Platelet-Rich Plasma Inhibits Mechanically Induced Injury in Chondrocytes

Xuetao Xie, M.D., Ph.D., Veronica Ulici, Ph.D., Peter G. Alexander, Ph.D., Yangzi Jiang, Ph.D., Changqing Zhang, M.D., Ph.D., and Rocky S. Tuan, Ph.D.

Purpose: To investigate the effect of platelet-rich plasma (PRP) on mechanically injured chondrocytes. **Methods:** PRP from bovine whole blood was activated to prepare platelet-rich plasma releasate (PRPr). Bovine articular chondrocytes were subjected to 16%, 0.5-Hz biaxial cyclic tensile strain (CTS) for 48 hours and cultured for another 24 hours without cell stretching as an in vitro model of mechanically injured chondrocytes. Culture medium in the 3 PRP- and CTS-treated groups was supplemented with 10% PRPr at the start of CTS, after 24 hours of CTS, and after 48 hours of CTS, respectively. Gene expression levels of type II collagen, aggrecan, matrix metalloproteinase (MMP)-3, MMP-13, inducible nitric oxide synthase, and cyclooxygenase 2 were quantitatively evaluated. Changes in the content of nitric oxide (NO), prostaglandin E2 (PGE_2), MMP-3, and tissue inhibitor of metalloproteinase 1 in the culture medium were also measured. **Results:** PRPr increased type II collagen and aggrecan messenger RNA expression; diminished CTS-dependent up-regulation of MMP-3, inducible nitric oxide synthase, and cyclooxygenase 2 gene expression; and reduced CTS-induced overproduction of NO and PGE_2 when PRPr was applied early at the start of CTS. The addition of PRPr after 24 hours of CTS only inhibited MMP-3 gene up-regulation and the increase of NO and PGE_2 induced by CTS. These changes were not observed when PRPr was supplemented after 48 hours of CTS. PRPr mitigated the increased MMP-3 production and decreased tissue inhibitor of metalloproteinase 1 secretion resulting from CTS in a time-dependent manner. **Conclusions:** PRP treatment ameliorated multiple CTS-mediated catabolic and inflammatory responses in chondrocytes. More beneficial effects were observed with early PRP application. **Clinical Relevance:** Intra-articular PRP injections at the beginning of strenuous exercises may be used to protect chondrocytes from mechanical injury, thus preventing joints from increased wear.

Cartilage homeostasis is a requirement of healthy joints. As the singular cellular component of articular cartilage, chondrocytes are responsible for the maintenance of dynamic cartilage metabolism in a low-turnover state. Mechanical damage to articular cartilage, such as in sports or recreational activities, may transiently disrupt the balance between the anabolic and catabolic states.[1] The inability of chondrocytes to tolerate this stress will result in inhibition of extracellular matrix (ECM) production and accumulation of mediators associated with matrix degradation, including nitric oxide (NO), prostaglandin E_2 (PGE_2), and matrix metalloproteinases (MMPs).[2-5] If injurious mechanical load persists and the metabolic imbalance cannot be corrected in time, progressive cartilage degeneration will likely occur.[1,6]

In the past decade, platelet-rich plasma (PRP) has been frequently applied for the purpose of stimulating matrix accumulation and inhibiting cartilage degradation because of abundant anabolic and anti-inflammatory cytokines in its releasate (PRPr).[7,8] Several preclinical and clinical studies have been reported with encouraging results using PRP for the treatment of osteoarthritis (OA), which is characterized by high catabolism and low anabolism of articular cartilage.[7,8] More importantly, a few randomized controlled trials have recently confirmed the effectiveness of PRP in relieving OA symptoms and improving

From the Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine (X.X., V.U., P.G.A., Y.J., R.S.T.), Pittsburgh, Pennsylvania, U.S.A.; and Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital (X.X., C.Z.), Shanghai, China.

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Address correspondence to Rocky S. Tuan, Ph.D., Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, 450 Technology Dr, Room 221, Pittsburgh, PA 15219, U.S.A. E-mail: rst13@pitt.edu

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joint function.[9-11] As for the working mechanism of PRP in the osteoarthritic environment, PRP may mitigate the metabolic imbalance, as reported in chondrocytes in an interleukin (IL) 1β—induced OA model.[12] Therefore it seems an appealing option to use autologous PRP to correct the emerging metabolic imbalance in acute cartilage injury, thereby preventing injured cartilage from progressive degeneration. However, few reports are available concerning the effect of PRP on acute cartilage injury.[13,14] Because the superficial layer of articular cartilage is typically subjected to high levels of cyclic shear and tensile stress during joint motion and because overloading observed in continual strenuous exercises results in a physiological imbalance,[15] an ideal in vitro model should involve a continuous mechanical load exerting injurious effects. A number of studies have investigated the effect of cyclic tensile strain (CTS) on chondrocytes.[3,4,16] Our previous study showed that 16%, 0.5-Hz biaxial CTS induced injury in chondrocytes by means of upregulation of a number of catabolic genes and elevation of pro-inflammatory cytokine levels.[4]

The purpose of this study was to investigate the effect of PRP on mechanically injured chondrocytes. We hypothesized that PRP would inhibit CTS-induced injury in chondrocytes.

Methods

PRP and PRPr Preparation

Anticoagulated whole blood from 3 healthy adult cows (Lampire Biological Laboratories, Pipersville, PA) was acquired. PRP was enriched by a 2-step centrifugation method described elsewhere,[17] with a few modifications. The modifications included the following: (1) 500 mL of blood from each cow was evenly divided into 10 tubes before centrifugation, and (2) all centrifugation steps were completed at 4°C. Fifty milliliters of whole blood in each tube was separated into a platelet-containing fraction and a cell-containing fraction by centrifugation at 250*g* for 10 minutes. The platelet-containing fraction was then transferred to new tubes. After a second, higher-speed centrifugation at 1,000*g* for 10 minutes, platelets were precipitated and resuspended in one-tenth of the supernatant plasma. Aliquots of this PRP preparation and whole blood were used to determine the concentration of platelets and mononuclear cells. On activation with the addition of 22.8-mmol/L calcium chloride (vol/vol, 1:10), the PRP preparation started to clot and the supernatant released was designated as PRPr. After incubation at 37°C for 2 hours, PRPr was collected through centrifugation at 1,000*g* for 10 minutes at 4°C. All the PRPr prepared from 3 cows was pooled together and then stored in aliquots at −80°C until use.

Chondrocyte Isolation and Culture

Chondrocytes were isolated from adult bovine knees according to protocols published elsewhere.[4] Cartilage slices were diced finely and digested in a stirring flask containing 1-mg/mL type II collagenase (Worthington, Lakewood, NJ) overnight in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Grand Island, NY) supplemented with antibiotic-antimycotic at 37°C. The next day, fetal bovine serum (FBS) (Invitrogen) was added to stop enzymatic digestion. After filtration through 40-μm nylon mesh cell strainers, the cells were centrifuged, resuspended, pooled, and subsequently plated into T150 flasks (cell density, 40,000/cm^2) in DMEM supplemented with 10% FBS and antibiotics for 3 days and were then collected at around 80% confluency and cryo-preserved in liquid nitrogen until use. The cells were precultured before freezing to increase the survival rate and the number of cells available for subsequent experiments.

Application of CTS

On the day before the application of CTS, chondrocytes were seeded onto 6-well Bioflex plates (Flexcell, Hillsborough, NC) at a density of 40,000 cells/cm^2 in DMEM with 10% FBS. The plates were precoated with 5-μg/mL bovine plasma fibronectin (Sigma, St. Louis, MO) in 0.1-mol/L sodium bicarbonate solution for 2 hours at room temperature, followed by rinsing with Hank's Balanced Salt Solution (Invitrogen) 3 times. The wells were coated to facilitate cell adhesion during cyclic stretching of the Bioflex membranes. On the day of CTS application, after medium was replaced with DMEM containing 1% FBS, cells were subjected to biaxial CTS using a custom-designed vacuum-operated loading device (Fig 1).[4] A pulsed waveform from 0% to 16% elongation at a 0.5-Hz frequency was continuously applied for 48 hours. After another 24-hour incubation without CTS application, chondrocytes and supernatant medium were collected. For real-time polymerase chain reaction (PCR) experiments and biochemical analyses of NO and PGE_2 in the culture medium, cells were divided into the following 6 groups: chondrocytes without CTS or PRP treatment (control group); chondrocytes treated with CTS alone (CTS group); chondrocytes treated with 10% PRPr alone (PRP group), which was introduced simultaneously with CTS application in other groups; chondrocytes treated with CTS and 10% PRPr introduced immediately before CTS (early-PRP group); chondrocytes treated with CTS and 10% PRPr after 24 hours of CTS (mid-PRP group); and chondrocytes treated with CTS and 10% PRPr after 48 hours of CTS (late-PRP group) (Fig 2). The 10% concentration of PRPr was chosen from previous studies.[12,17] Each experiment was performed 3 times.