Alcoholic hepatitis is a drug-induced disorder

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3 Reasons Why Alcohol is the Most Dangerous Drug

Make no mistake... alcohol is deadly. Very deadly.

We have always made a point to make everyone aware that we do not have a problem with alcohol. Alcohol has existed for at least 10,000 years. We are by no means taking a position that alcohol should be abolished, or that if you drink alcohol you are doing something wrong. Everyone has the right to make their own choices.

We just want to make people aware of the truth...

1 – Alcohol Kills More Than All Drugs Combined

While the “War on Drugs” portrays the message that drugs like heroin and cocaine are the dangers, it fails to mention that alcohol contributes to 100,000 deaths per year, while all other drugs combine to contribute to 20,000 deaths per year.

2 – Drunk People Do Crazy Things

3 – Alcohol Use is Celebrated
Clinical Progression of ALD

- Fatty liver
- Steatohepatitis
- Fibrosis
- Mortality

Years

- Acute alcoholic hepatitis
- Cirrhosis
- HCC

Years
Pathomechanism of alcoholic liver disease

Szabo et al, unpublished
Alcohol Metabolism

Alcohol dehydrogenase (ADH)
Aldehyde Dehydrogenase (ALDH)
APAP metabolism

**Acetaminophen**
- Glucuronide moiety (Non-toxic)
- Conjugation
- P450 (2E1)
- Sulfate moiety (Non-toxic)

**N-acetyl-p-benzo-quinone imine (NAPQI) (TOXIC)**
- Conjugation
- Glutathione
- Cysteine and mercapturic acid conjugates (non-toxic)
- NAC
Working model of CYP2E1-dependent oxidative stress and cytotoxicity

Circulating miRNAs

- Regulate genes both at the post-transcriptional and transcriptional levels.

- Present in cell-free body fluids including serum, plasma, urine, amniotic fluid, milk etc.

- Found in vesicles (exosomes, microvesicles, apoptotic bodies) and lipoproteins (HDL, LDL). complexes with proteins (Ago’s).

- The stability of circulating miRNAs make them **attractive new non-invasive biomarkers**.

- Circulating miRNAs signatures in various diseases.

- Contribute to cell-cell communication.

miRNA-122, a liver specific miRNA

- miR-122 accounts for ~70% of the total miRNA population in hepatocytes (Chang et al., RNA Biology, 2004).

- miR-122 regulates genes involved in cholesterol biosynthesis and facilitates HCV replication (Catherine et al., Science 2005).

- Increased plasma/serum levels of miR-122 in humans in:
  - Drug-induced liver injury (Starkey et al., Hepatology, 2012).
  - Chronic hepatitis C infection or non-alcoholic fatty-liver disease or HCV-induced fibrosis (Cermelli et al., Plos one, 2011, Trebicka et al., J Hepatol: 2013).
  - Hepatocellular carcinoma (Xu, Mol Carci, 2011).
Serum miR-122 increase correlates with liver injury and ALT increase

Correlation between serum miR-122 and ALT

Correlation between plasma miR-122 and ALT

Bala et al, Hepatology 2012
J Translational Medicine 10:151 (2012)
miR-122 is an early plasma marker of APAP-induced liver injury

C57BL/6 mice received saline or acetaminophen (500mg/kg).

Chronic alcohol decreases miR-122 in the liver and AAV8-TuD mediated miR-122 inhibition increases liver injury

n=8-14/group, *p<0.05, **p<0.005

miR-122

fold change/U6

WT PF

WT Et

PF

EtOH

Satischandran & Szabo
Under revision
Proposed model for miR-122 in alcohol-induced sensitization to APAP toxicity

EtOH
↓
decreased liver miR-122

+ 

Decreased GSH
↓
increased ROS

Hepatocyte injury/death

miR-122
Restorative with rAAV-miR-122 or miR-122 mimic

Hepatocyte damage

miR-122
Inhibition with rAAV-TuD

Decreased liver miR-122

? GSH
?ROS

Increased hepatocyte injury

Satischandran & Szabo
unpublished
IRF3 has a pro-apoptotic BH3-like domain

- dsRNA-induced apoptosis in fibroblasts
  IRF3 can bind to and activate cytosolic Bax, resulting in Bax translocation to the mitochondria and initiation of the intrinsic apoptotic pathway
  - Chattopadhyay, EMBO J 2010;29:1762-73
  - Vince, Tschopp, EMBO J 2010;29:1627-28

IRF3 phosphorylation is directly involved in hepatocyte death in ALD

Petrasek and Szabo, PNAS 2013, PMID: 24052526
IRF3 associates with the ER adaptor STING

- ER stress is a homeostatic response to
  - accumulation of misfolded proteins
  - dysregulation of calcium signaling
  - hypoxia
- ER stress is a known determinant of liver diseases

IRF3 is activated by ER stress via STING in ALD

Source of activation?

Endoplasmic reticulum (ER)
- Platform for activation of IRF3 by cytosolic RNA or DNA of viral origin
- In certain viral infections, IRF3 associates with ER via STING (Stimulator of interferon genes)


Petrasek and Szabo, PNAS 2013, PMID: 24052526
The role of IRF3 and STING in ALD

- Ethanol
  - leaky gut
  - Endoplasmic reticulum stress (hepatocytes)
  - STING

- LPS
  - TLR4 (Kupffer cells)
  - TRAM/TRIF
  - p-FADD

- IRF3
  - p-TBK1
  - phospho-IRF3
  - Binding to IFN-β promoter
  - Co-operation with NF-κB
  - Association with Caspase-8 and Bax
  - Mitochondrial apoptotic pathway
    - Hepatocytes
      - Hepatocyte death
      - ALD

- Type-I IFNs
  - Hepatocytes
  - Liver immune cells
    - Anti-inflammatory and anti-fibrotic

- Inflammatory cytokines
  - Liver immune cells
    - Pro-inflammatory

Petrasek et al, 2013 PNAS, 110(41):16544-16549. PMID: 24052526
APAP liver injury results in phosphorylation of IRF3

APAP dose 500 mg/kg; Saline: n = 3; APAP: n = 7 - 9

Iracheta-Velle & Szabo unpublished
Summary

APAP-induced acute liver failure results in phosphorylated IRF3, liver injury and inflammasome activation.

STING deficiency is attenuates serum ALT increase, inflammasome activation and cleaved Caspase-3 in the liver after APAP overdose.

STING deficiency protects from APAP-induced liver necrosis and mortality.
Summary

miR-122 inhibition in the liver results in steatohepatitis and replacement of miR-122 is protective from alcohol-induced liver disease.

miR-122 inhibition in the liver increases CyP2E1 and reduces the GSH:GSSG ratio.

STING deficiency is protective from hepatocyte apoptosis, liver injury and inflammation after alcohol or APAP overdose.

IRF3 and STING collectively link hepatocyte death and inflammation in acute alcohol/drug-induced liver injury.
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