Liver Toxicants: Synthetic and Naturally Occurring, Classic and Emerging

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Disclosures

I have nothing to disclose
A case...

- Over a 4 month period, 5 workers in an industrial waste disposal plant present with acute hepatitis.
- The workers had been working in the high-vapor-generating area of the plant.
- Pathologic examination showed typical features of acute toxic hepatitis.
- Several chemicals with hepatotoxic potential, including pyridine, dimethylformamide, dimethylacetamide, and methylenedianiline were detected in the plant.


Outline

- Review of hepatic physiology and mechanisms of hepatic injury
- Synthetic Toxicants
- Naturally Occurring Toxicants
- Classic Toxicants
- Emerging Toxicants
- Management of exposed patients
Function of the Liver

- **Energy metabolism and substrate interconversion**
  - Gluconeogenesis, glycogenolysis, glycolysis
  - Cholesterol synthesis

- **Protein synthetic functions**
  - Albumin, clotting factors, binding proteins, apolipoproteins, angiotensinogen, and insulin-like growth factor

- **Solubilization, transport, and storage functions**
  - Biotransformation through phase I and phase II
  - Storage of vitamins A, D, and B12 and folate

- **Protective and clearance functions**
  - Detoxification
  - Synthesis and export of glutathione

Phase I and phase II reactions

- Phase I reactions may precede phase II reactions.
Biotransformation

<table>
<thead>
<tr>
<th>Phase I Enzymes - (Preparatory) – add/expose polar groups</th>
<th>Phase II Enzymes - (Synthetic) – conjugation of polar groups – increase hydrophilicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>dihydropyrimidine dehydrogenase (DPYD)</td>
</tr>
<tr>
<td>Esterase, Peptidase, epoxidase</td>
<td></td>
</tr>
<tr>
<td>Oxidation (loss of an electron)</td>
<td>glutathione-S-transferase (GST)</td>
</tr>
<tr>
<td>P450, alcohol dehydrogenase, monoamine oxidase</td>
<td></td>
</tr>
<tr>
<td>Reduction (gain of an electron)</td>
<td>N-acetyltransferase (NAT)</td>
</tr>
<tr>
<td>Azo, Nitro, Carbonyl, Quinone</td>
<td></td>
</tr>
<tr>
<td>Dealkylation</td>
<td>sulfotransferase (SULT)</td>
</tr>
<tr>
<td></td>
<td>thiolpurine methyltransferase (TPMT)</td>
</tr>
<tr>
<td></td>
<td>UDP-glucuronosyltransferase (UGT)</td>
</tr>
</tbody>
</table>

Example of metabolism of phenytoin

Phenytoin

4-Hydroxy-phenytoin

4-Hydroxy-phenytoin glucuronide

Phenytoin: Highly lipophilic

4-Hydroxy-phenytoin: Slightly soluble in water

4-Hydroxy-phenytoin glucuronide: Very soluble in water


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Contributions of various CYP450 enzymes and different phase II pathways

DPYD, dihydropyrimidine dehydrogenase; GST, glutathione-S-transferase; NAT, N-acetyltransferase; SULT, sulfotransferase; TPMT, thiopurine methyltransferase; UGT, UDP-glucuronosyltransferase.

Multiple Mechanisms of Hepatic Injury

- Covalent binding
- Lipid peroxidation
- Thiol group changes
- Enzyme inhibition
- Ischemia
- Depletion of ATP
- Damage to intracellular organelles
- DNA damage
- Apoptosis

Liver Lobule

http://illuminationstudios.com/archives/150/structure-of-a-hepatic-lobule
### Metabolic Zones of the Liver

<table>
<thead>
<tr>
<th>Location (Zone)</th>
<th>Biochemistry</th>
<th>Types of Injury</th>
<th>Example</th>
</tr>
</thead>
</table>
| **Periportal (1)** | High oxygen content  
High glutathione content | Oxygen free radical-mediated necrosis | Phosphorus, Iron |
| **Mid-zonal (2)** | Shared functions, zones 1 and 3 | Shared functions, zones 1 and 3 | Beryllium |
| **Centrilobular (3)** | Low oxygen content  
High capacity of glucuronidation and sulfation  
High CYP2E1, alcohol dehydrogenase | Necrosis caused by toxic metabolites of CYP2E1  
Increased CCl₄ and ethanol injury caused by reducing environment | APAP, ethanol, halothane, carbon tetrachloride, paraquat, Bromobenzene, chlorinated hydrocarbons |
# Factors in the Site-Specific Injury of Representative Hepatotoxicants

<table>
<thead>
<tr>
<th>Site</th>
<th>Representative Toxicants</th>
<th>Potential Explanation For Site-specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Periportal</strong> - Zone 1 hepatocytes (versus zone 3)</td>
<td>Fe (overload)</td>
<td>Preferential uptake and high oxygen levels</td>
</tr>
<tr>
<td><strong>Centrilobular</strong> - Zone 3 hepatocytes (versus zone 1)</td>
<td>CCl₄, Acetaminophen, Ethanol</td>
<td>More P450 isozyme for bioactivation, More P450 isozyme for bioactivation and less GSH for detoxification, More hypoxic and greater imbalance in bioactivation/detoxification reactions</td>
</tr>
</tbody>
</table>

- **Bile duct cells**
  - Methylene dianiline
  - Exposure to the high concentration of reactive metabolites in bile

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### Normal Liver Histology

![Image of normal liver histology](http://www.ouhsc.edu/histology/Glass%20slides/88_03.jpg)

### Centrilobular Necrosis

![Image of centrilobular necrosis](http://faculty.swosu.edu/scott.long/txcl/hepatotx.jpg)
Steatosis (Fatty Liver)

- Microvesicular fatty liver
- Macrovesicular fatty liver

The cytoplasm is replaced by bubbles of fat that do not displace the nucleus.

The cytoplasm is replaced by a large bubble of fat that displaces the nucleus to the edge of the cell.

Synthetic toxicants
Haloalkanes

- Trichloromethane (chloroform), trichloroethylene, carbon tetrabromide, tetrachloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, and hydrochlorofluorocarbons
- Hepatotoxic potential is inversely proportional to chain length and bond energy and directly proportional to the number of halogen atoms in the molecule and to the atomic number of the halogen
Aromatic Hydrocarbons

- Most aromatic hydrocarbons are relatively low in hepatotoxic potential
- Some evidence for acute hepatic injury caused by benzene, toluene, xylene, and styrene.

Naturally occurring toxicants
Comfrey

- Marketed as herbal teas, root powders and as capsules used to treat wounds and to decrease pain and inflammation.
- Oral comfrey has been **banned or restricted** in most countries, but topical forms (ointments, creams and liniments) are available.
- Pyrrolizidine alkaloids and, when taken orally can damage hepatic endothelial cells and **can cause sinusoidal obstruction**, resulting in severe liver injury.
- The injury usually arises within 1 to 2 months of starting the comfrey product.
Penny Royal

- Used in aromatherapy, as a bath additive and as an insect repellent
- Has a strong fragrance similar to spearmint and was used for centuries to flavor food
- Contains the volatile oil pulegone
- Highly toxic, and even small doses (one tablespoon, 15 mL)
- Pulegone and its products deplete glutathione levels


Classic toxicants
The Usual Suspects

- Obesity - nonalcoholic fatty liver disease is the **most common liver disease** in the United States.
- Ethanol – responsible for 11.9% of the 6729 liver transplants nationwide in 2014, **second only to Hepatitis C (18.3%)**. **Industrial Exposure was 0.2% of liver transplants.**
- Acetaminophen - was the leading cause of acute liver failure in the United States, 1998 to 2003.


*Organ Procurement and Transplantation Network, US Department of Health and Human Services


DIMETHYLFORMAMIDE (DMF)

- Termed **“the universal solvent”**
- A colorless, water-soluble liquid. Odorless or may have fishy smell depending on its purity
- Exposure can occur during the use of ink coatings, adhesives, in the synthetic leather industry, and in the repair of aircraft. Over 2 million pounds used in the United States in 2014*
- Reported facial flushing and palpitations after ingesting alcohol.

*US EPA Toxic Release Inventory
**Dimethylacetamide (DMA)**

- Colorless, water miscible, has a high boiling point, and is commonly used as a polar solvent.
- Used as a solvent in the production of X-ray contrast media; a solvent in the production process of antibiotics like cephalosporins.

**Trichloroethylene**

- A clear nonflammable liquid with a sweetish smell resembling chloroform.
- Used in the food industry for the decaffeination of coffee and the preparation of flavoring extracts from hops and spices. Over 2 million pounds used in the United states in 2014*
- Previously used as an inhaled anesthetic, known to precipitate cardiac arrhythmias which are exacerbated by catecholamines.

*US EPA Toxic Release Inventory
Tetrachloroethylene

- A volatile, highly stable, and nonflammable, used as a solvent in dry cleaning and metal cleaning.
- It is also used as a veterinary anti-helmintic, processing and finishing in the textile industry, as an extraction solvent, grain fumigant, heat-exchange fluid, and in the manufacture of fluorocarbons.
- It is also used to degrease metal parts in the automotive and other metalworking industries and appears in certain consumer products including spot removers, paint strippers, silicone lubricants, and food.

Chloroform

- Chloroform is used to make other chemicals and can also be formed in small amounts when chlorine is added to water, nearly 400,000 pounds used in the United States in 2014.
- Byproduct of water chlorination and the bleaching of paper.

*US EPA Toxic Release Inventory
Epping Jaundice

- February 1965 – a medical student presents to St. Margaret’s Hospital, Epping, England with abdominal pain and jaundice.
- 84 people were affected
- The outbreak was tied to a bakery that had received contaminated flour.
- The flour was transported in a van that also carried chemicals including 4, 4’-diaminodiphenylmethane (MDA) for a large chemical manufacturing company.


Emerging toxicants
Dietary Supplements

- 2013 – Outbreak of acute non-viral hepatitis originating in Hawaii, affecting 97 people; 47 were hospitalized, 3 received a liver transplant, and 1 death was reported. Linked to OxyElite Pro resulting in an FDA recall.

- 2015 - Oxy ELITE Pro Super Thermogenic found to contain fluoxetine.

Kava

- Herbal derived from roots of the plant Piper methysticum ("intoxicating Pepper" plant)
- The active ingredients are kavapyrones, which have effects similar to alcohol, such as relaxation, talkativeness, and euphoria.
- Patients typically present with fatigue, nausea, elevations in serum aminotransferase levels, and jaundice 2 to 24 weeks after starting use of the product.
- The cause of hepatotoxicity from kava is unclear. Hepatotoxicity may be due to idiosyncratic or immunological pathogenesis.
- Liver injury subsides within 1 to 3 months of discontinuing the herbal product.
Herbal Supplements

- Not regulated in the same way as medications
- FDA only requires post marketing surveillance (manufacturers are not required to do any premarketing testing to ensure the supplements are safe or effective as they are for medications)
- **Over half of all FDA recalls** from 2004 – 2014 were dietary supplements, 274 dietary supplements recalled from 2009 - 2012
- In a single-center study from China, 138 cases hepatotoxicity from a population of > 21,000 patients in Shanghai over the 2-year period, April 2008 – 2010. The leading causes were herbals, accounting for 53.6% of cases, similar to incidences previously reported in other eastern cultures.


Management
Surveillance

- Routine medical surveillance should only be conducted only when exposure assessment suggests a potential for hepatic injury.
- **Poor predictive value of an routine screening** may lead to many costly diagnostic evaluations for nonoccupational liver disease.
- Preemployment measurement of baseline liver enzymes may be helpful in establishing causality for purposes of workers’ compensation.

Clinical Evaluation of a worker with suspected occupational liver injury

- The most common causes of elevated liver enzymes are ingestion of **ethanol and obesity**.
- Other causes of liver disease should be ruled out; infectious or medication induced hepatitis.
- If a history of excessive ethanol ingestion is suspected, the liver enzymes should be repeated after 4 weeks of abstinence
- Persistent serum transaminase elevation may represent chronic exposure.
Physical Examination

- Mild or early hepatotoxicity may cause few physical findings.
- Acute liver disease may present with right upper quadrant tenderness, hepatosplenomegaly, or jaundice.
- Chronic liver disease may result in stigmata such as spider angiomata, palmar erythema, testicular atrophy, ascites, and gynecomastia.

Diagnostics

- Liver Injury Tests – enzymes released from injured hepatocytes
  - aspartate aminotransferase (AST)
  - alanine aminotransferase (ALT)
    - Both can be elevated due viral, alcoholic, or ischemic hepatitis, extrahepatic obstruction, obesity, or medications
- Liver Function Tests
  - Serum Bilirubin
  - Clotting Factors (PT/INR)
- Imaging: Ultrasound
Management

- Removal from exposure
- Frequency of monitoring will depend on suspected level of exposure/injury (can range from every 6 hours to every 4 weeks)
- Referral to higher level of care (range in treatment such as glutathione replacement to liver transplant)

Question 1: Which of the following has the highest potential to cause liver injury?

- A) Methane
- B) Chlorobutane
- C) Carbon Tetrachloride
- D) Benzene
Question 2: Which zone of the hepatocyte has the least oxygen content?

- A) Portal Tract
- B) Zone 1
- C) Zone 2
- D) Zone 3

Question 3: The following slide is consistent with:

- A) Microvesicular steatois from dimethylformamide exposure
- B) Macrovesicular steatosis from hydrazine exposure
- C) Centrilobular Necrosis from carbon tetrachloride exposure
- D) Cirrhosis from ethanol exposure