



TREHALOSE STABILIZED FREEZE DRIED HUMAN PLATELETS, THROMBOSOMES®, REDUCE BLOOD LOSS IN THROMBOCYTOPENIC RABBIT EAR BLEED MODEL BY AS MUCH AS 89.5%
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ABSTRACT

Thrombosomes® are a lyophilized derivative of human platelets, is being developed as a hemostatic product for treatment of civilian or military combat related trauma and shock conditions associated with life-threatening bleeding. Historical and recent data from Afghanistan and Iraq indicate that 35% of fatalities caused by hemorrhage on the battlefield could be prevented with improved hemostatic methods. Thrombosomes® is produced by a proprietary process that includes trehalose, other carbohydrates and a custom lyophilization process. Thrombosomes® maintains certain platelet functions such as adhesion, aggregation, thrombin generation, and clot stabilization. The efficacy of Thrombosomes® has been demonstrated in in-vivo models of uncontrolled bleeding; 1) such as an external injury produced over a background of busulfan-induced thrombocytopenia (rabbit), 2) lethal hemorrhage, arterial cut (rat), the former showing significant reduction in blood loss and in the latter significant yet transient improvement in survival time. Furthermore, Thrombosomes® has been subjected to safety studies in two species (rabbit and canine) where no test article associated adverse events (AE) or severe adverse events (SAE) have been observed during and after 2 and 14 days of observation. Histopathological studies at necropsy also resulted in negative findings. Thrombosomes® provides a unique hemostatic profile, which when combined with a long-shelf life, ambient storage conditions, and stability in extreme conditions, could meet a critical medical unmet need enabling treatment of life-threatening bleeding conditions in diverse circumstances and especially where fresh platelets are not available.

MATERIALS AND METHODS

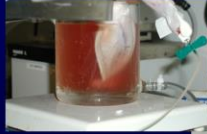
Rabbits were made thrombocytopenic following the method of Kuter and Rosenberg (Blood, 1995, 85: 2720-30). The bleeding study was performed if the platelet count on average was < 20%. An ear artery catheter was placed in one ear for withdrawal of 3ml of blood for Technetium-99m (Tc-99m) labeling of RBC using Ultratag kit. Labeled RBC were i.v. injected back in the same ear.

Thrombosomes® administration was performed 10 minutes after infusion of Tc-99m-labeled RBC. The Thrombosomes® or control preparations (vehicle, saline) were infused intravenously at 1ml/min using a syringe pump. The incision and bleeding study was initiated 15 minutes after administration. The other ear was pre-warmed by immersion in a 37°C saline bath as described by Lee et al (Transfusion Medicine, 2000, 96: 3630-3636). A site devoid of macroscopically visible vessels was identified in the ear, and a full-thickness incision (5 mm) was made. The ear was re-immersed in the saline bath for 30 minutes. Aliquots from the saline bath (1 ml) were removed periodically and simultaneous blood samples from the systemic circulation were obtained. The radioactivity was counted in each sample to determine the amount of Tc-99m-RBC lost over time.

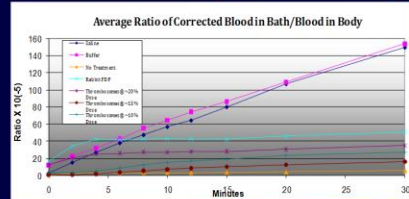
Sprague Dawley Rats were subjected to a (lethal) 50% tail amputation and allowed to bleed for ten minutes. A dose of Thrombosomes® (20% top load), in-date stored human platelets, or an equal volume of saline was then administered and the animals observed for 4 hours.

BLEEDING REDUCTION IN THROMBOCYTOPENIC NZWR

Control animal bleeding into saline bath

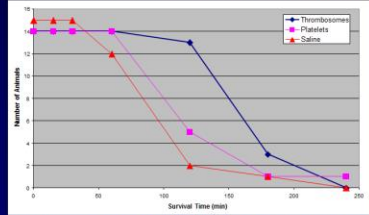


Thrombosomes® treated animal



Animals receiving doses of 10%, 15%, and 20% lost 83%, 90%, and 78% less blood than the control animals, respectively, those receiving rabbit FDP experienced a 75% reduction. All three dose groups were significantly different from the vehicle and saline control animals (p<0.5), however there was no significant difference in blood loss between dose levels. (N = 6 in each arm)

DOUBLED SURVIVAL TIME IN A LETHAL HEMORRHAGE MODEL



Thrombosomes® doubled survival time in a Sprague Dawley Rat lethal hemorrhage model from 60 minutes to 120 minutes.

GLP SAFETY

- Rabbits, 3 arms (6 in each arm)
 - 10X the 20% dose (~70mL in 70 min)
 - Thrombosomes®
 - Vehicle Control
 - Human 7 Day PLT*
 - Animals sacrificed at 2 and 14 days
 - No test article related AE, SAE, hematologic, blood chemistry or histopathological changes observed in the Thrombosomes® or Vehicle Control arms
 - Animals receiving human 7 day old PLT were observed to have SAE and histopathological changes
 - Anti-A,B from human O plasma caused a hemolytic transfusion reaction
- Canine, 3 arms (6 in each arm)
 - 10X the 20% dose (~130mL @ 1mL/min)
 - Canine Thrombosomes®
 - Vehicle Control
 - Fresh Canine Platelets
 - Animals sacrificed at 2 and 14 day
 - No test article related AE, SAE, hematologic, blood chemistry or histopathological changes observed

CONCLUSIONS

- The studies regarding the in vivo safety and efficacy of Thrombosomes® suggest the following:
 - Thrombosomes® delivers hemostatic efficacy in uncontrolled lethal arterial bleed and prolongs survival (rat)
 - Thrombosomes® delivers hemostatic efficacy following tissue injury associated with uncontrolled bleeding in NZWR with busulfan induced thrombocytopenia
 - Thrombosomes® exposure at multiples (10X) of the efficacy dosing regimens in the models described, (vide supra), failed to elicit test article associated adverse events in either the rabbit or canine.
 - These studies support continued investment in the development of Thrombosomes® for suitable indications in the military and civilian treatment of bleeding.

