TOXIC RESPONSES
OF THE LIVER

Toxicology Journal Club
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INTRODUCTION TO THE LIVER

- Major target organ of many toxins
- Understanding of hepatotoxicity:
  
  (1) MAJOR FUNCTIONS OF THE LIVER
  (2) ITS STRUCTURAL ORGANISATION
  (3) BILE FORMATION
(1) LIVER FUNCTIONS

Complex organ - many vital functions:

- Carbohydrate/Fat/Protein metabolism
- Drugs & hormone metabolism
- Immunologic function
- Bilirubin formation and excretion
(2) STRUCTURAL ORGANISATION

KEY ROLE IN INTERMEDIARY METABOLISM
The lobule

Anatomical view from periphery to central vein

A. Oxygenated blood arrives in branch of hepatic artery

B. Branch of hepatic portal vein brings blood from intestine

C. Central veins take away processed blood

D. Bile duct takes bile to gall bladder
The acinus

Figure 13-2. Schematic of liver operational units, the acinus.

The lobule is centered around the terminal hepatic vein (THV) and is divided into three zones (zones 1, 2, 3) by the penetrating vessels. The acinus has as its base the penetrating vessels, where blood flows down the acinus past the cords of hepatocytes. Zones 3 and 2 are regions that are increasingly distant from the blood supply.
Sinusoids
Sinusoid Cells

Sinusoidal Lumen

Space Of Disse

Hepatocytes

Kupffer Cells

Ito Cells

Endothelial Cells
Endothelial Cells

Fenestrated endothelium
Separated from hepatocytes by Space of Disse
Semipermeable membrane – materials exchange
Lipoprotein metabolism – process LDL, HDL, VLDLs
Antigen presentation
Stellate cells
Differentiate into myofibroblasts
Cytokine secretion
Secrete ECM proteins
Kupffer Cells

Non-specific host defence
Inflammation/Phagocytosis
Antigen presentation
Cytokine secretion
Senescent and damaged RBCs
Tumour cell surveillance
(3) BILE FORMATION

Composed of bile salts, glutathione, phospholipids, cholesterol, bilirubin, organic anions, proteins, metals, ions, xenobiotics

Bile formation essential for:
- Lipid uptake from small intestines
- Protection of small intestine from oxidative injury
- Excretion of endogenous and xenobiotic compounds
Bile Excretion

- Driving Force of Bile Formation -
  - Active Transport of Bile Salts

- ATP-dependent exporters
  - MDR (multiple-drug resistance)
  - cMOAT (canalicular multiple organic ion transporter)
Bile Excretion

- Metals Added
  - Diffusion v receptor
  - Excretion by lysosomes
  - Cu, Mn, Cd, Se, Au, Ag, As
Bile Formation

- Conjugates of glutathione, glucuronide, sulphate
- Bile Salts
- Bilirubin
- Metals
- Drugs, hormones, xenobiotics
- Organic cations, drugs, phospholipids
- Bile Canaliculi
- cMOAT
- MDR
FACTORS INVOLVED IN LIVER INJURY

Susceptible because:

■ ANATOMY:
  - Considerable cardiac output
  - Unusual sinusoidal architecture

■ LOCATION:
  - 1st organ perfused by things absorbed from GIT

■ FUNCTION:
  - Primary organ involved in biotransformation
BIOACTIVATION & DETOXIFICATION

**PHASE I**
- Cyt P450 (centrilobular)
  - Oxidation, reduction hydrolysis
- Xenobiotic → Reactive intermediate

**PHASE II**
- Glutathione/transaminases (periportal)
  - Conjugations
- → Stable metabolite

**Balance**

Cell Injury → Detoxification
Bioactivation

**Ethanol**
- EtOH $\rightarrow$ acetaldehyde
  (rapidly by alcohol dehydrogenase)
- Acetaldehyde $\rightarrow$ acetate
  (slowly by aldehyde dehydrogenase)

- Polymorphisms in Asian people $\rightarrow$ more “fast” & less “slow” $\rightarrow$ Build-up of acetaldehyde
Bioactivation

Cytochrome P450 is a Haem-containing protein

The basic reaction that it catalyses is monooxygenation - one atom of $O_2$ is incorporated into a substrate (RH), and the other is reduced to water using NADPH:

$$\text{SUBSTRATE (RH)} \rightarrow \text{PRODUCT (ROH)}$$

$$\text{NADPH + H}^+ \rightarrow \text{NADP}^+$$
Cytochrome P450 Enzymes

A superfamily of enzymes in the SER with wide substrate specificity - a major group responsible for drug metabolism

Two Important Points to Remember:

Each isozyme can metabolise MANY different drugs, and many drugs can be metabolised by more than one isozyme

Very few compounds are conjugated directly, so PHASE I metabolism is a very important line of defence - Unfortunately Cyt p450 can → reactive oxygen compounds that are hepatotoxic (esp CYP2E1 & CYP3A)
Bioactivation

Carbon Tetrachloride

$CCl_4 \xrightarrow{P450} \cdot CCl_3 \xrightarrow{CYP2E1} CCl_3OO\cdot$

Lipid peroxidation of fatty acid
Bioactivation

**Acetaminophen**

1st introduced into clinical medicine late 1900s - "back door" - no formal preclinical animal toxicity studies

Attracted little attention and was soon forgotten

Potential hepatotoxicity was not suspected until the first clinical reports of severe and fatal liver damage following OD in 1960s

Species differences in its metabolic activation.

Paracetamol is involved in 15 to 30% of deliberate self-poisonings in UK
**Acetaminophen**

- *Glutathione-S-transferase is important*
- Uses glutathione as a cofactor
- Acetaminophen metabolite conjugated by *Glutathione*
- Toxicity only revealed when GSH levels depleted below a certain level

- Overdose
- Fasting
Overdose

Acetaminophen

P450

NAPQI
(N-acetyl-p-benzoquinoneimine)

Sulphate/Glucuronide Saturated

Covalent Binding to Macromolecules

Cell Death (Zone 3)

Glutathione

Glutathione Conjugate
Sinusoidal Cells in Liver Toxicity

- Kupffer cells/Ito cells become activated after exposure to toxins

- Kupffer cells can be activated by Vit A \( \rightarrow \) this can in turn enhance acute toxicity of \( CCl_4 \)

- Activated Kupffer cells produce reactive oxygen species & reactive nitrogen species
Mechanisms of Liver Damage

**Cell skeleton**

- **Microcystin**:  
  - Covalently binds to cytoskeletal proteins  
  - Leads to hyperphosphorylation reactions  
  - Microtubular “scaffolding” collapses resulting in deformation of hepatocyte
Mechanisms of Liver Damage

**Cholestasis**
- Toxins can inhibit bile formation by various mechanisms
  - Transporter/Export function
  - Tight junction leakage
  - Concentration of reactive substances
Mechanisms

Mitochondrial damage

- Toxins can:
  - Inhibit mitochondrial DNA synthesis
  - Lead to free radical production by effects on electron transport chain
RESPONSE TO DAMAGE

In general tissues respond similarly:

- Inflammation
- Degeneration / Necrosis
- Recovery / Proliferation / Malignancy
Types of Liver Injury

Response relates to degree & duration of the insult as well as the population of cells affected

- Fatty change
- Hepatocellular death
- Canaliculare cholestasis
- Bile duct damage
- Sinusoidal damage
- Cirrhosis
- Neoplasia
Fatty Change

- Increased lipid in hepatocyte cytoplasm
- Due to altered lipid metabolism
- Common with acute toxins
- Potentially reversible
- Alcohol - most common cause
Cell Death

Necrosis

- Cell swells
- Leakage of cytoplasm
- Nuclear disintegration
- Eg/ Acetaminophen
Apoptosis

Cell shrinkage
Nuclear fragmentation
Formation of apoptotic bodies
Usually no/minimal inflammation
Cholestasis

- Reduced formation/secretion of bile

- So things normally excreted in bile, like bilirubin, begin to accumulate → icterus

- Cyclosporin, oestrogens
Bile Duct Damage

- Cholangiodestructive cholestasis
- Increased serum activity of gamma glutamyltransferase (GGT)
- Bile duct epithelial damage →
  - Necrosis, Inflammation, Fibrosis
  - Bile duct hyperplasia
  - Bile duct loss (Vanishing bile duct syndrome)
Biliary inflammation and fibrosis
Cirrhosis

- Progressive liver injury - chronic reaction
- Necrotic areas replaced by fibrous tissue
- Result of repetitive damage of liver cells
- Alcohol abuse
Cirrhosis
Neoplasia

- **Primary neoplasm**
  - Neoplasm from cells in the liver
    - Hepatocytes
    - Bile duct epithelium

- **Secondary neoplasm**
  - Neoplasm outside the liver
    - Invasion
    - Metastasis

- **Aflatoxins**
  - Hepatocellular carcinomas
Hepatocellular adenoma
Hepatocellular carcinoma
Bile duct adenoma
Biliary Carcinoma
LIVER SUMMARY

Metabolically important organ
  - Biosynthesis, nutrient metabolism
  - Detoxification & Biotransformation

Achilles Heel Effect
  - High cardiac output & optimum anatomic location
  - Biotransformation can produce harmful substances
  - Enterohepatic recirculation - re-exposure
THE END

WHAT AM I, CHOPPED LIVER?

SOON.