Veterinary Allogeneic Platelet-Rich Plasma Safety Overview

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1 Introduction

This review will present the results of two randomized placebo-controlled safety studies of allogeneic platelet-rich plasma (PrecisePRP™) and a literature review of the published data on allogeneic platelet-rich plasma. This manuscript is intended as a guide for veterinary practitioners on the safe application of allogeneic platelet-rich plasma (PRP) in canine and equine patients.

Platelet-rich plasma (PRP) has been studied in multiple species with varying use profiles. A thorough review of the platelet-rich plasma literature yielded over 30 references in support of PRP and its use as a topical, intra-articular, and/or intralesional therapy published since 2008. Meta-analysis in human clinical trials as well as multiple study reviews in canine and equine suggest that the most common concern for platelet-rich plasma effectiveness is associated with a lack of uniformity and standardization(Garbin et al., 2021;Everts et al., 2022). Important components of platelet-rich plasma have been debated; however, total platelet dose, growth factor content, and leucocyte count appear to be common factors for most authors when relating in vitro characterization and effectiveness outcomes(McCarrel and Fortier, 2009). A review of the veterinary literature was used to support both potential indications as well as dose and administration.

Platelets are provided to supply the growth factors and cytokines predominately located in the alpha granules. After administration, the growth factors and cytokines are released into the area of injection and provide anti-inflammatory cytokines and repair signaling. Platelets also release factors that attract or "home" mesenchymal stem cells, leucocytes, and other mononuclear immune cells to assist in the repair process. It has been reported that PRP injected into the joint or lesion reduces pain signaling.

Recently, the discussion of platelet phenotypes and their relationships to platelet function, circulation, and membrane characteristic have led to the exploration of new methods for platelet concentrate storage, including storage at 4-8°C. Current published uses of platelet-rich plasma in the canine and equine support that it can be used intra-articularly, intralesionally, or topically and should be activated to allow the release of dense and alpha granules. Cold-stored platelets have been characterized as moderately activated as compared to room temperature-stored platelets. Recent in vitro characterization of the cold-stored platelets supports that their phenotype is most consistent with the desired function of platelet-rich plasma(Zhao et al., 2022). Manipulating the storage parameters for platelet concentrates prior to pooling and lyophilization allows platelet lyophilization without cryopreservatives(VetStem data, 2022, 2023).

In using the commonly available PRP produced by mechanical device, the safety issues include sterility, variability in dose, contamination with unwanted cell types (wbc, rbc) and variability in potency. In moving from autologous mechanically-produced PRP to cGMP manufactured donor-

derived allogeneic PRP, many of these safety and product quality problems are eliminated or significantly reduced.

2 Platelet Therapy – Definition

The most common term for platelet therapy is "platelet-rich plasma." The term platelet-rich has been used loosely to describe solutions where the platelet concentration is higher than that found in whole blood and the platelets are suspended in plasma. The growth factors stored in the alpha granules in platelets(Knezevic et al., 2016) are most often cited as the active healing agents including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-B), and basic fibroblast growth factor (bFGF)(Fahie et al., 2013).

The most common method to concentrate platelets is centrifuge spinning. As stated by Dr. Sam Franklin (2016, Veterinary Product News), the majority of the systems on the market are designed for preparing human platelet concentrates and are sold into the veterinary markets without published data supporting suitability for veterinary species. In a search for published data on methods and clinical results, one may be surprised to find most systems have only testimonial data.

3 PrecisePRP™ Manufacturing Standards

PrecisePRP™ Equine is a leucoreduced allogeneic, pooled freeze-dried, platelet-rich plasma product from up to 18 equine donors. The biological source material for this product is in-date leucoreduced, apheresis-derived equine platelet concentrates and apheresis-derived frozen plasma. All horses are blood type AaCa positive and negative for plasma antibodies directed at blood types they are not. Donors are from a closed herd compliant with USDA regulations for production of licensed biologics. Selection of donors and evaluation for infectious disease is compliant with FDA CVM Guidance #254. Supplied in a 50 mL glass vial, this product is a sterile, nonpyrogenic white-to-tan powder. Once rehydrated with 8 mL of sterile water for injection, this product is a gold translucent fluid with 4.0×10^9 platelets per vial, less than 1500 white blood cells per µL, and less than 0.01% red blood cells.

PrecisePRP™ Canine is a leucoreduced allogeneic, pooled, freeze-dried, platelet-rich plasma product from up to 32 canine donors. The biological source material for this product is in-date leucoreduced, apheresis-derived canine platelet concentrates and apheresis-derived or whole blood-derived frozen plasma. Donors are blood typed for DEA 1, 4, and 7 as well as tested for plasma antibody negative to routine canine red blood cell antigens. Selection of donors and evaluation for infectious disease is compliant with FDA CVM Guidance #254. Supplied in a 50 mL glass vial, this product is a sterile, nonpyrogenic white-to-tan powder. Once rehydrated with 8 mL of sterile water for injection, this product is a light yellow translucent fluid with 4.0×10^9 platelets per vial, less than 1500 white blood cells per µL, and less than 0.01% red blood cells.

4 PrecisePRP™ Randomized Controlled Safety Study in the Equine

VetStem, Inc. conducted an equine safety study at request of FDA with a primary objective to evaluate the safety of leucoreduced equine allogeneic pooled freeze-dried platelet rich plasma (PRP, PrecisePRP™ Equine) for intra-articular injection in adult horses.

PrecisePRP™ Equine was studied in a randomized, masked, placebo-controlled target animal safety study (adult horses) at the label dose of 4 mL in comparison to a blinded control group injected with 4 mL of placebo saline. The horses were injected in one radiocarpal joint and one tarsocrural joint, with the injections being two weeks apart. Horses were followed for seven days after each injection

and monitored for clinical and laboratory safety of the PrecisePRP™ Equine injections. This study was conducted with a formal IACUC approval and was conducted at an independent veterinary school testing facility.

There were no treatment-related adverse reactions reported. The study included evaluations of daily health, temperature, pulse, respiration, clinical pathology, injection sites reactions (heat, swelling, passive flexion pain and joint circumference), veterinary physical exams, and lameness evaluations (including evaluation by a clinician and by the Lameness Locator® system). A single adverse event in the placebo group was reported as colic, which resolved within one day. The adverse event table below shows that four control horses and one treated horse had mild transient lameness attributed to the needle injection procedure.

Adverse Event	PrecisePRP™ Equine (N=6)	Saline Placebo (N=6)
Mild transient lameness post-injection	1	4
Colic	0	1

This study of the safety of the intraarticular administration of PrecisePRP™ Equine demonstrated a good margin of safety at the label dose of 4 mL. There were small excursions from normal ranges for some measurements, but no clinically significant excursions or trends were detected.

5 PrecisePRP™ Randomized Controlled Safety Study in the Canine

PrecisePRP™ Canine was studied in a randomized, masked, placebo-controlled safety study in adult beagles at request of the FDA. Design was intra-articular injection at the label dose of 2 mL in comparison to a blinded control group injected with 2 mL of placebo saline. The dogs were injected in one stifle joint and one hip joint, with the injections being two weeks apart. Dogs were followed for seven days after each injection and monitored for clinical and laboratory safety of the PrecisePRP™ Canine injections. This study was conducted with a formal IACUC approval and was conducted at an independent testing facility.

There were no adverse treatment-related effects on body weights, gait, daily health observations, temperature, pulse, respiration, clinical pathology, injection sites, no significant findings on veterinary physical exams, and no adverse events as a result of treatment. Specific evaluations of the injected joints included heat, joint swelling, and passive flexion pain as well as lameness evaluation.

6 Literature Review of Safety of Allogeneic PRP

Published safety data exists for both canine and equine allogeneic platelet-rich plasma. Clinical case reports, systemic literature reviews, preclinical safety analysis, and comparative studies with autologous platelet-rich plasma and mesenchymal stem cells are available for the horse and dog. In the safety evaluation published by Garbin(Garbin et al., 2022), a pooled allogeneic freeze-dried PRP was evaluated with autologous frozen products for safety and found to be statistically unremarkable from autologous products relating to inflammation and lameness post injection. In an Italian clinical trial in canine patients, no adverse events associated with immunogenicity were noted utilizing a pooled allogeneic platelet-rich plasma(Catarino et al., 2020).

In addition, there are safety articles on the use of allogeneic PRP in the human. In a 2021 review of the literature, Akbarzadeh found 2 laboratory animal studies and 8 human studies of allogeneic PRP safety in wound healing(Akbarzadeh et al., 2021). The authors concluded “none of the studies

identified any major side effects or adverse events" and "the treatment resulted in a reduced time to heal and/or reduced wound size in most cases." In a 2023 human meta-analysis of autologous PRP, as a reference for PRP efficacy and safety in osteoarthritis, the authors included 24 randomized controlled trials comprising 1,344 patients(Xiong et al., 2023). Their conclusion stated that PRP injection therapy can safely and effectively improve functional activity in patients with OA and produce positive analgesic effects in patients with knee OA, temporomandibular joint OA, and ankle OA. In addition, they found that the analgesic effect of leucocyte-poor PRP was greater than that of leucocyte-rich PRP.

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