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Treating Severe Knee Osteoarthritis with Combination of Intra-Osseous and Intra-Articular Infiltrations of Platelet-Rich Plasma: An Observational Study

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Abstract

Objective. Assessing the therapeutic effects of a combination of intra-articular and intra-osseous infiltrations of platelet-rich plasma (PRP) to treat severe knee osteoarthritis (KOA) using intra-articular injections of PRP as the control group. *Design*. In this observational study, 60 patients suffering from severe KOA were treated with intra-articular infiltrations of PRP (IA group) or with a combination of intra-osseous and intra-articular infiltrations of PRP (IO group). Both groups were matched for sex, age, body mass index, and radiographic severity (III and IV degree according to Ahlbäck scale). Clinical outcome was evaluated at 2, 6, and 12 months, using the Knee injury and Osteoarthritis Outcome Score (KOOS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaires. *Results*. At 2, 6 and 12 months after treatment, IO group had a significant improvement in all KOOS and WOMAC subscales ($P < 0.05$). On the contrary, patients of the IA group did not improve in any of the scores. Sixteen out of 30 IO group patients showed minimal clinically important improvement (MCII) whereas 8 out of 30 IA group patients showed this response at 6 months (26.7%; 95% CI −0.4 to 49.9; $P = 0.037$). At 12 months, 14 patients of IO group and 5 patients of the IA group showed MCII (30%; 95% CI 4.3 to 51.9; $P = 0.013$). No differences between groups were observed at 2 months. *Conclusions*. PRP intra-articular injections in severe KOA were not effective and did not provide any benefit. Combination of intra-articular and intra-osseous infiltrations of PRP was not clinically superior at 2 months, but it showed superior clinical outcomes at 6 and 12 months when compared with intra-articular injections of PRP.

Keywords

knee osteoarthritis, platelet-rich plasma, subchondral bone, intra-osseous infiltration

Introduction

Knee osteoarthritis (KOA) is an active, heterogeneous, and low-grade inflammatory condition leading to functional disability and pain.[1,2] Its costs are between 1.0% and 2.5% of gross domestic product, and obesity and aging are the 2 main risk factors.[3] Traditionally, KOA was considered a cartilage-driven "wear and tear" disease; however, an increasing body of evidence suggests the direct involvement of 2 well-vascularized synovial joint tissues, namely, the subchondral bone (SB) and synovial membrane (SM) in the degradation of articular cartilage (AC).[4-6] Intra-articular delivery is the conventional modality to reach AC, SM, and synovial fluid (SF) with platelet-rich plasma (PRP), and it has been shown to be safe and efficacious in reducing pain and improving joint function in patients with moderate KOA.[7-10] However, in patients with severe KOA, SB undergoes structural changes, including a progressive replacement of the subchondral marrow with fibroneurovascular mesenchymal tissue, an undermineralization of bone, bone marrow lesions (BMLs), osteophytes, sclerosis, and stiffness of SB.[5,11,12] In this context, intra-articular infiltrations of PRP are insufficient to reach the SB, thereby limiting their efficacy.[13,14] According to the promising results observed in animal[15,16] and humans treated with intra-osseous infiltrations of PRP,[17,18] this work aimed to assess the therapeutic effects of a novel approach by treating severe KOA with a combination of intra-articular

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and intra-osseous infiltrations of PRP, using intra-articular injections of PRP as the control group.

Since the combination of intra-articular and intra-osseous infiltration of PRP targets AC, SF, SM, and SB, we hypothesize that this new approach might improve the effectiveness of intra-articular infiltrations of PRP for severe KOA.

Methods

The study was designed as an observational study to analyze the combination of intra-articular and intra-osseous injections of PRP. Patients were enrolled from 2015 to 2016 and 30 patients with severe KOA treated with intra-articular infiltrations of PRP were used as a control group (IA group).

Patients

The study included a total of 60 patients aged between 40 and 80 years with severe KOA and diagnosed according to the American College of Rheumatology criteria and with radiographic severity III and IV degree according to Ahlbäck scale, who received intra-articular or intra-osseous infiltrations of PRP. The inclusion criteria were as follows: (1) patients of both sexes aged 40 to 80 years, (2) predominant medial tibiofemoral KOA, (3) radiographic severity degree III and IV according to the Ahlbäck scale. The exclusion criteria were (1) an excessive misalignment with a diaphyseal varus deformity of 4° and valgus of 16° that required osteotomy, (2) arthroscopy in the last year prior to treatment, (3) infiltration of hyaluronic acid or corticosteroids in the past 6 months and (4) systemic autoimmune rheumatic disease.

Each group included 30 patients, one who received 3 intra-articular infiltrations of PRP on a weekly basis (IA group), while the other group received a combination of 2 intra-osseous PRP infiltrations with the first intra-articular injection followed by 2 more intra-articular injections in the following 2 weeks after the intra-osseous infiltrations (IO Group) (**Fig. 1**). Regarding treatment allocation, the patients chose their preferred option after explaining the study and offering the two treatments. Patients in both groups were matched by age, gender, and body mass index (BMI) and by radiographic severity (same Ahlbäck grade III and IV). The baseline features of both groups are shown in **Table 1**. Patient sex, age, BMI, and baseline Knee injury and Osteoarthritis Outcome Score (KOOS) were not dissimilar between except for activities of daily living (ADL) of KOOS and function of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Concurrent medication such as paracetamol was forbidden 48 hours prior to assessment.

Platelet-Rich Plasma Preparation

Thirty-two milliliters or 90 mL of venous blood was extracted from the patient depending on whether infiltration was intra-articular or intra-osseous, respectively. Blood was withdrawn into 9-mL tubes containing 3.8% (w/v) sodium citrate and centrifuged at 580 × *g* for 8 minutes at room temperature. The 2-mL plasma fraction located just above the sedimented red blood cells, but not including the buffy coat, was collected in a tube and carried to the injection room for use. This plasma fraction preparation contained a moderate concentration of platelets (1.5 to 2.5 times the concentration of platelets compared with peripheral blood, depending on the platelet count and size as well as the hematocrit) and an absence of erythrocytes and leukocytes. The product received by patients of IA group contained a mean of 377.65 ± 74.60 platelets/mL (range 250-552 platelets/mL) and the PRP for the IO group a mean of 363.30 ± 71.13 platelets/mL (range 198-518 platelets/mL). To initiate the activation of platelet clotting, calcium chloride (10% w/v) was added to the liquid PRP aliquots just before injection. All procedures were performed under sterile conditions.

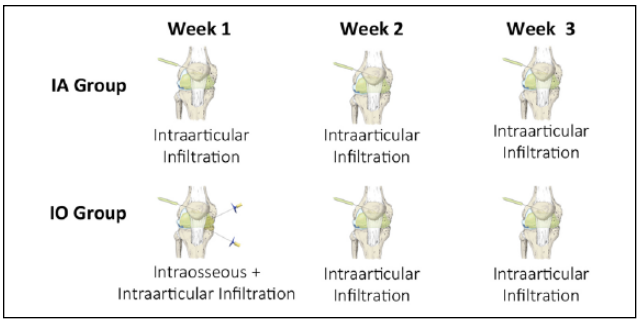

Figure 1. Schematic representation of the different treatment groups. IA—patients treated with intra-articular infiltrations of platelet-rich plasma; IO—patients treated with intra-osseous infiltrations of platelet-rich plasma.

Table 1. Baseline Characteristics of the Studied Patients.

	IA Group[a]	IO Group[b]	*P*
N	30	30	
Age, years	67.9 ± 7.3	63.4 ± 9.0	0.071
Males, %	56.0	60.0	0.755
Body mass index, kg/m^2	30.9 ± 5.6	30.7 ± 5.6	0.140
Ahlbäck grade (*n*)			0.275
III	29	27	
IV	1	3	

[a]IA group: intra-articular infiltrations of platelet-rich plasma.
[b]IO group: intra-osseous and intra-articular infiltrations of platelet-rich plasma.

Treatments

The first PRP administration of IO group patients included 3 different injections in different anatomical locations and conduced in the operating room. First, one PRP intra-articular injection was conducted, and afterward 2 PRP intra-osseous injections were performed according to the

technique described by Sánchez *et al*.[19] Briefly, under anesthesiologist surveillance, sedation of the patient was induced,[19] reaching a degree of sedation of −4 or −5 on the Richmond Sedation Scale. The patient was positioned supine and 2 marks were drawn in the medial region of the knee, one located 2 cm proximal and other located 2 cm distal to medial joint line. The infiltration area was prepared with a povidone-iodine solution and local anesthesia was conducted into the periosteum of condyle and tibial plateau. First, 8 M of PRP was infiltrated intra-articularly after evacuating the totality of the synovial fluid. This quantity of volume was administered due to the previous experience and studies with this protocol to treat KOA.[9,20] Eight milliliters of PRP is an adequate volume to soak the intra-articular space and cover the synovial membrane after coagulation,[21] in addition to being a well-tolerated volume for patients. Next, intra-osseous infiltrations were performed with a 13G trocar used for bone biopsy, which was manually introduced into the bone and inserted 2 cm into the medial tibial plateau and medial femoral condyle. Once the trocar was placed in the desired position 5 mL of PRP was infiltrated into SB. The control of trocar placements was facilitated using a fluoroscope. Intra-osseous infiltration did not focus on specific lesions but was performed at the same point in all interventions, since PRP allocates all over the subchondral area regardless of tissue lesions.[21] Two more intra-articular PRP infiltrations were performed 7 and 14 days after the first treatment.[19]

In contrast, patients of IA group received only 3 conventional intra-articular infiltrations of PRP with a weekly periodicity.[9]

Outcome Evaluation

Patients filled out KOOS at baseline and 2 months, 6 months, and 12 months after the third IA injection, and were evaluated by a different physician than the one who applied the treatment.

The primary efficacy criterion was a change from baseline in joint pain, measured using the KOOS pain subscale. Success rates were calculated according to a reduction in the pain score of at least 10 points from baseline (minimal clinically important improvement [MCII]).[22] Secondary efficacy variables included changes in KOOS subscales for symptoms, ADL, function in sport and recreation (Sport/Rec), knee-related quality of life (QOL), as well as the WOMAC subscales for pain, stiffness, and physical function. The evolution from baseline in overall knee pain after application of the visual analog scale (VAS) that ranged from 0 to 100 was determined by the WOMAC scale. In case of patients who failed to improve and underwent other treatments before 12 months, their basal values were included to obtain the score at this time-point.

Statistical Analysis

Power analysis was conducted to estimate the sample size needed to achieve 80% power at a 5% level of significance for the primary outcome measures to find as statistically significant a proportion difference, expected to be of 15% in IA group 1 and 50% in IO group. Demographic and medical variables (gender, age, BMI, and OA grade) were determined by the mean, standard deviation, range, and percentage. Success rate was assessed using χ^2 test. Comparisons were performed by Student *t* test for independent or paired-samples parametric data, Wilcoxon signed-rank test for paired-samples nonparametric data, and Mann-Whitney *U* test for independent samples nonparametric data; distribution of the samples was assessed by Saphiro-Wilk test. Data were considered statistically significant when $P < 0.05$. Statistical analysis was performed with SPSS 17.0 (SPSS, Chicago, IL).

Results

Intra-Articular Group (IA Group)

The percentage of patients who showed a pain reduction of at least 10 points (MCII) from baseline to 2 months and 6 months of follow-up was 43.3% (13 of 30 patients) and 26.7% (8 of 30 patients), respectively. The evolution of patients treated only with intra-articular PRP (IA group) is shown in **Table 2**. These patients did not experience significant pain improvement at 2 and 6 months according to the results of KOOS, WOMAC, and VAS scales. In the other KOOS subscales as well as in the WOMAC scores, there were no statistically significant differences at any point in the follow-up.

Before the 12 months, 10 patients left the follow-up, 8 of whom did not respond well to the treatment and underwent other interventions (26.7% of the 30 patients who were treated). The 2 remaining patients who were not monitored at 12 months were unreachable. Thus, at 12 months, 16.7% of patients in IA group showed a pain reduction of at least 10 points (MCII) from baseline (5 of 30 patients). At this time, the patients also did not experience a significant improvement in the results.

Intra-Osseous Group (IO Group)

Concerning the percentage of patients with MCII according to KOOS pain subscale, treatment applied in the IO group achieved a percentage of patient with a pain reduction of at least 10 points of 56.6% (17 of 30) at 2 months and 53.3% (16 of 30) at 6 months. **Table 3** shows the evolution of patients in IO group at 2, 6, and 12 months after treatment. In contrast to patients in the IA group, patients receiving intra-osseous PRP therapy had significant pain improvement ($P < 0.05$) at 2 and 6 months according to KOOS, WOMAC, and VAS scores. Moreover, this improvement was also obtained

Table 2. Evolution of IA Group[a] Patients at Time-Points.

	Baseline	2 Months		6 Months		12 Months	
	Score	Score	P	Score	P	Score	P
KOOS Pain	53.2 ± 14.8	58.3 ± 16.5	0.053	56.0 ± 19.0	0.335	53.2 ± 21.7	0.973
KOOS Symptoms	66.3 ± 20.8	69.2 ± 22.0	0.395	62.1 ± 21.3	0.311	63.0 ± 22.4	0.323
KOOS ADL	51.3 ± 14.5	55.8 ± 17.2	0.261	54.3 ± 20.7	0.376	51.7 ± 17.0	0.899
KOOS Sport/Rec	22.0 ± 25.6	18.5 ± 18.7	0.541	20.7 ± 17.3	0.843	19.0 ± 20.1	0.475
KOOS QOL	26.7 ± 18.1	31.3 ± 16.5	0.153	31.3 ± 18.1	0.170	29.2 ± 17.8	0.501
WOMAC Pain	8.8 ± 3.2	7.8 ± 3.3	0.062	7.9 ± 3.4	0.114	9.1 ± 4.1	0.612
WOMAC Stiffness	3.4 ± 2.04	3.3 ± 2.0	0.823	3.7 ± 1.7	0.291	3.7 ± 2.1	0.333
WOMAC Function	33.5 ± 11.8	30.0 ± 11.7	0.072	31.1 ± 13.4	0.288	33.9 ± 14.9	0.834
VAS	4.7 ± 1.60	4.2 ± 1.6	0.166	4.3 ± 1.8	0.271	5.0 ± 2.1	0.343

KOOS = Knee injury and Osteoarthritis Outcome Score; ADL = activities of daily living; Sport/Rec = function in sport and recreation; QOL = knee-related quality of life; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; VAS = visual analogue scale.
[a]IA group: intra-articular infiltrations of platelet-rich plasma.

Table 3. Evolution of IO Group[a] Patients at Time-Points.

	Baseline	2 Months		6 Months		12 Months	
	Score	Score	*P*	Score	*P*	Score	*P*
KOOS Pain	56.7 ± 15.2	67.6 ± 13.8	<0.001*	69.1 ± 17.4	<0.001*	67.7 ± 17.2	<0.001*
KOOS Symptoms	62.5 ± 16.1	69.2 ± 15.8	0.014*	72.5 ± 18.3	0.006*	72.1 ± 17.5	0.002*
KOOS ADL	61.5 ± 17.2	71.1 ± 19.5	0.002*	73.3 ± 17.5	0.003*	71.2 ± 16.1	0.001*
KOOS Sport/Rec	23.2 ± 20.2	30.2 ± 20.8	0.019*	37.2 ± 25.1	0.017*	28.5 ± 22.1	0.032*
KOOS QOL	30.4 ± 15.8	41.0 ± 19.0	0.001*	42.7 ± 20.3	0.001*	37.5 ± 16.0	0.004*
WOMAC Pain	7.7 ± 3.3	5.7 ± 2.8	0.001*	5.3 ± 3.3	0.001*	5.2 ± 2.9	<0.001*
WOMAC Stiffness	3.1 ± 1.7	3.3 ± 2.0	0.271	2.1 ± 1.7	0.010*	2.4 ± 1.5	0.010*
WOMAC Function	26.5 ± 11.9	19.6 ± 13.3	<0.001*	17.7 ± 11.5	0.001*	19.9 ± 11.4	0.001*
VAS	4.1 ± 1.6	3.2 ± 1.5	0.004*	2.9 ± 1.6	0.001*	3.0 ± 1.6	0.001*

KOOS = Knee injury and Osteoarthritis Outcome Score; ADL = activities of daily living; Sport/Rec = function in sport and recreation; QOL = knee-related quality of life; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; VAS = visual analogue scale.
[a]IO group: intra-osseous and intra-articular infiltrations of platelet-rich plasma.
*$P < 0.05$ with regard to baseline.

in the other variables assessed by the KOOS and WOMAC scales (except from stiffness at 2 months).

Eleven patients withdrew the follow-up before the 12 months, 5 of whom did not respond well to the treatment and underwent other interventions (16.6% of the 30 patients who were treated). Four other patients continued with the improvement and they did not consider it necessary to follow up. The 2 remaining patients who were not monitored at 12 months were unreachable. Therefore, 46.7% of patients showed a pain reduction of at least 10 points (MCII) from baseline (14 of 30 patients). At this time the patients showed a significant improvement ($P < 0.05$) in the results of KOOS, WOMAC, and VAS scores.

IA Group versus IO Group

When comparing the response of both treatments according to the percentage of patients with MCII on the pain scale, a statistically significant improvement was observed at the sixth month after treatment. The percentage of patients with MCII in pain reduction of IO group was 26.7 points of percentage higher than IA group patients (95% CI −0.4 to 49.9; $P = 0.037$). This significant improvement was maintained at 12 months with 30.0 points of percentage higher than patients of IA group (95% CI 4.3 to 51.9; $P = 0.013$). These differences are consistent when comparing the scores from baseline to time-points (δ), being statistically significant at 6 months after treatment (12.4 ± 15.9 vs 2.8 ± 15.5; $P = 0.021$) and at 12 months after treatment (11.6 ± 14.8 vs −0.1 ± 14.6; $P = 0.005$) (**Table 4**). This improvement was not observed when only 2 months elapsed after treatment. Concerning symptoms, the rate of response was also 26.7 points of percentage higher in patients of IO group at 6 months (95% CI −0.6 to 50.2; $P = 0.038$) and 23.4 points at 12 months (95% CI 0.4 to 44.4; $P = 0.029$). Similarly, IO group showed also, compared with IA group, a statistically

Table 4. Comparison of Patients with MCII and Improvement (δ) at Time-Points.

	MCII, n (%)				δ (mean ± SD)			
	IA Group[a]	IO Group[b]	Proportion/Mean Difference (95% CI)	*P*	IA Group[a]	IO Group[b]	Proportion/Mean Difference (95% CI)	*P*
Two months after treatment								
KOOS Pain	13 (43.3)	17.0 (56.7)	13.4 (–13.8 to 38.7)	0.303	5.4 ± 15.5	10.9 ± 12.0	5.5 (–1.6 to 12.6)	0.130
KOOS Symptoms	11 (36.7)	13.0 (43.0)	6.3 (–20.1 to 31.8)	0.621	3.2 ± 21.6	6.6 ± 12.5	3.4 (–5.7 to 12.5)	0.459
KOOS ADL	9 (30.0)	16.0 (53.3)	23.3 (–3.9 to 47.2)	0.069	5.1 ± 16.8	9.7 ± 15.5	4.6 (3.7 to 12.9)	0.275
KOOS Sport/Rec	6 (20.0)	13.0 (43.3)	23.3 (–2.7 to 46.1)	0.054	−2.6 ± 24.3	7.0 ± 14.7	9.6 (−0.7 to 19.9)	0.069
KOOS QOL	12 (40.0)	16.0 (53.3)	13.3 (–13.9 to 38.5)	0.305	5.2 ± 18.2	10.6 ± 14.6	5.4 (–3.1 to 13.9)	0.210
Six months after treatment								
KOOS Pain	8 (26.6)	16.0 (53.3)	26.7 (0.3 to 50.4)	0.037*	2.8 ± 15.5	12.4 ± 15.9	9.7 (1.5 to 17.8)	0.021*
KOOS Symptoms	9 (30.0)	17.0 (56.7)	26.7 (–0.6 to 50.2)	0.038*	−4.2 ± 22.1	9.9 ± 18.3	14.1 (3.6 to 24.6)	0.009*
KOOS ADL	10 (33.3)	14.0 (46.7)	13.4 (–13.3 to 38.3)	0.293	2.9 ± 18.2	11.9 ± 20.3	8.6 (–1.1 to 18.8)	0.081
KOOS Sport/Rec	9 (30.0)	17.0 (56.7)	26.6 (–0.7 to 50.1)	0.039*	−1.3 ± 22.6	14.0 ± 28.6	15.3 (2.0 to 28.7)	0.048*
KOOS QOL	12 (40.0)	18.0 (60.0)	20 (–7.4 to 44.5)	0.124	4.6 ± 17.3	12.3 ± 17.6	7.7 (–1.3 to 16.7)	0.060
Twelve months after treatment								
KOOS Pain	5 (16.7)	14 (46.7)	30.0 (4.3 to 51.9)	0.013*	−0.1 ± 14.6	11.1 ± 14.8	11.2 (3.5 to 18.8)	0.005*
KOOS Symptoms	3 (10)	10 (33.4)	23.4 (0.4 to 44.4)	0.029*	−3.3 ± 17.9	9.6 ± 15.2	12.9 (4.3 to 21.4)	0.004*
KOOS ADL	6 (20)	12 (40)	20.0 (–5.4 to 42.9)	0.094	0.4 ± 13.6	9.7 ± 14.8	9.4 (1.9 to 16.7)	0.014*
KOOS Sport/Rec	6 (20)	8 (26.1)	6.7 (–16.8 to 29.5)	0.543	−3.0 ± 22.7	5.4 ± 13.0	8.4 (–1.2 to 17.9)	0.085
KOOS QOL	8 (26.7)	11 (36.7)	10.0 (–15.5 to 34.2)	0.409	2.5 ± 20.7	7.1 ± 12.4	4.6 (–4.1 to 13.2)	0.292

MCII = minimal clinically important improvement; δ = difference in the improvement from baseline; CI = confidence interval; KOOS = Knee injury and Osteoarthritis Outcome Score; ADL = activities of daily living; Sport/Rec = function in sport and recreation; QOL = knee-related quality of life; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; VAS = visual analogue scale.

[a]IA group: intra-articular infiltrations of platelet-rich plasma.

[b]IO group: intra-osseous and intra-articular infiltrations of platelet-rich plasma.

*$P < 0.05$ with regard to baseline.

significant improvement in symptoms at 6 months (9.9 ± 18.3 vs −4.2 ± 22.1; $P = 0.009$) and 12 months (9.6 ± 15.2 vs −3.3 ± 17.9; $P = 0.004$).

None of these differences between both groups were observed at 2 months.

Patients who underwent intra-osseous infiltrations did not refer side effects and complications during the procedure. After the infiltration mild pain of short duration (24-48 hours) were reported with no other adverse effects.

Discussion

In this study, both groups were treated with 3 intra-articular infiltrations of PRP on a weekly basis, and only the IO group underwent a novel local therapy consisting of a combination of intra-articular and intra-osseous infiltrations of PRP. The combination of intra-articular and intra-osseous infiltrations of PRP did not show differences compared control treatment at 2 months, but it exerted significant pain reduction and improvement in knee joint functionality at 6 and 12 months after treatment in KOA patients of advanced degrees, with no severe adverse effects in both modalities of treatment.

During the past years, several clinical studies have reported controversial results about the use of intra-articular delivery of PRP for KOA. On one hand, some studies reported that in patients with mild to moderate KOA is safe and more efficacious than hyaluronic acid or normal saline in alleviating pain and improving patient functionality.[8,20,23] On the other hand, the non-superiority of PRP against other treatments also has been shown by several studies.[24,25] Analysis of the products used in the studies continues to suffer from inconsistencies both in its preparation and its application presenting many variables, namely number of platelets, activation method, dosage and presence of leukocytes. The latter has been one of the most studied as responsible for the safety and effectiveness of PRP. Both *in vitro* and *in vivo* studies that associates the presence of leukocytes within PRP with the detrimental effects on chondrocytes, human subchondral mesenchymal stem cells (MSCs), osteoblasts and synoviocytes[26] likely due to the release of catabolic (matrix metalloproteinase-9 [MMP-9]), and pro-inflammatory cytokines (interleukin-1β [IL-1β] and tumor necrosis fator-α [TNF-α]) mediated by the activation of the nuclear factor-κB (NF-κB) pathway.[27,28] Clinical studies using PRP with leukocytes reported both positive and negative results,[29-31] and the presence of leukocytes does not seem to cause a pro-inflammatory environment compared with PRP without leukocytes.[32] Nonetheless, recent works

conducted by Milants *et al*.[33] and Piuzzi *et al*.[34] seem to recommend the PRP poor in leukocytes for treatment of KOA, not for safety but for efficiency. These seemingly contradictory results between preclinical and clinical studies might partially be explained by the fact that a common denominator of these biological therapies appears to be their anti-inflammatory effect mediated by the inhibition of the NF-κB pathway.[35,36] This mismatch may well arise from redundancy as a basic information transfer principle of the regulatory pathways which operate on the whole animal during tissue repair. Rather than cells per se, it may be the secretion of versatile proteins that *in vivo* are used interchangeably, as may be the case of the NFkB inflammatory pathway, which seems to be backed up by several growth factors and cytokines released by platelets, monocytes, macrophages, or even MSCs acting as redundant components in cell information.[37]

Despite the controversy about the use of PRP for the treatment of KOA, there is more consensus that by increasing the severity of the pathology, the effectiveness of PRP decreases, regardless of the applied product. (Bottegoni, Dei Giudici, Salvemini, Chiurazzi, Bencivenga and Gigante, 2016),[13] (Jang, Kim and Cha, 2013)[14] Therefore, administration route could be a key element. Intra-articular drug delivery route is insufficient to tackle the SB, a tissue whose role in the pathophysiology and progress of KOA, mainly in the late stages, is increasingly recognized,[5,11,12] and it has been postulated as a pivotal target to treat severe KOA.[5,38] The significant improvement in the KOOS pain score and secondary outcome measure of KOOS and WOMAC subscales from baseline to 6 and 12 months shown by the IO group compared with the IA group in the present work, were attributed to the additional treatment modality, namely, intra-osseous infiltrations of PRP. The lack of significant difference at 2 months when comparing both groups despite the slight improvement in the IO group may be due to PRP begins its effect at the second month and its real effect is observed progressively over time.[39] These results are in accordance with the data reported by Sánchez *et al*.[19] that conducted a pilot study in 13 patients with severe KOA combining IA and IO infiltrations of PRP and reported a significant reduction in KOOS pain score, significant decrease of synovial fluid (MSC) after 1 week of treatment, and improvement in knee joint function.

Intra-osseous injections of PRP in humans have been proven to be efficacious in several conditions such as nonunion fractures or self-stimulating bone marrow of the iliac crest.[40-42] Despite these promising results, bone regeneration based on the use of PRP generates controversy due to studies with contradictory results. As in the case of cartilage, different variables that influence the preparation and application of PRP imply the use of different products and therefore different results.[43] A systematic review conducted by Roffi *et al*.[44] showed the benefit of PRP in preclinical studies while clinical studies presented more limitations in this regard, which suggests the difficult in translating and optimizing the use PRP for bone healing in the clinical practice.

However, intra-osseous infiltrations of PRP in this work do not expect to regenerate bone but also to stimulate the SB in order to improve the joint biological environment. Zhen *et al*.[45] showed that by inhibiting transforming growth factor-β (TGF-β) signaling in a specific population of MSCs present at the SB (nestin-positive MSC) the severity of OA was reduced.[45] In fact, previous studies have shown that the decrease in MSC in the SF, in low degree OA, suggests clinical improvement.[46] Therefore, it is reasonable to speculate that, by administering PRP directly into SB, the concurrent presence of platelet-secreted TGF-β1 and vascular endothelial growth factor (VEGF) as well as plasma growth factors such as insulin-like growth factor-I (IGF-I) and hepatocyte growth factor (HGF) could have a modulatory effect on TGF-β signaling pathway.[21,45] This might reduce the presence of MSCs and could likely be associated with the shrinking of fibroneurovascular tissue of KOA SB, an explanation which parallels the antifibrotic mechanism already reported in several cell phenotypes[47] thereby contributing to modulate the aberrant fibroneurovascular tissue and to alleviate pain and hyperalgesia.[48] In this regard, Muiños-López *et al*.[49] showed that intra-osseous infiltrations of PRP but not intra-articular infiltrations decreased the presence of synovial MSC. In addition to these effects that occurred in SB, intra-articular infiltration of PRP suppressed effect of NF-κB on intra-articular inflamed cells, which would lead to the reduction of proinflammatory cytokines that otherwise might contribute to pain by stimulating hyperalgesia and sensitizing joint nociceptors to other stimuli.[6,50] This anti-inflammatory effect not only favor a pain reduction but also could influence in other biological processes related to KOA such as cell senescence.[51,52] Finally, significant amount of endogenous cannabinoids within PRP might act as ligands for cannabinoid receptor 1 (CB1) and 2 (CB2) of chondrocytes, synovium cells, and bone cells of OA patients, thereby supporting both a pain and inflammation reduction by targeting the endogenous cannabinoid system.[53,54]

Regardless how much of the therapeutic effect of intra-articular and intra-osseous infiltration of PRP is placebo, there is ample *in vitro* and *in vivo* evidence to suggest that PRP intervention on KOA is something more than a sham intervention where PRP would meet the requirements of an ideal placebo.[26] As an example, bone marrow stimulating techniques, and intra-osseous infiltrations of PRP as one of them, have proven to induce a cartilage-like repair tissue and repair chondral defects,[55,56] which render PRP application an structure-modifying therapy. However, and prior to treatment of PRP, we first would remove the synovial fluid and only then infiltrated PRP intra-articularly. This raises the question about how much of the therapeutic effect of IA

infiltrations is placebo response or physiological effect after both the removal of synovial fluid with pain-signaling and mediating molecules in addition to the injection of a fluid by means of a needle into the knee joint.[57-60]

This study presents some limitations. First, the particularities of this new treatment have prevented a better study design that would have generated more solid results and conclusions. However, the achieved results allow us to consider new and deeper studies based on this field. Second, a relatively small number of patients were enrolled in the study. Third, from a researcher's point of view, there is a lack of follow-up of structural changes in SB throughout 3-T magnetic resonance imaging, histological, and immunohistochemistry studies, and flow cytometry, which might suggest a structure-modifying disease intervention with this novel approach.

In summary, PRP intra-articular injections in severe OA were not effective and did not provide any benefit, and the combination of intra-articular and intra-osseous infiltrations of PRP was not clinically superior to intra-articular infiltration at 2 months. However, the results display a higher pain reduction and improvement in knee joint functionality at 6 and 12 months in patients with severe KOA, with no severe adverse effects in both modalities of treatment. Therefore, further studies will be needed in order to increase our knowledge of intra-osseous infiltrations of PRP.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Sabino Padilla is a scientist at BTI Biotechnology Institute, a dental implant company that investigates the fields of oral implantology and PRGF-Endoret technology.

Ethical Approval

This study was carried out in accordance with the international standard on clinical trials: Declaration of Helsinki in its latest revised version (Fortaleza, Brazil; 2013), and Good Clinical Practice Regulations (International Conference for Harmonization). Ethical approval for this study was obtained from the Ethics Committee of the Basque Country (ceic.eeaa@euskadi.eus) (Protocol No. MIKPLA201501).

Informed Consent

Written informed consent was obtained from all subjects before the study.

Trial Registration

Not applicable.

References

1. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage. 2013;21(1):16-21.
2. Berenbaum F, Meng QJ. The brain-joint axis in osteoarthritis: nerves, circadian clocks and beyond. Nat Rev Rheumatol. 2016;12(9):508-16.
3. Glyn-Jones S, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H, *et al*. Osteoarthritis. Lancet. 2015;386(9991): 376-87.
4. Barr AJ, Campbell TM, Hopkinson D, Kingsbury SR, Bowes MA, Conaghan PG. A systematic review of the relationship between subchondral bone features, pain and structural pathology in peripheral joint osteoarthritis. Arthritis Res Ther. 2015;17:228.
5. Campbell TM, Churchman SM, Gomez A, McGonagle D, Conaghan PG, Ponchel F, *et al*. Mesenchymal stem cell alterations in bone marrow lesions in patients with hip osteoarthritis. Arthritis Rheumatol. 2016;68(7):1648-59.
6. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012;51(2):249-57.
7. Filardo G, Kon E, Pereira Ruiz MT, Vaccaro F, Guitaldi R, Di Martino A, *et al*. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. Knee Surg Sports Traumatol Arthrosc. 2012;20(10):2082-91.
8. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. Arthroscopy. 2016;32(3):495-505.
9. 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(8):1070-78.
10. Vaquerizo V, Plasencia MÁ, Arribas I, Seijas R, Padilla S, Orive G, *et al*. Comparison of intra-articular injections of plasma rich in growth factors (PRGF-Endoret) versus Durolane hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: a randomized controlled trial. Arthroscopy. 2013;29(10):1635-43.
11. Lajeunesse D. Subchondral bone involvement in the pathophysiology of osteoarthritis. In: Martel-Pelletier J and Pelletier JP, editors. Understanding osteoarthritis from bench to bedside. Trivandrum, Kerala, India: Research Signpost; 2011. p. 69-83.
12. Suri S, Walsh DA. Osteochondral alterations in osteoarthritis. Bone. 2012;51(2):204-11.
13. Bottegoni C, Dei Giudici L, Salvemini S, Chiurazzi E, Bencivenga R, Gigante A. Homologous platelet-rich plasma for the treatment of knee osteoarthritis in selected elderly patients: an open-label, uncontrolled, pilot study. Ther Adv Musculoskelet Dis. 2016;8(2):35-41.
14. Jang SJ, Kim JD, Cha SS. Platelet-rich plasma (PRP) injections as an effective treatment for early osteoarthritis. Eur J Orthop Surg Traumatol. 2013;23(5):573-80.
15. Liu HY, Huang CF, Lin TC, Tsai CY, Tina Chen SY, Liu A, *et al*. Delayed animal aging through the recovery of stem cell senescence by platelet rich plasma. Biomaterials. 2014;35(37):9767-76.
16. Tohidnezhad M, Wruck CJ, Slowik A, Kweider N, Beckmann R, Bayer A, *et al*. Role of platelet-released growth factors in detoxification of reactive oxygen species in osteoblasts. Bone. 2014;65:9-17.

17. Philippart P, Meuleman N, Stamatopoulos B, Najar M, Pieters K, De Bruyn C, *et al*. In vivo production of mesenchymal stromal cells after injection of autologous platelet-rich plasma activated by recombinant human soluble tissue factor in the bone marrow of healthy volunteers. Tissue Eng Part A. 2014;20(1-2):160-70.
18. Sánchez M, Delgado D, Sánchez P, Muiños-López E, Paiva B, Granero-Moltó F, *et al*. Combination of intra-articular and intraosseous injections of platelet rich plasma for severe knee osteoarthritis: a pilot study. Biomed Res Int. 2016;2016:4868613.
19. Sánchez M, Fiz N, Guadilla J, Padilla S, Anitua E, Sánchez P, *et al*. Intraosseous infiltration of platelet-rich plasma for severe knee osteoarthritis. Arthrosc Tech. 2014;3(6):e713-7.
20. Sánchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. Clin Exp Rheumatol. 2008;26(5):910-13.
21. Sánchez M, Anitua E, Delgado D, Sanchez P, Prado R, Goiriena JJ, *et al*. A new strategy to tackle severe knee osteoarthritis: combination of intra-articular and intraosseous injections of platelet rich plasma. Expert Opin Biol Ther. 2016;16(5):627-43.
22. Roos EM, Lohmander LS. The knee injury and osteoarthritis outcome score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 2003;1:64.
23. 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(2):356-64.
24. Filardo G, Di Matteo B, Di Martino A, Merli ML, Cenacchi A, Fornasari P, *et al*. Platelet-rich plasma intra-articular knee injections show no superiority versus viscosupplementation: a randomized controlled trial. Am J Sports Med. 2015;43(7):1575-82.
25. Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: a prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. Am J Sports Med. 2017;45(2):339-46.
26. Assirelli E, Filardo G, Mariani E, Kon E, Roffi A, Vaccaro F, *et al*. Effect of two different preparations of platelet-rich plasma on synoviocytes. Knee Surg Sports Traumatol Arthrosc. 2015;23(9):2690-703.
27. Xu Z, Yin W, Zhang Y, Qi X, Chen Y, Xie X, *et al*. Comparative evaluation of leukocyte- and platelet-rich plasma and pure platelet-rich plasma for cartilage regeneration. Sci Rep. 2017;7:43301.
28. Cavallo C, Filardo G, Mariani E, Kon E, Marcacci M, Pereira Ruiz MT, *et al*. Comparison of platelet-rich plasma formulations for cartilage healing: an in vitro study. J Bone Joint Surg Am. 2014;96(5):423-9.
29. 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.
30. Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. Am J Phys Med Rehabil. 2012;91(5):411-7.
31. Riboh JC, Saltzman BM, Yanke AB, Fortier L, Cole BJ. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. Am J Sports Med. 2016;44(3):792-800.
32. Mariani E, Canella V, Cattini L, Kon E, Marcacci M, Di Matteo B, *et al*. Leukocyte-rich platelet-rich plasma injections do not up-modulate intra-articular pro-inflammatory cytokines in the osteoarthritic knee. PLoS One. 2016;11(6):e0156137.
33. Milants C, Bruyère O, Kaux JF. Responders to platelet-rich plasma in osteoarthritis: a technical analysis. Biomed Res Int. 2017;2017:7538604.
34. Piuzzi NS, Chughtai M, Khlopas A, Harwin SF, Miniaci A, Mont MA, *et al*. Platelet-rich plasma for the treatment of knee osteoarthritis: a review. J Knee Surg. 2017;30(7):627-33.
35. 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(3):757-66.
36. Montaseri A, Busch F, Mobasheri A, Buhrmann C, Aldinger C, Rad JS, *et al*. IGF-1 and PDGF-bb suppress IL-1β-induced cartilage degradation through down-regulation of NF-κB signaling: involvement of Src/PI-3K/AKT pathway. PLoS One. 2011;6(12):e28663.
37. Gerhart J, Kirschner M. The theory of facilitated variation. Proc Natl Acad Sci U S A. 2007;104(Suppl 1):8582-9.
38. Dieppe P. Subchondral bone should be the main target for the treatment of pain and disease progression in osteoarthritis. Osteoarthritis Cartilage. 1999;7(3):325-6.
39. Anitua E, Sánchez M, Aguirre JJ, Prado R, Padilla S, Orive G. Efficacy and safety of plasma rich in growth factors intra-articular infiltrations in the treatment of knee osteoarthritis. Arthroscopy. 2014;30(8):1006-17.
40. Guadilla J, Fiz N, Andia I, Sánchez M. Arthroscopic management and platelet-rich plasma therapy for avascular necrosis of the hip. Knee Surg Sports Traumatol Arthrosc. 2012;20(2):393-8.
41. Kyllönen L, D'Este M, Alini M, Eglin D. Local drug delivery for enhancing fracture healing in osteoporotic bone. Acta Biomater. 2015;11:412-34.
42. Sanchez M, Anitua E, Cugat R, Azofra J, Guadilla J, Seijas R, *et al*. Nonunions treated with autologous preparation rich in growth factors. J Orthop Trauma. 2009;23(1):52-9.
43. Oryan A, Alidadi S, Moshiri A. Platelet-rich plasma for bone healing and regeneration. Expert Opin Biol Ther. 2016;16(2):213-32.
44. Roffi A, Di Matteo B, Krishnakumar G, Kon E, Filardo G. Platelet-rich plasma for the treatment of bone defects: from pre-clinical rational to evidence in the clinical practice. A systematic review. Int Orthop. 2017;41(2):221-37.
45. Zhen G, Wen C, Jia X, Li Y, Crane JL, Mears SC, *et al*. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med. 2013;19(6):704-12.
46. Sekiya I, Ojima M, Suzuki S, Yamaga M, Horie M, Koga H, *et al*. Human mesenchymal stem cells in synovial fluid

increase in the knee with degenerated cartilage and osteoarthritis. J Orthop Res. 2012;30(6):943-9.
47. Yuan XL, Meng HY, Wang YC, Peng J, Guo QY, Wang AY, *et al*. Bone-cartilage interface crosstalk in osteoarthritis: potential pathways and future therapeutic strategies. Osteoarthritis Cartilage. 2014;22(8):1077-89.
48. Seidel MF, Herguijuela M, Forkert R, Otten U. Nerve growth factor in rheumatic diseases. Semin Arthritis Rheum. 2010;40(2):109-26.
49. Muiños-López E, Delgado D, Sánchez P, Paiva B, Anitua E, Fiz N, *et al*. Modulation of synovial fluid-derived mesenchymal stem cells by intra-articular and intraosseous platelet rich plasma administration. Stem Cells Int. 2016;2016:1247950.
50. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010;6(11):625-35.
51. Toh WS, Brittberg M, Farr J, Foldager CB, Gomoll AH, Hui JH, *et al*. Cellular senescence in aging and osteoarthritis. Acta Orthop. 2016;87(Sup363):6-14.
52. Moussa M, Lajeunesse D, Hilal G, El Atat O, Haykal G, Serhal R, *et al*. Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage. Exp Cell Res. 2017;352(1):146-56.
53. Descalzi F, Ulivi V, Cancedda R, Piscitelli F, Luongo L, Guida F, *et al*. Platelet-rich plasma exerts antinociceptive activity by a peripheral endocannabinoid-related mechanism. Tissue Eng Part A. 2013;19(19-20):2120-9.
54. Richardson D, Pearson RG, Kurian N, Latif ML, Garle MJ, Barrett DA, *et al*. Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res Ther. 2008;10(2):R43.
55. Chiang CW, Chen WC, Liu HW, Chen CH. Application of synovial fluid mesenchymal stem cells: platelet-rich plasma hydrogel for focal cartilage defect. J Exp Clin Med. 2014;6(4):118-24.
56. Hunziker EB, Lippuner K, Keel MJ, Shintani N. An educational review of cartilage repair: precepts & practice—myths & misconceptions—progress & prospects. Osteoarthritis Cartilage. 2015;23(3):334-50.
57. Jalowiec JM, D'Este M, Bara JJ, Denom J, Menzel U, Alini M, *et al*. An in vitro investigation of platelet-rich plasma-gel as a cell and growth factor delivery vehicle for tissue engineering. Tissue Eng Part C Methods. 2016;22(1):49-58.
58. Altman RD, Devji T, Bhandari M, Fierlinger A, Niazi F, Christensen R. Clinical benefit of intra-articular saline as a comparator in clinical trials of knee osteoarthritis treatments: a systematic review and meta-analysis of randomized trials. Semin Arthritis Rheum. 2016;46(2):151-9.
59. Bannuru RR, Schmid CH, Kent DM, Vaysbrot EE, Wong JB, McAlindon TE. Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: a systematic review and network meta-analysis. Ann Intern Med. 2015;162(1):46-54.
60. Hunziker EB, Quinn TM, Häuselmann HJ. Quantitative structural organization of normal adult human articular cartilage. Osteoarthritis Cartilage. 2002;10(7):564-72.