Mechanisms Linking Blood Clotting to Liver Toxicity and Repair

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A Bit about Me

• BS, Biochemistry, Colorado State University (malaria research)
• PhD, Pharmacology/Toxicology, Michigan State University (liver toxicology)
• Postdoctoral, Scripps Research Institute (cell signaling and blood coagulation)
• Assistant Professor, University of Kansas Medical Center
• Associate Professor → Professor, Michigan State University
• @TFFVIIa: “Dad. Husband. Scientist. Chicken wing enthusiast. Dad joke influencer”

• Other notable stuff:
  • Society of Toxicology Secretary-Elect
  • MSU IACUC Vice-Chair
  • PI of an NIH grant focused on first-time summer undergraduate research
Acknowledgements and My Research Team

**Luyendyk Laboratory:**
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**Collaborators:**
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- Patrick Starlinger, MD (Vienna)

**Research support**

http://cvm.msu.edu/liverletdie
Intersection between Blood Coagulation and Liver Disease

- Healthy Liver
- Coagulation Proteases and Their Targets
- Disease Progression
- Repair and Regeneration
- Diseased Liver
Brief Overview and Objectives

• Essentials of blood clotting and its regulation

• How does a liver toxicologist develop a passion for blood clotting?
  • A bit on liver physiology and function . . . and how that road leads to clotting

• Essentials to connect liver damage to blood coagulation activation
  • Example: acetaminophen hepatotoxicity

• Examples of mechanistic toxicology impacting discoveries in hepatology and hemostasis and thrombosis
Very Likely That You Are Familiar with or Have Observed Blood Clotting
The Hemostatic System (Coagulation and Platelets) Plays an Important Role in Cessation of Bleeding after Vascular Injury (from Paper Cuts to Trauma)

Regulation of Blood Coagulation

Thrombosis: When a Blood Clot Stops Flow through a Blood Vessel

1 in 4 deaths worldwide are from conditions causing thrombosis

Pharmaceuticals
Platelet inhibitors
Anticoagulants

The dinnertime conversation might be: so-and-so is taking a “blood thinner.”

WORLD THROMBOSIS DAY
13 OCTOBER

https://www.worldthrombosisday.org/

https://www.cdc.gov/ncbddd/dvt/infographic-impact.html

https://www.worldthrombosisday.org/
“You Told Us You Were a Toxicologist”

“You Study the Liver”

“How Does a Liver Toxicologist End Up Interested in Blood Clotting???”
Liver Is the Primary Site of Coagulation Protein Production

1° site of synthesis

Proteins involved in blood coagulation
The Liver Performs Multiple Key Physiological Functions

**Coagulation factors**
- Albumin
- Angiotensinogen
- Thrombopoietin

**Protein synthesis**
- Albumin

**Nutrient homeostasis**
- Fat digestion/vitamin uptake (bile)
- Fatty acid/lipid metabolism
- Vitamin A storage
- Glucose metabolism and storage

**Detoxification**
- Drugs, environmental contaminants, endogenous chemicals
- Pathogen/bacterial product
- Clearance
  - Liver is rich in immune cells

**Blood pressure regulation**
- → Platelet production

**Liver is rich in immune cells**

Delicate Rebalancing of Coagulation in Patients with Liver Disease

1° site of synthesis

Procoagulant factors
Anticoagulant proteins

Intersection of Hemostasis and Liver Disease

Clinical Investigation (Human patients)

Discover basis for altered hemostasis in liver disease

TOXICOLOGY

Basic Research (Experimental models)

Define mechanistic impact of hemostatic system on liver toxicity and disease

TOXICOLOGY

Acetaminophen Hepatotoxicity

Clinically Relevant Liver Damage and Excellent Experimental Tool
Acetaminophen-Induced Liver Injury

• Acetaminophen (paracetamol; APAP) is a widely used over-the-counter analgesic and antipyretic (reduces pain and fever)
  • APAP is a component of more than 600 different medicines!!

• Acetaminophen causes dose-dependent hepatotoxicity
  • The therapeutic dose (3–4 grams/day) is not too far away from doses that are toxic (10-ish grams)!!
  • https://www.knowyourdose.org/know-your-dose-game/

• Acetaminophen overdose is not a small problem:
  • Leading cause of drug-induced liver injury and emergency department visits in the US
  • Around 1/2 of APAP overdose patients require hospitalization
  • APAP overdose is the leading cause of acute liver failure (ALF) in the United States, Canada, and throughout Europe and the UK
Mechanisms of Acetaminophen Hepatotoxicity in Mice

Adapted from Jaeschke et al., Drug Metabolism Reviews 44: 88-106, 2012.

300 mg/kg APAP, C57Bl/6J mice

Centrilobular necrosis

Adapted from Jaeschke et al., Drug Metabolism Reviews 44: 88-106, 2012.
Liver Damage and Liver Repair after APAP Overdose in Mice

300 mg/kg APAP, C57Bl/6J mice

Centrilobular necrosis

APAP overdose

APAP overdose

Necrosis

Repair

6 18 24 48 72 hours

MICHIGAN STATE UNIVERSITY
SPARTANS WILL.
Is Acetaminophen-Induced Liver Injury Linked to Changes in

1) Blood Coagulation?
or
2) Platelets?
APAP-Induced Liver Injury Drives Blood Coagulation

C57Bl/6J mice
300 mg/kg APAP

APAP overdose

Coagulation activation

Thrombin

Centrilobular necrosis

Graph showing time (hours) vs. TAT (ng/ml)

Blood Coagulation Promotes Initial APAP Hepatotoxicity

C57Bl/6J mice
300 mg/kg APAP

APAP overdose

Contact: Intrinsic (FXII : FXI)

Extrinsic (TF:FVIIa)

Thrombin

Centrilobular necrosis

Initial Hepatotoxicity

Anticoagulant drugs
Heparin
Lepirudin
Dabigatran


Blood Coagulation Promotes Initial APAP Hepatotoxicity

C57Bl/6J mice
300 mg/kg APAP

APAP overdose

Contact: Intrinsic
(FXII : FXI)

Extrinsic
(TF:FVIIa)

Thrombin

Centrilobular necrosis

???

Initial
Hepatotoxicity

“Inactive”

Active

Protease-activated receptors (PARs)

Thrombin

hPAR1

hPAR1*


APAP-Induced Liver Injury Is Linked to Blood Coagulation Activation

C57Bl/6J mice
300 mg/kg APAP

APAP overdose

Contact: Intrinsic (FXII:FXI)

Extrinsic (TF:FVIIa)

Thrombin

PAR-1
PAR-4

Platelets

Centrilobular necrosis

Early hepatotoxicity


Platelets Promote Progression of APAP-Induced Liver Injury

C57Bl/6J mice
300 mg/kg APAP
What Factors Drive Platelet-Dependent Liver Injury?

Can We Frame the Question on Observations in Patients with Acetaminophen-Induced Liver Injury?
Von Willebrand Factor (VWF) Bridges Platelets to Other Proteins

Mackman et al ATVB, Volume 27. 2007.1687-1693
Unbalanced VWF and ADAMTS13 in Acute Liver Failure Patients

Inadequate VWF regulation → Hepatic platelet accumulation → Liver injury

Hugenholtz...Lisman Hepatology 2013;58(2):752-61
VWF Drives Persistent Hepatic Platelet Accumulation in the APAP-Injured Liver

- VWF deposits in the APAP-injured liver
- VWF contributes to persistent platelet accumulation

VWF Deficiency or Inhibition Promotes Resolution of APAP-Induced Liver Damage

Wild-type mice vs. VWF KO mice

Necrosis (%)

24h 48h 72h

Wild-type mice
VWF KO mice

APAP 300 mg/kg

Wild-type mice

Liver necrosis (48 hours)

Antibody

APAP

Summary and Translational Potential

• VWF promotes persistent hepatic platelet accumulation that delays liver repair after APAP challenge in mice
  • Early evidence suggest blocking VWF-platelet interactions accelerates liver repair

• Elevation in plasma VWF concentration is associated with poor outcome in patients with ALI/ALF

### TABLE 3. Hemostatic Features of Patients With ALI/ALF According to 21-Day Outcome: Transplant-Free Survivors and Non-Transplant-Free Survivors (Death or Liver Transplantation)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Number Analyzed</th>
<th>21-Day TFS (n = 483)</th>
<th>Number analyzed</th>
<th>Non-21-Day TFS (n = 193)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF (%) (95% CI)*</td>
<td>480</td>
<td>403.6 (387.1-420.0)</td>
<td>192</td>
<td>559.0 (521.5-596.6)</td>
</tr>
<tr>
<td>ADAMTS13 (%) (95% CI)†</td>
<td>478</td>
<td>22.5 (20.3-24.6)</td>
<td>192</td>
<td>17.8 (15.0-20.7)</td>
</tr>
<tr>
<td>ETP (nM lla*min) (95% CI)</td>
<td>471</td>
<td>543.7 (511.8-575.6)</td>
<td>185</td>
<td>566.9 (498.9-635.0)</td>
</tr>
<tr>
<td>CLT &gt; 180 minutes (%) (95% CI)</td>
<td>460</td>
<td>65.7 (61.3-70.0)</td>
<td>181</td>
<td>61.3 (54.2-68.5)</td>
</tr>
</tbody>
</table>
Revisiting Our Objectives

• Cellular and molecular elements of hemostasis can play a key role in mechanisms of toxicity and tissue repair

• Normal liver function is essential to maintain the blood coagulation cascade

• Acetaminophen is a leading cause of drug-induced liver injury
  • How many medicines contain acetaminophen??

• Components of the hemostatic system (e.g., VWF-platelet engagement) might make nice targets to improve outcome after APAP overdose
Questions? Comments?

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Dear Students,

Welcome to the Fall edition of the SOTudent e-Letter, a biannual publication distributed by the Society of Toxicology’s Student Advisory Committee (SAC). In this edition you will find out a little about SAC and what we do, updates on student membership in your regional chapter, and how you can apply for SOT student member awards.

Also in this issue is a short preview of some exciting activities and opportunities waiting for students at the 2004 meeting in Baltimore! Be sure to write these down!

I look forward to seeing all of you in Baltimore!

James Luyendyk
SAC Chairperson