Toxicology for Industrial and Regulatory Scientists

Toxicology of Organ Systems

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Overview

• For liver, lung, brain/nervous system, kidney, heart and skin: cell types, organ-related toxicants, and methods of testing will be discussed.

• For eye and gastrointestinal tract, an abbreviated discussion of some relevant toxicants will be discussed.
Toxicology of the Liver
Drug-induced liver injury

• Most common reason to halt development of a drug
• “Currently, no serum biomarkers, including the biochemical gold standard alanine aminotransferase, can differentiate drug-induced from non-drug-related liver injury, can differentiate liver injury mediated by a specific drug or mechanism, or can accurately predict the progression and outcome of hepatic injury”

Cell types in the liver

- **Hepatocytes**
  - Majority of cells in the liver
  - Synthesize bile, contain metabolic enzymes

- **Kupffer cells**
  - Resident macrophages in liver
  - Ingest circulating particles

- **Ito (stellate) cells**
  - Main site of vitamin A storage in the body
  - Proposed to act as antigen presenting cells
  - Activation (e.g. by ethanol) induces secretion of collagen, other extracellular matrix proteins

- **Endothelial cells**
  - Line sinusoids
Liver - Structural Organization

Classic liver lobule consists of a portal tract (‘triad’)

1) portal vein  
2) hepatic artery  
3) bile duct  
4) central vein

- Hepatic blood flow and oxygen gradient are important factors affecting the metabolic activity of the liver

- Liver ‘zones’
  - Zone 1: Highest oxygen content; damage by direct acting agents; efficient extraction of bile salts; highest level of glutathione
  - Zone 2: Intermediate
  - Zone 3: ‘Hypoxic’; greatest concentration of cytochrome P450s (CYPs)
Organization of the liver

Blood drains from the portal tract toward the central vein

Bile flows toward the portal tract
Functions of the Liver

Function
- “First pass” metabolism of absorbed materials
  - Detoxification/degradation
  - Bioactivation
- Detoxification of endogenous toxins
  - Bilirubin, ammonia
- Activation of testosterone
  - Administration of CYP inhibitors -> loss of secondary male sex characteristics
- Phagocytosis of materials such as bacterial fragments/endotoxin
  - Kupffer cells
- Synthesis of clotting factors, albumin, transport proteins (VLDL)
- Synthesis and secretion of bile
  - Many transporters are involved in this process
- Dysfunction can occur without appreciable (histologically-evident) cell damage
Liver Cell Death
(applies to other tissues, too)

- **Necrosis (oncolytic cell death)**
  - Cell swelling, leakage of cellular contents, nuclear disintegration (karyolysis), and inflammatory cell infiltration
  - Generally affects many contiguous hepatocytes/parenchymal cells
  - Results in release of liver-specific enzymes into plasma (alanine [ALT] or aspartate [AST] aminotransferases)
  - Histologically, inflammatory cell infiltrates and lack of nuclei and clear cell membranes by H&E staining

- **Apoptosis (programmed cell death)**
  - A single-cell event that is programmed to eliminate aged or un-needed cells (e.g. during development)
  - Associated with cell shrinkage; no inflammation
  - Caspases intracellularly cleave DNA and nuclear structural proteins (apoptotic bodies)
  - Apoptotic bodies are phagocytosed by adjacent Kupffer cells or hepatocytes
    - Hence, no release of intracellular contents
## Types of liver injury and representative agents

<table>
<thead>
<tr>
<th>Fatty liver</th>
<th><strong>CCl₄, ethanol, valproic acid, fialuridine, high fat diet</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte death</td>
<td>Acetaminophen, allyl alcohol, copper, dimethylformamide</td>
</tr>
<tr>
<td>Immune-mediated response</td>
<td>Halothane, diclofenac</td>
</tr>
<tr>
<td>Canalicular cholestasis</td>
<td>Chlorpromazine, estrogens, manganese, cyclosporin A, 1,1-dichloroethylene</td>
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<tr>
<td>Bile duct damage</td>
<td>Amoxicillin, methylene diamine, sporidesmin, α-naphthylisocyanate</td>
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<tr>
<td>Sinusoidal disorders</td>
<td>Anabolic steroids, microcystin, cyclophosphamide</td>
</tr>
<tr>
<td>Fibrosis, cirrhosis</td>
<td><strong>CCl₄, ethanol, vitamin A, vinyl chloride, thioacetamide</strong></td>
</tr>
<tr>
<td>Tumors</td>
<td>Aflatoxin, androgens, arsenic, vinyl chloride, hepatitis infection</td>
</tr>
</tbody>
</table>
Fatty liver
Assessment of Hepatic Toxicity

- Hepatocyte necrosis
  - Histopathological evaluation
  - Clinical pathology assay for increases in liver enzyme activity (ALT)
- Bile duct damage
  - elevated alkaline phosphatase
- Cholestasis
  - Jaundice, increase in total bilirubin, histological evaluation
- Steatosis (fatty liver)
  - Histopathological evaluation (paraffin), Oil red O staining (frozen)
- Cirrhosis
  - Histopathological evaluation, special stains to detect fibrotic tissue
- Other potentially useful, emerging serum biomarkers:
  - PON1
  - Arginase 1
  - Glutamate dehydrogenase
  - Serum F protein
  - Regucalcin
Lung
Overview of the Respiratory tract

From Molecular and Biochemical Toxicology, RC Smart and E Hodgson, eds. p. 640.
Function of the lung

- Function: Gas exchange
  - Dependent upon intimate contact between the very thin processes of type 1 epithelial cells and blood endothelial cells in the alveolar walls.

Micrograph of 4 alveoli (A) separated by alveolar septum. C=capillaries.
Cell types in the lung

• Type 1: cells: gas exchange

• Type 2 cells
  – Surfactant production
  – Division to repair lung injury

• Clara cells
  – Cytochrome P450-mediated bioactivation of compounds to toxic metabolites in the lung happens most often in Clara cell
  – Examples: naphthalene, styrene, 3-methylindole
  – Distribution of Clara cells varies greatly from species to species

• Pulmonary macrophages
  – Phagocytosis
  – Cytokine release
Particle deposition in the respiratory tract

Directional change

Very abrupt
(impaction; 5-30 µm)

Less abrupt
(sedimentation; 1-5 µm)

Mild
(diffusion; 1 µm and less)

Air velocity

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+++  ++

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Toxicant Damage to the Lung

• Pathogenesis of lung damage caused by chemicals
  – Bioactivation to reactive intermediates
    • Clara cells > Type 2 cells
  – Free radicals, oxidative stress
    - ozone, NOx, tobacco smoke constituents
  – Particle ingestion by macrophages
    • Cytokine release, inflammatory cell recruitment
  – Water solubility of gases define the pattern of toxicity of the gas
    • Highly soluble gases (chlorine gas, formaldehyde sulfur dioxide, ammonia) do not penetrate further than the nose
    • Highly insoluble gases (phosgene, nitrogen oxides, ozone) penetrate deeply into the lung responses

– Particles
  • Depending on particle size, they are deposited by interception (fibers), impaction, sedimentation, and diffusion
CC10 immunohistochemistry of Clara cells in terminal bronchioles of mice

(A) Control mice shows intense and evenly distributed Clara cell-specific CC10 expression (black arrows).

(C) Consistent with Clara cell necrosis, a single dose of coumarin caused a significant diminution in CC10.

(E) Clara cell recovery as indicated by re-expression of CC10 (black arrow).

Lung Injury

• Acute responses to injury
  – Pulmonary edema produces a thickening of the alveolar capillary barrier
    • Interferes with gas exchange at alveolar level
    • Recovery is dependant on the severity of the initial injury
  – Acute airway reactivity can be provoked by cholinergic drugs (acetylcholine) or other mediators (histamine, prostaglandins)
    • Causes decrease in airway diameter and increase in resistance to air flow
Lung Injury

- Chronic responses to lung injury
  - Fibrosis: fibrotic lungs contain increased amounts of collagen, and decreased surface area available for gas exchange.
  - Emphysema: lungs become larger and too compliant, with destruction of the gas exchange surfaces.
  - Asthma: caused by narrowing of the large conducting airways.
  - Cancer: major causes are tobacco smoke; asbestos fibers; crystalline silica; metallic dust fumes (arsenic, beryllium, cadmium, chromium, nickel); and radon.
Phospholipidosis

- Relatively common finding in drug discovery
- Does not preclude clinical use of a drug
- Can be found in many organ systems in rodents
  - Lung, spleen, heart, brain, kidney, thymus, gut, eye.....
- Most often manifests in the lung in humans
- At light microscopic level, cells appear to have inclusions (displaced nuclei, clear material in cytoplasm)
- Electron microscopy is used as confirmation
Drug-induced PLD

• May have pathological consequences, or not

• Many examples:
  – Amiodarone (anti-arrhythmic)
  – Perhexiline (anti-angina)
  – Gentamicin (antibiotic)
  – Statin drugs (lipid lowering drugs)
  – Fluoxetine (antidepressant)

Assessment of Lung Toxicity--humans

• Pulmonary function tests
  – Evaluate constrictive or obstructive airway changes

• Sputum analysis
  – Evaluate bacterial pathogens

• Bronchoalveolar Lavage
  – Evaluate inflammatory cells, pathogens, extracellular protein

• Histopathological evaluations
  – Tumors

• Radiological exam
  – Tumors, fibrotic lesions
Example of pulmonary function test

**Spirometry**

**Some #s:**

- Total lung capacity =~ 6L
- Residual volume =~ 1.2L
- Vital capacity =~ 4.5L
- Tidal volume =~ 0.5L

- FEV1 = amount of air that can be forcibly exhaled in one second (values range for ~3.5 L for healthy adult females to ~5L for healthy adult males)

C&D 6th edition Chapter 15
Assessment of Lung Toxicity—lab animals

- Histology
  - “Inflation fixation” is critical
  - Morphometric reconstruction of airways
- Pulmonary function
  - plethysmograph
- Isolated perfused lung
- Lung slices
- Microdissection of certain cell populations
- Pulmonary lavage
  - Cell counts, protein content, etc.
- Isolated lung cell populations
  - Isolated Clara cells ->
Brain/Central Nervous System
Neurotoxicity

• Any adverse effect in the structure or function of the nervous system: brain, spinal cord, peripheral nerves

• What is an adverse effect?
  – Hallucinations
  – Convulsions
  – Ischemic injury (e.g. stroke)
  – Loss of sensory function (vision, touch, olfaction, taste, hearing)
  – Hyperactivity/excessive nervousness
  – Decreased motor activity
  – Decreased I.Q./impaired learning
  – Memory loss
  – Headaches
  – Decreased motor function
  – Lethargy
Cell types in the nervous system

• Neurons
• Glial cells
  – Oligodendroglial cells
    • synthesize myelin in the central nervous system
  – Schwann cells
    • synthesize myelin in the peripheral nervous system
  – Astrocytes
    • form, together with brain endothelial cells, the blood-brain barrier
    • ‘reactive gliosis’ in response to neuronal loss
  – Microglia
    • phagocytic cells in the nervous system
Neurons

• Neurons are the cells of the nervous system that conduct neurotransmission
• Communication with target cells occurs at the synapse
• Two very important structural proteins
  – Neurofilaments (3 subunits)
  – Tubulin (microtubules)
• Structurally, most neurons consist of
  – a nerve cell body
    • Protein synthesis occurs only in the nerve cell body
  – an axon
  – one or more dendrites

From Fishbach GD (1994) Scientific American Special Report “Mind and Brain”
Nerve cell body

Axon

Myelin

neurofilaments

microtubules
Targets for neurotoxic damage

- Nerve cell body (neuron)
- Axon
- Myelin
- Synapse
Nerve cell body toxicants

- **Organo-mercury compounds**
  - E.g. methyl mercury—prenatal exposure can cause congenital brain malformation
- **Manganese**
  - Toxic to neurons of substantia nigra—link to Parkinson’s?
- **Aluminum**
  - Once thought to cause Alzheimer’s disease
- **Glutamate receptor agonists**
  - E.g. domoic acid
- **MPTP**
  - Heroin contaminant that caused Parkinson’s disease in addicts in the San Francisco area in the 1980s
  - Mitochondrial toxicant, bioactivated by monoamine oxidases, substrate of dopamine transporter
- **Noise, various solvents**
  - Hearing loss due to damage to hair cells in the inner ear
  - Many solvents cause selective loss of hearing only at certain frequencies
Myelin toxicants

- Hexachlorophene
  - Used in antimicrobial soaps
- Triethyl tin
  - Used in marine paints to prevent algal growth
- Lead
- Amiodarone
  - Potassium channel blocker, used as an antiarrhythmic drug
- Disulfiram

Myelin toxicants cause ‘blebs’ in myelin
Can result in complete loss of myelin
Clinical manifestation: slowed nerve conduction velocity
Examples of axonal toxicants
(many affect cytoskeletal proteins)

- Taxol
- Colchicine
- Vincristine
- n-Hexane
- 2,5-Hexanedione
- Carbon disulfide (CS₂)
- Acrylamide
- Pyridinethione
Taxol and colchicine: microtubule toxicants

Stabilizes m’tubules

Inhibits polymerization of m’tubules
Crosslinking agents*

*crosslinking of neurofilaments in the axon causes axonal swellings and impairs axonal transport

C&D 5th p. 474
Events at the Synapse

• Neurotransmission is initiated by the release of neurotransmitters into a synapse
  – Examples of neurotransmitters include dopamine, serotonin, acetylcholine

• After signal has been sent, neurotransmitter molecule must be removed from the synapse
  – Enzymatic degradation
  – Reuptake

*Fishbach GD (1994) Scientific American Special Report “Mind and Brain”*
Toxic Events at the Synapse - examples

• Organophosphorous insecticides inhibit the enzyme acetylcholinesterase, which typically degrades the neurotransmitter acetylcholine, leading to prolonged signal transmission
  – Twitching muscles, excess glandular (salivary, tear) secretion

• Cocaine inhibits the dopamine transporter (DAT), which normally facilitates the re-uptake of dopamine from the synapse, into the pre-synaptic cell
  – Excess CNS dopamine receptor activation
Evaluation of neurotoxicity

• Histopathology
  – H&E
  – Special stains: GFAP, Nissl, cresyl violet

• Teased nerve preparations
  – Nerve fibers are pulled apart with small forceps to examine individual nerve fibers
    • Demyelination, axonal swellings, inflammatory cell infiltration

• Electrophysiological tests
  – E.g. nerve conduction velocity

• Biochemical
  – E.g. cholinesterase inhibition (chickens)

• Functional/behavioral tests
  – Functional observational battery (motor activity + histopathology)
  – Spatial memory
  – Anxiety/fear
  – Operant tasks
Kidney
Glomerulus
Prox convoluted tubule
Prox straight tubule
Descending limb of loop of Henle
Thin ascending limb of loop of Henle
Cortex
Cortical collecting tubule
Medulla
Medullary collecting tubule
Papillary duct
Adapted from C&D 6th p. 492
Kidney Functions

• Excretion
• Extracellular fluid volume regulation
• Electrolyte homeostasis
• Acid-base balance
• Metabolism
  – Vitamin D3 $\rightarrow$ 1,25-dihydroxy vitamin D3
• Synthesis and release of hormones
  – Renin
  – Erythropoietin
Kidney - Susceptibility to Toxic Injury

• Renal blood flow is high compared to organ weight
  – Kidney ~1% of body weight but receives ~25% of cardiac output

• Blood flow to kidney is uneven
  – Cortex receives highest blood flow and hence highest exposure to toxicants

• Process of concentrating urine also concentrates toxicants
  – Toxicants can be concentrated by up to 200-fold
  – Precipitates can cause tubular obstruction

• Potential for bioactivation of toxicants
  – CYPs in proximal and distal tubules
  – Prostaglandin synthetase in medullary and papillary cells
Kidney – Site-specific Susceptibility

- **Glomerular toxicants**
  - Antigen-antibody complexes
  - Aminogluicoside antibiotics (gentamycin, tobramycin)
  - Adriamycin

- **Proximal tubule:**
  - Antibiotics (aminoglycosides, cephalosporins)
  - Antineoplastics
  - Radiographic contrast agents
  - Halogenated hydrocarbons (carbon tetrachloride, chloroform, tetrachloroethylene)
  - Heavy metals (mercury, uranium, cadmium, chromium)

- **Distal tubule/collecting duct**
  - Lithium
  - Tetracyclines
  - Fluoride ions
  - Amphotericin
  - Methoxyflurane

- **Papilla**
  - Aspirin
  - Phenacetin
  - Acetaminophen
  - Bromoethlamine
  - Other NSAIDs
Kidney - Pathologic Responses

• Acute renal failure
  – Most common nephrotoxic damage
  – Characterized by abrupt decline in glomerular filtration rate (GFR) resulting in azotemia (high nitrogen content in the blood)

• Chronic renal failure
  – Progressive deterioration of renal function may occur with long-term exposure to a variety of chemicals, including analgesics, lithium, and cyclosporine

• Adaptation following toxic insult
  – The kidney can compensate for loss of renal functional mass
  – Following a nephrotoxic insult, cells that are non-lethally injured may undergo cell repair/adaptation
  – Uninjured cells may undergo compensatory hypertrophy, cellular adaptation, and proliferation, all contributing to the structural and functional recovery of the kidney
Renal toxicity testing

• Histopathology
• Classical urinary markers of renal damage: albumin, total protein, glucose, pH
• More current (& sensitive?) biomarkers of renal toxicity
  – KIM-1: kidney injury molecule 1
  – Cystatin C: low MW protein; normally filtered by kidney
  – beta-2-microglobulin: low MW; normally filtered, then partially resorbed
  – NAG: β-N-acetylglycosaminidase activity
  – Clusterin: secreted glycoprotein synthesized in response to tubular injury
  – Trefoil factor 3: one or more 38- or 39-amino acid domains in which 6 cysteine residues form 3 disulfide bonds to create a characteristic three-leafed structure
  – NGAL: neutrophil gelatinase-associated lipocalin
Cardiovascular System
Anatomy of the Heart

Electrical System of the Heart

- Sinoatrial (SA) Node
- Anterior Internodal Tract
- Middle Internodal Tract
- Posterior Internodal Tract
- Atroventricular (AV) Node
- Bachmann's Bundle
- Left Bundle Branch
- Conduction Pathways
- Right Bundle Branch

www.bing.com/images
Cardiovascular System

• Function
  – Circulation of blood to supply tissues of the body with nutrients, respiratory gases, hormones, and metabolites, while removing waste products of cellular metabolism and foreign matter

• Cardiac muscle, like nerve cells, is an ‘excitable’ tissue

• Cells in the heart:
  – Cardiac muscle cells = myocytes (25% of cells in heart)
    • Myocyte contraction occurs as a result of an action potential causing release of calcium from the sarcoplasmic reticulum
  – Cardiac fibroblasts (67% of cells in heart)
  – Other connective tissue cells
  – Purkinje cells
  – Vascular cells
Cardiac ‘rhythm’

- Normally set by sinus P-node (pacemaker) cells
- Arrhythmia: Deviation from the normal cardiac rhythm
  - Tachycardia: excessively fast heart rate
  - Bradycardia: slowed heart rate
  - Atrial fibrillation
Electronic cell-to-cell coupling in the heart

- Assessed by electrocardiography
  - ECG

- Important deflections and intervals in an ECG
  - PR interval: time from onset of atrial activation to the period of ventricular activation
  - QRS: conduction pathways through the ventricles
  - ST: interval during which the entire ventricular myocardium is depolarized
  - QT: period of ‘electrical systole’; reflects the action potential duration
    - Prolonged QT interval is recognized as a life-threatening condition induced by some drugs
Cardiac Toxic Responses

- Primary indicator of cardiac toxicity is decreased cardiac output, resulting in tissue hypoperfusion
  - Normal adult resting cardiac output is 5 L/min
- Cardiac hypertrophy
  - Associated with re-expression of fetal genes
  - Enlargement of existing cardiac myocytes
    - Developing: cardiac workload exceeds output
    - Compensatory: cardiac output is maintained
    - Decompensatory: ventricular dilation develops and output declines
- Heart failure
  - Inability of the heart to maintain sufficient cardiac output
  - Clinical analysis of right vs. left ventricular dysfunction can predict prognosis
QT Prolongation and Sudden Cardiac Death

• Drug-induced QT-prolongation leads to ventricular arrhythmia and ‘Torsade de Pointes’ (TdP)
• This is considered a severe cardiac toxic event
• Testing for QT prolongation is required by the U.S. FDA for drug development
• Drugs associated with TdP:
  – Cyclooxygenase-2 (COX-2) inhibitors
    • Vioxx, Bextra
  – Antibacterials
    • Fluoroquinolone
  – H1 receptor antagonists (Antihistamines)
    • Terfenadine, astemizole
http://www.youtube.com/watch?v=1ccTO6fNobU
Additional Cardiovascular System Toxicants

• Anthracyclines and other antineoplastic agents
  – Doxorubicin, 5-fluorocil, cyclophosphamide

• CNS drugs
  – Tricyclic antidepressants
    • Ventricular arrhythmias, hypotension
  – Antipsychotic drugs
    • Hypotension, ECG changes
  – General anesthetics
    • Decrease myocyte contractility, arrhythmias, sensitization to other cardiac arrhythmogens

• Local anesthetics
  – Cocaine, procainamide
    • extremely high doses cause slowed conduction speed, decreased rate of depolarization
More Cardiovascular System Toxicants

- Alcoholic beverages
- Heavy metals
  - Cobalt (‘beer drinkers cardiomyopathy’)
  - Cadmium (some evidence)
  - Lead (hypertension)
- Industrial chemicals
  - carbon disulfide, styrene, 1,3-butadiene

**Oil red O** staining of lipid on aortic valves from mice exposed to

A. Control diet (C)
B. HF diet (HF)
C. CS₂ + C
D. CS₂ + HF

Assessment of Cardiac Toxicity

• Abnormal heart rhythm
  – Pulse rate determination
    • Tachycardia, bradycardia

• ECG analysis
  – AV Block
  – Atrial, ventricular fibrillation
  – QT Prolongation (Torsade de pointes)

• Chest Pain (angina)

• Blood pressure changes
  – Hypotension
  – Hypertension

• Myocardial degeneration/necrosis
  – Myocardial degeneration/necrosis
    • Histopathological examination
Biomarkers of Cardiac Toxicity

- **Creatine Kinase**
  - Multiple isoforms; CK-MB is considered a reasonably specific biomarker of acute myocardial infarction (MI)

- **Myoglobin**
  - Present in all muscle; serum levels increase dramatically 1-4 hr after MI

- **B-type Natriuretic peptide**
  - Cardiac neurohormone; secreted in response to volume and pressure overload; accepted as biomarker of congestive heart failure in Europe.

- **C-reactive protein**
  - Marker of systemic and vascular inflammation; appears to predict future cardiac events in asymptomatic individuals.

- **Cardiac troponins**
  - Cardiac troponin T (cTnT) and I (cTnI) are constituents of myofilaments; expressed exclusively in cardiac myocytes
  - cTn measurement has become the gold standard for diagnosis of acute MI
Skin
Four primary functions of skin

- Prevention of water loss
- Permeability barrier to the environment
- Protection from invasion of microorganisms
- Protection against abrasive action

General structure:
The Epidermis

Stratum corneum

Stratum granulosum

Stratum basale

Stratum compactum

Stratum spinosum

Epidermal Keratinocytes

- **Stratum corneum**
  - Outermost layer of the epidermis
  - Consists of several layers of completely flattened, keratinized cells without cytoplasm or nuclei
  - Varies in thickness across the body, and by species
  - Resistant to water loss and pathogen invasion

- **Stratum lucidum**
  - Found on parts of body with very thick skin and no hair (e.g. palmar and plantar surfaces)

- **Stratum granulosum**
  - Irregularly-shaped cells containing profilaggrin
  - Release lamellar granules that release lipid into extracellular space beneath the stratum cornium
    - These lipids, including ceramides, cholesterol, fatty acids, cholesterol esters, provide a barrier to chemical absorption across the skin

- **Stratum spinosum**
  - Several layers of irregularly shaped cells containing tonofilaments
  - Connected by desmosomes to adjacent cell layers
Epidermal Keratinocytes, con’t

• Stratum Basale
  – Single layer of cuboidal/columnar cells attached to the basement and to each other.
  – Undergoes continual mitosis
  – Self replacement of human (and pig) skin takes ~30 days

Epidermal Non-keratinocytes

• Melanocytes
  – Located within basal cell layer of epidermis, hair follicles, sweat glands, sebaceous glands
  – Dendritic process intercalate between keratinocytes
  – Melanosomes move to tip of melanocyte process and are phagocytized by adjacent keratinocytes

• Merkel cells
  – Associated with neurons in skin
  – Act as slow-adapting mechanoreceptors for touch

• Langerhans cells
  – Found in upper stratum spinosum and have long dendritic processes that extend into the granulosum cell layer
  – Antigen presenting cells
  – Initiate some forms of immune-mediated dermatologic reactions
The Dermis

- Forms the bulk of the skin
- Composed primarily of filamentous, fibrous, and amorphous connective tissue
- Determines the tensile strength and elasticity of the skin
- Provides physical support for nerve and vascular networks
- Site of origin of skin appendages
  - Hair follicles
  - Sebaceous glands
  - Sweat glands
Toxic responses of the skin

• Contact dermatitis
  – Irritant dermatitis
  – Allergic contact dermatitis
• Chemical burns
• Granulomatous disease
  – Typically foreign body reactions; relatively infrequent
• Phototoxicology
  – Sunburn
  – Phototoxicity
  – Photoallergy
• Chloracne
• Urticaria
• Skin cancer
Contact dermatitis

• Irritant dermatitis
  – Often cause damage/discomfort as a result of cumulative irritation
    • Soaps, detergents, solvents, cutting oils
  – Can increase likelihood of allergic response by increasing penetration
  – Often accompanied by minimal inflammation

• Allergic contact dermatitis
  – Delayed (T-cell-mediated) response
  – Chemical penetrates through skin, binds to an endogenous molecule, which is presented as an antigen by Langerhans cells to T-helper cells
    • Examples: poison ivy (urushiol), 2,4-dinitrochlorobenzene
Chemical burns

• Typically associated with strong acids or bases (alkalis)
  – *Obvious examples*: ammonia, calcium oxide, HCl, HF, sodium hydroxide, toluene diisocyanate, hydrogen peroxide
  – Not-so-obvious examples: ethylene oxide, methyl bromide

• Lesion is typically localized to site of exposure
  – One exception: HF can cause systemic hypocalcemia
Phototoxicology

• Phototoxicity
  – Skin becomes red/blistered upon topical or systemic exposure to certain chemicals and sunlight
  – Chemicals causing phototoxicity most often absorb light in the UVA range (320-400 nm)
  – Examples: Psoralens, PAHs, tetracyclines, eosin, sulfonamides, sulfonylureas, thiazides, acridine orange

• Photoallergy
  – Chemical elicits an allergic response by forming a complete antigen upon absorbing UV or visible light
  – Examples: halogenated salicylanilides (antimicrobials), p-aminobenzoate (former sunscreen ingredient), some fragrances
Chloracne

• Rare; occurs upon exposure to ligands for the aryl hydrocarbon receptor (AhR) and inducers of CYP1A1

• Skin lesions are precipitated by transition of sebaceous glands to keratinizing cells

• Chemicals: polychlorinated biphenyls/furans naphthalenes/dioxins
  • TCDD, 2,4,5-T
Uticaria

• A.k.a hives
• Elicited by allergens to which IgE antibodies have been elicited

Skin Cancer

• Extensively studied in the ‘initiation-promotion’ paradigm
• Known causes in humans include radiation (sunlight, X-rays), PAHs, arsenic
Measuring skin toxicity/responses

• In vitro
  – Diffusion and Flow-through cells
    • Biopsy or cadaver skin is placed in a cell such that a ‘donor’ fluid and flux of a test compound is monitored across the skin under static or moving fluid conditions
      – E.g. Bronough cells, Franz cells
  – 3-D skin models
    • Skin fibroblasts differentiate into a fully-differentiated epidermis and respond to inflammatory cytokines.
Diagram of a Franz diffusion cell.

Measuring skin toxicity/responses

• In vivo
  – Patch tests—allergy testing
  – Percutaneous absorption and penetration
    • Species and body site considerations are important
  – Transepidermal water loss (TEWL)
  – Histology (‘punch biopsies’)
  – High-frequency ultrasound
  – Fourier Transform Raman Spectroscopy
Toxicology of the Gastrointestinal (GI) System
The GI System

• Extends from the mouth through the rectum; many associated glands and structures

• Function
  – Primarily to adsorb nutrients and water
  – One of the most important sites where toxicants are absorbed
    • Transporter facilitated, as well as through and between epithelial cells
  – Specialized transport systems facilitate the absorption of nutrients and electrolytes in the upper, middle, and lower small bowel
    • No ‘specific’ transporters for toxicants
    • Xenobiotics can ‘hijack’ transporters for physiological substrates and nutrients
      – E.g. the same transporter that facilitates calcium uptake also permits lead transport
Microscopic Anatomy of the Small Intestine

**Villi:** absorption

**Crypt:** proliferation

**Muscularis mucosa**

**Submucosa**

Common structure throughout the gut

Wheater PR et al. Functional Histology (text), 1979
Toxicology of the GI System

- Xenobiotics can affect many GI functions
  - Transit (GI motility, neurological interference)
  - Absorption (transporter-mediated and paracellular transport)
    - Transporters: metal transporters, drug transporters, drug effluxors, etc.
  - Digestion (enzyme inhibition)
  - Secretion (ions, hormones, enzymes)
  - Microflora
    - Depletion of microflora due to antibiotic administration
    - Overgrowth causes altered coagulation due to bacterial secretion of vitamin K2
  - Immunity (abundant submucosal lymphatic tissue; mucosal secretions)
  - Pre-absorption biotransformation
    - ‘the grapefruit syndrome’: Furanocoumarins in grapefruit inhibit intestinal CYP3A4, thereby increasing the amount absorbed of some drugs
## Drugs exerting adverse effects on the GI tract

<table>
<thead>
<tr>
<th>Constipation</th>
<th>Diarrhea</th>
<th>Ileus</th>
<th>Ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticholinergics</td>
<td>Antibiotics</td>
<td>Loperamide</td>
<td>NSAIDS</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>Prostaglandins</td>
<td>Tricyclics</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>Diuretics</td>
<td>Fatty acids</td>
<td>Phenothiazines</td>
<td>KCl</td>
</tr>
<tr>
<td>Tricyclic antidepressants</td>
<td>Cholinergic agonists</td>
<td>Anticholinergics</td>
<td>Iron preparations</td>
</tr>
<tr>
<td>Iron preparations</td>
<td>Cholinesterase inhibitors</td>
<td>Opiates</td>
<td></td>
</tr>
</tbody>
</table>
Ocular
Ocular Toxicity - Anatomy of the Eye

http://www.webvision.med.utah.edu
Compartments of the Visual System

• Eye
  – Cornea
  – Lens
  – Retina
  – Retinal pigment epithelium

• Central visual pathway
  – Optic nerve
  – Optic tract

• Central processing areas
  – Lateral geniculate nucleus
  – Visual cortex
## Ocular Toxicants

<table>
<thead>
<tr>
<th>Cornea</th>
<th>Lens (cataracts)</th>
<th>Retina</th>
<th>Optic nerve and tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV radiation $&lt;310$ nm</td>
<td>Corticosteroids</td>
<td>Inorganic lead</td>
<td>Acrylamide</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Sugars (diabetes mellitus)</td>
<td>Methanol</td>
<td>Carbon disulfide</td>
</tr>
<tr>
<td>Organic solvents</td>
<td>UV radiation</td>
<td>Organic solvents</td>
<td>Ethambutol</td>
</tr>
<tr>
<td>Acids (pH $&lt;2.5$)</td>
<td>Oxidative stress</td>
<td>Organophosphates</td>
<td></td>
</tr>
<tr>
<td>Bases</td>
<td>Phenothiazine drugs</td>
<td>Many drugs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroquine tamoxifen</td>
<td></td>
</tr>
</tbody>
</table>
Assessment of Ocular Toxicity

• Ocular irritancy and toxicity
  – Traditionally based on Draize et al., 1944
    • In vivo assay, using albino rabbits; criticized because of pain to the animals, interlaboratory variability, poor predictive value for human irritants
  – Alternatives assays are under development/consideration
    • Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
    • Some alternatives include use of isolated rabbit, chicken, or bovine eyes; hen egg chorioallantoic membrane
Assessment of Ocular Toxicity

• Clinical signs of ocular changes
  – Jaundice, conjunctivitis

• Direct or indirect ophthalmoscopy
  – Specialized expertise is required! Detects cataracts, retinal changes

• Electrophysiological techniques
  – Flash-evoked electroretinogram
    • Electrodes record from the cornea after a light stimulus
  – Visual-evoked potentials
    • Electrodes record from the visual cortex after stimulation
  – Electro-oculogram
    • Used to assess retinal pigment epithelium status and eye movements

• Behavioral and Psychophysical methods
  – Assessment of whether stimuli can be discriminated or detected
    • E.g. contrast sensitivity, luminescence threshold; visual acuity, color discrimination
Resources
(aside from the usual well-respected toxicology textbooks)

• Thoolen B et al. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol* 2010 38:5S.


• Bolon B and Butt MT, eds. Fundamental Neuropathology for Pathologists and Toxicologists. 2011.


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