Slounase, a Modified Snake Venom Hemocoagulase Combined with FXa, Enhances Hemostasis and Limits Bleeding in Both Normal and Hypocoagulant Conditions

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Abstract

Background: Uncontrolled post-traumatic bleeding is the leading cause of preventable death. Hemocoagulase, a thrombin-like serine protease from snake venom, converts fibrinogen to fibrin and thus may reduce blood loss in hemorrhagic medical conditions. A modified hemocoagulase combined with activated factor X (FXa), may potentially improve the hemostatic effect of hemocoagulase. However, the *in vivo* hemostatic effect of these reagents is not well characterized due to lack of reliable *in vivo* hemostasis models.

Aims: To determine the efficacy of slounase *and* hemocoagulase on hemostatic clot formation and bleeding at the site of vascular injury i*n vivo*.

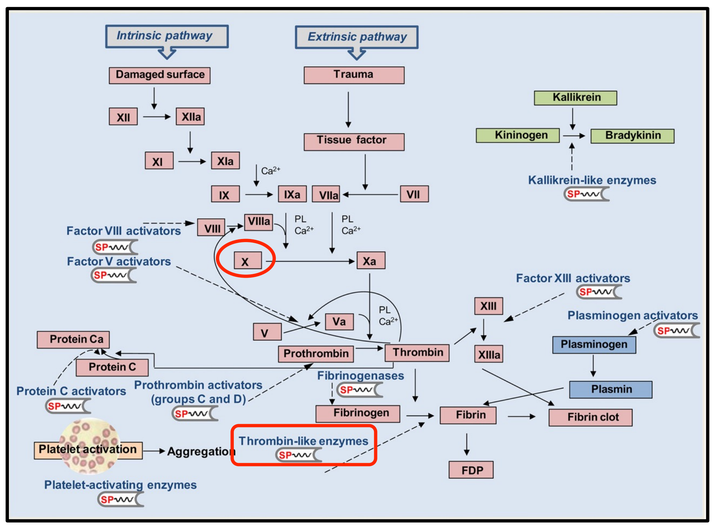
Methods: Effect of slounase, hemocoagulase, and FXa on human platelet function was assessed *in vitro*. *In vivo* hemostatic efficacy was determined in mice using intravital microscopy laser ablation hemostasis models and liver puncture hemostasis model.

Results: Both slounase and FXa, but not hemocoagulase, enhanced human platelet aggregation induced by thrombin. As expected intravenous injection of 25U of heparin into WT mice prevented the hemostatic clot formation at the site of vascular injury and FXa treatment did not restore clot formation *in vivo.* Hemocoagulase treatment in heparin-treated hypocoagulant mice resulted in detectable fibrin formation but no notable change in platelet recruitment in the clot. Interestingly, slounase treatment resulted in a significant enhancement of both fibrin and platelet content in control WT mice and restored the clot formation in heparin-treated hypocoagulant mice. Similar results were also observed in the laser-ablated saphenous vein hemostasis model. The bleeding time was shorter in slounase-treated hypocoagulant mice both in vascular injury models and using liver pricking injury model.

Conclusions: Slounase enhances hemostasis in normal mice and restores hemostasis in hypocoagulant conditions via both enhancing fibrin formation and platelet activation while hemocoagulase only enhances fibrin formation *in vivo.*

Background

Schematic of physiological hemostatic pathways and the sites of action of snake venom serine proteinases



Batroxobin: a serine proteinase from South American lancehead snakes of the genus Bothrops.

- Batroxobin from B. atrox snake venom is used as a hemostatic drug (Reptilase).
- Converts fibrinogen to fibrin I at the site of vascular injury independent of thrombin.

Serrano SM. Toxicon. 2013 Feb;62:19-26 .

Figure 1. Uncontrolled post-traumatic bleeding is the leading cause of potentially preventable death. While the use of the currently available anti-platelet agents has significantly improved cardiovascular outcomes, they are associated with increased risk of bleeding. Thus, the development of novel hemostatic reagents and reliable *in vivo* hemostasis models that can assess their hemostatic efficacy are warranted. Snake venom serine proteinases (SVSPs) are a group of extensively studied toxins affecting mainly the hemostatic system.

Rationale

Modified snake venom hemocoagulase combined with activated factor X (FXa) may have a better hemostatic effect in vivo

Results

Slounase enhances thrombin-induced human platelet aggregation *in vitro*

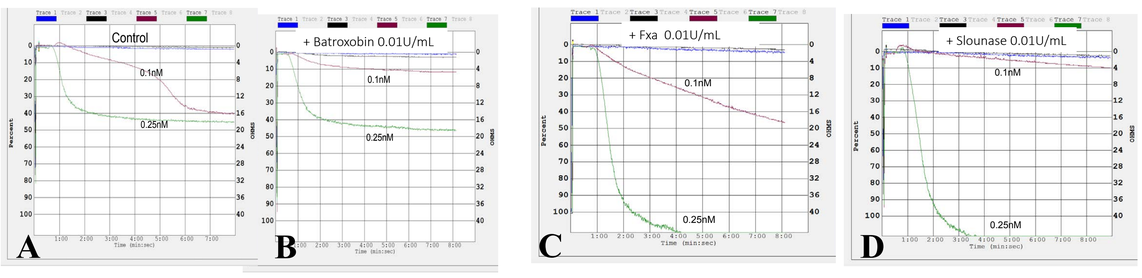


Figure 2. Washed human platelets incubated with either control buffer (A), 0.01U/mL of Batroxobin (B), FXa (C) or Slounase (D) for 5 min. Platelets aggregation was induced by thrombin (0, 1 and 0.25 nM) and aggregation was recorded with light transmission aggregometry.

FXa, batroxobin and slounase enhance *in vivo* hemostasis in WT mice

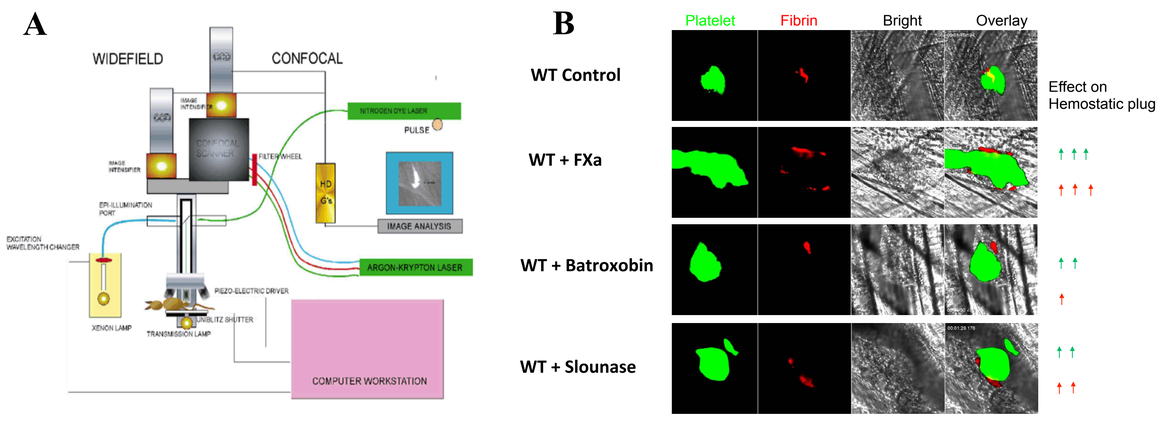


Figure 3. A: Schematic diagram of the confocal–widefield high-speed microscope used for real-time intravital microscopy of the microcirculation of a living mouse. B: Mice were anesthetized, and tail vein injected with anti-platelet (green) and anti-fibrin(red) antibodies followed by i.v. treatment of 1U/Kg of FXa, Batroxobin or Slounase for 5 min prior to microscope study. The cremaster muscle arterioles were exteriorized and visualized under intravital microscopy. A high intensity laser pulse from the laser ablation system was used to puncture a hole in the cremaster muscle arteriole wall as visualized by red blood cell (RBC) leakage from the vessel. Images of hemostatic plug formation were acquired in real-time with a fluorescent microscope. Data is representative of 6 independent experiments.

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Impaired hemostasis in heparin-treated hypocoagulant mice

Cremaster arterial rupture model of hemostasis

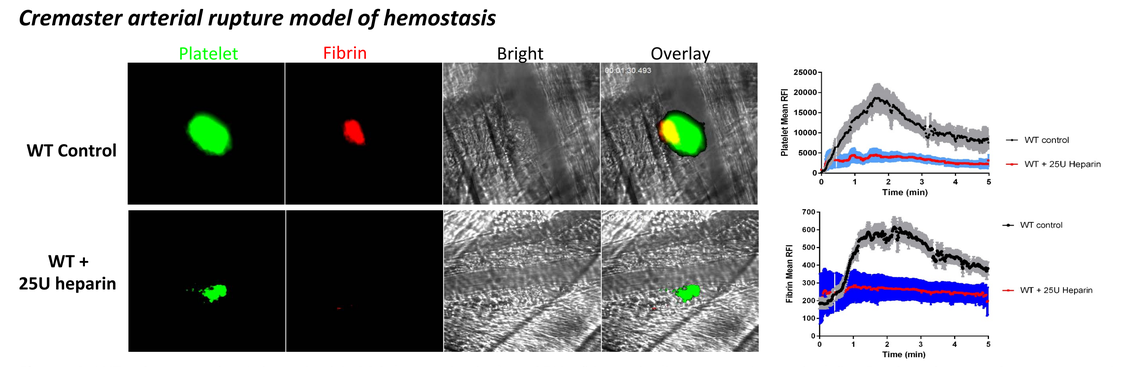

Figure 4. WT mice were i.v. Injected with PBS (control mice) or 25U of heparin (hypocoagulant mice) for 5 min prior to microscope study. Cremaster muscle arteriole wall was ruptured by laser injury to form hemostatic plug at the site of injury. Representative picture of platelet (green) and fibrin (red) formation within the clot were recorded in real time in vivo under microscope (left). Quantitate analysis of platelet recruitment and formation of fibrin in the clot over time (Right).

Slounase enhanced both platelet recruitment and fibrin formation within the hemostatic plug in heparin-treated hypocogulant mice

A ***Cremaster arterial rupture model of hemostasis***

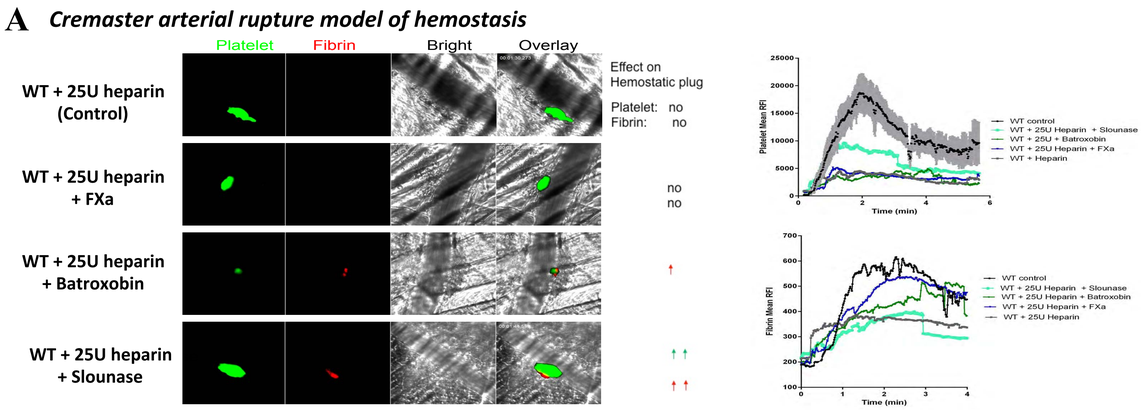

Figure 5. WT mice were i.v. treated with 25U of heparin (hypocoagulant mice) followed by i.v. treatment of 1U/Kg of FXa, Batroxobin or Slounase for 5 min prior to microscope study. Cremaster muscle arteriole wall was ruptured by laser injury to form hemostatic plug at the site of injury. Representative picture of platelet (green) and fibrin (red) formation within the clot was recorded in real time in vivo under an intravital microscope (left). Quantitate analysis of platelet recruitment and formation of fibrin in the clot over time (Right).

B ***Laser ablation saphenous vein hemostasis model***

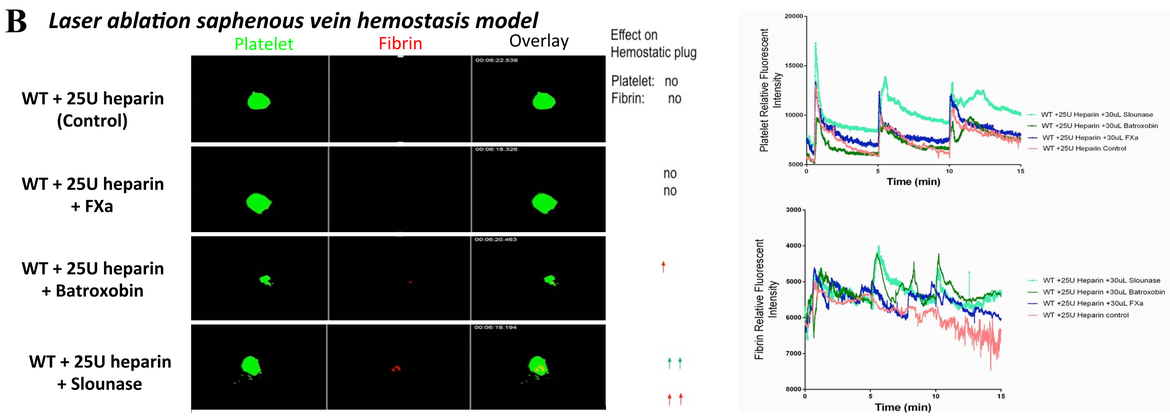

Figure 6. WT mice were i.v. treated with 25U of heparin (hypocoagulant mice) followed by i.v. treatment of 1U/Kg of FXa, Batroxobin or Slounase for 5 min prior to microscope study. The saphenous vein was prepared and exposed under intravital microscopy. A penetrative injury on the saphenous vein wall was induced by maximal laser injury at 30 seconds followed by repetitive injury at 5 min and 10 min. Hemostatic plug formation in saphenous vein was monitored and recorded in real time. Representative picture of platelet (green) and fibrin (red) formation within the clot after the 2nd injury was shown (Left). Quantitate analysis of platelet recruitment and formation of fibrin in clot over time (Right).

Summary

- Both batroxobin and slounase enhance thrombin-induced human platelet aggregation.
- Batroxobin, FXa and slounase treatment enhance hemostasis in normal WT mice.
- Slounase enhance hemostasis in heparin-treated hypocoagulant mice *in vivo*.
- Our data strongly indicates that slounase has a better hemostatic effect compared to batroxobin in both normal and hypocoagulant states via enhancing platelet recruitment and fibrin formation in the clot.

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