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

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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 HEPATOCELLULAR 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
In Model Systems

Upstream Danger

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 → Lethal or Nonlethal

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

Mitochondria ER

Adaptive Responses

Innate + Adaptive Immunity

DAMPs

susceptibility
Adaptation to Hepatocellular Stress

**Stress**

- ↑NRF2 → transcription of antioxidant genes
- \( \text{UPR}_{\text{ER}} \) → ↑chaperones and ↓client proteins
- \( \text{UPR}_{\text{MT}} \) → ↑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)

↑Bile Acid → **FXR** → Adaptive responses

- ↑BSEP (canalicular export)
- ↓NTCP (↓ uptake)
- ↑MRP 3/4 (sinusoidal export)
- ↓CYP7A1 (↓ BA synthesis)

**others**

- PXR
- NRF2
- NF-κB

Bile acid detoxification (sulfation, hydroxylation)

Indirect – survival and antioxidant response
ER Stress

- Covalent binding
- Misfolded protein
- ROS, bile acids

Unfolded Protein Response

(Arp78 displaced)

Activation of ER memb sensors

Apoptosis

- Inflammation
- P-JNK
- Mitoch ROS

Inflammation

- Bcl-2
- CHOP

Adaptive

- Chaperones
- ERAD proteins
- 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 ↔ ROS (mitoch)
- Others: bile acids, ER stress
Adaptation to Mitochondrial Stress

- **UPR<sup>MT</sup>**
  - 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
  - Ubiq. p62

- **Mitochondrial biogenesis**
  - retrograde signaling
  - mitochondrial stress → CRTC3 → CREB → PGC1α → nuclear genes
    - co-activator → transcription factor → co-activator (master regulator)

- **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
GR
GP
GSSG

Intensity and Duration of ROS Determine Outcome

“Canary in the mine” (stress warning and maintenance of defense)
Effect of JNK1 versus JNK2 on isolated mitochondria

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

**Graph:**
- **X-axis:** Time (min)
- **Y-axis:** Fluorescent intensity
- **Legend:**
  - ▲: [Ca$^{2+}$] 50nM + JNK(a) + ATP
  - ○: [Ca$^{2+}$] 50nM + JNK(m) + ATP
  - △: [Ca$^{2+}$] 0nM + JNK(a) + ATP
  - ●: [Ca$^{2+}$] 0nM + JNK(m) + ATP

**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

APAP

APAP

Mito ROS → MPT → Necrosis

P-JNK

Sab

OCR

Sustained

ROS

Bcl family

mediated MOMP

Apoptosis

Win S. et al.  JBC, 286: 35071, 2011
Win S. et al.  Cell Death Dis, 5:, e989, 2014
Protection against liver injury in TAM-inducible albumin-CRE deletion of floxed Sab

Tam – Sab \( ^{ff} \)  
Tam – Sab \( ^{\Delta \text{Hep}} \)

APAP 300mg/kg 24hr  
APAP 300mg/kg 24hr

GalN/TNF 6hr  
GalN/TNF 6hr

TUNEL stain  
TUNEL stain

Hepatocytes

Serum ALT (U/L) 1000X

APAP 300mg/kg 24hr  
GalN/TNF 6hr

Cytoplasm

Mitochondria
Two Mitochondrial Scaffold Proteins (outer membrane Sab and inner membrane DOK-4) Mediate JNK signaling

Susceptibility to P-JNK determined by level of expression of Sab and Dok4

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ROS → MPT → NECROSIS
- e.g. APAP toxicity

Bcl → MOMP → APOPTOSIS
- e.g. TNF/galactosamine
- Palmitic acid lipotoxicity
- Tunicamycin-ER stress induced apoptosis

ETC

P-Src

SHP1 (PTPN6)

Sustained P-JNK

OM

IM

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

**Mitochondria are central mediators of hepatocyte apoptosis.**

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

**Death Receptors** → caspase 8 → ROS → Bax → cyt.c → caspase 3

- Survivin genes
- NF-kB
- MKPase
- Bcl-X₇
- Mcl1

**Survival responses**
- Caspase inhibitors
  - cFLIP
  - XIAP
- Anti-apoptosis Bcl members
  - Bcl-X₇
  - Mcl1
- Anti-oxidant response
  - NF-kB
  - JNK
  - NRF2
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.