Intra-articular Injection of Pure Platelet-Rich Plasma Is the Most Effective Treatment for Joint Pain by Modulating Synovial Inflammation and Calcitonin Gene-Related Peptide Expression in a Rat Arthritis Model

Naoko Araya,* MD, Kazumasa Miyatake,*† MD, PhD, Kunikazu Tsuji,‡ PhD, Hiroki Katagiri,* MD, PhD, Yusuke Nakagawa,‡ MD, PhD, Takashi Hoshino,* MD, PhD, Hiroaki Onuma,* MD, Saisei An,* MD, Hirofumi Nishio,§ MD, PhD, Yoshitomo Saita,§ MD, PhD, Ichiro Sekiya,‖ MD, PhD, and Hideyuki Koga,* MD, PhD
Investigation performed at Tokyo Medical and Dental University, Tokyo, Japan

Background: Platelet-rich plasma (PRP) has emerged as a treatment for osteoarthritis (OA). However, the effect that leukocyte concentrations in PRP have on OA remains unclear.

Purpose: To clarify the optimal PRP formulation for OA treatment by comparing pure PRP, leukocyte-poor PRP (LP-PRP), and leukocyte-rich PRP (LR-PRP) in a rat arthritis model.

Study Design: Controlled laboratory study.

Methods: Knee arthritis was induced bilaterally in male Wistar rats with intra-articular injections of monosodium iodoacetate (MIA) on day 0. Rats were randomly assigned to 1 of 3 treatment groups (pure PRP, LP-PRP, and LR-PRP). On day 1, allogenic PRP was injected into the right knee of rats and phosphate-buffered saline was injected into the left knee as a control. Weight distribution on the hindlimbs was measured for 14 days to assess pain behavior. Rats were euthanized at day 5 or 14 for histological assessment of synovial tissue and cartilage. Immunohistochemical staining of calcitonin gene-related peptide (CGRP) and α-smooth muscle actin was performed to determine the mechanism of pain relief induced by the PRP preparations.

Results: In all groups, PRP increased the load-sharing ratio on PRP-injected knees, with pure PRP eliciting the largest effect among the 3 kinds of PRP ($P < .05$). Structural changes in the synovial tissue were significantly inhibited in the pure-PRP group compared with the control group after both 5 and 14 days ($P < .001$ and $P = .025$, respectively), whereas no significant difference was found between the control, LP-PRP, and LR-PRP groups. An inhibitory effect on cartilage degeneration was observed only in the pure-PRP group on day 14. Pure PRP also significantly inhibited expression of CGRP-positive nerve fibers in the infrapatellar fat pad compared with the other groups ($P < .05$).

Conclusion: In an MIA-induced arthritis model, pure PRP injection was the most effective treatment for reduction of pain-related behavior and inhibition of synovial inflammation and pain sensitization.

Clinical Relevance: PRP formulations should be optimized for each specific disease. This study shows the superiority of pure PRP for treatment of arthritis and joint pain.

Keywords: platelet-rich plasma; leukocyte; osteoarthritis; pain; monosodium iodoacetate (MIA); incapacitance test; calcitonin gene-related peptide; α-smooth muscle actin

Intra-articular administration of platelet-rich plasma (PRP) has recently emerged as a treatment for osteoarthritis (OA).[5,14] Several clinical trials, with evidence gathered from both in vitro and in vivo studies, have demonstrated that intra-articular PRP injections have a beneficial effect on pain relief and functional improvement in patients with symptomatic knee OA.[9,14,33] Reports suggest that PRP has both anti-inflammatory and antinociceptive effects on the synovium and cartilage co-culture systems in vitro.[29,35] Moreover, some in vivo studies in animals indicate that intra-articular PRP injections prevent cartilage degeneration[42] and attenuate synovial inflammation and joint pain.[19,22]

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Despite these promising results, other studies have shown less favorable outcomes.[12,20] Because of the variety of PRP preparation techniques, there are conflicting results, and this makes the comparison between articles difficult.[13,40] Hence, there is growing interest in performing studies comparing different cellular compositions of PRP to identify the ideal PRP composition.

Leukocyte concentrations in PRP have been a focus given that leukocytes are related to inflammatory factors.[3,13,21,31,40] Leukocytes cause an increase in proinflammatory factors and down-regulate anticatabolic factors in cultured synoviocytes.[3,7] Additionally, intra-articular injection of leukocyte-rich PRP (LR-PRP) increases inflammatory factors in the synovial fluid compared with leukocyte-free, pure PRP in rabbit OA models.[40,42] In contrast, some in vitro studies of cartilage and synovium culture have suggested that both leukocyte-poor PRP (LP-PRP) and LR-PRP decrease proinflammatory markers and that LR-PRP maintains a higher concentration of anti-inflammatory cytokines than pure PRP.[29,31] Only 1 clinical trial has made a direct comparison of the 2 PRP preparations and demonstrated that both pure-PRP and LR-PRP treatments improved clinical outcome scores.[13] Therefore, the effect of the leukocyte concentration in PRP treatments on the outcomes of patients with OA remains unclear. Furthermore, no reports have compared the effect of different leukocyte concentrations of PRP on pain and synovitis in an animal OA model.

In most previous studies, PRP with leukocytes contained more platelets than pure PRP, and the difference in platelet concentration may have affected the results. In this study, to determine the effect of leukocyte concentration in PRP more clearly, we adjusted the platelet concentration in each PRP preparation so that they were all equal. Furthermore, to analyze the mechanism underlying the pain relief associated with PRP administration, we assessed the density of calcitonin gene-related peptide (CGRP)–positive nerve fibers and α-smooth muscle actin (α-SMA)–positive cells in the infrapatellar fat pad (IFP). CGRP is a pain-related neuropeptide that is expressed in sensory nerves.[23] α-SMA is expressed in myofibroblasts and smooth muscle cells lining the blood vessel wall.[25] Reports have indicated that blood vessels and nerves have physiological interactions that contribute to pain in OA.[23] The purpose of the current study was to clarify the mechanism by which leukocytes in 3 kinds of PRPs (pure PRP, LP-PRP, and LR-PRP) affect OA-associated pathophysiological processes and to identify the optimal PRP preparation for the treatment of OA.

METHODS

Preparation of the PRP

The Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University approved this study (approval No. A2019-037).

Approximately 10 mL of whole blood was collected from 12-week-old male Wistar rats (Charles River) via cardiac puncture and anticoagulated with 2 mL of acid-citrate dextrose solution A. We used 1 mL of anticoagulated whole blood for whole blood analysis, and the remaining 10 mL of blood was collected in a Ycellbio PRP tube (Ycellbio Medical Co). In brief, according to the manufacturer's instructions, the PRP tube was centrifuged at 1200*g* for 2 minutes. After the level of buffy coat was adjusted via the control lever in the bottom of the tube, it was further centrifuged at 1600*g* for 4 minutes. The plasma and the upper half of the buffy coat were isolated as pure PRP and the lower half was collected as LP-PRP or LR-PRP. The platelet concentration in the different PRPs was adjusted to 3- to 5-fold of the whole blood. PRP was activated by freezing at –80°C for 24 hours and incubated at 37°C for 1 hour. After incubation, activated PRPs were centrifuged at 12,000*g* for 2 minutes to separate the debris. The supernatant was collected and stored at –80°C until use.

Induction of Arthritis and Treatment

The present study included 36 male Wistar rats (Charles River) that were 12 weeks old and weighed 360 to 420 g each. Knee arthritis was induced bilaterally by intra-articular injections of 1 mg of monosodium iodoacetate (MIA) (Sigma-Aldrich) in 30 μL of sterile saline on day 0 as described previously.[37]

Rats were randomly assigned to 3 treatment groups: pure-PRP treatment, LP-PRP treatment, or LR-PRP treatment (n = 12 per group). On day 1, the right knee joint received an intra-articular injection of 30 μL of allogenic PRP and the left knee joint received an injection of 30 μL of phosphate-buffered saline (PBS) (Figure 1). All injections were administered under isoflurane anesthesia. The rats were kept under a 12/12-hour light/dark cycle with food and water. They were euthanized on day 5 or day 14 (n = 6 per group at each time point), and both knee joints were harvested for histological evaluation.

Analyses of Hindlimb Weightbearing

Weightbearing distribution was measured with an incapacitance tester (Linton Instrumentation) to evaluate

†Address correspondence to Kazumasa Miyatake, MD, PhD, Department of Joint Surgery and Sports Medicine, Graduate School, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8510, Japan (email: miyatake.orj@tmd.ac.jp).
*Department of Joint Surgery and Sports Medicine, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.
‡Department of Cartilage Regeneration, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.
§Department of Orthopaedics, Juntendo University, Faculty of Medicine, Tokyo, Japan.
‖Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan.
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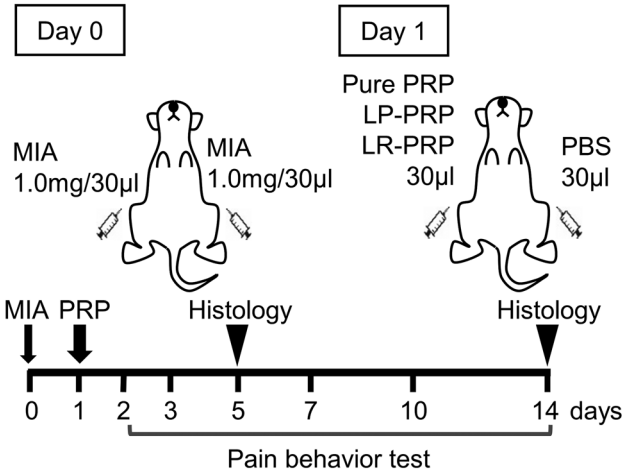

Figure 1. Study design. Knee arthritis was induced bilaterally by intra-articular injections of MIA at day 0. Rats received a specific PRP injection in the right knee and PBS in the left knee. The pain behavior test was performed as indicated. Histological findings were evaluated at days 5 and 14. LP, leukocyte-poor; LR, leukocyte-rich; MIA, monosodium iodoacetate; PBS, phosphate-buffered saline; PRP, platelet-rich plasma.

pain-related behavior.[6,11,15,16,27] The rats were placed in a prismatic plexiglass case, where each hindlimb rested on a separate force plate. The measurement was performed on day 0, before injection of MIA, and on days 2, 3, 5, 7, 10, and 14. At least 100 measurements per rat were recorded at each time point. The assessor was blinded to group allocation and covered the tester's display to blind the measured values during measurement. The percentage of weight on the right limb (PRP injection side) was calculated through use of the average of these measurements. The difference between the load ratio on day 0 and at each time point was used for statistical analysis.

Histological and Immunohistochemical Analysis

The knee joints were fixed with 4% paraformaldehyde for 7 days, decalcified in 20% ethylenediaminetetraacetic acid solution for 21 days, and then embedded in paraffin wax. The specimens were cut into 5-μm sagittal sections.

To assess the severity of synovial inflammation and structural changes in the IFP, sections were stained with hematoxylin and eosin. Inflammation severity was semiquantitatively evaluated by using the IFP inflammation score on a scale of 0 to 6 points (normal 0, worst 6) according to previously described methods.[37]

For evaluation of cartilage quality, sections were stained with Safranin-O. The cartilage degeneration of the medial compartment was scored via the Osteoarthritis Research Society International (OARSI) score on a scale of 0 to 24 points (normal 0, worst 24).[30] The average score for the femoral condyle and tibial plateau was used for statistical analysis.

The expression of CGRP-positive nerve fibers and α-SMA in the synovial membrane was analyzed through use of immunohistochemical staining. The sections were deparaffinized in xylene, rehydrated in graded alcohol, and rinsed with PBS. Endogenous peroxidases were quenched by use of 0.3% hydrogen peroxidase in methanol for 15 minutes. Heat-induced antigen retrieval was performed for CGRP by placing the slides in 98°C citrate buffer for 30 minutes. Antigen retrieval for α-SMA was performed by placing the slides in citrate buffer at room temperature for 30 minutes. The sections were blocked with 5% normal goat serum (Vector Laboratories) and were then incubated overnight with primary antibodies against CGRP (dilution 1:250) (#T-4238; Peninsula Laboratories LLC) and α-SMA (dilution 1:500) (#19245; Cell Signaling Technology) at 4°C. After rinsing in PBS, they were incubated in biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories) for 30 minutes. Immunostaining was detected through use of the Vectastain ABC reagent (Vector Laboratories) followed by diaminobenzidine staining. The sections were counterstained with hematoxylin. CGRP-positive nerve fibers and α–SMA positive cells in IFP were counted, and the entire area of the IFP was measured by Zeiss AxioVision software. The number of positive cells per square millimeter was calculated and expressed as a density.[36]

Statistical Analysis

To derive the sample size, we performed power calculations based on a pilot study. We hypothesized that at day 14, the difference in mean change of load-sharing ratio between the pure-PRP group and the other PRP groups would be at least 4%, with an SD of approximately 3. Our sample size per group for analysis of variance was 5 rats per group (power = 0.80; $\alpha = .05$). For histological assessment in the short term, we hypothesized that at day 5, the difference in IFP score between the pure-PRP group and the other groups would be at least 2 points, with an SD of approximately 0.8. Our sample size per group for analysis of variance was 5 rats per group (power = 0.80; $\alpha = .05$). At each measurement point 1 rat was added, and 12 rats per group (total 36 rats) were included in this study.

Analysis of load-sharing ratio was carried out on post-PRP injection data of rats observed for 14 days through use of a 2-way repeated-measures analysis of variance (ANOVA) followed by post hoc Tukey honestly significant difference (HSD) test. A 1-way ANOVA with post hoc Tukey HSD test was performed to compare the histological scores and CGRP and α-SMA density between the groups. Pearson correlation analysis was used to assess the correlation between load-sharing ratios and histological scores. For histological analysis, sections of the left knee (PBS injection side) were tested as controls and 1 representative slice was evaluated. The histological scores were evaluated by 2 independent observers, where 1 observer evaluated samples in a blinded manner. Interclass correlations for the interobserver variability between the 2 observers were 0.903 (IFP inflammation score) and 0.911 (OARSI

TABLE 1
Platelet and Leukocyte Concentrations in Pure PRP, LP-PRP, and LR-PRP[a]

	Platelets $\times 10^4/\mu\mu L$			Leukocytes per $\mu\mu L$		
	Whole Blood	PRP	PRP/Whole Blood	Whole Blood	PRP	PRP/Whole Blood
Pure PRP (n = 3)	61.4 ± 10.1	230.9 ± 30.4	3.76 ± 0.29	4067 ± 1815	ND	ND
LP-PRP (n = 1)	79.7	302	3.79	3000	1700	0.57
LR-PRP (n = 3)	68.2 ± 23.0	240.9 ± 66.4	3.67 ± 0.69	4400 ± 572	14,166 ± 6604	3.38 ± 1.88

[a]Values are expressed as mean ± SD. Leukocytes in pure PRPs were undetected. LP, leukocyte-poor; LR, leukocyte-rich; ND, not detected; PRP, platelet-rich plasma.

score). The statistical analyses were performed via SPSS software (version 26; SPSS Inc). P values less than .05 were considered significant.

RESULTS

Components of PRPs

The components of the PRP preparations are shown in Table 1. The mean platelet concentration in the PRP preparations was approximately 3.7-fold higher than that in whole blood. The mean leukocyte concentration of LR-PRP was higher than that of whole blood, whereas the LP-PRP concentration was lower than in whole blood. Leukocytes were undetectable in pure PRP. The 3 kinds of PRP conformed to the criteria of respective PRP preparations, as previously reported.[21]

Pure PRP Is the Most Effective Treatment for Pain-Related Behavior

Figure 2 describes the change of load-sharing ratios on the PRP-injected side. In all groups, the mean load-sharing ratios on PRP-injected limbs increased at all measurement points until day 14 compared with day 0. No interaction was detected between group and time (2-way ANOVA; $F = 1.5$; $P = 0.15$) and the main effect by group was significant ($F = 12.3$; $P = .001$). A post hoc Tukey HSD test showed that the mean change of load-sharing ratio in the pure-PRP group increased significantly more than in the other 2 groups (pure vs LP, $P = .027$; pure vs LR, $P = .001$).

Pure PRP Is the Most Effective Treatment for Prevention of Synovitis and IFP Structural Change

Because there was a strong effect on pain relief with PRP injections, histological assessment was performed to examine the mechanism of pain relief. On day 5, although infiltration of inflammatory cells into the synovial tissue and fibrosis in the IFP was inhibited in all PRP treatment groups, pure PRP had the strongest effect of all (Figure 3A). IFP inflammation scoring was used to analyze the severity of inflammation, where a higher score represented more severe inflammation. The scores in the pure-PRP group (2.1 ± 1.0) were significantly lower than those in the control and LR-PRP groups

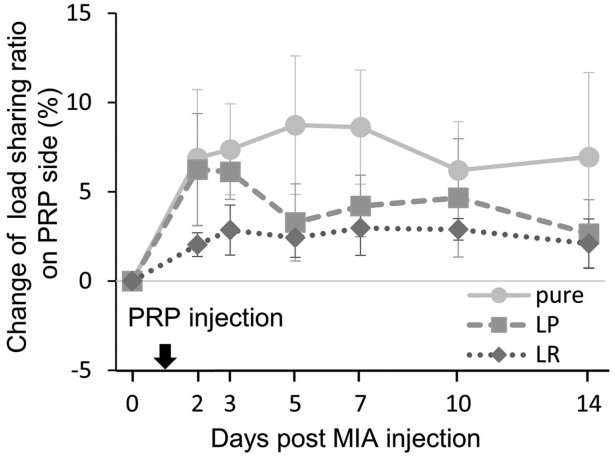

Figure 2. Change of load-sharing ratio on PRP-injected limbs measured by an incapacitance tester. Means and SDs are presented. LP, leukocyte-poor; LR, leukocyte-rich; MIA, monosodium iodoacetate; PRP, platelet-rich plasma.

on day 5 (control, 4.0 ± 0.8, $P < .001$; LR-PRP, 3.5 ± 0.9, $P = .042$) (Figure 3C). In contrast, the mean scores in the LP-PRP and LR-PRP groups tended to be lower than the scores in control knees; however, no significant difference was seen between groups. On day 14, synovial inflammation and fibrosis in the IFP had progressed in all groups compared with day 5. Nevertheless, fat tissue in the IFP was partially observed in the pure-PRP group only. In contrast, IFPs were almost filled with fibrotic lesions in the control group and other PRP groups (Figure 3B). The IFP inflammatory scores in the pure-PRP group were significantly lower than those in the control group, although there were no significant differences between the other PRP groups (Figure 3D).

Pure PRP Reduces the Progression of Cartilage Degeneration

On day 5, the dyeability of articular cartilage via Safranin-O staining was reduced in the control and LR-PRP groups compared with the pure-PRP and LP-PRP groups (Figure 4, A and B). Cartilage degeneration progressed from day 5 to 14 in all groups. However, dyeability of the tibial plateau cartilage was maintained only in the pure-PRP group at day 14.

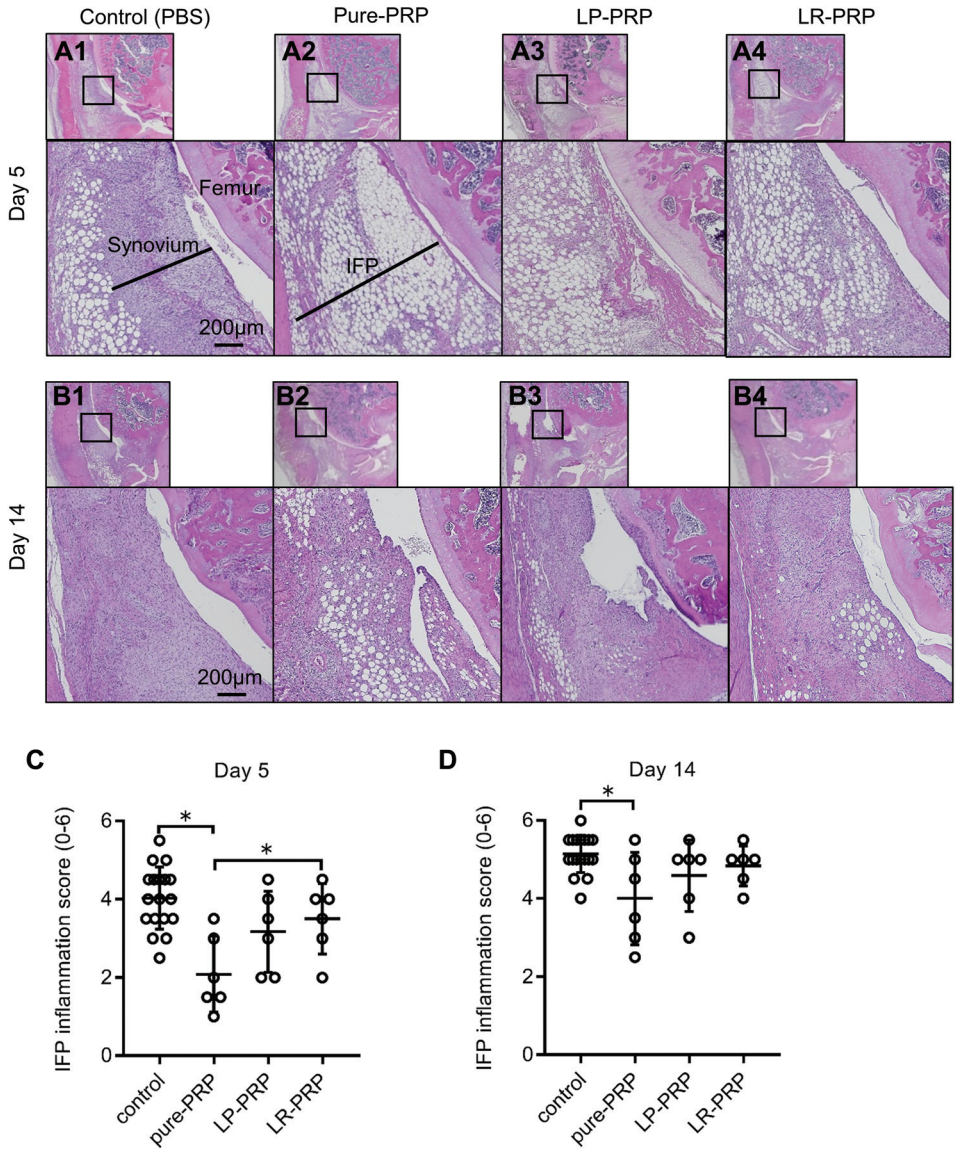

Figure 3. Histological analysis of synovial tissue and the IFP. (A, B) Representative images of each group stained with hematoxylin and eosin. Images at (A, 1-4) day 5 and (B, 1-4) day 14. IFP inflammation scores at (C) day 5 and (D) day 14. Bars represent the means and SD. *$P < .05$. IFP, infrapatellar fat pad; LP, leukocyte poor; LR, leukocyte-rich; PBS, phosphate-buffered saline; PRP, platelet-rich plasma.

OARSI scores were applied and plotted (Figure 4, E and F). No significant difference was seen between the groups on day 5. The scores for knees injected with pure PRP were significantly lower than for control knees on day 14 (pure PRP, 8.0 ± 2.1; control, 12.0 ± 1.9; $P = .002$). In contrast, the scores in the LP-PRP and LR-PRP groups were not significantly different from the control group on day 14.

Density of CGRP-Positive Fibers and α–SMA Positive Cells Is Lower in Pure–PRP Injected Knees

To further analyze the mechanism of pain relief from a pain-related molecule and neovascularization perspective, the density of CGRP-positive nerve fibers and α–SMA positive cells in the IFP body was assessed through use of immunohistochemical staining (Figure 5). On day 5, the density of both CGRP-positive fibers and α–SMA positive cells in knees injected with pure PRP was significantly lower than that in control knees (Figure 5, A2 and B2). On day 14, pure PRP–injected knees showed significantly lower CGRP-positive fiber density than the control and LP–PRP- and LR–PRP injected knees (Figure 5, A3). In contrast, no significant differences were found in the density of α–SMA positive cells between groups on day 14 (Figure 5, B3). No significant differences were seen in CGRP and α-SMA expression in LP- and LR-

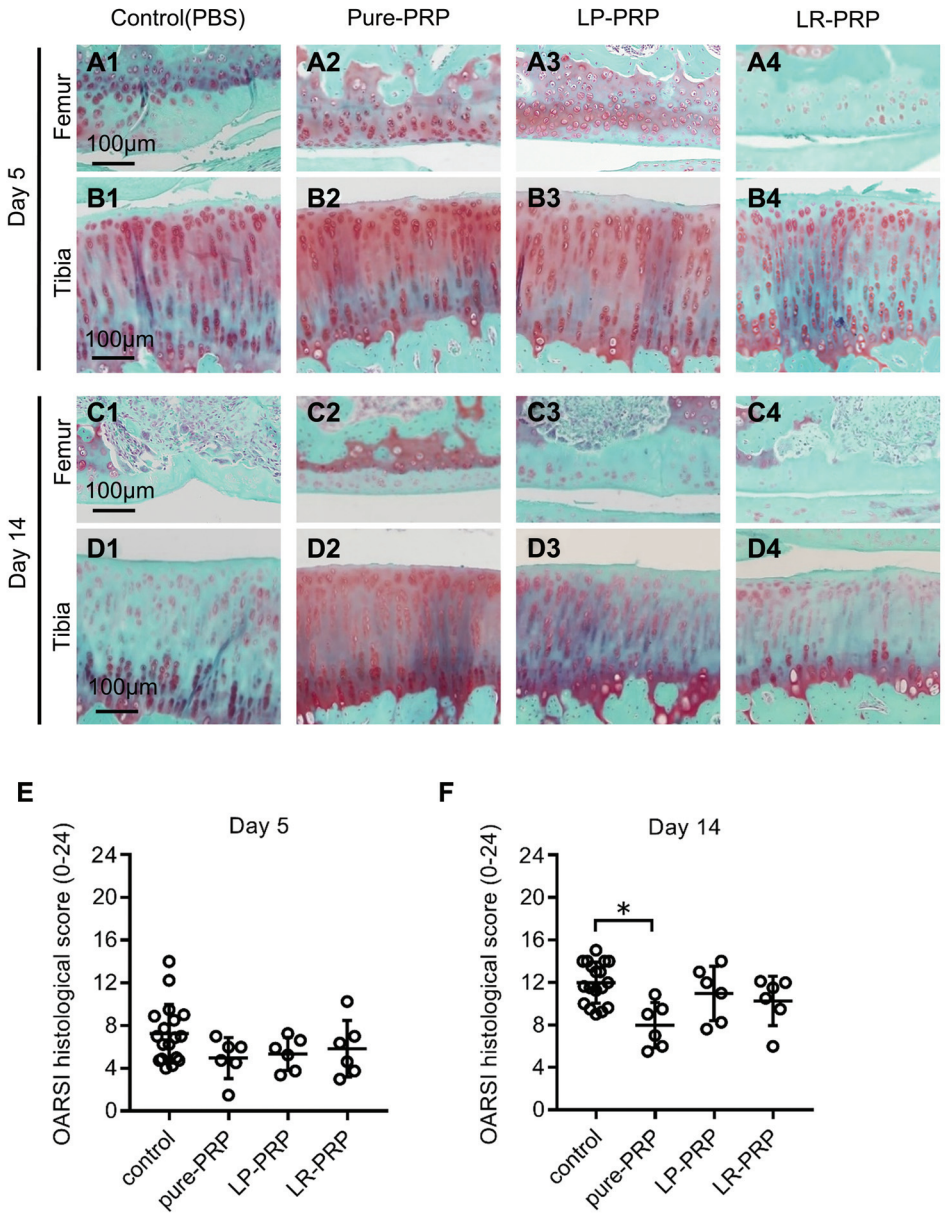

Figure 4. Histological analysis for cartilage. (A-D) Representative images of medial compartment stained with Safranin-O. (A, 1-4) Femoral condyle and (B, 1-4) tibial plateau at day 5. (C, 1-4) Femoral condyle and (D, 1-4) tibial plateau at day 14. Average of OARSI histological scores for medial femoral condyle and tibial plateau at (E) day 5 and (F) day 14. Bars represent the means and SD. *$P < .05$. LP, leukocyte-poor; LR, leukocyte-rich; OARSI, Osteoarthritis Research Society International; PBS, phosphate-buffered saline; PRP, platelet-rich plasma.

PRP–injected knees compared with control knees on days 5 and 14.

DISCUSSION

Our results showed that intra-articular injection of pure PRP was the most effective treatment for reduction of pain and inhibition of the progression of synovitis, IFP structural change, and cartilage degeneration in the short term. Moreover, CGRP-positive nerve fibers in the IFP were significantly lower in knees injected with pure PRP, suggesting that pure PRP is beneficial for inhibiting pain sensitization.

In this study, pure PRP was the most effective PRP for pain relief. Similarly, pure PRP inhibited synovitis and structural changes in the IFP significantly more effectively than did PBS and LR-PRP. Leukocyte concentration is positively correlated with interleukin 1β (IL-1β), tumor necrosis factor α, matrix metalloproteinase 9, and vascular endothelial growth factor concentrations in PRP.[21,34,40,41]

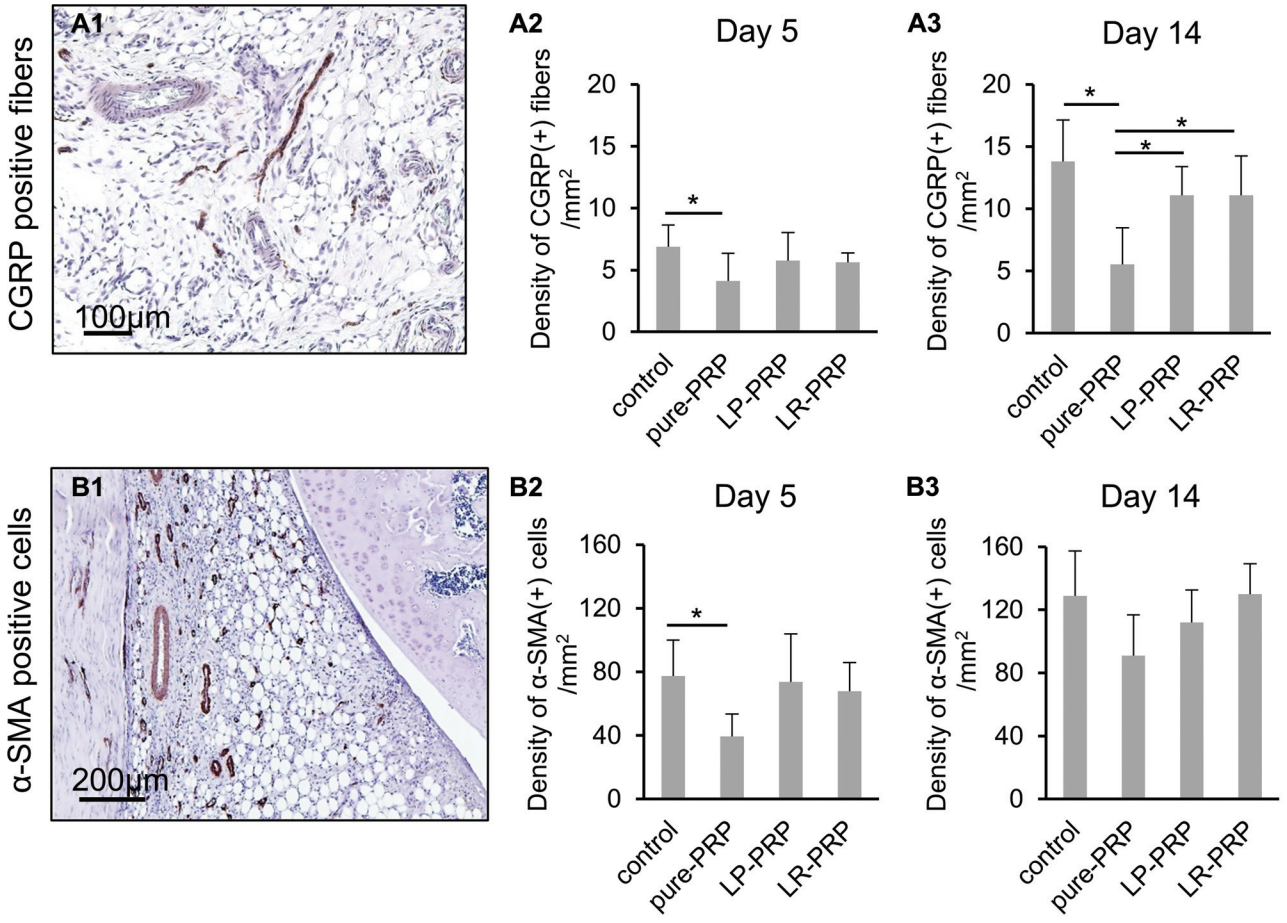

Figure 5. Immunohistochemical analysis of CGRP and α-SMA. (A1) Representative images of CGRP-positive nerve fibers in the infrapatellar fat pad. (A2, A3) The density of CGRP-positive fibers. (B1) Representative images of α–SMA positive cells in IFP. (B2, B3) The density of α–SMA positive cells. Bars represent the means and SD. *$P < .05$. α-SMA, α-smooth muscle actin; CGRP, calcitonin gene-related peptide; LP, leukocyte-poor; LR, leukocyte-rich; PRP, platelet-rich plasma.

Likewise, studies on synoviocyte cultures have demonstrated that IL-1β, IL-6, and IL-8 levels are significantly increased after treatment with LR-PRP compared with those after treatment with leukocyte-reduced PRP.[3,7] These cytokines are well-known proinflammatory and/or catabolic factors that are associated with OA pathogenesis.[24,39] Our results support the previous in vitro findings by demonstrating the superior effect of pure PRP on synovitis.

In addition, several studies have reported an association between joint pain and inflammation of the synovium and IFP. In clinical studies, synovitis detected by magnetic resonance imaging has been associated with a lower pressure pain threshold at the patella, indicating increased pain sensitivity and worse subjective pain scores.[28,43] Previous data also suggested that irreversible structural changes in the IFP after joint inflammation play a critical role in persistent pain in a rat arthritis model.[16,17] Therefore, suppressing the progression of structural changes in the IFP by early administration of pure PRP may contribute to continuous pain relief.

Regarding cartilage degeneration, a significant difference in OARSI scores was observed only between pure PRP and PBS on day 14. Moreover, no correlation was seen between pain behavior tests and OARSI scores (day 5, $r = 0.037$, $P = .94$; day 14, $r = –0.35$, $P = .15$). Similarly, previous in vivo and clinical studies have suggested that PRP injection reduces pain independent of the severity of cartilage damage.[8,19] Therefore, the protective effect of PRP injection on cartilage may not be associated with pain relief.

To explain the discrepancy between OA severity and pain relief, we focused on the expression of CGRP-positive fibers and α–SMA positive cells in the IFP. Our aim here was to elucidate potential mechanisms of pain relief after PRP administration. CGRP is one of the major neuropeptides, and it is present in sensory nerve fibers and implicated in OA peripheral sensitization.[4] CGRP-positive fibers have been found in the IFP and synovial tissue of OA patients, and expression in the IFP increases with OA progression.[1] In addition, the continuous upregulation of CGRP was associated with persistent pain in a rat arthritis model.[16] Likewise, in an inflammatory situation,

α-SMA is expressed in myofibroblasts and smooth muscle cells lining the blood vessel wall and is an indicator of fibrosis and angiogenesis.[18,25] Sensory nerves grow along blood vessels in the joint, which suggests that nerve growth is regulated by adjacent blood vessels.[23] In addition, CGRP stimulates endothelial cell proliferation, migration, and tube formation, which enhances angiogenesis.[10,38] Thus, the physiological interaction between angiogenesis and nerve growth may contribute to the progression of OA pain.[23,38]

In this study, the density of CGRP-positive fibers in the pure-PRP group was significantly lower than in the control group on day 5. The difference in CGRP density in the pure-PRP group and the other groups increased on day 14, and pure PRP significantly suppressed CGRP-positive fibers compared with PBS, LP-PRP, and LR-PRP. In contrast, the density of α–SMA positive cells was significantly lower in the pure-PRP group than in the control group on day 5, although there was no significant difference between the groups on day 14. Therefore, pure PRP may suppress nerve growth independently of angiogenesis.

Prostaglandin E_2 (PGE_2), which is the enzymatic product of cyclooxygenase 2 (COX-2), reportedly regulates CGRP expression in the synovium and IFP.[2,26] In addition, leukocyte-containing PRP is a more potent upregulator of COX-2 expression and a stronger enhancer of PGE_2 production compared with pure PRP.[40,41] The attenuated induction of PGE_2 and CGRP-positive nerve fiber growth by pure PRP may explain its superior efficacy to LP-PRP and LR-PRP with regard to inhibition of pain sensitization.

The rodent MIA-induced arthritis model has been used to assess pathological progression of the disease, pain behavior, and peripheral nerve sensitization.[16,17,32,37] Injection of high-dose MIA (1.0 mg per joint) into the knee induces irreversible structural changes in the IFP and persistent pain, whereas low-dose MIA (0.2 mg per joint) does not induce structural changes or persistent pain.[16,37] Therefore, we considered that the levels of pain in the high-dose MIA-induced arthritis model are highly reproducible and that the model is suitable for assessing the effect of anti-inflammatory therapies on pain and histological changes of synovium and IFP. In addition, we confirmed the high accuracy of intra-articular injection technique in preliminary experiments and therefore can assume with high confidence that the Wistar rats had equal levels of bilateral knee arthritis and joint pain upon high-dose MIA injection.

This study had some limitations. First, the changes of load-sharing ratio reflect not subjective pain but rather disability of hindlimbs by arthritis and joint destruction. Second, values for weightbearing variables had high within-group variation so that statistical analysis may have been underpowered to detect a statistically significant difference between LP-PRP and LR-PRP. In future study, a clinical trial is required to compare the effect of 3 kinds of PRP on OA pain.

In conclusion, this study has demonstrated that among 3 PRP preparations with equal platelet concentrations, pure PRP was the most effective in terms of reduction of pain-related behavior and inhibition of the progression of structural changes in the synovium and IFP. Moreover, there were fewer CGRP-positive nerve fibers in the IFP of knees injected with pure PRP, suggesting that pure PRP might be beneficial for inhibiting pain sensitization. Our findings add to the body of knowledge about the mechanisms underlying pain relief via PRP injections and support the appropriate use of PRPs for the treatment of arthritis and joint pain.

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