The liver in poisoning: what can we learn from animal models?

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Outcome and causes of acute liver failure

• All patients with ALF in the USA between 1994-1996 registered

• 295 patients: 74 survived spontaneously, 121 were transplanted, 99 died

• *Drugs:* The most important cause for ALF → paracetamol, NSAIDs, halothane, INH, pyrazinamide, antidepressants, valproate, nucleoside reverse transcription inhibitors (NRTI), herbals

• *Miscellaneous:* particularly vascular (Budd-Chiari, shock liver)
Traditional classification of hepatic toxicants

**Intrinsic (predictable) toxicants**
- Dose-dependent, supratherapeutic exposure, mechanisms known
- Animal models often available
- Examples: ethanol, methotrexate, **paracetamol**, salicylates

**Idiosyncratic (unpredictable) toxicants**
- Rare, toxicity occurs any time at therapeutic dose
- Non-predictable, no clear dose dependency
- Mechanisms mostly not known and no animal models
- Examples
  - Allergic: abacavir, flucloxacillin, …
  - Non-allergic (metabolic): **valproate**, amiodarone, …
Paracetamol-associated liver injury – micropathology

- Centroacinar necrosis is the hallmark of liver pathology
- This localization is explained by the fact that NAPQI is the toxic agent
- NAPQI is an electrophile formed in CYP2E1 and CYP1A2-dependent way
- CYP2E1 and 1A2 are primarily located in centroacinar hepatocytes
Paracetamol-associated hepatotoxicity – animal models

- Mice fed a diet with a normal vitamin E content (Vit E+/+) develop higher ALT levels than rats after treatment with paracetamol (300 mg/kg).
- Treatment with ally alcohol (AA) is associated in much higher MDA concentrations than paracetamol with comparable ALT levels.
- Mice are a better model for paracetamol-associated hepatotoxicity than rats.

Mechanisms of paracetamol-associated liver failure

- Production of NAPQI starts immediately after ingestion of paracetamol
- At the same time, the cellular (in particular the mitochondrial) GSH content starts to drop
- NAPQI binds to cellular proteins and cell injury starts
- There is sterile inflammation and reparative processes

CYP2E1 and paracetamol-associated liver injury

- Mice die of liver injury; effect on survival is dose dependent
- CYP2E1 knockout mice (-/-) survive better than wild type mice (+/+)
- Other CYPs than CYP2E1 may be involved in paracetamol metabolism
Paracetamol-associated drop in liver GSH

- Mice were treated with paracetamol (350 mg/kg i.p.).
- Within 1 hour after application, the liver GSH content has dropped to <10% of normal and has recovered at 8 h.
- The GSSG content drops initially and is higher than in control livers at 8 h.
- Cyclosporin does not affect the liver GSH or GSSG content.

J Hepatol 2005;42:110-116
Paracetamol-associated mitochondrial damage

- Mice were treated with paracetamol (350 mg/kg i.p.)
- 8 hours after treatment, mitochondrial GSH is still decreased by paracetamol
- The decrease is prevented by cyclosporin
- The ATP content has dropped by >90%; this drop is partially prevented by cyclosporin

J Hepatol 2005;42:110-116
Paracetamol-associated induction of MPT

- Mice were treated with paracetamol (350 mg/kg i.p.)
- 8 hours after treatment, mitochondria from paracetamol treated rats showed rapid swelling in the presence of 20 µM CaCl$_2$
- Cytochrome c in cytoplasm was higher in paracetamol-treated livers
- Cyclosporin was partially preventive

J Hepatol 2005;42:110-116
Paracetamol-associated nuclear DNA fragmentation

- Mice were treated with paracetamol (300 mg/kg i.p.) alone or in combination with GSH or NAC.
- 6 hours after treatment, livers were subjected to H&E staining, nitrotyrosine or TUNEL staining.
- GSH and NAC partially prevent protein oxidation and DNA damage associated with paracetamol.

Hepatology 2010;51:246-254
Mechanisms of paracetamol-associated liver failure

- CYP-associated metabolism produces the electrophile NAPQI
- NAPQI reacts with glutathione and with mainly mitochondrial proteins
- Activation of ASK1 and JNK which enhances mt ROS production
- Formation of MPT and drop in mt membrane potential
- Release of AIF and other factors
- Cell necrosis due to low ATP content

Paracetamol-associated liver failure & inflammation

- Initial event: formation of NAPQI → mitochondrial toxicity → circulating mtDNA and extracellular ATP
- Secondary event: activation of innate immune system → up-regulation of inflammatory cytokines, activation of NK cells and neutrophils
- The inflammasome may be involved
Paracetamol-induced hepatotoxicity and TLR-9

- TLR-9 deficient mice have less IL-1β activation, less ALT and better survival
- TLR-9: Toll-like receptor 9
- Similar findings with NLRP3⁻/⁻, ASC⁻/⁻ or pro-caspase 1⁻/⁻ mice

J Clin Invest 2009;305-314
Paracetamol-associated hepatotoxicity – principle mechanisms

1. Metabolite formation and mitochondrial damage

- APAP → NAPQI → GSH → mt DNA
- ROS → ONOO⁻ → NO + O₂ → mt DNA
- pJNK → JNK → ASK1 + Trx → pJNK

2. Inflammasome activation

- mt DNA
- extracellular ATP
- TLR
- MyDBB
- NLRP3
- ASC
- pro-caspase-1
- IL-1β
- IL-18

3. Inflammation and reparation

- K⁺ efflux, ROS lysosomal damage
- NF-κB
- pro-IL-1β
- caspase-1
- IL-1β
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Valproate-associated liver failure

Frequency
• 1:6,500 polytherapy, 1:40,000 monotherapy
• more frequent in children than in adults

Onset
• in 70% of patients during the first 3 months after start of treatment

Clinical picture, pathology
• seizures, thrombocytopenia, DIC, fulminant liver failure
• microvesicular steatosis
Metabolism and toxicity of valproate

**Proposed mechanism of toxicity**
- Depletion of CoASH and carnitine
- Toxic metabolites: inhibition of β-oxidation, respiratory chain
- Risk factor: impaired mitochondrial function, e.g. POLG mutations
Mouse model for valproate toxicity

- JVS\(^{-/-}\) mice are carnitine deficient due to a mutation in the gene coding for OCTN2
- Homozygous JVS\(^{-/-}\) mice hardly survive
- Heterozygous JVS\(^{+/}\) mice have reduced carnitine tissue stores and may be a model to demonstrate toxicity of drugs affecting β-oxidation
In vivo β-oxidation by JVS+/- mice and VPA

- JVS +/- treated with VPA have higher AST and alk phos activities than WT mice
- JVS +/- treated with VPA have impaired β-oxidation compared to wild-type or JVS +/- mice

J Pharmacol Exp Ther 2008;324:568-75
Hepatic fat accumulation during starvation

- Mice had free access to food and were starved for 24 hours
- JVS\(^{+/-}\) mice treated with VPA had the most accentuated fat accumulation
- JVS\(^{+/-}\) mice treated with VPA also showed increased transaminases

A: Wild type
B: Wild type plus VPA
C: JVS\(^{+/-}\) mice
D: JVS\(^{+/-}\) plus VPA

J Pharmacol Exp Ther 2008;324:568-75
Apoptosis in JVS mice

- Besides fat accumulation and increased transaminase, JVS\(^{+/−}\) mice treated with VPA had increased expression of caspase 3.
- Increased transaminases are were explained by apoptosis.
- JVS\(^{+/−}\) mice appear to be more sensitive to VPA than wild type mice.
Valproate-associated fulminant liver failure

- **Effect**
- **Log dose**

- **Pharmacological effect**
- **Toxicity in the presence of susceptibility factors**
- **Toxicity in the absence of susceptibility factors**

- **Susceptibility factors**: mitochondrial diseases (for instance reduced activity of DNA-Polymerase γ (POLG)), impaired β-oxidation

- **Consequences**: patients are more susceptible for liver injury induced by valproate

- Can be simulated in animal models
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Conclusions

• Animal models are useful to find out mechanisms and susceptibility factors for hepatotoxicity

• Mechanisms of idiosyncratic toxicities are currently investigated and more and more understood; animal models can be created

• Knowledge of susceptibility factors will allow for excluding susceptible patients from critical therapies and/or for designing less toxic drugs