VIA MEDICA

ORIGINAL PAPER

Folia Histochem Cytobiol
Vol. 62, No. 3, 2024, pp. 154–163

DOI: 10.5603/fhc.100179
ISSN 0239-8508, e-ISSN 1897-5631

Platelet-rich plasma ameliorates cartilage degradation in rat models of osteoarthritis *via* the OPG/RANKL/RANK system

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ABSTRACT

Introduction. Osteoarthritis (OA) is one of the most common degenerative joint diseases in the elderly, which is featured by the degradation of articular cartilage. Recently, platelet-rich plasma (PRP) injection into the affected joint has become one biological therapy for OA treatment. The OPG/RANKL/RANK signalling has been reported to mediate OA progression. Our study aimed to confirm whether PRP injection retards OA development through the regulation of the OPG/RANKL/RANK system.

Material and methods. The OA rat models were induced by medial menisci resection combined with anterior cruciate ligament transection. Four weeks after surgery, OA-induced rats received intra-articular injection with 50 μL PRP once a week for 6 weeks. Rats were euthanised one week after the 6th injection. Rat knee joints were subjected to histopathological examination by haematoxylin-eosin (H&E) and safranin O staining. Osteoprotegerin (OPG), receptor activator of nuclear factor kappa B (RANK), and RANK ligand (RANKL) in the articular cartilage of rats were tested through immunofluorescence staining and western blotting. Serum interleukin-1β (IL-1β) and interleukin-6 (IL-6) levels were measured by enzyme-linked immunosorbent assay (ELISA). Matrix metalloproteinase-13 (MMP-13), aggrecan, collagen α, IL-1β, IL-6, tumour necrosis factor-alpha (TNF-α), and nuclear factor kappa-B (NF-κB) mRNA and protein expression in rat articular cartilage were examined by real-time quantitative polymerase chain reaction (RT-qPCR) and western blotting, respectively.

Results. Intra-articular injections of PRP significantly improved the structural integrity of the articular cartilage and enhanced the synthesis of glycosaminoglycans. PRP reduced MMP-13 protein level but increased aggrecan and collagen α protein levels in articular cartilage of OA rats. OA-induced increase in serum IL-1β, IL-6, and TNF-α concentrations as well as increased MMP-13, and decreased collagen II mRNA levels were reversed by the administration of PRP. OA increased IL-1β, TNF-α, and NF-κB mRNA expression in rat articular cartilage whereas PRP administration ameliorated these changes. Moreover, in the articular tissue of OA-induced rats the increased OPG protein level was further elevated by PRP injections whereas the protein level of RANK did not change. The increase in the protein level of RANKL in OA-induced articular tissue was offset by PRP administration. PRP elevated OPG mRNA expression and the OPG/RANKL mRNA ratio, but reduced RANKL mRNA expression and the RANKL/RANK mRNA ratio in the articular tissue of OA-induced rats.

Conclusions. PRP mitigates cartilage degradation and inflammation in experimental knee OA by regulating the OPG/RANKL/RANK signalling system.

Keywords: platelet-rich plasma; osteoarthritis; cartilage degradation; inflammation; extracellular matrix; OPG/RANKL/RANK system

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Submitted:
9 April, 2024
Accepted after reviews:
2 August, 2024
Available as Online first:
14 August, 2024

INTRODUCTION

Osteoarthritis (OA) is one of the most common degenerative joint diseases in the elderly, and severe OA can lead to disability in patients [1]. The degradation of articular cartilage is a hallmark of OA pathogenesis, which is a major reason for chronic pain and dysfunction in affected joints [2]. The progressive loss of articular cartilage can be caused by an imbalance in extracellular matrix (ECM) homeostasis [3]. Chondrocytes are the only cell type of cartilage, which are responsible for the synthesis and renewal of the ECM as well as the maintenance of matrix integrity and homeostasis [4]. When chondrocyte metabolic activity is increased, chondrocyte matrix synthesis is accelerated, which further induces a synovial inflammatory response and increases the release of lysosomal enzymes required for cartilage degradation, such as matrix metalloproteinases (MMPs), leading to a decrease in the amount of type II collagen in cartilage and ultimately joint cartilage erosion and destruction [5, 6]. Current treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) can only relieve the associated symptoms but cannot reverse the course of the disease [7]. This highlights the urgent need to develop new effective interventions to combat cartilage degeneration.

Over the past dozen years, a treatment called platelet-rich plasma (PRP) has emerged in the field of medical research and has subsequently become popular treatment in professional sports and athletics [8]. PRP is obtained from whole blood by gradient centrifugation and contains 3 or more times the concentration of platelets than regular whole blood [9]. Platelets contain more than 800 proteins and molecules, including cytokines, chemokines, membrane proteins, metabolites, messenger molecules, growth factors, and many other factors [10]. In addition to their role in coagulation and haemostasis, platelets are involved in vasoconstriction, inflammation, immune response, angiogenesis, and tissue regeneration [11]. Accordingly, they are involved in many physiological signalling mechanisms and are associated with a variety of pathologies [12]. As suggested by basic science and clinical research, PRP injections into the affected joint have become one biologic therapy for OA treatment [13]. PRP promotes the repair of damaged articular cartilage through various bioactive factors and proteins in the plasma released after activation [14]. In addition, PRP was reported to stimulate mesenchymal stem cell proliferation, migration, and differentiation into articular chondrocytes, thereby modulating the repair and regeneration of damaged articular cartilage and delaying cartilage degeneration [15].

Bone remodeling, especially subchondral bone remodeling, is tightly controlled by a molecular triad consisting of osteoprotegerin (OPG), receptor activator of nuclear factor kappa B (RANK), and RANK ligand (RANKL) [16]. RANK, which belongs to the tumour necrosis factor (TNF) receptor family, is expressed on mature osteoclasts and osteoclast progenitors and can be activated by cell membrane-anchored or soluble RANKL [17]. RANKL, also called OPG ligand (OPGL), is expressed by osteoblasts, which plays a vital role in mediating bone resorption by regulating osteoclastogenesis and activating mature osteoclasts [18]. RANKL binds to the cell surface receptor RANK, thereby stimulating osteoclastogenesis and osteoclast activity [19]. OPG is secreted as a soluble decoy receptor by osteoblasts and bone marrow stromal cells. OPG prevents RANK activation and subsequent osteoclastogenesis through inhibiting the interaction of RANKL-RANK [20]. Numerous studies have suggested that the imbalance of the OPG/RANKL/RANK system can result in the collapse of cartilage and subchondral bone and cartilage degradation, which is closely associated with the pathogenesis of OA [21, 22].

Based on the aforementioned literature, our study aimed to investigate whether PRP prevents cartilage degeneration and hinders OA progression through regulating the OPG/RANKL/RANK signalling. The effects of PRP intra-articular injection on the cartilage matrix metabolism, inflammatory response, and OPG, RANKL, and RANK expression in OA rat models were evaluated. Our findings might reveal a novel mechanism through which PRP exerts its therapeutic effects in treating OA in the clinic.

MATERIALS AND METHODS

Animals

Twenty-eight male Sprague-Dawley (SD) rats (8 weeks old, 250–300 g) purchased from SLAC Laboratory Animal Company (Shanghai, China) were used in the current investigation. Animals were maintained in individually ventilated cages under specific pathogen-free (SPF) conditions, with free access to standard laboratory chow and water *ad libitum*. All animal experimental procedures were approved by the Animal Care and Use Committee of the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology.

PRP preparation

Based on the method of Asjid *et al.* [23], PRP was prepared from rat blood by using the double spin method. In brief, blood samples were collected from 10 healthy SD rats by heart puncture exsanguinations under sterile conditions after anaesthesia with 3% pentobarbital sodium (30 mg/kg) and stored in tubes with anticoagulant (3.8% sodium citrate). To separate red blood cells, white blood cells, and platelet cells, blood samples were centrifuged at 1000 rpm for 10 min. The upper part of the supernatant, equivalent to plasma and platelets, was collected into new tubes, which were further centrifuged at 2000 rpm for 10 min. The supernatant was

disposed, and only the lower 20% of the plasma was reaped as PRP or plasma rich in platelets. Then, the PRP considered suitable for the study was produced through resuspension of the residual material including the platelet pellet. The prepared PRP was used within 6 h. Before being intra-articularly injected into rats, PRP was activated by mixing it with 10% calcium gluconate (LABiTec GmbH, Ahrensburg, Germany) at a dilution of 1:9.

Establishment of OA rat model

Eighteen SD rats were randomised into 3 groups (n = 6/group): Control, OA, and OA + PRP groups. The OA model was induced by medial menisci resection combined with anterior cruciate ligament transection as previously described [24, 25]. Briefly, rats were anaesthetised *via* intraperitoneal injection of ketamine (80 mg/kg) and xylazine (8 mg/kg), and a longitudinal medial parapatellar incision was made in both knees of each rat. Patellar dislocation was performed, followed by full flexion of the knee to expose the joint cavity. The anterior cruciate ligament was exposed after excising the synovial membrane and bending the knee joint, and it was then transected. A surgical scissor was used to completely remove the medial meniscus. The patella was relocated back to its original position post-surgery. After irrigating the knee with physiological saline, the articular capsule and skin were closed with a 4–0 nylon suture. For the sham-operated group, the knee joint cartilage surface was exposed, and the wounds were then sutured, but without ligament transection or meniscectomy. Four weeks after surgery, rats in the OA + PRP group received intra-articular injection with 50 μL PRP once a week for 6 weeks. At the same time, rats in the sham and OA groups received intra-articular injection of 50 μL physiological saline. Rats were euthanised one week after the sixth injection, the blood samples were collected from the jugular vein, and the joint tissues were obtained for further examination.

Histological stainings and pathological assessment

The knee joints were immersion-fixed in 4% paraformaldehyde for 24 h before being embedded in paraffin and sectioned (5 μm thickness) for histology. The paraffin sections were deparaffinised in xylene and rehydrated using gradient alcohol, followed by H&E and safranin O staining using the corresponding kits (Solarbio, Beijing, China). Representative changes were photographed by light microscopy (Leica Microsystems, Wetzlar, Germany) and assessed by experienced pathologists who were blinded to the experimental treatment conditions and the assignment of the animals. The pathological scores were calculated based on the Osteoarthritis Research Society International (OARSI) system [26].

Immunofluorescence staining

After deparaffinisation and hydration, the paraffin-embedded slides of rat articular cartilage were boiled in a pressure cooker containing 6.5 mmol/L sodium citrate (pH 6.0) for epitope retrieval. Afterwards, the slides were permeabilised with 0.2% Triton X-100, washed with phosphate-buffered saline (PBS), and blocked with 10% normal donkey serum to eliminate the nonspecific fluorescence, followed by incubation with the primary antibodies against OPG (ab73400; 5 μg/mL; Abcam), RANK (KL-2695R; 1:200; Shanghai Kanglang Biotechnology Co., Ltd, Shanghai, China), and RANKL (66610-1-Ig; 1:400; Proteintech) overnight at 4°C in a humidity box and with the fluorescence-labelled secondary antibodies for 1 h at room temperature. 4′,6-diamidino-2-phenylindole (DAPI) was used for the counter-staining of nuclei. All sections were observed under an automatic fluorescence microscope (Olympus, Tokyo, Japan) and analysed by Image-Pro Plus 6.0 (Media Cybernetics, Rockville, MD, USA).

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were centrifuged at 2000 rpm and 4°C for 10 min. The supernatant was collected and stored at –80°C. Serum interleukin-1β (IL-1β; #RLB00) and interleukin-6 (IL-6; #R6000B) levels were measured by using the corresponding ELISA kits (R&D systems, Los Angeles, CA, USA) following the operation manuals.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Rat articular cartilage tissues were ground in liquid nitrogen, and TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was then added for total RNA extraction. The PrimeScript RT reagent kit with gDNA Eraser (Takara, Dalian, China) was used to obtain the complementary DNA (cDNA). RT-qPCR was conducted using a TB Green Premix Ex Taq II (Takara) with a PIKORed 96 RT-PCR detection system (Thermo Fisher Scientific, Waltham, MA, USA) under the following thermocycling conditions: Initial denaturation at 94°C for 5 min was followed by 40 cycles of denaturation at 94°C for 20 sec, annealing at 65°C for 20 sec, and elongation at 70°C for 30 sec. Relative gene expression levels were determined using the comparative $2^{-\Delta\Delta Cq}$ method with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control. The primer sequences used in our study are listed in Table 1.

Western blotting

The articular cartilage tissues of rats were homogenised in radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) containing a protease inhibitor, and the protein concentrations were determined by Bicinchoninic Acid Protein Assay kit (Beyotime).

Table 1. Primer sequences used for RT-qPCR

Gene	Primer sequences
MMP-13	Forward: 5′-TTTCCTCCTGGACCAAACC-3′ Reverse: 5′-AGTTGTAGCCTTTGGAGCT-3′
Collagen II	Forward: 5′-GCACCCATGGACATTGGAGG-3′ Reverse: 5′-AGCCCCGCACGGTCTTGCTT-3′
IL-1β	Forward: 5′-AAACAGATGAAGTGCTCCTTCCAGG-3′ Reverse: 5′-TGGAGAACACCACTTGTTGCTCCA-3′
TNF-α	Forward: 5′-CGATGAACCACGCCAGTCGCC-3′ Reverse: 5′-GGATGAACACGCCAGTCGCC-3′
NF-κB	Forward: 5′-CGACGTATTGCTGTGCCTTC-3′ Reverse: 5′-TTGAGATCTGCCCAGGTGGTA-3′
OPG	Forward: 5′-GCTTGAAACATAGGAGCTG-3′ Reverse: 5′-GTTTACTTTGGTGCCAGG-3′
RANK	Forward: 5′-ATGCGGTTTGCAGTTCTTCTC-3′ Reverse: 5′-ACTCCTTATCTCCACTTAGG-3′
RANKL	Forward: 5′-CACTATTAATGCCACCGAC-3′ Reverse: 5′-GGGTATGAGAACTTGGGATT-3′
GAPDH	Forward: 5′-AACTCCCATTCTTCCACCT-3′ Reverse: 5′-TTGTCATACCAGGAAATGAGC-3′

Abbreviations: GAPDH — glyceraldehyde 3-phosphate dehydrogenase; IL-1β — interleukin-1β; MMP-13 — Matrix metalloproteinase-13; NF-κB — nuclear factor kappa-B; OPG — osteoprotegerin; TNF-α — tumour necrosis factor-alpha.

Thereafter, proteins (20 μg) were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA) after separation by 10% sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE). Subsequently, the membranes were blocked by 5% non-fat milk dissolving in tris-buffered saline containing 0.1% Tween 20 (TBST) for 2 h at room temperature, followed by incubation with primary antibodies against MMP-13 (18165-1-AP; Proteintech, Rosemont, IL, USA), collagen II (28459-1-AP; Proteintech), aggrecan (13880-1-AP; Proteintech), OPG (ab73400; Abcam, Cambridge, UK), RANK (ab305233; Abcam), and RANKL (23408-1-AP; Proteintech) at the dilution of 1:1000. The next day, the membranes were washed thrice by TBST (5 min each time) and incubated with goat anti-rabbit secondary antibodies (Abcam) for 1 h at room temperature. The protein bands were scanned using the Odyssey Infrared Imaging System (Licor, Lincoln, NE, USA), and the relative band intensity was quantified by Quantity One 4.6.2 software (Bio-Rad, Hercules, CA, USA). All values were normalised to GAPDH.

Statistical analysis

Results were analysed by GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego CA, USA) and presented as mean ± standard deviation (SD). All data were tested for normal distribution using the Shapiro-Wilk test. The significance of differences was determined with Student's *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's test. $P < 0.05$ was considered statistically significant.

RESULTS

PRP ameliorates symptoms of OA in rats

To investigate the potential effects of PRP on OA, an OA rat model was constructed by medial meniscectomy and anterior cruciate ligament transection of both knee joints. After 4 weeks, the rats were intraarticularly injected with PRP for 6 consecutive weeks. The knee joints of rats were harvested for histopathological staining. As shown by H&E staining, the articular cartilage possessed a regular morphological structure in the Control group. In contrast, the OA group exhibited irregular articular cartilage surface, badly eroded articular cartilage, and thinner cartilage matrix. Intra-articular injection of PRP significantly improved the structural integrity of the articular cartilage, as shown by increased thickness of articular cartilage and reduced severity of lesions and osteophytes (Fig. 1A). Safranin O staining revealed that the OA group had a markedly lower content of glycosaminoglycans than the Control group, while intra-articular injection of PRP evidently enhanced the synthesis of glycosaminoglycans (Fig. 1A). Furthermore, the OARSI score was considerably lower in the OA + PRP group than in the OA group, suggesting that PRP mitigated cartilage damage in OA (Fig. 1B).

PRP alleviates the degradation of articular cartilage in OA rat models

Articular cartilage degradation is a hallmark of OA pathogenesis, which can be caused by excessive loss of cartilage extracellular matrix (ECM). As shown by western blotting, OA induction led to a marked increase in the articular cartilage MMP-13 protein level and a decrease in collagen type II and aggrecan protein levels in rat articular cartilage, while these changes were partially or totally reversed by intra-articular injection of PRP (Fig. 2). This indicated that PRP alleviated articular cartilage degradation in OA rat model by inhibiting excessive loss of ECM.

PRP mitigates inflammation and improves cartilage matrix metabolism in OA rat models

The expression of key inflammatory cytokines in the serum was measured to evaluate whether PRP plays an anti-inflammatory role in OA rat model. The increased serum IL-1β and IL-6 levels in OA rat model were antagonised by injection of PRP (Fig. 3A, B). There was no difference in IL-6 serum concentrations between the Control and OA + PRP groups. As manifested by RT-qPCR analysis,

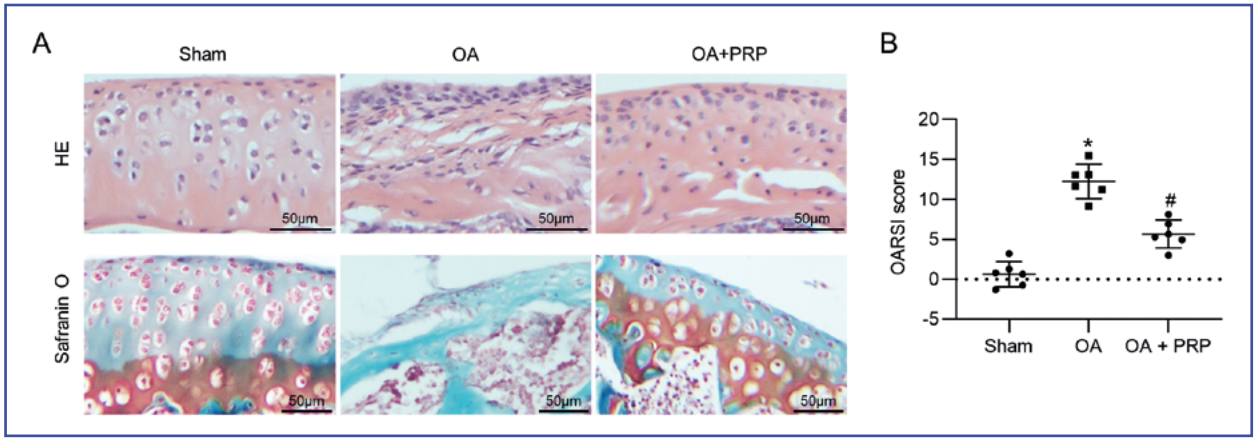

Figure 1. PRP ameliorates morphological features of OA in rat' knee joint. **A.** Upper panel: Representative images of H&E staining show the morphological changes of rat articular cartilage induced by OA induction. Scale bars = 50 μm. Lower panel: Representative images of safranin O staining reveal the content of glycosaminoglycans in rat articular cartilage. Scale bars = 50 μm. **B.** The OARSI scores of the Control, OA, and OA + PRP groups were calculated, respectively. N = 3, $^{*}P < 0.05$ *vs.* Control, $^{\#}P < 0.05$ *vs.* OA. Abbreviations: H&E — haematoxylin-eosin; OA — osteoarthritis; OARSI — Osteoarthritis Research Society International; PRP — platelet-rich plasma.

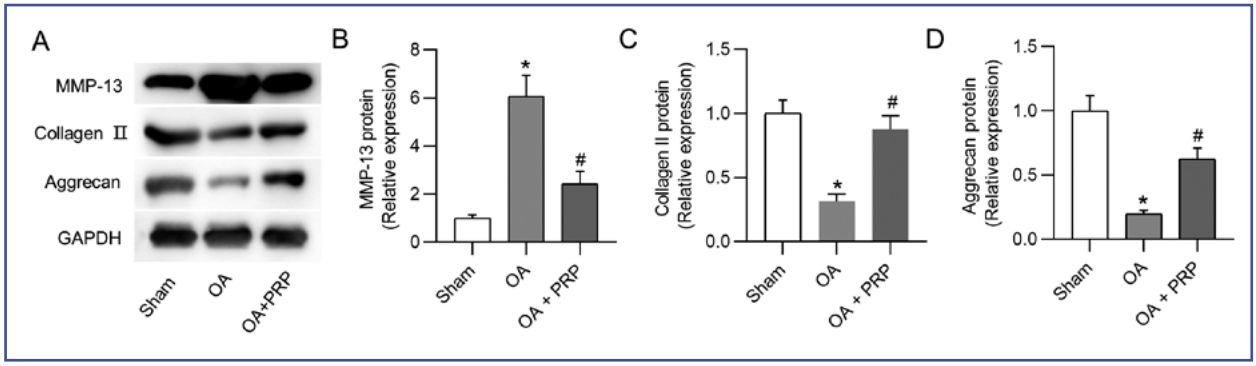

Figure 2. PRP alleviates the degradation of articular cartilage in OA rat models. **A.** Protein levels of ECM catabolic (MMP-13) and anabolic (collagen II, aggrecan) mediators in rat articular cartilage were assessed by western blotting. **B–D.** Semi-quantitative analysis of MMP-13, collagen II, and aggrecan protein content in rat articular cartilage. N = 6. $^{*}P < 0.05$ *vs.* Control, $^{\#}P < 0.05$ *vs.* OA. Abbreviations: ECM — extracellular matrix; OA — osteoarthritis; PRP — platelet-rich plasma.

OA induction resulted in a significant increase of IL-1β and TNF-α mRNA expression in rat articular cartilage (Fig. 3C, D). The mRNA expression of the transcription factor NF-κB, whose activation can induce the production of large amounts of pro-inflammatory cytokines and the occurrence of inflammatory response, was also upregulated in the OA group compared with the Control group (Fig. 3E). The PRP administration ameliorated the increase in IL-1β, TNF-α, and NF-κB mRNAs' expression in the articular cartilage of OA rats (Fig. 3C–E). Furthermore, the OA group presented evidently higher MMP-13 mRNA expression and lower collagen IImRNA expression than the Control group, whereas injections of PRP reduced MMP-13 and elevated collagen IImRNA levels in the articular tissue of OA rats (Fig. 3F, G). These results verified that PRP suppressed local and systemic inflammatory response and improved cartilage matrix metabolism in the OA rat model.

PRP regulates the OPG/RANKL/RANK system in OA rat models

Western blotting analysis demonstrated that the protein level of OPG was notably higher in the articular cartilage of OA-induced rats than in that of control rats, which was further increased by the administration of PRP (Fig. 4A). In contrast, neither OA induction nor PRP injections influenced the protein levels of RANK in rat articular cartilage (Fig. 4B). Besides, the increase in the protein level of RANKL in OA-induced rat models was offset by PRP (Fig. 4C). The changes in the ratios of OPG/RANKL and RANKL/RANK mRNAs were assessed through RT-qPCR analysis, which manifested that the ratio of OPG mRNA/RANKL mRNA was reduced while the ratio of RANKL mRNA/RANK mRNA was elevated in the OA group *versus* the Control group. However, PRP injection reversed OA-induced changes in the mRNA ratios OPG/RANKL and RANKL/RANK (Fig. 4D, E).

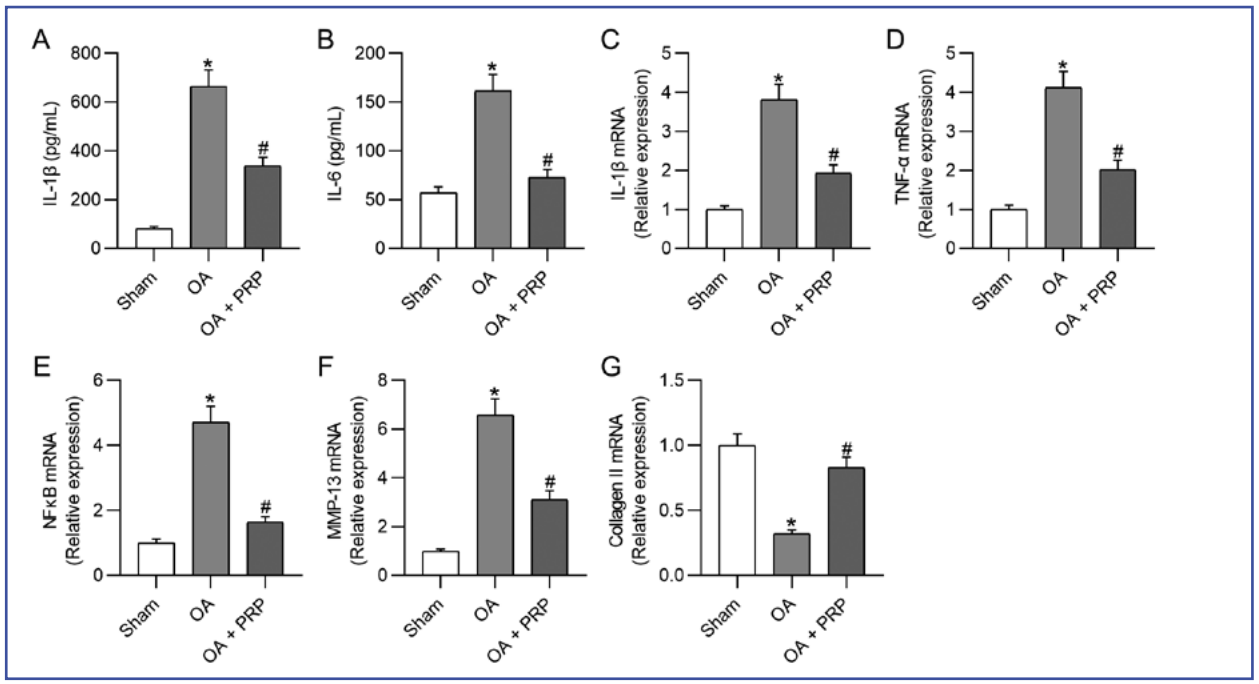

Figure 3. PRP mitigates the inflammation in osteoarthritis (OA) rat model. **A, B.** Serum levels of IL-1β and IL-6 in rats were measured by ELISA. **C–G.** Evaluation of IL-1β, TNF-α, NF-κB, MMP-13, and collagen IImRNA expression in rat articular cartilage by RT-qPCR. N = 6 *per* group. *P < 0.05 *vs.* Control, #P < 0.05 *vs.* OA.

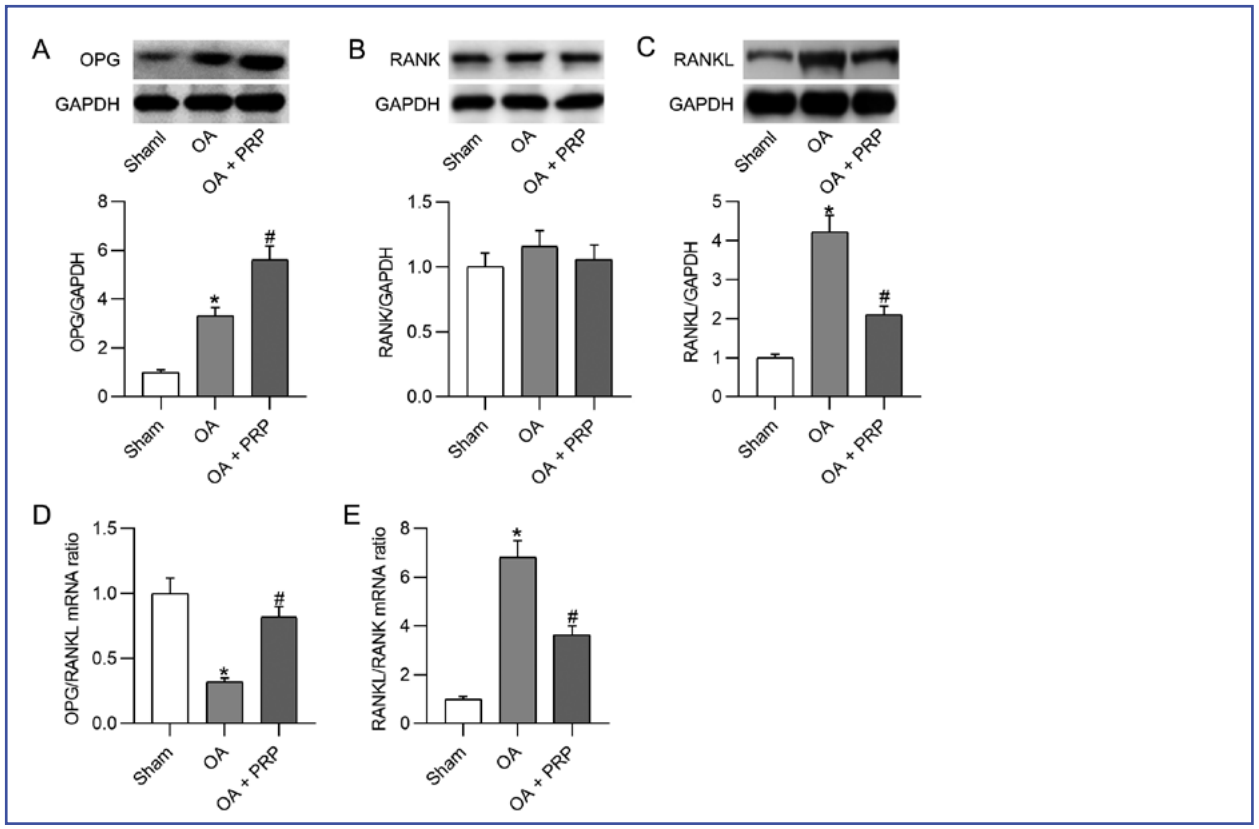

Figure 4. PRP regulates the OPG/RANK/RANKL system in OA rat model. **A–C.** OPG, RANK, and RANKL protein levels in rat articular cartilage were determined by western blotting analysis. **D, E.** OPG/RANKL and RANKL/RANK mRNA ratios in rat articular cartilage were determined by RT-qPCR. N = 6, *P < 0.05 *vs.* Control, #P < 0.05 *vs.* OA. Abbreviations: OA — osteoarthritis; OPG — osteoprotegerin; PRP — platelet-rich plasma; RANKL — RANK ligand.

PRP increases OPG expression but decreases RANKL expression in OA rat model

The expression of the OPG/RANKL/RANK system in rat articular cartilage was further confirmed by immunofluorescence staining (Fig. 5A). The results were consistent with the western blotting analysis, which showed that the increased expression of OPG in OA-induced rats was further enhanced after injection of PRP, as shown by the increased OPG-positive area in the OA + PRP group compared with the OA group (Fig. 5B). There was no significant change in RANK expression either by OA induction or PRP administration (Fig. 5C). In addition, the OA group displayed markedly more RANKL-positive area than the Control group, but less RANKL-positive area than the OA + PRP group, indicating that the increase in RANKL expression caused by OA induction was abolished after injection of PRP (Fig. 5D).

DISCUSSION

In recent years, intra-articular injection of PRP has been reported as an effective method in the treatment of OA [27]. The purpose of this study was to confirm whether PRP injection ameliorates cartilage degradation in an OA animal model by regulating the OPG/RANKL/RANK signalling. Our results showed that PRP injection alleviated OA progression in rat model by improving cartilage matrix metabolism and suppressing inflammation. In addition, PRP injection increased OPG expression and the OPG/RANKL mRNA ratio but decreased RANKL expression and the RANKL/RANK mRNA ratio in OA rat models.

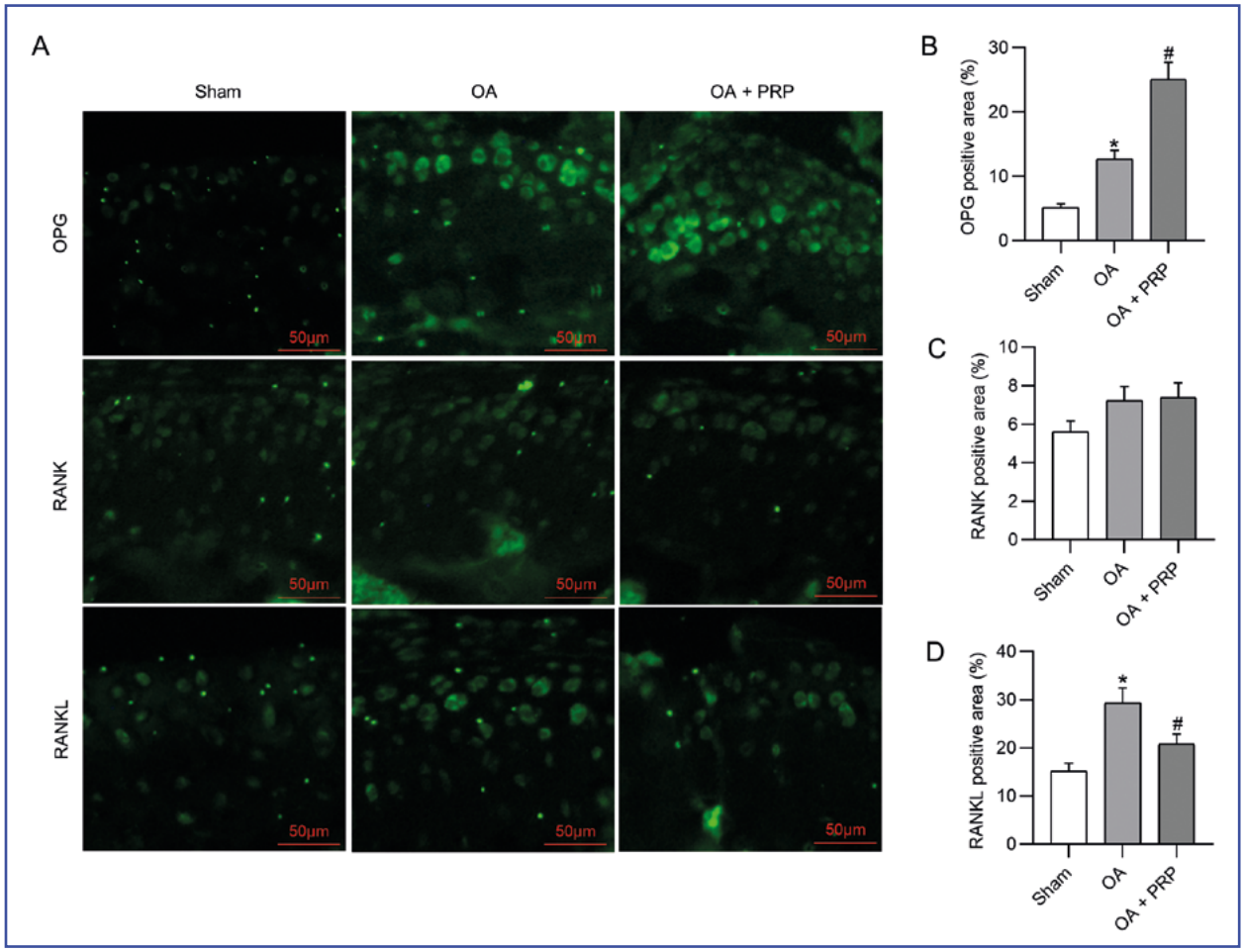

Figure 5. PRP increases OPG expression and decreases RANKL expression in the OA rat model. **A.** Representative immunofluorescence images showing OPG, RANKL, and RANK presence in rat articular cartilage. Scale bars = 50 μm. **B–D.** Quantification of OPG-positive, RANK-positive, and RANKL-positive areas according to the immunofluorescence results. N = 6, $^{*}P < 0.05$ *vs.* Control, $^{\#}P < 0.05$ *vs.* OA. Abbreviations: OA — osteoarthritis; OPG — osteoprotegerin; RANKL — RANK ligand.

The main pathological change of osteoarthritis is cartilage degeneration, which is related to synovitis, and early studies have suggested that synovitis is considered as the secondary pathological change that occurs in the early stage of OA [28]. Further research on OA showed that synovial inflammation can directly lead to the change of cartilage structure and affect the intraarticular environment. The proinflammatory cytokines (such as IL-1β, IL-6, and TNF-α) were found to be abnormally expressed in animal models and patients with OA [29, 30]. Inflammation is mainly involved in the pathological process of cartilage degeneration by destroying chondrocytes and ECM dynamic balance through several signalling pathways mediated by cytokines, including the NF-κB signalling pathway [31]. Likewise, the progression of age-related OA is associated with NF-κB activation in OA chondrocytes caused by the accumulation of advanced glycation end products [32]. It has been shown that silencing the expression of NF-κB p65 reduces TNF-α and IL-1β levels in synovial fluid, mitigates synovial inflammation, and alleviates cartilage degradation in experimental OA models [33]. Studies have revealed that PRP exerts anti-inflammatory effects by inhibiting the NF-κB signalling [34, 35]. Previously, Yin *et al.* reported that PRP decreased IL-1β and TNF-α levels as well as attenuated the severity of cartilage degenerative changes in an OA rabbit model [36]. Herein, we discovered that OA-induced upregulation of IL-1β, TNF-α, and NF-κB expression in rat articular cartilage was abolished by repeated administration of PRP, indicating that PRP effectively alleviates OA-associated inflammation.

Collagen is the most abundant protein in human body with different functions. At present, there are at least 16 types of collagens that have been confirmed, among which type II collagen is the main protein in cartilage [37]. Type II collagen, which is almost unique to cartilage and accounts for 90–95% of collagens in the cartilage ECM, is interwoven with proteoglycan aggregates to form fibres and endows cartilage with a semi-rigid structure [38]. Under normal conditions, the synthesis and degradation of the cartilage matrix maintain a balanced state [39]. Nevertheless, there is excessive collagen II degradation in the pathogenesis of OA, which further results in the progressive loss of matrix proteins and joint integrity [40]. The 2 main targets of degradation are type II collagen and the proteoglycan aggrecan, whose loss substantially contributes to OA progression [41]. All components of the ECM can be degraded by MMPs, among which collagenase MMP-13 is preferentially expressed in articular chondrocytes and degrades type II collagen [42]. In normal joint tissues, MMP-13 is expressed at low levels, while in arthritic joint tissues, MMP-13 expression is greatly increased [43]. Existing studies demonstrate that collagen II and MMPs have become potential therapeutic targets for OA [44, 45]. The pathological changes of OA can be reduced by inhibiting collagen II degradation [46]. Besides, suppressing MMP-13 activity maintains the cartilage ECM structure and mitigates morphological damage [47]. Previously, Yin *et al.* discovered that the mRNA expression of collagen II in human articular chondrocytes was increased by PRP treatment [48]. Treatment with PRP releasate reduces collagen II and aggrecan expression but enhances MMP-13 expression in IL-1β-induced human osteoarthritic chondrocytes [49]. In the present study, the decrease in collagen II and aggrecan expression and increase in MMP-13 expression in OA rat models were abrogated by PRP injections, suggesting that PRP effectively ameliorates cartilage matrix metabolism in OA.

The OPG/RANKL/RANK system is an important pathway that regulates bone metabolism [50, 51]. Even though OPG, RANK, and RANKL have been fully studied in the development of inflammatory joint disorders, including psoriatic arthritis and rheumatoid arthritis [52], little is known about the detailed role of the OPG/RANKL/RANK system in cartilage degradation in OA. It has been shown that OPG and RANKL are not only expressed in osteoarthritic cartilage but are also involved in the pathway that affects subchondral bone tissue [53]. The OPG/RANKL ratio is also altered in osteoarthritic chondrocytes and synovial fluid, in which RANKL exerts deleterious effects while OPG exerts positive effects [54]. As reported by Bolon *et al.* progressive loss of cartilage matrix and thinning of the cartilage layer were observed in OPG knockout mice, which was accompanied by low collagen II synthesis [55]. RANKL null mice exhibit disrupted columnar organisation of chondrocytes along with the structure of the cartilage growth plate [54]. Severe osteopetrosis was discovered in mice with a destroyed OPG gene [56, 57]. The role of RANKL in bone and cartilage destruction has been demonstrated in experimental arthritis models and human rheumatoid arthritis [58, 59]. Cartilage destruction and bone loss can be prevented by OPG treatment in rats with adjuvant arthritis [60]. In IL-1β--stimulated SW1353 chondrosarcoma cells, an increased RANKL/OPG ratio was related to enhanced MMP-13 synthesis [61]. Furthermore, in a mixed rabbit osteoporosis-osteoarthritis model, low OPG and high RANKL expression are linked to upregulated MMP-9 expression [62]. Compared with patients with femoral-neck fractures, patients with knee OA present increased IL-6 and RANK mRNA levels and high MMP-13 mRNA expression [63]. Based on the above observations, the up-regulation of catabolic factors such as MMP-1, -3, -9, and -13, or aggrecanase-1 and -2 produced by chondrocytes may be attributed to the high RANKL/OPG ratio. Our study revealed that PRP enhanced OPG expression and the OPG/RANKL mRNA ratio whereas attenuated RANKL expression and the RANKL/RANK mRNA ratio in an OA rat model.

Collectively, our study validated that PRP injection ameliorated cartilage degradation and inflammation in rat models of OA by affecting the OPG/RANKL/RANK system and amelioration of catabolic action of RANKL and RANK. Our findings might reveal a new mechanism of action for PRP in the clinical treatment of OA.

Article information and declarations

Data availability statement

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Ethics statement

All animal experimental procedures were approved by the Animal Care and Use Committee of the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology.

Author contributions

Qun Wu, Xianbao Yao, and Nan Shan were the main designers of this study. Qun Wu, Xianbao Yao, Nan Shan, Yi Cai, and Yongzhi Fan performed the experiments and analysed the data. Qun Wu, Xianbao Yao, Nan Shan, Yi Cai, and Yongzhi Fan drafted the manuscript. All authors read and approved the final manuscript.

Funding

The work was supported by Wuhan Municipal Health Commission Chinese Medicine Research Project (WZ22Q47) and Natural Science Foundation Project of Hubei Province (2022CFC025).

Acknowledgments

The authors appreciate all the participants providing support for this study.

Conflict of interest

The authors declare no conflict of interest.

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