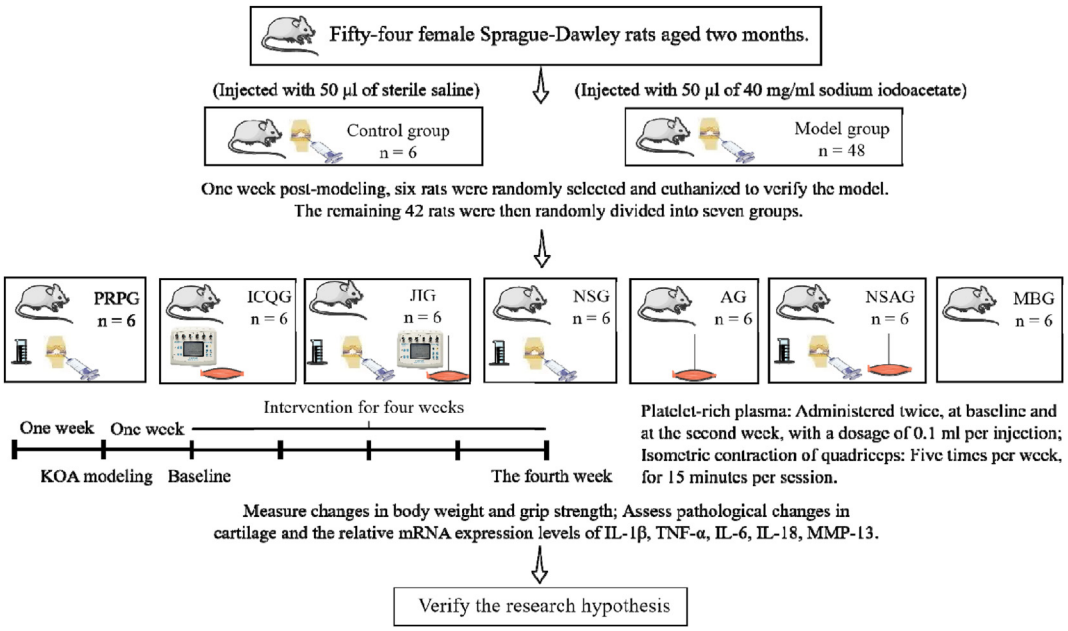

Fig. 1. Experimental design and grouping.
PRPG: Platelet-Rich Plasma Group, ICQG: Isometric Contraction of Quadriceps Group, JIG: Joint Intervention Group, NSG: Normal Saline Group, AG: Acupuncture Group, NSAG: Normal Saline and Acupuncture Group, MBG: Model Blank Group, CG: Control Group.

2.4. Electrical stimulation to simulate isometric quadriceps contraction

The intensity of muscle isometric contraction was determined by the current level, with a current strength of 0.5–2 mA (starting at 0.5 mA in the first week, increasing by 0.5 mA each week, up to 2 mA by the fourth week) [33–35] and a frequency of 1 Hz [36,37]. The electrical stimulation lasted for 15 min per session [35,36], five times per week, over 4 weeks. Rats were restrained throughout the electrical stimulation process.

2.5. Testing methods

2.5.1. Hindlimb grip strength and body weight tests

A YLS-13A rat grip strength meter made in China was used to measure the hindlimb grip strength of the rats. Measurements were taken before modelling, at 7 days post-modelling (baseline) and once a week after the intervention for a total of six times (three measurements were averaged). Body weight was also recorded.

2.5.2. Pathological and mRNA expression testing of knee cartilage

Rats were euthanised (by intraperitoneal injection of pentobarbital sodium, 150–200 mg/kg), and knee joint cartilage was collected. HE staining and transmission electron microscopy were used to observe the ultrastructure of the cartilage, and Mankin scoring [38] was used to assess the degree of cartilage damage. Relative mRNA expression level of IL-1β, TNF-α, IL-6, IL-18 and MMP-13 in the cartilage was detected by RT-PCR (Table 1).

Table 1
The primers and their corresponding nucleotide sequences.

Primer Name	Upstream	Downstream
β-actin	gggaaatcgtgcgtgacatt	gcggcagtggccatctc
IL-1β	aatctcacagcagcatctcgacaag	tccacgggcaagacataggtagc
IL-6	acttccagccagttgccttcttg	tggtctgttgtgggtggtatcctc
IL-18	cgaccgaacagccaacgaatcc	gtcacagccagtcctcttacttcac
MMP-13	cagccctatcccttgatgccattac	gggtgcagacgccagaagaatc
TNF-α	caccacgctcttctgtctactgaac	tgggctacgggcttgtcactc

3. Statistical analysis

All measured data were processed for mean ± standard deviation using SPSS 20.0. The Shapiro–Wilk test and Levene's tests were used to check for normal distribution and homogeneity of variances. If the data met the criteria for normal distribution and homogeneity of variances, then one-way ANOVA was used to analyse differences between two groups. If the data did not meet these criteria, then the Kruskal–Wallis H test was used to compare differences between two groups. Post-hoc comparisons were adjusted using the Bonferroni method to ensure that the overall type I error rate for each ANOVA did not exceed 0.05 [39,40]. The level of significance was set at $\alpha = 0.05$.

4. Results

4.1. Validation of the KOA model

HE staining (Fig. 2) showed that the cartilage layer of the randomly selected six rats was damaged throughout the entire layer, with the disappearance of the cartilage structure, a remarkable reduction in the number of chondrocytes and the injured area extending to the subchondral layer. The damaged area was replaced by fibrous tissue, and the number of osteoclasts increased. The Mankin score was 9.67 ± 0.47 points, indicating that the KOA model induced by sodium iodoacetate in this study is reliable.

4.2. Body weight and hindlimb grip strength in KOA rats

Compared with the CG, the body weight of the MBG and NSG decreased ($P < 0.05$) during the first and second weeks. The hindlimb grip strength of all model groups (seven groups) decreased ($P < 0.05$) in the first week (Fig. 3).

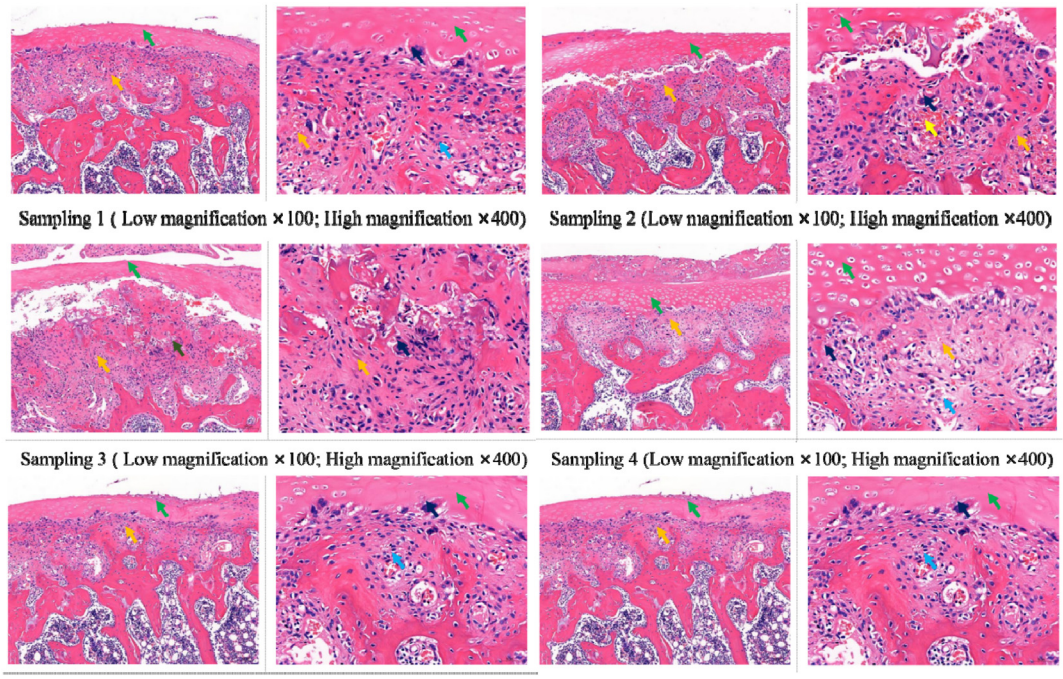

Fig. 2. HE staining results of articular cartilage from 6 randomly selected knee joint samples one week post-induction of osteoarthritis model.
Damage to the cartilage layer (↑); proliferation of fibrous tissue (↑); increased osteoclasts (↑); formation of new capillaries (↑); hemorrhage (↑); reduction in the number of trabeculae (↑).

4.3. Therapeutic effects of combined intervention on KOA rat cartilage

HE staining and transmission electron microscopy showed severe cartilage damage in the MBG, AG, NSAG and NSG, followed by the PRPG and ICQG. The JIG had a more intact cartilage structure, and the CG had a normal cartilage structure. Compared with the JIG, the Mankin scores increased in the PRPG, ICQG, AG, NSAG and NSG ($P < 0.01$; Figs. 4 and 3I).

4.4. Combined intervention reduces inflammatory cytokines and MMP-13-relative mRNA expression in KOA rats

The Shapiro–Wilk test showed that the data met the normal distribution criteria ($P > 0.05$). Levene's test for homogeneity of variances found equal variances for IL-1β, IL-6, IL-18, MMP-13 and TNF-α. ANOVA revealed significant differences ($P < 0.001$) in the expression level of IL-1β ($F_{(7,40)} = 8.218$), IL-6 ($F_{(7,40)} = 33.213$), IL-18 ($F_{(7,40)} = 8.460$), MMP-13 ($F_{(7,40)} = 8.548$) and TNF-α ($F_{(7,40)} = 14.176$) amongst the groups.

Post-hoc comparisons using the Bonferroni adjustment were made to assess inter-group differences. Compared with the MBG, the JIG and PRPG showed the downregulation of IL-1β, IL-6, IL-18, MMP-13 and TNF-α ($P < 0.05$), and the ICQG showed the downregulation of IL-18, MMP-13 and TNF-α ($P < 0.01$). Compared with the JIG, the ICQG, NSAG and NSG showed the upregulation of IL-1β ($P < 0.05$), and the AG and NSAG showed the upregulation of IL-6, IL-18 and MMP-13 ($P < 0.05$). The PRPG, ICQG and NSG showed the upregulation of IL-6 ($P < 0.05$), and the NSG showed the upregulation of MMP-13 ($P < 0.05$). In addition, the NSAG and NSG showed the upregulation of TNF-α ($P < 0.01$). Compared with the PRPG, the ICQG, AG, NSAG and NSG showed the upregulation of IL-1β and IL-6 ($P < 0.05$); AG showed the upregulation of IL-18 ($P < 0.01$), and the NSAG showed the upregulation of IL-18, MMP-13 and TNF-α ($P < 0.05$). Compared with the ICQG, the NSAG showed the upregulation of IL-1β, IL-18, MMP-13 and TNF-α ($P < 0.05$), and AG showed the upregulation of IL-1β and IL-18 ($P < 0.05$, Fig. 3II–VI).

5. Discussion

This study focused on a rat model of KOA to explore whether combined intervention has a synergistic effect. We validated our research hypothesis that combined intervention has a synergistic effect on KOA compared with single interventions.

5.1. Validation of the KOA model

This study induced KOA in rats by intra-articular injection of sodium iodoacetate, causing degradation, loss and changes in the cartilage matrix of the knee joint. One week after modelling, HE staining was performed on the knee cartilage of six randomly selected rats from the modelling group. The results indicated full-thickness damage to the cartilage layer, disappearance of the cartilage structure, a remarkable reduction in the number of chondrocytes, involvement of the subchondral layer in the damage area, replacement of the damaged area by fibrous tissue and an increase in the number of osteoclasts. The Mankin score was 9.67 ± 0.47 points, indicating that the KOA modelling in this study was reliable.

Sodium iodoacetate-induced KOA in rats mimics the pathological changes observed in human osteoarthritis, and it is a commonly used experimental model for studying the disease in clinical research [24–26]. Naveen et al. [41] compared the histological, biochemical and biomechanical characteristics of cartilage in rats with KOA induced by sodium iodoacetate injection and transection of the anterior cruciate ligament, and the results showed that sodium iodoacetate injection induces degenerative changes in rat cartilage 1 week after injection, which is comparable to KOA induced by transecting the anterior cruciate ligament and human

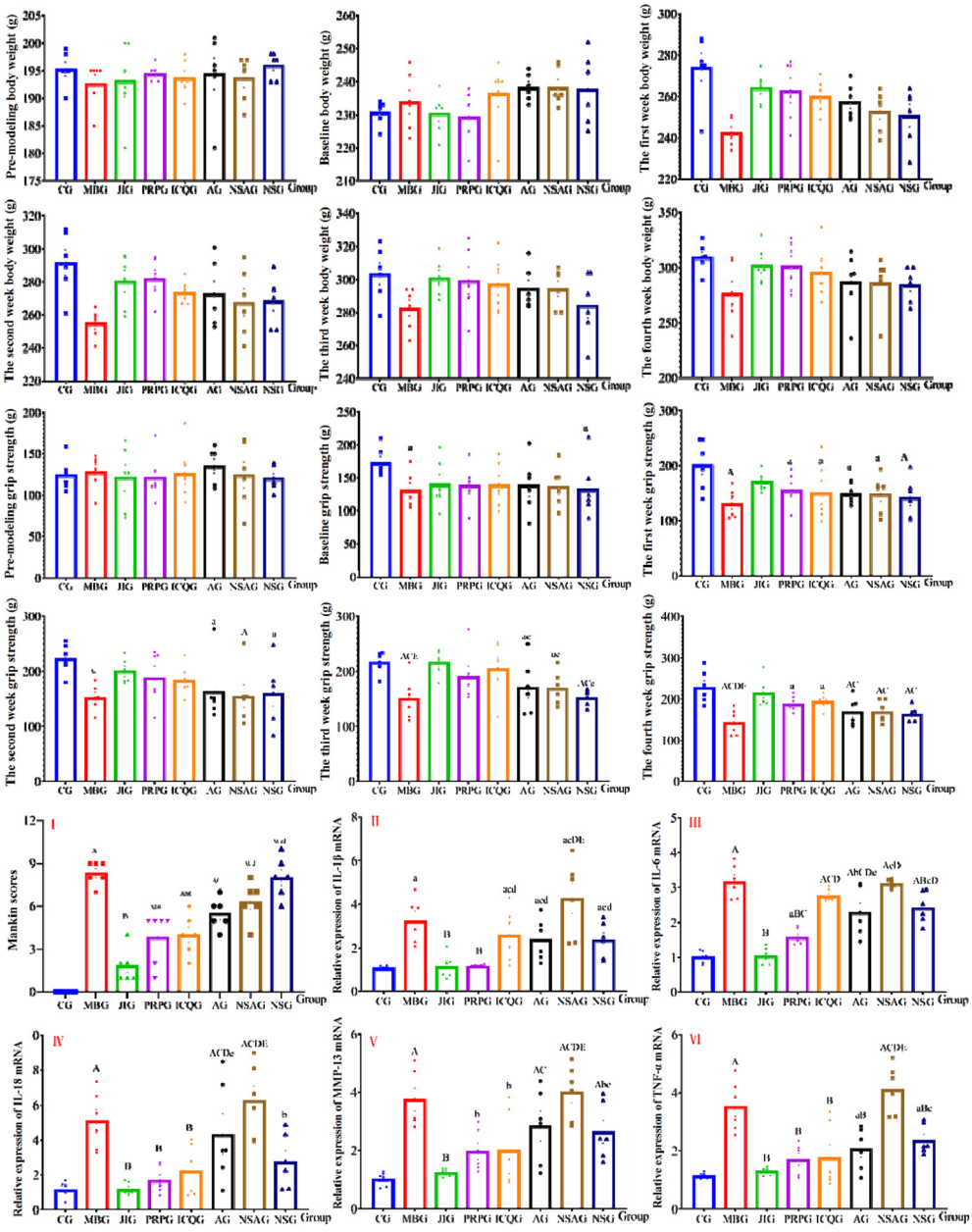

Fig. 3. Body weight, hind limb grip strength, Mankin scores, and relative expression of inflammatory cytokine mRNA in articular cartilage tissue of different groups of rats. PRPG: Platelet-Rich Plasma Group, ICQG: Isometric Contraction of Quadriceps Group, JIG: Joint Intervention Group, NSG: Normal Saline Group, AG: Acupuncture Group, NSAG: Normal Saline and Acupuncture Group, MBG: Model Blank Group, CG: Control Group. Compared with CG, $^{a}p < 0.05$, $^{A}p < 0.01$; Compared with MBG, $^{b}p < 0.05$, $^{B}p < 0.01$; Compared with JIG, $^{c}p < 0.05$, $^{C}p < 0.01$; Compared with PRPG, $^{d}p < 0.05$, $^{D}p < 0.01$; Compared with ICQG, $^{e}p < 0.05$, $^{E}p < 0.01$.

KOA. Other studies have found that sodium iodoacetate injection increases the weight-bearing of rat hind limbs after 3 days, followed by the thinning of the articular cartilage and subchondral bone lesions by the 7th day [42]. This study used the same modelling method as previous studies [24–26,42], and HE staining and Mankin scoring confirmed the success of KOA modelling.

5.2. Body weight and hindlimb grip strength

The changes in body weight and hindlimb grip strength were used to verify the research hypothesis. Initially, the body weight in the MBG and NSG groups decreased compared with the control group probably because of post-modelling hind limb pain. This finding is consistent with that of previous studies [27]. The greater reduction in MBG might be attributed to less severe KOA inflammation compared with the JIG. By the third and fourth weeks, the lack of weight difference amongst the groups may reflect the progressive KOA inflammation restricting physical activity. As hypothesised, the initial decrease in body weight could be due to pain-induced inactivity, whereas the subsequent weight increase in severely inflamed rats may be due to reduced mobility over time [43]. The grip strength data corroborate these observations, indicating an overall therapeutic response to intervention.

Hindlimb grip strength in all model groups was reduced post-modelling probably because of KOA-induced joint pain [44,45], thereby affecting test outcomes. After intervention, the combined intervention group and the PRPG showed grip strength comparable

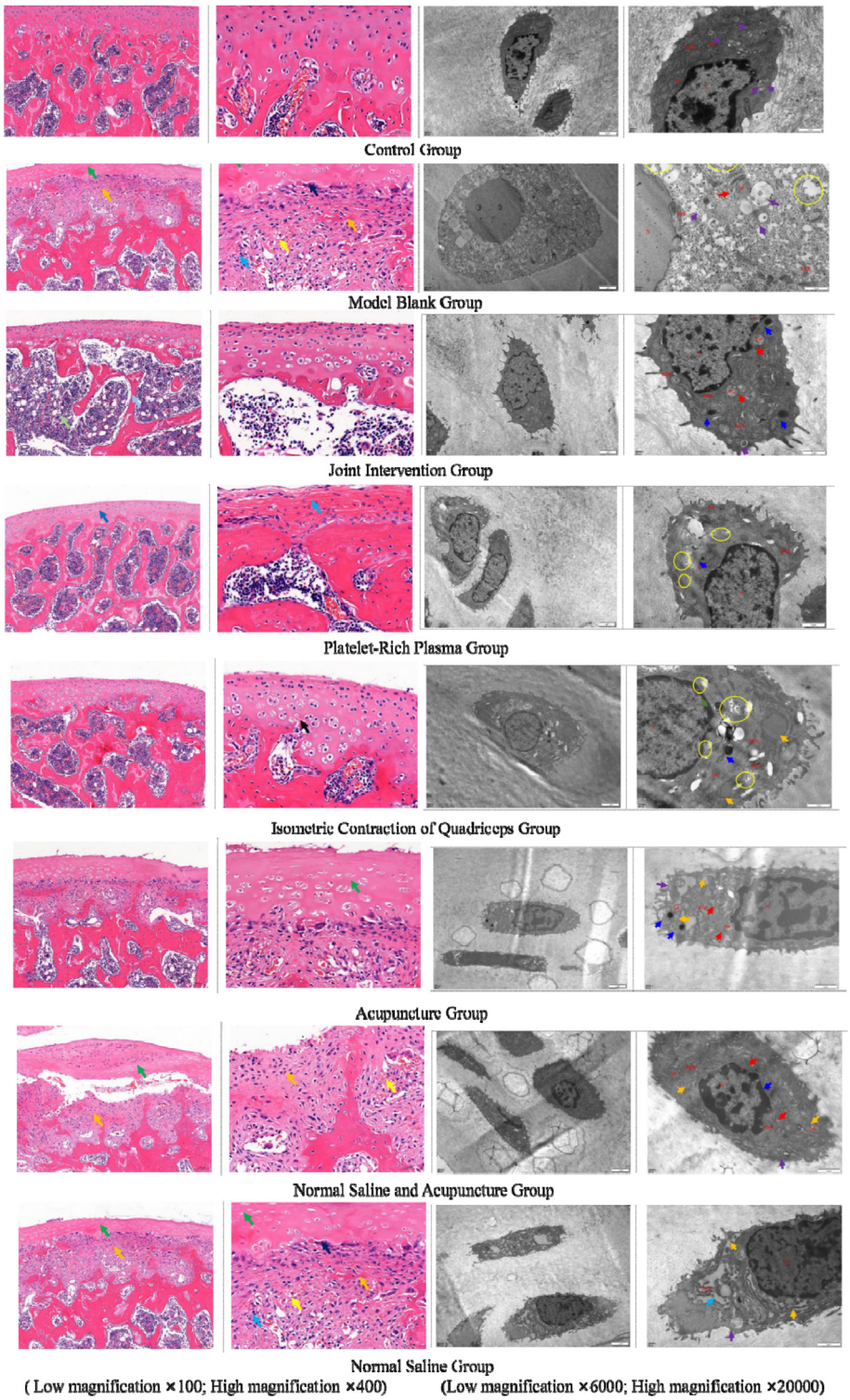

Fig. 4. Representative HE staining and transmission electron microscopy images from one sample per group.
HE staining: damage to the cartilage layer (↑), proliferation of fibrous tissue (↑), increased osteoclasts (↑), formation of new capillaries (↑), hemorrhage (↑), reduction in the number of trabeculae (↑); **Transmission electron microscopy:** expansion of the rough endoplasmic reticulum (↑), primary lysosomes (↑), autophagic lysosomes (↑), lipid droplets (↑), mitochondrial swelling (↑), ribosome loss (○).

to the control group, suggesting the positive effect of both treatments on the strength of KOA rats [44]. The ICQG also demonstrated a non-significant difference, indicating a potential therapeutic benefit. Pain alleviation, a critical aspect of KOA management, can improve grip strength [44]. Although this study did not measure pain indicators, the observed changes in grip strength support the hypothesis that pain reduction leads to improved physical function in KOA rats. Future studies should explore the underlying mechanisms linking pain, body weight and grip strength in KOA.

5.3. Cartilage tissue pathology

The results of cartilage tissue pathology verified the research hypothesis. Consistent conclusions were reached through qualitative observation by HE staining and transmission electron microscopy, as well as quantitative analysis by Mankin scoring: MBG, AG, NSAG and NSG had severe cartilage damage, followed by PRPG and ICQG. The JIG had intact cartilage structure, and the control group had normal cartilage structure.

Previous studies have reported the positive impact of PRP on the histopathology of KOA rats induced by sodium iodoacetate. Transplanting PRP-treated chondrocytes into KOA rats found that PRP remarkably promoted chondrocyte proliferation [46]. Compared with KOA rats, the PRP intervention group had more chondrocyte counts, higher cartilage height, PRP-inhibited chondrocyte apoptosis and reduced matrix loss [28]. Injecting PRP delayed the adverse changes in the histopathology of the knee joint of KOA rats [47]. Another study also found that PRP greatly improved the tissue structure of the knee joint in KOA model rats [48]. The results of this study are consistent with those of previous studies [28,46], indicating that PRP has a positive impact on the pathological morphology of KOA. In addition, the positive impact of exercise therapy on the histopathology of KOA rats induced by sodium iodoacetate has been reported. Most studies used treadmill exercise and found that 4 weeks of treadmill exercise had a remarkable protective effect on the knee joint cartilage of KOA rats [49]. Different speeds of single treadmill intervention can also reduce the degree of joint cartilage lesions in KOA rats [50]. Therefore, regular exercise can prevent bone loss caused by KOA [51]. Exercise intervention of KOA rats with a wheel and treadmill found that both exercise methods can reduce cartilage inflammation [52]. This study used electrical stimulation to induce rat ICQ, and the pathological results of KOA rats are consistent with previous studies [49–52], indicating that ICQ has a positive impact on the pathological morphology of KOA.

5.4. Gene expression of inflammatory cytokines and MMP-13 in knee cartilage

The gene expression of inflammatory cytokines and MMP-13 in knee cartilage verified the research hypothesis. IL-1β is a pro-inflammatory cytokine involved in various autoimmune inflammatory reactions and various cellular activities [53]. IL-1β and joint cartilage damage are positively correlated, causing the degradation of the cartilage matrix [54]. The role of IL-6 in the occurrence and development of KOA is also prominent [55]. Compared with normal human joint cartilage, the expression level of IL-6 in the cartilage of patients with KOA increased, causing an imbalance in the immune state of joint cartilage tissue and leading to the destruction of chondrocytes [56]. IL-18 is a pro-inflammatory cytokine [57], and the severity of KOA is positively correlated with the expression level of IL-18 [58]. MMP-13 is a key enzyme for the pathological destruction of cartilage, and it plays an important role in the pathological progression of KOA [20]. Reducing the expression of MMP-13 in joint cartilage can reduce the proliferation and hypertrophic differentiation of chondrocytes, thereby delaying cartilage degeneration [59]. Inhibiting the expression of MMP-13 can protect the cartilage of patients with KOA [60]. TNF-α is involved in the degradation of the cartilage matrix and bone destruction, inducing degeneration and homeostatic imbalance of joint cartilage, and is considered an important inflammatory factor related to the pathogenesis of KOA [61,62].

Previous study has reported the impact of PRP on cartilage tissue in rats with KOA induced by sodium iodoacetate. Compared with the model group, the expression level of IL-1β, IL-6, IL-18, MMP-13 and TNF-α was downregulated in the PRP group, indicating that PRP possesses anti-inflammatory properties and cartilage repair potential [29]. Another study has also demonstrated that PRP injection downregulated the expression level of inflammatory factors (IL-1β, IL-6 and IL-18) and MMP-13 in the cartilage [63]. The results of this study are consistent with previous research [29,63], showing that PRP reduced the expression levels of IL-1β, IL-6, IL-18, MMP-13 and TNF-α in the cartilage tissue of KOA rats, thereby alleviating KOA symptoms. In addition, the impact of exercise therapy on cartilage tissue in KOA rats induced by sodium iodoacetate has been reported. Compared with KOA rats, 12 weeks of moderate-intensity treadmill exercise downregulated the expression level of IL-1β, MMP-13 and MMP-3 in the joint cartilage [50]. Four weeks of moderate-intensity treadmill exercise downregulated the expression level of MMP-13 in the cartilage, providing a positive protective effect [49]. This study used electrical stimulation to induce ICQ, and the effects on inflammation and MMP-13 in KOA rats were consistent with previous studies [49,50], indicating that ICQ has a positive impact on KOA.

5.5. Mechanism of combined intervention for KOA

Compared with single PRP or ICQ interventions, the combined intervention has a synergistic effect in reducing the inflammatory response of KOA, promoting chondrocyte regeneration and repairing matrix tissue because of the following reasons: synergistic action, anti-inflammatory effect, cartilage protection, tissue repair effect and biological effects.

Firstly, PRP contains a variety of growth factors and cytokines that can promote tissue repair and regeneration [5]. ICQ, through muscle contraction stimulation, may enhance local blood circulation and nutrient supply, thereby promoting the distribution and action of growth factors in PRP [21]. Secondly, the platelet-released factors contained in PRP have anti-inflammatory properties, which can reduce the expression level of inflammatory factors such as IL-1β, IL-6, IL-18 and TNF-α [8,9]. ICQ may indirectly reduce the inflammatory response by improving muscle function and reducing the load on the joint. Thirdly, the bioactive molecules in PRP help protect the cartilage from further damage and promote the proliferation and matrix synthesis of chondrocytes [10]. ICQ may protect the cartilage by enhancing muscle strength and stability, thereby reducing mechanical pressure on the joint [21]. Fourthly, PRP can promote cell proliferation, differentiation and matrix synthesis, accelerating tissue repair [11]. ICQ improves joint stability by enhancing muscle strength [21], which provides a better mechanical environment for cartilage repair. Fifthly, various growth factors and cytokines in PRP positively affect the biological behaviour of chondrocytes, such as promoting cell migration, proliferation and differentiation [5]. ICQ may improve muscle function, enhance joint stability and flexibility and facilitate cartilage repair.

In summary, the combined intervention of PRP and ICQ may play an important role in reducing inflammation, promoting cartilage repair and protecting joints through multiple mechanisms of interaction. The combined intervention provides a comprehensive treatment method for preventing KOA, combining the advantages of bio-therapy and physical therapy, and treats KOA from different angles and levels to achieve better therapeutic effects. However, these hypotheses need to be further verified by clinical and basic research.

5.6. Limitations of the study

This study has certain limitations. Firstly, the study period is relatively short. Thus, evaluating the long-term effects and safety of the combined intervention is not possible, and the potential side effects or complications that the combined intervention may bring

cannot be fully assessed. Secondly, the most effective components and their concentrations in PRP were not determined, and different frequencies and durations of the ICQ protocol were not explored. Finally, in-depth research on the molecular biological mechanisms of the combined intervention is lacking.

6. Conclusion

This study indicates that the combined intervention of PRP and ICQ can alleviate the inflammatory response of joint cartilage in KOA rats, promote the regeneration of chondrocytes and repair matrix tissue. Moreover, compared with single interventions, the combined intervention is more effective for KOA.

Ethics approval

This study was approved by the Animal Experiment Ethics Committee of Chengdu Sport University (Approval No. 202213).

Funding

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Author contributions

LC, KW, SWC, YJT and BXH: designing this study, writing initial draft and revision, revising language and content, supervision, project administration, and funding acquisition. KW and SWC: making figure and table. LC, YJT and BXH: rechecking the manuscript and putting forward suggestions for amendment. All authors contributed to the article and approved the submitted version.

Availability of data and material

All data relevant to the study are included in the article or uploaded as supplementary information.

Consent to participate

This study recruited elderly female participants diagnosed with KOA from the Sichuan Provincial People's Hospital. This study complied with the Declaration of Helsinki, and informed consent was obtained from all participants.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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