Veterinary Allogeneic Platelet-Rich Plasma Efficacy and Safety Overview

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1 Abstract

PrecisePRP™ Canine and PrecisePRP™ Equine are allogeneic pooled leucoreduced and freeze-dried platelet-rich plasma products. They are FDA-reviewed and intended as species-specific sources of platelet-rich plasma for intra-articular use. This white paper is a review of the safety and efficacy of platelet-rich plasma as well as a direct review of the safety and efficacy of the PrecisePRP™ products for veterinary use. The FDA Center for Veterinary Medicine has reviewed both products and published their risk based review on the FDA website stating, "The FDA concluded that the developer of PrecisePRP properly identified and appropriately mitigated the potential risks associated with the product, and the FDA has no additional safety concerns." According to the FDA, these products are the first FDA-reviewed platelet-rich plasma products for horses and dogs available to veterinarians. This review of the safety and efficacy of the PrecisePRP™ products is intended to provide guidance for use by clinical veterinarians.

2 Introduction

This review will present an overview of the safety and efficacy of PrecisePRP™ in the canine and equine along with supporting published literature on platelet-rich-plasma. This publication will include the results of two randomized placebo-controlled safety studies of allogeneic platelet-rich plasma (PrecisePRP™) as submitted to the Center for Veterinary Medicine at the FDA and a literature review of the published data on autologous and allogeneic platelet-rich plasma. This manuscript is intended as an educational tool for veterinary practitioners on the application of platelet-rich plasma (PRP) in canine and equine patients with an emphasis on the utilization of PrecisePRP™, an off-the-shelf allogeneic platelet-rich plasma product.

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, red cell contamination, 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 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 a joint or lesion reduces pain signaling (Knezevic et al., 2016).

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 directly addressed by the manufacturing process and the quality testing for lot release.

3 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.

4 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 x 10^9 platelets per vial, less than 1500 white blood cells per µL, and less than 0.01% red blood cells.

5 Regulatory

The FDA Center for Veterinary Medicine has reviewed both the PrecisePRP™ Canine and Equine products and has stated, "The FDA concluded that the developer of PrecisePRP properly identified and appropriately mitigated the potential risks associated with the product, and the FDA has no additional safety concerns." You may find these statements, a description of the FDA CVM program for animal cells, tissues, and cell- and tissue-based products (ACTPs), and a listing of the products that successfully completed the review process on the FDA website at https://www.fda.gov/animal-veterinary/cvm-updates/fda-announces-decision-tissue-based-product-use-dogs?utm_medium=email&utm_source=govdelivery.

6 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 intra-articular 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.

7 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.

In addition to the above randomized controlled safety study in canines, a field open label study of injection in multiple joints in canines was conducted at the request of the FDA to evaluate any safety concerns with multi-joint injections. A total of 12 dogs aged 1.6-13 years were enrolled and injected in 2-8 joints at the same clinic visit. The average number of joints injected was 3.6. All enrolled dogs were monitored for a minimum of 17 days after injection for adverse events. No adverse events were report in this study.

8 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.

9 Pilot Efficacy of PrecisePRP™ in the Canine

As part of the FDA review process, VetStem was allowed to enroll canine patients in an open-label field study prior to FDA final review. A total of 59 dogs were enrolled and completed approximately

60-90 days post-injection evaluation using a 5-point quality of life score. Below are the outcomes per type of lesion treated in this study:

Condition Treated	Overall Quality of Life at 90 Days				
	Signif Improved	Mildly Improved	No Change	Mildly Decreased	Signif Decreased
Cranial Cruciate Lig with Surgery	25	4	1		
Cranial Cruciate Lig w/o Surgery	9	1			1
Tendon/Ligament Other	2				
Osteoarthritis	6	4			
OCD Shoulder	1				
Fracture Augment	2	1			
MPL with Surgery	1				
Avulsion Repair Augment	1				
Totals	47	10	1	0	1
Total Completed by 9/24/24	59				

# Doses Administered/Dog	# Dogs
1	32
2	23
3	4

The efficacy using a 5-point QOL score was demonstrated with 47/59 (79.66%) of the dogs significantly improved and 10/59 (16.95%) of the dogs mildly improved. One dog worsened in the 90-day time period due to reinjury.

The number of doses administered per dog (single visit) are listed above with 54% of the dogs receiving a single dose of PrecisePRP™ and 46% receiving multiple doses (at the initial clinic visit). The data collection form also requested the veterinarian to state whether, based on their clinical experience with PrecisePRP™, they would recommend use in the future. A total of 58/59 stated they would recommend PrecisePRP™. Additional data collection is underway and future studies are planned and will be published.

10 Acknowledgements

The authors are grateful to the study investigators and their dedicated staff at Auburn University (Lindsay Boone, DVM, PhD, DACVS-LA) and at Colorado Animal Research for their diligence and care in conducting these safety studies as well as the multiple veterinarians who participated in the canine field efficacy study.

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