Intra-Articular Platelet-Rich Plasma Combined With Hyaluronic Acid Injection for Knee Osteoarthritis Is Superior to Platelet-Rich Plasma or Hyaluronic Acid Alone in Inhibiting Inflammation and Improving Pain and Function

Zhe Xu, M.D., Zhixu He, Ph.D., Liping Shu, Ph.D., Xuanze Li, M.D., Minxian Ma, M.D., and Chuan Ye, Ph.D.

Purpose: To evaluate the effectiveness and explore the therapeutic mechanisms of platelet-rich plasma (PRP) combined with hyaluronic acid (HA) as a treatment for knee osteoarthritis (KOA). **Methods:** In total, 122 knees were randomly divided into HA (34 knees), PRP (40 knees), and PRP+HA (48 knees) groups. Platelet densities in whole blood and PRP were examined using Wright–Giemsa staining. Visual analogue scale, Lequesne, Western Ontario and McMaster Universities Osteoarthritis Index, Lysholm scores, and postoperative complications were evaluated. High-frequency color Doppler imaging was used to observe the synovium and cartilage. Enzyme-linked immunosorbent assays were used to quantify interleukin-1β, tumor necrosis factor-α, matrix metalloproteinase-3, and tissue inhibitor of metalloproteinase-1 levels in synovial fluid. **Results:** The platelet density in PRP was 5.13-times that in whole blood ($P = .002$). At 24 months, pain and function scores in the PRP+HA group were better than those in the HA-alone and PRP-alone groups ($P_{pain} = .000$; $P_{function} = .000$). At 6 and 12 months, synovial hyperplasia in the PRP and PRP+HA groups was improved ($P < .05$). After 6 and 12 months, the synovial peak systolic velocity, synovial end-diastolic velocity, systolic/diastolic ratio, and resistance index were improved in the PRP+HA group ($P < .05$). Complications were greatest in the PRP group ($P = .008$). After 6 and 12 months, interleukin-1β, tumor necrosis factor-α, matrix metalloproteinase-3, and tissue inhibitor of metalloproteinase-1 in the PRP and PRP+HA groups decreased ($P < .05$), with more apparent inhibition in the PRP+HA group ($P < .05$). **Conclusions:** PRP combined with HA is more effective than PRP or HA alone at inhibiting synovial inflammation and can effectively improve pain and function and reduce adverse reactions. Its mechanism involves changes in the synovium and cytokine content. **Level of Evidence:** Level II, Prospective cohort study.

See commentary on page 916

Knee osteoarthritis (KOA) is the predominant cause of knee joint pain and dysfunction in the elderly population,[1,2] and the global incidence is growing at an annual rate of 4.7% to 6.0%.[3] KOA affects patient quality of life and also imposes serious psychological and economic burdens on families and society.[4,5] Currently, total knee arthroplasty is the ultimate treatment for KOA. However, conservative treatments are administered most frequently, including nonsteroidal anti-inflammatory drugs, hyaluronic acid (HA),

From the Department of Orthopaedics, The Affiliated Hospital of Guizhou Medical University (Z.X., X.L., C.Y.), Key Laboratory of Adult Stem Cell Transformation Research, Chinese Academy of Medical Sciences (Z.X., Z.H., L.S., M.M., C.Y.), National-Local Joint Engineering Laboratory of Cell Engineering and Biomedicine (Z.X., L.S., M.M., C.Y.), and Center for Tissue Engineering and Stem Cell Research, Guizhou Medical University (Z.X., L.S., X.L., M.M., C.Y.), Guiyang; and China Orthopaedic Regenerative Medicine Group (CORMed), Hangzhou (C.Y.), China.

The authors report that they have no conflicts of interest in the authorship and publication of this article. Full ICMJE author disclosure forms are available for this article online, as supplementary material.

Received February 4, 2020; accepted October 11, 2020.

Address correspondence to Chuan Ye, Ph.D., Department of Orthopaedics, The Affiliated Hospital of Guizhou Medical University, Guiyang, China. E-mail: yechuanchina@hotmail.com

0749-8063/20118/$36.00

https://doi.org/10.1016/j.arthro.2020.10.013

and steroids. Among these treatments, HA is widely used. The advantage of HA is that it can achieve short-term pain relief but does not affect the natural course of KOA,[6-8] although administering multiple injections increases the economic burden for patients.[9] Some studies suggest that multiple HA injections may increase the risk of infection during future total knee arthroplasty procedures.[10,11] Thus, HA fails to meet the requirements of an efficacious KOA treatment.

In recent years, platelet-rich plasma (PRP) has received considerable attention as a possible treatment for KOA. PRP contains at least 7 growth factors, including platelet-derived growth factor, vascular endothelial growth factor, and transforming growth factor, and can promote chondrocyte regeneration and induction of adipose-derived mesenchymal stem cells into chondrocytes.[12-15] In some clinical cases, satisfactory treatment results have been achieved,[16,17] but PRP treatment is not free of complications. Evidence of the treatment mechanism obtained from high-quality research is still lacking. Early postoperative joint pain, exacerbation of swelling, rash, proteinuria, vomiting, and other adverse reactions have been reported.[18-20]

Most previous studies have focused on the advantages and disadvantages of PRP and HA in the treatment of KOA,[6,16] and few studies have focused on the clinical efficacy and treatment mechanism of a combination of PRP and HA. In recent years, increasing evidence, both in vitro and in vivo, has supported the clinical use of PRP combined with HA therapy in the treatment of articular pathology, including KOA.[21-26] Furthermore, Marmotti et al.[27] and Yan et al.[28] found that the addition of HA to PRP could effectively promote the proliferation of chondrocytes and improve cartilage repair. More recently, a systematic review and meta-analysis including 653 trials also provided information about the therapeutic trajectory of PRP combined with HA for KOA. Interestingly, it revealed that PRP combined with HA did provide better overall clinical improvement than HA in terms of symptom-function improvement at every follow-up visit or in terms of duration of effect.[29] In this study, the improvement of inflammation and joint function and pain for KOA was investigated in depth. Therefore, the goal of this study was to evaluate the effectiveness and explore the therapeutic mechanisms of PRP combined with HA as a treatment for KOA. We hypothesized that PRP combined with HA would have a better clinical effect at inhibiting inflammation of the synovium than PRP or HA alone.

Methods

Patient Selection

The study protocol was approved by the Ethics Committee and was publicly accessible before enrollment of the first patient. We performed the study in accordance with the ethical standards outlined in the 2013 revision of 1975 Declaration of Helsinki, and we report the results according to the 2010 Consolidated Standards of Reporting Trials statement. The potential benefits and risks of PRP injection and follow-up were explained to each study patient. All patients provided written informed consent for participation in the study. The enrollment period was from June 1, 2016, to June 1, 2017, and the trial was registered (registration number: ChiCTR1800017731). Patient screening was performed in the outpatient department, where the chairman of the orthopaedic department (C.Y.) evaluated patients' eligibility for study inclusion through history collection, imaging examination, and laboratory testing, and patients were included in this study only if they met all the inclusion and exclusion criteria shown in Table 1.

All patients were assessed with visual analog scale (VAS) scores, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores, Lequesne scores, and Lysholm scores before and at 4 time points (1, 6, 12, and 24 months) after all 3 injections. Synovial thickness; cartilage thickness; synovial blood flow; and matrix metalloproteinase-3 (MMP-3), tissue inhibitor of metalloproteinase-1 (TIMP-1), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) contents in the knee fluid before and 6 and 12 months after injection and complications within 2 months after the first injection were recorded.

PRP Preparation and Platelet Count

A 36-mL peripheral blood sample (bilateral knees, 72 mL) was collected from each patient, and 4 mL (bilateral knees, 8 ml) of the anticoagulant acid citate dextrose was added to 50-mL centrifuge tubes (Corning, Lowell, MA). At room temperature, the blood was centrifuged (TGL-16Gr, Anting Scientific Instrument Factory, Shanghai,

Table 1. Subject Eligibility Criteria

Inclusion criteria
Ability to provide informed consent
Aged between 42 and 79 years
Diagnosis of Kellgren–Lawrence stage II∼III
No previous injection therapy (HA, PRP, steroid, etc.)
No previous surgical history (HTO, arthroscopy, internal fixation of a knee fracture, etc.)
No previous pain medication (NSAIDs, etc.) in the past month
Exclusion criteria
Kellgren–Lawrence stage IV
Allergy or contraindication to the study drugs
Secondary osteoarthritis (infectious arthritis, rheumatoid arthritis, hemophilic arthritis, traumatic knee osteoarthritis, etc.)
Synovial fluid could not be extracted before and after injection
Severe cardiocerebrovascular disease, liver or kidney disease, or endocrine disease
Endocrine disease (poor control of type II diabetes, uncontrolled hyperthyroidism, etc.)
Poor skin condition at the puncture site

HA, hyaluronic acid; ns, not significant; HTO, high tibial osteotomy, NSAID, nonsteroidal anti-inflammatory drug; PRP, platelet-rich plasma.

China) at 160*g* for 10 minutes. After the first spin, the blood was separated into 3 components: erythrocytes at the bottom, a buffy coat in the middle, and platelet-containing plasma at the top. The platelet-containing plasma was gently aspirated and transferred to a new tube and centrifuged again at 250*g* for 15 minutes. After the second spin, the platelet-poor supernatant plasma was discarded by gentle aspiration. The leukocyte-poor PRP was resuspended in the residual supernatant, which was collected and measured for volume by gentle aspiration with a 5-mL sterile injection syringe (Jet Biofil, Guangzhou, China).[30] Whole-blood and PRP specimens from all patients were stained using Wright–Giemsa staining. Platelet concentrations were measured using a hematology analyzer (MEK-6400, Nihon Kohden, Japan), which was completed by the senior examiner of the clinical laboratory before injection.

Procedure

Each intra-articular injection was performed by an independent orthopaedic physician who was not involved in the assessments. To keep the patients blinded to their type of injection, we used a curtain to separate the patient from the injector. With knee flexion at 90°, a lower lateral patellar approach and a 25-G needle (outer diameter 0.50 mm, inner diameter 0.25 mm, length 90 mm) were used to inject 2 mL of HA (SOFAST, 2 mL/20 mg, 2500 kDa, Shandong, China), 4 mL of PRP, and 4 mL of PRP+2 mL HA in 3 groups: group HA, group PRP, and group PRP+HA, respectively. Then, 0.5 mL of lidocaine was injected as local anesthesia into the skin and was not injected into the knee cavity to avoid a possible deleterious effect on platelets.[31] Every knee received 3 injections, and the interval was half a month. After injection, we passively flexed the knees for 20 seconds to achieve an adequate intra-articular distribution. Ten minutes after injection, the patients were sent home with written instructions, mainly including avoiding strenuous exercise for 48 hours and applying ice for 15 minutes 3 times a day.

Outcome Measures

Neither the patients nor researchers knew the group assignments for the trial. All data were evaluated by independent physicians who remained blind to the study. Through a simple random remainder grouping method, the knees of subjects who met all the inclusion criteria were randomly assigned to the HA, PRP, and PRP+HA groups.

Efficacy Evaluation

Each patient received a booklet about the VAS, WOMAC, Lysholm, and Lequesne score questionnaires, and the questionnaires had to be completed by the patients before injection and at 1 month, 6 months, 12 months, and 24 months after all three injections. Paracetamol was the only drug permitted in the study but had to be discontinued 72 hours before each follow-up assessment.

Ultrasound Evaluation

Ultrasound examinations were completed by 3 physicians with more than 20 years of clinical experience who were unaware of the study. Their main specialty is skeletal muscle ultrasound detection. We performed a pilot study before the study and found that there was no difference in the accuracy of ultrasound detection among the 3 physicians. A color ultrasound instrument (iu22; Philips, Andover, MA) was used, the linear array probe was 5∼12 MHz, the musculoskeletal low-speed blood flow condition was selected, the pulse Doppler sampling volume was 1 mm, and the angle between the sound speed and the blood flow was less than 45°. Each patient was in the supine position, fully exposing the knee joint, and the knee was bent at 30°.[32,33] The suprapatellar capsule (the probe was not pressurized), anterior patellar capsule, infrapatellar capsule, and medial and lateral synovia of the femoral condyle were examined. The thicknesses of the synovium and cartilage, the exudation depth, and the blood flow of the synovium (the synovial peak systolic velocity [PSV], synovial end-diastolic velocity [EDV], systolic/diastolic ratio [S/D], and resistance index [RI]) were observed before and at 6 months and 12 months after injection. S/D and RI were the most important indexes to observe the synovial blood flow resistance, which increases with decreased synovitis.

Synovial Fluid Evaluation

Before injection, an independent orthopaedic physician extracted approximately 2 to 3 mL of joint fluid and stored it at −80°C. MMP-3, TIMP-1, IL-1β, and TNF-α were detected by a senior researcher blinded to this study using a double-antibody sandwich enzyme-linked immunosorbent assay. The kit was provided by Neobioscience, and a microplate reader (BioTek, Synergy H1, Stem Cell Engineering Laboratory) was used for detection.

Complication Evaluation

Within 2 months after the first injection, the occurrence of systemic and local complications (nausea, vomiting, pain, swelling, rash, and hematoma) was recorded. The complication evaluation of pain mainly depends on the VAS scores at 3, 7, 14, 30, and 60 days after the first injection.

Power Calculation and Data Analysis

To calculate the adequate number of knees for the study, we performed a power analysis using software (PASS 20.0; NCSS, Kaysville, UT). A minimum sample size of 87 knees was required (or 29 knees per group) based on a study power of 80% ($\beta = 0.20$), a false-positive rate of 5% ($\alpha = 0.05$), and effect size (Cohen $f = 0.14$) in prior therapy for VAS scores and synovial

thickness versus post-therapy, according to previous studies.[3,34] Predicting a 10% dropout rate, we enrolled approximately 40 knees per group at baseline.

All data were analyzed using SPSS 25.0 software for statistical analysis (IBM Corp., Armonk, NY). All data were normally distributed. All measurement data are expressed as the means ± standard deviation and confidence intervals (CIs). A repeated-measures analysis of variance was used for comparisons between various time points in the same group. A least significant difference (Bonferroni) test or Tamhane's test was used for between-group comparisons. A paired-samples t test was used for pairwise comparisons. The significance level was set at $P = .05$.

Results

In total, 122 knees (78 patients, with 44 patients receiving a bilateral injections) were randomly divided into 3 groups. The follow-up ended on October 1, 2019. The study included 23 male patients and 55 female patients ranging in age from 42 to 79 years with a body mass index between 22.0 and 25.0. Overall, 46 left knees and 76 right knees had a Kellgren–Lawrence grade of II-III, and the duration of joint pain was less than 1 year. Due to additional procedures, 98 knees were excluded from the analysis (Fig 1). No significant differences in baseline characteristics (Table 2) were found among the 3 groups ($P > .05$).

Platelet Density in Whole Blood and PRP

The platelet density was $18.5 \pm 4.5 \times 10^4/\mu L$ in whole blood and $95.0 \pm 17.3 \times 10^4/\mu L$ in PRP (Fig 2 A and B). The platelet density (Fig 2C) in PRP was 5.13 times that in whole blood ($P = .002$).

Knee Pain Score

After 1 month, the VAS score in the HA group (Fig. 3A) decreased from 4.23 ± 0.70 to 2.82 ± 0.83 ($P = .000$), but there was no significant difference after

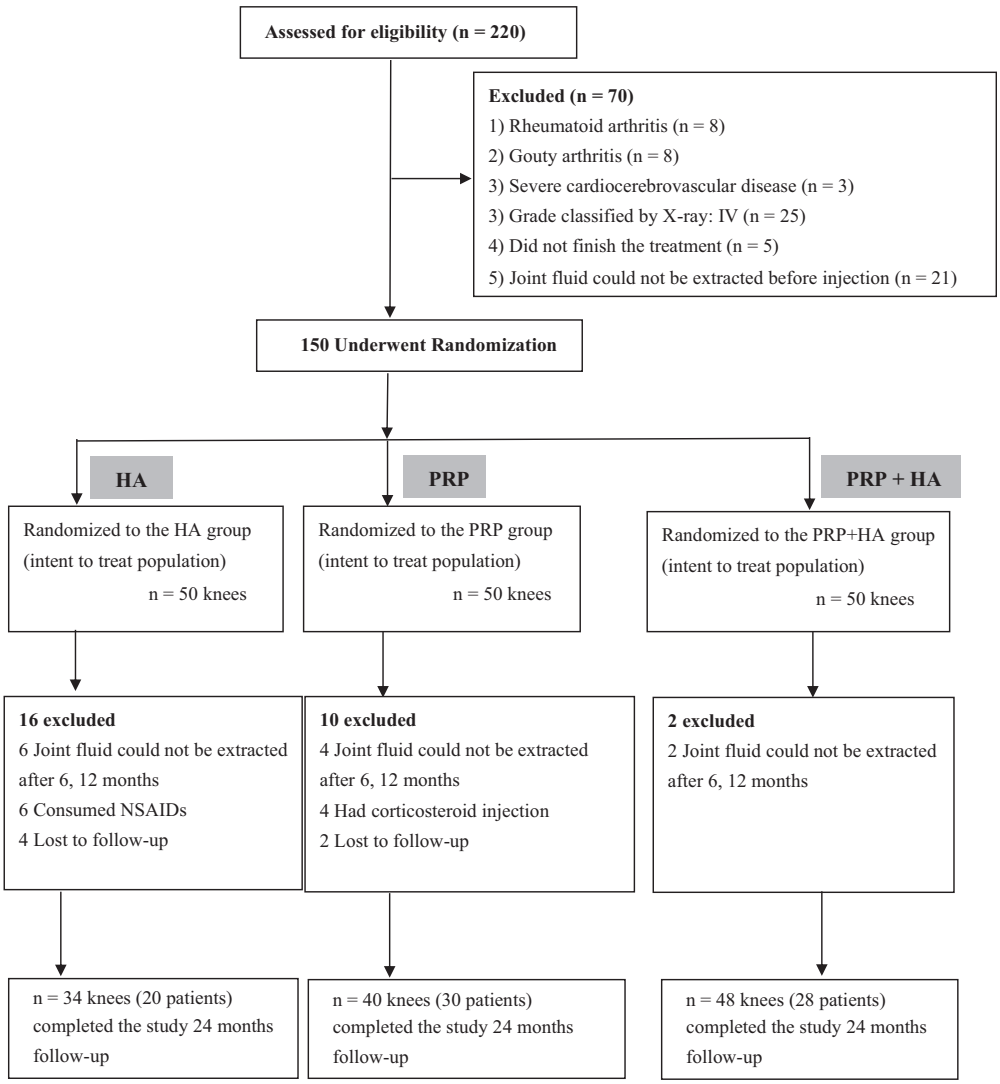

Fig 1. Flow diagram of the clinical trial (HA, hyaluronic acid; NSAID, nonsteroidal anti-inflammatory drug; PRP, platelet-rich plasma.)

Table 2. Baseline Characteristics of the Study Patients in the Three Groups

VVariable	HA	PRP	PRP+HA	*P* Value
Age, y	57.1 ± 3.4	56.9 ± 4.2	57.9 ± 4.1	.64
Sex, male/female	5/15	10/20	8/20	.81
BMI	22.8 ± 2.1	22.5 ± 2.3	21.5 ± 2.5	.12
Ipsilateral, left/right	15/19	11/29	20/28	.26
Duration, mo	10.5 ± 2.0	11.5 ± 2.6	11.1 ± 2.5	.32
Kellgren–Lawrence grade				.63
Grade II	20	19	25	ns
Grade III	14	21	23	ns
Comorbidities				.83
Essential hypertension	3	3	2	ns
Type II diabetes	0	0	2	ns
Coagulopathy	0	0	0	ns
Renal insufficiency	0	0	0	ns
Severe heart disease	0	0	0	ns

NOTE. Data are presented as the means ± standard error (95% confidence interval) unless otherwise indicated.
BMI, body mass index; HA, hyaluronic acid; ns, not significant; PRP, platelet-rich plasma.

6, 12, or 24 months ($P > .05$). After 1 month, the VAS score in the PRP group increased from 4.33 ± 0.66 to 4.85 ± 0.62 ($P = .000$), and significant improvement was noted after 6 and 12 months ($P = .000$; $P = .000$). However, no significant difference was observed after 24 months ($P = .48$). PRP+HA and PRP treatments resulted in better pain scores than HA treatment ($P = .000$). At 24 months after injection, PRP+HA was more effective than HA and PRP alone at relieving pain ($P = .000$). Significant improvement was observed in the PRP+HA group after 1, 6, 12, and 24 months ($P < .001$).

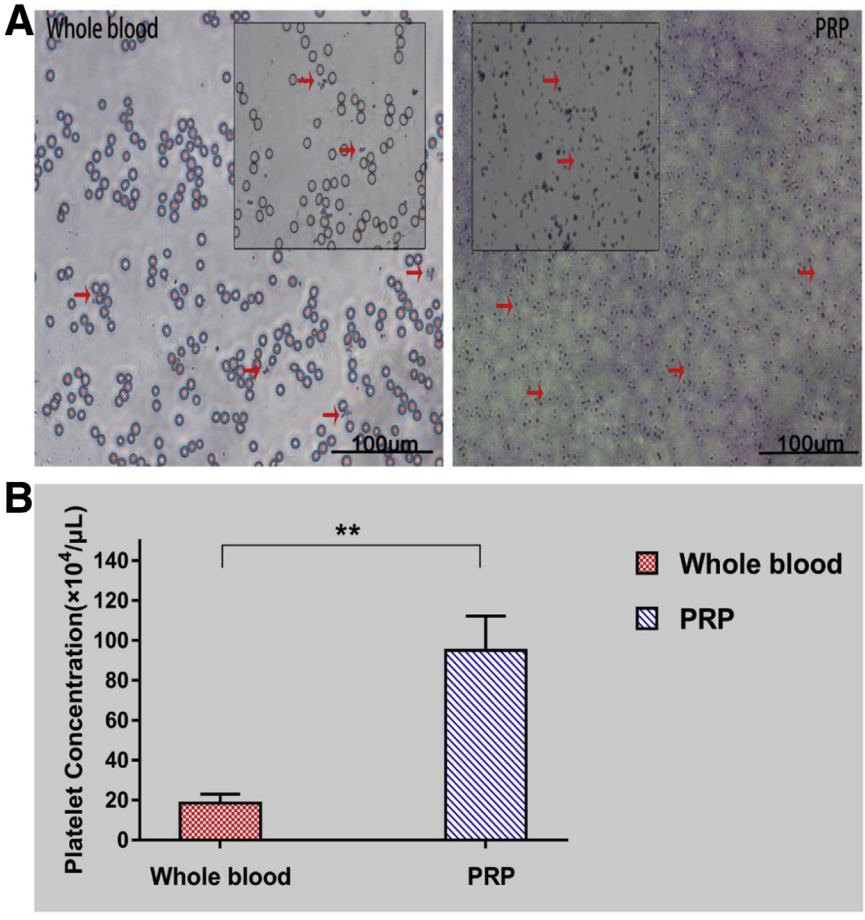

Fig 2. (A) Whole-blood and PRP smears stained using Wright–Giemsa. (B) Platelet densities in whole blood and PRP were determined using a hematology analyzer. The platelet density (arrow) in PRP was 5.13 times that in whole blood ($P = .002$). (PRP, platelet-rich plasma.)

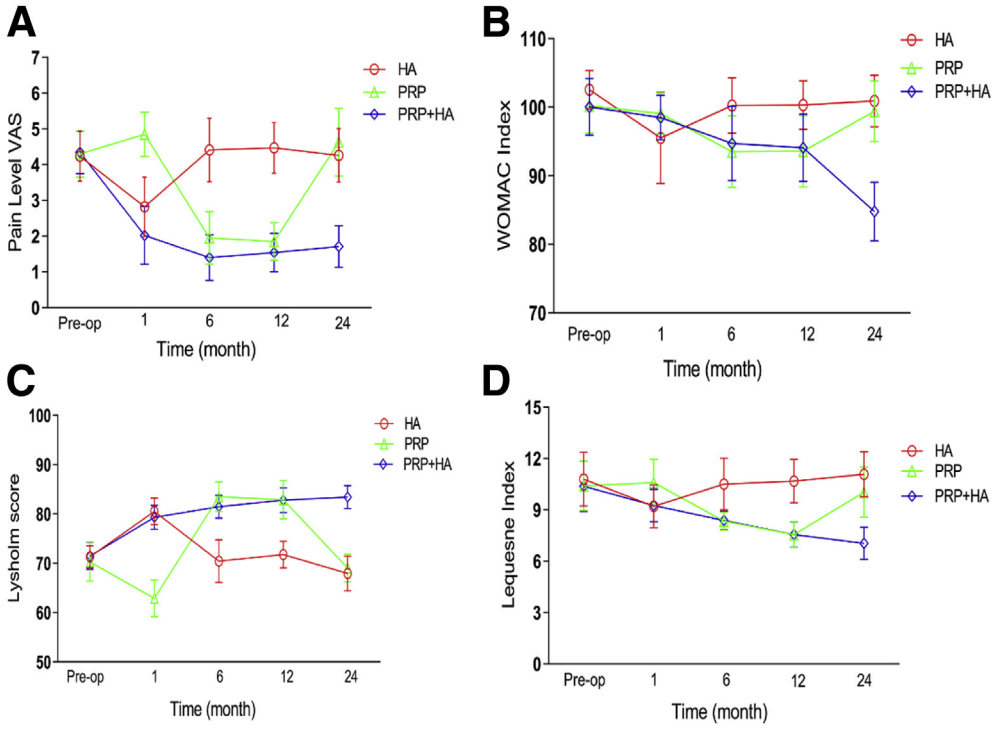

Fig 3. (A, B, C, D) The VAS, WOMAC, Lequesne, and Lysholm scores at each follow-up time point. (HA, hyaluronic acid; PRP, platelet-rich plasma; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.)

Functional Score

At 1 month, significant improvements in the Lysholm, WOMAC, and Lequesne scores were noted (Fig 3 B-D) in the HA group ($P = .000$; $P = .000$; $P = .000$, respectively), whereas at 6, 12, and 24 months after the injection, the functional scores were not better than the preoperative scores ($P > .05$). At 6 and 12 months after the injection, the PRP and PRP+HA groups had significantly better functional scores than the HA group ($P < .001$). The PRP+HA group had better functional scores than the PRP group after 24 months ($P = .000$).

Cartilage, Synovial Thickness, and Effusion Changes

Six months after injection, the synovial thicknesses (Fig 4A) of the medial and lateral femoral condyles were significantly improved in the PRP+HA and PRP

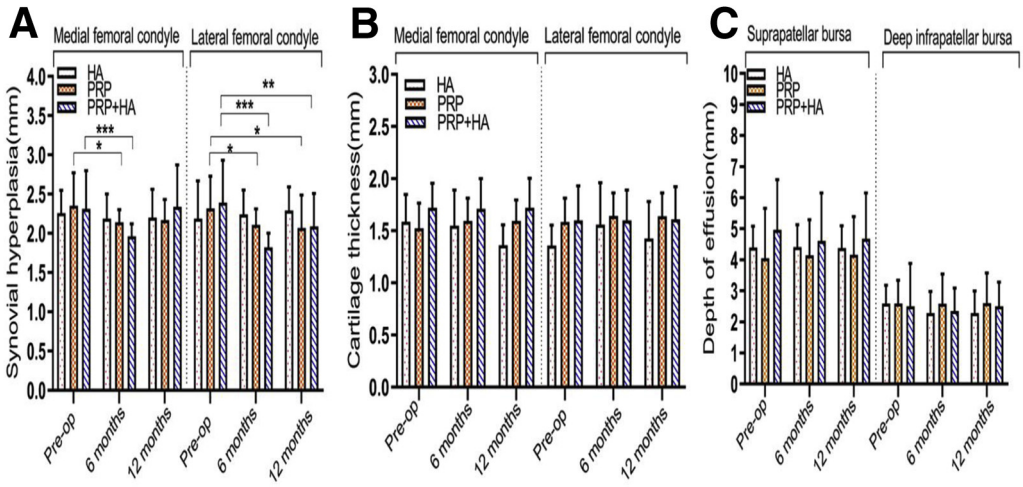

Fig 4. (A) High-frequency color Doppler imaging results demonstrated an improvement in synovial hyperplasia of the medial and lateral femoral condyle in the PRP and PRP+HA groups 6 months after injection ($P < .05$), with more obvious improvement in the PRP+HA group ($P < .05$). (B) No significant change in the thickness of the medial or lateral femoral condyle cartilage was observed in the 2 groups ($P > .05$). (C) No significant change in the depth of effusion in the suprapatellar bursa or deep infrapatellar bursa was observed in the 3 groups ($P > .05$). (HA, hyaluronic acid; PRP, platelet-rich plasma.)

groups ($P < .05$). At 12 months, the synovial thickness of the lateral femoral condyle was partly improved in the PRP+HA group ($P < .05$). The improvement effect of PRP+HA was more obvious than that of PRP ($P < .05$). In addition, no significant changes in the thickness of the medial or lateral femoral condyle cartilage (Fig 4B) or in the depth of effusion in the suprapatellar bursa or deep infrapatellar bursa (Fig 4C) were observed in the 3 groups ($P > .05$).

PSV, EDV, S/D, and RI Changes

Six months after injection, the synovial PSV (from 6.88 [95% CI 6.19-7.56] to 5.42 [95% CI 4.89-5.94]; $P = .003$), EDV (from 4.44 [95% CI 3.89-4.99] to 2.52 [95% CI 2.29-2.75]; $P = .000$), S/D (from 1.74 [95% CI 1.57-1.92] to 2.30 [95% CI 2.06-2.54]; $P = .001$), and RI (from 0.37 [95% CI 0.32-0.42] to 0.51 [95% CI 0.47-0.56]; $P = .000$) values (Fig 5 A-D) of the medial condyle had improved significantly, and the lateral synovial blood flow values (PSV, EDV, S/D, and RI) were also significantly improved in the PRP+HA group ($P < .05$). The synovial blood flow values (PSV, EDV, S/D, and RI) of the medial and lateral condyles had improved significantly in the PRP group ($P < .05$). After 12 months, the synovial PSV (from 6.17 [95% CI 5.57-6.76] to 5.11 [95% CI 4.74-5.49]; $P = .011$), EDV (from 4.16 [95% CI 3.72-4.59] to 3.32 [95% CI 3.07-3.57]; $P = .004$), S/D (from 1.56 [95% CI 1.41-1.71] to 1.95 [95% CI 1.74-2.16]; $P = .010$), and RI (from 0.31 [95% CI 0.27-0.36] to 0.42 [95% CI 0.36-0.47]; $P = .013$) values of the lateral condyle in the PRP+HA group had improved ($P < .05$).

Complications

Joint swelling was measured 1 cm proximal of the base of the patella with a tape measure. Pain after injection was evaluated using VAS scores by video phone to patients. No systemic complications including nausea and vomiting were found in any of the 78 patients. No increased pain after injection was found in the HA group, 5 cases in the PRP group, and 2 cases in the PRP+HA group. The local complications of all 122 knees in the PRP group were higher than in the HA and PRP+HA groups ($P = .008$), and no significant differences were identified between the HA and PRP+HA groups ($P = 1.00$) (Table 3).

MMP-3, TIMP-1, IL-1β, and TNF-α Changes

Six months after injection, IL-1β (from 59.71 [95% CI 57.37-62.06] to 51.41 [95% CI 49.33- 53.49]; $P = .000$), TNF-α (from 174.89 [95% CI 155.97-193.81] to 143.53 [95% CI 133.11-153.95]; $P = .014$), MMP-3 (from 171.59 [95% CI 160.05-183.13] to 151.38 [95% CI 142.99-159.78]; $P = .018$), and TIMP-1 (from 3282.67 [95% CI 3169.78-3395.57] to 2935.91 [95% CI 2754.44-3117.37]; $P = .005$) were decreased in the

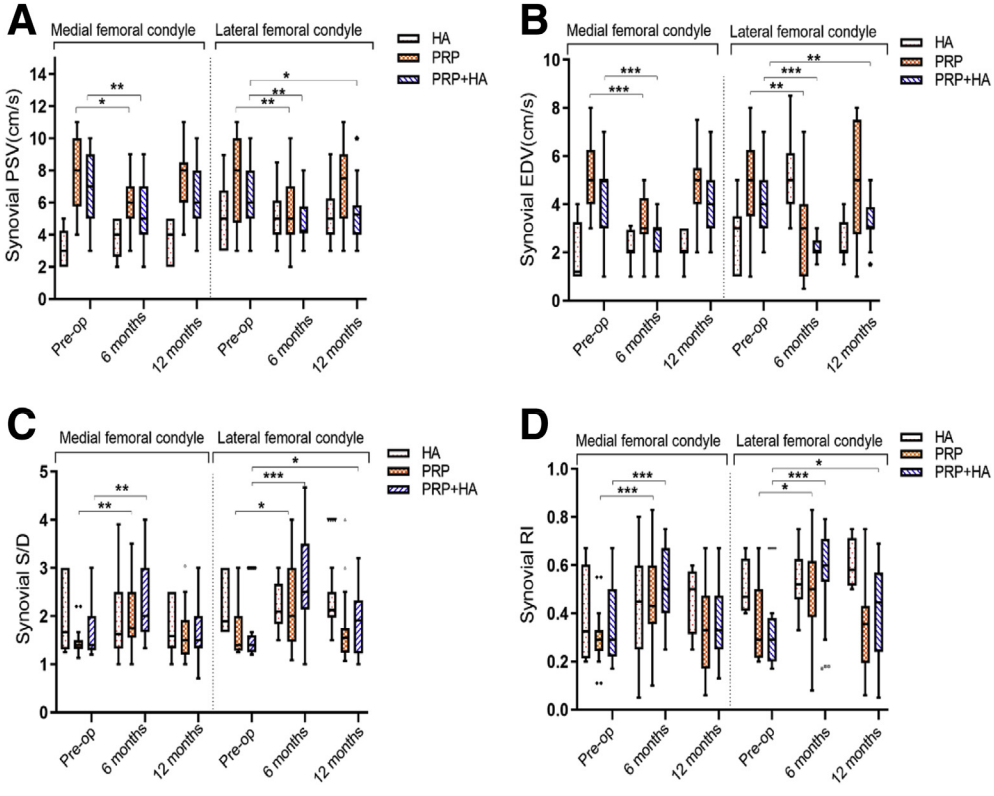

Fig 5. (A, B) No significant change in the synovial PSV or EDV values of the medial and lateral condyles was observed in the HA group ($P > .05$). After 6 months, the synovial PSV and EDV values of the medial and lateral femoral condyle in the PRP+HA and PRP groups decreased significantly ($P < .05$). (C, D) Significant increases in the S/D and RI of the medial and lateral condyle were observed in the PRP and PRP+HA groups ($P < .05$). At 12 months, the synovial PSV, EDV, S/D, and RI of the lateral condyle were significantly improved in the PRP+HA group ($P < .05$). Boxes indicate the 25% and 75% percentiles, whiskers indicate the minimum to maximum values, and bars indicate the median. (EDV, end-diastolic velocity; HA, hyaluronic acid; PRP, platelet-rich plasma; PSV, peak systolic velocity; RI, resistance index; S/D, systolic/diastolic ratio.)

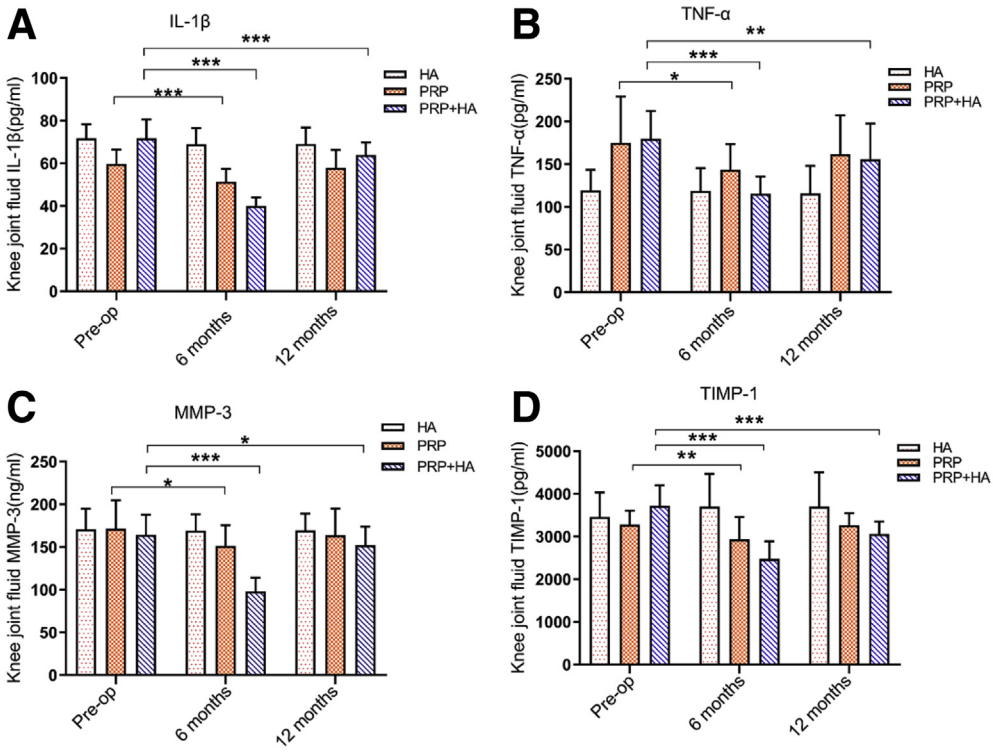

Fig 6. (A, B, C, D) Six months after injection, ELISA showed that postoperative IL-1β, TNF-α, MMP-3, and TIMP-1 levels in synovial fluid were unchanged in the HA group ($P > .05$) and that the IL-1β, TNF-α, MMP-3, and TIMP-1 levels in the PRP and PRP+HA groups were lower than those before injection ($P = .000$). After 12 months, the PRP+HA group still showed inhibition of IL-1β, TNF-α, MMP-3, and TIMP-1 ($P < .05$), and the inhibition was significantly weaker than that before 6 months ($P < .001$). (ELISA, enzyme-linked immunosorbent assay; HA, hyaluronic acid; IL-1β, interleukin-1 β; MMP-3, matrix metalloproteinase-3; PRP, platelet-rich plasma; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-α, tumor necrosis factor-α.)

PRP group (Fig 6 A-D) but were more significantly decreased in the PRP+HA group ($P < 0.001$). At 12 months after injection, the PRP+HA group still showed inhibition of IL-1β (from 71.68 [95% CI 69.06-74.29] to 63.98 [95% CI 62.28-65.68]; $P = .000$), TNF-α (from 179.62 [95% CI 170.18-189.07] to 155.65 [95% CI 143.49-167.81]; $P = .007$), MMP-3 (from 164.31 [95% CI 157.46-171.16] to 152.23 [95% CI 145.95-158.51]; $P = .031$), and TIMP-1 (from 3723.80 [95% CI 3584.88-3862.74] to 3059.15 [95% CI 2974.30-3143.99]; $P = .000$).

Table 3. Comparison of Treatment Complications Among the Three Groups

Complications	HA	PRP	PRP+HA	*P* Value
Infection	0	0	0	
Fever	0	0	0	
Joint swelling	1	4	0	
Pain after injection	0	5	2	
Hematoma	0	0	0	
Rash	0	0	0	
Muscle atrophy	0	0	0	
Venous thrombosis	0	0	0	
Incidence	1/34	9/40	2/48	$P = .008$

NOTE. Comparison of treatment complications among the 3 groups (Fisher exact test = 9.12, $P = .008$). The incidence of complications in the PRP group was greater than that in the HA and PRP+HA groups ($P = .02$; $P = .02$, respectively), and there was no significant difference between the HA group and PRP+HA group ($P = 1.00$).

HA, hyaluronic acid; PRP, platelet-rich plasma.

Discussion

This study demonstrated that PRP combined with HA improved local synovial hyperplasia and blood flow and better inhibited nonbacterial inflammation of the synovium than HA or PRP alone. Furthermore, the combination treatment effectively improved pain and function scores and reduced the incidence of adverse reactions. PRP combined with HA and PRP alone partially reduced the level of inflammatory factors (IFs) and MMPs in the synovial fluid, reflecting the potential therapeutic mechanism of the 2 treatments.

This study showed that HA had short-term clinical efficacy in the treatment of KOA,[35-37] but its 6 months postoperative effect was not as good as those of PRP and PRP combined with HA. Twenty-four months after injection, PRP combined with HA still had a good clinical effect and clear advantages over PRP alone. In recent years, animal experimental studies have shown that PRP combined with HA has a better clinical effect than PRP alone at repairing cartilage defects.[27,28] Especially in cell and animal models in vitro, HA combined with PRP could rescue proinflammatory cytokine-induced degeneration through chondrogenic signaling recovery.[23] Currently, few clinical studies on the treatment of KOA support these basic experiments.[21,24,25] However, excellent results of the PRP+HA association have been reported in the healing of pressure ulcers and surgical wounds and in Morton neuroma surgery,[38-40] but these anecdotal findings need confirmation by controlled trials, and the mechanism of treatment needs to be

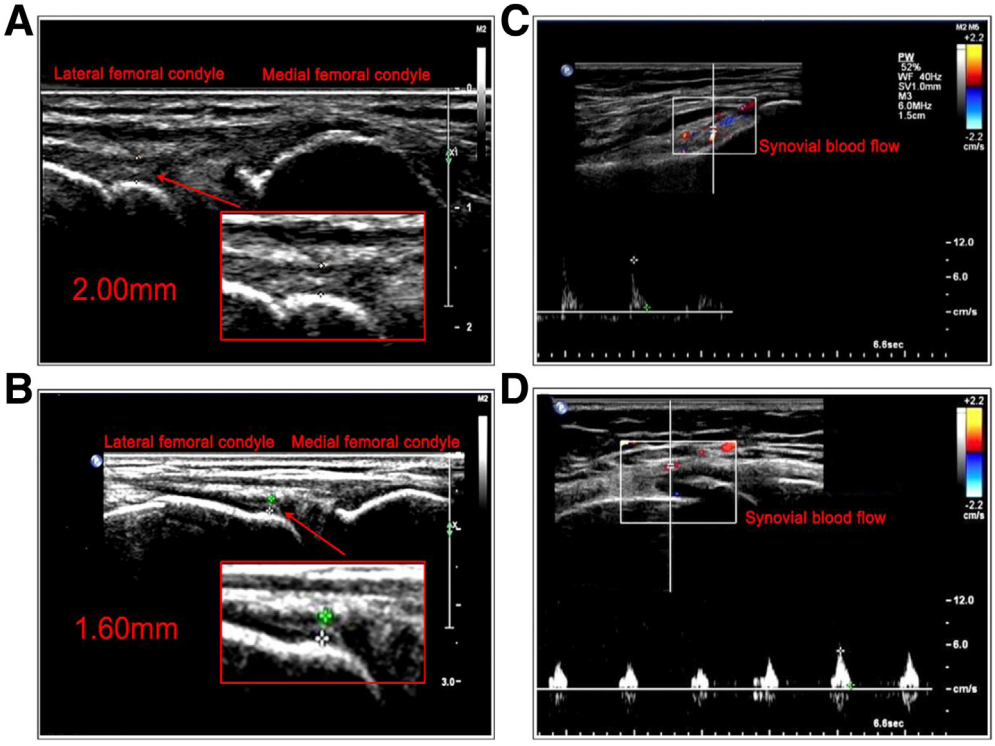

Fig 7. (A, B) High-frequency color Doppler images showing significant improvement of the synovial hyperplasia of the lateral femoral condyle. (C, D) High-frequency color Doppler images demonstrating a decrease in the synovial blood flow volume of combined treatment with platelet-rich plasma and hyaluronic acid.

discussed in depth. The positive result showed that the addition of HA might offer a better environment for cartilage regeneration. Moreover, studies have used HA as a scaffold material for cartilage repair and as a carrier for the adhesion of stem cells.[41] These experiments taken together suggest that the association of HA and PRP can influence and facilitate cell division, migration, and differentiation, which may explain the better clinical effect of PRP combined with HA.

Marx[42] suggested that the platelet density of PRP should be approximately 4～5 times that of whole blood to provide a sufficient platelet reserve for the release of various active biological factors. The PRP used in this study complies with this standard.

In this study, we used high-frequency color Doppler imaging as an objective KOA evaluation method. Although magnetic resonance imaging is a mainstream evaluation method and has unquestionable performance,[43-46] it is expensive and often requires a long wait time for appointments, increasing the psychological and economic burden on patients. In contrast, high-frequency color Doppler imaging reduces the patient wait time as well as the cost of treatment. Moreover, this method can effectively detect synovial hyperplasia, blood flow, and cartilage thickness under the guidance of physicians experienced with ultrasound.[47,48]

Some studies have shown that the synovium plays an important role in the symptoms and structural changes in KOA.[3,48-50] High-frequency color Doppler imaging can detect microvascular flow changes,[51] and this technique was used to demonstrate that both PRP combined with HA and PRP alone can significantly improve synovial hyperplasia of the femoral condyle and decrease the synovial PSV and EDV of the femoral condyle of the knee joint. However, PRP combined with HA was more advantageous for controlling the synovial S/D and RI of the femoral condyle. With high-frequency color Doppler imaging, blood flow in the healthy synovium is very difficult to detect, but inflammatory stimuli can cause blood vessels to dilate. PRP combined with HA or PRP alone can inhibit synovial inflammation. In turn, the dilated synovial blood vessels constrict, their inner diameter decreases, and the blood flow volume inside the blood vessels is reduced. Therefore, when nonbacterial inflammation of the synovium is relieved, the PSV and EDV show a decline to varying degrees, whereas the S/D and RI show increases due to increased blood flow resistance.[52] PRP combined with HA inhibits synovial inflammation more effectively than PRP alone (Fig 7 A-D).

All patients included in this study were diagnosed with KOA in the medial compartment, and the quality of the synovium is closely related to the severity of KOA.[53,54] Some studies have suggested that the medial compartment in KOA bears the most pressure load,[55-58] which leads to lower quality of the synovium of the medial femoral condyle than that of the lateral compartment. The injection of PRP+HA provided a suitable microenvironment for the growth of synovium but did not change the force line of the lower limbs. Thus, the pressure load on the medial compartment

persisted. Although the medial condyle of the femur has been improved to some extent, the quality of the medial condyle is not as good as that of the lateral condyle,[59] which led to a medial to lateral difference 12 months after injection. Because the knee joint has a complex anatomical structure, PRP and HA may not achieve an even distribution inside the joint, and a concentration gradient may occur. This may be another factor underlying the differential improvement in synovial hyperplasia and blood flow in different regions of the same knee joint. The results did not demonstrate significant changes in the cartilage thickness of the femoral condyle or the depth of joint effusion. The present study focused on short-term changes, which may account for the discrepancy in results from previous studies with a longer observation period.[16]

In this study, we found that the incidence of PRP-induced adverse reactions increased significantly, which is not clearly explained at present, but white blood cells may play an important role in the occurrence of adverse reactions because they can stimulate oxidative stress reactions and toxicity of proteolytic enzymes in the knee joint.[20] PRP is rich in platelets, which release bioactive factors that can antagonize the adverse effects of white blood cells; these effects may explain why pain and swelling occur only in the early stage of injection.[60] There have been few clinical controlled trials of PRP combined with HA for KOA, and these studies did not focus on the complications of PRP combined with HA.[21,24,25] Only one controlled experiment, including 360 patients, systematically stated the complications of PRP combined with HA, but its main concern was the complications the treatment-emergent adverse events (hypertension and proteinuria) caused by different doses of PRP and HA.[22] However, this current study focused on the systematic and local complications of PRP combined with HA and that of PRP and HA alone. The combination of PRP and HA significantly reduced the local complications (pain and joint swelling). The reasons for these results are not very clear. HA might weaken the oxidative stress reaction and proteolytic enzymes induced by leukocytes and effectively improve the microenvironment of the knee joint.

This study found that there was variability among preoperative values, and the main reason was the strong correlation between the inflammatory level of the grade of KOA and the pathologic condition of the synovium.[61,62] This study found that PRP and PRP combined with HA could effectively inhibit the level of inflammation, and the inhibition function of PRP combined with HA was more apparent and persistent. Several studies have confirmed that IFs and MMPs play an important role in KOA pathogenesis and progression.[63-65] In particular, IL-1β and TNF-α can promote the expression of MMPs in cartilage and synovial tissue. PRP and PRP combined with HA had an obvious inhibitory effect on IFs and MMP 6 months after injection, because PRP obtained a variety of growth factors and bioactive cells, which could effectively inhibit synovitis and provide a suitable microenvironment for cartilage regeneration. However, the inhibition of PRP combined with HA was more clear, and the inhibition time was longer. The main reason may be that PRP combined with HA can release growth factors and active cells more gently, form a material scaffold of HA,[41] cause PRP to better adhere in the synovium and cartilage, and inhibit the release of IFs and MMPs.

Limitations

This study has several limitations. Some patients' joint fluid could not be extracted 6 months or 12 months after injection and a small proportion of patients consumed nonsteroidal anti-inflammatory drugs or steroids, resulting in different sample sizes of the 3 groups. The main reason for consuming nonsteroidal anti-inflammatory drugs and steroids is that the long-term effect of PRP or HA alone is not as good as that of PRP+HA. In contrast, some enrolled patients had received bilateral injection, which increased the heterogeneity of the population. However, this is also a pragmatic reflection of the outpatient department. Thus, by including patients with bilateral KOA, our study design closely reflects actual clinical practice and further validates the application of our results to a larger clinical patient population.[31] Moreover, the significant differences between the HA, PRP, and PRP+HA groups in the preoperative values of synovial blood flow and IFs have obvious correlations with the grade of KOA and the pathologic condition of the synovium.[61] This is a true reflection of clinical practice, and a random method could not effectively avoid this difference. In addition, PRP combined with HA could inhibit synovitis of the lateral compartment better than that of the medial compartment because of the stress of the medial compartment. Lastly, high-frequency color Doppler imaging was performed by highly experienced physicians; due to practical restrictions, we could not ensure that the examination was completed by the same doctor in each case. The potential lack of accuracy due to different U/S operators might have introduced bias into the experimental data.

Conclusions

PRP combined with HA is more effective than PRP or HA alone at inhibiting synovial inflammation and can effectively improve pain and function and reduce adverse reactions. Its mechanism involves changes in the synovium and cytokine content.

Acknowledgments

The authors thank Zhixu He, Ph.D., and Liping Shu, Ph.D., from the Key Laboratory of Adult Stem Cell Transformation Research, Chinese Academy of Medical Sciences, for their assistance with initial project planning and study design.

References

1. Stewart HL, Kawcak CE. The importance of subchondral bone in the pathophysiology of osteoarthritis. *Front Vet Sci* 2018;5:178.
2. Sakata R, Reddi AH. Platelet-rich plasma modulates actions on articular cartilage lubrication and regeneration. *Tissue Eng Part B Rev* 2016;22:408-419.
3. Ahmad HS, Farrag SE, Okasha AE, et al. Clinical outcomes are associated with changes in ultrasonographic structural appearance after platelet-rich plasma treatment for knee osteoarthritis. *Int J Rheum Dis* 2018;21:960-966.
4. Zhang W. Risk factors of knee osteoarthritis—excellent evidence but little has been done. *Osteoarthritis Cartilage* 2010;18:1-2.
5. Raeissadat SA, Rayegani SM, Forogh B, et al. Intra-articular ozone or hyaluronic acid injection: Which one is superior in patients with knee osteoarthritis? A 6-month randomized clinical trial. *J Pain Res* 2018;11:111-117.
6. Görmeli G, Görmeli CA, Ataoglu B, et al. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: A randomized, double-blind, placebo-controlled trial. *Knee Surg Sports Traumatol Arthrosc* 2017;25:958-965.
7. Jevsevar D, Donnelly P, Brown GA, et al. Viscosupplementation for osteoarthritis of the knee: A systematic review of the evidence. *J Bone Joint Surg Am* 2015;97:2047-2060.
8. Gomoll AH, Filardo G, de Girolamo L, et al. Surgical treatment for early osteoarthritis. Part I: Cartilage repair procedures. *Knee Surg Sports Traumatol Arthrosc* 2012;20:450-466.
9. Bedard NA, Dowdle SB, Anthony CA, et al. The AAHKS Clinical Research Award: What are the costs of knee osteoarthritis in the year prior to total knee arthroplasty. *J Arthroplasty* 2017;32:S8-S10.e1.
10. Cancienne JM, Werner BC, Luetkemeyer LM, et al. Does timing of previous intra-articular steroid injection affect the post-operative rate of infection in total knee arthroplasty. *J Arthroplasty* 2015;30:1879-1882.
11. Bedard NA, Pugely AJ, Elkins JM, et al. The John N. Insall Award: Do intraarticular injections increase the risk of infection after TKA. *Clin Orthop Relat Res* 2017;475:45-52.
12. Qian Y, Han Q, Chen W, et al. platelet-rich plasma derived growth factors contribute to stem cell differentiation in musculoskeletal regeneration. *Front Chem* 2017;5:89.
13. Krüger JP, Ketzmar AK, Endres M, et al. Human platelet-rich plasma induces chondrogenic differentiation of subchondral progenitor cells in polyglycolic acid-hyaluronan scaffolds. *J Biomed Mater Res B Appl Biomater* 2014;102:681-692.
14. Shen J, Gao Q, Zhang Y, et al. Autologous platelet-rich plasma promotes proliferation and chondrogenic differentiation of adipose-derived stem cells. *Mol Med Rep* 2015;11:1298-1303.
15. van Pham P, Bui KH, Ngo DQ, et al. Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage. *Stem Cell Res Ther* 2013;4:91.
16. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: Intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sports Traumatol Arthrosc* 2010;18:472-479.
17. Kanchanatawan W, Arirachakaran A, Chaijenkij K, et al. Short-term outcomes of platelet-rich plasma injection for treatment of osteoarthritis of the knee. *Knee Surg Sports Traumatol Arthrosc* 2016;24:1665-1677.
18. Gobbi A, Karnatzikos G, Mahajan V, et al. Platelet-rich plasma treatment in symptomatic patients with knee osteoarthritis: Preliminary results in a group of active patients. *Sports Health* 2012;4:162-172.
19. Vaquerizo V, Plasencia MÁ, Arribas I, et al. Comparison of intra-articular injections of plasma rich in growth factors (PRGF-Endoret) versus Durolane hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: A randomized controlled trial. *Arthroscopy* 2013;29:1635-1643.
20. Huang G, Hua S, Yang T, et al. Platelet-rich plasma shows beneficial effects for patients with knee osteoarthritis by suppressing inflammatory factors. *Exp Ther Med* 2018;15:3096-3102.
21. Lana JF, Weglein A, Sampson SE, et al. Randomized controlled trial comparing hyaluronic acid, platelet-rich plasma and the combination of both in the treatment of mild and moderate osteoarthritis of the knee. *J Stem Cells Regen Med* 2016;12:69-78.
22. Yu W, Xu P, Huang G, et al. Clinical therapy of hyaluronic acid combined with platelet-rich plasma for the treatment of knee osteoarthritis. *Exp Ther Med* 2018;16:2119-2125.
23. Chen WH, Lo WC, Hsu WC, et al. Synergistic anabolic actions of hyaluronic acid and platelet-rich plasma on cartilage regeneration in osteoarthritis therapy. *Biomaterials* 2014;35:9599-9607.
24. Abate M, Verna S, Schiavone C, et al. Efficacy and safety profile of a compound composed of platelet-rich plasma and hyaluronic acid in the treatment for knee osteoarthritis (preliminary results). *Eur J Orthop Surg Traumatol* 2015;25:1321-1326.
25. Chen SH, Kuan TS, Kao MJ, et al. Clinical effectiveness in severe knee osteoarthritis after intra-articular platelet-rich plasma therapy in association with hyaluronic acid injection: three case reports. *Clin Interv Aging* 2016;11:1213-1219.
26. Saturveithan C, Premganesh G, Fakhrizzaki S, et al. Intra-articular hyaluronic acid (HA) and platelet rich plasma (PRP) injection versus hyaluronic acid (HA) injection alone in patients with Grade III and IV knee osteoarthritis (OA): A retrospective study on functional outcome. *Malays Orthop J* 2016;10:35-40.
27. Marmotti A, Bruzzone M, Bonasia DE, et al. One-step osteochondral repair with cartilage fragments in a

composite scaffold. *Knee Surg Sports Traumatol Arthrosc* 2012;20:2590-s601.
28. Yan W, Xu X, Xu Q, et al. Platelet-rich plasma combined with injectable hyaluronic acid hydrogel for porcine cartilage regeneration: A 6-month follow-up. *Regen Biomater* 2020;7:77-90.
29. Zhao J, Huang H, Liang G, et al. Effects and safety of the combination of platelet-rich plasma (PRP) and hyaluronic acid (HA) in the treatment of knee osteoarthritis: A systematic review and meta-analysis. *BMC Musculoskelet Disord* 2020;21:224.
30. Yin W, Xu H, Sheng J, et al. Optimization of pure platelet-rich plasma preparation: A comparative study of pure platelet-rich plasma obtained using different centrifugal conditions in a single-donor model. *Exp Ther Med* 2017;14:2060-2070.
31. Lin KY, Yang CC, Hsu CJ, et al. Intra-articular injection of platelet-rich plasma is superior to hyaluronic acid or saline solution in the treatment of mild to moderate knee osteoarthritis: A randomized, double-blind, triple-parallel, placebo-controlled clinical trial. *Arthroscopy* 2019;35:106-117.
32. Greis C. Quantitative evaluation of microvascular blood flow by contrast-enhanced ultrasound (CEUS). *Clin Hemorheol Microcirc* 2011;49:137-149.
33. D'Agostino MA, Conaghan P, Le Bars M, et al. EULAR report on the use of ultrasonography in painful knee osteoarthritis. Part 1: Prevalence of inflammation in osteoarthritis. *Ann Rheum Dis* 2005;64:1703-1709.
34. Duymus TM, Mutlu S, Dernek B, et al. Choice of intra-articular injection in treatment of knee osteoarthritis: platelet-rich plasma, hyaluronic acid or ozone options. *Knee Surg Sports Traumatol Arthrosc* 2017;25:485-492.
35. Housman L, Arden N, Schnitzer TJ, et al. Intra-articular hylastan versus steroid for knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc* 2014;22:1684-1692.
36. Lo GH, LaValley M, McAlindon T, et al. Intra-articular hyaluronic acid in treatment of knee osteoarthritis: A meta-analysis. *JAMA* 2003;290:3115-3221.
37. Bellamy N, Campbell J, Robinson V, et al. Viscosupplementation for the treatment of osteoarthritis of the knee. *Cochrane Database Syst Rev* 2006;2:CD005321.
38. Ramos-Torrecillas J, García-Martínez O, De Luna-Bertos E, et al. Effectiveness of platelet-rich plasma and hyaluronic acid for the treatment and care of pressure ulcers. *Biol Res Nurs* 2015;17:152-158.
39. Cervelli V, Lucarini L, Spallone D, et al. Use of platelet-rich plasma and hyaluronic acid in the loss of substance with bone exposure. *Adv Skin Wound Care* 2011;24:176-181.
40. De Angelis B, Lucarini L, Orlandi F, et al. Regenerative surgery of the complications with Morton's neuroma surgery: Use of platelet rich plasma and hyaluronic acid. *Int Wound J* 2013;10:372-376.
41. Wang AT, Zhang QF, Wang NX, et al. Cocktail of hyaluronic acid and human amniotic mesenchymal cells effectively repairs cartilage injuries in sodium iodoacetate-induced osteoarthritis rats. *Front Bioeng Biotechnol* 2020;8:87.
42. Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 2004;62:489-496.
43. Zhang M, Driban JB, Price LL, et al. Development of a rapid knee cartilage damage quantification method using magnetic resonance images. *BMC Musculoskelet Disord* 2014;15:264.
44. Maschek S, Wirth W, Ladel C, et al. Rates and sensitivity of knee cartilage thickness loss in specific central reading radiographic strata from the osteoarthritis initiative. *Osteoarthritis Cartilage* 2014;22:1550-1553.
45. Alizai H, Roemer FW, Hayashi D, et al. An update on risk factors for cartilage loss in knee osteoarthritis assessed using MRI-based semiquantitative grading methods. *Eur Radiol* 2015;25:883-893.
46. Roemer FW, Guermazi A, Trattnig S, et al. Whole joint MRI assessment of surgical cartilage repair of the knee: cartilage repair osteoarthritis knee score (CROAKS). *Osteoarthritis Cartilage* 2014;22:779-799.
47. Sarmanova A, Hall M, Moses J, et al. Synovial changes detected by ultrasound in people with knee osteoarthritis—a meta-analysis of observational studies. *Osteoarthritis Cartilage* 2016;24:1376-1383.
48. Sarmanova A, Hall M, Fernandes GS, et al. Association between ultrasound-detected synovitis and knee pain: A population-based case-control study with both cross-sectional and follow-up data. *Arthritis Res Ther* 2017;9:281.
49. Hügle T, Geurts J. What drives osteoarthritis? Synovial versus subchondral bone pathology. *Rheumatology (Oxford)* 2017;56:1461-1471.
50. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: Latest findings and interpretations. Ther Adv Musculoskelet *Dis* 2013;5:77-94.
51. Oo WM, Linklater JM, Bennell KL, et al. Superb microvascular imaging in low-grade inflammation of knee osteoarthritis compared with power Doppler: Clinical, radiographic and MRI relationship. *Ultrasound Med Biol* 2020;46:566-574.
52. Ren JH, Guo FJ, Han XJ, et al. Assessment of synovitis in the knee of patients with newly-diagnosed rheumatoid arthritis using high frequency and color Doppler ultrasonography. *Zhonghua Yi Xue Za Zhi* 2011;91:1630-1632.
53. Ene R, Sinescu RD, Ene P, et al. Synovial inflammation in patients with different stages of knee osteoarthritis. *Rom J Morphol Embryol* 2015;56:169-173.
54. Mathiessen A, Conaghan PG. Synovitis in osteoarthritis: Current understanding with therapeutic implications. *Arthritis Res Ther* 2017;19:18.
55. Kim KI, Seo MC, Song SJ, et al. Change of chondral lesions and predictive factors after medial open-wedge high tibial osteotomy with a locked plate System. *Am J Sports Med* 2017;45:1615-1621.
56. Jung WH, Takeuchi R, Chun CW, et al. Second-look arthroscopic assessment of cartilage regeneration after medial opening-wedge high tibial osteotomy. *Arthroscopy* 2014;30:72-79.
57. Yang ZY, Chen W, Li CX, et al. Medial compartment decompression by fibular osteotomy to treat medial compartment knee osteoarthritis: A pilot study. *Orthopedics* 2015;38:e1110-1104.
58. Baldini T, Roberts J, Hao J, et al. Medial compartment decompression by proximal fibular osteotomy: A biomechanical cadaver study. *Orthopedics* 2018;41:e496-e501.

59. Niemeyer P, Schmal H, Hauschild O, et al. Open-wedge osteotomy using an internal plate fixator in patients with medial-compartment gonarthritis and varus malalignment: 3-year results with regard to preoperative arthroscopic and radiographic findings. *Arthroscopy* 2010;26:1607-1616.
60. Louis ML, Magalon J, Jouve E, et al. Growth factors levels determine efficacy of platelets rich plasma injection in knee osteoarthritis: A randomized double blind noninferiority trial compared with viscosupplementation. *Arthroscopy* 2018;34:1530-1540.e2.
61. Scanzello CR. Role of low-grade inflammation in osteoarthritis. *Curr Opin Rheumatol* 2017;29:79-85.
62. Wang SL, Zhang R, Hu KZ, et al. Interleukin-34 synovial fluid was associated with knee osteoarthritis severity: A cross-sectional study in knee osteoarthritis patients in different radiographic stages. *Dis Marker* 2018;2018: 2095480.
63. van der Goes M, Jacobs J, Bijlsma J. Rediscovering the therapeutic use of glucocorticoids in rheumatoid arthritis. *Curr Opin Rheumatol* 2016;28:289-296.
64. Wang J. Efficacy and safety of adalimumab by intra-articular injection for moderate to severe knee osteoarthritis: An open-label randomized controlled trial. *J Int Med Res* 2018;46:326-334.
65. Lin J, Li X, Qi W, et al. Isofraxidin inhibits interleukin-1β induced inflammatory response in human osteoarthritis chondrocytes. *Int Immunopharmacol* 2018;64: 238-245.