Commentary

Do lyophilized platelets hold promise for treatment of hemorrhagic diseases in wild animals?

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Bleeding as a result of trauma, hemorrhagic diseases, or primary platelet-related abnormalities is a major cause of morbidity and mortality in humans and animals.[1–5] In humans, for example, hemorrhage is the most common cause of preventable death following traumatic injury in patients < 65 years of age and is a leading cause of potentially survivable deaths during military operations.[1,3,6] Treatment for hemorrhage, in addition to supportive care and volume resuscitation, frequently includes IV administration of whole blood or platelet concentrates to counteract the effects of a rapid decrease in platelet numbers associated with platelet consumption and loss of blood volume.[1,4,7–9]

In domestic dogs and cats, platelet transfusions have been used to treat a variety of conditions associated with low platelet numbers, including primary immune-mediated thrombocytopenia, drug-induced thrombocytopenia, infectious diseases (eg, *Ehrlichia* and viral infections), incompatible blood transfusions, parasitic infections (eg, *Dirofilaria immitis*), bone marrow abnormalities, neoplasia, disseminated intravascular coagulation, blood loss, and vasculitis.[4,10] In addition to their general hemostatic and thrombotic properties, platelets have multiple other complex functions that are poorly understood but involve modulation of fibrinolysis, inflammation, vascular tone, and cellular growth.[8,11,a] Platelets carry a substantial number of pro- and antiangiogenic factors, including adhesive proteins, growth factors, and cytokines, that are released following platelet activation.[8,11,a] In addition, platelets can recognize bacteria, recruit immune cells, secrete bactericidal mediators, and absorb viral particles.[6,11,12]

Platelet concentrates used for transfusion are usually collected by means of apheresis and contain platelet concentrations at least 8 times the typical blood concentration. However, for maximal platelet function, fresh platelet concentrates must be stored at room temperature on a rotator. In addition, the US FDA has limited the shelf life of human platelet concentrates to 5 days because of the high risk of bacterial contamination.[4,8] Given these concerns, stockpiling fresh platelet concentrates is impractical without a steady supply of donors and ready access to the infrastructure needed for routine processing. These limitations are particularly pressing when dealing with wildlife and zoo animals because blood collection typically requires substantial coordination and processing infrastructure that is frequently unavailable.

Cold storage of platelets in additive solution eliminates the need for constant rotation and prolongs the shelf life,[13,14] and the low storage temperature slows the growth of bacterial contaminants[15] while maintaining platelet function. However, refrigeration needed for cold storage of platelets may not be available in remote environments, and the same sourcing and processing limitations associated with fresh platelet concentrates remain.

Cryopreservation of platelets allows for convenient, long-term storage of concentrated platelets.[4,8] However, the cryopreservation process is long,[16] and cryopreserved platelets have substantially reduced function and lifespan after being thawed. In addition, cryopreserved platelets must be stored in a freezer.[4]

The challenges associated with fresh, cold-stored, and cryopreserved platelet concentrates have driven the development of lyophilized (ie, freeze-dried) platelet-derived hemostatic agents that can overcome

many of the logistic disadvantages associated with other hemostasis products.[1,7,8,17-19,b] At least 2 lyophilized platelet preparations have been described; both have a shelf life longer than that for cryopreserved platelet products, while also having the benefit that they can be stored at room temperature or under standard refrigeration.[10,20,21,c]

Lyophilized platelet products are prepared for injection by rehydrating the dried platelets with sterile water. One formulation uses trehalose, a disaccharide used in a variety of plant and animal organisms to survive desiccation and withstand extreme seasonal temperature fluctuations, to stabilize the platelets.[1,16] These trehalose-stabilized platelets have a shelf life of 24 to 36 months at room temperature and a reported recovery rate > 85%[1,16] with preservation of hemostatic function following reconstitution.[1,7,13,14,22,b]

In studies involving animals with experimentally induced hemorrhage, the efficacy of lyophilized platelet products has varied. In thrombocytopenic rabbits, preemptive infusion of a lyophilized platelet product 15 minutes prior to induction of cutaneous hemorrhage resulted in a nearly 90% reduction in blood loss and significantly decreased mean coagulation time.[d] In splenectomized dogs undergoing cardiopulmonary bypass, administration of a single, large bolus of a canine-origin lyophilized platelet product resulted in increased hemostatic effects for at least 3 hours after the procedure, compared with a control solution.[8,20] Dose-safety evaluations involving administration of a human-derived lyophilized platelet product to cynomolgus monkeys (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*) did not reveal any evidence of intravascular coagulation or other inflammatory reactions.[1,8,23]

Given their long shelf life and ease of use, and the fact that they can be stored at room temperatures or in a refrigerator, lyophilized platelet products have obvious advantages in human medicine over other platelet products, but their potential in veterinary medicine, especially given the limited availability of fresh whole blood and fresh platelet concentrates, may be even greater. Studies[2,10,23,c,d] in animals with thrombocytopenia or noncompressible hemorrhage suggest that these products are efficacious and safe. Thus, they may be attractive for treatment of a variety of disease conditions in domestic animals.

In contrast, use of lyophilized platelet products in wildlife and zoo animals remains largely unexplored. Platelet evaluation in these species has been hindered by the multistep collection process required for apheresis and the impracticalities of storing fresh or cryopreserved platelets in a zoological setting. Nevertheless, there are many diseases of captive wild animal species that may benefit from treatment with lyophilized platelet products. For example, the massive internal hemorrhage associated with elephant endotheliotropic herpesvirus (EEHV) infection is a leading cause of death in young (1- to 8-year-old) captive Asian elephants (*Elephas maximus*).[5] Given that most Asian elephants living outside North America reside in southeastern Asian countries that frequently lack the requisite infrastructure and equipment to properly provide healthcare to elephants in the field, the acute internal hemorrhage associated with EEHV infection can be untreatable. Many facilities do not have access to the electricity and space required for other hemostatic agents, such as cold-preserved or cryopreserved platelets, to be used in the treatment of EEHV-associated hemorrhage. As the current treatment options for EEHV are not routinely successful, the hope is that the addition of a new hemostatic supportive modality to the current treatments for elephants with EEHV-associated hemorrhage will reduce the number of deaths associated with this disease.

Evaluating the in vivo efficacy and safety of human-origin versus species-specific lyophilized platelet products in a variety of zoological taxa is impractical. Thus, research should be prioritized on treatment of diseases for which current treatments have proven unsuccessful, as is the case with EEHV infection, and a focus should be placed on developing additional lyophilized platelet products for use in endangered species that have been documented to be at risk for developing difficult-to-treat hemorrhagic conditions. Along these lines, clinical trials evaluating the efficacy and safety of Asian elephant–derived lyophilized platelet products are of high importance. The evaluation process includes testing for blood types, growth factors, and cell marker expression and for the potential for transfusion reactions. This may necessitate collection of large volumes of blood for testing and quality control as well as for the eventual production of commercially available products. Especially for endangered species, therefore, this may be limited to large specimens that can be trained to routinely donate blood.

Despite the numerous benefits potentially associated with using lyophilized platelet products, these products do have some limitations. Most importantly, reconstituted lyophilized platelets persist in the circulation for relatively short periods.[1,7,8] Mean lifespan of endogenous platelets in humans is reportedly approximately 8 days, whereas reconstituted lyophilized platelets survive in the circulation for only 10 minutes to 2 hours.[1,22] This short lifespan may make lyophilized platelet products better for treatment of acute hemorrhagic events and for use prior to surgery, and less ideal for treatment of prolonged bleeding or chronic thrombocytopenia. Also, the efficacy of lyophilized platelet products in inducing hemostasis differs among species, and further research is needed to assess the mechanisms of these variations.[24]

Lyophilized platelet products have been in development for > 50 years with promising results for efficacy and safety in animal studies. Advantages of lyophilized platelet products include their prolonged shelf life and ease of storage and use. With retention

of > 85% of hemostatic activity following reconstitution, lyophilized platelet products may be able to overcome some of the challenges associated with treating animals with life-threatening hemorrhage, from trauma or otherwise. Further species-specific evaluations of lyophilized platelet products in wild animal species with hemorrhagic or thrombocytopenic conditions are needed. Results of in vitro and in vivo studies in multiple animal species suggest that lyophilized platelet products function as intended, but the variations in response among species requires further evaluation. A commercial canine-origin lyophilized platelet product has recently been released, and human-origin lyophilized platelet products are currently in development, providing the basis for continued research and development in many other species and applications. Identification of lyophilized platelet products as safe and effective will no doubt have a major impact on current care in veterinary medicine.

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Footnotes

a. Ramachandran N, Hiles MC. Use of sterilized, lyophilized platelets for multiple applications (abstr). *Cryotherapy* 2014;16(suppl):S60.

b. Fitzpatrick G, Dee J, Cliff R. Trehalose stabilized freeze dried human platelets, Thrombosomes, express surface markers, thromboelastogram (TEG) values and size distribution similar to two to three day old stored platelets (abstr). *Vox Sanguinis* 2010;99(suppl 1):262.

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