Original Article
Platelet-Rich-Plasma alleviates pathological symptoms in a rabbit model of osteoarthritis

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Abstract: To investigate the effect of PRP on pathological changes in articular cartilage and IL-1β expression in rabbit knee osteoarthritis. 60 specific pathogen free New Zealand white rabbits were randomly evenly divided into four groups. The left knee joint cavities of animals in the sham, model and PRP group were surgically opened and a model of osteoarthritis was established in model and PRP groups following the improved Hulth protocol. Fifteen rabbits without any operation were set as normal group. One week after osteoarthritis-modeling, the PRP group received injection of PRP (harvested from ear vein blood following the Aghaloo protocol) into the joint cavity. An equal amount of saline was injected into sham and model groups once weekly for 6 consecutive weeks. At week seven the left knee was visualized and symptoms of osteoarthritis were assessed. In comparison to the model group, Kellgren-Lawrence, Pelletier and Mankin scoring was significantly improved in rabbits administered PRP (P<0.01), although still differed significantly from the normal and sham groups (P<0.05). Ultrastructure-level cartilage lesions were relatively moderate after PRP treatment. The IL-1β content of joint fluid, blood serum and cartilage was significantly lower in the PRP group than in the model group (P<0.01), but still higher than in the sham group (P<0.05). The IL-1β level in the serum was positively correlated with that in joint fluid (R²=0.9702). PRP can alleviate cartilage lesions in a rabbit model of knee osteoarthritis. Its effects may be related to downregulation of pro-inflammatory factor IL-1β.

Keywords: Platelet rich plasma, osteoarthritis, cartilage, Interleukin-1β

Introduction

Osteoarthritis, a type of whole joint degenerative disease, is the most commonly observed joint disease. Osteoarthritis is typified by articular cartilage degeneration and reactive hyperplasia of the joint edge and cartilage bone, subchondral bone remodeling, and inflammation of the synovial membrane. Osteoarthritis mainly affects those over 60 years of age, and can cause joint swelling and pain, restricting activities of daily living and posing an increasing burden to the health care system as the population ages. However, since the molecular mechanisms underlying osteoarthritis are not clear, few effective therapies have been developed to treat osteoarthritis, and none have been developed to prevent osteoarthritis [1, 2].

Physiological and biochemical factors thought to contribute to the development of osteoarthriti-
Peripheral blood is centrifuged to obtain a highly concentrated sample of platelets. PRP has been used in many different medical fields, including orthopedics, sports medicine, ophthalmology, stomatology, dermatology and plastic surgery, to aid tissue reconstruction and regeneration [7-10]. PRP is thought to support tissue repair by providing a high concentration of growth factors in the most appropriate physiological proportion [7-10]. However, the precise role of individual cytokines in the treatment of osteoarthritis remains to be determined.

In this study, we established a rabbit model of osteoarthritis to which we administered PRP. We monitored the effect of PRP on gross morphology and IL-1β levels in the joint. Our finding provides a theoretical basis for treating osteoarthritis with PRP, and implicates inhibition of IL-1β expression in the therapeutic effect of PRP.

Material and methods

Animals and grouping

60 specific pathogen free New Zealand white rabbits (Wanqianjiahe Lab Animal, Licence: 0001647) aged between 4 and 5 months, weighing 2.5 to 3.0 kg were randomly divided into four groups of 15 animals (male 7, female 8): the normal, sham, model and platelet rich plasma (PRP) group. All rabbits were maintained in the Core Animal Facility of Hubei Technology University, 2 rabbits per cage (80 × 80 × 60 cm³). The animal experiments were approved by the Experiment Ethics Committee of Southern Medical University, Guangzhou, China.

Rabbits were anesthetized with 10 mg/kg ketamine (Fujian Gutian Pharmaceutical Co., Ltd.), and the left knee joint cavities of animals in the sham, model and PRP group were surgically opened and a model of osteoarthritis was established in model and PRP groups following the improved Hulth protocol [11]. A 4 cm-longitudinal incision was made along the knee. After confirming no primary lesions, the anterior and posterior cruciate ligament and medial collateral ligament were cut and the medial meniscus was completely removed without injuring the cartilage surface. After thorough hemostasis and saline-flushing of the articular cavity, if the drawer test and medial stress test were positive, the capsule and skin were sutured layer by layer, then dressed with bandage without fixation. The sham group only received articular cavity-opening, then capsule and skin were sutured. The normal group did not receive any treatments. Animals were administered 400 kU/Kg penicillin (North China Pharmaceutical Co., Ltd.) daily by intramuscular injection. The dressings were changed, and the wound was inspected every two days for the first week. As osteoarthritis was reported to be made more severe by fatiguing activity [12], all rabbits were forced to move for 30 mins every day for twice within one week after surgery to establish the model of osteoarthritis [6, 13].

PRP preparation and administration

All materials were prepared under sterile conditions. Ear vein blood (10 ml) was extracted with a needle pre-immersed in 1 ml 10% sodium citrate and collected in 15 ml tubes. PRP was extracted following the Aghaloo protocol [10]. Briefly, after centrifuging at 215 ×g for 10 mins, the plasma layer above the white membrane was aspirated and centrifuged again at 863 ×g for 10 mins. The platelet-depleted supernatant was then aspirated, and the remaining 0.8 ml was PRP (1,958.33±316.41 × 10⁹/L).

One week after surgery, rabbits in PRP group received 0.5 ml PRP through articular cavity injection once per week for 6 weeks. An equal volume of saline was injected into sham and model group animals.

Sample preparation and index detection

Kellgren-Lawrence scaling: After 7 weeks, the gross morphology of rabbits’ knees was evaluated by x-ray at the Image center of Central Hospital of Xian Ning. The soft tissue around the joint, joint space, joint surface and osteophyte presence were graded using the Kellgren-Lawrence scale [14], as follows: Grade 0, normal; Grade I, suspected narrowing of joint space with possible osteophyte; Grade II osteophytes were present and the joint space was slightly narrowed; Grade III: some osteophytes and obvious narrowing of joint space, slight and restricted sclerosis of bone under cartilage; Grade IV, osteophytes affected the cartilage surface, obvious narrowing of joint space, obvious sclerosis and obvious joint hypertrophy and deformity.
Measurement of IL-1β in the joint fluid and serum: After X-ray examination the left knee joint was shaved, and disinfected with alcohol, and joint puncture was carried out. Sterile saline (1 ml) was injected into the joint space and after repetitive washing was aspirated, and collected in a 2 ml centrifuge tube. The fluid samples were centrifuged at 90,000 ×g for 15 min, and supernatants were collected for further analysis. Middle ear vein blood (5 ml) was collected and centrifuged at 90,000 ×g for 15 min, then supernatant serum was collected for analysis. The IL-1β content was measured using the following protocol (Boster Bio, Wuhan, China).

Histological observation: After collecting joint fluid and blood samples, animals were sacrificed via blood depletion. The articular cavity was opened along the medial knee joint, femoral condylar cartilage was dissected and was assessed via Pelletier scoring under light microscope (Olympus, Japan) [15]. Then sections were fixed in 10% PFA. After serial dehydration, decalcification, slicing (RM2315, Leica) and HE staining, the cartilage was assessed via Mankin scoring under light microscope (Olympus) [16]. Femoral condylar cartilage was cut into 0.2 × 0.3 × 0.3 cm³ pieces, which were fixed in 3% glutaraldehyde for 8 hr, then post-fixed with osmic acid, dehydrated, embedded in epoxy resin, longitudinal sectioned ultra-thinly and observed with TEM (Hitachi, Japan).

Immunohistochemistry: The level of IL-1β in the cartilage was assessed by immunohistochemistry. Femoral condylar cartilage was cut into 0.2 cm × 0.3 cm × 0.3 cm pieces which were fixed in 10% PFA. After serial dehydration, decalcification and slicing (RM2315, Leica), IL-1β was stained following the protocol (Boster Bio, Wuhan, China). The density of IL-1β staining was assessed via Pelletier scoring under light microscope (Olympus) [15].

Table 1. Kellgren-Lawrence scoring of rabbits after modeling

<table>
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<tr>
<th>Group</th>
<th>Kellgren-Lawrence scoring</th>
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<tr>
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<td>Normal (n=15)</td>
<td>14</td>
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<td>Sham (n=14)</td>
<td>13</td>
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<td>PRP (n=15)</td>
<td>0</td>
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<td>Model (n=14)</td>
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Figure 1. X-ray image of left knee of rabbits in different groups. X-ray image of left knee of rabbits in (A) normal group (n=15), (B) sham group (n=14), (C) RPP group (n=15) and (D) model group (n=14). Left, vertical plan; right, coronal plane.
PRP alleviates rabbit model of osteoarthritis

Results

Gross morphology of osteoarthritis knee model

In this study, we established a rabbit model of osteoarthritis, and while the joints of animals in the normal and sham group appeared healthy (internal and external joint spaces were normal with smooth joint surfaces, and without osteophyte, and uniform bone density and cartilage distribution, Figure 1A, 1B), the joint spaces of animals in the model group were narrowed, their joint surfaces were roughened, osteophytes were visible and the density of cartilage increased substantially (Figure 1D). All animals in the normal and sham groups were graded with Kellgren-Lawrence scores of 0 or I. In contrast almost all animals in the model group were graded with Kellgren-Lawrence scores of III or IV (Table 1).

One rabbit in the model group died as a result of pyogenic infection of the knee joint 3 days after modeling, and 1 rabbit in the sham group dies as a result of diarrhea 22 days after modeling.

Capacity of PRP to ameliorate symptoms of osteoarthritis

To investigate the capacity of PRP to ameliorate the symptoms of osteoarthritis, we administered PRP for six weeks to animals in which this model of osteoarthritis was established. In animals administered PRP, slightly less severe symptoms of osteoarthritis were observed. Joint spaces were less narrowed, joint surfaces were less severely roughened, few osteophytes were visible and the and density of cartilage increased only a little (Figure 1C). Animals administered PRP also had slightly improved Kellgren-Lawrence scores, between I and III, and none received a score of IV (Table 1).

Statistics

SPSS11.9 (IBM, NY, USA) was used for statistical analysis. Quantitative results were represented by mean ± standard error (SE); rank test was used to compare grading results. Gross scoring among groups, histological Mankin scoring, IL-1β levels in the joint fluid and serum, and ratio of IL-1β positive cells in joint cartilage were compared using one-way ANOVA and post-hoc q test. Correlation was analyzed with Spearman test. P<0.05 was accepted as significant.
PRP alleviates rabbit model of osteoarthritis

Pelletier scoring of joints

Seven weeks after modelling, visual examination of the joints of animals in the normal and sham groups revealed no obvious changes in femur condyles or tibial plateau cartilage (Figure 2A, 2B). In the model group rupture of the internal condyle of the femur and cartilage stripping was observed in four of 14 animals (Figure 2D). In animals administered PRP, cartilage erosion and roughening was evident and centered in the Loading area of the femoral condyle, and fibrochondrogenesis was observed in two of 15 animals (Figure 2C). Pelletier scoring revealed no significant differences between the normal group and sham group (P=0.6077), both of which were scored significant lower than PRP (P=0.0496 and 0.0431, respectively), and model group (P=0.0027 and 0.0030, respectively). The scores in the PRP group were significantly lower than in the model group (P=0.0052, Figure 2E).

Mankin scoring of cartilage

Microscopy of the joints of animals in the normal and sham groups revealed no obvious changes in the artrodial cartilage (Figure 3A, 3B). In the model group, most cartilage surfaces were deteriorated, chondrocytes were substantially depleted, cartilage tidal lines were disturbed or even disappeared, and fractured cells were observed, substrate was exposed, the collagen framework was not uniformly arranged and the staining was uniformly pink (Figure 3D). In animals administered PRP, chondrocytes in surface and middle layers were clustered, cartilage tidal lines were disturbed with fracture and/or ulceration of the surface and middle layer. Cartilage matrix was exposed, the collagen framework was not uniformly arranged and the staining was uniformly pink (Figure 3C). Mankin scoring revealed no significant difference between normal and sham group (P=0.7105), both of which were scored significant lower than PRP (P=0.0398 and 0.0406, respectively) and model group (P=0.0026 and 0.0038, respectively). The score was significantly lower in PRP group than the model group (P=0.0081) (Figure 3E).

Ultrastructure of articular cartilage

Electron microscopy revealed the cell body and membrane of chondrocytes to be intact in the normal and sham groups. Stretching microvillii, intact nuclear membranes; oval cells with abundant cellular organelles, and rough surfaced endoplasmic reticulum was observed. The matrix was not observed, and collagenous

Figure 3. Histological observation of left knee joints and Mankin score of rabbits in different groups. A. Normal group, B. Sham group, C. RPP group, D. Model group, E. Mankin score of joints in different groups. *P<0.05 vs. normal and sham group. #P<0.05 vs. model group (400 ×).
PRP alleviates rabbit model of osteoarthritis

Figure 4. Transmission electron microscopic imaging of left knee joints of rabbits in different groups (5000 ×). A. Normal group, B. Sham group, C. RPP group, D. Model group.

Figure 5. IL-1β level in joint fluid and serum of rabbits in different groups. A. IL-1β level in joint fluid after 6-week treatment. B. IL-1β level in serum after 6-week treatment. C. Correlation of IL-1β level in joint fluid and in serum. *P<0.05 vs. normal and sham group. #P<0.05 vs. model group.
fibers were uniformly arranged containing abundant polysaccharide proteins (Figure 4A, 4B). In the model group, chondrocyte shrinkage and abundant necrolysis was observed. The nuclear membranes were not evident, and number of cell surface bulges decreased significantly. Cellular organelles were condensed in sheet-like structures with high electron density. Vacuoles containing lipid droplets were evident in the cytoplasm. The matrix was exposed, and the collagenous fibers were disordered with obvious fractures (Figure 4D). In the PRP group, the cell membrane of chondrocytes were relatively intact, and abundant cellular organelles were observed. Most cell surface bulges were ameliorated. Nuclear morphology was roughly normal and cytoplasmic staining was relatively uniform. Rough surfaced endoplasmic reticulum were relatively abundant and some vacuoles were observed. In the matrix, the collagenous fibers were not entirely uniformly arranged with some fractures; the polysaccharide proteins were unevenly distributed (Figure 4C).

Serum- and junction-IL-1β

ELISA revealed the level of IL-1β in the serum and joint fluid to not differ significantly between the normal and sham group (P=0.8692 and 0.9305 in serum and joint fluid, respectively), but to be significantly higher in the PRP (P=0.0175 and 0.0202, respectively in the serum, and P=0.0366 and 0.0283, respectively in the joint fluid) and and model groups (P=0.0008 and 0.0014, respectively in the serum and P=0.0015 and 0.0021, respectively in joint fluid). However, the levels of both were significantly lower in the PRP group than the model group (P=0.0086.
and 0.0079 in serum and joint fluid, respectively, Figure 5A, 5B). Serum-IL-1β was positively correlated with junction-IL-1β (r²=0.9702, P=0.0000, Figure 5E).

Articular cartilage IL-1β

Immunohistochemistry revealed that the joint cavities of animals in the normal and sham groups contained only few IL-1β positive chondrocytes (Figure 6A, 6B). In the model group, some IL-1β positive chondrocytes were observed across the four layers of sample (Figure 6D). In the PRP group few IL-1β positive chondrocytes were found across the four layers of sample (Figure 6C). The number of IL-1β positive chondrocytes (Figure 6E) was significantly lower in normal and sham groups than in RPR and model group (P=0.0458 and 0.0394, RPP vs. normal and sham group, and P=0.0002 and 0.0013, model vs. normal and sham group, respectively). The density of IL-1β staining (integral optical density, IOD, Figure 6F) was also significantly lower in normal and sham groups than in RPR and model group (P=0.0375 and 0.0408, RPP vs. normal and sham group, and P=0.0037 and 0.0043, model vs. normal and sham group, respectively). The number of IL-1β positive chondrocytes (P<0.05, Figure 6E) and the signal density of IL-1β (IOD, Figure 6F) were also significantly lower in the RPR group than the model group (P=0.0068 and 0.0073, respectively).

Discussion

We successfully established a model of osteoarthritis in the left knee of rabbits following an improved Hulth protocol. In comparison to the PRP group, Kellgren-Lawrence, Pelletier and Mankin scoring was significantly worsened in model group rabbits. Ultrastructure-level cartilage lesions were observed by electron microscopy. The IL-1β content of joint fluid, blood serum and cartilage was significantly elevated. These observations suggested successful establishment of the model. Only one animal in model group of 15 died as a result of pyogenic infection of the manipulated knee.

To investigate the capacity of PRP to ameliorate the symptoms of osteoarthritis, we administered PRP for six weeks to animals in which this model of osteoarthritis was established. In animals administered PRP, slightly less severe symptoms of osteoarthritis were observed, including aspects of gross morphology, cellular structure and cartilage ultrastructure. Furthermore, we characterized the possible involvement of IL-1β in osteoarthritis by quantifying IL-1β levels in the serum and joint fluid, and also its expression in chondrocytes. We found that the level of IL-1β to be higher in the groups that experienced most severe symptoms of osteoarthritis.

The role of growth factors in the therapeutic effect of PRP has been extensively studied [17, 18]. PRP contains levels of PDGF, EGF and TGF that are 30, 10 and 7 fold higher than those found in normal blood. PRP has been widely used in clinical orthopedics-related research [19]. Marx et al. found that PRP can accelerate morphogenesis and regeneration of the reconstructed jawbone [20]. Intra articular cavity injection of PRP was also found to reduce degenerative cartilage wear [21]. In this study, autologous PRP was found to exert anti-inflammatory effects, alleviate pathological injury of chondrocytes and the matrix, and to lower the level of IL-1β, a pro-inflammatory factor, in the joint fluid and serum. In combination with our pathological observations, these results suggest that PRP may improve the symptoms of osteoarthritis, although in this model, six weeks of once weekly injections did not fully ameliorate the symptoms of osteoarthritis.

IL-1 is a classic pro-inflammatory factor [22] and IL-1β was previously reported to be the predominant form of IL-1 in the supernatants of cultured synovial cells with osteoarthritis [23]. IL-1β expression is reported to be upregulated in chronic osteoarthritis [24-26]. Under inflammatory pressure, granulocytes and macrophages secrete GM-CSF, promoting IL-1β expression. IL-1β promotes expression of matrix metalloproteinases in the cartilage and synovial tissues, which causes destruction of chondrocytes, inhibition of cartilage glycoprotein synthesis promoted fibroblast and matrix-degradation. Degradation product of the cartilage matrix can induce secondary inflammation in synovium, and inflammation in the synovium will further promote IL-1β expression. The Secondary inflammation-induced cascade can create a deleterious loop that further aggravates arthritis [27-29]. IL-1β can induce bone resorption and stimulate the proliferation of...
osteoblast like cells to form osteophytes, a typical symptom of cartilage sclerosis [30]. Thus IL-1β inhibitors have been administered to treat osteoarthritis with some clinical success [31]. Here we examined the levels of IL-1β in the joint fluids and serum, and found administration of PRP ameliorated the elevated IL-1β observed in this model of osteoarthritis. Immunohistochemical staining of IL-1β in the cartilage also indicated that PRP ameliorated expression of IL-1β in these tissues. Thus, PRP may act as an inflammatory agent interrupting IL-1β expression, or another component of the previously described cascade that promotes IL-1β expression.

Although no remedies that effectively reverse the progression of osteoarthritis have been identified [32], our experiments indicate that PRP can alleviate the symptoms of osteoarthritis, protecting articular cartilage and inhibiting IL-1β expression. Thus we highlight a new potential treatment for osteoarthritis, the efficacy of which will require careful study in the clinic.

Disclosure of conflict of interest

None.

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