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Platelet rich plasma use in veterinary medicine

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Platelet rich plasma (PRP) has been a successful tool for the treatment of osteoarthritis in both small animal and equine patients. Platelets provide a naturally occurring stockpile of growth factors (GFs) and signaling molecules that govern healing and tissue regeneration[1]. The same bio-reactive properties that allow PRP to mitigate the vicious cycle of osteoarthritis and ameliorate sequelae associated with its presence in joints, may be leveraged to do the same in several other conditions. . This white paper is intended to provide evidence-based guidance in the evaluation of PRP as a therapy for veterinary patients.

Platelet Rich Plasma

Growth factor peptides, clotting factors, and other bio-reactive molecules are stored in intracellular vesicles known as alpha-granules. Growth factors released by activated platelets include platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and transforming growth factor beta (TGF-beta). The effects of these molecules on coagulation and neovascularization are suspected to improve healing in damaged epithelial cells and connective tissues. Platelet derived growth factor is responsible for collagen synthesis, proliferation and chemotaxis of fibroblasts, and macrophage activation. TGF-beta enhances type 1 collagen, promotes angiogenesis, and stimulates chemotaxis of immune cells. VEGF is primarily responsible for angiogenesis, changes in vessel permeability, and acts as a mitogen for endothelial cells. bFGF governs the proliferation and differentiation of chondrocytes, osteoblasts, and mesenchymal cells[2].

In addition to alpha-granules, platelets contain dense bodies (or delta granules). When activated, dense bodies release adenosine, histamine, serotonin, and calcium; important contributors to tissue regeneration[1]. Adenosine acts a cytoprotectant, preventing tissue damage and has been shown to have immunomodulatory effects on macrophages resulting in an alteration of interleukin production. Histamine causes a vasodilatory effect at the level of the capillary endothelium. This increased permeability allows increased migration of leukocytes into the local tissues. Serotonin is a neurotransmitter that causes an even greater effect on capillary permeability than histamine as well as recruiting fibroblasts and macrophages to the area. Finally, calcium governs proliferation and differentiation of keratinocytes[3].

PRP, through the release of granular cytokines, supports pain modulation in osteoarthritis. Both alpha and lambda granules have anti-inflammatory cytokines like TGF, multiple growth factors, IGF-1 and PF-4[4]. Shifting the joint biome from a catabolic environment to an anabolic one with PDGF, VEGF and TGF-Beta allows pain control through healing, revascularization, and

chemotactic recruitment of MSC. These attributes of this biologic provide opportunities in soft tissue as well as osteoarthritis. Promising treatment for the conditions listed below are examples of the potential expansion of use for PRP.

Partial Cranial Cruciate Ligament Tears

Injury to the cranial cruciate ligament (CCL) is one of the most common musculoskeletal disorders of the dog, and a frequent cause of hind limb lameness. Progressive disease of the CCL is associated with inflammatory changes in the synovial fluid of the stifle and increased expression of collagenolytic protease molecules, such as matrix metalloproteinases (MMPs), that contribute to the degradation of collagen and lead to progressive CCL degeneration and rupture[5, 6]. In a blinded, randomized study, Cook *et. al*. demonstrated that 5 intra-articular injections of PRP injected over 8 weeks following a partial tear resulted in improved clinical outcome compared to the control group[7]. In this study, the PRP patients had an increased range of motion, decreased pain, and improved function for the duration of the 6-month study. There is also evidence suggesting a synergistic effect between adipose-derived mesenchymal stem cells and PRP when used in combination for treatment of partial CCL tears[8].

Corneal Ulcers

Medical management of chronic or complicated ulcers that penetrate the stroma of the eye has historically required treatment with anti-collagenase therapy combined with antibiotics and in more severe or refractory cases, conjunctival flap surgery. Currently, autologous serum, administered as a topical drop, is used in cases of refractory ulcers. The serum contains both broad anti-collagenase activity and growth factors to promote healing[9]. PRP contains a higher concentration of growth factors than autologous serum, and therefore may be more beneficial in promoting cellular proliferation[10]. Several growth factors present in PRP have been identified and used in treatment of complicated corneal ulcers. PDGF, FGF, and hEGF all stimulate cellular proliferation and bFGF is suspected to increase migration of epithelial cells to the defect and promote corneal endothelial cell proliferation[11]. In a prospective, comparative human study, Alio *et. al.* showed a significant improvement (characterized by reduction in size or depth of a corneal defect and a reduction in pain and inflammation) in 24 out of 26 eyes with chronic non-healing ulcers following PRP as the stand-alone treatment[12].

PRP has been shown to have strong antimicrobial properties against methicillin resistant and methicillin sensitive *Staphylococcus aureus* species as well as Group A *Streptococcus* species[13]. In a prospective, controlled cohort equine study, Rushton *et. al.* revealed an increase in cellular migration and proliferation of corneal epithelial cells when treated with PRP compared to serum or plasma rich in growth factors in vitro[14]. Additional research is needed to determine if the efficacy will carry over to domestic animals, but PRP shows promise as a therapeutic option for complicated corneal ulcers.

Wound Care/Degloving Injuries

Wound healing is a multi-faceted process that takes place in response to cellular, chemical, and physical signals, culminating in the repair and reorganization of damaged tissue. The initial response begins with hemostasis and progresses though the inflammatory, proliferative, and remodeling phases[15]. Each phase is mediated by a complex interaction of growth factors and cytokines/chemokines, notably VEGF, TGF-beta, EGF, and PDGF[16]. These growth factors are found in high concentrations in PRP. It is suggested that the increased concentration of growth factors promotes cellular regeneration, stimulates angiogenesis, increases collagen formation, and thus accelerates wound healing[10, 17, 18]. In a recent nonrandomized, comparative, experimental study, Jee *et. al.* showed intralesional injection of PRP resulted in increased granulation, increased angiogenesis, more rapid epithelialization, and more collagen deposition than control wounds after 14 days[17]

In some amenable animal cases and throughout the human literature, PRP is often being combined with an activator consisting of thrombin and calcium chloride to create a platelet gel that is applied to wounds in conjunction with a bandage[19, 20]. In a 10 year, retrospective, meta-analysis of human wounds treated with PRP gel, complete and partial wound healing was improved compared to controls[21]. Similarly, a published case report of the use of PRP gel on a non-healing wound in a 10 year old Shih-Tzu showed a significant clinical improvement following therapy[20]. The clinical use of PRP mirrors the natural wound healing process through application of multiple growth factors in their natural biologic ratio. While randomized and blinded studies of PRP use in wound healing are rare, there is both experimental and clinical evidence to suggest that PRP is a viable therapy in treatment of acute and chronic wounds.

Dental Extractions

Periodontal disease is reported in 80% of canines by just 2 years of age, which when left untreated can culminate in the need for tooth extraction[22]. Tooth extraction results in the alteration of normal periodontal structures and triggers a cascade of physiologic events that result in healing of the extraction socket. The defect is filled by a blood clot and coated in a fibrin network, the epithelium begins to proliferate, and finally new bone is formed to fill in the alveolus[23]. When this process fails and there is no granulation tissue, patients develop a condition known as alveolar osteitis or dry socket. This is reported as a severe throbbing pain in humans and is often refractory to analgesia[24]. A randomized, controlled, human clinical trial by Alissa *et. al.* suggests that PRP therapy can be beneficial in reducing post-operative alveolar osteitis and improving soft tissue healing[25]. Several comparative experimental studies using multiple mammalian models have shown statistically significant improvement in bone density following treatment with PRP in surgically induced defects[26, 27].

In the randomized, controlled study by Messora *et. al.*, ten dogs underwent surgically induced mandibular defects. The defects treated with autologous PRP in combination with cancellous

bone allograft experienced statistically greater mineralized bone density and greater mineralized tissue area than those treated with bone graft alone[26].

Surgical Closures

Similar to its uses in wound healing, PRP application to surgical sites aims to accelerate the natural healing process through introduction of high concentrations of growth factors, adhesion molecules, and clotting molecules released during platelet degranulation[10, 17, 18]. In a randomized, blinded, 30 patient study featuring humans with sternal incisions from a cardiopulmonary bypass surgery, patients receiving PRP treatment reported less pain at the incision sites and had subjectively less bruising during convalescence. While the study had a small sample size, it suggests that PRP therapy may provide improved clinical outcomes compared to controls[28]. This conclusion is also supported by a retrospective, comparative study looking at the use of PRP in cardiothoracic surgery in humans in which Khalafi *et.* al. compared 571 patients receiving PRP therapy post operatively to 557 patients receiving no PRP and found significant reduction in rates of infection and drainage at the incision site for those who received PRP application during surgical closure[29].

Reproductive Therapy

The use of PRP in the field of reproduction is still relatively new. Platelet rich plasma contains high concentrations of growth factors and cytokines that modulate the surrounding tissue environment. Introduction of increased concentrations of PDGF, IGF-1, EGF, and TGF-beta is thought to regulate the balance between endometrial proliferation and cellular differentiation[30]. Several small studies have been performed to evaluate the effects of PRP on uterine inflammation in equids and on endometrial thickness and viability of IVF implantation in humans. In a study comprised of 21 mares, 13 with chronic degenerative endometritis and 8 with a healthy endometrium, Reghini *et. al.* demonstrated a significant reduction in uterine inflammation following artificial insemination with use of intrauterine PRP infusion[31]. Chang *et. al.* infused autologous PRP into the uterus of 5 women in whom hormonal infertility therapy was unsuccessful due to exceptionally thin, poor quality endometrium. Intrauterine PRP infusion in conjunction with normal hormone replacement therapy resulted in 4 out of 5 women's pregnancies progressing normally[32]. As research continues, the therapeutic role of PRP in reproductive pathology will become clearer, but currently there is evidence that it may be beneficial in treating inflammation and endometritis.

Tendinopathy/Myopathy

Muscle and tendon injuries are not uncommon in dogs and horses, especially those involved in sporting activities. PRP has long been used for such injuries in horses but is relatively new in small animal medicine. Type I collagen is a large component of connective tissue such as muscles and tendons and is also a potent stimulator of platelets and granulocytes[33]. When platelets are activated, alpha granules release growth factors and cytokines, the downstream effects of which is thought to be extracellular matrix synthesis and epithelial cell proliferation. A prospective, non-randomized pilot study looked at the effects of a single intralesional injection

of PRP in 10 dogs with supraspinatus tendinopathy. While the study was small, it showed a subjective improvement in lameness and function in 40% of the dogs, as well as an improvement in tendon lesion heterogeneity and echogenicity on ultrasound in 60% of dogs[34].

PRP has also been combined with mesenchymal stem cells for treatment of canine supraspinatus tendinopathy with moderate success[35]. Similarly, Ricco *et. al.* found that 17 of 19 (89%) sport horses with acute or sub-acute superficial digital flexor (SDF) tendinopathy returned to their previous level of performance after being treated with a single intralesional injection of adipose derived MSCs and PRP. Moreover, all of the affected SDF tendons demonstrated appropriately structured longitudinal tendon fibers and a greater echogenicity sonographically starting from 60-90 days post-injection[36].

Additionally, PRP and the growth factors found therein are being investigated for use in treating acute and chronic myopathies. An in vivo, controlled study involving surgical laceration of the gastrocnemius muscle in a mouse model showed that repeated injections of bFGF, IGF and NGF into the damaged muscle resulted in improved healing time, increased tetanic strength of the muscle fibers and decreased fibroblast infiltration compared to controls[37]. Likewise, Hammond *et. al.* demonstrated a shortened recovery time in rats with experimentally induced repetitive stress myopathies treated with a single intra-muscular injection of PRP[38].

In summary, platelet rich plasma is presented here as a potentially useful tool to consider as a stand-alone therapy or in conjunction with stem cells for many veterinary diseases and injuries as well as adjunctive therapy with surgical procedures. This white paper is intended to provide guidance when considering PRP therapy for veterinary patients. The peer-reviewed references provided are intended to help guide veterinary practitioners in the evidence-based selection of appropriate therapies for their patients.

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