Role of Inflammation in DVT: Its Relationship with Thrombosis

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Disclosures

Nothing To Disclose

Virchow’s Triad (1856)

Circulatory stasis
Hypercoagulability
Endothelial injury

Stewart’s Original Hypothesis
Venous Thrombosis

- Thrombus Forms
- Leukocytes/Platelets Activate
- Coagulation Proceeds on Platelet Surfaces
- Leukocytes/Platelets Layer - Amplification

Selectins

Selectins are glycoproteins found primarily on endothelial cells, leukocytes and platelets.
They are involved in trafficking of leukocytes in acute and chronic inflammatory processes, including:
- Post-ischemic inflammation in muscle, kidney and heart
- Scar formation
- Atherosclerosis
- Glomerulonephritis
- Lupus Erythematosus
- DVT

Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets

P-selectin
- Earliest Elevated Glycoprotein Expressed by Activated Platelets and Endothelial Cells
- Mediates Adhesion of Leukocytes, Platelets and Cancer Cells to Endothelial Cells
- P-selectin:PSGL-1 Triggers Release of Procoagulant Microparticles - Fibrin Formation and Thrombus Growth
- Increases on Monocytes Expression of Tissue Factor, Phosphatidylserine Exposure Surface-dependent Thrombin Generation

E-selectin
- Enhances endotoxin induced tissue factor (TF) mediated coagulation in humans carrying the S128R E-selectin allele
- Patients homozygous for the S128R E-selectin allele have an increased risk for VTE recurrence
- E-selectin raises the affinity and avidity of 2 (CD18) integrins which support neutrophil trafficking to sites of acute inflammation and recruit platelets and red blood cells (Chase SD et al, Ann Biomed Eng 2012)

Delta-CT (^CT) Mouse
- Gene Deletions Exons 13 and 14
- P-selectin Cytoplasmic Tail
- Homozygous Mice Have Normal Phenotype
- Express Circulating Plasma P-selectin 3-4 Fold Increase vs. Wild Type Mice
- Hypercoagulable from Microparticles

Thrombus Mass Day 2

- ^CT vs. WT, PKO, EPKO, P<.01
- 50% vs. 16%
Neutrophil Extracellular Traps

Endothelium
- Co-culture of ECs and Neutrophils promote NET formation; in turn, NETs induce EC death.
- NETs bind platelets and support their aggregation; Histone (H4) activates platelets through TLR2 and TLR4-dependent mechanisms, which then release polyphosphates which augment coagulation enhancing Factor XI and prothrombin activation complex.

Platelets
- NETs bind platelets and support their aggregation; Histone (H4) activates platelets through TLR2 and TLR4-dependent mechanisms, which then release polyphosphates which augment coagulation enhancing Factor XI and prothrombin activation complex.

Red Blood Cells
- RBCs avidly bind to NETs and RBCs may promote coagulation by exposing PS and altering blood viscosity.

Coagulation
- NETs stimulate fibrin formation and deposition in-vitro, and fibrin co-localizes with NETs in thrombi.
- NETs provide a scaffold for thrombi resistant to tPA-induced thrombolysis.

P-selectin Inhibition Therapeutically Promotes Thrombus Resolution and Prevents Vein Wall Fibrosis Better than Enoxaparin and an Inhibitor to von Willebrand Factor

Hypothesis
- By limiting thrombus amplification either by inflammatory or platelet inhibition with P-selectin or VWF inhibition, thrombosis will be treated better than standard LMWH anticoagulation.

Baboon Model and Experimental Design

Netosis induced by thrombin-activated platelets rosetting with neutrophils is inhibited by anti-P selectin aptamer, and antibody to PSGL-1

NET formation from stimulated neutrophils in ^CT mice is significantly enhanced, suggesting that NET formation may contribute to increased thrombosis in these mice.

References
### Baboon Groups

#### n=3 Control

**Treatments**
- n=3 Anti-P-selectin Treatment (ARC5462)
  - 2mg/kg IV on day 2. Then 1mg/kg Sub Q on day 3, followed by 1mg/kg Sub Q bid until euthanasia on day 21 after thrombosis.

- n=3 LMWH Treatment
  - 1.5mg/kg SQ once on day 2, then 1.5mg/kg Sub Q once daily until euthanasia on day 21 after thrombosis.

- n=3 Anti-VWF Treatment (ARC15105)
  - Started on Day 2. 250µg/kg IV, on day 2. Repeat single dose of 250µg/kg IV on Days 6, 10, and 14 after testing platelet aggregates with PFA-100 test. Euthanasia occurred on day 21 after thrombosis.

### Baboon Groups (Prophylactic Groups)

#### n=4 Anti-P-selectin Prophylactic (ARC5692)

Begun the day of surgery 3mg/kg (2mg/kg IV and 1mg/kg Sub Q) and continued 1mg/kg Sub Q for 6 days; Euthanized on day 21 after thrombosis.

#### n=3 Anti-VWF-Prophylactic (ARC15105)

Dosed pre-surgery. 250µg/kg IV, once. Dose repeated on day 2 (after testing platelet aggregates with PFA-100 test) for 6 days; Euthanized on day 21 after thrombosis.

### Magnetic Resonance Venography (Including Prophylactic Groups)

#### Ascending Phlebography

- **CTR**
  - B0: 80%
  - B2: 50%
  - B6: 13%
  - B14: 0%
- **LMWH-Tx group**
  - B0: 80%
  - B2: 50%
  - B6: 13%
  - B14: 0%
- **Anti-VWF-Aptamer-Tx group**
  - B0: 80%
  - B2: 50%
  - B6: 13%
  - B14: 0%
- **Anti-Psel-Aptamer-Tx group**
  - B0: 80%
  - B2: 50%
  - B6: 13%
  - B14: 0%

### Duplex ultrasound analysis: iliac valve function.

- **CTR group**
  - Valve competent
  - Valve incompetent
- **LMWH-Tx group**
  - Valve competent
  - Valve incompetent
- **Anti-VWF-Aptamer-Tx group**
  - Valve competent
  - Valve incompetent
- **Anti-Psel-Aptamer-Tx group**
  - Valve competent
  - Valve incompetent

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Coagulation Tests (Bleeding risk)

E-Selectin Inhibitor GMI-1271

- E-selectin antagonist, GMI-1271
- Small molecule antagonist that specifically inhibits E-selectin and mimics the bioactive conformation of the sialyl-Lex carbohydrate ligand
- Presently, the compound is being evaluated for use in blood cancers and other cancers that are also associated with elevated risk of metastasis

Myers DD et al, unpublished data