Cardiovascular Biomarkers Working Group: Solving the Problem of Drug-Related Thrombosis

Presentation by Marjory Brooks, DVM

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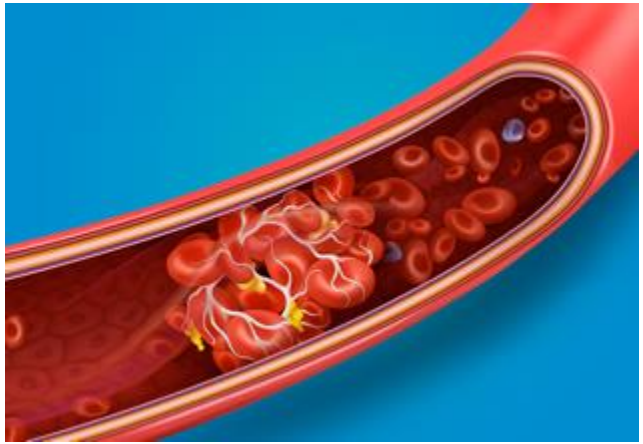
DRUG RELATED THROMBOSIS

- Problem Statement:
  - Preclinical studies have proven unreliable predictors of thrombotic complications.
- Objective:
  - Identify translational biomarkers suitable for early preclinical drug pipeline



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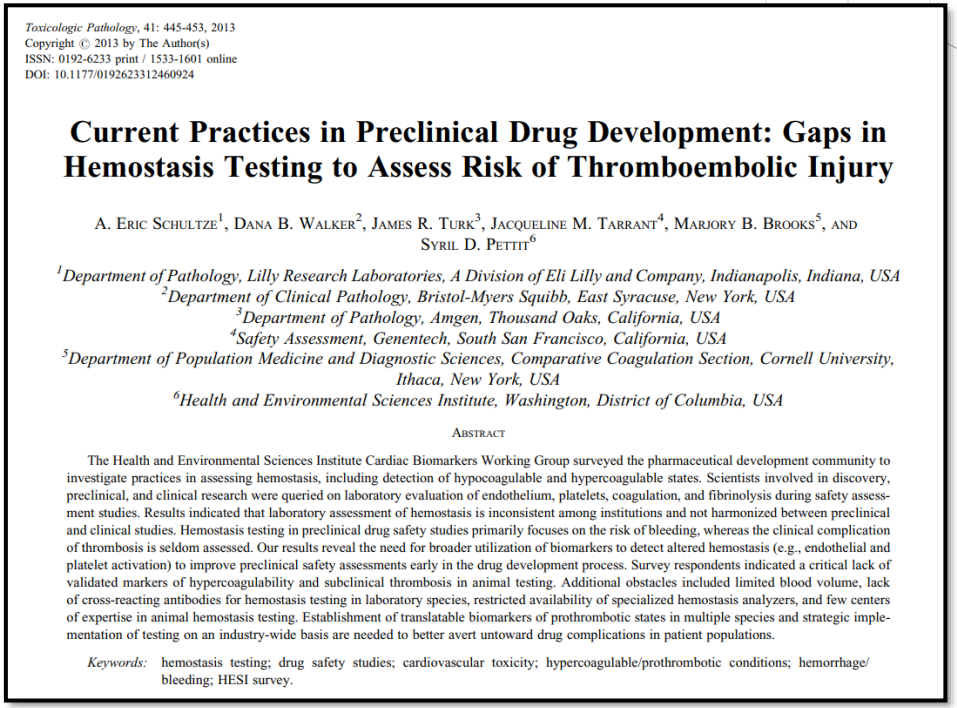
Drug Related Thrombosis- The Problem

- Thrombotic Complications
  - Myocardial infarction
  - Ischemic stroke
  - Deep vein thrombosis
  - Pulmonary embolism
  - Microangiopathy
  - Thrombotic thrombocytopenia

- Implicated drugs/biologics
  - COX2 Inhibitors
  - Hormones/growth factors
    - Oral contraceptives, HRT
    - Erythropoietin
    - Testosterone
  - Antineoplastics
    - Anti-estrogens
    - Tyrosine kinase inhibitors
    - Mitomycin
    - L-asparaginase
  - Heparin
  - COVID19 Vaccine

Cardiovascular Biomarkers Working Group

- Why does preclinical testing fail?
- Industry Gap Analysis
  - Mismatch in endpoint selection
    - Over-reliance on overt thrombosis
  - Test focus on hemorrhagic complications
  - Lack of thrombotic risk biomarkers



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Current Practices in Preclinical Drug Development: Gaps in Hemostasis Testing to Assess Risk of Thromboembolic Injury

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Abstract

The Health and Environmental Sciences Institute Cardiac Biomarkers Working Group surveyed the pharmaceutical development community to investigate practices in assessing hemostasis, including detection of hypocoagulable and hypercoagulable states. Scientists involved in discovery, preclinical, and clinical research were queried on laboratory evaluation of endothelium, platelets, coagulation, and fibrinolysis during safety assessment studies. Results indicated that laboratory assessment of hemostasis is inconsistent among institutions and not harmonized between preclinical and clinical studies. Hemostasis testing in preclinical drug safety studies primarily focuses on the risk of bleeding, whereas the clinical complication of thrombosis is seldom assessed. Our results reveal the need for broader utilization of biomarkers to detect altered hemostasis (e.g., endothelial and platelet activation) to improve preclinical safety assessments early in the drug development process. Survey respondents indicated a critical lack of validated markers of hypercoagulability and subclinical thrombosis in animal testing. Additional obstacles included limited blood volume, lack of cross-reacting antibodies for hemostasis testing in laboratory species, restricted availability of specialized hemostasis analyzers, and few centers of expertise in animal hemostasis testing. Establishment of translatable biomarkers of prothrombotic states in multiple species and strategic implementation of testing on an industry-wide basis are needed to better avert untoward drug complications in patient populations.

Keywords: hemostasis testing; drug safety studies; cardiovascular toxicity; hypercoagulable/prothrombotic conditions; hemorrhage/bleeding; HESI survey.

Schultze et al., 2012. Toxicologic Pathology, https://doi.org/10.1177/0192623312460924

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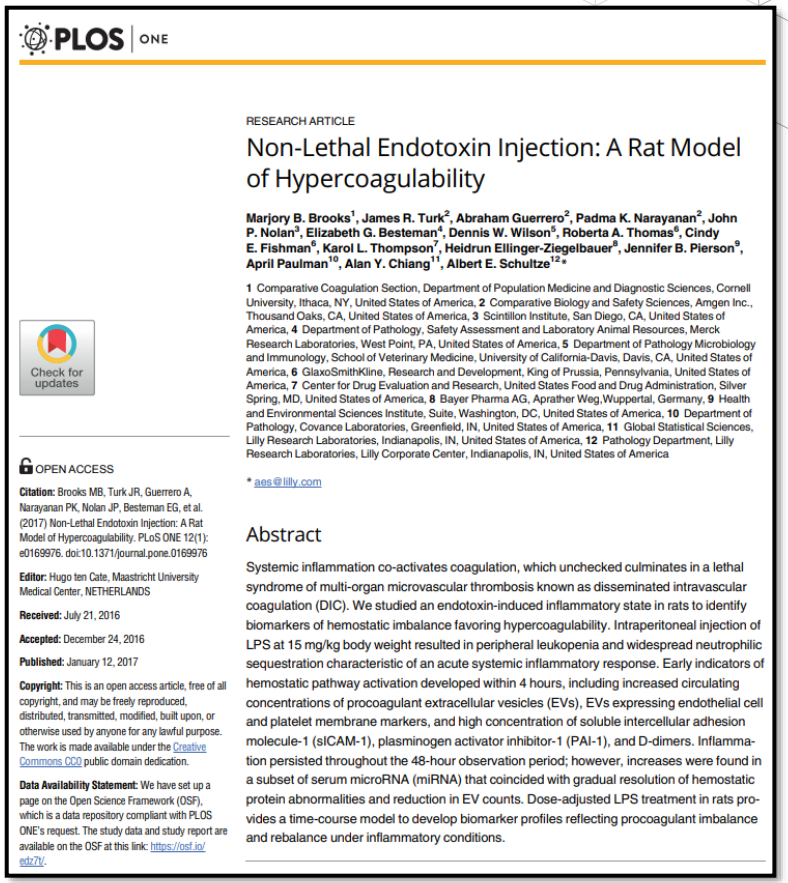
Proof of Concept Studies: Modeling Pre-Thrombotic States

- Study 1: Non-overt (incipient) thrombosis
  - Endotoxin induced
  - Time course: serial sample analyses
- Acute thrombo-inflammatory model
  - Endothelial cell activation
  - Leukocyte activation
  - Procoagulant response

Brooks et al., 2017. PLOS One, https://doi.org/10.1371/journal.pone.0169976



PLOS ONE

RESEARCH ARTICLE

Non-Lethal Endotoxin Injection: A Rat Model of Hypercoagulability

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Data Availability Statement: We have set up a page on the Open Science Framework (OSF), which is a data repository compliant with PLOS ONE's request. The study data and study report are available on the OSF at this link: https://osf.io/edz7t/.

Abstract

Systemic inflammation co-activates coagulation, which unchecked culminates in a lethal syndrome of multi-organ microvascular thrombosis known as disseminated intravascular coagulation (DIC). We studied an endotoxin-induced inflammatory state in rats to identify biomarkers of hemostatic imbalance favoring hypercoagulability. Intraperitoneal injection of LPS at 15 mg/kg body weight resulted in peripheral leukopenia and widespread neutrophilic sequestration characteristic of an acute systemic inflammatory response. Early indicators of hemostatic pathway activation developed within 4 hours, including increased circulating concentrations of procoagulant extracellular vesicles (EVs), EVs expressing endothelial cell and platelet membrane markers, and high concentration of soluble intercellular adhesion molecule-1 (sICAM-1), plasminogen activator inhibitor-1 (PAI-1), and D-dimers. Inflammation persisted throughout the 48-hour observation period; however, increases were found in a subset of serum microRNA (miRNA) that coincided with gradual resolution of hemostatic protein abnormalities and reduction in EV counts. Dose-adjusted LPS treatment in rats provides a time-course model to develop biomarker profiles reflecting procoagulant imbalance and rebalance under inflammatory conditions.

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Proof of Concept Studies: Study 1

- Results
  - Early procoagulant excess = clot formation
  - Later rebalance = clot resolution
- Stimulus/response biomarkers
  - Procoagulant markers
    - Plasma proteins (DD, sICAM, PAI-1)
    - Procoagulant extracellular vesicles
  - Regulatory pathways
    - microRNA analyses

Study 2: Dietary Induced Thrombo-Inflammation

- Background
  - Thrombosis = multifactorial & complex
  - Comorbidities increase thrombotic risk
    - Age
    - Inactivity
    - Cardiac disease
    - Obesity & metabolic syndrome

- Project approach
  - Preclinical model of increased thrombotic risk
  - Drug “stress test”

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Study 2: Dietary Induced Thrombo-Inflammation

- Fat-induced hypercoagulability
  - ZDF rat model
  - Type 2 diabetes & obesity
- Study design
  - Feed to target weight
    - Low fat vs. high fat diet
  - Monitor
    - Metabolic derangements
    - Hemostatic balance

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Use of the ZDF rat to model dietary fat induced hypercoagulability is limited by progressive and fatal nephropathy

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ARTICLE INFO

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Thrombin
Thromboxane

ABSTRACT

Introduction: Zucker diabetic fatty (ZDF) rats are used widely as an animal model of metabolic syndrome and insulin resistance. Our study focused on the effects of high versus low dietary fat on the development of Type 2 diabetes in obese male ZDF rats (*fa/fa*), including biomarkers to detect early signs of hypercoagulability and vascular injury in the absence of overt thrombosis. Methods: In this study, male (5/group) 10-week-old CRL: ZDF370(obese) rats were fed low (LFD, 16.7% fat) or high fat (HFD, 60% fat) diet for 12 or 15 weeks. Cohorts of 5 rats within diet groups were scheduled for sample collection after weeks 12 and 15. Results: HFD–fed ZDF rats had oily coats, lower rates of food consumption, more accelerated weight gain and increased serum cholesterol (+15%) and triglyceride concentrations (+75%) vs. LFD–fed ZDF rats. Urinary ketones were observed only in HFD–fed ZDF rats and greater urine glucose and protein concentrations in HFD–fed ZDF vs. LFD–fed ZDF rats were seen. Hemostasis testing showed ~2-fold greater fibrinogen concentration, increased von Willebrand factor concentration, and high thrombin generation in HFD–fed ZDF vs LFD–fed ZDF rats. Increased mortality in the HFD–fed ZDF rat was attributed to exacerbations of altered carbohydrate metabolism as evidenced by ketonuria and nephropathy leading to renal failure. Discussion: This characterization shows that the ZDF rat at the age, sex and weight used in this study is highly sensitive to dietary fat content that can exacerbate prothrombotic, metabolic and renal disturbances and increase mortality.

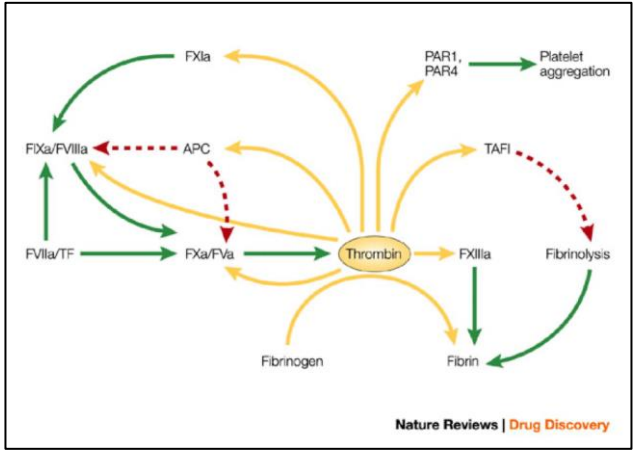
Pugsley et al., 2021. Journal of Pharmacological and Toxicological Methods
https://doi.org/10.1016/j.vascn.2020.106933

Study 2: Dietary Induced Thrombo-Inflammation

- Results
- High fat diet induced:
  - Metabolic syndrome & diabetes
    - Accelerated weight gain
    - Hypercholesterolemia & triglyceridemia
    - Glucosuria, ketonuria
  - Prolonged procoagulant stimulus
    - Hemo-inflammatory protein increase
      - Fibrinogen, von Willebrand factor, PAI-1
    - High thrombin generating capacity

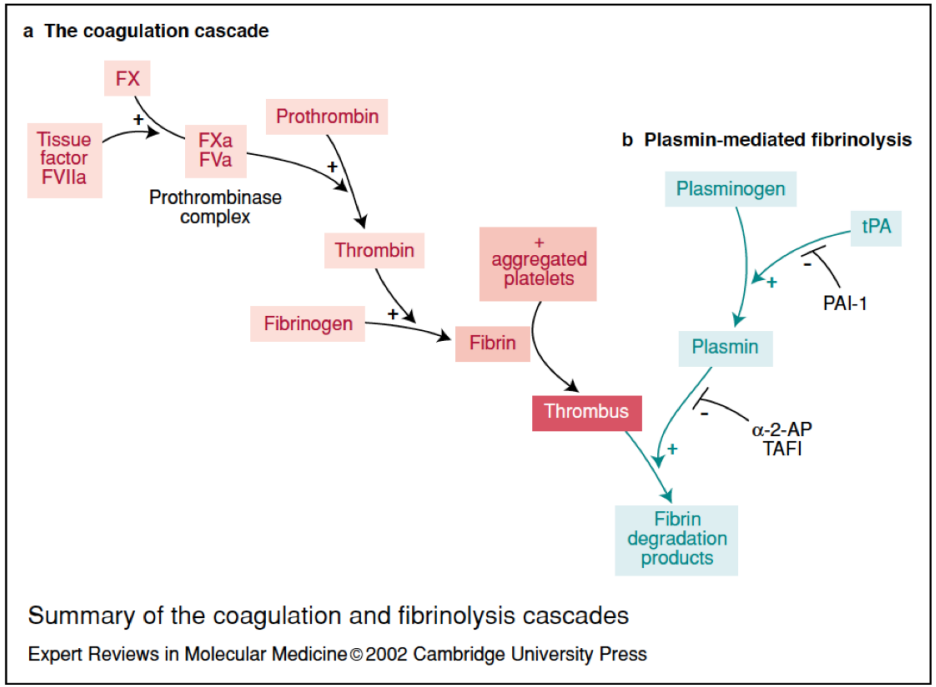
Study 2: Dietary Induced Thrombo-Inflammation

- Thrombin Generation
  - Thrombin = key hemostatic protein
    - Fibrin clot formation & platelet activation
    - Feedback regulation
- ZDF model response to high fat
  - High peak thrombin & overall thrombin generating potential
  - Risk factors for venous thrombosis in people



Active - Study 3: Defined Stimulus Models

- Simplified prothrombotic imbalance
  - Procoagulant excess
    - Tissue factor stimulus
  - Ineffective fibrinolysis
    - Plasminogen inhibitor
- Pilot study
  - Optimize infusion protocol
  - Dose and time selection

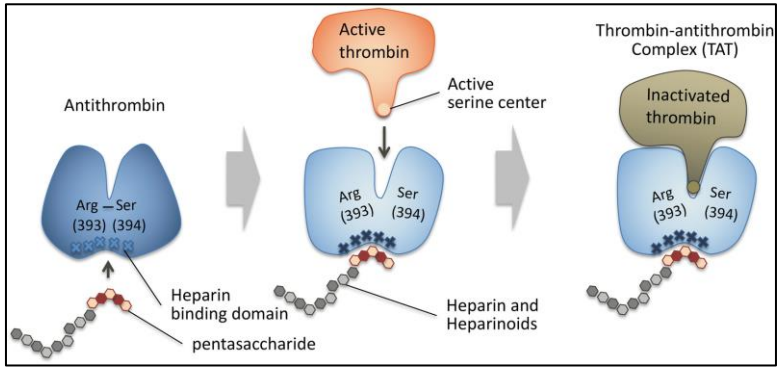


Summary of the coagulation and fibrinolysis cascades

Expert Reviews in Molecular Medicine©2002 Cambridge University Press

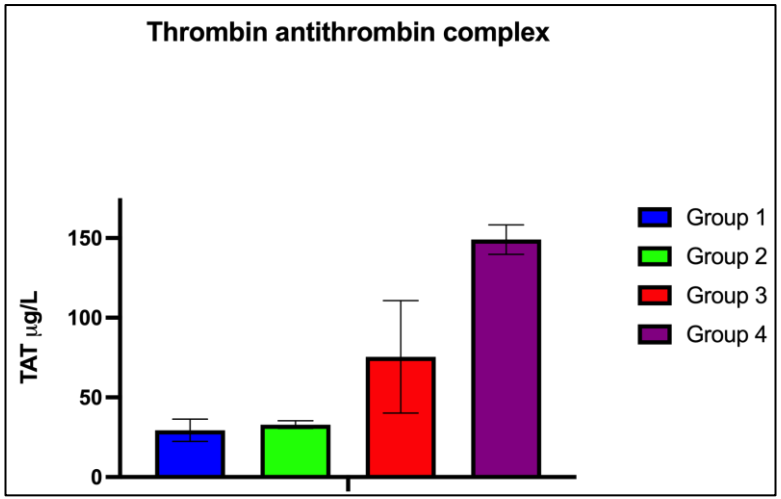
Active Study 3: Defined Stimulus Model

- Tissue factor infusion
- Biomarker = TAT complex



Iba T. (2021) Thrombin-Antithrombin System. In: Moore H.B., Neal M.D., Moore E.E. (eds) Trauma Induced Coagulopathy. Springer, Cham. https://doi.org/10.1007/978-3-030-53606-0_4

- Preliminary results
  - Dose-related procoagulant excess



Cardiovascular Biomarkers Working Group: Goals 2021

- Prothrombotic Signature Development
  - Translational across species
  - Early indicators of procoagulant imbalance
    - Combined functional & quantitative biomarkers
- Applications
  - Drug & biologic testing
  - Disease modeling

- Cross Collaborations
  - Vascular biology
  - Immunology
  - Hematology
  - Biostatistics/computational biology

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Call for Participation & Expertise:

Please reach out to co-managers if interested in partnering or joining our Workgroup, as well as if you have access to resources or have expertise in the following:

- Expertise in:
  - Coagulation & Cardiac Biomarkers
  - Platelet physiology
  - Microfluidics
  - Proteomics
  - Tissue Factor & Tranexamic Acid
  - Study design parameters
- Resources
  - Animals & models
  - Aid in conducting assays
  - Statistical analysis

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Questions?