Hepatic Toxicity

MSc in Molecular Pathology and Toxicology 2001

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THE LIVER

• The liver constitutes about 5% of the body mass of a rodent or human.

• It has many functions eg.
  - Carbohydrate storage and metabolism
  - Synthesis of fibrinogen and albumin etc.
  - Fat metabolism
  - Synthesis of bile acids
  - Metabolism of hormones
  - Formation of urea from amino acids

• Blood supply is about 20% arterial-80% venous.

• May contain 10-15% of blood volume
INFLUENCE OF TOXIC CHEMICALS ON THE LIVER

• The liver is the most common site of damage in laboratory animals administered drugs and other chemicals. There are many reasons including the fact that the liver is the first major organ to be exposed to ingested chemicals due to its portal blood supply.

• Although chemicals are delivered to the liver to be metabolized and excreted, this can frequently lead to activation and liver injury.

• Study of the liver has been and continues to be important in understanding fundamental molecular mechanisms of toxicity as well as in assessment of risks to humans.
LIVER
HEPATIC PORTAL VEIN
VENA CAVA
SMALL AND LARGE INTESTINE
HEPATIC VEIN
LIVER
BILE DUCT
SMALL AND LARGE INTESTINE
LOBULE

HEPATIC ARTERY

PORTAL VEIN

CENTRAL VEIN

PORTAL TRIAD

BLOOD FLOW

BILE FLOW

BILE DUCT
TYPES OF LIVER CELLS

Hepatocytes- Not all the same; depends on lobular site
  Zone I   Higher in respiratory enzymes (periportal)
  Zone III Higher in cytochrome P450 (centrilobular)

Endothelial cells
Bile duct cells
Oval cells- Possibly stem cells
Kupffer cells- Phagocytic cells Important role in inflammation
Ito cells- Fat storing or stellate cells
TYPES OF HEPATIC INJURY OR RESPONSES

Each of the different cell types may respond to a toxic insult. If severe, toxicity can result in cell death but hepatocytes have remarkable adaptive responses. These responses depend on the chemical and dose.

• Necrosis
• Lipidosis (fatty liver)
• Infiltration and Pigmentation
• Cholestasis
• Cirrhosis
• Hepatitis
• Vascular injury
• Neoplasia
### EXAMPLES OF ACUTE HEPATOTOXIC CHEMICALS

<table>
<thead>
<tr>
<th></th>
<th>Necrosis</th>
<th>Fatty Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon tetrachloride</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dimethylnitrosamine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pyrrolizidine alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Beryllium</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Diquat</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
NECROSIS

- Cell death of hepatocytes. Damage occurs in different parts of the liver lobule depending on oxygen tension or levels of particular drug metabolizing enzymes.
- Allyl alcohol causes periportal necrosis (III) because the enzyme metabolizing it is particularly located there.
  \[ \text{CH}_2=\text{CHCH}_2\text{OH} \rightarrow \text{CH}_2=\text{CHCHO} \]
- Carbon tetrachloride causes centrilobular necrosis - endothelial and Kupffer cells adjacent to hepatocytes may be normal - with diethylnitrosamine, endothelial cells are also killed. Due to activation by higher concentrations of cytochrome P450 in zone I.
- Some cells undergo apoptosis which may be increased by agents such as ethanol. Unlike necrosis there is no release of cellular contents and thus no inflammatory cells.
LIPIDOSIS

- Many chemicals cause a fatty liver. Sometimes associated with necrosis but often not. Not really understood but essentially is due to an imbalance between uptake of fatty acids and their secretion as VLDL.
- Carbon tetrachloride can cause lipidosis by interfering in apolipoprotein synthesis as well as oxidation of fatty acids.
- Other chemicals can cause lipidosis by interfering with export via the Golgi apparatus.
- Ethanol can induce increased production of fatty acids.
INFILTRATION & PIGMENTATION

• Some chemicals such as CS$_2$ cause hydropic degeneration (water accumulation in hepatocytes) possibly related to failure of sodium balance.

• Other chemicals, especially the so called peroxisome proliferators, induce lipofuscin (age pigment) accumulation due to poorly metabolized lipid probably accumulating in lysosomes.

• Some drugs eg griseofulvin, cause the deposition of the lipophilic haem precursor protoporphyrin.
CHOLESTASIS

- Accumulation of bile pigments and other products in the bile canaliculi interfering with bile flow. Can occur without damage to hepatocytes. 
  \(\alpha\)-naphthylisocyanate disrupts hepatocyte junctions forming caniculi.

CIRRHOSIS

- Hepatic fibrosis and hypoxia leading to nodular regeneration.
- Can occur after repeated insult and may lead to hepatic failure. Iron in gerbils is a good example. This mimics human disease of secondary haemochromatosis.
- Also carbon tetrachloride.
Damage to other cell types

• Damage to hepatocytes is not always observed. In some circumstances bile duct necrosis is observed (alpha-naphthylisocyanate). Other chemicals cause bile duct hyperplasia, oval cell proliferation and cholangiofibrosis (2-acetylaminofluorene).

• Other toxins can affect endothelial cells, Ito cells and Kupffer cells. Ricin and beryllium can initially first attack Kupffer cells.
## Consequences of impaired hepatic function

<table>
<thead>
<tr>
<th>Type of function</th>
<th>Example</th>
<th>Malfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient homeostasis</td>
<td>Glucose storage &amp; synthesis</td>
<td>Hypoglycemia, confusion</td>
</tr>
<tr>
<td></td>
<td>Cholesterol uptake</td>
<td>Hypercholesterolemia</td>
</tr>
<tr>
<td>Filtration of particulates</td>
<td>Endotoxin from bacteria</td>
<td>Endotoxemia</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Clotting factors</td>
<td>Bleeding</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>Hypoalbuminemia</td>
</tr>
<tr>
<td></td>
<td>Transport proteins</td>
<td>Fatty liver</td>
</tr>
<tr>
<td>Biotransformation</td>
<td>Bilirubin</td>
<td>Jaundice</td>
</tr>
<tr>
<td></td>
<td>Xenobiotics</td>
<td>Low drug metabolism</td>
</tr>
<tr>
<td></td>
<td>Steroids</td>
<td>Loss 2\textsuperscript{nd} male</td>
</tr>
<tr>
<td></td>
<td></td>
<td>characteristics</td>
</tr>
<tr>
<td>Bile and biliary excretion</td>
<td>Bilirubin,</td>
<td>Jaundice</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Hypercholesterolemia</td>
</tr>
<tr>
<td></td>
<td>Xenobiotics</td>
<td>Drug clearance</td>
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</tbody>
</table>
NEOPLASIA

• Many chemicals that cause neoplasms in rodents cause hepatocellular neoplasia (often more than 50%) although in humans, in the Western world, this is a relatively rare cancer. On the other hand, it does occur with alcohol abuse in humans and is much more common elsewhere in the world e.g. as caused by aflatoxin.

• In rodent liver definition of neoplastic lesions has proved immensely difficult. Some strains of mice have high spontaneous incidences (30-50%) whereas in others it is extremely low (<5%).
As far as regulatory toxicology is concerned, liver neoplasia is a very important and controversial topic.

Most rodent tumours are hepatocyte derived but bile duct neoplasms (cholangiocarcinoma etc.) are observed with some carcinogens such as $N$-nitrosomorpholine.

Haemangiomas and haemangiosarcomas also occur.

Often with any one carcinogen e.g. aflatoxin B$_1$ a range of tumours can be observed.

Hepatic chemical-induced carcinogenesis is a topic which is studied in great detail as a model for neoplasia in other organs.
NEOPLASTIC PROGRESSION

CHEMICAL INSULT

INITIATED CELLS

Clonal expansion

FOCI

PROMOTION

ADENOMAS/NODULES

PROGRESSION

CARCINOMAS

May come from alkylated cells or from proliferation due to injury
FOCI OF HEPATOCELLULAR ALTERATIONS AND NEOPLASIA

- CYTOLOGIC PHENOTYPES
  - Clear cell
  - Acidophilic cell
  - Basophilic cell
  - Tigroid cell

- HISTOCHEMICAL PHENOTYPES
  - Glycogen storage
  - Iron loading resistance
  - Enzyme induction
    - \(\gamma\)-glutamyl transpeptidase
    - GSH-transferase
    - Glucose 6-phosphate dehydorgenase
  - Enzyme Deficiency
    - ATPase
    - Glucose-6 phosphatase
    - Serine dehydratase
# Mechanisms in hepatocellular injury

<table>
<thead>
<tr>
<th>Aspects of mechanisms</th>
<th>Components identified</th>
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<tbody>
<tr>
<td>Metabolic activation of chemical</td>
<td>Cytochrome P450s</td>
</tr>
<tr>
<td></td>
<td>Glutathione transferases</td>
</tr>
<tr>
<td></td>
<td>Alcohol dehydrogenases</td>
</tr>
<tr>
<td>Effect of chemical activation</td>
<td>Covalent binding to protein, lipid, DNA</td>
</tr>
<tr>
<td></td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>Critical targets</td>
<td>Membrane peroxidation</td>
</tr>
<tr>
<td></td>
<td>Protein thiol depletion</td>
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<td></td>
<td>Calcium transport</td>
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For many chemicals the exact mechanisms are unknown.
Chemicals and drugs carried to the liver are metabolized initially by the cytochrome P450 haemoprotein system. This is usually to render them more polar i.e. more hydrophilic, and more excretable by conjunction with glutathione and by formation of glucuronides.

Many cytochrome P450 isoenzymes are constitutive in rat liver which is probably the most active of all tissues in this respect.

This is a vast subject and cannot be covered here but some essential points can be made.
• Although some isoenzymes will only catalyse certain monooxygenase steps - isoenzymes in the hepatic endoplasmic reticulum can catalyse oxidation of many chemicals to varying degrees including drugs, chemicals, pesticides.

• Substrate specificities, amino acid sequences and inducibilities are highly conserved across species. This has allowed a universal classification system to be constructed which often enables direct comparisons to be made between experimental systems and human isozymes.

• For some studies of drug metabolism human cytochrome P450 isozymes have been inserted into cell lines either singly or in combinations to determine the exact role of each isozyme
DIFFERENT DRUGS / CHEMICALS WILL INDUCE SYNTHESIS OF A VARIETY OF P450 ISOENZYMES

<table>
<thead>
<tr>
<th>Type</th>
<th>CYP1A1</th>
<th>CYP1A2</th>
<th>CYP1B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘3-Methylcholanthrene’ type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Phenobarbital’ type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Ethanol’ type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Steroid drug’ type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Hypolipidaemic drug’ type</td>
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</tr>
</tbody>
</table>
SUICIDAL INACTIVATION OF CYTOCHROME P450

Many chemicals when being metabolized by P450 react with these ‘detoxifying enzymes.’

CS$_2$ and other sulphur-containing drugs and chemicals react with the protein.

CC$_1$$_4$ and halothane destroy cytochrome P450 by reacting with the haem moiety at the meso positions.

Many unsaturated chemicals e.g. ethylene, griseofulvin, diethylnitrosamine alkylate pyrrole nitrogens of the haem moiety with the loss of iron. If the substituent is only a methyl or ethyl group, the resulting N-methylproto-porphyrin acts as an inhibitor of the crucial haem biosynthesis enzyme ferrochelatase.
Compounds such as diquat or menadione undergo one-electron reduction to yield the semiquinone radical perhaps catalysed by cytochrome P450 reductase. Auto-oxidation by $O_2$ gives the parent chemical + $O_2^-$. Chemicals such as bromobenzene probably react with glutathione very rapidly either with/without prior activation. This can deplete the cell of reductive capacity to critical levels.
• One explanation for the hepatic toxicity of paracetamol is that N-acetyl-p-benzoquinone imine both depletes GSH directly and cycles to produce $O_2^-$.

• At first the imine is reacting with the GSH but eventually activation by P450 overwhelms the GSH and the imine reacts with critical proteins. This then become critical and possibly irreversible.

• KO 1A2/2E1 mice are protected from paracetamol action.

• N-acetylcysteine protects, however the precise mechanism is unknown.
CONJUGATION OF METABOLITES

• GLUTATHIONE TRANSFERASES

These conjugate many hydrophobic and electrophilic compounds and their metabolites with glutathione. The conjugates are eventually cleaved to the mercapturates for excretion. Some glutathione conjugates of halogenated olefins are reactive and toxic.

Both cytosolic and microsomal GSTs have been demonstrated with overlapping substrate specificities and can be induced although not to the extent of the cytochrome P450 isoenzymes.
• **GLUCURONIDASES**

Usually conjugation of hydroxy metabolites of drugs to increase hydrophilicity for excretion. Again there are a number of these with some inducibility and some products which increase toxicity.

• **SULPHATASES**

Many hydroxylated endogenous chemicals (e.g. steroids) and drug metabolites are excreted as the sulphates.

In some circumstances, as with the carcinogen N-hydroxyacetylamino-fluorene N,O-sulphate is very unstable and probably more carcinogenic than the parent molecule.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Pathway</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 1</strong></td>
<td></td>
<td><strong>NON-TOXIC</strong></td>
</tr>
<tr>
<td>Sulfur dioxide</td>
<td>Sulfite oxidase</td>
<td>Sulphate</td>
</tr>
<tr>
<td>Phenol</td>
<td>UDP-GT</td>
<td>X-glucuronide</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>Epoxide hydrolase</td>
<td>Ethyleneglycol</td>
</tr>
<tr>
<td><strong>Type 2</strong></td>
<td></td>
<td><strong>TOXIC</strong></td>
</tr>
<tr>
<td>Chloroform</td>
<td>P450</td>
<td>Phosgene</td>
</tr>
<tr>
<td>Di-(2-ethylhexyl) phthalate</td>
<td>non-specific esterases</td>
<td>mono-ester</td>
</tr>
<tr>
<td>Red 2G</td>
<td>Azo reductase</td>
<td>Aniline</td>
</tr>
</tbody>
</table>
• AFLATOXIN B₁

This is a mould metabolite well established as a hepatic toxin and carcinogen in rats and humans.

The crucial reaction is cytochrome P450 activation of aflatoxin B₁ to the 2,3-epoxide. Elevated levels of particular glutathionetransferases will protect by conjugating the epoxide with glutathione. If no, alkylation of guanine at N⁴ or O⁶ leads to neoplasia.

Although reactions with DNA appear to be simple it is a complex process which determines whether DNA alkylation actually leads eventually to cancer.
CONSEQUENCES OF TOXIC MECHANISMS

• PEROXIDATION OF MEMBRANE LIPIDS
  Well known as a parameter of cell damage but is it before/after other critical cellular damage? Blebbing of plasma membrane

• PROTEIN THIOL DEPLETION
  Depletion of protein thiols can precede loss of viability.

• ALTERATIONS IN CALCIUM HOMEOSTASIS
  Both alkylation and oxidative toxicities. The plasma membrane is a prime regulator of calcium concentrations. In addition, calcium is sequestered in organelles.
• MITOCHONDRIAL DAMAGE

Loss of ATP synthesis, superoxide generation, rupture and loss of cytochrome c leading to apoptosis.

• ENDOPLASMIC RETICULUM DAMAGE

Dilation, hypertrophy

• DNA DAMAGE

Clearly DNA damage both by alkylation or by oxidation can lead to cell death e.g. strand breaks.

More subtle changes contribute to neoplasia. Depends on repair, site and growth status of the cells.

Chromatin disruption.

Activation of endonucleases.
CC1₃⁺ + R-SH → RS⁻, R-S-CC1₃, CHL₃,
CC1₃⁺ + protein, lipid → covalent binding
CC1₃⁺ + polyunsaturated lipid → lipid peroxidation
Lipid peroxidation → membrane damage,
enzyme inactivation,
toxic aldehyde and peroxide products
Aldehydes and peroxides → protein cross linking,
capillary permeability,
dNA damage
decreased enzyme activities
OXIDATIVE STRESS

Toxic agents may oxidatively injure liver cells in two general ways.

1. They may cause the formation of excess $O_2^-$ and $H_2O_2$.

2. They may deplete cells of protective constituents such as glutathione or vitamin E, so that cells are more susceptible to normal levels of reactive oxygen species.

3. Some chemicals may cause $O_2^-$ formation and react with glutathione.

Whether OH. is the key ultimate toxicant is still open to debate.
• Probably an important consideration is the role of iron.

\[
\begin{align*}
\text{Fe}^{3+} + \text{O}_2^- & \rightarrow \text{Fe}^{2+} + \text{O}_2^- \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-
\end{align*}
\]

or related iron oxygen species.

However, there may be circumstances where iron release from ferritin becomes a critical event. Xenobiotics are starting to be implicated in iron pathobiology.

Haemochromatosis is a disease in which the liver has very high amounts of iron and suffers, necrosis, cirrhosis and liver cancer.
Factors that influence toxicity

- Dosage
- Degree and route of absorption
- Nutrition
- Disease
- Sex
- Age
- Genetic propensity
Autoimmune responses

- Sometimes it seems that in the metabolism of a drug protein-drug adducts are produced which are expressed on the hepatocyte cell surface and act as antigens.
- Hepatocytes can then be destroyed by cell-mediated immune responses. Accompanying inflammation.
- May occur in humans after multi exposures and no dose response.
- Classic cases of halothane hepatitis in humans. Rare but high mortality.
### Genetic variation in drug metabolism enzymes

<table>
<thead>
<tr>
<th>Population</th>
<th>Percentage</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eskimos</td>
<td>95-100%</td>
<td>INH</td>
</tr>
<tr>
<td>Japanese</td>
<td>88%</td>
<td>INH</td>
</tr>
<tr>
<td>White Americans</td>
<td>48%</td>
<td>INH</td>
</tr>
<tr>
<td>Afro-Caribbean Americans</td>
<td>52%</td>
<td>INH</td>
</tr>
<tr>
<td>South Indians</td>
<td>39%</td>
<td>INH</td>
</tr>
<tr>
<td>Britons</td>
<td>38%</td>
<td>SMZ</td>
</tr>
<tr>
<td>Egyptians</td>
<td>18%</td>
<td>INH</td>
</tr>
</tbody>
</table>

**INH** - isoniazid  
**SMZ** - sulfamethazine
DETECTING HEPATIC INJURY

- Plasma ALT
- SDH, AST
- Hepatocellular
- Plasma bilirubin
- Plasma ALP
- GGT
- Cholestasis

Drug and dye clearance tests
Analysis of urine constituents by NMR
Liver imaging
Genomic and proteomic approaches

• Soon we will be able to apply global gene expression and protein profiles to understanding mechanisms of liver injury from gene activation through to pathology.

• This will allow us to predict responses to agents much more accurately and focus treatments.

• Multigene responses to hepatotoxic and carcinogenic agents are now under investigations in many labs.
End stage liver disease

In the end liver injury may be very serious leading to

- Jaundice
- Cirrhosis and ruptured portal hypertension
- Anorexia
- Ascites
- Hepatic encephalopathy
- Liver failure
- Renal failure
- Hepatocellular carcinoma