Hepatotoxicity

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Photomicrographs courtesy of the National Toxicology Program (http://ntp.niehs.nih.gov)
Outline

• Overview/Classification
• Assessment
  • Clinical signs
  • Clinical chemistry
  • Gross pathology
  • Organ weights
  • Histopathology
• Considerations/Influences/Factors/Tissue repair
• Case examples
Hepatotoxicity

• Predictable hepatotoxins
  – Acetaminophen
  – Allyl alcohol
  – Carbon tetrachloride

• Idiosyncratic hepatotoxins
  – Traglitazone
  – Bromfenac
FIGURE 2.—The concept of idiosyncratic hepatocellular injury. For the majority of treated patients, the drug is entirely safe. A subset develop elevated serum aminotransferases (ALT), but most of these patients will have resolution of the liver injury with continued treatment. A generally accepted theory is that a subset of these patients with elevated serum ALT will not “adapt” and will go on to develop progressive liver injury.

## Categories of Hepatotoxicity

<table>
<thead>
<tr>
<th>Histologic Lesion/Type of injury</th>
<th>Mechanisms</th>
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<tbody>
<tr>
<td>• Degeneration/necrosis/cytotoxicity</td>
<td>• Ca homeostasis disruption</td>
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<td>• Cholestasis</td>
<td>• Canicular/cholestatic</td>
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<tr>
<td>• Inflammation</td>
<td>• Metabolic bioactivation</td>
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<tr>
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<td>• Autoimmunity</td>
</tr>
<tr>
<td></td>
<td>• Increased apoptosis</td>
</tr>
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<td>• Mitochondrial injury</td>
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<td>• Non-hepatocyte mediated</td>
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MECHANISMS OF HEPATOTOXICITY

- Definitive (single) etiology versus multi-hit
  Adverse effects often occur together (domino effect)
  - hepatocellular degeneration, necrosis
  - biliary cell degeneration, necrosis
  - cholestasis
  - hepatitis (often not a primary finding)
  - fibrosis, cirrhosis
  - vascular, sinusoidal effects
  - pre-neoplastic lesions (altered hepatocellular foci, others), mitogenesis
Assessment of Hepatotoxicity

• Clinical signs
• Clinical chemistry
• Gross pathology
• Organ weight
• Histopathology
Clinical Chemistry

• Advantages
  – Serial sampling
  – Detection of metabolic injury
  – Detection of organ specific effects
  – Help establish NOEL
  – Help determine toxic mechanism

• Act within specific cellular localization
  – Cell membrane
  – Cytosol
  – Mitochondria
Cellular Localization

- **Cholephilic analytes & enzymes**
  - Bile acids
  - Alkaline phosphatase
- **Cytosolic enzymes**
  - Alanine aminotransferase
  - Sorbitol dehydrogenase
- **Membrane enzymes**
  - Gamma glutamyl transpeptidase
  - Alkaline phosphatase
- **Mitochondrial enzymes**
  - Glutamate dehydrogenase
Recommended Clinical Chemistry for Hepatotoxicity

• Hepatocellular toxicity
  – Alanine aminotransferase (ALT)
    • Cytosolic
    • Liver specific
    • Half-life 48 to 60 hours
      • Glucocorticoids can increase ALT in rats up to 13X
  – Sorbitol dehydrogenase (SDH)
    • Good for hepatocellular injury in all species
    • Short half-life (<6 hours)
• Aspartate aminotransferase (AST)
• Lactate dehydrogenase
  – Total bile acids
    • Affected by altered enterohepatic circulation and altered hepatic function
    • Also good indicator of cholestasis
Recommended Clinical Chemistry for Hepatotoxicity

• Hepatobiliary toxicity
  – Alkaline phosphatase
    • Fairly ubiquitous – membranes & brush borders
    • Bone and placenta
    • Good marker of cholestasis
    • Minimal increase in hepatocellular damage
  – 5’-Nucleotidase
    • Membrane enzyme
    • Ubiquitous – kidney and intestine high
    • Good marker for cholestasis
  – Total bile acids
    • Relatively sensitive indicator of cholestasis
  – Bilirubin, direct and total
    • Total bilirubin (direct from cholestasis; indirect from hemolytic disease)
    • Measure total and direct and determine indirect by subtraction
Evaluation of Liver

Alanine Aminotransferase (ALT, SGPT)
- Greatest activity - hepatocytes; also found in skeletal/cardiac muscle
- Biological half-life - varies (~48-60 hours)
- Can be induced (eg., glucocorticoids – up to 13X increase)
- Increased - hepatocellular injury, induction, muscle injury
- Decreased - enzyme inhibition (cyclosporin)

Sorbitol Dehydrogenase (SDH)
- Greatest activity - hepatocytes; also found in testes
- Biological half-life - short (≤6 hours)
- Sample stability - not as stable; in rats, stable refrigerated (~2 days)
- Not known to be induced
- Only known cause for serum increase - hepatocellular injury or leakage
- Good indicator for all species
Evaluation of Liver - cont.

Aspartate Aminotransferase (AST, SGOT)
- Greatest activity - found in numerous tissues (not specific for liver injury)
- Biological half-life - short (~15-24 hours)
- Red blood cells contain significant amounts (hemolysis - falsely elevates)
- Used in past to detect hepatocellular injury (still used for large animals); used for muscle injury

Alkaline Phosphatase (ALP)
- Greatest activity - liver, bone intestine, kidney, placenta
- Biological half-life - isoenzymes of different tissues highly variable
- Can be induced (eg., glucocorticoids, phenobarbital, dieldrin)
- Increased - cholestasis, drug induction, increased osteoblastic activity, cancer
- Decreased - decreased food intake (rats)
Evaluation of Liver - cont.

Bilirubin, direct (conjugated) and indirect (unconjugated)
- Breakdown product of hemoglobin
- Direct: conjugation carried out by liver
- Increased indirect – Increased hemolysis; decreased hepatic uptake
- Increased direct - cholestasis

Total Bile Acids (TBA)
- Produced by liver - cholic and chenodeoxycholic (primary bile acids)
- Taurine or glycine conjugated and secreted into bile
- Intestinal bacterial modification produces deoxycholic and lithocholic acids
- Increased - cholestasis, decreased hepatic uptake/conjugation, hepatic injury
- Decreased - altered enterohepatic recirculation
**Clinical Chemistry Case Examples**

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**Decreased ALP - decreased food intake**
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Increased ALT, SDH, TBA – suspect hepatocellular injury
## Clinical Chemistry Case Examples

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Increased ALT, SDH, ALP, TBA, T & Dbili – suspect biliary obstruction
Interpreting Clinical Chemistry

• Know the reference range
• Know the sampling interval
• Enzyme induction versus cellular damage
• Magnitude of the change
• Alteration of single versus multiple analytes
• Correlation with other changes
  – Clinical signs
  – Organ weights
  – Histopathology
Outline

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  - Histopathology
- Considerations/Influences/Factors/Tissue repair
- Case examples
Hepatomegaly
**Liver weights (g) in rats treated with flame retardant containing polybrominated diphenyl ethers**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Male rat</th>
<th>Female rat</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>10.1±0.5</td>
<td>5.6±0.5</td>
</tr>
<tr>
<td>0.01</td>
<td>11.2±1.0</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>5</td>
<td>12.3±1.4*</td>
<td>6.5±0.4*</td>
</tr>
<tr>
<td>50</td>
<td>16.0±1.6*</td>
<td>8.7±0.5*</td>
</tr>
<tr>
<td>100</td>
<td>17.4±1.4*</td>
<td>9.8±0.8*</td>
</tr>
<tr>
<td>500</td>
<td>20.0±1.8*</td>
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* p<0.05.
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Cellular degeneration

- Glycogen depletion
- Fatty change
- Phospholipidosis
- Amyloidosis
- Mineralization
- Pigment deposition
- Crystals
- Inclusion bodies
- Hypertrophy, hepatocellular
- Atrophy, hepatocellular
Functional Structure of Hepatic Parenchyma

- Classical lobule
- Portal lobule
- Central vein
- Liver acinus
- Portal canals
Heterogeneity of Liver

- Hepatocytes (80% of parenchyma)
- Biliary epithelium
- Endothelia
  - sinusoids
  - blood vessels (arteries and veins)
  - lymphatics
- Kupffer cells
- Hepatic stellate cells
- Lymphocytes (Pit cells)
5 major players

- Sinusoid
- Hepatic Stellate Cell
- Endothelial cell
- Bile canaliculus
- Hepatocyte
- Space of Disse
- Kupffer cell
- Microvilli
- Nucleus
Hepatic stellate cell
(5-8% of liver cells)

Tissues and Organs: a text of scanning electron microscopy, Kessel, RG and Kardon, RH, 1979
Heterogeneity of Liver

- Progenitor cells
  - Oval cell – rodent models
  - Hepatoblasts – humans Fibroblasts
- Smooth muscle cells (blood vessels)
- Mesothelia
- Nerves (unmyelinated)
- Neuroendocrine cells
- Hematopoietic cells
- Blood
- Extracellular matrix
  - 5-10% of liver is collagen
Histologic Liver Responses

- Cytoplasmic alteration
  - Glycogen deposition and depletion
  - Fatty change
  - Pigmentation
  - Degeneration
  - Cell death
    - Apoptosis
    - Necrosis
  - Hypertrophy
  - Karyomegaly
  - Atrophy

- Inflammatory cell infiltrates
- Angiectasis
- Proliferative responses
  - Non-neoplastic
  - Neoplastic
- Biliary cysts
- Phospholipidosis
- Amyloidosis
- Crystals
- Inclusion bodies
Normal Rodent Liver – Fasted Animal
Glycogen accumulation
PAS

PAS with diastase
Glycogen
Glycogen

Normal glycogen accumulation

Glycogen depletion
Glycogen

Normal glycogen accumulation

Excessive glycogen
Histologic Liver Responses

- Cytoplasmic alteration
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Macrophesicular Fatty Change
Fatty change and glycogen accumulation
Microvesicular Fatty Change
Macrovesicular fatty change

Microvesicular fatty change
Oil-red-O stain for lipid
Mouse Liver
Cytoplastic Alteration

Glycogen

Microvesicular Fat
Glycogen and Fatty Change

• Glycogen common & often not diagnosed
  – Treatment effects not common
• Fatty change reflects a treatment effect
  – Typically reversible
  – Macrovesicular – altered lipid metabolism
  – Microvesicular – possible mitochondrial effect
• Combinations of glycogen and fatty change
Histologic Liver Responses

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Hepatic Pigments

- In hepatocytes, canaliculi, bile ductules, bile ducts, Kupffer cells
  - Bile (cholestasis)
  - Lipofuscin
  - Hemoglobin, methemoglobin
  - Thorotrast / other drugs
Pigment Deposition
Cholestasis
Cholestasis (bile)

cytoplasmic
canalicular
Detection of bile

Hall’s stain for bile
Histologic Liver Responses

- Cytoplasmic alteration
  - Glycogen
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  - Pigmentation
  - Degeneration
- Cell death
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Degeneration and Fatty Change in a Mouse Given Benzene Hexachloride
Hydropic Degeneration
Cystic Degeneration
(Spongiosis hepatis)
Pigmentation & Degeneration

• In general, pigmentation and degeneration are treatment-related
  – Cystic degeneration is usually not treatment-related
• Severity of change is important
• Both are reversible but pigment may take a long time to be removed
Histologic Liver Responses

- Cytoplasmic alteration
  - Glycogen depletion
  - Fatty change
  - Pigmentation
  - Degeneration
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Apoptosis
Apoptosis in a Liver with Increased Peroxisomes in Hepatocytes
Single Cell Necrosis or Apoptosis?
Necrosis (Oncosis)
PERIPORTAL

MID-LOBULAR

CENTRILOBULAR

Classical lobule
Centrilobular Hepatocyte Necrosis
Centrilobular Hepatocyte Necrosis
Centrilobular Hepatocyte Necrosis
Bridging Centrilobular Hepatocyte Necrosis
Centrilobular necrosis

CENTRILOBULAR

PORTAL
Centrilobular Necrosis with Hemorrhage and Mineralization
Centrilobular necrosis & inflammation
Midlobular necrosis
Acetaminophen – lobe variation in necrosis
3-D reconstruction

MRI study - acetaminophen

Control

Treated
MRI study - acetaminophen

Control

Treated

Portal vein

Hepatic vein
Necrosis & Apoptosis
Outline

• Overview/Classification
• Assessment
  • Clinical signs
  • Clinical chemistry
  • Gross pathology
  • Organ weights
  • Histopathology
• Considerations/Influences/Factors/Tissue repair
• Case examples
Histologic Liver Responses

- Cytoplasmic alteration
  - Glycogen depletion
  - Fatty change
  - Pigmentation
  - Degeneration
  - Cell death
    - Apoptosis
    - Necrosis
  - Hypertrophy
- Karyomegaly
- Atrophy

- Inflammatory cell infiltrates
- Angiectasis
- Proliferative responses
  - Non-neoplastic
  - Neoplastic
- Biliary cysts
- Phospholipidosis
- Amyloidosis
- Crystals
- Inclusion bodies
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Hepatomegaly

- Increase cell size (hypertrophy)
- Increase cell number (hyperplasia)
- Increase blood
**Liver weights (g) in rats treated with flame retardant containing polybrominated diphenyl ethers (PBDEs)**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Male rat</th>
<th>Female rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.1±0.5</td>
<td>5.6±0.5</td>
</tr>
<tr>
<td>0.01</td>
<td>11.2±1.0</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>5</td>
<td>12.3±1.4*</td>
<td>6.5±0.4*</td>
</tr>
<tr>
<td>50</td>
<td>16.0±1.6*</td>
<td>8.7±0.5*</td>
</tr>
<tr>
<td>100</td>
<td>17.4±1.4*</td>
<td>9.8±0.8*</td>
</tr>
<tr>
<td>500</td>
<td>20.0±1.8*</td>
<td>12.2±1.1*</td>
</tr>
</tbody>
</table>

* p<0.05.

**Microsomal Enzyme Induction**
Enzyme Induction
Smooth Endoplasmic Reticulum (SER) Proliferation

Central Vein
Smooth Endoplasmic Reticulum (SER) Proliferation
CYP3A1 Immunohistochemistry

Constitutive

Induced

Clayton, et al. 2007
Common enzyme inducers

- CAR (Constitutive Androstane Receptor)
  - Phenobarbitone & CYP2B

- PRX (Pregnane X receptor)
  - Cyproterone acetate & CYP3A

- AhR (Aryl hydrocarbon receptor)
  - Dioxin & CYP1A1, 1A2, 1B1

- PPAR (Peroxisome Proliferator-Activated Receptor)
  - Fibrates and Peroxisomes
Peroxisome Proliferation
Peroxisome Proliferation Ultrastructure

Control

Treated
Peroxisome Proliferators

- Hypolipidemics
  - Clofibrate
  - Gemfibrozil
- Methaphenilene
- Ibuprofen
- Diethylhexyl phthalate
Toxicologic significance of enzyme inducers

• Increased degradation of hormones
• Increased deactivation or activation of xenobiotics
• Altered metabolism of drugs
Adverse?
When is hepatic enzyme induction adverse?

• Degeneration
• Necrosis / apoptosis
• Hyperplasia
  • Hepatocellular
  • Bile duct
• Steatosis / lipidosis
• Cholestasis
• Karyomegaly
Persistent & Excessive Enzyme Induction
Persistent & Excessive Enzyme Induction
Karyomegaly
Multinucleated Hepatocytes
Cholestasis
Hepatic Enzyme Induction

• Common response to xenobiotic exposure
• Considered adaptive and non-adverse in the absence of indications of toxicity
  – No associated histopathology or markedly abnormal clinical chemistry
• May have secondary effects (e.g., thyroid adenomas)
• Can be adverse when extreme
  – Can produce toxicity, can generate oxygen radicals
Hypertrophy and Cancer
Hypertrophy and Liver Cancer in Mice

- enzyme induction > 140%

- liver weight >150% at 1 year

- induction of p450 enzymes may lead to the formation of oxygen radicals or other electrophilic reactive species capable of causing DNA damage

- hyperplasia and degeneration

- polyploidy, aneuploidy

- high probability of liver cancer at 2 years
Histologic Liver Responses

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Inflammation

- Acute
- Chronic
- Chronic active
- Granulomatous
- Mononuclear cell aggregates
- Inflammation, peribiliary
- Fibrosis (cirrhosis)
Focal Necrosis and Inflammation
Granulomatous Inflammation
Extramedullary hematopoiesis?
Extramedullary hematopoesis?
Portal Fibrosis
“Cirrhosis”

Diallyl phthalate F344 Rat
Some Summary Points

- Liver has remarkable reserve capacity
- Responses can be adaptive
  - Non-adverse
  - Adverse
- Adverse effects often occur together
- Rat liver has a secondary lobular structure that may explain unusual distribution of lesions
- Nodular lesions and aggressive proliferative changes in the liver are not necessarily neoplasms
Histologic Liver Responses

- Cytoplasmic alteration
  - Glycogen depletion
  - Fatty change
  - Pigmentation
  - Degeneration
  - Cell death
    - Apoptosis
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- Amyloidosis
- Crystals
- Inclusion bodies
Foci of Cellular Alteration
Foci of cellular alteration occur spontaneously in older rodents and may be induced by treatment and occur in younger rodents. They are relatively uncommon in young rodents. It is recommended that the occurrence of foci of cellular alteration in prechronic studies be documented and classified into appropriate subtypes.
Progression of Proliferative Liver Lesions

Basophilic Focus

Hepatocellular adenoma

Metastatic carcinoma

Hepatocellular carcinoma
Relationship of Foci and Liver Tumors in F344 Rats

• Liver tumor negative studies
  – No increased incidence of foci at study termination

• Liver tumor positive studies – genotoxic agent
  – 3 to 10-fold increase in multiple foci
  – Eosinophilic (3-10X); Clear (2-9X); Mixed (3x); Basophilic (4-8X)

• Liver tumor positive studies – non-genotoxic agent
  – 2 to 15-fold increase in foci
  – Eosinophilic (4-15X); Mixed (2-5X)
Regenerative Hyperplasia; Nodular hyperplasia
Oval Cell Proliferation
Bile Duct Hyperplasia
Outline

• Overview/Classification
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  • Clinical chemistry
  • Gross pathology
  • Organ weights
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• Considerations/Influences/Factors/Tissue repair
• Case examples
Ability of liver to regenerate is well known
Following injury a cascade of promitogenic signals is triggered
Tissue repair follows a dose response
Tissue repair increases with dose up to a threshold dose
Promitogenic signaling is inhibited by doses above the threshold
Chemicals That Induce Tissue Repair

- Acetaminophen
- Allyl alcohol
- Carbon tetrachloride
- Choroform
- 1,2-Dichlorobenzene
- Thioacetamide
- Trichloroethylene
Considerations in Interpretation of Bioassay Data

<table>
<thead>
<tr>
<th>Neoplasia</th>
<th>Non-neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Modifying factors</td>
<td>• Modifying factors</td>
</tr>
<tr>
<td>• Dose relationships</td>
<td>• Dose relationships</td>
</tr>
<tr>
<td>• Trans-sex &amp; trans-species</td>
<td>• Trans-sex &amp; trans-species</td>
</tr>
<tr>
<td>• Common vs. unique lesions</td>
<td>• Common vs. unique lesions</td>
</tr>
<tr>
<td>• Lesion progression</td>
<td>• Lesion progression</td>
</tr>
<tr>
<td>• Species/strain susceptibility</td>
<td>• Species/strain susceptibility</td>
</tr>
<tr>
<td>• Controls</td>
<td>• Controls</td>
</tr>
<tr>
<td>• Lumping &amp; Splitting</td>
<td>• Lumping &amp; Splitting</td>
</tr>
<tr>
<td>• Direct vs. indirect causality</td>
<td>• Direct vs. indirect causality</td>
</tr>
<tr>
<td>• Benign vs. malignant</td>
<td>• Adaptive vs. adverse</td>
</tr>
<tr>
<td>• Latency</td>
<td>• Severity</td>
</tr>
<tr>
<td>• Multiplicity</td>
<td>• MTD, NOEL and NOAEL</td>
</tr>
<tr>
<td>• Levels of evidence of carcinogenicity</td>
<td></td>
</tr>
</tbody>
</table>
## Dose and Dose Relationships

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mg/kg</td>
<td>10 mg/kg</td>
<td>20 mg/kg</td>
<td>40 mg/kg</td>
</tr>
<tr>
<td>Liver lesion response</td>
<td>3/50</td>
<td>6/50</td>
<td>13/50</td>
<td>25/50</td>
</tr>
</tbody>
</table>
Figure 1. Serum ALT levels 24 hours after dosing with APAP (300mg/kg) or vehicle (0.5% methylcellulose).

From I. Rusyn, University of North Carolina
Non-neoplastic Severity Responses

Grades of lesions severity used by the NTP:

- Minimal (1+)
- Mild (2+)
- Moderate (3+)
- Marked (4+)
- Severe (5+)
MTD, NOEL and NOAEL

MTD = Maximum tolerated dose

NOEL = No observable effect level. Highest dose administered that does not produce toxic effects.

NOAEL = No observable adverse effect level. Highest dose administered that does not produce an adverse effect.
Case 1 - Chronic hepatic inflammation in a 6-month study. 
N=20 per group.

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Is there a real effect here? At what dose?
Case 1 - Chronic hepatic inflammation in a 6-month study.
N=20 per group.

<table>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>16*</td>
</tr>
</tbody>
</table>

* p = 0.059

Is there a real effect here? At what dose? Is there a NOAEL?

If this is the only change in the study, what is the MTD for purposes of setting a high dose for a 2-year cancer bioassay?
Case 2

Liver necrosis in a 6-month monkey study. N=4 per group. The company wants to market this new anti-inflammatory drug for use in humans.

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Control</th>
<th>Low Dose</th>
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<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis, mild</td>
<td>0/4</td>
<td>0/4</td>
<td>2/4</td>
<td>1/4</td>
</tr>
</tbody>
</table>
Liver necrosis in a 6-month monkey study. N=4 per group. The company wants to market this new anti-inflammatory drug for use in humans.

<table>
<thead>
<tr>
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<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis, mild</td>
<td>0/4</td>
<td>0/4</td>
<td>2/4</td>
<td>1/4</td>
</tr>
</tbody>
</table>

What is the NOAEL?

You are a new toxicologist just hired by this company. Would you recommend that they take this drug into human clinical trials?

What top dose would you recommend that they use?

Any additional suggestions for your new employer regarding this new drug candidate?
Case 3 - Liver inflammation in a 6-month rodent toxicity study.

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation, acute, focal, mild</td>
<td>0/20</td>
<td>0/20</td>
<td>2/20 (10%)</td>
<td>3/20 (15%)</td>
</tr>
<tr>
<td>Inflammation, acute, focal, severe</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
<td>3/20 (15%)</td>
</tr>
<tr>
<td>Inflammation, chronic, focal, mild</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Inflammation, granulomatous, focal, mild</td>
<td>1/20 (5%)</td>
<td>0/20 (0%)</td>
<td>1/20 (5%)</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Fibrosis, focal, minimal</td>
<td>0/20 (0%)</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
</tr>
</tbody>
</table>

Is there a real effect? Is there a good dose response?
<table>
<thead>
<tr>
<th>Diagnoses</th>
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<th>High dose</th>
</tr>
</thead>
<tbody>
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<td>0/20</td>
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<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Inflammation, granulomatous, focal, mild</td>
<td>1/20 (5%)</td>
<td>0/20 (0%)</td>
<td>1/20 (5%)</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Fibrosis, focal, minimal</td>
<td>0/20 (0%)</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
</tr>
<tr>
<td>Inflammation, chronic, active</td>
<td>2/10</td>
<td>2/10</td>
<td>5/20</td>
<td>11/20*</td>
</tr>
</tbody>
</table>

* p=0.0497

What is the NOEL? What is the NOAEL? What is the MTD?
Specific hepatic changes often do not occur in isolation