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Establishment of an Induced Hemophilia A Model in Anesthetized Cynomolgus Monkeys

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ABSTRACT

Hemophilia A is a bleeding disorder due to deficiency of Factor VIII (FVIII), an essential blood-clotting protein. A tail bleeding model in cynomolgus monkeys with antibody-induced hemophilia was initially developed by Prisys Biotech, then further refined at Altasciences, to evaluate the hemostatic effect of pro-coagulant compounds. Vehicle or polyclonal anti-FVIII antibody was intravenously administered 60 minutes prior to the initiation of the tail bleeding procedure under full anesthesia. Blood samples were collected prior to treatment, after administration of the vehicle or anti-FVIII antibody, and before euthanasia at the end of the experiment. Comparison of Activated Partial Thromboplastin Time (APTT) and Thromboelastography (TEG) was performed. Bleeding time and hemoglobin loss were evaluated at four intervals from 0 through 40 minutes post initial tail bleeding with challenge procedure every 10 minutes included. No changes in APTT were observed in the vehicle control group. Prolongation of APTT approximately doubled by the sheep anti FVIII induction treatment. Reaction (R) time, Clot formation (K) value and α-angle in TEG for vehicle control animals were stable at all time points, while induction of hemophilia significantly increased the mean R time, elevated mean K value and lowered the α-angle. The mean total bleeding time and loss of hemoglobin through 40 minutes post vein puncture increased approximately 3.7 and 2.6- fold, respectively, in the hemophilia induced animals. Induction of hemophilia A with sheep anti-FVIII at a dose level of 16.4 mg/kg resulted in expected perturbations in blood coagulation and clot formation (APTT prolongation, R and K value increase, and α-angle decrease), and substantially increased bleeding time and blood loss, confirming successful establishment of an experimentally induced hemophilia A model in cynomolgus monkeys.

INTRODUCTION

The present study was undertaken to refine a tail bleeding model in anesthetized cynomolgus monkeys with antibody-induced hemophilia and to evaluate the hemostatic effect of pro-coagulant compounds.

MATERIALS AND METHODS

Animals and Their Treatment

Male cynomolgus monkeys (2.7 to 3.8 years old, 2.0 to 3.7 kg) were given either vehicle (histidine buffer, CA) or sheep-anti FVIII (16.4 mg/kg, TA1) 60 minutes prior to initiation of tail bleeding procedures followed by a second dose of vehicle or Recombinant Activated Factor VII (rFVIIa) (1.8 mg/kg, TA2) 5 minutes prior to initiation of tail bleeding procedures.
Blood samples were collected prior to treatment, after administration of the vehicle or anti-FVIII antibody, and before euthanasia at the end of the experiment from either a peripheral vein or a tail vein from chemically-restrained anesthetized animals.
Animals were anesthetized during the entire bleeding procedure and euthanized after procedures before regaining consciousness. All animal-related procedures were approved by the IACUC.

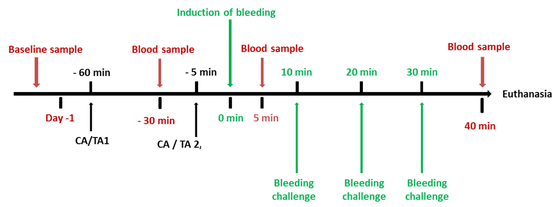

Figure 1. Experimental Design. Animals were anesthetized prior to induction of bleeding.

Tail Surgical Procedure and Blood Collections for Evaluation

- Animals were sedated with ketamine/xylazine, intubated and placed on isoflurane gas.
- The distal tail was prepped aseptically with ~ 3 cm line marked on the middle of the tail.
- A surgitron with a needle electrode was used to make a skin incision with ~ 1.5 cm of the tail vein isolated.
- The tail was submerged in saline at 37ºC in a glass jar for at least 10 minutes.
- A double vein puncture was performed with 21 gauge needles to induce bleeding.
- The tail was placed back into 3 L saline with the incision site entirely submerged and positioned so that the bleeding could be observed through the side of the glass jar.
- The tail was monitored for 10 minutes. If bleeding stopped during that period, the time was recorded. If bleeding reoccurred, the time of start and stop was recorded.
- Ten minutes after the vein puncture, the site was swabbed 3 times to reestablish bleeding. The tail was then placed in a fresh jar of saline to record the bleeding time.
- These steps were performed at the following intervals: 0 to 10, 10 to 20, 20 to 30 and 30 to 40 minutes post puncturing the vein.

Activated Partial Thromboplastin Time (APTT) Analysis

Coagulation samples were analyzed using a STA Compact coagulation analyzer.

Thromboelastography (TEG) Analysis

TEG was performed using kaolin-activated citrated whole blood samples and a TEG 5000® thromboelastograph hemostasis analyzer. The following parameters were measured:

1. Reaction time (R)
2. Clot formation time (K)
3. Angle (α)

Quantification of Blood Loss via Hemoglobin Evaluation

One 50 mL sample from each 3.5 L saline portion was collected for later hemoglobin analysis at Novo Nordisk A/S. The 50 mL were centrifuged with supernatant discarded, whereafter the tube contents were reconstituted in 250 µL and frozen. The tubes were thawed and a 1.75 ml of lysis buffer (ABX Lysebio, ref. 0906013, Horiba ABX SAS, Montpellier, France) added, resulting in a new total volume of approximately 2.0 mL. Hemoglobin concentration of each fraction was measured by spectrophotometry on an ACLTOP 500 (Instrumentation Laboratory, Bedford, MA). Under assumption of homogeneity, the total hemoglobin content in the original 3.5 L saline portion was calculated by multiplication by 70.

RESULTS AND DISCUSSION

Summary of Activated Partial Thromboplastin Time (APTT) Data

No change in APTT was noted in the control animals (Group 1; CA/CA). APTT approximately doubled by the sheep anti FVIII (TA1) induction treatment and remained similarly prolonged through the end of the monitoring interval in the hemophilia animals (Group 2; TA1/CA). A significant procoagulant effect was apparent after administration of rFVIIa (TA2) to Group 3 (TA1/TA2). Compared with the TA1-driven 30-minute post dose prolongation, APTT was reduced by approximately 50% at both 10 minutes post TA2 administration and 40 minutes Post Initial Tail Bleed (PTB) and was comparable to the acclimation value (**Figure. 2**).

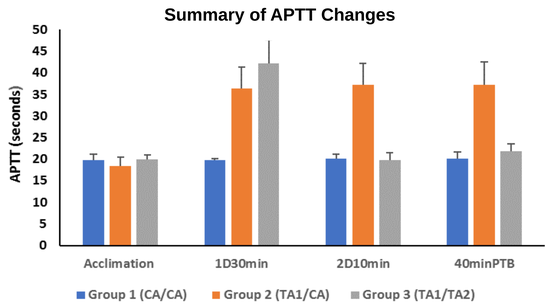

Figure 2. Effect of sheep-anti FVIII (16.4 mg/kg, TA1) and rFVIIa (1.8 mg/kg, TA2) on APTT. APTT values are mean ± SEM. 1D30min: 30 min post Dose 1; 2D10min: 10 min post Dose 2; 40minPTB: 40 min post initiation of tail bleed

Summary of TEG Data

TEG is a method for testing the overall efficiency of blood coagulation, inclusive of coagulation factor function, platelet function, clot strength and fibrinolysis. Four values that represent clot formation are determined by the test: the R value and the speed of clot formation (K value and angle).
At 30 minutes after TA1 administration, the mean R value increased 6.2- fold (**Figure. 3**), the mean K value increased 5.7- fold (**Figure. 4**), the mean angle value decreased to 49% (**Figure. 5**), compared to the pre dose values in Group 2. These changes were correlated with APTT alterations, which confirmed the sheep anti-FVIII antibody potential to induce hemophilia in cynomolgus monkeys. A procoagulant effect of TA2 (rFV11a) was apparent at 10 minutes after TA2 dose administration, as full recovery to acclimation values or similar to those of the concurrent control group occurred.

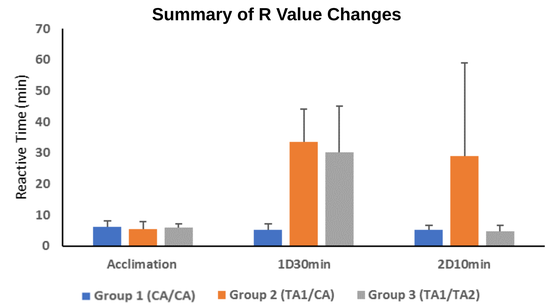

Figure 3. Effect of sheep-anti FVIII (16.4 mg/kg, TA1) and rFVIIa (1.8 mg/kg, TA2) on R value in TEG. Reactive time values are mean ± SEM. 1D30min: 30 min post Dose 1; 2D10min: 10 min post Dose 2

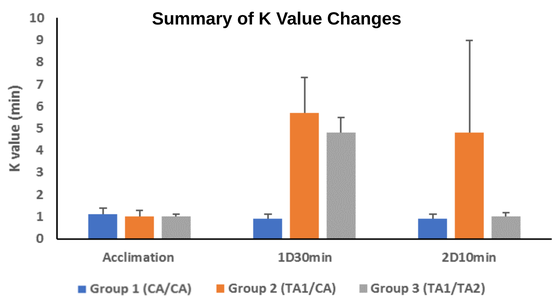

Figure 4. Effect of sheep-anti FVIII (16.4 mg/kg, TA1) and rFVIIa (1.8 mg/kg, TA2) on K value in TEG. K values are mean ± SEM. 1D30min: 30 min post Dose 1; 2D10min: 10 min post Dose 2

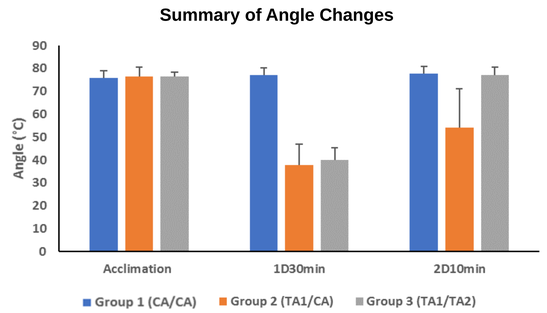

Figure 5. Effect of sheep-anti FVIII (16.4 mg/kg, TA1) and rFVIIa (1.8 mg/kg, TA2) on angle change in TEG. Angle values are mean ± SEM. 1D30min: 30 min post Dose 1; 2D10min: 10 min post Dose 2

Visual Observation of Blood Loss via Bleeding Times

The average bleeding time in the control animals (Group 1) was 520 seconds (8.67 minutes), or 27% of the bleeding time in the hemophilia animals (Group 2) after induction by sheep anti FVIII (TA1). The average bleeding time in Group 3 (TA1/TA2) was 33% of that in Group 2 which indicated a substantial procoagulant effect of rFVIIa (TA2).

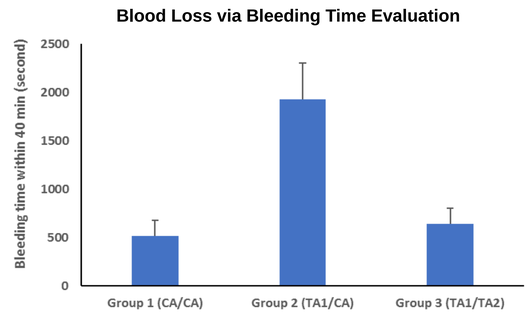

Figure 6. Effect of sheep-anti FVIII (16.4 mg/kg, TA1) and rFVIIa (1.8 mg/kg, TA2) on mean bleeding time through 40 minutes post vein puncture. Animals were anesthetized during bleeding.

Quantification of Blood Loss via Hemoglobin Evaluation

Blood loss within 40 minutes of the initial vein puncture was quantified by the group averages of the sum of hemoglobin concentrations in collected blood at 0–10 minutes, 10–20 minutes, 20–30 minutes, and 30–40 minutes post vein puncture. The average hemoglobin value was 69437, 190963 and 67714 nMol, in Groups 1, 2 and 3, respectively. The average hemoglobin value in Groups 3 (TA1/TA2) was 35% of average value in the hemophilia animals in Group 2. The mean hemoglobin value in Group 3 is comparable with that of the control group (CA/CA). The calculated maximum bleed for any animal in the HA control group was no more than 50 mL (typical 30–40 mL).

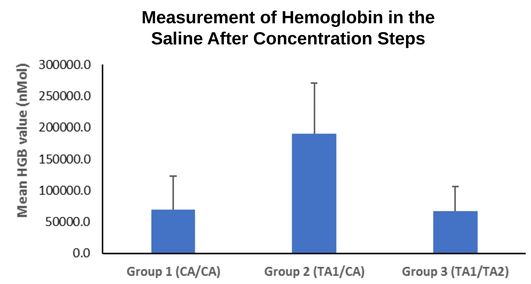

Figure 7. Effect of sheep-anti FVIII (16.4 mg/kg, TA1) and rFVIIa (1.8 mg/kg, TA2) on mean hemoglobin value within 40 minutes post vein puncture. Animals were anesthetized during bleeding.

CONCLUSION

Induction treatment with sheep anti-FVIII at a dose level of 16.4 mg/kg resulted in expected perturbations in blood coagulation and clot formation (APTT prolongation, R and K increase, and angle decrease), confirming successful establishment of experimentally induced hemophilia A model in cynomolgus monkeys. Increased tail bleeding under anesthesia further authenticated the induced hemophilic phenotype.

Administration of rFVIIa at dose level of 1.8 mg/kg reversed the APTT and TEG changes and decreased the bleeding time and blood loss to levels comparable to those of the control article group.

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