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The effect of intra-articular platelet-rich plasma, bio-physically activated PRP and mesenchymal stem cell administration for interleukins in dogs with osteoarthritis

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ABSTRACT

The aim of this study was to determine the levels of cytokines (TNF-α, PGE_2, IL-1β, IL-6, IL-10) in synovial fluid samples from joints affected by osteoarthritis (OA), and to assess the efficacy of a single intraarticular (IA) injection of an autologous platelet rich plasma (PRP), mesenchymal stem cells (MSCs), bio-physically activated platelet rich plasma (B-PRP) and their combinations for treatment of OA in dogs. Thirty-six different breeds, gender, age and weight dogs affected by OA were used as materials. The groups were divided into: PRP, MSCs, PRP + MSCs, B-PRP, B-PRP + MSCs and SC (Saline Control). Only one dose was injected. The primary inclusion criterion for the dogs was that they had no systemic disease. The Genesis Cell System was used for preparation of autologous PRP, and the platelets were counted before injection. The Genesis Autologous Cell System 2 (30ml) was used as the bio-physical activator in preparation of the PRP. The bio-PRP physical activator device was mounted on one end and the other end of the activator was left empty. Biovalda Health Technology Inc. was used for producing allogeneic adipose stem cells. Cytokines (TNF-α, PGE_2, IL-1β, IL-6, IL-10) in terms of quantities were measured using enzyme-linked immunosorbent assay (ELISA) from the synovial fluid samples before treatment and on the 0, 15^{th}, 30^{th}, 60^{th} and 90^{th} days of treatment. In all cases, clinic and radiographic examinations were performed on the 0, 15^{th}, 30^{th}, 60^{th} and 90^{th} days. The results obtained from the PRP + MSCs combination group were more successful compared to the other groups. It was noted that successful results could be obtained with PRP alone or in combination with stem cells, especially if repeated intra-articular injections are required. Also future studies are needed to understand the effectiveness of B-PRP. Only the B-PRP and MSCs combination were effective on many enzymes, but varying results were obtained in all cases.

Key words: allogeneic adipose stem cells; cytokines; growth factor; pain score

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Introduction

Osteoarthritis (OA, degenerative joint disease) is a common problem in dogs, particularly in old and large breeds. Although there is no cure for this progressive condition, identifying the problem in the early stages and initiating appropriate management can help to keep the dog active and improve its quality of life (TAMURA et al., 2002; SCHULZ, 2007; ARICAN, 2014). By most estimates, 20% of all dogs are affected by OA, making it the most common chronic disease in dogs (ROUSH et al., 2002).

In addition to the biomechanical and age-related alterations in chondrocyte function, inflammation and the accompanying dysregulated cytokine activities probably contribute to the disruption of the balance between anabolism and catabolism of cartilage (BRENN et al., 2007). Thus, chondrocyte division, matrix synthesis and destruction may decrease or increase. A single cytokine can initiate or terminate the action of another cytokine. The cytokines TNF-α, IL-1β, IL-6 and PGE_2 have an important role in both joint inflammation and cartilage destruction. In addition, IL-10 (anabolic cytokine) has a role in suppression of IL-1β, and TNF-α synthesis (AOKI et al., 2004; SOMMER and KRESS, 2004; BENITO, 2005; BRENN et al., 2007).

Recent OA treatments are intended not only to treat joint pain and inflammation, but also increase the anabolic activity of chondrocytes and improve tissue repair by decreasing tissue degeneration. Intraarticular (IA) injections of platelet rich plasma (PRP), bio-physically activated PRP (B-PRP) and mesenchymal stem cells (MSCs) are thought to have the potential to slow down the progression of OA by stimulating cartilage anabolism with growth factors (STIEF et al., 2011; KNOP et al., 2016; ARICAN et al., 2019; PARLAK and ARICAN, 2020).

The aim of this study was to determine the levels of catabolic cytokines (IL-1β, IL-6, TNF alfa, PGE_2) and anabolic cytokines (IL-10) in synovial fluid samples from joints affected by OA, and to assess the efficacy of a single IA injection of MSCs, autologous PRP, B-PRP, and their combinations for treatment of OA in dogs.

Materials and methods

Study Design. Thirty-six dogs (weight range: 25 to 50 kg, mean age: 8.6 years) with a unilateral stifle joint affected by OA were evaluated in this study. The study design was approved by the institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine and followed the published guidelines. Thirty-six dogs were randomly divided into 6 groups: Group I (PRP), Group II (MSCs), Group III (PRP + MSCs), Group IV (B-PRP), Group V (B-PRP + MSCs) and Group VI (SC) (0.9% isotonic saline). The joint fluid analysis, and clinical and radiographic examinations were performed in each group on days 0, 15, 30, 60 and 90.

Selection Criteria. The primary inclusion criterion for the dogs was that they had no systemic disease. Dogs that had undergone any kind of surgical procedure in the previous six months, any intra-articular injection in the previous three months, or had received any parenteral steroidal anti-inflammatory drugs and nutritional supplements in the previous month were excluded from the study.

Clinical Examination. For the routine clinical examination of the dogs (0, 15^{th}, 30^{th}, 60^{th} and 90^{th} days), tibial compression and cranial drawer sign tests were performed on the affected joint after sitting, walking, climbing a ladder, going down a ladder, and running and jumping activities. Pain rating tests (Canine Brief Pain Inventory (CBPI)) and walking rating tests (Hudson Visual Analog Scale (HVAS)) were performed (HUDSON et al., 2004; BROWN et al., 2008). Repeated walking and pain rating tests were performed and evaluated by the same veterinarian.

Radiographic Examination. Affected joints were examined radiographically (Regius 110; Konica Minolta, Tokyo, Japan) on days 0, 15, 30, 60 and 90. Radiographic examinations (standing latero-medial (LM), cranio-caudal (CrCa), tibial compression LM) of the dogs in the study were performed on the specified days. Repeated radiographic examinations were performed and evaluated by the same veterinarian. Radiographic interpretation was evaluated according to Kellegren-Lawrence scoring; Grade 0. (no radiographic findings),

Grade 1 (narrowing of the joint cavity, possible osteophytic proliferation), Grade 2 (significantly osteophytic proliferation, severe narrowing of the joint cavity), Grade 3 (severe narrowing in the joint cavity, sclerosis, severe osteophytic proliferation, deformity in the subchondral bone) and Grade 4 (severe sclerosis, wide osteophytic proliferation, marked subchondral deformity) (KELLGREN and LAWRENCE, 1957).

Force Plate Analysis. A pressure analyzer (PetSafe Stance Analyzer, Kruuse, Germany) was used to measure the weight distribution in the dogs' extremities. This is based on the principle of proportionally calculating the body weight falling at four different measurement points and transferring the data to the computer. In practice, the analyzer was prepared by weight calibration before the examination. Subsequently, the dogs were placed on the analyzer one by one and held fixed with one extremity in each square compartment. Meanwhile, by taking weight measurements (at least 15 times) from an auxiliary analyzer control, the average pressures were recorded automatically.

Preparation of Platelet-Rich Plasma. Genesis Autologous Cell System 2 (Neo Genesis, Seoul, Republic of Korea) PRP (30 ml) preparation kits were used in the study to obtain the standardised platelet counts at the desired level. The acid citrate dextrose (ACD) (3 ml) from the kit was added to a 50 ml injector in order to prevent blood clotting, and 27 ml of the blood was collected from the jugular vein. A 30 ml mixture of the blood and ACD was injected into the Genesis tube. The tubes were centrifuged in a Genesis centrifuge instrument at 1700 × g for 5 minutes. The anticoagulated blood revealed three layers after centrifugation: a bottom layer (red blood cells, density = 1.09); middle layer (platelets and white blood cells (buffy coat), density = 1.06); top layer (plasma, density = 1.03). The bottom lid of the tube was replaced with a buffy coat controller cap and the pusher was assembled. The buffy coat controller was turned counter-clockwise until the layer of the red blood cells reached the "0" line. The platelet poor plasma portion was removed, the pusher was turned until the layer of red blood cells reached the top, and the PRP portion (3–5 ml) was pushed into a Luer lock injector. The average platelet counts in injected PRP for each dog ranged between 1.000.000-1.200.000/μL. The injection was performed immediately.

Preparation of Bio-Physically Activated Platelet-Rich Plasma. The injector with the prepared PRP was assembled at one end of the Biophysical activator instrument and an empty Luer lock injector was assembled at the other end of the instrument. The activation process involved injecting the platelets from one injector into the other injector through the activator 30 times. The injection was performed immediately. The platelet concentrate (PRP and B-PRP) was injected into the joint until sufficient resistance was obtained to push the syringe plunger back.

Allogeneic Adipose Mesenchymal Stem Cell (MSCs) Isolation and Expansion. The allogeneic adipose stem cells preparation was produced by Biovalda Ltd. MSCs were isolated from the allogeneic origin of the adipose tissue and reproduced. Adipose tissue donors were the dogs (n=3) included in the study. Approximately 1,000,000-1,300,000 cells were injected intraarticularly.

Sampling of Joint Fluid. The joint fluids taken from each dog in the study groups on the specified days were centrifuged at 2000 rpm for 5 minutes and stored at -80°C. The joint fluids were dissolved shortly before examination. Some cytokines that act as inflammatory mediators (IL-1β, IL-6, IL-10, TNF-α, PGE_2) were measured by ELISA.

Measurement of Inflammatory Mediators by Enzyme-Linked Immunosorbent Assay (ELISA). A Canine TNF-α ELISA Kit (No: 201-15-0019, sensitivity; 0.028 pg/ml, measuring range; 0.03-9 pg/ml), a Canine PGE_2 ELISA Kit (No: 201-15-1983, sensitivity; 2.857 pg/ml, measuring range; 3-900 pg/ml), a Canine IL-1β ELISA Kit (No:201-15-0171, sensitivity; 0.223 pg/ml, measuring range; 0.3-70 pg/ml), a Canine IL-6 ELISA Kit (No:201-15-0128, sensitivity; 0.047 pg/ml, measuring range; 0.05-15 pg/ml) and a Canine IL-10 ELISA Kit (No:201-15-0125, sensitivity; 0.423 ng/L, measuring range; 0.5–150 ng/L) from Sunred Biological Technology Co., Ltd. (Shanghai, China) were used. Canine commercial kits were used, based on the dual antibody sandwich ELISA

principle. ELISA kits were analyzed in an ELISA reader device (MWGt Lambda Scan 200, Bio-Tek Instruments, Winooski, VT, USA).

Statistical Analysis. The data obtained from the HVAS and CBPI tests were subjected to analysis using SPSS 20.0 (IBM, USA). The Mann Whitney U test was used to evaluate the data. The results of the joint fluid ELISA tests run on the six groups were evaluated by Tukey's test, comparing the single and double administration groups, and also comparing the results within the single and double administration groups with themselves.

Results

Clinical Examination. In the study, there were no infections due to IA injections. All components of the HVAS (mood, attitude, comfort, activity, playfulness, exercise, walking comfort) and CBPI (pain, general activity, the ability to enjoy life, rise, walk, run and climb) were significantly different between pre-treatment and the 30th ($P<0.05$), 60th ($P<0.05$), and 90th days of the treatment ($P< 0.05$) in the PRP, B-PRP and MSCs groups. Improvements in treatment outcomes were noted in almost all groups.

Radiographic Examination Findings. Second grade OA was determined in 30 cases, third grade in 4 cases and fourth grade in 2 cases. There were no differences in radiographic scores on the 0, 15th, 30th, 60th and 90th days according to the Kellegren-Lavrence score.

Force Plate Analysis Findings. In the study, weight distribution in the extremities was evaluated by compression analysis and recorded. Results from pre-treatment and the 15th, 30th, 60th and 90th days of treatment were compared individually. The affected extremities of the 36 dogs were easy to distinguish from the other limbs as their compression force was deficient due to inadequate weight distribution. There were no differences between the groups of PRP, B-PRP and MSCs as a result of the treatments.

ELISA Analysis of Results of Cytokines in Joint Fluid Samples.

TNF-α. The effect of single and combined administration of PRP, B-PRP and MSCs on TNF-α levels in the joint fluid of dogs affected by OA is presented in Figure 1. The TNF-α level was compared with day 0 in the in-group evaluation. While it increased significantly ($P<0.05$) in the SC group on the 60th and 90th days, and on the 30th day in the MSCs and B-PRP+MSCs groups, it decreased significantly on the 90th day in the MSCs-PRP group ($P<0.05$).

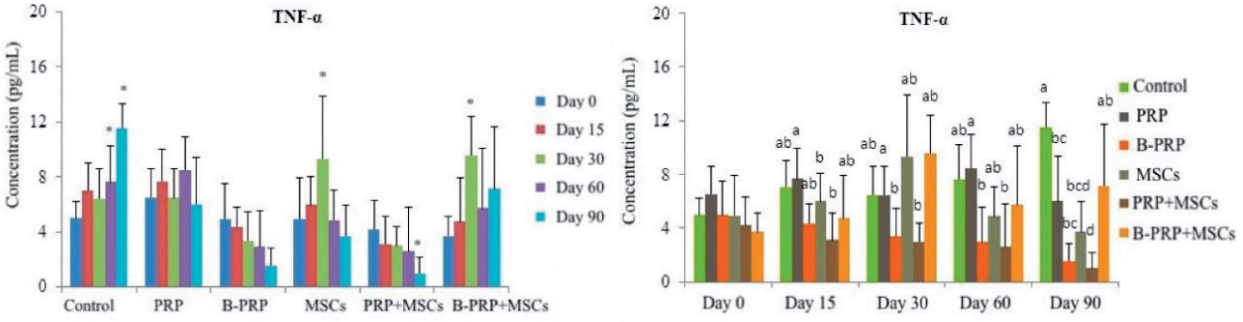

Fig. 1. The effect of single and combined administration of PRP, B-PRP and MSCs on joint fluid TNF-α (pg/mL) levels in dogs affected by OA. (Left (*); In-group, Right (a,b,c,d); between groups)

IL-1β. The effect of single and combined administration of PRP, B-PRP and MSCs on the IL-1β levels in the joint fluid of dogs affected by OA is presented in Figure 2. There was no significant difference between groups in the statistical evaluation at IL-1β level. ($P>0.05$) In the in-group statistical analysis, a significant increase was found on the 60th and 90th days in the SC group, and on the 15th day in the PRP group ($P<0.05$), and a significant decrease on the 15th and 90th days in the PRP+MSCs group compared to day 0 ($P<0.05$).

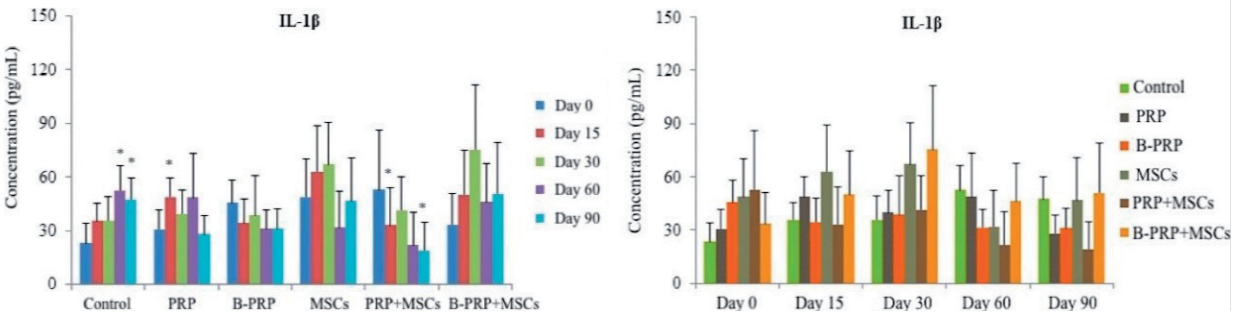

Fig. 2. The effect of single and combined administration of PRP, B-PRP and MSCs on joint fluid IL-1β (pg/mL) levels in dogs affected by OA. (Left (*); In-group)

IL-6. The effect of single and combined administration of PRP, B-PRP and MSCs on IL-6 levels in the joint fluid of dogs affected by OA is presented in Figure 3. In the in-group evaluation, IL-6 levels increased significantly ($P<0.05$) in the SC group on the 90th day, and on the 15th and 30th days in the B-PRP+MSCs group compared to day 0 ($P<0.05$), it was lower on the 60th day after MSCs administration ($P<0.05$). In the statistical evaluation of the groups, it was found that the application of PRP, B-PRP, MSCs and PRP+MSCs on the 90th day significantly decreased the IL-6 level ($P<0.05$) compared to the SC group, while no significant difference was found between the groups on the other days ($P>0.05$).

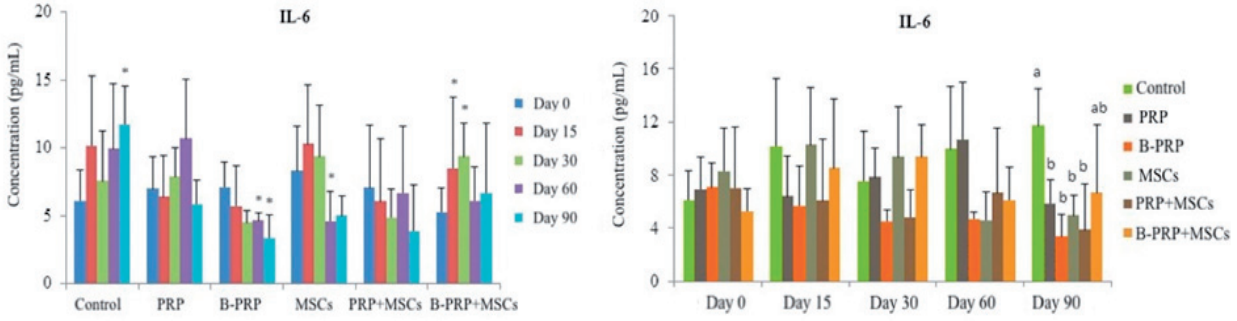

Fig. 3. The effect of single and combined administration of PRP, B-PRP and MSCs on joint fluid IL-6 (pg / mL) levels in dogs affected by OA. (Left (*); In-group, Right (a,b,c,d); between groups)

IL-10. The effect of single and combined administration of PRP, B-PRP, and MSC$_s$ on IL-10 levels in the joint fluid of dogs affected by OA is presented in Figure 4. While IL-10 level, which is an anti-inflammatory cytokine, increased significantly on the 60th and 90th days in the SC group, on the 15th and 30th days in the MSCs group, and on the 15th and 30th days in the B-PRP+MSCs group compared to day 0 in the statistical evaluation ($P<0.05$), it had decreased significantly on the 90th day in the B-PRP group ($P<0.05$). When the IL-10 level was compared between the groups, it was observed that B-PRP+MSCs administration increased IL-10 levels significantly on the 30th day compared to the SC, PRP, B-PRP and PRP+MSCs groups, and in the MSCs group compared to the B-PRP group, on the 90th day of PRP. It was determined that PRP, MSCs, PRP+MSCs and B-PRP+MSCs administration significantly decreased IL-10 levels ($P<0.05$).

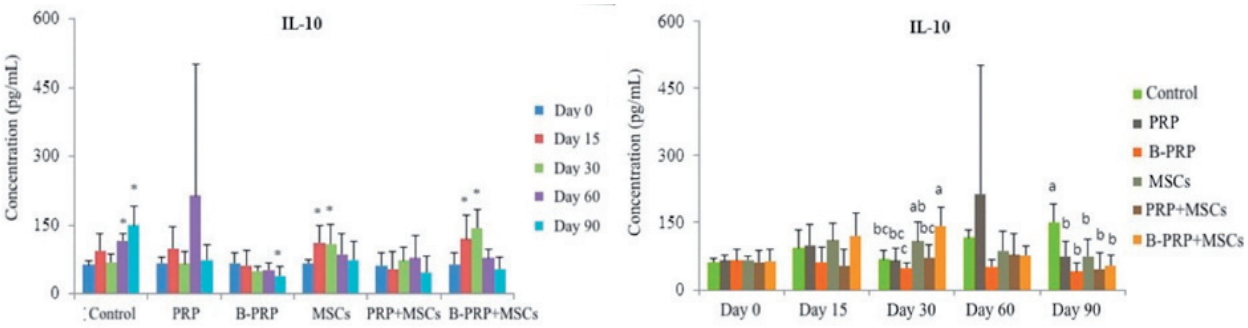

Fig. 4. The effect of single and combined administration of PRP, B-PRP and MSCs on joint fluid IL-10 (pg/mL) levels in dogs affected by OA. (Left (*); In-group, Right (a,b,c,d); between groups).

PGE_2 The effect of single and combined administration of PRP, B-PRP and MSC_s on PGE_2 of the joint fluid levels of dogs affected by OA is presented in Figure 5. There was no statistically significant difference in the PGE_2 level in all groups compared to day 0 ($P>0.05$). PRP+MSCs administration in dogs affected by OA significantly decreased the PGE_2 level on the 15th and 60th days, and with PRP, B-PRP, PRP+MSCs and B-PRP+MSCs administration, on the 90th day compared to the SC group ($P<0.05$).

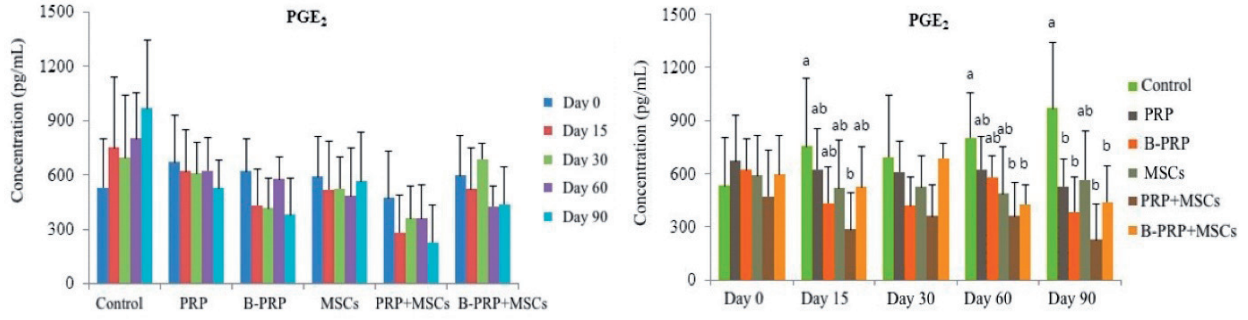

Fig. 5. The effect of single and combined administration of PRP, B-PRP and MSCs on joint fluid PGE_2 (pg/mL) levels in dogs affected by OA. (Left (*); In-group, Right (a,b,c,d); between groups)

Discussion

It is more difficult to associate lameness and pain with OA in dogs than humans. Walking and pain tests have been used in previous studies and are accepted as subjective methods (HUDSON et al., 2004; BROWN et al., 2007, 2008; HJERMSTAD et al., 2011). It was considered acceptable to have the dogs checked by the same physician. When the subjective HVAS and CBPI questionnaires were compared between the 0th and 90th days, positive progress was observed in all groups except the SC group. A compression analyzer is an objective method for determining the degree of lameness. In this examination method, unequal weight distribution was measured in the extremities of dogs with serious orthopedic disorders, such as arthritis, elbow and hip dysplasia, and cruciate ligament ruptures. It has been reported in previous studies that it is possible to evaluate surgical procedures and medical treatment results using treadmills (BUDSBERG et al., 1993; BUDSBERG, 2001). In other studies, statistical improvements were shown at the end of the 12th week in dogs treated

with PRP (FAHIE et al., 2013). The affected extremities of the 36 dogs used in this study were easy to distinguish from other extremities, as their compression force was insufficient due to the inadequacy of weight. As a result of the treatments, no difference was observed between the groups by force plate analysis. According to the results of the paired T test within the group, a statistical ($P<0.05$) difference was found when comparing day 0 (before treatment) and the 15th, 30th, 60th and 90th days of the treatment.

Chondrocytes are the only cellular component in hyaline cartilage, and their anabolic and catabolic activities are in balance. In OA cases, this balance is disrupted and catabolic activity increases (GOLDRING and GOLDRING, 2004). TNF-α and IL-1β are proinflammatory cytokines secreted by many cells, such as chondrocytes, osteoblasts, synovium infiltrated immune system, adipocyte and synoviocyte in the degenerative joint. In the case of OA, the level of TNF-α and IL-1β increases significantly in the synovial fluid (MACCOUX et al., 2007; WOJDASIEWICZ et al., 2014). The increase in TNF-α and IL-1β levels causes the release of mediators such as IL-6, PGE_2 and MMPs that have inflammatory and chondrolytic effects, causing an increase in catabolic activity and further progression of damage (WOJDASIEWICZ et al., 2014). In previous studies, it was stated that MSCs and PRP treatment significantly decreased TNF-α and IL-1β levels in OA (YUN et al., 2016). A possible mechanism of action of PRP in OA is due to the inhibition of Nuclear Factor kappa B (NF-κB), which plays an important role in the release of TNF-α and IL-1β, and increases proteoglycan and type II collagen synthesis, inducing the proliferation of chondrocytes and mesenchymal stem cell differentiation (KWON and PARK, 2012; TEXTOR, 2014). In the present study, TNF-α and IL-1β levels were decreased in the treatment groups compared to the SC group on the 90th day. Among the treatment groups, the lowest value was obtained in the PRP+MSCs group. In other studies conducted with OA in dogs, it was stated that combined PRP+MSCs treatment was more effective than single administration (YUN et al., 2016). This may be due to the stronger effects of combined therapy on cartilage degeneration and regeneration.

IL-6 is one of the proinflammatory cytokines involved in the formation of OA. IL-6 is released from chondrocyte, osteoblast, macrophage, and adipocyte cells in the joint and induced by TNF-α and IL-1β (GOLDRING and GOLDRING, 2004; WOJDASIEWICZ et al., 2014). It has been reported that the level of IL-6 in the joint fluid significantly increases in dogs affected by OA (MACCOUX et al., 2007; WOJDASIEWICZ et al., 2014; MUIR et al., 2016). Together with TNF-α and IL-1β, IL-6 causes an increase in inflammation and cartilage degeneration since it has an inflammatory and chondrolytic effect (GUERNE et al., 1989). In this study, PRP and MSCs administration significantly decreased IL-6 levels compared to the SC group. This decrease in IL-6 levels may be due to the suppression of TNF-α and IL-1β release by PRP and MSCs administration.

IL-10 is an anti-inflammatory cytokine that shows protective activity by stimulating proteoglycan synthesis in the joints and preventing the apoptosis of chondrocytes (GOLDRING and GOLDRING, 2004; WOJDASIEWICZ et al., 2014). It is inhibitory to IL-10, TNF-α and IL-1β. It has been reported that when IL-10 is applied in vitro to snovial samples taken from patients affected by OA, it suppresses TNF-α and IL-1β levels by 60% and 83%, respectively (JANSEN et al., 2008). In addition, IL-10 suppresses inflammatory mediators such as PGE_2 while stimulating the release of growth factors, such as bone morphogenetic proteins (BMPs) and SOX, which regulate chondrogenesis (WOJDASIEWICZ et al., 2014). In this study, IL-10 levels increased in the SC group. Similarly, it was found in the other studies that the IL-10 level increased in patients affected by OA (MACCOUX et al., 2007). Although it has been said that MSCs and PRP treatment increases IL-10 level in patients affected OA, the failure to achieve a significant increase in the treatment groups in this study may be due to the stage or severity of the disease, or the age of the dogs, since the study was conducted in patients with natural OA. The reduction of TNF-α and IL-1β secretion by MSCs and PRP administration may eliminate the stimulation of

IL-10 secretion by eliminating the retrostimulating effect.

In dogs affected by OA, there is an increase in PGE_2 level, which is induced by proinflammatory cytokines from chondrocytes, and has an important role in inflammation and pain (ROBINSON et al., 2016). PGE_2 as a proinflammatory mediator, causes further progression of the inflammatory process and increases joint damage. In addition, PGE_2 increases nociceptor sensitivity, leading to hypersensitivity and therefore pain (ULMANN et al., 2010). In the present study, it was determined that PRP+MSCs, B-PRP, B-PRP+MSCs and PRP administration significantly decreased PGE_2 levels compared to the SC group. It was also reported in previous studies that MSCs and PRP administration decreased the PGE_2 levels in patients affected by OA (FORTIER and TRAVIS, 2011; KHATAB et al., 2018). The decrease in PGE_2 levels with PRP and MSCs administration may be due to suppression of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6.

In the present study, variable results were obtained for TNF- α, IL-1β, IL-6, IL-10 and PGE_2 levels in the PRP, B-PRP, MSCs, B-PRP+MSC, and PRP+MSCs groups, especially in the PRP+MSCs group. TNF-α, IL-1β, IL-6 and PGE_2 levels decreased, but the amount of IL-10 remained constant. IL-10 levels increased in the PRP and MSCs groups, but their combination did not show this effect. Generally, the PRP+MSCs group was found to be the most effective in inflammatory activity among the groups.

In conclusion, it was revealed that PRP+MSCs combination results were more effective in all parameters, although positive progress was achieved in all groups as seen in the evaluation of the clinical scoring, radiological and pressure analysis and cytokine measurement results. We believe that in cases where reversible conservative methods are insufficient in the treatment of the OA, cheap, practical and repeatable PRP-MSCs combinations, as an effective, minimally invasive and restorative treatment method, can reduce the need for invasive treatment methods and surgery.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Study design, survey development, data analyses and manuscript preparation performed by all authors. Mustafa Arıcan and Kurtuluş Parlak: conception and design of the study, acquisition, analysis and interpretation of data, drafting and critical revision of the article; Kamil Üney: analysis of the data and critical revision of the article. Elgin Orçum Uzunlu and Mustafa Yalçın: acquisition of the data. The authors report no financial or other conflicts related to this report.

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PARLAK, K., K. ÜNEY, E. O. UZUNLU, M. YALÇIN, M. ARICAN: Učinak intraartikularne plazme obogaćene trombocitima, biofizički aktivirane autologne plazme obogaćene trombocitima (PRP-a) i primjene mezenhimnih matičnih stanica na interleukine u pasa s osteoartritisom. Vet. arhiv 92, 459-468, 2022.

SAŽETAK

Cilj istraživanja bio je odrediti razine citokina TNF-α, PGE_2, IL-1β, IL-6 i IL-10 u uzorcima sinovijske tekućine iz zglobova zahvaćenih osteoartritisom (OA) te procijeniti učinkovitost pojedinačne intraartikularne injekcije (IA) (PRP), mezenhimnih matičnih stanica (MSC), biofizički aktivirane plazme obogaćene trombocitima (B-PRP) i njihovih kombinacija u liječenju osteoartritisa u pasa. U istraživanju je upotrijebljeno 36 pasa s osteoartritisom, različitih pasmina, spola, dobi i tjelesne mase. Oni su podijeljeni u skupine PRP, MSCs, PRP + MSCs, B-PRP, B-PRP + MSCs i SC (Saline Control). Injicirana je samo jedna doza. Primarni je kriterij za uključivanje pasa bio da nisu imali sistemsku bolest. Autologni stanični sustav Genesis 2 (30 mL) upotrijebljen je kao biofizički aktivator u pripremi PRP-a. Uređaj za biofizičko aktiviranje PRP-a montiran je na jednom kraju, a drugi je kraj aktivatora ostao prazan. Alogene adipozne matične stanice proizvedene su prema uputama a Biovalda Health Technology Inc. Količina citokina TNF-α, PGE2, IL-1β, IL-6 i IL-10 dobivena je testom ELISA iz uzoraka sinovijalne tekućine prije liječenja te 0., 15., 30., 60. i 90. dan liječenja. U svim su slučajevima klinički i radiološki pregledi obavljeni 0., 15., 30., 60. i 90. dan. Rezultati dobiveni u skupini PRP + MSC pokazali su veću učinkovitost u usporedbi s drugim skupinama. Zapaženo je da se dobri rezultati mogu postići samo uz PRP ili u kombinaciji PRP-a i matičnih stanica, posebno u slučaju ponovljene intraartikularne injekcije. Potrebna su i daljnja istraživanja kako bi se objasnila učinkovitost B-PRP-a. Vezano za enzime, samo je kombinacija B-PRP-a i MSC-a bila učinkovita, ali s različitim rezultatima.

Ključne riječi: alogene adipozne matične stanice; citokini; čimbenik rasta; procjena boli