In Vivo Efficacy of a Novel Oral, Small Molecule Composition in the Hemophilia A Murine Tail Clip Bleeding Assay

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Introduction

- An oral, small molecule, low-cost and shelf-stable therapy for hemophilia A currently does not exist
- As a result, large populations of patients in the developing world lack reliable access to essential hemophilia A medications
- HemoSavin Pharma is currently developing such a therapy with the aim to give every hemophilia A patient access to prophylactic treatment

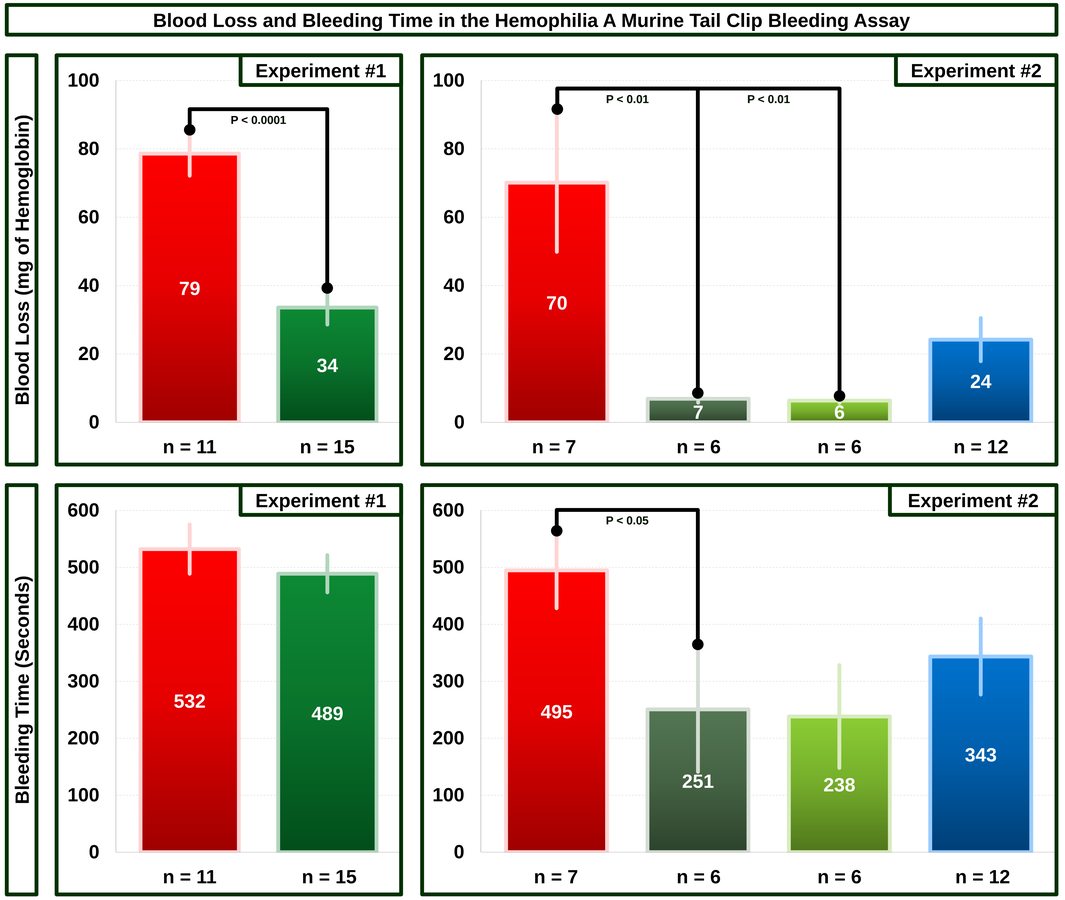
Objectives

- The hemophilia A mouse model is among the most commonly used animal models for drug development research
- These mice exhibit impaired coagulation times and bleeding into joints and soft tissues
- In this study, we sought to demonstrate the efficacy of our novel oral, small molecule composition and its variants in reducing blood loss and bleeding time in this model

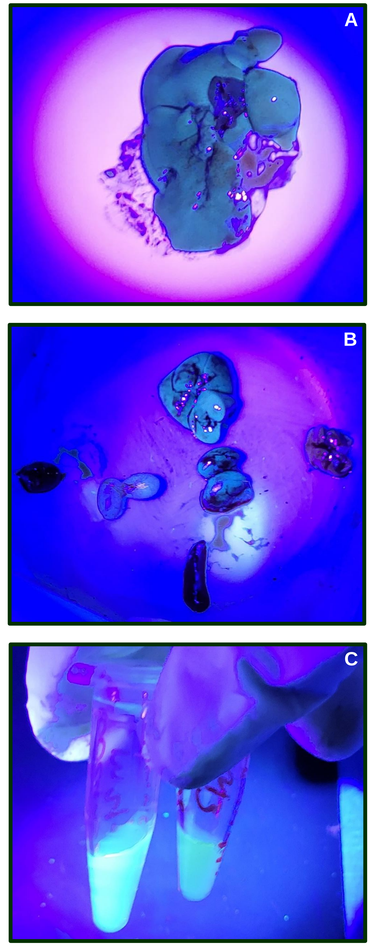
Methods

- Hemophilia A mice of strain B6;129S-F8tm1Kaz/J were sourced from Jackson Laboratory and bred at the University of Michigan's (UM's) Unit for Laboratory Animal Medicine (ULAM) to provide a sufficient number of animals for the study
- Mice were administered 250 mg/kg bodyweight of YSPKP, YSPKP-0.45>3K or YSPKP-Lys dissolved in distilled water via oral gavage once every day for three days prior to the tail clip
- Each mouse's weight and behavior was recorded during the gavage period
- After one hour following the final administration, mice were anesthetized with 2.5-5% isoflurane delivered via a vaporizer and distal segments of their tails corresponding to a diameter of 1.67 mm were removed with surgical scissors
- The tails were allowed to bleed freely into a 50 mL tube containing warmed phosphate-buffered saline for ten minutes, and the bleeding times and hemostatic plug formations were observed
- Tails were then cauterized and organs were removed and examined
- Blood loss was quantified via the Drabkin's hemoglobin assay

Results



Blood loss and bleeding time for YSPKP, YSPKP-0.45>3K, and YSPKP-Lys in the hemophilia A murine tail clip bleeding assay. In the first experiment, YSPKP reduced blood loss and bleeding time by 57% and 8% when compared to the water control group. In the second experiment, YSPKP-0.45>3k reduced blood loss and bleeding time by 90% and 49% while YSPKP-Lys reduced blood loss and bleeding time by 91% and 52%. The YSPKP-0.45>3K and YSPKP-Lys results were comparable to those from the normal control group.



Distribution of YSPKP in (**A**) the liver, (**B**) the heart, testes, liver, kidneys, spleen, lungs and (**C**) urine. A 395 nm light was used to visualize the organs.

Summary

- All three of the tested compositions exhibited a statistically significant reduction in blood loss when compared with the water control
- The original YSPKP composition reduced blood loss by 57% when compared to the water control, while YSPKP-0.45>3K and YSPKP-Lys reduced blood loss by 90% and 91%
- Corresponding reductions in bleeding time were also observed for the three compositions but only the 49% reduction of YSPKP-0.45>3K was statistically significant
- Necropsy did not reveal any abnormalities in major organs and behavioral analysis did not suggest any toxic effects
- The distribution of the compositions into major organs was visible under a 395 nm UV light

Conclusion

- We have demonstrated the in vivo efficacy of our novel oral, small molecule composition, YSPKP, and its two variants, YSPKP-0.45>3K and YSPKP-Lys in reducing blood loss in the hemophilia A murine tail clip bleeding assay
- We have also shown that these compositions do not appear to cause death or toxic effects

Acknowledgments

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- Arno Scheller served as the laboratory supervisor
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