Adaptation to Cellular Stress: Role in severity and susceptibility to DILI?

Neil Kaplowitz, MD
University of Southern California, Research Center for Liver Diseases, Keck School of Medicine of USC
Los Angeles, CA
Conflict of Interest

I have no conflicts with regard to this presentation.

Consulting Agreements: GSK, Merck, Roche, Takeda, Pfizer, Acorda

Collaborations: Ionis, Genentech

SAB: Hepregen, Dili sym
Current understanding of IDILI

There are known knowns

There are known unknowns

There are unknown unknowns

D. Rumsfeld
WHAT IS HEPATOCHELULAR STRESS?

Liver disease promoting triggers (drugs, viruses, alcohol, fatty acids), often mediated by organelle and oxidative stress, activate signal transduction pathways and transcription factors which promote gene expression programs or post-translation modifications which mitigate or promote injury, the final outcome being cell survival, death, or an inflammatory response.
Susceptibility to occurrence and/or severity of idiosyncratic DILI (IDILI)?

- IDILI mainly mediated by adaptive immunity.
- Most patients with susceptible HLA polymorphisms do not develop IDILI or develop only mild injury.
  - Immune Tolerance
  - Why?
  - Hepatocellular adaptation to stress
- Most drugs associated with IDILI cause organelle/biochemical stress in model systems (cell culture, isolated organelles).
- Predictive in vitro toxicology vs. clinical experience??
Significance of Hazards in Immune DILI

DRUG → HAZARDS

Upstream Danger

In Model Systems

Downstream Sensitization to Immune Killing

? Mechanistically Integral to Development of Immune DILI

? Surrogate for Immunogenic Parent Drug or Reactive Metabolites
Hepatocellular Stress and IDILI: Conceptual Framework

Drugs
- (reactive metabolites)
- or
- Parent drug

→ Organelle stress

- Mitochondria
- ER

→ Adaptive Responses

- Covalent binding (ER, etc)
- Sequestration (mitochondria, lysosomes)
- Transporter inhibition – bile acid retention
- ROS

Susceptibility

Innate + Adaptive Immunity
- DAMPS

Lethal
- or
- Nonlethal
Adaptation to Hepatocellular Stress

- **Stress**
  - ↑NRF2 → transcription of antioxidant genes
  - UPR\textsubscript{ER} → ↑chaperones and ↓client proteins
  - UPR\textsubscript{MT} → ↑chaperones, import machinery
  - Autophagy/mitophagy → remove damaged organelles
  - Mitochondrial biogenesis
  - Mitochondrial fission/fusion
  - Post-translational Modifications
    - Redox: disulfides, sulfenic acid, glutathionylation, nitrosylation, methylation, acetylation, phosphorylation, ubiquitinylation (and reversal)
  - MicroRNAs - unexplored
Transcription Responses to Bile Acid retention (stress)

$\uparrow$Bile Acid $\rightarrow$ FXR $\rightarrow$

- Adaptive responses
  - $\uparrow$BSEP (canalicular export)
  - $\downarrow$NTCP ($\downarrow$ uptake)
  - $\uparrow$MRP 3/4 (sinusoidal export)
  - $\downarrow$CYP7A1 ($\downarrow$ BA synthesis)

- Bile acid detoxification
  - (sulfation, hydroxylation)

Indirect – survival and antioxidant response
ER Stress

covalent binding, misfolded protein
ROS, bile acids

Unfolded Protein Response

(Grp78 displaced)
Activation of ER memb sensors

Apoptosis
Inflammation

A
P-JNK
↓↑
Mitoch ROS

Inflammation
\[ \text{NF-kB} \]

↓
Bcl-2
↑
CHOP

Adaptive

\[ \text{NF-kB} \]

↑
chaperones
↑
ERAD proteins

\[ \text{NF-kB} \]

↑
chaperones
+ \[ \text{Nrf2} \]

translational inhibition

\[ \text{NF-kB} \]

↑
ERAD proteins

↑
chaperones

↑
ERAD proteins

\[ \text{NF-kB} \]

↑
chaperones
+ \[ \text{Nrf2} \]

translational inhibition

\[ \text{NF-kB} \]

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translational inhibition

\[ \text{NF-kB} \]

↑
ERAD proteins

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chaperones

↑
ERAD proteins

\[ \text{NF-kB} \]

↑
chaperones
+ \[ \text{Nrf2} \]

translational inhibition
Triggers of Mitochondrial Stress

- Inhibition of mitoch DNA synthesis (e.g. nucleosides)
- Drug (cationic) accumulation (e.g. amiodarone)
- Reactive metabolites (covalent binding)
  (e.g. valproic acid, APAP)
- Signal transduction (e.g. APAP)
  e.g. P-JNK $\leftrightarrow$ ROS (mitoch)
- Others: bile acids, ER stress
Adaptation to Mitochondrial Stress

- **UPR\textsuperscript{MT}**
  
  ATFS1 transcription factor:
  
  - taken up and degraded by mitochondria
  - mitoch depolarization → diverts ATFS1 to nucleus
  
  ➤ mitochondrial chaperones + protein import machinery

- **Mitophagy** (removal of ROS producing mitochondria)
  
  - stress/damage → stabilization of PINK1 which then binds + activates Parkin → mitophagy

- **Mitochondrial biogenesis**
  
  - retrograde signaling
  
  - mitochondrial stress → CRTC3 → CREB → PGC1\textalpha → nuclear genes

- **Fission** (*needed for mitophagy*)
  
  - Mitoch stress → stabilizes MFF → target of DRP1 in outer membrane
  
  - destabilizes OPA1 (fusion factor) in inner membrane

- **Fusion**
  
  - mediated by outer membrane MFN 1/2 and inner membrane OPA-1
  
  - enhances mitochondrial function
Mitochondrial ROS in Health and Disease

Complex I + III

O₂

O²⁻

H₂O₂

H₂O

Signaling
Activate kinases (ERK)

Antioxidant Defense
KEAP, Nrf2, ARE

Hypoxia response
HIF-1α

MAPK (JNK)
Inflammation
Cell death

GSH

GP

GR

GSSG

Intensity and Duration of ROS Determine Outcome

“Canary in the mine” (stress warning and maintenance of defense)

Physiological (Adaptive)

Pathological
Effect of JNK1 versus JNK2 on isolated mitochondria

Hepatology in press 2016
$\text{Ca}^{2+}$ amplifies ROS production induced by P-JNK/ATP

Fluorescent intensity

Time (min)

Reaction mixture:
Mitochondria = 20ug
Volume = 100ul
JNK (total) = 100ng
ATP = 600uM

Model of JNK-Sab Mediated Mitochondrial Impairment and Cell Death

APAP
TNF/galactosamine
Tunicamycin
Palmitic Acid

Mito ROS $\rightarrow$ MPT $\rightarrow$ Necrosis

P-JNK
Sab
OCR

Bcl$_2$ family mediated MOMP

Apoptosis
Protection against liver injury in TAM-inducible albumin-CRE deletion of floxed Sab
Two Mitochondrial Scaffold Proteins (outer membrane Sab and inner membrane DOK-4) Mediate JNK signaling

**Diagram:***

- **OM** (Outer Membrane) with **Sab** (Scaffold Protein) and **DOK-4** (Dok Protein 4)
- **P-Src** and **SHP1 (PTPN6)**
- **Sustained P-JNK** leads to **ROS** → **MPT** → **NECROSiS**
  - e.g. APAP toxicity
- **Bcl-2** → **MOMP** → **APOPTOSIS**
  - e.g. TNF/galactosamine, Palmitic acid lipotoxicity, Tunicamycin-ER stress induced apoptosis

**Legend:**
- **↑** indicates increase
- **↓** indicates decrease

**Text:**

- Susceptibility to P-JNK determined by level of expression of Sab and Dok4
Regulation of Apoptosis: Dominant Form of Cell Death in Liver Disease

Death Receptors → caspase 8

ROS → Bax → cyt.c → caspase 3

NF-κB → survival genes

Intrinsic Stress
Mitoch ER DNA (ROS, palmitic acid, bile acids)

Survival responses
Caspase inhibitors cFLIP XIAP [NF-κB]
Anti-apoptosis Bcl members Bcl-X<sub>L</sub> Mcl1
Anti-oxidant response NF-κB JNK NRF2

Mitochondria are central mediators of hepatocyte apoptosis.
Conclusions

1. Chemicals stress hepatocytes in a variety of ways.

2. Many intricate adaptive responses dampen the adverse effects of stress and protect hepatocytes.

3. Stress can affect the “FITNESS” of hepatocytes leading to increased susceptibility to the lethal consequences of IMMUNE ATTACK (or generate sublethal DANGER SIGNALS).

4. The balance of injurious versus adaptive responses to drug-induced stress may be modulated by genetic and environmental factors.

5. Analogous to the yin-yang nature of immunity, the injurious stress versus adaptive responses in hepatocytes may be an important contributor to the occurrence of IDILI, even if immune mediated.