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Treatment of canine osteoarthritis with allogeneic platelet-rich plasma: review of five cases

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Abstract

Background: Osteoarthritis (OA) is a major cause of chronic pain and lameness in dogs. Platelet-rich plasma (PRP) is a concentrate of growth and differentiation factors from the blood, which can be used in regenerative medicine strategies.

Aim: The main aim of this study was to evaluate the effect of allogeneic PRP on the treatment of canine OA.

Methods: Five dogs from several breeds, between 6 and 12 years old, and from both genders were studied. Clinical and imageological examinations diagnosed OA in the knee, tibiotarsal, elbow, and intercarpal joints. These dogs were refractory to medical therapy and to physical rehabilitation protocols that included shockwave therapy, laser therapy, electrostimulation, hydrotherapy, and diathermy.

Animals were treated with allogeneic PRP obtained from the blood of the five dogs, which was processed in a pool. Echoguided intra-articular PRP injection was administered under sedation and after aseptic field preparation. Lameness at walk and trot (five grades) and pain (five scores) were evaluated before treatment and 30, 60, and 90 days post-treatment.

Results: All animals presented improvements at 30 and 60 days in both parameters. Four dogs showed a decrease of three grades of lameness after 90 days and there was complete absence of lameness in 2 days. Pain was reduced from severe and moderate to mild in all the dogs after 30 days, and among them, three revealed no pain after 90 days.

Conclusion: This study sheds light on the applicability and safety of a single administration of allogeneic PRP in osteoarthritic dogs.

Keywords: Dog, Lameness, Osteoarthritis, Pain, Platelet-rich plasma..

Introduction

Osteoarthritis (OA) is a common chronic degenerative disease of synovial joints in both human and veterinary patients. The inflammatory process causes slow and progressive destruction of the subchondral bone, formation of osteophytes, thickening of the joint capsule, and synovitis (Malek *et al.*, 2012; Glyn-Jones *et al.*, 2015). Ultimately, this condition leads to significant pain and associated lameness in companion animals, including dogs (Brown *et al.*, 2007; Schaible, 2012).

At the present moment, there is no effective therapeutic protocol, either medical or surgical, that allows clinical resolution of OA (Bland, 2015). Therefore, the main focus includes pain control by using nonsteroidal anti-inflammatory drugs, opioid analgesics, as well as gabapentin or amantadine for chronic pain (Malek *et al.*, 2012; KuKanich, 2013). Acupuncture is also referred to have a positive effect in pain management in veterinary patients as a part of a multimodal plan (Fry *et al.*, 2014). This approach includes physical rehabilitation for the improvement of joint function and hindrance of the joint's degenerative processes (Alvarez *et al.*, 2016). Weight reduction is referred to have a positive effect on the control of clinical signs of lameness in dogs with hip OA (Impellizeri *et al.*, 2000). Dietary oral supplementation with chondroitin/glucosamine sulfate (McCarthy *et al.*, 2007), omega-3 fish oil fatty acids (Fritsch *et al.*, 2010), and beta-1,3/1,6-glucans (Beynen and Legerstee, 2010) are examples of adjuvant strategies to reduce clinical signs of OA and to prevent progression of the degenerative process. Other options include intraarticular injections of hyaluronan (Brandt *et al.*, 2004) and intramuscular administration of polysulfated glycosaminoglycan (Fujiki *et al.*, 2007).

Regenerative Medicine has seen a rapid evolution during recent years with new therapies based on the application of innovative biomaterials, adult stem cells,

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and different sources of growth and differentiation factors (GDF). One of the first-known regenerative approaches proposed in clinical practice was platelet-rich plasma (PRP) (De Coppi, 2012; Guercio *et al.*, 2012; Gato-Calvo *et al.*, 2019).

PRP is a blood-derived biological product with a high concentration of platelets in a small volume of plasma. Methodologies to prepare PRP can rely on single centrifugation, double centrifugation, or selective blood-filtration procedures, and on manual or automatic systems operated in open or closed circuits (Gato-Calvo *et al.*, 2019). After activation with calcium chloride, several GDFs are released from alpha granules stored inside platelets, which include platelet-derived growth factor, transforming growth factor β1, transforming growth factor β2, growth factor vascular endothelial growth, fibroblast growth factor and epidermal growth factor, insulin-like growth factors 1 and 2, interleukin 8, keratinocyte growth factor, and connective tissue growth factor (Gnecchi *et al.*, 2008; Alves *et al.*, 2011; Cuervo *et al.*, 2020). In addition, PRP also contains adhesion molecules, such as fibrinogen, fibronectin, and vitronectin (Sánchez-González *et al.*, 2012). These bioactive molecules influence the processes of mitosis, chemotaxis, cell differentiation, growth of undifferentiated mesenchymal cells and the production of extracellular matrix, thus promoting tissue regeneration (Mata, 2013).

PRP can be categorized based on its composition as increased leukocytes with no activation, increased leukocytes with activation, minimal leukocytes with no activation, and minimal leukocytes with activation (Mishra *et al.*, 2012). In order to have a therapeutic effect, the PRP should contain at least 1,000,000 platelets per microliter of plasma to provide the adequate release of GDF in the application site (Sánchez-González *et al.*, 2012).

The therapeutic efficacy of PRP, either from commercial brands or in-house protocols, has been tested *in vitro* and in preclinical and clinical trials for oral and maxillofacial surgery, ophthalmology, dermatology, and musculoskeletal conditions in humans (Gato-Calvo *et al.*, 2019). In dogs, PRP has been used for the treatment of different osteoarticular diseases, including OA (Bland, 2015; Karayannopoulou *et al.*, 2015; Vilar *et al.*, 2018; Cuervo *et al.*, 2020).

The aim of this study is to evaluate the effect of allogeneic PRP therapy on pain and joint function in dogs with OA, and that are refractory to pharmacological and physical rehabilitation conventional approaches.

Materials and Methods

Study population and evaluated parameters

The studied series comprised five dogs treated at the Arrábida Veterinary Hospital with clinical and imageological diagnosis of OA. They were aged between 6 and 14 years, and two were Labrador Retrievers, one was a mixed breed dog, one was a German Shepherd, and one was a German Pointer (Table 1).

The orthopedic examination of the animals included the assessment of lameness grade and pain score on days 0, 30, 60, and 90 after PRP application. Lameness was categorized from grade 1 to grade 5. Pain score was assessed by palpation and was categorized as follows: grade 0, absence of pain and without clinical manifestation; grade 1, mild pain and the animal bends the limb, but does not vocalize or looks at the limb; grade 2, moderate pain and the animal bends the limb, does not vocalize, but looks at the limb; grade 3, severe pain, the animal bends limb, vocalizes, and looks at limb; and finally, grade 4, severe pain, the animal pulls away the limb, vocalizes, shows aggressive behavior, and looks at limb.

Therapeutic approach before PRP therapy

Prior to the study, the dogs were subjected to multidisciplinary protocols for pain management and to improve gait biomechanical function. Pharmacological treatment included meloxicam (0.1 mg/kg, *per os*, SID) for 8–15 days used in acute pain episodes, tramadol (2–4 mg/kg, *per os*, BID or TID), and gabapentin (5 mg/kg, *per os*, BID or TID) for chronic pain control. The animals were supplemented with chondroitin and glucosamine sulfate orally and no specific medical diet was prescribed.

In addition to the referred medical treatment, animal 1 was subject to shockwave therapy using the equipment PulseVet, Alpharetta, GA, USA; animals 3 and 4 were prescribed laser therapy (Lite Cure, New Castle, DE), electrostimulation (BTL 4000 Premium, Famões, Portugal), and hydrotherapy (Hidro Physio, Shropshire, United Kingdom); and animal five was treated with diathermy therapy (BTL 6000, Famões, Portugal) (Fig. 1).

Table 1. Summary of cases included in this study regarding the breed, age, and affected joint.

Animal	Breed	Age (years)	Joint
1	Mixed breed	12	Knee
2	Labrador Retriever	10	Tibiotarsal
3	Labrador Retriever	13	Knee
4	German Shepherd	14	Elbow
5	German Pointer	6	Carpal

After therapy, the animals did not reveal any clinical improvement and, during a 6-months period, they had not undergone any pharmacological or physical rehabilitation procedures.

PRP therapy

The osteoarthritic joints of the five studied animals were targeted for allogeneic PRP therapy. The PRP gel was provided by Vetherapy (Coimbra, Portugal). In order to obtain PRP, venous blood was collected in a 250-ml transfusion bag with citrate phosphate dextrose. The blood samples were sent to the laboratory and were analyzed to make sure animals were vaccinated. Within 12 hours, the blood samples were processed under Vetherapy's proprietary protocol by the dual centrifugation technique (one low-speed and one high-speed centrifugation) with the standardization of platelet counts per ml (minimum of 1 million platelets per microliter). The obtained PRP was pooled together, stored and frozen, and sent to Arrábida Veterinary Hospital in 1.5-ml vials on dry ice. In all the dogs from which blood was obtained using an allogeneic approach, echoguided intraarticular PRP injection was administered under sedation with a combination of 0.2 mg/kg of midazolam, 0.2 mg/kg of methadone, and 0.6 mg/kg of propofol, intravenously and after aseptic field preparation (Fig. 2). After this procedure, the animals were evaluated after 30, 60, and 90 days.

Statistical analysis

The data obtained were subject to descriptive statistical analysis that included the calculation of absolute and relative frequencies, means, and standard deviation using the Microsoft Office Excel 2013 software (Microsoft, United States of America).

Ethical approval

There were no ethical issues to address.

Results

It can be seen that all animals improved after 90 days of treatment. In this scenario, two clinical parameters were evaluated: the grade of lameness on days 0, 30, 60, and 90 (Table 2) and the score of pain on days 0, 30, 60, and 90 (Table 3).

All animals showed a decrease in lameness grade after 90 days. Animal 2, after day 90, presented a normal gait. The two animals with grade III lameness, after 90 days of PRP administration, improved for grades I and II, respectively. The two animals that presented grade II lameness evolved to grade 0, i.e., without lameness. The only animal with grade IV lameness, after PRP application decreased to a grade II.

For pain evaluation, there were positive results, with the first three animals becoming painless, while animals 4 and 5 went from having severe and moderate pain to mild pain after day 90, respectively.

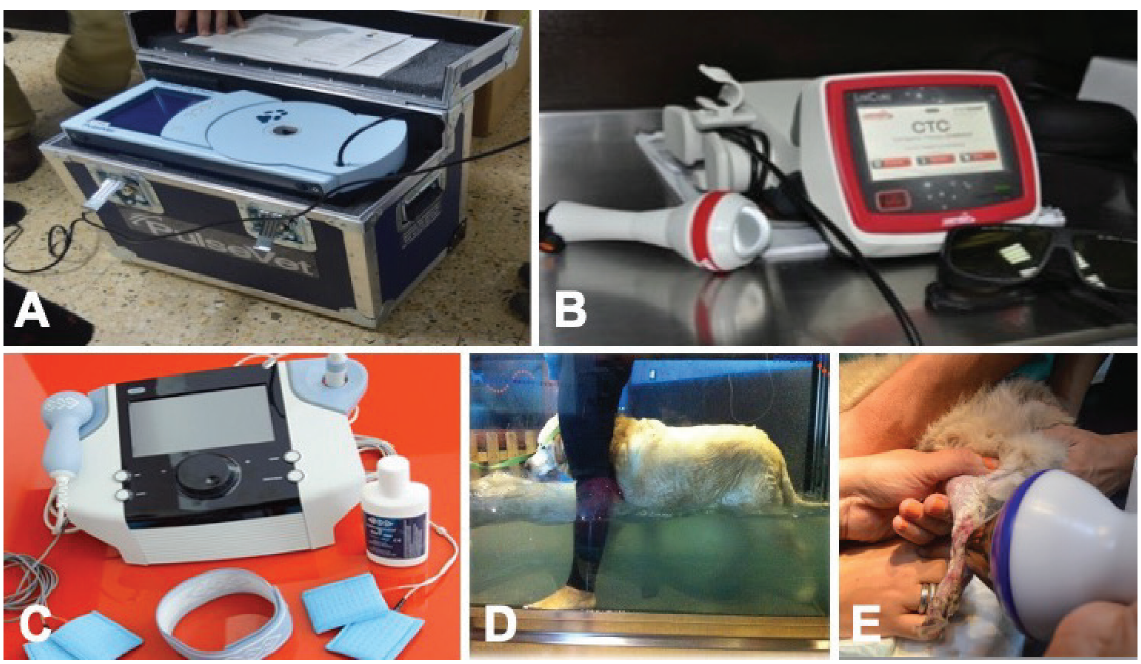

Fig. 1. Equipment used in the physical rehabilitation protocol. (A) Extracorporeal shockwaves. (B) Class IV laser therapy. (C) Electrostimulation therapy. (D) Hydrotherapy. (E) Diathermy therapy.

Discussion

One of the purposes of using therapies, such as PRP, is to enhance healing and stimulate the regeneration of injured tissues that have a weak natural regenerative capacity, such as tendons, ligaments, menisci, and cartilages (Gutierrez-Nibeyro, 2011). It is believed that platelets can mediate the release of high concentrations of growth factors. It is described that these growth factors can favorably induce analgesia, soft tissue healing, regulation of anti-inflammatory signals and vascularization, and innervation of autografts, making PRP an interesting therapeutic approach to OA (Vilar *et al.*, 2018)

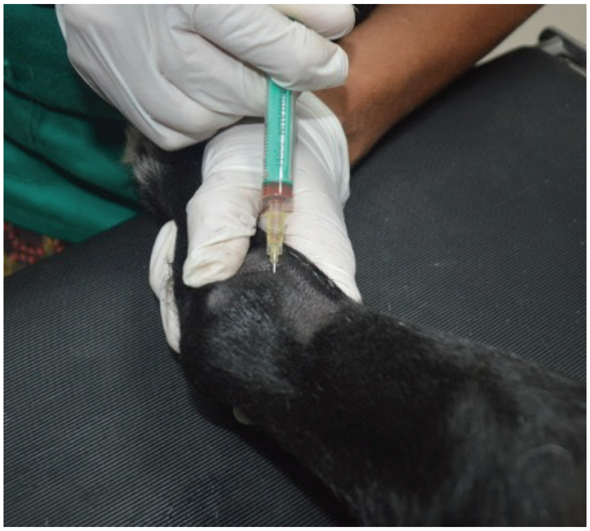

Fig. 2. Intraarticular injection of PRP after echoguided localization of the articular cavity.

In this work, our study population comprised mainly adult to geriatric large breed dogs (Labrador Retriever, German Shepherd, and German Pointer), described as predisposed to the development of OA (Bland, 2015). The clinical results obtained were promising because PRP administration was well tolerated by these types of patients and clinical improvement was documented. These results are in agreement with the published scientific literature.

A recent study on dogs showed promising results on the management of OA caused by cruciate ligament rupture using minimal leukocyte PRP. The studied population demonstrated an increase in many different gait associated parameters, such as peak vertical forces, angular range of motion, and stance time without associated side effects (Vilar *et al.*, 2018). Studies on horses have shown that intralesional treatment of PRP promoted resolution of tendinitis associated with decreased lameness, edema, local sensitivity, as well as improved ultrasound image of evaluated structures (Carmona *et al.*, 2007). Abellanet and Prades (2009) studied 42 sport horses with OA lesions, wherein 70% of horses receiving PRP therapy resumed their normal work routine and only 9.5% of the animals relapsed when compared to the control group, wherein 33% of the horses relapsed. In the latter two studies, no local or systemic side effects were reported following PRP treatment (Carmona *et al.*, 2007; Abellanet and Prades, 2009).

The combined application of PRP with stem cells, such as adipose-derived stem cells (ASCs), in osteoarticular diseases in dogs has also demonstrated promising

Table 2. Lameness grades at day 0 and at days 30, 60, and 90 after PRP administration.

Animal	Day 0	Day 30	Day 60	Day 90
1	Grade III	Grade II	Grade II	Grade I
2	Grade II	Grade II	Grade I	No lameness
3	Grade IV	Grade III	Grade II	Grade II
4	Grade III	Grade III	Grade II	Grade II
5	Grade II	Grade II	Grade I	No lameness

Table 3. Pain scores at day 0 and at days 30, 60, and 90 after PRP administration.

Animal	Day 0	Day 30	Day 60	Day 90
1	Intense	Moderate	Mild	Painless
2	Moderate	Mild	Mild	Painless
3	Intense	Moderate	Mild	Painless
4	Severe	Intense	Mild	Mild
5	Moderate	Mild	Mild	Mild

results for its significant effectiveness in improving animal welfare during the recovery period. Although a relatively new concept, the strategy is appealing because the resulting regenerative matrix delivers a potent trilogy of undifferentiated cells, fibrin matrix, and differentiation factors (Sánchez-González *et al.*, 2012). The concomitant application of ASCs and bone marrow is considered as one of the most promising therapies (De Coppi, 2012). ASC-based therapies have regenerative potential because of their ability to differentiate into damaged tissue cells and the ability to chemically recruit other undifferentiated cells from the recipient organism to the site of tissue regeneration. In addition, they are immunoprivileged cells, they do not trigger a rejection reaction by the body, they have a very beneficial immunomodulatory effect on the treatment of inflammatory and immune-mediated diseases (Requicha *et al.*, 2016) and are a useful alternative to prolonged glucocorticoid and non-steroidal anti-inflammatories. These drugs can induce nefarious side effects, such as immunosuppression, iatrogenic hyperadrenocorticism, gastrointestinal ulceration, and renal or hepatic failure (Johnston and Budsberg, 1997)

The use of PRP in humans for OA treatment is still a bit controversial and lacks standardization (Gato-Calvo *et al.*, 2019). For authors, this can be seen as an opportunity for further comparative studies in order to contribute to a better understanding of this type of therapeutics in companion animals and helping with standardization and future translation to human applications.

Conclusion

PRP has therapeutic applicability in different animal species, resulting in a decrease of clinical signs associated with osteoarticular lesions.

At the end of the treatment, clinical evaluation showed that, in some cases, there were no signs of joint pain in the treated joints, as well as no lameness. The encouraging results of this study indicate the need for more comprehensive studies with larger numbers of animals in order to validate the method, aiming for it to become commonly administered to companion animals with degenerative joint disease.

Allogeneic administration of PRP has consistently been shown to be a simple, rapid, cost-effective, and safe way to assist the treatment of this disease. It is believed that the therapeutic use of this procedure can help in obtaining clinical improvement of diseased animals, which results in better quality of life and animal welfare.

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Conflict of interest

The authors declare that there is no conflict of interest. PC is the founder and CEO of Vetherapy who provided the PRPs for this study but had no involvement in the PRP administration and monitoring of the animals throughout the study.

Authors contributions

JR conceived the idea; JR and PC designed the study; AM was responsible for the clinical cases; JC, AM, and SS analyzed the data; JC, PC, and JR wrote the paper with input from all the authors.

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