의학석사 학위논문

Allogeneic Pure Platelet-Rich Plasma Therapy for Adhesive Capsulitis

A Bed to Bench Study

동종 혈소판 풍부혈장을 이용한 유착성 관절낭염 치료의 중개연구

2020 년 02 월

서울대학교 대학원

의학과 중개의학전공

이 민 지

Allogeneic Pure Platelet-Rich Plasma Therapy for Adhesive Capsulitis

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지도 교수 조 현 철

이 논문을 의학석사 학위논문으로 제출함

2020 년 02 월

서울대학교 대학원
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이민지의 석사 학위논문을 인준함
2020 년 02 월

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Abstract

Introduction: Whereas platelet-rich plasma (PRP) has been widely studied in musculoskeletal disorders, few studies to date have reported for adhesive capsulitis (AC). A fully characterized and standardized allogeneic pure PRP may provide clues to solve the underlying mechanism of PRP with respect to synovial inflammation and thus may clarify its clinical indications. The aim of the study was to evaluate the safety and efficacy of a fully characterized pure PRP injection in patients with adhesive capsulitis in a clinical study, and to assess the effects of pure PRP on synoviocytes with or without inflammation in vitro.

Methods: In the clinical study, a total of 15 patients with adhesive capsulitis received ultrasonography-guided intra-articular PRP injection and were followed for 6 months. Pain, range of motion, muscle strength, shoulder function, and overall satisfaction in patients were compared with the results in a propensity score-matched control group who received corticosteroid (triamcinolone acetonide 40mg). In the vitro study, synoviocytes were cultured with or without interleukin 1β (IL-1β) and PRPs. Gene expression of pro- and anti-inflammatory cytokines, matrix enzymes and their inhibitors were evaluated.

Results: PRP did not cause adverse events but rather decreased pain and improved shoulder ROMs and functions to a comparable extent to steroid injection in patients with adhesive capsulitis. PRP induced inflammation in absence of inflammation, however significantly ameliorated IL-1β induced synovial inflammatory condition by regulating cytokines such as IL-1β, tumor necrosis factor-α, cyclooxygenase-2,

microsomal prostaglandin E synthase-1, vasoactive intestinal peptide, matrix enzymes and their inhibitors.

Conclusion: This study showed that allogeneic pure PRP acts in pleiotropic manner and decreased pro-inflammatory cytokines only in the inflammatory condition. Therefore, allogeneic PRP could be a treatment option for inflammatory stage of adhesive capsulitis.

Keywords: Allogeneic platelet-rich plasma; pleiotropic effects; adhesive capsulitis; frozen shoulder; synovitis

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List of Abbreviations

AC, adhesive capsulitis

ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs

ASES, American Shoulder and Elbow Surgeons

bFGF, basic fibroblast growth factor

COX-2, Cyclooxygenase-2

CTGF, connective tissue growth factor

DASH, the Disabilities of the Arm, Shoulder, and Hand Questionnaire

EGF, epidermal growth factor

GF, growth factor

IGF, insulin-like growth factor

IL, interleukin

IL-1Ra, interleukin-1 receptor antagonist

MMP, matrix metalloproteinase

mPGES-1, microsomal prostaglandin E synthase-1

OA, osteoarthritis

PDGF-AB, platelet-derived growth factor-AB

PPP, platelet-poor plasma

PRP, platelet-rich plasma

RA, rheumatoid arthritis

ROMs, range of motions

SANE, single assessment numeric evaluation

SPADI, Shoulder Pain and Disability Index

SST, Simple Shoulder Test

TGF-β1, transforming growth factor-β1

TIMP, tissue inhibitor of metalloproteinase

TNF-α, tumor necrosis factor-α

UCLA, University of California at Los Angeles

VAS, visual analog scale

VEGF, vascular endothelial growth factor

VIP, vasoactive intestinal peptide

Introduction

Study Background

Adhesive capsulitis (AC), also known as frozen shoulder, is a common shoulder problem with the prevalence of 2~5% in the general population.[24] Patients with AC experience intense shoulder pain and limited range of motion of the glenohumeral joint with contracture of the capsule, especially with external rotation and abduction.[73] The onset of the disease falls between 30 and 70 year-old, and the disease is especially predominant in women.[24]

Adhesive capsulitis progresses through 4 stages and therapeutic approaches could be different with the stage of the disease.[54] Stage 1 is described by a gradual onset of pain. Pain at night is common and inability to sleep on the affected side is frequently reported. At the stage 2 (freezing stage) is represented to acute synovitis and progressive capsular contracture. In the stage 3 (frozen stage; adhesive phase), pain may be still present, and significant stiffness occurs. In the stage 4 (thawing stage; resolution phase), pain is minimal and a gradual improvement in motion can occur. Although AC is considered to be a self-limited disease, 20% to 50% of patients show little or no improvement with residual limited range of motion.[47]

Adhesive capsulitis without an obvious preceding cause is classified into primary AC, whereas secondary AC is associated with local or systemic disorders such as diabetes mellitus, Dupuytren disease, shoulder trauma, various cardiac, endocrine, and neurologic disorders.[15, 24, 61] Pathogenesis of primary AC is still unknown,

however, many studies indicate that the main pathology of primary adhesive capsulitis is a preceding synovitis followed by the contracture of the glenohumeral joint capsule.[61] Cytokines such as interleukins (ILs) and tumor necrosis factor-α (TNF-α) are considered to be involved in synovitis.[33] Although synovitis is an important contributor to AC, most studies with respect to synovial inflammation have focused on rheumatoid arthritis (RA) or osteoarthritis (OA).

Despite the reduction of function and quality of life caused by AC, there is still no consensus on the most effective methods of managing the disease.[56] Current treatments for AC include physical therapy, oral medication (NSAIDS or steroid), steroid injection and surgical capsular release.[40, 47] Among them, corticosteroid injection is the most common treatment in clinical practice.[40] Although the effect of corticosteroid in pain relief is quick and apparent, this effect does not last long and adverse events may arise in adjacent body structures, such as tendon and bone. In use of triamcinolone acetonide, tendon properties became weaker and consequently increasing the rupture rate.[64] Disorganization of the collagen in vivo and reduced mechanical properties of tendon were also reported.[19, 21] Furthermore, corticosteroid exposure causes loss of bone mass by altering the fragile balance between osteoclast and osteoblast activity, thus increasing the risk of fracture.[9, 44] All these factors remind physicians to be cautious in using steroids, and the alternative may need to avoid side effects of steroids but effective in controlling inflammation.

Platelet-rich plasma (PRP), a natural reservoir of cytokines and growth factors (GFs), has been widely used to treat musculoskeletal disorders.[2, 25, 53] PRP is known

to modulate anabolic and anti-inflammatory effect on damaged tissue. [3, 68] However, its mechanism and clinical indication are still not clearly known. Many studies of rotator cuff diseases, rheumatoid arthritis and osteoarthritis have evaluated the efficacy of PPR on the diseases.[3, 25, 26, 41, 53, 67] Furthermore, molecular basis of PRP have been investigated mostly on the target cell of tendon, cartilage and bone.[31] PRP enhances proliferation of tenocytes, and rescues tenocytes from IL-1β or a corticosteroid induced apoptosis and senescence.[29] PRP has anti-inflammatory effects on the chondrocytes and synoviocytes from OA patients via suppressing the activation of NF-κB signaling.[3] To date, few studies have reported on adhesive capsulitis and the synoviocytes from AC patients.

Autologous PRP therapies have been safely used in many medical fields, but it is challenging or even impossible for some patients to use their own blood. Administering allogeneic PRP to infants, the elderly, or patients with hematological diseases, anti-platelet medication, and/or diabetes may be a better option.[4] Moreover, PRP from older males with knee OA is reported to suppress chondrocyte matrix synthesis and upregulates inflammation.[48] Considering the fact that the old is more vulnerable to AC, autologous PRP from old people may negatively affect their shoulder structure. In contrast, these drawbacks in autologous PRP treatment could be removed by using allogeneic PRP from healthy donors which is be prepared and analyzed using a completely standardized system.

Growing number of standardization system have been proposed with respect to the components in PRPs.[45, 51] Recent studies reported that PRP inhibited the release

of pro-inflammatory cytokines in synoviocytes from OA patients,[57, 59, 65] while increased level of pro-inflammatory cytokines were investigated using leukocyte-rich PRP.[6, 11] These inconsistent results are attributed to characteristics of PRP, not only the concentrations of white blood cells (WBCs), but also the concentrations of platelets, red blood cells (RBCs), fibrinogen, growth factors and cytokines could resulted in various outcomes.[35, 43, 58, 72] Nonetheless, characteristics of PRP are not fully described in many studies.

Here we presented pure PRP (leukocyte poor PRP) with full descriptions on concentration of blood cells and bioactive materials such as GFs and PRP were controlled with respect to '4Ds' to remove interference of other factors such as leukocytes or inconsistent preparation tools in PRP therapy on AC.[28, 35] 4Ds: Drug, delivery, donor, and disease were controlled through previous standardized system.[28]

Purpose of Research

The purposes of the study were to evaluate the safety and efficacy of a fully characterized allogeneic pure PRP injection in patients with AC compared with steroid injection and to investigate the effect of pure PRP on synoviocytes with or without inflammation. Our hypothesis was that pure PRP would have anti-inflammatory effects on synoviocytes, therefore PRP would have therapeutic effects in patients with AC.

Methods

Preparation and Characterization of PRPs

Platelet-rich plasma (n=2) was prepared using a plateletpheresis system with a leukoreduction set (COBE Spectra LRS Turbo, Caridian BCT, Lakewood, CO, USA) from patients undergoing arthroscopic rotator cuff repair with PRP who were otherwise healthy according to a previously described. To confirm the safety of the PRP, tests for hepatitis B (HBV), hepatitis C (HCV), human immunodeficiency virus (HIV), and syphilis (VDRL) were assessed and confirmed negative. For the application study, the number of platelets in PRP were first concentrated to 4,000 x 10^3 platelets/μL (PRP 4,000), and then further diluted to 1,000 x 10^3 (PRP 1,000) and 200 X 10^3 (PRP 200) platelets/μL. Complete blood counts of above PRPs were assessed using a fully automated analyzer (XE-2100, Sysmex Corp, Kobe, Japan) and the concentrations of fibrinogen were counted by an automated coagulation analyzer (CA-7000, Sysmex Corp). For the platelets activation, 10% calcium gluconate with 166.7 IU/mL thrombin (Reyon Pharmaceutical, Seoul, Korea) was added to platelet-poor plasma (PPP) and PRP at 1:10 (vol/vol) for the in vitro study, and 10% calcium gluconate was added for the clinical study.

Concentrations of 7 growth factors, epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor-AB (PDGF-AB), and insulin-like growth factor (IGF-I) in the PRPs and PPP were determined by enzyme-linked immunosorbent assay (ELISA)

according to the manufacturer's protocol. The activation status of the platelets was confirmed using flow cytometry with markers CD61 and CD62P.

Quantification of growth factors using ELISA

The levels of EGF (Human EGF Quantikine ELISA Kit, DEG00; R&D Systems, Minneapolis, Minnesota), TGF-β1 (Human TGF-β1 Quantikine ELISA Kit, DB100B; R&D Systems), VEGF (Human VEGF Quantikine ELISA Kit, DVE00; R&D Systems), bFGF (Human FGF basic Quantikine HS ELISA Kit, HSFB00D; R&D Systems), PDGF-AB (Human PDGF-AB Quantikine ELISA Kit, DHD00C; R&D Systems), IGF-1 (Human IGF-1 Quantikine ELISA Kit, DG100; R&D Systems), and CTGF (Human CTGF ELISA Kit, SK00726-01; Aviscera Bioscience, Santa Clara, California) in PPP and PRPs were measured. All experiments were performed in duplicate according to manufacturer's instructions.

The optical densities of the microplate wells were measured with a microplate reader (SpectraMax Plus384; Molecular Devices, Sunnyvale, California). Sample concentrations were obtained by interpolating from the standard curve.

Patient Enrollment and Selection of the Control using Propensity Score Matching

This retrospective study was approved by our institutional review board. From June 2012 to October 2013, patients were enrolled in this study according to the following inclusion and exclusion criteria. All participants provided written informed consent (SMG-SNUBMC 06-2012-78).

To compare the efficacy of PRP injection, a propensity-score-matched analysis was performed to select control patients with adhesive capsulitis who had been treated with intra-articular steroid injection under ultrasonography guidance in our database, which can reduce selection bias and confounders in an observational study. For matching, one-to-one nearest neighbor match between PRP and steroid groups was conducted based on the propensity scores. To derive propensity scores, the following variables were included in a multivariable logistic regression: sex, age, dominance, and symptom duration.

Inclusion & Exclusion Criteria

Inclusion Criteria

Participants should meet all the inclusion criteria. Patients must consent in writing to participate in the study by signing and dating an informed consent document approved by IRB indicating that the patient has been informed of all pertinent aspects of the study prior to completing any of the screening procedures. (1) Male or female 18 years of age and older; (2) Patients who have had pain less than 12 months[14]; (3) limitation of both active and passive movements of the glenohumeral joint of ≥25% in at least 2 directions (abduction,flexion,external rotation,internal rotation), as compared with the contralateral shoulder in the scapular plane and in progressive degree of horizontal adduction

Exclusion Criteria

Participants who met a single condition were excluded from the study. (1) Patients with concurrent bilateral shoulder pain; (2) Patients with Diabetes mellitus; 3) Patient with overt hypothyroidism or hyperthyroidism; (4) Patients who received any drug by intra-articular injection for treatment within 6 months prior to this enrollment; (5) Patients who have a history of shoulder trauma including dislocation- subluxation- and fracture; (6) Patients who have a history of breast cancer- or surgery around shoulder- neck and upper back; (7) Patients with neurological deficit; (8) Patients who have a history of allergic adverse reactions to corticosteroid; (9) Patients with secondary adhesive capsulitis; (10) Patients with systemic inflammatory disease including rheumatoid arthritis; (11) Patients with

degenerative arthritis, infectious arthritis of shoulder joint; (12) Patients taking anticoagulants; (13) Patients who have a full-thickness rotator cuff tear (evidenced by magnetic resonance imaging (MR) or ultrasonography); (14) Patients who have difficulty participating in data collection due to communication problem and serious mental illness; (15) Pregnant women or lactating mother; (16) Patients with cerebrovascular accident; (17) Patients with symptomatic cervical spine disorders; (18) Patients with serious condition which can affect this study such as severe cardiovascular diseases- renal diseases- liver diseases- endocrine diseases- and cancers

Ultrasonography guided PRP and Steroid injection

Under ultrasonography guidance, all of the injections were administered in a seated position with the arm internally rotated in front of the abdomen (Figure 1A). The transducer and the patients' skin were sterilized with 2% chlorhexidine and 10% povidone-iodine solution. After applying sterile gel to the transducer, the glenohumeral joint was visualized. A 25G needle was introduced under the transducer while visualizing it in real-time as a thin hyperechoic line. Four ml of allogeneic PRP 1,000 was injected into the glenohumeral joint space (Figure 1B). In the propensity-score-matched controls, 1ml of triamcinolone acetonide (40mg/ml) in 3ml of saline was injected.

Postinjection Home Exercise Program

After injection, a home exercise program for shoulder and scapular stretching was encouraged twice a day for 20 minutes each session. Strengthening exercises were encouraged when stretching exercise and active elevation did not cause pain in the shoulder. All pain medications except the rescue analgesics, a combination tablet of 18.5 mg of tramadol and 162.5 mg of acetaminophen, were discontinued.

Outcome assessments

The outcome were assessed using our previously described protocol.[28] For the safety evaluation, the general symptoms or signs related to infection and immune responses such as fever, chills, pruritus, dyspnea, urticaria or rash were observed. Local wounds were also evaluated to determine the presence of zones of erythema, swelling, or abnormal discharge after injection and at each visit.

For the clinical evaluation, each patient completed a questionnaire that consisted of standardized outcome assessments at baseline and at 1 week, and 1, 3, and 6 months after injection. Clinical outcome measures include (1) pain, (2) muscle strength, (3) ROMs, (4) functional scores, and (5) overall satisfaction and function. A visual analog scale (VAS) was used to evaluate pain at rest, during motion and at night. The patients were instructed to use a 10-centimeter scale marked from ‘no pain’ to ‘unbearable pain’. The mean pain scores were calculated and compared. The worst pain was also recorded. The strength of the supraspinatus, infraspinatus, and subscapularis muscle was measured using a hand-held electronic scale (CHS,

CAS, Yangju, Korea). Range of motion was measured with a goniometer in active forward flexion, abduction, external rotation with the arm at the side, and internal rotation. Internal rotation was measured using the vertebral levels, and these were translated into numbers from 1 for the buttocks to 17 for T2.

Six common measurements for functional outcome were used, since their prior parameters are different and their correlation is significantly different.[55] The functional scores used were the Constant system, the Shoulder Pain and Disability Index (SPADI) system, the American Shoulder and Elbow Surgeons (ASES) system, the Disabilities of the Arm, Shoulder, and Hand Questionnaire (DASH), the University of California at Los Angeles (UCLA) system, and the Simple Shoulder Test (SST). Constant system is a comprehensive and comparable assessment of shoulder function which includes parameters for activities of daily living, pain, strength, and ROMs. SPADI and ASES system includes questionnaires for severity of pain and daily living. Physical functions, symptoms and social/ psychological function are included in DASH system. The parameters in UCLA system include function, strength, pain and satisfaction. SST systems simplify process with yes/no question. Higher scores are associated with improved function except SPADI and DASH scores.[70]

To evaluate overall function and satisfaction, we assessed the overall function and satisfaction from “I cannot use it” to “I feel normal” for function (the single assessment numeric evaluation, SANE), and overall satisfaction from ‘never satisfied’ to ‘very satisfied’ using 10-centimeter scale.

Isolation and Culture of Degenerative Synoviocytes from Human Rotator Cuff Tear

This study was conducted in accordance with the institutional review board (SMG-SNUBMC 26-2014-28). All tissue specimens were collected with the consent of the patients. Synoviocytes from patients undergoing arthroscopic rotator cuff repair with the mean ages of 58.67 ± 10.8 years (n = 6) were harvested, washed twice with DPBS (Dulbecco's Phosphate-Buffered Saline; Welgene) and minced into 1 x 1mm in size. Cells were isolated by treating with 0.3% collagenase II (Sigma; C0130) for 3 hours in High-Glucose Dulbecco's modified Eagle medium (HG-DMEM) containing antibiotic solution (100 U/mL penicillin and 100 mg/mL streptomycin) with gentle agitation in 37℃. Undigested synovium was filtered with 100 ㎛ cell strainer (SPL; Cell strainer), then isolated cells were centrifugated and washed twice with DPBS. All cells were seeded in 100-mm tissue culture dish (SPL; 90 x 20mm) at 37℃ in a humidified 5% CO_2 atmosphere. Media was changed twice a week, and cells were split into one forth at 60% to 80% confluence. Human synoviocytes in passages two to five were used in this study.

Treatment of Synoviocytes with IL-1β and PRP for the Evaluations of Gene Expression

After allowing to attach for 24 hours, cells were treated with 1ng/mL IL-1ß (recombinant human IL-1 beta/IL-1F2 Protein, CF, 201-LB/CF, R&D Systems,

Minneapolis, MN, USA), 1μM dexamethasone (Sigma), PPP (10% vol/vol) or PRPs (10% vol/vol) for 24 hours. Non-treated cells were used as a control.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR).

Genes with three categories were assessed, 1) Pro-inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), cyclooxygenase-2 (Cox-2), microsomal prostaglandin E synthase-1 (mPGES-1), and tumor necrosis factor- α (TNF-α) 2) Degradative enzymes and their inhibitors, matrix metalloproteinase-1, -3, -9, and -13 (MMP-1, -3, -9 and -13), tissue inhibitor of metalloproteinase-1, and -3 (TIMP-1 and -3), a disintegrin and metalloproteinase with thrombospondin motifs-4, and -5 (ADAMTS-4 and -5) 3) Anti-inflammatory cytokines, interleukin-4 and -10 (IL-4 and -10), vasoactive intestinal peptide (VIP), and interleukin-1 receptor antagonist (IL-1Ra).

Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from synoviocytes seeded at a density of 2 x 10^4 cells/cm^2 in the 6-well plate (SPL Lifesciences, Pocheon, Korea) using a HiYield Total RNA mini kit (Real Biotech Corporation, Taiwan) quantified using a NanoDrop ND-100 spectrophotometer (NanoDrop, Wilmington, Delaware). First-strand complementary DNA (cDNA) was synthesized using the Superscript III Reverse Transcription kit (Invitrogen, Carlsbad, California). Briefly, first-strand

cDNA was synthesized from cellular RNAs (1 ug) by heating a mixture (1 ug RNA, 1 uL Oligo(dT)20 [50 uM], 1 uL dNTP [10 mM], and up to 10 uL DW) to 65℃ for 5 minutes, cooling on ice for 2 minutes, and then adding a mixture containing 2 uL 10X RT buffer, 4 uL $MgCl_2$ (25 mM), 2 uL DTT (0.1 M), 1 uL RNaseOut (40 U/mL), and 1 uL Superscript III Reverse Transcriptase (200 U/mL) (Invitrogen). The reaction mixture was held at 50℃ for 50 minutes to promote cDNA synthesis, and the reaction was terminated by heating to 85℃ for 5 minutes and then cooling on ice for 2 minutes. Finally, RNase H (1 uL, 2 U/mL) was added and incubated at 37℃ for 20 minutes to remove RNA strands from RNA-cDNA hybrids. Synthesized cDNA was used for real-time RT-PCR. To perform real-time PCR utilizing a LightCycler 480 (Roche Applied Science, Mannheim, Germany), Taq-Man Gene Expression Assays (Applied Biosystems, Foster City, California) were used as a probe/primer set specified for IL-1β (assay ID: Hs99999029_m1), IL-6 (assay ID: Hs99999032_m1), COX-2 (assay ID: Hs00153133_m1), mPGES-1 (assay ID: Hs00610420_m1), TNF-α (assay ID: Hs99999043_m1), MMP-1 (assay ID: Hs00899658_m1), MMP-3 (assay ID: Hs00968308_m1), MMP-9 (assay ID: Hs00957555_m1), MMP-13 (assay ID: Hs00233992_m1), TIMP-1 (assay ID: Hs99999139_m1), TIMP-3 (assay ID: Hs00165949_m1), ADAMTS-4 (assay ID: Hs00192708_m1), ADAMTS-5 (assay ID: Hs00199841_m1), IL-4 (assay ID: Hs00174122_m1), IL-10 (assay ID: Hs00961622_m1), VIP (assay ID: Hs00175021_m1), IL-1RN (assay ID: Hs00893626_m1).

The PCRs were performed in a final volume of 20 uL containing 10 uL 2X LightCycler480 Probes Master (FastStart Taq DNA polymerase, reaction buffer,

dNTP mix [with dUTP instead of dTTP], and 6.4 mM MgCl2) (Roche Applied Science), 1 uL TaqMan Gene Expression Assay , 5 uL cDNA as the template, and 4 uL H_2O using the following program: 95℃ for 10 minutes, 60 cycles at 95℃ for 10 seconds, and 60℃ for 1 minute, followed by 72℃ for 4 seconds, and a final cooling at 40℃ for 30 seconds. Gene expressions were normalized versus GAPDH as follows: the cycle number at which the transcript of each gene was detectable (threshold cycle, Ct) was normalized against the Ct of GAPDH, which is referred to as ∆Ct. Gene expressions relative to GAPDH are expressed as $2^{-\Delta Ct}$, where ∆Ct = CT gene of interest – CT GAPDH.

Statistical analysis

The data values are shown as the mean ± standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables. For the propensity-score-matched set, the clinical variables between the groups were compared using two sample t-test for the continuous variables and chi-square test or Fisher's exact test for categorical variables. The assessment of clinical variable after PRP and steroid injection was evaluated using Wilcoxon signed rank test. A Paired t-test was used to determine the changes from baseline in all scale variables. For the in vitro study, the significance of difference was determined using Kruskal–Wallis test and Mann–Whitney test with Bonferroni correction for multiple comparisons. All of the statistical analyses were conducted using IBM SPSS Statistics version 20.0 (IBM Corp., Chicago, IL, USA). R version 3.4.0 (http://www.r-project.org)

was used for the propensity score matched analysis. P-value < 0.05 was considered to be statistically significant.

Results

Characteristics of the PRPs

Pure PRPs with low concentrations of RBCs and WBCs were used in this study. Mean platelets, RBCs, WBCs, and fibrinogen counts are shown in Table 1.The mean fibrinogen concentrations of the PPP and PRPs were similar regardless of the numbers of platelets. The concentrations of growth factors increased along with the increased number of platelets, except for IGF-1 which is a normal component of the plasma.[50] For the clinical study, characteristics of PRP 1000 were described with the concentrations, activation, and method of application (CAM) classification.[30] The mean concentration of the platelet was 1,154.50 ± 43.13 x 10^3/μL, and the mean percentage of the activated platelets after preparation was 4.47% ± 0.35.

TABLE 1. Characteristics of PRPs Used[1.]

Characteristics of PRPs Used

Clinical Study							
Counts of platelets, RBCs, and WBCs; concentration of fibrinogen; and activation status							
	Platelets, $\times 10^6/\mu L$	RBCs, $\times 10^6/\mu L$	WBCs, $\times 10^6/\mu L$	Fibrinogen, mg/dL		Activation Status[2], %	
PRP 1000	1154.50 ± 43.13	0.15 ± 0.04	0.01 ± 0.01	157.30 ± 13.29		4.47% ± 0.35	
Activation							
Status	Supernatant						
Method	Calcium alone						
Method of application							
State	Liquid						
Volume. mL	4						
Number	1						
Interval, days	0						
Concentrations of growth factors							
	EGF, (pg/ml)	TGF-β (ng/ml)	VEGF (pg/ml)	CTGF (pg/ml)	bFGF (pg/ml)	PDGF-AB (ng/ml)	IGF-1 (ng/ml)
PRP 1000	2,880 ± 780	52.63 ± 2.99	1,190 ± 70	54,850 ± 30,960	4.93 ± 0.63	66.26 ± 7.54	165.45 ± 4.74

In Vitro Study

Counts of platelets, RBCs, and WBCs; concentration of fibrinogen

	Platelets, $\times 10^6/\mu L$	RBCs, $\times 10^6/\mu L$	WBCs, $\times 10^6/\mu L$	Fibrinogen, mg/dL
PPP	3.71 ± 1.25	0.00 ± 0.00	0.01 ± 0.01	216.78 ± 12.07
PRP 200	205.33 ± 12.66	0.04 ± 0.01	0.01 ± 0.01	178.50 ± 39.33
PRP 1000	909.00 ± 92.77	0.17 ± 0.02	0.01 ± 0.01	193.13 ± 15.66
PRP 4000	3440.67 ± 1000.24	0.51 ± 0.08	0.02 ± 0.03	181.58 ± 25.23

Concentrations of growth factors

	EGF, (pg/ml)	TGF-β (ng/ml)	VEGF (pg/ml)	CTGF (pg/ml)	bFGF (pg/ml)	PDGF-AB (ng/ml)	IGF-1 (ng/ml)
PPP	0.72 ± 0.19	6.28 ± 0.20	0.00 ± 0.00	58.13 ± 27.10	1.21 ± 0.46	0.02 ± 0.00	160.13 ± 38.91
PRP 200	469.23 ± 54.78	21.90 ± 2.50	258.41 ± 65.79	69.35 ± 28.60	5.65 ± 2.03	17.02 ± 2.53	184.67 ± 81.35
PRP 1000	1,426.42 ± 131.76	78.47 ± 12.82	949.84 ± 80.90	155.01 ± 22.07	9.96 ± 2.17	77.49 ± 13.84	176.60 ± 53.70
PRP 4000	2,623.41 ± 831.91	202.47 ± 17.19	2,586.26 ± 953.24	501.76 ± 124.54	21.30 ± 3.79	228.38 ± 24.97	173.23 ± 66.53

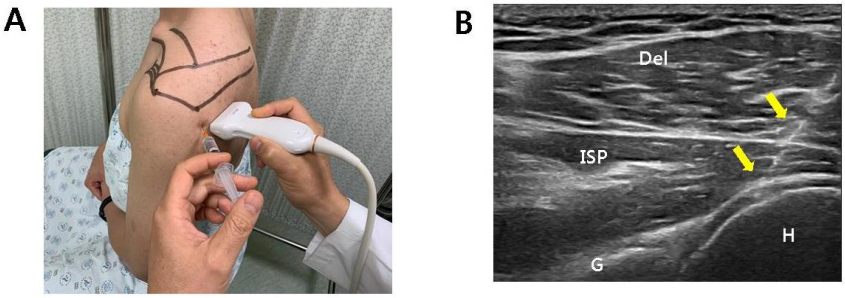
[1]Data are presented as mean ± SD otherwise specified. Note: bEGF, basic fibroblast growth factor; CTGF, connective tissue growth factor, EGF, epidermal growth factor; IGF-1, insulin-like growth factor 1; PDGF-AB, platelet-derived growth factor AB; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell; TGF-β1; VEGF, vascular endothelial growth factor; WBC, white blood cell. Flow cytometry with CD64 and CD62P was used to measure activation status[2] of PRP1000. Results were expressed by the percentage of CD62P-positive counts over CD61-positive counts.

TABLE 2. Comparison of baseline characteristics between the PRP and propensity score-matched steroid groups[1]

Variable	PRP (n=15)	Steroid (n=15)	*P* value
Age, y	60.3 ± 9.3	58.4 ± 3.9	.481
Sex, n (%)			.456
Male	7 (46.67)	5 (33.33)	
Female	8 (53.33)	10 (66.67)	
Dominant side affected, n (%)	6 (40.0)	8 (53.3)	.464
Symptom duration, mo	10.8 ± 11.8	6.5 ± 4.0	.206
Symptom aggravation, mo	2.4 ± 1.8	2.5 ± 1.9	.906
Previous treatment history, n (%)			
Surgery	0	0	
Pharmaceutical	15 (100.0)	15 (100.0)	1.000
Injection	5 (33.3)	3 (20.0)	.409
Physiotherapy	8 (53.3)	8 (53.3)	1.000
Acupuncture	9 (60.0)	1 (6.7)	.002
VAS pain			
At rest	3.2 ± 2.5	3.2 ± 2.3	1.000
On motion	6.3 ± 2.1	5.7 ± 2.7	.302
At night	6.2 ± 2.7	5.1 ± 2.9	.538
Mean	5.2 ± 1.8	4.7 ± 2.3	.475
Worst	8.1 ± 1.5	8.3 ± 1.4	.803
Constant score	42.5 ± 15.6	41.4 ± 11.6	.836
SPADI score	52.8 ± 17.9	54.1 ± 23.2	.867
ASES score	48.0 ± 16.9	49.6 ± 18.6	.808
DASH score	35.4 ± 16.2	37.8 ± 19.0	.714
UCLA score	16.7 ± 4.9	15.3 ± 3.1	.360
SST score	5.5 ± 3.3	4.1 ± 2.7	.189
Range of motion, deg			
Flextion	122 ± 21	117 ± 16	.459
Abduction	104 ± 27	106 ± 19	.849
External rotation	27 ± 15	26 ± 9	.940
Internal rotation	5 ± 3	4 ± 2	.201
Imaging for Enrollment, n (%)			.283
US	12 (80.0)	14 (93.3)	
MRI	3 (20.0)	1 (6.7)	
Finding with US or MRI, n (%)			1.000
Intact	1 (6.7)	0	
Fraying or tendinopathy	11 (73.3)	12 (80.0)	
partial-thickness tear	3 (20.0)	3 (6.7)	
full-thickness tear	0	0	

[1]Values are expressed as mean ± SD unless otherwise specified. Individual patients were asked whether he or she received prior therapy during the past 3 months (yes or no). VAS, visual analog scale; ASES, American Shoulder and Elbow Surgeons; UCLA, University of California, Los Angeles; DASH, Disabilities of the Arm, Shoulder and Hand; SST, Simple Shoulder Test; SPADI, Shoulder Pain and Disability Index. NA, not available; PRP, platelet-rich plasma.

Figure 1. Ultrasonography-guided intra-articular injection and clinical evaluation of PRP and corticosteroid injection. (A) A 25-gauge needle was introduced into the posterolateral corner of the acromion (Acr) with the guidance of transducer. (B) The needle was approached into the intra-articular space under real-time ultrasonography guidance.



Safety and efficacy of ultrasonography-guided intra-articular PRP injections in patients with Adhesive capsulitis: a propensity score-matched case-control study

Patient characteristics & adverse events

Fifteen patients with adhesive capsulitis received either allogeneic PRP1000 or steroid injection (Figure 1, A and B). After propensity score matching, no significant difference was found in the baseline characteristics between the PRP and steroid groups except for previous treatment histology on acupuncture (Table 2). No general or local adverse events were observed in the PRP group during the immediate post-injection or follow-up periods.

Pain

Both before and at any time points after allogeneic PRP or steroid injection, VAS pain scores at rest, during motion, and at night and the mean and worst pain scores were not significantly different between the 2 groups (Figure 2, A-E; table 3). However, there was a difference in the changing pattern of pain between the groups. In the PRP group, all of the 5 VAS pain scores gradually decreased over time up to 6 months. If the minimal clinically important difference (MCID) and patient acceptable symptomatic state (PASS) for VAS pain for rotator cuff diseases, 1.4 and 3.0, respectively, were adapted for adhesive capsulitis,[63] all of the 5 VAS pain decreased beyond the MCID and achieved PASS in the PRP group. In the steroid group, generally all the 5 VAS pain more promptly declined at 1 week and faster reached the lowest scores at 3 months than in the PRP group. However, all the pain

scores tended to increase again after that up to 6 months, and the worst pain at 6 months became 3.7±2.6 that did not achieve PASS. These results would indicate that PRP injection is slow and long-acting whereas steroid injection is a quick and short-acting treatment which is consistent with previous results of allogeneic PRP injection for rotator cuff disease.[28]

Strength

Before injection, the strength of the supraspinatus, infraspinatus and subcapularis muscles was not significantly different between the 2 groups (Figure 3, A-C; table 4). After injection, in the PRP group, strength of the supraspinatus significantly increased by 1.28-fold at 1 month (P = .030), and that of the infraspinatus and subscapularis increased by 1.41- and 1.26-fold at 3 months, respectively (P = .041, and .018, respectively). In the steroid group, strength of rotator cuff significantly increased in sequence by 1.24-fold at 1 week (P = .028) for the supraspinatus, by 1.25-fold at 1 month (P = .013) for the infraspinatus, and by 1.25-fold at 3 months (P = .036), respectively.

Range of motion

Both before injection, active forward flexion, abduction, external rotation with the arm at the side, and internal rotation were not significantly different in the 2 groups (Figure 4, A-D; table 5). After injection, all the ROMs significantly increased at 1 week and further increased up to 6 months in both groups except for the abduction and internal rotation at 1 week in the PRP group both of which

significantly improved at 1 month. Active forward, flexion, abduction, external rotation with the arm at the side, and internal rotation increased by 1.30-, 1.50-, 1.38-, and 2.20-fold in the PRP group, and 1.35-, 1.51-, 1.48-, and 2.54-fold in the steroid group at 6 months, respectively (all $P < .001$ except for external rotation with the arm at the side in the PRP group, $P = .005$). However, ROMs did not reach the comparable levels of the contralateral side in both groups at any time points (table 6).

Functional scores

Before injection, all of the shoulder functional scores, Constant, SPADI, ASES, DASH, UCLA, and SST, were not different between the 2 groups (Figure 5, A-F; table 7). After injection, in the PRP group, all the scores promptly improved at 1 week gradually improved with time up to 6 months compared with before injection except for DASH at 3 months and SST at 1 month. In the steroid group, all the scores significantly improved at 1 week, reached the best at 3 months, and then rebounded thereafter except for the Constant score. There were temporary greater improvement was seen in the steroid group in the Constant ($P = .042$), and ASES ($P = .041$) scores at 1 month, and DASH score at 1 week ($P = .046$), and 1 month ($P = .005$). Nonetheless, all the scores were better in the PRP group at 6 months while no statistically significant differences were found. All of the 5 scores for which MCIDs have been known improved beyond the MCID at 6 month in both groups: Constant (10.4), SPADI (15.4), ASES (up to 16.9), DASH (10.2), and SST (2).[23, 37, 62, 69]

Overall function and satisfaction

Before injection, overall function of the affected shoulder measured with the Single Assessment Numeric Evaluation was not different between 2 groups (Figure 6, A and B; table 8). After injection, overall function in the PRP group improved gradually, became significantly greater than at the baseline at 3 months, and reached the highest at 6 months (76.7 ± 16.8, $P < .001$). In the steroid group, faster improvement was found at 1 week, reached the highest at 3 months (75.3 ± 19.6, $P < .001$), and then slightly decreased at 6 months (71.7 ± 23.0, $P = .001$). While overall function was better in the PRP group at 6 moths, no significant difference was found. Overall satisfaction also showed similar change pattern in both groups. In the PRP group, overall satisfaction gradually increased after injection, and reached the highest at 6 months (79.0 ± 22.69). In the steroid group, it reached its highest at 3 months (81.67 ± 21.19), and decreased to 69.0 ± 30.37 at 6 months. There was no significant difference between the 2 groups at any time points.

Figure 2. (A-E) Changes in pain after PRP or steroid injection

n=15 for each group. Scores measured before injection (baseline), 1 week, 1month, 3 months, 6 months after injection, except for overall satisfaction. A paired t-test was used for the comparison between baseline and each time point. Two sample t-test was used to compared between groups.

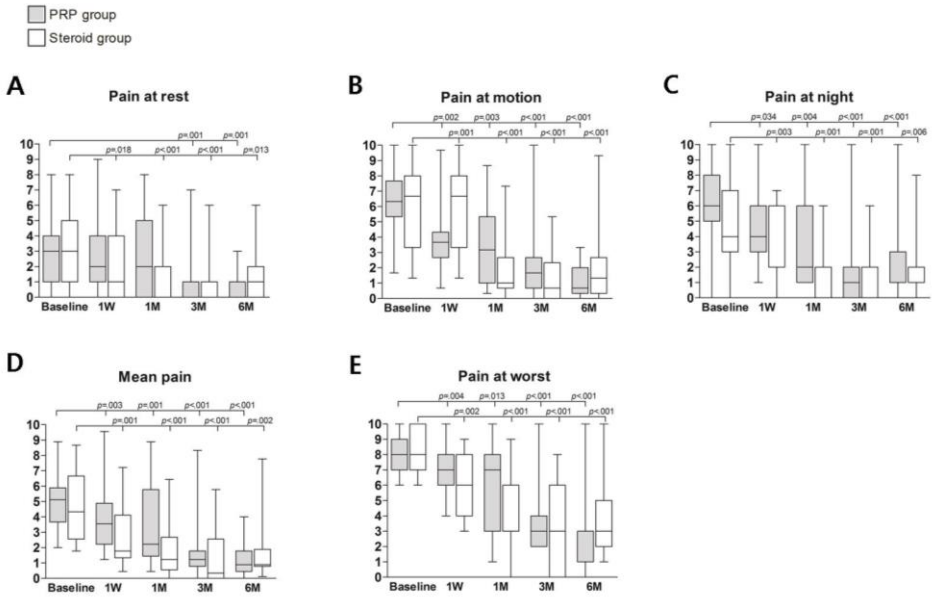


Table 3. Change of pain after PRP or steroid injection

Variable	**PRP (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	**Steroid (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	*P* Value[5] (between groups)
Pain at rest											
Preinjection	3.2 ± 2.5					3.2 ± 2.3					1.000
1W	2.5 ± 2.5	.117				2.1 ± 2.4	.018				.630
1M	2.5 ± 2.50	.177	1.000			1.1 ± 1.7	<.001	.030			.075
3M	1.5 ± 2.0	.001	.033	.104		0.8 ± 1.7	<.001	.036	.389		.338
6M	0.7 ± 0.9	.001	.011	.013	.082	1.1 ± 1.6	.013	.200	1.000	.685	.403
Pain on motion											
Preinjection	6.3 ± 2.1					5.7 ± 2.7					.538
1W	4.0 ± 2.2	.002				3.1 ± 2.1	.001				.278
1M	3.5 ± 2.5	.003	.409			2.0 ± 2.0	<.001	.050			.088
3M	2.5 ± 2.6	<.001	.017	.055		1.5 ± 1.9	<.001	.020	.213		.255
6M	1.1 ± 1.1	<.001	<.001	.001	.024	2.0 ± 2.4	<.001	.153	.936	.452	.207
Pain at night											
Preinjection	6.2 ± 2.7					5.1 ± 2.9					.302
1W	4.9 ± 2.7	.034				3.1 ± 2.4	.003				.059
1M	3.4 ± 2.8	.004	.045			2.3 ± 2.2	.001	.151			.238

3M	2.1 ± 2.9	<.001	<.001	.059		1.5 ± 2.1	.001	.058	.177		.517
6M	2.1 ± 2.5	<.001	.007	.181	1.000	2.0 ± 2.1	.006	.208	.660	.418	.876
Mean pain											
Preinjection	5.2 ± 1.8					4.7 ± 2.3					.475
1W	3.8 ± 2.2	.003				2.8 ± 2.1	.001				.200
1M	3.1 ± 2.4	.001	.194			1.8 ± 1.8	<.001	.035			.098
3M	2.0 ± 2.4	<.001	.002	.048		1.3 ± 1.7	<.001	.021	.168		.338
6M	1.3 ± 1.2	<.001	.001	.012	.235	1.7 ± 2.0	.002	.166	.851	.485	.524
Worst pain											
Preinjection	8.13 ± 1.46					8.3 ± 1.4					.803
1W	6.67 ± 1.60	.004				5.9 ± 2.1	.002				.284
1M	5.83 ± 2.64	.013	.201			4.1 ± 2.5	<.001	.007			.080
3M	3.87 ± 2.26	<.001	<.001	.003		3.3 ± 2.9	<.001	.003	.293		.574
6M	2.87 ± 2.56	<.001	<.001	.002	.271	3.7 ± 2.6	<.001	.008	.594	.729	.401

Values are expressed as mean ± SD unless otherwise specified.
[1]Comparison between the baseline and each time point
[2]Comparison between 1W and each time point
[3]Comparison between 1M and each time point
[4]Comparison between 3M and each time point
[5]Comparison of the mean difference between PRP and steroid group at each time point.

Figure 3. (A-C) Changes in strength of rotator cuff muscles after PRP or steroid injection

A paired t-test was used for the comparison between baseline and each time point. Two sample t-test was used to compared between groups.

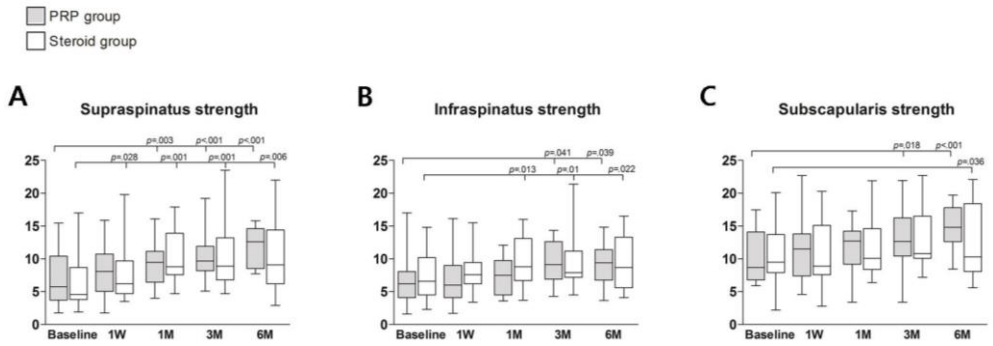


Table 4. Change of strength after PRP or steroid injection

Variable	**PRP (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	**Steroid (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	*P* Value[5] (between groups)
Supraspinatus, 1b											
Preinjection	6.7 ± 4.4					6.7 ± 4.3					.990
1W	7.3 ± 4.8	.369				8.3 ± 5.0	.028				.579
1M	8.6 ± 4.1	.030	.025			10.0 ± 3.8	.001	.045			.320
3M	10.4 ± 3.6	<.001	.001	.012		10.3 ± 4.8	.001	.036	.678		.945
6M	11.8 ± 3.1	<.001	<.001	<.001	.104	10.3 ± 5.2	.006	.075	.809	.809	.335
Infraspinatus, 1b											
Preinjection	6.7 ± 4.0					7.4 ± 3.6					.659
1W	7.1 ± 4.1	.388				8.4 ± 3.5	.082				.355
1M	7.6 ± 3.0	.301	.493			9.2 ± 3.5	.013	.254			.202
3M	9.5 ± 3.2	.041	.033	.025		9.7 ± 4.5	.010	.178	.549		.890
6M	9.2 ± 3.0	.039	.054	.062	.480	9.0 ± 4.1	.022	.348	.864	.287	.888
Subscapularis, 1b											
Preinjection	10.3 ± 3.9					10.2 ± 4.3					.938
1W	11.3 ± 4.7	.101				10.3 ± 5.1	.955				.586
1M	12.0 ± 3.6	.058	.480			12.0 ± 5.2	.134	.024			.986
3M	13.0 ± 4.7	.018	.021	.213		18.6 ± 21.9	.161	.003	.111		.350
6M	15.0 ± 3.3	<.001	.007	<.001	.027	12.8 ± 5.5	.036	.103	.487	.946	.189

Values are expressed as mean ± SD unless otherwise specified.
[1]Comparison between the baseline and each time point
[2]Comparison between 1W and each time point
[3]Comparison between 1M and each time point
[4]Comparison between 3M and each time point
[5]Comparison of the mean difference between PRP and steroid group at each time point.

Figure 4. (A-D) Changes in range of motions after PRP or steroid injection

A paired t-test was used for the comparison between baseline and each time point. Two sample t-test was used to compared between groups.

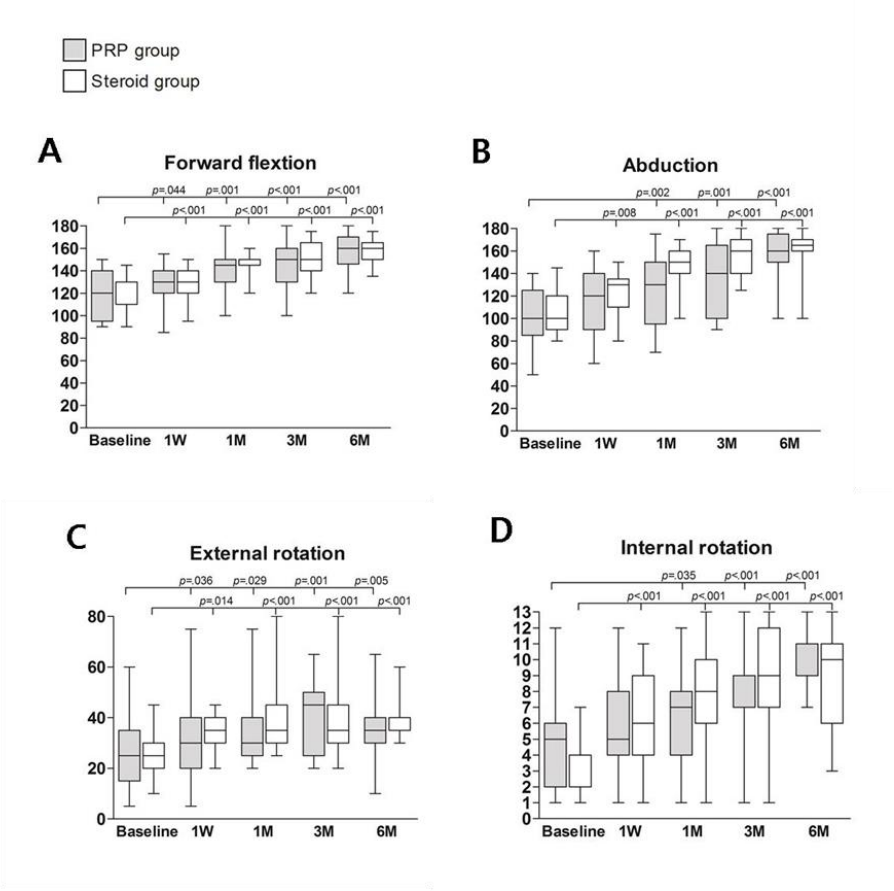


Table 5. Change of ROMs after PRP or steroid injection

Variable	**PRP (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	**Steroid (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	*P* Value[5] (between groups)
Forward flexion, deg											
Preinjection	122 ± 21					117 ± 16					.459
1W	127 ± 20	.044				130 ± 15	<.001				.687
1M	141 ± 19	.001	.002			147 ± 9	<.001	.001			.257
3M	145 ± 19	.001	<.001	.027		151 ± 15	<.001	.004	.245		.395
6M	158 ± 17	.001	<.001	.003	.008	157 ± 12	<.001	<.001	.029	.175	.890
Abduction, deg											
Preinjection	104 ± 27					106 ± 19					.849
1W	114 ± 28	.064				123 ± 19	.008				.352
1M	126 ± 29	.002	.013			147 ± 18	<.001	<.001			.024
3M	135 ± 31	.001	.002	.169		156 ± 15	<.001	<.001	.128		.026
6M	156 ± 21	<.001	<.001	<.001	.010	159 ± 21	<.001	<.001	.094	.582	.707
External rotation with arm at the side, deg											
Preinjection	27 ± 15					26 ± 9					.940
1W	33 ± 16	.036				33 ± 8	.014				.944
1M	35 ± 16	.029	.262			39 ± 15	<.001	.139			.555
3M	41 ± 15	.001	.009	.052		40 ± 15	<.001	.109	.567		.855
6M	37 ± 15	.005	.212	.666	.118	39 ± 8	<.001	.039	.923	.827	.591
Internal rotation, vertebral level											
Preinjection	5 ± 3					4 ± 2					.201
1W	6 ± 3	.084				7 ± 3	<.001				.339
1M	6 ± 3	.035	.152			8 ± 3	<.001	.025			.145
3M	8 ± 3	<.001	<.001	.008		9 ± 3	<.001	.025	.284		.767
6M	10 ± 2	<.001	<.001	<.001	.012	9 ± 3	<.001	.019	.263	.779	.154

Values are expressed as mean ± SD unless otherwise specified.
[1]Comparison between the baseline and each time point
[2]Comparison between 1W and each time point
[3]Comparison between 1M and each time point
[4]Comparison between 3M and each time point
[5]Comparison of the mean difference between PRP and steroid group at each time point.

Table 6. Change of the ROMs of affected side and the ROMs of contralateral side

Variable	Affected side (n=15)	Contralateral (n=15)	P-value[1]
Forward flexion, deg (PRP group)			
Preinjection	122 ± 21	167 ± 12	<.001
1W	127 ± 20	170 ± 11	<.001
1M	141 ± 19	169 ± 9	<.001
3M	145 ± 19	169 ± 9	<.001
6M	158 ± 17	167 ± 11	.017
Abduction, deg (PRP group)			
Preinjection	104 ± 27	170 ± 15	<.001
3W	114 ± 28	176 ± 9	<.001
1M	126 ± 29	176 ± 6	<.001
3M	135 ± 31	177 ± 4	<.001
6M	156 ± 21	175 ± 6	.002
External rotation with arm at the side, deg (PRP group)			
Preinjection	27 ± 15	56 ± 12	<.001
1W	33 ± 16	58 ± 12	<.001
1M	35 ± 16	56 ± 11	<.001
3M	41 ± 15	59 ± 10	<.001
6M	37 ± 15	57 ± 13	<.001
Internal rotation, vertebral level (PRP group)			
Preinjection	5 ± 3	12 ± 1	<.001
1W	6 ± 3	12 ± 1	<.001
1M	6 ± 3	12 ± 2	<.001
3M	8 ± 3	11 ± 1	.001
6M	10 ± 2	12 ± 1	.002

Forward flexion, deg (Steroid group)			
Preinjection	117 ± 16	175 ± 6	<.001
3W	130 ± 15	175 ± 6	<.001
1M	147 ± 9	175 ± 6	<.001
3M	151 ± 15	176 ± 6	<.001
6M	157 ± 12	176 ± 6	<.001
Abduction, deg (Steroid group)			
Preinjection	106 ± 19	177 ± 5	<.001
1W	123 ± 19	176 ± 6	<.001
1M	147 ± 18	177 ± 6	<.001
3M	156 ± 15	178 ± 5	<.001
6M	159 ± 21	178 ± 4	.004
External rotation with arm at the side, deg (Steroid group)			
Preinjection	26 ± 9	56 ± 12	<.001
1W	33 ± 8	53 ± 11	<.001
1M	39 ± 15	55 ± 13	<.001
3M	40 ± 15	55 ± 13	<.001
6M	39 ± 8	50 ± 16	.060
Internal rotation, vertebral level (Steroid group)			
Preinjection	4 ± 2	12 ± 1	<.001
3W	7 ± 3	12 ± 1	<.001
1M	8 ± 3	12 ± 1	<.001
3M	9 ± 3	12 ± 1	<.001
6M	9 ± 3	12 ± 1	<.001

Values are expressed as mean ± SD unless otherwise specified.
[1]Comparison of the mean difference between affected side and contralateral side.

Figure 5. (A-F) Changes in commonly used functional scores after PRP or steroid injection

SPADI, Shoulder Pain and Disability Index; ASES, American Shoulder and Elbow Surgeons score; DASH, Disabilities of the Arm, Shoulder and Hand; UCLA, University of California, Los Angeles score; SST, Simple Shoulder Test. A paired t-test was used for the comparison between baseline and each time point. Two sample t-test was used to compared between groups.

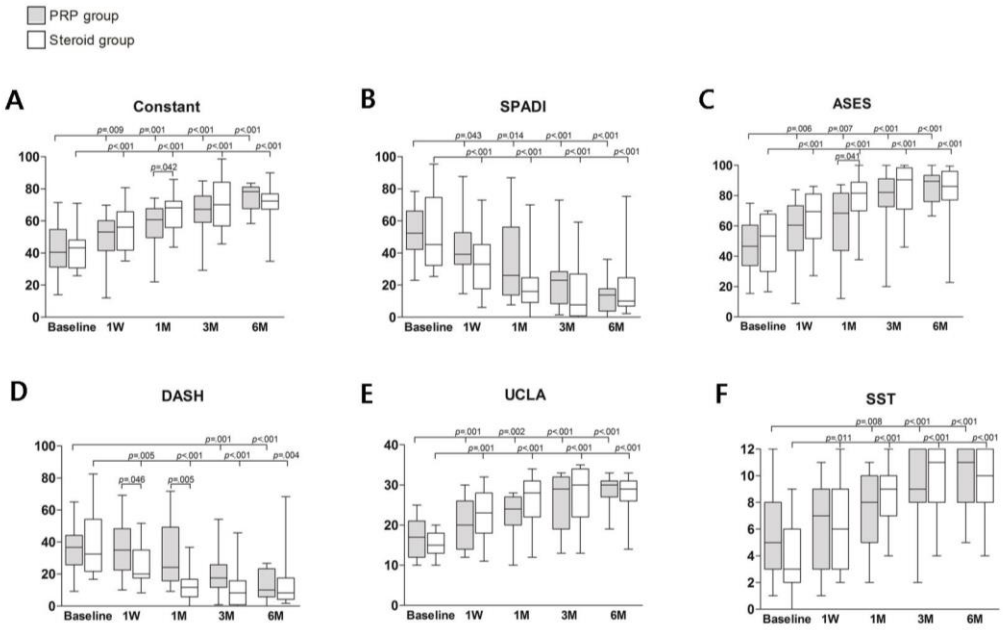


Table 7. Change of Constant, SPADI, ASES, DASH, UCLA and SST Scores after PRP or steroid injection

Variable	**PRP (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	**Steroid (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	*P* Value[5] (between groups)
Constant											
Preinjection	42.5 ± 15.6					41.4 ± 11.6					.836
1W	50.2 ± 15.1	.009				56.6 ± 14.5	<.001				.246
1M	57.0 ± 13.9	.001	.013			66.8 ± 11.2	<.001	.016			.042
3M	66.7 ± 13.8	<.001	<.001	<.001		70.4 ± 16.1	<.001	.022	.329		.506
6M	74.1 ± 8.9	<.001	<.001	<.001	.024	70.9 ± 12.2	<.001	.006	.364	.911	.410
SPADI											
Preinjection	52.8 ± 17.9					54.1 ± 23.2					.867
1W	43.9 ± 18.2	.043				33.8 ± 16.8	<.001				.128
1M	35.8 ± 24.4	.014	.089			21.2 ± 19.1	<.001	.009			.078
3M	23.3 ± 18.8	<.001	<.001	.010		16.3 ± 18.7	<.001	.004	.195		.313
6M	13.3 ± 10.9	<.001	<.001	.001	.011	17.3 ± 18.2	<.001	.019	.572	.844	.476
ASES											
Preinjection	48.0 ± 16.9					49.6 ± 18.6					.808
1W	56.6 ± 19.8	.006				66.6 ± 17.1	<.001				.151
1M	63.7 ± 22.0	.007	.088			79.0 ± 16.8	<.001	.006			.041
3M	77.4 ± 21.1	<.001	<.001	.006		84.0 ± 16.9	<.001	.007	.215		.352
6M	85.9 ± 10.7	<.001	<.001	.001	.097	83.1 ± 19.0	<.001	.025	.538	.862	.627

DASH											
Preinjection	35.4 ± 16.2					37.8 ± 19.0					.714
1W	36.8 ± 16.5	.569				25.4 ± 13.1	.005				.046
1M	31.6 ± 19.4	.441	0.125			13.6 ± 10.5	<.001	.001			.005
3M	19.4 ± 13.7	.001	0.001	.014		11.7 ± 12.9	<.001	.005	.478		.123
6M	12.3 ± 8.7	<.001	<.001	.001	.023	13.1 ± 16.4	.004	.043	.915	.785	.881
UCLA											
Preinjection	16.7 ± 4.9					15.3 ± 3.1					.360
1W	20.5 ± 5.8	.001				22.7 ± 6.1	.001				.321
1M	22.2 ± 6.1	.002	.211			26.7 ± 5.9	<.001	.032			.050
3M	25.7 ± 6.8	<.001	.012	.048		28.0 ± 7.1	<.001	.037	.376		.367
6M	28.7 ± 4.0	<.001	<.001	.003	.046	27.9 ± 4.7	<.001	.017	.525	.943	.622
SST											
Preinjection	5.5 ± 3.3					4.1 ± 2.7					.189
1W	6.2 ± 3.2	.375				6.1 ± 3.2	.011				.910
1M	7.4 ± 2.4	.008	.098			8.5 ± 2.5	<.001	.010			.217
3M	9.1 ± 3.1	<.001	<.001	.011		9.9 ± 2.4	<.001	.002	.046		.400
6M	10.1 ± 2.1	<.001	<.001	<.001	.072	9.8 ± 2.4	<.001	.003	.194	.878	.686

Values are expressed as mean ± SD unless otherwise specified.
[1]Comparison between the baseline and each time point
[2]Comparison between 1W and each time point
[3]Comparison between 1M and each time point
[4]Comparison between 3M and each time point
[5]Comparison of the mean difference between PRP and steroid group at each time point.

Figure 6. (A,B) Changes in overall function and satisfaction after PRP or steroid injection

A paired t-test was used for the comparison between baseline and each time point. Two sample t-test was used to compared between groups.

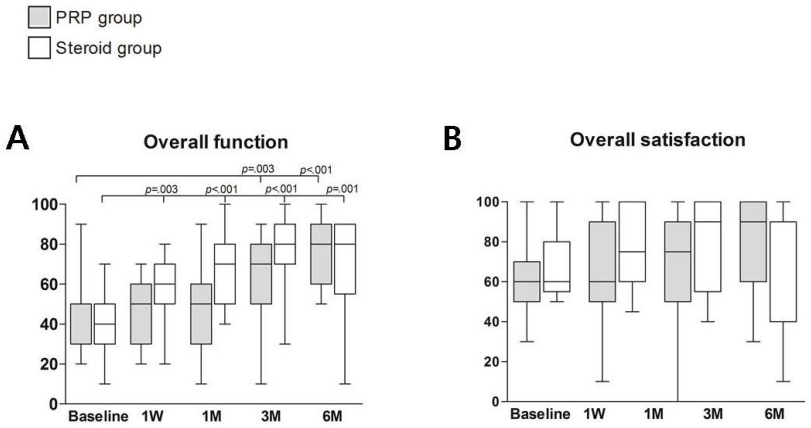


Table 8. Overall function and satisfaction after PRP or steroid injection

Variable	**PRP (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	**Steroid (n=15)**	*P* Value[1] (baseline)	*P* Value[2] (1W)	*P* Value[3] (1M)	*P* Value[4] (3M)	*P* Value[5] (between groups)
Overall function											
Preinjection	44.7 ± 1.92					40.7 ± 1.53					.534
1W	43.7 ± 1.65	.837				57.7 ± 1.61	.003				.026
1M	49.3 ± 2.02	.482	.212			66.3 ± 1.80	<.001	.138			.021
3M	64.0 ± 2.05	.003	<.001	.001		75.3 ± 1.96	<.001	.002	.089		.132
6M	76.7 ± 1.68	<.001	<.001	<.001	.001	71.7 ± 2.30	.001	.055	.439	.606	.501
Overall satisfaction											
1W	60.33 ± 19.22	NA				67.00 ± 14.98	NA				.334
1M	61.67 ± 26.30	NA	.827			77.00 ± 19.53	NA	.033			.084
3M	68.67 ± 26.35	NA	.281	.187		81.67 ± 21.19	NA	.015	.415		.193
6M	79.00 ± 22.69	NA	.016	.014	.097	69.00 ± 30.37	NA	.760	.299	.045	.248

Values are expressed as mean ± SD unless otherwise specified.
[1]Comparison between the baseline and each time point
[2]Comparison between 1W and each time point
[3]Comparison between 1M and each time point
[4]Comparison between 3M and each time point
[5]Comparison of the mean difference between PRP and steroid group at each time point.

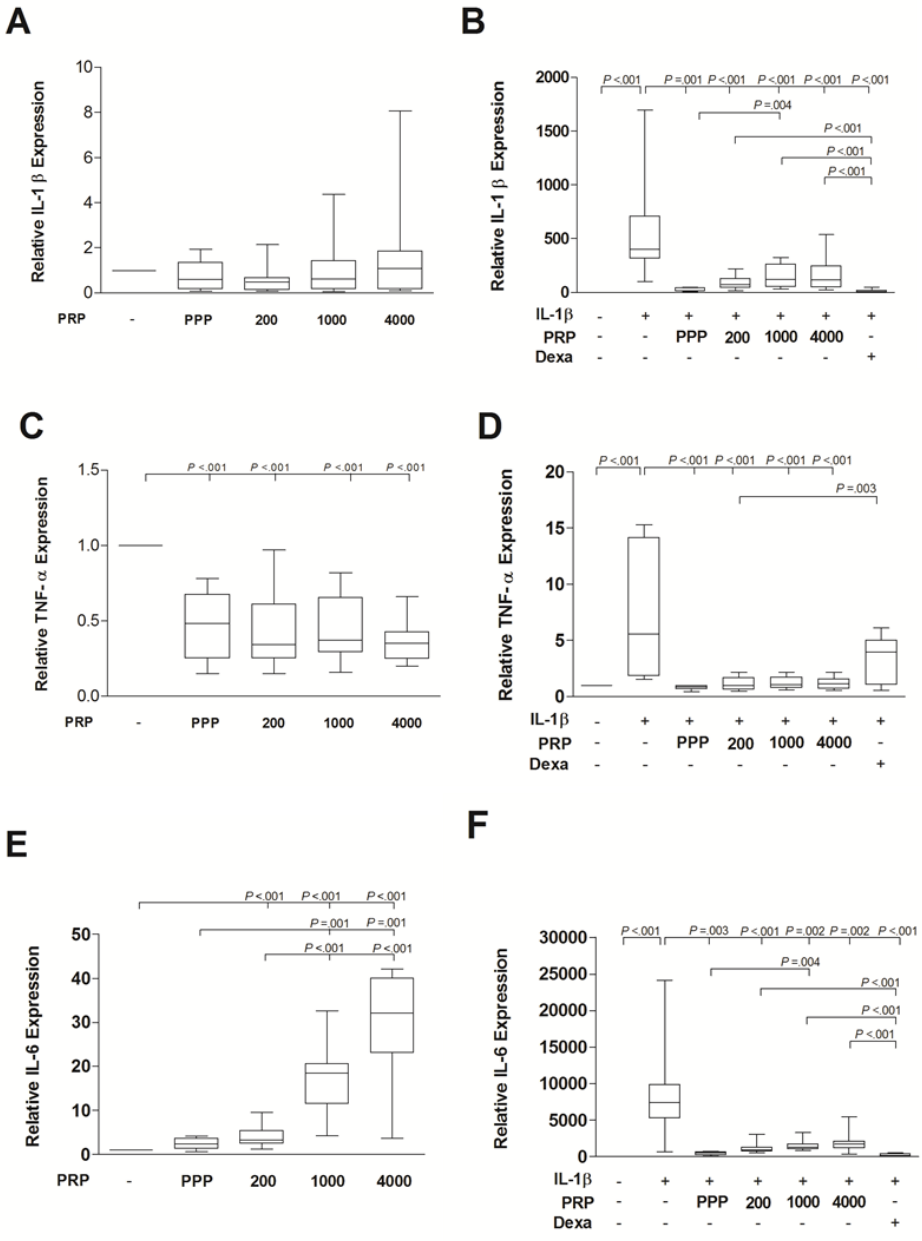
Effects of PRP on gene expression of pro-inflammatory cytokines in synoviocytes with or without IL-1β treatment

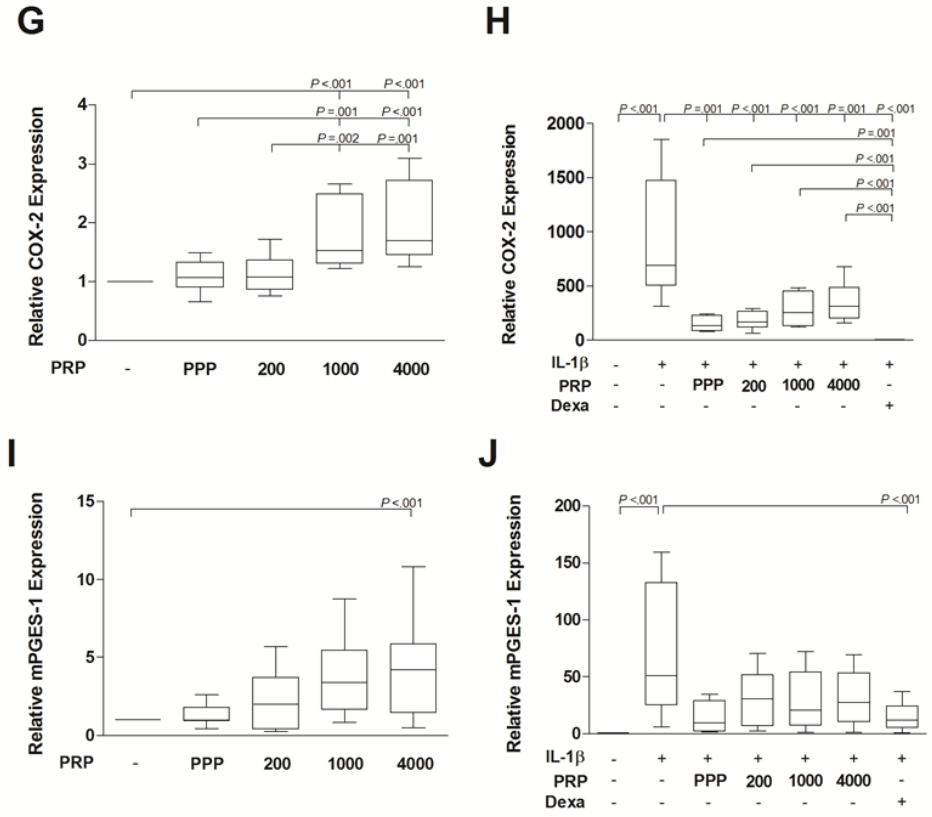
Gene expression of pro-inflammatory cytokines in synoviocytes responses in a pleiotropic manner upon the presence of the inflammatory factor, IL-1β, or not. Without IL-1β stimulation, PRP 1000 and PRP 4000 significantly induced the gene expression of IL-6, Cox-2, and mPGES-1(Figure 1, E, G, I), while inhibited that of TNF-1α (Figure 1C). There is no significant difference between using PRP or not for the IL-1β gene expression (Figure 1A).

With IL-1β stimulation, IL-1β significantly upregulated the gene expression of IL-1β, TNF-α, IL-6, COX-2, and mPGES-1 by 596-, 7-, 10078-, 914-, and 74-fold, respectively (Figure 1, B, D, F, H, and J). Dexamethasone treatment significantly downregulated the gene expression of IL-1β, IL-6, COX-2, and mPGE-1 by 14-, 276-, 3-, and 16-fold, respectively. PPP and PRP treatment significantly downregulated the gene expression of IL-1β, TNF-α, IL-6, and COX-2, whereas no significant difference was shown in TNF-α expression by dexamethasone.

Figure 7. Effects of PRP on gene expression of pro-inflammatory cytokines with or without IL-1β treatment

Synoviocytes were treated with PPP and PRP 200, PRP 1000, and PRP 4000 (10% vol/vol) with 1ng/ml recombinant human IL-1β for 24 hours. Non-treated cells were used as a negative control, and 1μM dexamethasone-treated cells were used as a positive control. (A, C, E, G, I) were performed without IL-1β, (B,D,F,H,J) were performed with IL-1β. Kruskal-Wallis test and Mann-Whitney test with Bonferroni correction were used for statistics. α-level < .005 was determined to be statistically significant.


A
Relative IL-1 β Expression
PRP - PPP 200 1000 4000
B
Relative IL-1 β Expression
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
C
Relative TNF- α Expression
PRP - PPP 200 1000 4000
D
Relative TNF- α Expression
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
E
Relative IL-6 Expression
PRP - PPP 200 1000 4000
F
Relative IL-6 Expression
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +


G
Relative COX-2 Expression
P<.001
P<.001
P=.001
P<.001
P=.002
P=.001
PRP - PPP 200 1000 4000
H
Relative COX-2 Expression
P<.001
P=.001
P<.001
P<.001
P=.001
P<.001
P=.001
P<.001
P<.001
P<.001
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
I
Relative mPGES-1 Expression
P<.001
PRP - PPP 200 1000 4000
J
Relative mPGES-1 Expression
P<.001
P<.001
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +

Effects of PRP on gene expression of degradative enzymes and their inhibitors in synoviocytes with or without IL-1β treatment

Gene expression of the degradative enzymes and their inhibitors in synoviocytes responses the other way depending on the condition with or without IL-1β, except for MMP-9 and TIMP-1. These genes follow the same tendency with the pro-inflammatory cytokines. In the absence of IL-1β, PPP, and PRP, PRP 1000 and PRP 4000 induced the gene expression of MMP-3, TIMP-3, and ADAMTS-4 (Figure 2, C, K, and M). PPP decreased the gene expression of MMP-1, whereas the PRP increased the gene expression of MMP-1 (Figure 2A). The gene expression of MMP-9 decreased by PRP (Figure 2E) and the gene expression of ADAMTS-5 significantly decreased by the PPP and PRP (Figure 2O). There was no significant difference between the groups in the gene expression of TIMP-1 (Figure 2I)

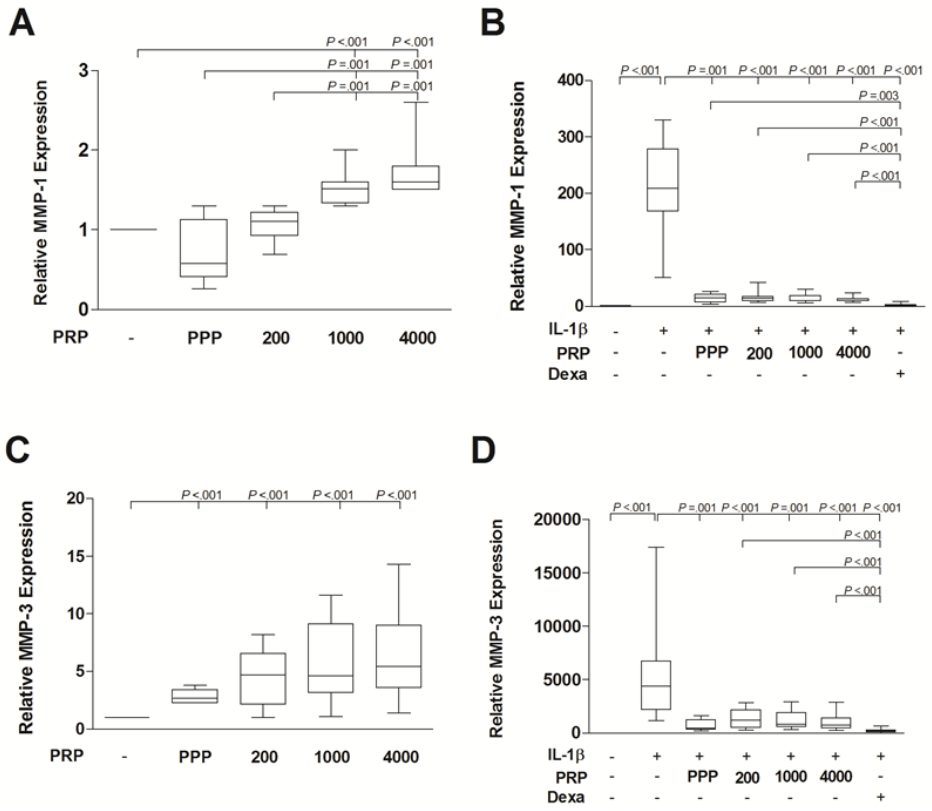
IL-1β significantly upregulated the gene expression of MMP-1, -3 ,-9, -13, and ADAMTS-4 and -5 by 182-, 5948-, 2-, 606-, 53-, and 2-fold, respectively (Figure 2, B, D, F, H, N, and P). Dexamethasone significantly downregulated the gene expression of MMP-1, -3, -9, and -13 and ADAMTS-4 by 1-, 239-, 0.8-, 1-, and 5-fold, respectively. It upregulated the gene expression of ADMATS-5 by up to 4-fold. However, PRP 1000 treatment decreased the gene expression of ADMATS-5 by 1.27-fold ($P = .003$). The gene expression of MMP-1, -3, and -13 and ADAMTS-4 was significantly downregulated, whereas no significant change was shown in MMP-9 with the PPP or PRP.

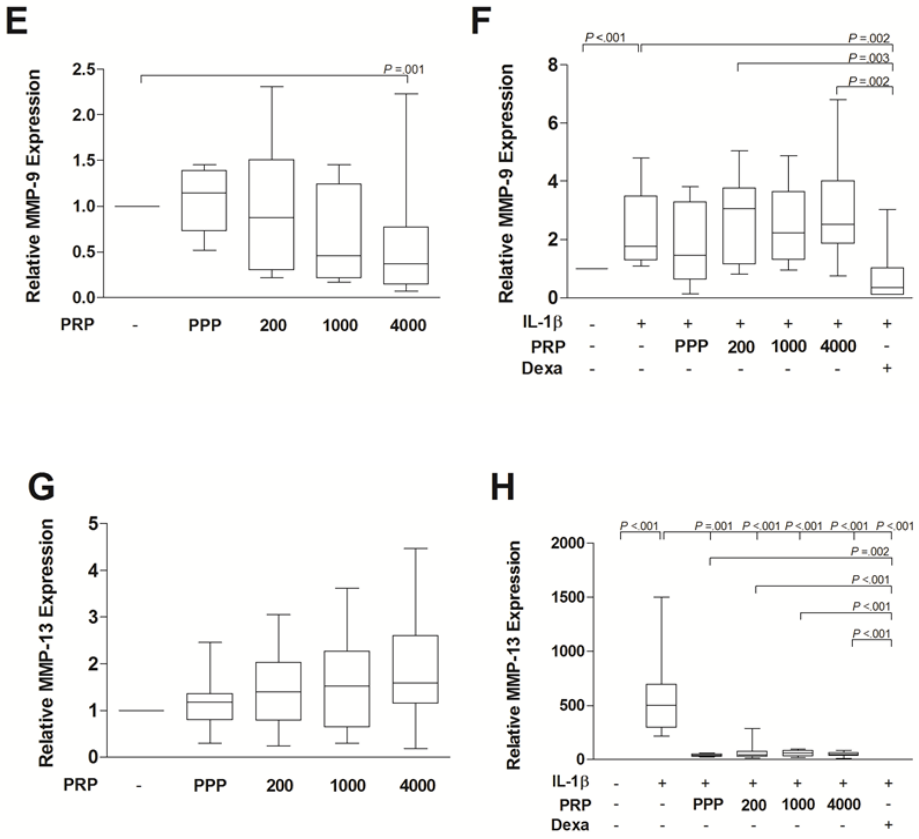
IL-1β upregulated the gene expression of TIMP-1 and -3 by 1.9- and 3-fold, respectively ($P < .001$). The gene expression of TIMP-3 significantly decreased with

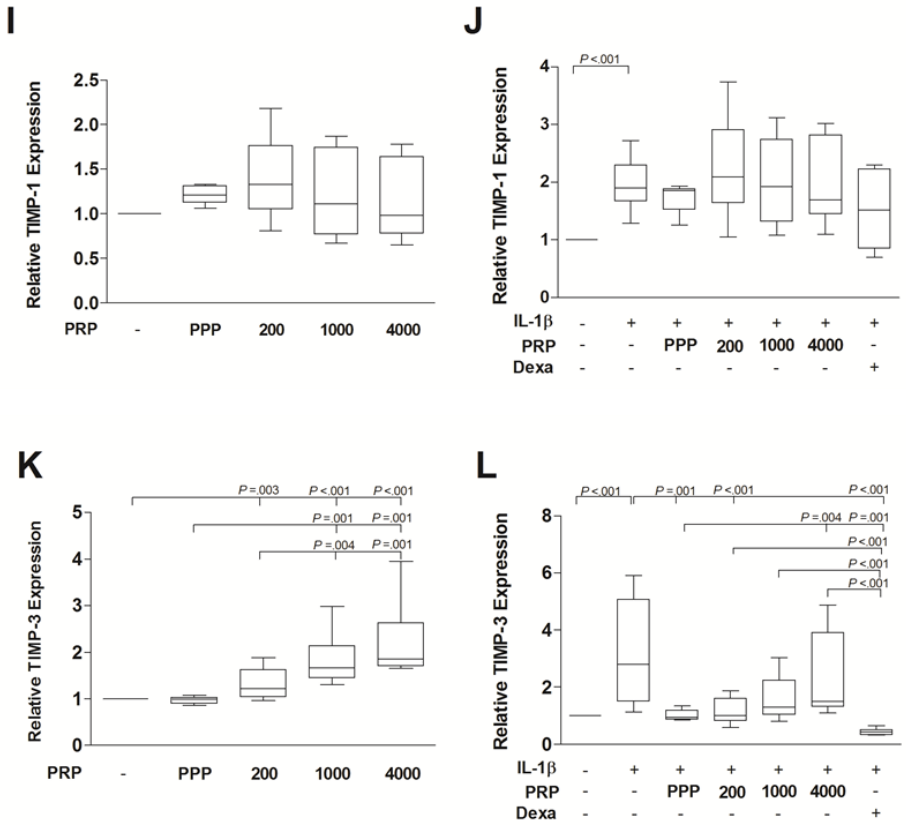
dexamethasone, PPP, and RPR200. There were no significant changes in the gene expression of TIMP-1 with the PPP, PRP, and dexamethasone.

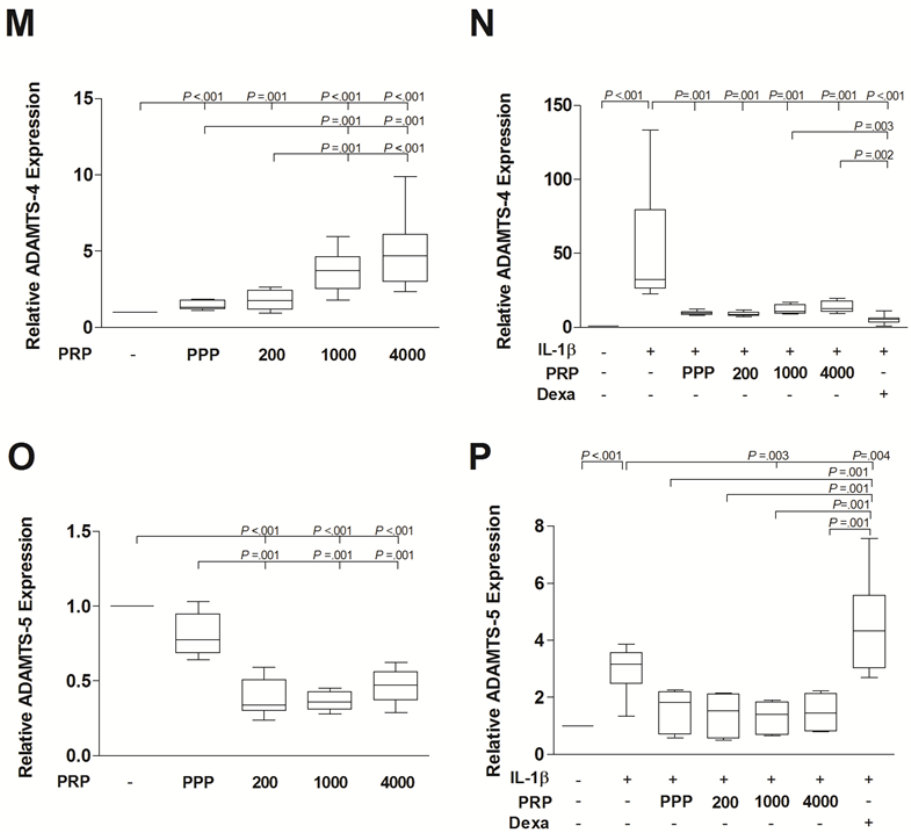
Figure 8. Effects of PRP on gene expression of degradative enzymes and their inhibitors in synoviocytes with or without IL-1β treatment

Procedure was same as above. (A, C, E, G, I, K, M, O) were performed without IL-1β, (B, D, F, H, J, L, N, P) were performed with IL-1β. Kruskal-Wallis test and Mann-Whitney test with Bonferroni correction were used for statistics. α-level < .005 was determined to be statistically significant.




E
Relative MMP-9 Expression
P = .001
PRP - PPP 200 1000 4000
F
Relative MMP-9 Expression
P < .001
P = .002
P = .003
P = .002
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
G
Relative MMP-13 Expression
PRP - PPP 200 1000 4000
H
Relative MMP-13 Expression
P < .001
P = .001
P < .001
P < .001
P < .001
P < .001
P = .002
P < .001
P < .001
P < .001
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +


I
Relative TIMP-1 Expression
2.5
2.0
1.5
1.0
0.5
0.0
PRP - PPP 200 1000 4000
J
Relative TIMP-1 Expression
P <.001
4
3
2
1
0
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
K
Relative TIMP-3 Expression
P =.003 P <.001 P <.001
P =.001 P =.001
P =.004 P =.001
5
4
3
2
1
0
PRP - PPP 200 1000 4000
L
Relative TIMP-3 Expression
P <.001 P =.001 P <.001 P <.001
P =.004 P =.001
P <.001
P <.001
P <.001
8
6
4
2
0
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +


M
Relative ADAMTS-4 Expression
15
10
5
0
P < .001
P = .001
P < .001
P < .001
P = .001
P = .001
P = .001
P < .001
PRP - PPP 200 1000 4000
N
Relative ADAMTS-4 Expression
150
100
50
0
P < .001
P = .001
P = .001
P = .001
P = .001
P < .001
P = .003
P = .002
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
O
Relative ADAMTS-5 Expression
1.5
1.0
0.5
0.0
P < .001
P < .001
P < .001
P = .001
P = .001
P = .001
PRP - PPP 200 1000 4000
P
Relative ADAMTS-5 Expression
8
6
4
2
0
P < .001
P = .003
P = .004
P = .001
P = .001
P = .001
P = .001
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +

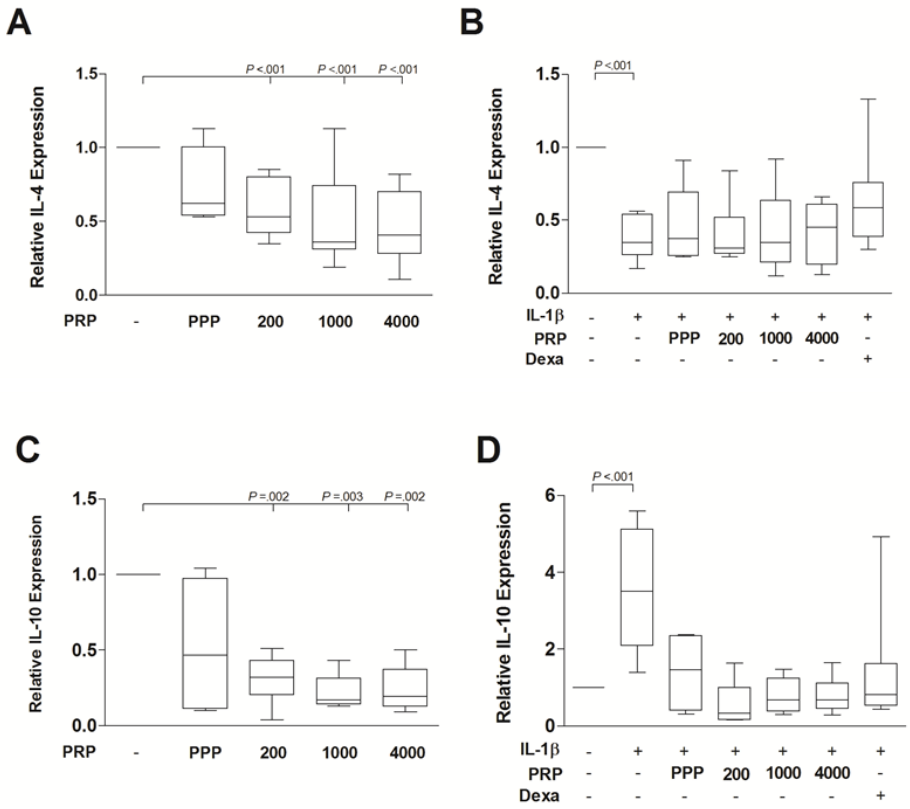
Effects of PRP on gene expression of anti-inflammatory cytokines in synoviocytes with or without IL-1β treatment

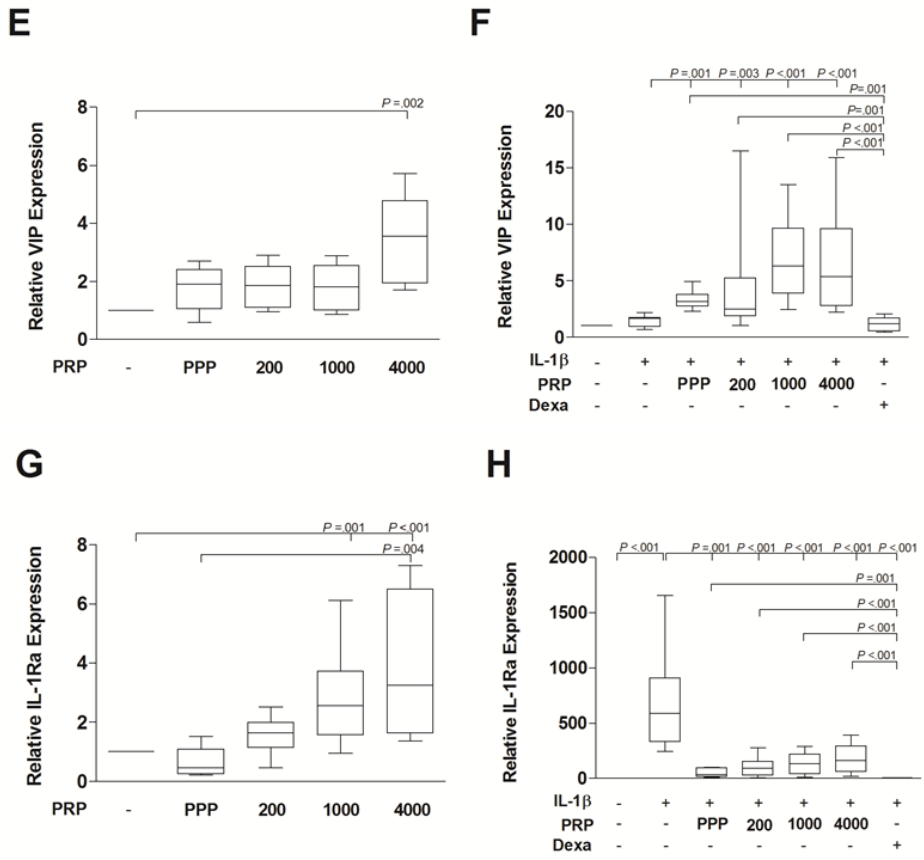
Gene expression of anti-inflammatory cytokines in synoviocytes reponses differently with or without IL-1β. Without IL-1β, the PRP reduced the gene expression of IL-4 and IL-10 (Figure 3, A and B) and PRP 4000 increased the gene level of VIP and IL-1Ra (Figure 3, E and G).

IL-1β treatment significantly inhibited gene expression of IL-4 by 0.37-fold and increased the gene expression of IL-10, VIP, and IL-1Ra by 3.5-, 1.4-, and 704-fold, respectively (Figure 3, D, F, and H). For VIP, treatment with the PPP or PRP significantly increased its expression, whereas no changes occurred with dexamethasone. There were no significant changes in IL-4 and IL-10 by treatment with PPP, PRP, or dexamethasone. Although the gene level of IL-1Ra failed to increase with the PPP or PRP, it had significantly higher expression levels (47.8-fold to 174-fold) in the PPP and PRP groups compared to t00he steroid group.

Figure 9. Effects of PRP on gene expression of anti-inflammatory cytokines in synoviocytes with or without IL-1β treatment

Procedure was same as above. (A,C,E,G) were performed without IL-1β, (B,D,F,H) were performed with IL-β. Kruskal-Wallis test and Mann-Whitney test with Bonferroni correction were used for statistics. α-level < .005 was determined to be statistically significant.




E
Relative VIP Expression
P = .002
PRP - PPP 200 1000 4000
F
Relative VIP Expression
P = .001 P = .003 P < .001 P < .001
P = .001
P = .001
P < .001
P < .001
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +
G
Relative IL-1Ra Expression
P = .001 P < .001
P = .004
PRP - PPP 200 1000 4000
H
Relative IL-1Ra Expression
P < .001 P = .001 P < .001 P < .001 P < .001 P < .001
P = .001
P < .001
P < .001
P < .001
IL-1β - + + + + + +
PRP - - PPP 200 1000 4000 -
Dexa - - - - - - +

Discussion

The most important findings of the study are that Pure PRP significantly relieved pain and improved shoulder strength, ROMs and function, in a manner comparable with steroid treatment, in patients with adhesive capsulitis at 6 months of follow-up without adverse events. Furthermore, pain measurements, strength of shoulder, and functional scores were promptly improved at first to third months after injection in steroid group but did not maintain up to 6 months. However, aforementioned parameters improved slow but steadily in PRP group and reached its peak at 6 months. Pure PRP has pleiotropic effects on synoviocytes depending on inflammatory condition induced with IL-1β by reducing the gene expression of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and COX-2 and their downstream enzymes such as MMPs and increased that of anti-inflammatory cytokine (eg. VIP). Taken together, the results of this clinical study and in vitro study suggest that PRPs may modulate inflammation-related cytokines to improved pain, ROMs and function in AC patients.

AC is known to be associated with IL-1α, IL-1β, -6, -8, and TNF-α, COX-1 and -2, and these cytokines may play an important role in synovial inflammation.[17, 20, 52] Several studies showed the effect of PRP on synoviocytes in RA or OA. Tohidnezhad et al. demonstrated that platelet-released growth factor significantly reduced TNF-α and IL-1β in synoviocytes under TNF-α stimulation.[65] Tong et al. showed that PRP inhibited the production of IL-1β, TNF-α and IL-6 in LPS-stimulated synoviocytes.[66] On the contrary, study of Olivotto showed no significant

change of IL-1β expression in TNF-α stimulated fibroblast-like synoviocyte with PRP.[49] Cell lines were used in these studies, and TNF-α or LPS was used to mimic the inflammatory condition of RA or OA. In present study, synoviocytes were isolated from shoulder with inflammation, this is distinct from previous studies in that primary cells from similar shoulder condition were used instead of using cell lines. Take cell lines into account that it do not behave identically with primay cells[34], the cells isolated from shoulder with inflammation may represent better understanding of synovitis. Furthermore, the cells were stimulated with IL-1β, one of the elevated pro-inflammatory cytokines in synovitis, to mimic synovial inflammation.[61] The gene expression of pro-inflammatory cytokines was downregulated by PRP, whereas in the absence of IL-1β, PRP induces inflammation by upregulating pro-inflammatory cytokines and MMPs. This pleiotropic effects of PRP was investigated in our previous study on tenocytes, in the similar manner, PRP exerts its anti-inflammatory effects on synoviocytes only under inflammatory condition induced with IL-1β.[28]

Imbalance of matrix synthesis and degradation occurs in AC, resulting in a failure of matrix remodeling.[13, 17] Remodeling of matrix is controlled by the MMPs and their inhibitors,[13] and failure in maintaining its homeostasis of MMPs and TIMPs ratio may be associated with fibrosis of the joint capsule. In the glenohumeral joint capsule of the AC patients or a rat AC model, levels of MMP-2, -3, -9 were significantly overexpressed.[16, 33] Overexpression of MMPs is detrimental in that MMPs not only degrade the extracellular matrix, but also mediate the downstream signaling of inflammation and apoptosis.[18] Furthermore, synoviocytes are major

contributors to the composition and function of synovioal fluid[1], thus MMPs released from synoviocytes could affect adjacent tendon, cartilage and muscles, therefore contribute to a vicious circle in shoulder anatomy. In a similar vein to our result, Sundman et al. reported that using PRP on synovium from OA patients reduced the gene expression of MMP-13.[59] In our study, treating PRP on IL-1β stimulated synoviocytes, the level of matrix enzymes (MMP-1,-3 and, -13 and ADAMTS-4 and, -5) were significantly decreased while no significant changes were detected in their inhibitor (TIMP-1). This may suggest that PRP modulates the homeostasis in matrix remodeling by downregulated the overexpressed level of MMPs that were induced by IL-1β, but also reduces deleterious effects on adjacent cells or tissues as well.

Although PRPs failed to increase anti-inflammatory cytokines of IL-4, IL-10, and IL-1Ra, significantly higher level of VIP was shown in PRP treated synoviocytes compared to IL-1β treated control as well as to corticosteroid group. While the role of VIP on AC has yet to be understood, it has been reported to inhibit the pro-inflammatory cytokines and MMPs in RA or OA specimens.[27, 32, 60] Jiang et al. found that decreased VIP levels may stimulate the production of pro-inflammatory cytokines, NO, and PGE2 in OA.[27] Juarranz et al. reported that VIP modulates the production of pro-inflammatory cytokines in human synovial cells from RA or OA. Synoviocytes were stimulated with TNF-α, and decreased mRNA levels of CCL-2, CXCL-8, and IL-6 were shown by treating VIP. [32] Takeba et al. reported that VIP inhibited pro-inflammatory cytokine and MMP production.[60] In this study, PRP decreased the gene expression of IL-6 and MMPs while increased that of VIP.

Considering all these factors, increased level of VIP may positively reduce pro-inflammatory cytokines. Therefore, this study shows VIP could be one of the anti-inflammatory factors in PRP treatment.

The present study is in line with the finding of our previous study on tenocytes in terms of the pleiotropic effects of allogeneic PRP. This finding indicates that using PRP in presence of inflammation is appropriate; otherwise it increases pro-inflammatory cytokines and their downstream enzymes to cause detrimental results. This may provide an important guidance for clinical use of PRP in that PRP is more appropriate in inflammatory conditions, than in health or non-inflammatory condition. Management of AC could be differently depending on which stages of the disease is, and herein treating PRP on AC may be used in the early stage (also referred as freezing stage) of disease which accompanies unmanageable pain induced by synovial inflammation.[47, 54]

Despite corticosteroid injection acts in rapid with respect to pain and range of motion on AC, the effects do not maintain beyond six weeks.[7, 10, 12] Furthermore, use of corticosteroid should be cautious, since experimental evidences support its deleterious effects on shoulder tissue, such as tendon and bone.[29, 44] In meta-analysis of rotator cuff repair, current evidences indicate PRP improved healing rates, pain levels and functional outcomes and PRP could be an alternative option for steroid.[25, 28] However, the evidences of PRP on AC are limited to four studies.[5, 8, 36, 71] These studies showed improvement in symptoms with no adverse effects. Nonetheless, durations of these studies were limited to 12 weeks, whereas 12 week-study may

not show distinct comparison between use of corticosteroid and PRP in that effectiveness of corticosteroid generally lasts 1 month to 3 months. The present study showed no significant differences between steroid and PRP injection groups, however in the steroid group, pain scores, shoulder strength, functional scores, overall satisfaction reached their peak at 3 months and tend to decreased at 6 months. The pattern of healing showed differently in PRP injection group, although PRP improved aforementioned parameters slower than steroid, those improved steadily up to 6 months. Second, limited range of motion is an important indicator of AC, however only Kothari et al. compared the changes in ROMs among groups (PRP, steroid, and ultrasonic therapy groups).[36] The present study compared the ROMs of PRP group to the steroid or contralateral shoulder groups, and showed whereas PRP failed to improved ROMs of affected shoulder upon that of the contralateral side, it significantly improved the ROMs of affected shoulder at any time point after intervention compared to the baseline. Third, autologous PRPs were used in the previous studies, whereas allogeneic PRP was used in this study. Using autologous PRP may be challenging or even impossible to whom with hematological disease or debilitated patients. Through preparing PRP from healthy one and examining the safety and its components before applying to patients could ensure its properties. Therefore, one step process with thawing could shorten the treatment time. Lastly, analysis of characteristics of PRP ($696 \times 10^6/\mu L$) was not investigated in above studies, except for the concentration of PRP was demonstrated in the study of Barman. Evidences of efficacy in PRP preparation did not reach a consensus, since compositions of PRP, clinical indication and preparations vary, and

lack of standardized system describing PRP therapy.[39, 45, 51] Since cellular composition of PRP is responsible for the concentration of growth factor and catabolic cytokine, the concentration of blood cells, growth factors or cytokines should be precisely monitored with a standardized protocol in PRP preparation.[58 22, 38, 42, 46] This would provide a better understanding regarding the mechanism and clinical indication of PRP. We provide "4Ds" to eliminate the controversial findings in interstudy differences of PRP therapy.[28] 4Ds: Drug (PRP), Delivery (application method), Donors (patients), and Disease (stage of adhesive capsulitis). 4Ds can be monitored to optimize the use of PRP for patients for whom this therapy is appropriate and optimal. In the current study, the 4Ds were controlled: drug (PRP properties and activation methods), delivery (injection location, number of injections, interval, and volume), donor (same allogeneic PRP from healthy donors without AC) and disease (early stage in AC). In present study, allogeneic PRP was prepared from healthy donors with a fully automated plateletpheresis system and its components, growth factors were fully characterized. All the injections were performed by a single physician with specialized expertise in ultrasonography guided shoulder injection. The diagnosis and stage of adhesive capsulitis was established clinically by a shoulder surgeon and radiologically by a fellowship-trained musculoskeletal radiologist. Although the stage of disease may vary among patients in detail, the stage of disease could be regarded as freezing or inflammatory stage in that most of the patients presented with unmanageable pain which was mainly associated with inflammation. In accordance with 4Ds, most of the confounding factors could be mitigated.

There are several limitations to this study. First, the synoviocytes were isolated from patients with rotator cuff disease, not from adhesive capsulitis. Synoviocytes from AC patients are difficult to be collected, since surgical operation is not always the best choice for AC. However, the synoviocytes from rotator cuff disease had been exposed to inflammatory condition in similar manner in joint capsule in AC, and they share same anatomical location in shoulder. Second, the study cohort was in small size and not randomly controlled, and long-term follow-up may be necessary since AC has long symptom duration. Long-term follow-up may show more clear distinction between PRP and steroid group by the reason of the short term efficacy of steroid.[10] Third, the group with no intervention or placebo may need, since the disease could be improved spontaneously in some patients. However, giving no treatment to the patients with severe pain could be controversial. Forth, these in vitro findings provide molecular mechanism on inflammatory stage of AC, but lack of evidences on fibrosis stage of AC.

Conclusion

This study demonstrated that allogeneic pure PRP acts in pleiotropic manner and decreased pro-inflammatory cytokines only in the inflammatory condition, and allogeneic pure PRP has comparable efficacy for pain relief and improving shoulder function with steroid injection in the patients with adhesive capsulitis. This provided the molecular evidences regarding allogeneic PRP that reduces inflammation in synoviocytes from shoulder, and therefore allogeneic PRP could be a treatment option for inflammatory stage of adhesive capsulitis.

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국문초록

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배경: 혈소판 풍부혈장(PRP; platelet-rich plasma)은 근골격계 질환에서 많이 연구된 생체 활성 단백질로 재생을 촉진시키는 치료법으로 사용되고 있다. 하지만, 유착성 관절낭염에 대한 임상보고는 매우 적다. 본 연구에서는 pure PRP 를 표준화된 방법으로 제조하고, 동종 PRP 에 함유된 사이토카인과 성장인자를 분석하므로써, PRP 가 유착성 관절낭염의 대표적 증상인 윤활막염에 대한 임상적 적응증과 기전을 연구하고자 하였다. 본 연구에서 유착성 관절낭염 환자를 대상으로 표준화된 pure PRP 를 관절강내 주사하여 안전성과 효력을 평가하였고, 활막세포에 염증환경을 조성한 환경 또는 아닌 환경에서 pure PRP 의 효과를 평가하였다.

방법: 임상 연구로는, 30 명의 유착성 관절낭염 환자에게 초음파 유도하에 관절강내로 PRP 또는 corticosteroid (triamcinolone acetonide 40mg)를 주사하고 6 개월 동안 추적검사 하였으며, 통증 및 근력, 관절운동범위, 어깨 기능을 측정하였다. PRP 군과 스테로이드 군에 대해서는 propensity score-matching 을 시행하였다. 인비트로 실험으로는, 활막세포에 인터루킨 1 베타와 PRP 를 농도 별로 처리하고,

전염증성 사이토카인, 기질분해 효소와 그의 억제제, 항염증성 사이토카인의 유전자 발현 변화을 분석하였다.

결과: 임상연구에서, PRP 주사로 인한 전신 또는 국소 부작용은 없었으며, 6 개월 추적검사에서 통증 및 근력, 관절운동범위, 어깨기능이 주사 전과 비교하였을 때 유의하게 회복되었다.

인비트로에서, PRP 는 활막세포의 염증이 결여된 상황에서는 오히려 전염증성 사이토카인 양을 증가시켰고, 염증 환경을 조성하였을 때에는 인터루킨 1 베타, 종양괴사인자 알파, 시클로옥시게나아제 −2, 프로스타글란딘 E2 합성효소, 혈관작동성 장폡타이드, 기질 분해효소와 기질 분해효소의 억제제들을 조절하므로써 항염증 효과를 보였다.

결과: 본 연구에서는 동종 pure PRP 는 유착성 관절낭염에 부작용 없이 스테로이드와 견줄 만한 강력한 치료 효과를 나타내었으므로 동종 pure PRP 는 스테로이드의 대체치료법으로의 사용 가능성을 보여준다. 또한, 염증환경에서 활막세포의 염증관련 유전자가 감소하는 것을 확인하므로, 동종 pure PRP 가 초기 유착성 관절낭염에 작용하는 분자생물학적 근거를 제시한다.

keywords : 동종 혈소판 풍부 혈장; 다면발현성; 유착성관절낭염; 동결견; 활막염

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