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**ESTIMATING THERAPEUTIC PLASMA LEVELS OF AMINOCAPROIC ACID IN
THE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)**

FINAL REPORT for the International Elephant Foundation

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2. Overall and specific conservation needs addressed with this project

Infection with Elephant Endotheliotropic Herpesvirus (EEHV) can result in devastating loss of young elephants and this disease severely impacts conservation efforts for maintaining self-sustaining captive populations. One cause of mortality following infection with this virus is uncontrolled hemorrhage and exhaustion of clotting elements. This project addresses the need for novel treatment options for hemostatic disorders in elephants, including EEHV.

Our research focuses on the process of fibrinolysis, or blood clot breakdown. Every blood clot made in the body is eventually broken down by fibrinolytic pathways; in many bleeding disorders, accelerated fibrinolysis results in rapid breakdown of clots, exacerbating hemorrhage. Anti-fibrinolytic drugs help control hemorrhage by preventing clot breakdown. We determined the effective plasma concentration of one anti-fibrinolytic drug, aminocaproic acid (EACA), to investigate its potential to treat bleeding disorders in Asian elephants. The main indication of this drug would include prevention of the hemorrhage related to EEHV or other causes of uncontrolled hemorrhage.

3. Goals and objectives of the project

Our goal is to establish plasma levels of EACA required to inhibit fibrinolysis in the Asian elephant. Our objectives are 1) to improve critical care of elephants, 2) to develop new therapies for hemorrhagic EEHV cases, and 3) to contribute to the sustainability of the captive Asian elephant population by decreasing mortality due to hemorrhagic disorders, which include EEHV and reproductive tract disease.

4. Specific actions of each objective:

We have collected plasma samples from six captive Asian elephants, and developed an *in vitro* model of hyperfibrinolysis to use in this project. We have also determined that aminocaproic acid is effective in inhibiting fibrinolysis in Asian elephant plasma *in vitro*. This *in vitro* work confirms that aminocaproic acid has potential benefits for elephants suffering from hemorrhagic disease.

5. Potential changes in original protocol

No major changes have been made to the protocol, but a few technical changes were made:

a) Complete blood count (CBC) and chemistry panels were not collected from the elephants at the time of coagulation sampling. Though this would have been ideal, additional blood samples were being collected for other ongoing research projects, which limited samples available to us. Blood for CBC and chemistry panels were collected at a separate time for all elephants in the study and these results have been evaluated for any parameters that may affect our results.

b) Initially we planned to run plasma and whole blood TEG on each individual elephant, to ensure each individual had normal coagulation before inclusion in the study. Instead, we used

plasma TEG and traditional coagulation tests (activated partial thromboplastin time, prothrombin time, and fibrinogen) to screen elephants for inclusion in the study. On the basis of these tests, one elephant was excluded.

c) In response to trial-and-error refinement of the *in vitro* TEG assay, concentrations of reagents used were different from those predicted in the grant protocol.

6. Major findings and accomplishment to date

We have completed the proposed study and achieved our goal of determining the effective concentration of EACA in elephant plasma. Final data analysis has been completed.

The first step of the project involved development of an *in vitro* model of hyperfibrinolysis. We induced a fibrinolytic state in elephant plasma *in vitro*, through addition of tissue Plasminogen Activator (tPA). The elephant plasma was sensitive to tPA; a concentration of 500 U/ml tPA resulted in complete fibrinolysis within 15 minutes of maximum clot formation.

We subsequently added increasing amounts of EACA to the hyperfibrinolytic plasma, to estimate the drug's therapeutic plasma concentration. Final statistical analysis of our results found an effective plasma concentration of 61.5 µg/ml (95% CI = 34.6 - 88.5 µg/ml). This concentration is markedly higher than the estimated therapeutic concentration for horses (5.82 µg/ml), and lower than the estimated therapeutic concentration for humans (130 µg/ml).

These data demonstrate the sensitivity of elephant plasma to pro-fibrinolytic mediators *in vitro* (which has not previously been described) and also confirms that EACA is effective in inhibiting fibrinolysis in elephant plasma. Doses used in companion animals may be inappropriate for elephants due to differences in therapeutic plasma concentration. Aminocaproic acid has the potential to be a useful therapy for hemostatic disorders in elephants, but further research is needed to determine safe oral dosage. A manuscript reporting our findings has been submitted to the Journal of Zoo and Wildlife Medicine, and is currently in revisions.

7. Problems encountered

No problems have been encountered.

8. Measure of success of project

This project has been successful in achieving our goal of determining the effective plasma concentration of EACA in elephant plasma.

9. "Next steps"

The ideal next step would be a pharmacokinetic trial of EACA in Asian elephants, to determine the most effective oral or parenteral dosage of EACA required to achieve effective plasma concentrations. This would require a new study.

10. Human interest story.

We have seven elephants at our zoo, including a new baby, so we are all keenly interested in maintaining the health and welfare of captive and free-ranging Asian elephants, and in

supporting a self-sustaining captive population. There is still a lot more for us to learn about coagulation and fibrinolysis in Asian elephants, and how disorders of coagulation may contribute to elephant morbidity. We are pleased to be able to contribute to the field of elephant medicine and hope to make continued research contributions in the future.

11. Summary

Our goal was to determine if aminocaproic acid (EACA), a drug used to prevent uncontrolled bleeding, could be effective in Asian elephants. Elephants can experience uncontrolled bleeding from a variety of causes, including following an infection with the elephant endotheliotropic herpesvirus (EEHV). Due to their large size and limited blood banking resources, treatment options for an elephant with uncontrolled bleeding are severely limited. Aminocaproic acid is widely used in human and domestic animal medicine to stabilize blood clots and prevent hemorrhage, and may have applications to elephants as well. We collected plasma samples from the elephants at our zoo and used a tool called thromboelastography to investigate fibrinolysis (blood clot breakdown) and to estimate the amount of EACA required to prevent clot breakdown, and thus prevent bleeding. We succeeded in developing an *in vitro* model of hyperfibrinolysis, mimicking a diseased state. We then succeeded in reversing this diseased state through addition of EACA. The elephant plasma required a lower concentration of EACA to inhibit fibrinolysis, compared to humans and dogs. Our results suggest that EACA may be effective in preventing bleeding in elephants *in vivo*, and may be a valuable treatment option for a variety of bleeding disorders, including animals with EEHV infection presenting with hemorrhage.

12. Short summary

Using a test called thromboelastography, we created an *in vitro* model of an elephant bleeding disorder (hyperfibrinolysis) and successfully inhibited hyperfibrinolysis *in vitro* using aminocaproic acid. These results provide important information on the potential for aminocaproic acid to treat bleeding disorders in elephants, including infection with EEHV.

13. Organizations associated with this project:

International Elephant Foundation: The IEF provided the necessary funds to complete this project through a generous gift

Rosamond Gifford Zoo: The Rosamond Gifford Zoo in Syracuse, New York, houses the six Asian elephant sampled for use in this project.

Cornell University: The researchers involved in this project are members of different specialty at the Cornell University College of Veterinary Medicine and have formed a team dedicated to the advancing the health and care of elephants.

14. Financial report:

Budget Item	Amount granted	Amount spent through 12/1/15	Balance
Venipuncture supplies	\$100	\$58.60	\$41.40
CBC/Chemistry/PT/Fibrinogen panels	\$468	\$670	-\$202
TEG assays	\$2,100	\$2,000	\$100
Transportation between Cornell and the Zoo	\$123	\$0	\$123
Total	\$2791	\$2728.60	\$64.40

15. Images attached separately.

16. Video attached separately.

17. Publications

A manuscript entitled “Effect of ϵ -aminocaproic acid on fibrinolysis in plasma of Asian elephants (*Elephas maximus*)” has been submitted to the Journal of Zoo and Wildlife Medicine (pending revisions).

18. No websites, blogs or social media accounts are maintained by any of the investigators.