Nitric Oxide Anticoagulation Preserves Systemic Thrombin Generation during Polytrauma in 72-hour Swine Model

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Introduction

- Systemic anticoagulation is used to avoid thrombosis during extracorporeal life support (ECLS) but causes bleeding. Strategies to minimize systemic anticoagulation may enable safer ECLS in combat wounded patients.
- Liquid-infused nitric oxide (NO)-releasing biocompatible ECLS circuit tubing in combination with NO gas added to the oxygenator sweep gas is a promising new therapy to avoid systemic anticoagulation.
- The Calibrated Automated Thrombogram (CAT) assay provides unique functional insight into plasma thrombin generation (**Fig 1**).

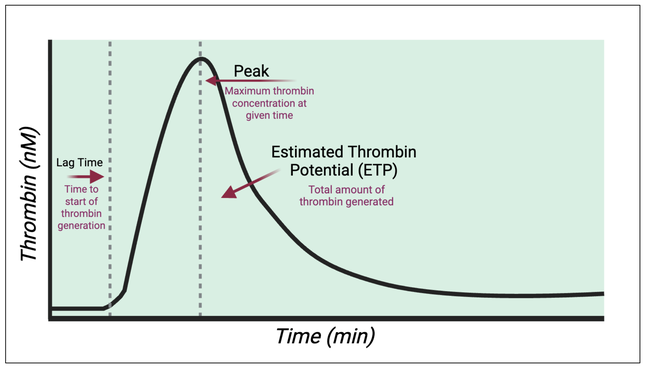


Figure 1. Thrombin generation curve illustrating key parameters of CAT assay including lag time, peak thrombin concentration, and estimated thrombin generation.

- Pre-clinical translational research studies at the AREVA Research Laboratory provide the unique opportunity to study injured animals, observing the impact of heparinization versus nitric oxide therapy on coagulation.

Objective

- **Objective**: Compare thrombin generation in systemic blood from swine that received NO-based anticoagulation versus traditional heparin.
- **Hypothesis**: NO-based, heparin-free ECLS demonstrates reduced thrombin generation compared to heparinized controls.

Methods

- Anesthetized (TIVA), mechanically ventilated Yorkshire swine (45-60kg) underwent polytrauma injury and were cannulated for VV-ECLS after a 30-min shock period (**Fig 2**).

Methods (cont'd)

- CAT was performed on platelet-poor plasma (PPP) using PPP-Reagent HIGH (Stago). Samples were analyzed on a Fluoroskan Ascent™ fluorometer following addition of hexadimethrine bromide (Polybrene) to neutralize residual heparin.
- **Statistical Methods**: Between- and within-group time dependent changes were assessed via mixed models with linear regression used for graphical representation. All tests were two-sided with α=0.05.

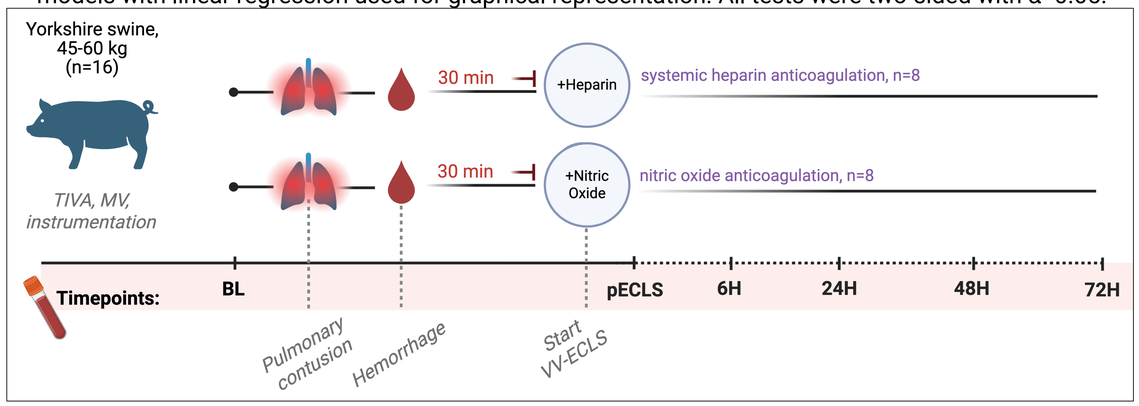


Figure 2. Timeline of sample collection and experimental design.

VV-ECLS Specifications	+Heparin Group	+NO Group
• 23 Fr dual-lumen cannula, R internal jugular vein • Novalung with XLUNG Kit • Blood Flow target: 1.5 L/min • Sweep Gas target: 3.0 L/min	• 2000 U UFH bolus at ECLS initiation • Continuous UFH Dose: administered to ACT target of 150% baseline	• No systemic heparin sparing 2000 U cannulation bolus • NO-releasing ECLS circuitry with NO (80ppm) added to oxygenator sweep gas

Results

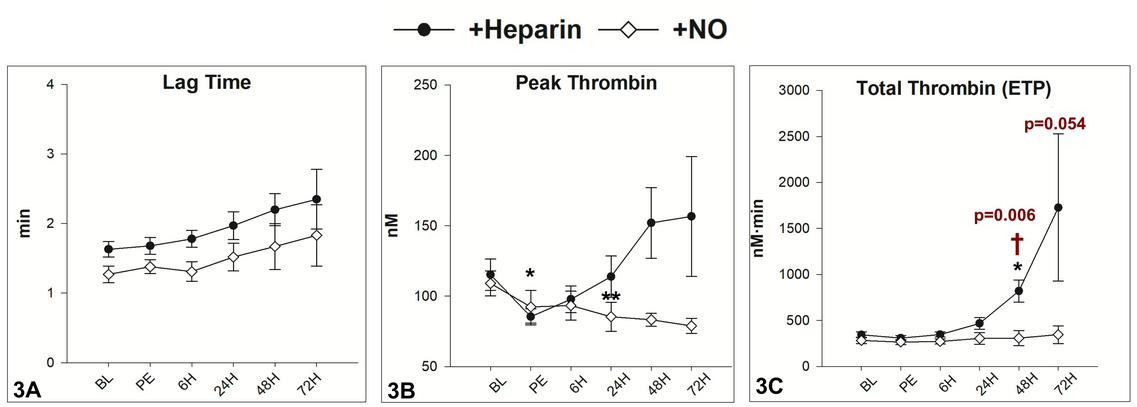
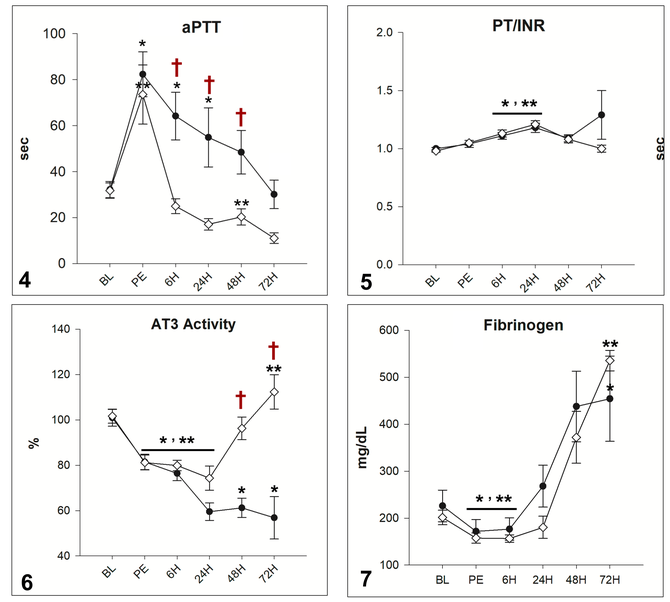


Figure 3. CAT group differences. *Significant with +Heparin compared to BL. **Significant within +NO compared to BL. † Significant +Heparin versus +NO. All tests two-sided with p<0.05 considered significant.

- No group differences in thrombin lag time and peak thrombin detected (**Fig 3A, 3B),** although peak thrombin was reduced in +NO at 24 hours versus BL (p=0.010), but unchanged in +Heparin (**Fig 3B**).
- Total thrombin generated (ETP) was significantly elevated in +Heparin compared to +NO at 48-hours (p=0.006) and continued to trend higher compared to +NO (n.s.) at 72-hours (p=0.054) (**Fig 3C**).

Results (cont'd)



Figures 4-7. Group differences between PT (**4**), PTT (**5**), AT3 (**6**), Fibrinogen (**7**). *Significant with +Heparin compared to BL. **Significant within +NO compared to BL. † Significant +Heparin versus +NO.

- No group differences in PT **(Fig 4).** PTT was elevated in +Heparin group starting at PE after heparin initiation **(Fig 5).**
- AT3 concentration decreased in both groups until 24H. +NO group recovered to BL by 48H while +Heparin group remained below BL through end of study **(Fig 6**).
- Fibrinogen concentration increased in both groups following injury and ECLS initiation (**Fig 7**).

Conclusions

- NO-based anticoagulation during ECLS avoided elevated thrombin generation that was observed in heparin anticoagulated controls.
- This study explores a novel mechanism for NO-mediated mitigation of circuit thrombosis, separate from platelet-specific effects.
- Further investigation to elucidate therapeutic safety and efficacy of NO-based anticoagulation in context of trauma is ongoing in our laboratory.

Acknowledgments

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