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ORIGINAL RESEARCH ARTICLE

Treatment of Knee Joint Osteoarthritis with Autologous Platelet-Rich Plasma in Comparison with Hyaluronic Acid

ABSTRACT

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Objective: This study aimed to find a simple, cost-effective, and time-efficient method for the preparation of platelet-rich plasma (PRP), so the acquired benefits will be readily available for multiple procedures in smaller outpatient clinics and to explore the safety and efficacy of the application of PRP in the treatment of degenerative lesions of articular cartilage of the knee.

Design: The study was designed as a prospective, cohort study with a control group. A total of 120 patients with Grade 1, 2, or 3 osteoarthritis according to the Kellgren and Lawrence grading scale were enrolled in the study. One group of patients was treated using three intra-articular applications of PRP, and the second group of patients was given three injections of hyaluronic acid. Outcome measures included the Western Ontario and McMaster Universities Osteoarthritis Index and the 11-point pain intensity Numeric Rating Scale.

Results: On average, a 4.5-fold increase in platelet concentration was obtained in the PRP group. No severe adverse events were observed. Statistically significantly better results in the Western Ontario and McMaster Universities Osteoarthritis Index and Numeric Rating Scale scores were recorded in a group of patients who received PRP injections after a 3- and 6-mo follow-up.

Conclusions: Our preliminary findings support the application of autologous PRP as an effective and safe method in the treatment of the initial stages of knee osteoarthritis. Further studies are needed to confirm these results and to investigate the persistence of the beneficial effects observed.

Key Words: Platelet-Rich Plasma, Growth Factors, Knee Osteoarthritis, Intra-Articular Injection

Osteoarthritis (OA) is a common and debilitating condition associated with pain and the loss of mobility that undermines quality-of-life. This multifactor disease is characterized by a destruction of the articular cartilage, subchondral bone alterations, and synovitis. The mechanisms responsible for OA progression are very complex and poorly understood.[1,2] A balance between anabolic and catabolic mechanisms maintains the homeostasis of extracellular matrix in the articular cartilage, and shifts toward degradation are associated with OA.

Currently available drugs for the treatment of OA, such as analgesics, nonsteroid and steroid anti-inflammatory drugs, glucosamine, chondroitin sulphate, and hyaluronic acid (HA), are predominantly directed toward the symptomatic relief of pain and inflammation, but they do little to reduce joint cartilage degeneration.[3] Regarding this fact, current research is investigating new methods of promoting cartilage repair, such as cytokine inhibitors, gene therapy, artificial cartilage substitutes, and applications of growth factors.[4]

Growth factors have been extensively studied for OA and cartilage repair because of their ability to enhance matrix synthesis. The efficacy of growth factors in cartilage repair is related to the recruitment of chondrogenic cells, stimulation of proliferation, and enhancement of cartilage matrix synthesis.[5] Growth factor therapy could be an attractive method for stimulation of the repair of damaged cartilage matrix. The platelet alpha-granules contain a range of important growth factors; therefore, they provide an obvious and readily accessible source of autogenous growth factors. The important growth factors contained in platelets and their actions are summarized in Table 1.

Platelet-rich plasma (PRP) is defined as an autologous concentration of human platelets in a small volume of plasma.[7] PRP is also a concentration of several fundamental protein growth factors proved to be actively secreted by platelets to initiate mesenchymal tissue healing. These growth factors stimulate cell proliferation, migration, differentiation, and matrix synthesis and can affect chondrocyte metabolism, chondrogenesis and improve cartilage healing in vivo.[8,9] PRP also contains plasmatic proteins, such as fibrin, fibronectin, and vitronectin, which act as mesenchymal cell adhesion molecules. It is believed that PRP can augment or stimulate healing with the same biologic healing process that normally occurs in the human body after injury.

The abundance of growth factors contained in platelets led to an obvious clinical idea: platelets can be concentrated and delivered to promote the healing process. Most studies used variants of PRP that were prepared using expensive and time-consuming techniques that limited the use and benefits of PRP use to larger sophisticated medical centers. Therefore, in this study, our first aim was to find a simple, low-cost manual protocol for PRP preparation and to confirm its reliability for the treatment of joint destruction in knee OA by aiding the regeneration of tissue that otherwise has low healing potential. The second aim of the study is to analyze the short-term results obtained and to determine the safety, feasibility, indication criteria, and application modalities for further studies.

TABLE 1 Growth factors present in platelets and its function[6]

Growth Factor	Function
Transforming growth factor–beta	Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic, and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates the mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation.
Basic fibroblast growth factor	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenic for mesenchymal stem cells, chondrocytes, and osteoblasts.
Platelet derived growth factor	Mitogenic for mesenchymal stem cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis.
Epidermal growth factor	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis.
Vascular endothelial growth factor	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells.
Connective tissue growth factor	Promotes angiogenesis, cartilage regeneration, fibrosis, and platelet adhesion.

METHODS

Setting

The study was performed in an outpatient orthopedic clinic in collaboration with the Associated Tissue Bank in Kosice, Slovakia.

PRP Preparation

The procedure for preparing PRP that is described here was modified after several trials from Landesberg et al.[10] The blood (27 ml of venous blood) sample was drawn into three 10-ml vacutainer tubes (S-Monovette, Sarstedt) containing either 1 ml of 0.106 M sodium citrate. The samples were gently agitated to thoroughly mix the anticoagulant with the blood. An aliquot was removed to determine the initial platelet count. The blood sample was then centrifuged for 15 mins at 3200 rpm at 22°C (Labofuge 400R, Heraeus) resulting in the three following layers: the inferior layer composed of erythrocytes, the intermediate layer composed of leukocytes, and the superior layer made up of plasma. The buffy coat layer together with the plasma layer was collected and centrifuged for another 10 mins at 1500 rpm to separate the leukocytes. The plasma layer was collected, and the third centrifugation step at 3200 rpm for 10 mins was performed to obtain a two-part plasma: the upper part consisting of platelet-poor plasma and the lower part consisting of PRP. The platelet-poor plasma was first discarded to avoid its mixing up with the PRP. The tubes were shaken vigorously for 30 secs to suspend platelets. The buffy coat layer, consisting of platelets, was then gently aspirated into a syringe in a volume of 3 ml of plasma and used for the intra-articular injection within 30 mins. An aliquot of product was sent to the laboratory for analysis of platelet concentration (Sysmex Automated Hematology Analyzer) and was quality tested (microbiologic control). All procedures were performed in the same office setting. All open procedures were performed in a class 10000 high-efficiency particulate air-filtered laminar flow hood.

Patients and Study Design

One hundred twenty patients with OA of the knee joint were enrolled in a prospective cohort study. The patients were 63 men and 57 women with a mean age of 53 yrs (range, 19–77 yrs). The mean body mass index was 27.9 ± 4.1 (range, 20.8–34.5) kg/m^2 in the PRP group and 28.3 ± 4.0 (range, 22.2–36.9) kg/m^2 in the HA group. This clinical trial was approved in accordance with the ethical standards of our hospital committee on human experimentation, and the informed consent of all patients was obtained before the treatment. The procedure was in accordance with the ethical standards of our hospital committee on human experimentation.

The following inclusion criteria for patient selection were used: the history of chronic pain of the knee lasting at least 12 mos and the radiologic signs of knee OA Grade 1, 2, and 3 according to Kellgren and Lawrence classification.[11] All patients had previously been treated conservatively using analgesics and nonsteroidal anti-inflammatory drugs without success for at least 6 mos. The exclusion criteria were thrombocytopenia (platelet count, $<100 \times 10^9$/liter), anemia (hemoglobin, <10 g/dl), systemic disease, hematologic disease, history of tumor or active tumor or hematologic malignant disease, severe cardiovascular disease, infection, immunosuppressive status, active anticoagulant therapy, and application of intra-articular depot glucocorticoid injection or HA within 3 mos before application of tested substance. Using anti-inflammatory drugs was not permitted from 5 days before the beginning of treatment to 7 days after the last treatment dose of PRP or HA.

Before the treatment, all patients underwent basic laboratory and hematologic screening and were evaluated with non–weight-bearing x-rays and magnetic resonance imaging of the affected knee.

Grade 1 in 2 cases, Grade 2 in 39 cases, and Grade 3 in 19 cases, according to Kellgren and Lawrence radiographic classification of OA, were determined in the PRP group. In the control group, Grade 1 OA was determined in 2 patients; Grade 2, in 37 patients; and Grade 3, in 21 patients. Grade 4 OA did not occurred in either group.

Treatment Procedure

Patients were randomly divided into two groups. The first group of 60 patients was treated using intra-articular application of autologous PRP (PRP group). The 60 patients of the control group (HA group) were treated with HA (Erectus 1.2%; CSC Pharmaceuticals Handels GmbH), which represents an increasingly popular form of treatment of knee OA based on the physiologic importance of hyaluronate in synovial joints. The medium molecular weight of Erectus has been described as the best for interaction with and activation of the receptor CD44, which confers to Erectus visco-inductive properties that may not occur with products of different molecular weights. Intra-articular injection of HA is used as standard therapeutic procedure for patients with OA in our outpatient clinics. Injections in both groups were administered three times in a weekly interval. In

the case of HA, the recommended application is a cycle of three intra-articular injections in weekly intervals. We decided to use the same conditions for the application of PRP injections in our clinics. It is well known that under normal conditions, the mean lifetime of platelets is about 8–10 days. Platelets begin secreting their growth factors immediately after application. Within 10 mins, they secrete 70% of their stored growth factors and close to 100% within the first hour. They then synthesize additional amounts of growth factors for about 8 days until they are depleted and die. Application of injections was performed after disinfection of skin through the lateral approach to the knee joint. No activities were prohibited. In the case of worsening of knee pain, the use of paracetamol (acetaminophen) was recommended up to maximum daily dose of 4 g. Our patients did not participate in physical therapy after the injections to exclude the synergistic effect of therapy, and there was no postprocedure exercise or range of motion routine.

Outcome Evaluation

The clinical outcome was measured at baseline and 3 and 6 mos after the last treatment dose using the Western Ontario and McMaster Universities Osteoarthritis Index and the 11-point pain intensity Numeric Rating Scale. All complications and adverse events were documented.

Statistical Analysis

The results were expressed as mean ± SD. A P value of 0.05 or less was considered statistically significant. A paired t test and Tukey multiple comparisons test were used to test for significant differences between the baseline band and various follow-up measurements.

RESULTS

The total time needed to prepare the platelet concentrate was approximately 60 mins. Complete blood count analysis was performed on whole blood and platelet-rich plasma samples from each study participant. The number of platelets in PRP increased with respect to the number of platelets in the whole blood sample. The platelet count in the whole blood had a mean value of $150 \pm 30 \times 10^6$ platelets/ml, and platelet count in PRP had a mean value of $680 \pm 132 \times 10^6$ platelets/ml. The mean platelet density increased by 450% in average when compared with the whole blood.

Additional blood parameters measured by the complete blood count illustrated that the centrifugation procedure increased the white blood cell concentration 3.6-fold ($6.4 \pm 2.3 \times 10^3/\mu l$ to $23.2 \pm 7.6 \times 10^3/\mu l$) and decreased the red blood cell concentration by 40% ($3.8 \pm 0.6 \times 10^6/\mu l$ to $1.4 \pm 0.9 \times 10^6/\mu l$).

There were no significant differences between both groups of patients according to age, sex, grade of OA, a mean score in the Western Ontario and McMaster Universities Osteoarthritis Index and the 11-point pain intensity Numeric Rating Scale (Table 2).

In the PRP group, we found improvement in the mean score of the Western Ontario and McMaster Universities Osteoarthritis Index, from 38.76 ± 16.50 to 14.35 ± 14.18 points at 3-mo follow-up and to 18.85 ± 14.09 points at 6-mo follow-up. The mean score of the 11-point pain intensity Numeric Rating Scale was 5.27 ± 1.87 at baseline, 2.06 ± 2.02 at 3-mo follow-up, and 2.69 ± 1.86 at 6-mos follow-up. The statistically significant improvement was achieved in both clinical evaluation schemes at the 3- and 6-mo follow-up periods with respect to baseline.

An improvement in the mean score of Western Ontario and McMaster Universities Osteoarthritis Index from 43.21 ± 13.70 to 26.17 ± 17.47 points at 3-mo follow-up and to 30.90 ± 16.57 points at 6-mo follow up was obtained in the HA group. The mean score of the 11-point pain intensity Numeric Rating Scale was 6.02 ±1.77 at baseline, 3.98 ± 2.27 points at 3-mo follow-up, and 4.3 ± 2.07 at 6-mo follow-up. The statistically significant improvement was achieved in both clinical evaluation schemes at the 3- and 6-mo follow-up periods with respect to the basal level.

The results remained stable from the end of thetherapy to the 6-mo follow-up, but they became nonsignificantly worse at the end of the 6-mo follow-up with respect to the 3-mo follow-up. Comparison of the results in the PRP and HA groups showed

TABLE 2 Group characteristics at the baseline

	PRP Group	HA Group
Mean age	52.80 ± 12.43	53.20 ± 14.53
Male/female ratio	33:27	31:29
Grade of OA (according to Kellgren and Lawrence Radiographic Grading Scale[10])		
1	2	2
2	39	37
3	19	21
4	0	0
WOMAC	38.76 ± 16.50	43.21 ± 13.70
NRS	5.27 ± 1.87	6.02 ± 1.77

Values are mean ± SD.

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; NRS, Numeric Rating Scale.

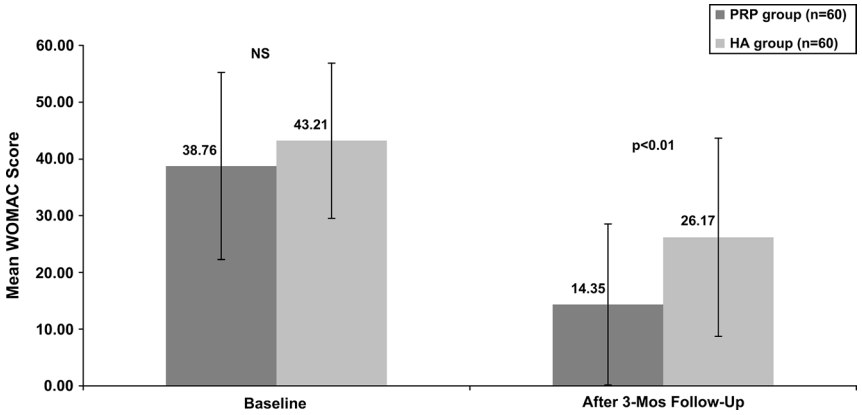

FIGURE 1 *A mean value of the WOMAC Osteoarthritis Index at baseline and at the 3- and 6-mo follow-up in the PRP and HA groups. P < 0.01 between groups. WOMAC, Western Ontario and McMaster Universities; NS, nonsignificant; HA, hyaluronic acid; PRP, platelet-rich plasma.*

statistically significantly better results in the PRP group in each of the follow-up periods in both clinical schemes (Figs. 1 and 2).

No major adverse events or complications were observed in both groups. We documented temporary mild worsening of pain in the knee joint after application of PRP in six cases, which was spontaneously resolved after 2 days.

DISCUSSION

Improved centrifugation techniques have led to the ability to concentrate platelets as PRP, with the goal of delivering these concentrates as sources of growth factors to accelerate and support the healing of hard and soft tissue injuries naturally without subjecting the patient to significant risk.

One potential advantage of platelet-rich preparations is that they are easily obtained from the patient's blood after a simple centrifugation process. There is a wide range of variation in the intensity and duration of centrifugation among research studies. For that reason, the lack of a suitable optimization and standardization of PRP preparation has provoked the appearance of many different platelet-rich products with controversial therapeutic effects.[6]

Our centrifugation procedure for preparing PRP increased the white blood cell concentration by 3.6-fold. Some authors even recommend, without any scientific evidence, the elimination of leucocytes.[12] However, several studies have already pointed out the key role of leucocytes in PRP for their anti-infectious action, immune regulation, and promotion of angiogenesis.[13] The leucocyte content did not seem to induce negative effects or to impair the potentially beneficial effects of PRP, even when used in joints.[14] However, we cannot conclusively claim that increased white blood cells in PRP have positive effect on knee joint, because we have no comparative data at this time.

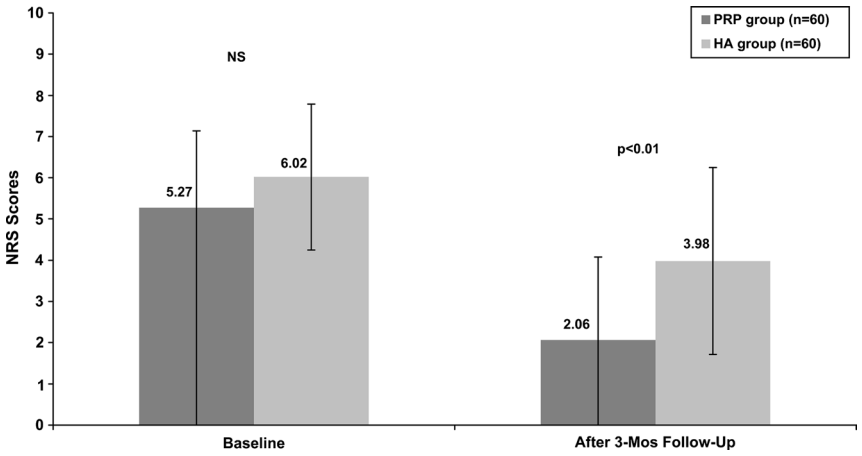

FIGURE 2 *A mean value of NRS at baseline during the 3- and 6-mo follow-up in the PRP and HA groups. P < 0.01 between groups. NS, nonsignificant; NRS, 11-point pain intensity Numeric Rating Scale; PRP, platelet-rich plasma.*

Studies have shown that clinical efficacy can be expected with a minimum increase in platelet concentration of 4- to 6-fold from whole blood baseline (1 million platelets/μl).[15,16] We developed a reliable and low-cost manual protocol for preparing autologous PRP, in which we achieved average an platelet concentration 4.5-fold increased over baseline platelet count.

Considering all the positive effects described for PRP in various clinical applications, we aimed to use nonactivated PRP according to Mishra and Pavelko.[17] They used nonactivated PRP for the treatment of chronic elbow tendinosis with significant improvement. Recently published study demonstrated the positive effect of nonactivated PRP on mesenchymal stem cells proliferation in vitro.[18] Treatment with 10% inactivated PRP results in a 5-fold increase in proliferation after 7 days. In another study, it has been shown that thrombin activation of PRP inhibited chondrogenesis and osteogenesis when compared with PRP alone. Nonactivated PRP resulted in increased formation of bone and cartilage in vitro and in vivo.[19] Calcium chloride was not used in the activation protocol to prevent increased osteogenic differentiation.[20] In future investigations, the pH of the PRP and its activation status need to be reported so that comparison across studies and therapy modalities may be possible.

PRP was first used in cardiac surgery by Ferrari et al.[21] in 1987 as an autologous transfusion component after open-heart surgery to reduce blood loss. PRP is now being widely tested in different fields of medicine, including orthopedics, sports medicine, dentistry, dermatology, and ophthalmology, as well as in plastic, maxillofacial, and cosmetic surgery.[8,9,22]

To our knowledge, there are limited studies on the clinical application of PRP via multiple injections to favor knee OA healing. In a recent study of Sampson et al.[23] including 14 patients with primary and secondary knee OA, treatment with PRP injections significantly improved function and reduced pain. Most of the patients expressed a favorable outcome at 12 mos after treatment. The study by Sanchez et al.[24] presented the preliminary results of the effectiveness of intra-articular injections of PRP in retrospective cohort study of 30 patients treated with PRP and 30 patients treated with HA. They suggested that knee OA treated with the application of PRP could present new and safe possibilities for enhanced outcomes. Kon et al.[25] published the results of prospective study of 100 patients (115 treated knees) affected by chronic degenerative condition of the knee, who were treated with three PRP intra-articular injections. These preliminary results showed that treatment with PRP injections is safe and has the potential to reduce pain and improve function and quality-of-life in patients with a low degree of articular degeneration. Giannini et al.[26] published similar results in their study of 46 patients. The conclusion of this study was that intra-articular injections of PRP showed a reduction of pain and recover function of the knee joint in patients affected by severe chondropathies of the knee.

PRP is prepared from autologous blood, so any concerns of allergic reactions or disease transfer are eliminated. PRP does not promote hyperplasia, carcinogenesis, or tumor growth.[27] There is possibility of temporary worsening symptoms after application of PRP. We documented mild worsening of knee pain after injections of PRP in six cases. The pain disappeared spontaneously in all cases inside of 2 days.

The clinical results of our pilot study are encouraging and suggest that this method may be successfully used for the treatment of initial stages of knee OA. Our clinical study is one of the first in vivo studies in our clinic in human investigation of the use of autologous growth factors to treat knee OA through PRP injections, but it has some limitations, including the lack of placebo control and shorter follow-up period. Despite these limitations, we demonstrate here that this is a potentially safe, simple, and low-cost method to improve articular joint healing, with promising results. Further study of PRP in combination with visco-supplementation should also be performed in the future to determine whether there is a synergistic effect. We are preparing a placebo-controlled prospective multicenter trial, which should help evaluate PRP as an effective treatment of knee OA before wide clinical application. Successful clinical trials will open new perspectives on autologous treatments for joint diseases.

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