Intra-articular platelet-rich plasma (PRP) injections for treating knee pain associated with osteoarthritis of the knee in the Japanese population: a phase I and IIa clinical trial

Yu Taniguchi1, Tomokazu Yoshioka1,2, Akihiro Kanamori1, Katsuya Aoto1, Hisashi Sugaya1,2, and Masashi Yamazaki1

1Department of Orthopedic Surgery, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan
2Division of Regenerative Medicine for Musculoskeletal System, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

ABSTRACT

Intra-articular platelet-rich plasma (PRP) injection has been found to be effective for treating osteoarthritis in patients from Western countries; however, the safety and efficacy of PRP have not been sufficiently investigated in Japanese patients. The present study aimed to evaluate the safety and feasibility of intra-articular PRP injection in Japanese patients with knee osteoarthritis. PRP without white blood cells was prepared using a single-spin centrifuge (PRGF-Endoret; BTI Biotechnology Institute, Vitoria, Spain). A 6-mL PRP volume was injected in the knee joint three times at 1 week intervals. All patients were prospectively evaluated before intervention and at 1, 3, and 6 months after the treatment. Adverse events, the Visual Analog Scale (VAS) pain score, Japanese Knee Osteoarthritis Measure (JKOM) score and Japanese Orthopedic Association score were evaluated. Ten patients (all women; average age, 60.6 years) were treated. Only minor adverse events after injection were noted, and symptoms resolved within 48 hours after the injection. The average VAS pain scores were 71.6 mm and 18.4 mm at baseline and the 6-month follow-up, respectively (P < 0.05). At the 6-month follow-up, 80% of patients had a decrease in the VAS pain score of 50% or more. The average JKOM scores were 35.2 and 14.3 at baseline and at the 1-month follow-up, respectively (P < 0.05). Intra-articular PRP injection likely represents a safe treatment option for Japanese patients with mild-to-moderate knee osteoarthritis, and has the potential to relieve pain for up to 6 months, but further study is needed to verify the efficacy.

Keywords: platelet-rich plasma, knee osteoarthritis, treatment safety, intra-articular injection

This is an Open Access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (http://creativecommons.org/licenses/by-nc-nd/4.0/).

INTRODUCTION

Osteoarthritis of the knee is one of the main causes of musculoskeletal disability, and is characterized by gradual abrasion of the articular cartilage, osteophyte formation, subchondral bone sclerosis, and inflammation of the joint. The number of patients with osteoarthritis continues to increase as the world population ages. In the USA, knee osteoarthritis affects more than...
20% of individuals older than 45 years. In Japan, the prevalence of age-associated diseases of the musculoskeletal system has also increased, and it was estimated that, in 2009, radiographic osteoarthritis was present in 25,300,000 individuals (8,600,000 men and 16,700,000 women) aged 40 years or older, while symptomatic osteoarthritis was present in approximately 7,800,000 individuals. In addition, the Japanese lifestyle differs from that in Western countries, and activities of Japanese individuals generally require a greater range of motion. Common movements used in daily life in Japan include floor-sitting, squatting, and kneeling (e.g., seiza-sitting).

Clinical guidelines recommend various non-surgical procedures to treat knee osteoarthritis. Non-pharmacological treatments include: patient education and self-management with exercise; weight reduction; use of crutches and bracing; shoe and insole modification; and local cooling. Pharmacologic therapies include the use of paracetamol, non-steroidal anti-inflammatory drugs, and opioids. If orally administered drugs are ineffective, intra-articular (IA) injection of corticosteroids and hyaluronic acid (HA) is the next preferred non-operative treatment. IA-HA is widely used in Japan because it is strongly recommended in the guideline for the management of knee osteoarthritis, published by the Japanese Orthopedic Association (JOA). However, IA-HA is not recommended in the corresponding guideline published by the American Academy of Orthopedic Surgeons. Currently, the efficacy of IA-HA for osteoarthritis of the knee is considered controversial.

Therapy with platelet-rich plasma (PRP) has emerged as a biological, conservative treatment for knee osteoarthritis. PRP is broadly defined as a plasma fraction containing abundant platelets, although no clear definition is given in terms of platelet counts or platelet concentration. As PRP has high concentrations of autologous growth factors and secretory proteins that may enhance cellular-level healing processes, it has been used in the clinical setting to enhance healing. Several studies have shown that treatment involving IA-PRP is superior to the use of IA-HA in terms of improving the Western Ontario & McMaster Universities (WOMAC) total score and other parameters. However, these studies were conducted in Europe and the USA, while the efficacy of IA-PRP therapy has not been sufficiently evaluated in Japanese patients with knee osteoarthritis. Recent research has shown that platelet function differs among ethnicities. Therefore, it is important to evaluate the safety and efficacy of IA-PRP among Japanese patients with knee osteoarthritis.

The primary objective of the present study was to verify the feasibility and safety of IA injections with PRP manufactured in our facility. We hypothesized that the use of IA-PRP represents a safe and feasible biological approach for treating pain due to osteoarthritis of the knee.

MATERIALS AND METHODS

Patient selection

The study protocol was approved by the appropriate institutional review board (application no.: H25-040). We recruited patients with early-phase knee osteoarthritis to confirm the safety of IA-PRP. All patients provided written informed consent to participate. The inclusion criteria were as follows: age between 50 and 75 years; a history of chronic (for at least 3 months) knee joint pain defined as a Visual Analog Scale (VAS) score >35 mm on a 0–100-mm scale; radiographically documented knee osteoarthritis of grades 1 to 3, according to the Kellgren-Lawrence (K-L) radiographic classification scale; body mass index ranging between 20 and 32 kg/m², so that the study sample reflected the average physique in the Japanese population; need for infiltration of the knee on one side. The exclusion criteria were as follows: polyarticular disease; knee arthroscopy within the previous year; HA or steroid IA infiltration within the previous 3 months; a history
of infectious disease and systemic disorders such as diabetes, rheumatoid arthritis, hematological diseases (coagulopathy), severe cardiovascular diseases, infections, or immunodepression; therapy with anticoagulants or an anti-aggregating agent; use of non-steroidal anti-inflammatory drugs 2 weeks before blood sampling; and hemoglobin levels <10 g/dL.

**PRP preparation**

The PRP required for injection was prepared in compliance with good manufacturing practices at the Cell Processing Factory at our hospital (Fig. 1). To avoid the effect of food intake on purified PRP, the patients were instructed to fast for 4 hours before blood collection on the day of injection. Water intake was not restricted. Using an aseptic technique, approximately 36 mL of venous blood were drawn from the antecubital vein in an effort to avoid irritation and trauma to the platelets. The blood was collected in four extraction tubes containing 3.8% sodium citrate as an anticoagulant. Subsequently, the tubes were centrifuged at 2100 rpm for 8 minutes at room temperature (PRGF System IV centrifuge; BTI Biotechnology Institute, Vitoria, Spain) to separate the blood in each tube into the plasma, the buffy coat, and residual red blood cells (RBCs).

![Platelet-rich plasma (PRP) preparation workflow](image)

(a) Centrifugation at 2100 rpm for 8 minutes using the single-spin system (PRGF System IV; BTI Biotechnology Institute, Vitoria, Spain).

(b) Photograph of the blood-collecting tube after centrifugation and scheme for fraction separation. The safety margin was set to exclude red blood cells and white blood cells. The lower half of the supernatant was defined as PRP, while the upper half was defined as platelet-poor plasma (PPP).

(c) Aspirating PPP and PRP using an exclusive pipette in a clean bench.

(d) Under aseptic conditions, 6 mL of PRP were injected into the suprapatellar pouch using the superolateral approach.
Hereafter, the procedure was performed entirely inside the biosafety cabinet. The platelet-poor plasma (PPP), which represented the uppermost 2-mL fraction in each tube, was aspirated with a pipette and sent for hematological analysis. The PRP, which corresponded to the lower 2-mL fraction from each tube, located just above the selectively precipitated RBCs but not including the buffy coat, was meticulously aspirated from each tube using a pipette. For each patient, four 2-mL PRP samples were collected (from the four blood-collecting tubes) and mixed into an 8-mL preparation, from which 6 mL were used for the IA injection\textsuperscript{14,15}. The remaining 2 mL of PRP preparation were divided into two small units of 1-mL each; one unit was sent to the laboratory for hematological analysis, while the other unit was stored in Eppendorf tubes at −80°C for subsequent determination of growth factor concentrations.

**Procedures**

The patient was placed in supine position with the knee in 20° flexion. Under aseptic conditions, 6 mL of PRP were injected into the suprapatellar pouch through the superolateral approach, using a 21-gauge needle. No local anesthetic was used. Blood pressure, heart rate, and body temperature were measured before and at 30 minutes after the injection. After the injection, the patients were instructed to refrain from physical exercise for at least 24 hours, but no restriction was specified regarding activities of daily living. Three IA-PRP injections were administered at 1-week intervals. The interval between injections, as well as the number of injections, was determined based on previous studies.\textsuperscript{11,14} The injections were performed by the same physician who was involved in recruitment and assessment of participants.

**Hematological analysis**

The white blood cell (WBC), RBC, and platelet counts of the whole-blood samples, as well as those of the PPP and PRP fractions, were determined using an automated cell count analyzer (Sysmex KX-21N; Sysmex Corp., Kobe, Japan).

**Measurement of growth factor concentrations**

A single freeze-thaw cycle was used to induce platelet activation and the release of growth factors. The samples were thawed and centrifuged for 10 minutes at 10,000 rpm, and the supernatants were tested. Quantitative determination of platelet-derived growth factor BB (PDGF-BB), tissue growth factor (TGF)-β1, and vascular endothelial growth factor (VEGF) levels in the PRP fraction was performed using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer’s instructions. All evaluations were conducted in multi-well plates, and all measurements were performed in duplicate. The color intensity of each well was measured using a spectrophotometer (Varioskan; Thermo Fisher Scientific, Yokohama, Japan) at 450 nm and a wavelength correction of 570 nm.

**Outcome measures**

To ascertain the safety of the prepared PRP, part of the prepared PRP was subjected to a bacterial culture test. For assessing the safety of IA-PRP, adverse events occurring during the treatment and follow-up period were documented at each visit. The onset, duration, and severity of events such as knee pain, stiffness, swelling, and burning sensation near the injection site were recorded in detail. The only permitted medication throughout the clinical trial was acetaminophen.

For assessing the feasibility of IA-PRP in managing knee osteoarthritis, all patients were evaluated at baseline (before the infiltration procedure) and at 1, 3, and 6 months after the last injection. The following measures were evaluated: VAS pain score, Japanese Knee Osteoarthritis Measure (JKOM) score,\textsuperscript{17} and Japanese Orthopedics Association (JOA) score\textsuperscript{18-20}, which represent
standard tools for assessing knee function in Japan. The reliability and validity of both the JOA score and the JKOM score have been demonstrated in the past by comparison against other measures such as the WOMAC score and the 36-Item Short Form Health Survey (SF-36) score. With respect to the VAS pain score, we recorded the percentage of patients with a decrease of at least 50% in the total score between the baseline and the 6-month follow-up measurements. Radiographs were obtained at baseline (before injection) and at the 1-month, 3-month, and 6-month follow-up visit. Radiographs in the supine anteroposterior view, lateral view, and skyline view were obtained during the same visits. Two authors (YT and TY) reviewed all radiographs and graded each case using the K-L radiographic classification scale. Serum hyaluronic acid (sHA) was measured as a known biomarker of knee osteoarthritis.\(^{21,22}\)

**Statistical analysis**

Differences between outcome measures at various time points (within-group differences) were evaluated using analysis of variance with Bonferroni correction. Between-group differences were evaluated using the Student t-test. A \(P\)-value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS Statistics 21.0 (International Business Machines Co., New York, NY, USA).

**RESULTS**

**Patients**

Eleven patients participated in this study, but one patient was excluded during the follow-up period because of fever (attributed to upper airway inflammation) and the presence of cloudy PRP due to a high-fat diet; thus, 10 patients were included in the analysis. The demographic and clinical characteristics of the patients are shown in Table 1. All patients were women, with a mean age of 60.6 years and a mean body mass index of 23.4 kg/m\(^2\). Osteoarthritis affected the right knee in 6 patients and the left knee in 4 patients. Knee osteoarthritis was graded according to the K-L classification as grade I in 4 patients, grade II in another 4 patients, and grade III in the remaining 2 patients. Regarding the affected compartment, 8 knees had medial osteoarthritis,
4 had lateral osteoarthritis, and 4 knees showed osteoarthritic changes in the patella-femoral joint. Physical examination did not reveal hydrarthrosis of the knee in any patient, and no patient underwent synovial fluid aspiration. Over the course of the study (i.e., from baseline until the end of follow-up), no patient gained or lost more than 2 kg in weight.

**Blood and growth factor findings**

The findings of the hematological analysis and growth factor concentrations are presented in Table 2. The average platelet count in the PRP (39.4±13.1 × 10^4/µL) was significantly higher than those in peripheral blood (24.0±3.7 × 10^4/µL) and PPP (27.1±5.8 × 10^4/µL). The platelet count in the PRP was 1.7-fold higher than that in peripheral blood. Based on the platelet count and the presence of WBC and neutrophils, the PRP preparation was classified as P2-Bβ according to the Platelets, Activation, and White cells classification system. The mean PDGF-BB, VEGF, and TGF-β1 concentrations were 6.8 ± 3.4 ng/mL, 213±207 pg/mL, and 33.3±10.8 ng/mL, respectively.

**Safety of IA-PRP**

Bacterial cultures of the prepared PRP were negative for all specimens. The adverse events noted during the study period are described in Table 3. Twenty-two adverse events were reported in relationship to 30 injections in 10 patients. All events represented generally mild reactions, and no patients withdrew from the study because of a serious adverse event due to IA-PRP injection. All adverse events resolved spontaneously within 48 hours after the injection.

**Feasibility of IA-PRP**

The mean VAS pain score decreased from 71.6 mm at baseline to 12.5 mm at 1 month, 15.1 mm at 3 months, and 18.4 mm at 6 months after the last injection (Fig. 2). The benefit in pain reduction, as measured in terms of the VAS score, was significant (P < 0.001). Moreover, the improvement in VAS pain score was maintained from the last injection until the last follow-up visit (at 6 months after treatment). Specifically, at the 6-month follow-up, 80% of patients had a decrease in the VAS pain score of 50% or more. In addition, the mean JKOM total score decreased from 36.2 at baseline to 14.3 at 1 month, 13.3 at 3 months, and 15.5 at 6 months after the last injection (Fig. 3). The JKOM score decreased significantly from baseline to 1 month after the end of the treatment (P = 0.044). The mean overall JOA scores at the 6-month follow-up were significantly higher than the baseline values (Fig. 4; 70 at baseline versus 89.5 at 6 months after treatment; P = 0.002). Regarding JOA domains, there was significant improvement.

**Table 2** Properties of the autologous blood product of 10 patients with knee osteoarthritis

|                | PB     | PPP    | PRP    |
|----------------|--------|--------|--------|
| WBC (/µL)      | 5,803 ± 1,564 | 0      | 79 ± 162 |
| RBC (×10^4/µL) | 437 ± 38 | 1.1 ± 3.3 | 1.8 ± 0.6 |
| PLT (×10^4/µL) | 24.0 ± 3.7 | 27.1 ± 5.8 | 39.4 ± 13.1 |
| PDGF-BB (ng/mL)| 6.8 ± 3.4 |         |        |
| VEGF (pg/mL)   | 213 ± 207 |         |        |
| TGF-β1 (ng/mL) | 33.3 ± 10.8 |        |        |

PB, peripheral blood; PLT, platelet count; PRP, platelet-rich plasma; RBC, red blood cell; TGF, tissue growth factor; PDGF-BB, platelet-derived growth factor BB; VEGF, vascular endothelial growth factor; WBC, white blood cells
regarding pain while walking and pain while ascending or descending stairs ($P = 0.034$ and $P < 0.001$, respectively). Radiographic findings showed that there was no progression in the K-L grade from baseline to 6 months after the end of the treatment. The sHA levels were $48.2\pm23$ ng/mL before treatment, $69.0\pm58$ ng/mL at 1 month after injection, $98.2\pm64$ ng/mL at 3 months after injection, and $97.5\pm58$ ng/mL at the end of follow-up.

**Table 3** Adverse events in 10 patients with knee osteoarthritis who received intra-articular injections with platelet-rich plasma

| Patient | Injection | Adverse event                                                                 | Duration  |
|---------|-----------|-------------------------------------------------------------------------------|-----------|
| 1       | 1st       | Acute local pain, stiffness                                                   | Immediately |
|         | 2nd       | None                                                                         |           |
|         | 3rd       | None                                                                         |           |
| 2       | 1st       | Uncomfortable feeling                                                        | Immediately |
|         | 2nd       | Subcutaneous bleeding                                                        | ≤48 h     |
|         | 3rd       | None                                                                         |           |
| 3       | 1st       | Cold chill, pain during walking, itching in the knee                          | ≤48 h     |
|         | 2nd       | Expanding acute pain at injection site                                        | ≤48 h     |
|         | 3rd       | Pain during walking, itching in the knee                                     | ≤48 h     |
| 4       | 1st       | Tingling sensation in the knee                                                | Immediately |
|         | 2nd       | None                                                                         |           |
|         | 3rd       | Pain during walking, itching in the knee                                     | ≤24 h     |
| 5       | 1st       | None                                                                         |           |
|         | 2nd       | None                                                                         |           |
|         | 3rd       | None                                                                         |           |
| 6       | 1st       | Pain during walking                                                           | ≤24 h     |
|         | 2nd       | Pain during walking                                                           | ≤24 h     |
|         | 3rd       | Acute knee pain, stiffness                                                   | ≤24 h     |
| 7       | 1st       | Tingling sensation in the knee                                                | ≤24 h     |
|         | 2nd       | Stiffness                                                                     | Immediately |
|         | 3rd       | Acute pain in the area behind the knee joint                                 | 1 h       |
| 8       | 1st       | Itching in the knee                                                           | Immediately |
|         | 2nd       | Pain during walking, itching in the knee                                     | ≤24 h     |
|         | 3rd       | None                                                                         |           |
| 9       | 1st       | Pain during walking, itching in the knee                                     | 1 h       |
|         | 2nd       | Sharp acute pain in the knee                                                  | 1 h       |
|         | 3rd       | Feeling of pressure in the knee                                               | Immediately |
| 10      | 1st       | Acute pain in the knee                                                        | ≤48 h     |
|         | 2nd       | Acute pain in the knee                                                        | ≤48 h     |
|         | 3rd       | Stiffness                                                                     | ≤48 h     |
Fig. 2 Trends in the mean Visual Analog Scale (VAS) scores for pain recorded over a follow-up of six months. Ten patients with knee osteoarthritis received intra-articular injections with platelet-rich plasma, administered once per week for three weeks. VAS scores were assessed at baseline (before injection) and during subsequent follow-up visits. *$P < 0.05$. 

Fig. 3 Trends in the mean Japanese Knee Osteoarthritis Measure (JKOM) scores over a 6-month follow-up. Ten patients with knee osteoarthritis received intra-articular injections with platelet-rich plasma, administered once per week for three weeks. JKOM scores were assessed at baseline (before injection) and during subsequent follow-up visits. *$P < 0.05$. 
DISCUSSION

The most important finding of the present study is that, in Japanese patients with knee osteoarthritis, IA-PRP did not cause any serious adverse events during the first 6 months after the treatment ended. All adverse events observed were minor and included acute knee pain, stiffness, tingling sensation, and walking pain just after the injection. These adverse events were observed for 22 of the 30 injections administered (73%), but all symptoms resolved spontaneously within 48 hours. Sánchez et al.\textsuperscript{14) evaluated 176 patients (PRP group, 89 patients vs. HA group, 87 patients) with symptomatic knee osteoarthritis. In their study, adverse events due to IA injections were generally mild and evenly distributed between the two groups, and all events such as local pain around the infiltration site disappeared within 48 hours after injection. Patel et al.\textsuperscript{12) reported various adverse events such as syncope, dizziness, headache, nausea, gastritis, sweating, and tachycardia at the time of injection; however, none was severe enough to cause concern, and all subsided within half an hour when the patients were under observation. In a study by Filardo et al.,\textsuperscript{11) no major complications related to the PRP injections were observed, but the incidence of post-injection pain reaction was higher in the PRP group than in the HA group. Filardo et al.\textsuperscript{11) considered the deleterious effects of PRP as being related to proteases and reactive oxygen released from WBCs. The absence of serious adverse events, noted in previous studies and in the present investigation, suggests that IA-PRP represents a safe treatment option for patients with knee osteoarthritis.

Regarding the efficacy of IA-PRP for knee osteoarthritis, Meheux et al.\textsuperscript{24) conducted a systematic review of six randomized, controlled trials, and found that PRP injection resulted in significant clinical improvements for up to 12 months post-injection; moreover, the clinical outcomes and WOMAC scores were significantly better after treatment with leukocyte-poor PRP than after treatment with HA at 3–12 months post-injection. Despite the favorable outcomes reported

---

**Fig. 4** Mean Japanese Orthopedic Association (JOA) scores. The patients with knee osteoarthritis received intra-articular injections with platelet-rich plasma, administered once per week for three weeks. JOA scores were assessed at baseline (before injection) and at the 6-month follow-up visit. Different categories are represented by different filling patterns. *$P < 0.05$. ROM, range of motion.
with PRP treatment, the evaluated studies only included Caucasian patients, and their findings are not applicable to the Japanese population. As recent studies have claimed that platelet function differs with ethnicity, a study of the safety and efficacy of PRP in the Japanese population was warranted. Our present study represents the first report regarding the outcome of IA-PRP in Japanese patients with knee osteoarthritis, and showed that 8 of 10 patients had a decrease in the VAS pain score of ≥50% (compared to VAS score at baseline), with the beneficial effects persisting for 6 months. In this study, the JOA and JKOM scores, both of which are specific to Japanese patients, were used for evaluation. Regarding the JOA score, improvements in pain brought about improvements in total score, as well as in the scores for walking ability and for ascending and descending stairs. On the other hand, the JKOM score improved significantly after 1 month, but the improvements seen after 3 and 6 months were not statistically significant. The JKOM was specifically developed as a tool for evaluating quality of life in patients with knee osteoarthritis, and its reliability and validity were previously demonstrated by comparisons with other measures such as the SF-36 and WOMAC. The improvement in JKOM score from baseline (i.e., before treatment) to 1 month after PRP injection may indicate that PRP is effective to the extent that it improves the patients’ quality of life.

The pathogenesis of knee osteoarthritis is complex and driven by inflammatory mediators within the affected joint. Joint changes such as synovitis, subchondral bone remodeling (thickening, bone collapse, bone cysts), degeneration of ligaments and menisci, and hypertrophy of the joint capsule are involved in the pathogenesis of osteoarthritis. PRP represents an autologous biological therapy option in which the patient’s own plasma and platelet-derived growth factors are used during the wound-healing process for regenerative purposes, to stimulate processes such as angiogenesis, mitogenesis, cell proliferation, and cell migration to chemo-attractants. Several studies have reported that PRP affects joint formation and promotes restoration of joint homeostasis. Akeda et al. compared the response of adult porcine chondrocytes cultured in the presence of 10% PRP, 10% PPP, or 10% fetal bovine serum, and reported that PRP treatment was associated with significantly higher DNA and proteoglycan content, as well as enhanced collagen synthesis. Spreafico et al. argued that, compared to human serum, PRP had a stronger effect on cell proliferation and chondrocyte activity, which suggests that this preparation may be useful for the safe and economic engineering of cartilage constructs. Anitua et al. contended that IA administration of plasma rich in growth factors may be beneficial in restoring the HA concentration and balancing angiogenesis, but it does not counteract the effects of interleukin-1β on synovial cells. Bendinelli et al. suggested that expression of the chemokine receptor CXCR4 was reduced in chondrocytes exposed to activated PRP, which may have clinical significance in the management of inflamed synovia (for example, in terms of reducing the expression of matrix-metalloproteinases and other cartilage-matrix degrading enzymes). In the present study, we measured the change in sHA levels as a biomarker for knee osteoarthritis, and found that sHA levels increased from baseline (i.e., before treatment) to 3 months after treatment. It was previously reported that sHA is a biomarker of radiographic knee osteoarthritis and a burden-of-disease marker in severe knee osteoarthritis (K-L grade 4), but is not a useful marker in mild-to-moderate knee osteoarthritis. In this study, we did not observe any changes over time regarding plain X-ray findings, which did not correspond to the evolution of sHA levels. As our patients had mild-to-moderate knee arthritis of K-L grade 1–3, sHA may not have been an appropriate biomarker in such cases. The use of alternative biomarkers should be considered in subsequent clinical comparative trials. While the in vitro study by Bendinelli et al. showed that PRP had a beneficial effect on joint formation, the mechanism through which IA-PRP may improve VAS pain scores in patients with knee osteoarthritis remains uncertain. It can be presumed that PRP promotes the restoration of joint homeostasis by inhibiting synoviocyte production of
inflammatory mediators. Nevertheless, further investigation is needed to prove this hypothesis.

The present study has several limitations. First, this was an open-label study, so no comparison with a control group was performed. However, this study was a I/IIa clinical trial that verified safety and feasibility; furthermore, it did not verify the efficacy as there was no control group. Second, the follow-up period was relatively short as a clinical trial to verify the efficacy. According to the systematic review by Campbell et al.\(^{35}\), IA-PRP is a viable treatment for knee osteoarthritis, and can induce symptomatic relief for up to 12 months. However, this clinical study was not designed to verify effectiveness, but because it was a I/IIa test to verify safety and feasibility, the 6-month period was appropriate\(^{14}\). Third, our imaging evaluation was insufficient for verification of efficacy because only X-ray imaging was used, while magnetic resonance imaging (MRI) is recommended to evaluate the severity of intra-articular osteoarthritis and the extent of synovitis. MRI is useful for determining the pathophysiology when osteoarthritis is considered a whole joint disease\(^{36}\).

Subsequent trials following on from the present phase I/IIa clinical trial will include evaluation of intra-articular findings on MRI before and after the injections. Fourth, sHA is inadequate as a biomarker of knee osteoarthritis, as it is not specific for changes in the knee, but is affected by changes in joints throughout the body. Based on the experience of the present study findings; however, we intend to conduct a clinical trial to verify the efficacy of using IA-PRP for relief of knee pain for osteoarthritis. This future study will be a multi-center, double-blind, controlled clinical trial; the control group will receive hyaluronic acid injection.

CONCLUSIONS

This study was I/IIa clinical trial and was done to verify safety and feasibility. Our findings suggest that IA-PRP is safe for use in Japanese patients with knee osteoarthritis, and, while local and minor adverse events related to PRP injection occurred, all symptoms disappeared within 48 hours. This therapy has the potential to induce pain relief that is maintained for up to 6 months, but further study is needed to verify the efficacy.

ACKNOWLEDGEMENTS

This study received financial support from the Japan Orthopaedics and Traumatology Foundation, Inc. (grant No. 284).

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1) Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg, 2004; 62: 489–496.
2) Yoshimura N, Muraki S, Oka H, Mabuchi A, En-Yo Y, Yoshida M, et al. Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the research on osteoarthritis/osteoporosis against disability study. J Bone Miner Metab, 2009; 27: 620–628.
3) Fujita K, Makimoto K, Higo T, Shigematsu M, Hotokebuchi T. Changes in the WOMAC, EuroQol and Japanese lifestyle measurements among patients undergoing total hip arthroplasty. Osteoarthritis Cartilage,
25) Tourdot BE, Conaway S, Niisuke K, Edelstein LC, Bray PF, Holinstat M. Mechanism of race-dependent

24) Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of intra-articular platelet-rich

23) DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system.

22) Kaneko H, Ishijima M, Doi T, Futami I, Liu L, Sadatsuki R, et al

21) Elliott AL, Kraus VB, Luta G, Stabler T, Renner JB, Woodard J, et al

20) Yasuda K, Majima T, Tsuchida T, Kaneda K. A ten- to 15-year follow-up observation of high tibial

19) Okuda M, Omokawa S, Okahashi K, Akahane M, Tanaka Y. Validity and reliability of the Japanese

18) Aoki Y, Yasuda K, Mikami S, Ohmoto H, Majima T, Minami A. Inverted V-shaped high tibial osteotomy compared with closing-wedge high tibial osteotomy for osteoarthritis of the knee. Ten-year follow-up result. J Bone Joint Surg Br, 2006; 88: 1336–1340.

17) Akai M, Doi T, Fujino K, Iwaya T, Kurosawa H, Nasu T. An outcome measure for Japanese people with knee osteoarthritis. J Rheumatol, 2005; 32: 1524–1532.

16) Edelstein LC, Simon LM, Montoya RT, Holinstat M, Chen ES, Bergeron A, et al. Human platelet PAR4 reactivity reflect expression of PCTP and miR-376c. Nat Med, 2013; 19: 1609–1616.

15) Vaquerizo V, Plasencia MÁ, Arribas I, Seijas R, Padilla S, Orive G, et al. Comparison of intra-articular injections of hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. Am J Sports Med, 2012; 40: 2822–2827.

14) Sánchez M, Fiz N, Azofra J, Usabiaga J, Aduriz Recalde E, Garcia Gutierrez A, et al. A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. Arthroscopy, 2012; 28: 1070–1078.

13) Raeissadat SA, Rayegani SM, Hassanabadi H, Fathi M, Ghorbani E, Babae M, et al. Knee osteoarthritis injection choices: platelet-rich plasma (PRP) versus hyaluronic acid (a one-year randomized clinical trial). Clin Med Insights Arthritis Musculoskelet Disord, 2015; 8: 1–8.

12) Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med, 2013; 41: 356–364.

11) Filardo G, Kon E, Di Martino A, Di Matteo B, Merli ML, Cenacchi A, et al. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. BMC Musculoskelet Disord, 2012; 13: 229.

10) Cerza F, Carni S, Carcangiu A, Di Vavo I, Schiavilla V, Pecora A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. Am J Sports Med, 2012; 40: 2822–2827.

9) Jevsevar DS, Brown GA, Jones DL, Matzkin EG, Manner PA, Mooar P, et al. OARSI guidelines for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009. Osteoarthritis Cartilage, 2010; 18: 476–499.

8) Zhang W, Nuki G, Moskowitz RW, Abramson S, Altman RD, Arden NK, et al. OARSI recommendations for the management of hip and knee osteoarthritis: part I: Grading of evidence and recommendation strength of interventions. Osteoarthritis Cartilage, 2013; 21: 1023–1040.

7) McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, et al. Comparison of hyaluronic acid corresponding to the radiographic severity of knee osteoarthritis in women. Arthritis Care Res, 2005; 52: 105–111.

6) Mulholland SJ, Wyss UP. Activities of daily living in non-Western cultures: range of motion requirements for hip and knee joint implants. Int J Rehabil Res, 2001; 24: 191–198.

5) Kobayashi S, Eftekhar NS, Terayama K, Iorio R, Takaoka K. Primary Charnley total hip arthroplasty: a comparison of American and Japanese cohorts followed for 10–20 years. J Arthroplasty, 2001; 16: 340–350.

4) Hemmerich A, Brown H, Smith S, Marthandam SS, Wyss UP. Hip, knee, and ankle kinematics of high range of motion activities of daily living. J Orthop Res, 2006; 24: 770–781.

3) DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system.

2) Kaneko H, Ishijima M, Doi T, Futami I, Liu L, Sadatsuki R, et al. Primary Charnley total hip arthroplasty: a comparison of American and Japanese cohorts followed for 10–20 years. J Arthroplasty, 2001; 16: 340–350.

1) Hemmerich A, Brown H, Smith S, Marthandam SS, Wyss UP. Hip, knee, and ankle kinematics of high range of motion activities of daily living. J Orthop Res, 2006; 24: 770–781.
PRP injections for knee OA

Biol, 2014; 34: 2644–2650.

26) Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol, 2011; 23: 471–478.

27) Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum, 2012; 64: 1697–1707.

28) Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone, 2012; 51: 249–257.

29) Ayhan E, Kesmezacar H, Akgun I. Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World J Orthop, 2014; 5: 351–361.

30) Martel-Pelletier J, Boileau C, Pelletier JP, Roughley PJ. Cartilage in normal and osteoarthritis conditions. Best Pract Res Clin Rheumatol, 2008; 22: 351–384.

31) Akeda K, An HS, Okuma M, Attawia M, Miyamoto K, Thonar EJ, et al. Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthritis Cartilage, 2006; 14: 1272–1280.

32) Spreafico A, Chellini F, Frediani B, Bernardini G, Niccolini S, Serchi T, et al. Biochemical investigation of the effects of human platelet releasates on human articular chondrocytes. J Cell Biochem, 2009; 108: 1153–1165.

33) Anitua E, Sánchez M, Nurden AT, Zalduno MM, de la Fuente M, Azofra J, et al. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. Rheumatology, 2007; 46: 1769–1772.

34) Bendinelli P, Matteucci E, Dogliotti G, Corsi MM, Banfi G, Maroni P, et al. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-κB inhibition via HGF. J Cell Physiol, 2010; 225: 757–766.

35) Campbell KA, Saltzman BM, Mascarenhas R, Khair MM, Verma NN, Bach BR Jr, et al. Does intra-articular platelet-rich plasma injection provide clinically superior outcomes compared with other therapies in the treatment of knee osteoarthritis? A systematic review of overlapping meta-analyses. Arthroscopy, 2015; 31: 2213–2221.

36) Hunter, DJ, Guermazi A, Lo GH, Grainger AJ, Conaghan PG, Boudreau RM, et al. Evolution of semi-quantitative whole joint assessment of knee OA: MOAKS (MRI Osteoarthritis Knee Score). Osteoarthritis Cartilage, 2011; 19(8): 990–1002.