Acetaminophen-induced Liver Injury: Translating Toxicity Mechanisms in Animals to Humans

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Overview

• Clinical Relevance
• Intracellular Signaling Mechanisms of Cell Death in Mice
• Mechanisms of Toxicity in Human Hepatocytes
• Mechanisms of Toxicity in Humans
• Sterile Inflammation in Humans
Acetaminophen (Paracetamol) Hepatotoxicity

Clinical Relevance

Most consumed pain medication in the world: 35 billion doses/year sold

50-60,000 hospitalizations and 300-500 deaths/year in the US!

Largest cause of Acute Liver Failure in the US! (46%)
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Mechanisms of APAP Bioactivation

Acetaminophen → Glucuronosyl-transferases → Glucuronide → Bile

Acetaminophen → P450 → Reactive Intermediate NAPQI

Acetaminophen → Sulfo-transferases → Sulfate → Bile

Detoxification:
- Bile mrp2
- Plasma mrp3

Initiation of Toxicity:
- APAP-Glutathione Conjugate
- APAP-Protein Adduct

Glutathione S-transferases

Proteins

GSH

~ 50%

~ 30%

~ 5-10%
Mechanisms of APAP Hepatotoxicity in Humans

Human Hepatoma Cell Line HepaRG

McGill et al., Hepatology 53: 974-82, 2011
Mechanism of APAP Toxicity in HepaRG Cells

GSH

APAP-Cys

JC-1

LDH

McGill et al., Hepatology 53:974-82, 2011
Mechanism of APAP Toxicity in HepaRG Cells

MitoSOX Red: Mitochondrial Superoxide

McGill et al., Hepatology 53:974-82, 2011
Mechanism of APAP Toxicity in HepaRG Cells: Apoptosis

McGill et al., Hepatology 53:974-82, 2011
Mechanism of APAP Toxicity in HepaRG Cells:

**Necrosis**

PI Staining

Vehicle – 24 h

APAP - 20mM – 24 h
APAP-induced Nuclear Endonuclease G Translocation in HepaRG Cells
APAP-induced DNA Fragmentation in HepaRG Cells: TUNEL Assay

20 mM APAP, 24 h
Mechanisms of APAP Hepatotoxicity in Humans

Primary Human Hepatocytes

Xie et al., Toxicol Appl Pharmacol 279: 266-74, 2014
Mechanisms of APAP Hepatotoxicity in Primary Human Hepatocytes

Xie et al., Toxicol Appl Pharmacol 279: 266-74, 2014
APAP-induced JNK Activation in Human Hepatocytes

**Graphs and Images:**
- **APAP Time (h):** 0, 3, 6, 15, 24, 36, 48
  - P-JNK and Total JNK bands at 54 kDa and 46 kDa
- **Cytosol and Mitochondria bands:** P-JNK and Total JNK

**Post-treatment ALT (% of total ALT):**
- Time (h): 24, 48
  - APAP, APAP+Veh, APAP+JNK inh
  - Comparison with statistical significance indicated by * and # symbols

**References:**
Xie et al., Toxicol Appl Pharmacol 279: 266-74, 2014
Oxidant Stress in Cell Culture (Mouse Hepatocytes)

MitoSox Red: Mitochondrial Superoxide

LDH Release (% of total LDH)

Oxygen Concentrations

APAP 6 h

APAP 15 h

YC1 Fluorescence (% control)

Mechanisms of APAP Hepatotoxicity in Humans

Evaluation of mechanistic biomarkers in plasma of overdose patients
APAP in Humans: Mitochondrial Biomarkers
Mitochondrial Markers of APAP Toxicity in Humans: mtDNA

Marker of APAP Toxicity: Nuclear DNA Fragmentation

Acetaminophen – Apoptosis or Necrosis?

K18 – full length cytokeratin-18 (M65)
ccK18 – caspase-cleaved cytokeratin-18 (M30)

Human Pathophysiology is Equivalent to Mechanisms in Mice

- GSH depletion with protein binding
- Mitochondrial dysfunction and damage
- Nuclear DNA damage
- Cell necrosis with cell contents release

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Proposed Schematic of APAP-induced Inflammatory Liver Injury

Woolbright & Jaeschke, J Hepatol 66: 836-848, 2017
Pro-inflammatory Cytokine Formation in APAP Overdose Patients

IL-1β

TNF-α

Plasma Levels (pg/ml)
Proposed Schematic of APAP-induced Inflammatory Liver Injury

Primary necrosis of hepatocytes

Secondary neutrophil-mediated cell death

TLR9:mtDNA

DAMP release including ATP and mtDNA

Pro-IL-1β expression

Pro-Caspase-1

Caspase-1

Pro-IL-1β

IL-1β

IL-1β:IL-1R

ROS proteases

Cytokine/Chemokine gradient including IL-1β

Woolbright & Jaeschke, J Hepatol 66: 836-848, 2017
Neutrophil Activation in an APAP Overdose Patient

Neutrophil Activation during Recovery after APAP-induced Liver Injury in Patients

Circulating Levels of CCL2 (MCP-1) in APAP Overdose Patients

![Graph showing MCP-1 levels in different groups over time]

- **Ctrl**: Control group
- **NLT**: Non-Lethal Toxicity group
- **NS**: Non-Survivors
- **SV**: Survivors

MCP-1 (pg/mL) vs Time (D0 to D6)

- Non-Survivors: Tracked with red triangles.
- Survivors: Tracked with red triangles.

Significance markers:
- *: p < 0.05
- #: p < 0.01
- †: p < 0.001
Acetaminophen Overdose

Cell injury

Necrotic Hepatocytes

HMGB1

TLR4

DNA Fragments

mtDNA

HMGB1

MCP-1

RAGE

Neutrophils

TNF-α, IL-1α/β

IL-8

NF-κB → ICAM-1

Hepatocytes

Host defense function

Removal of necrotic cell debris

Monocyte-derived Macrophages

Necrotic Cell Death

Release of DAMPs

Innate Immune Cell Activation

Tissue Repair
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