Renal Toxicological Pathology

April 5, 2013
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Review of the urinary and reproductive tract anatomy
Normal mouse kidney

glomeruli

cortex

medulla

renal papilla
Renal Function

• Name 5 functions
Renal Function

- Filtration and waste excretion
- Homeostasis of water-soluble molecules
- Electrolyte homeostasis/acid-base balance
- Metabolism/detoxification
- Hormone production
Hormonal Activity

- Produces erythropoietin, which regulates RBC production
- Hydroxylates 25-OH-cholecalciferol (vitamin D metabolite) to promote bone resorption and calcium and phosphorus absorption from the gut
- Releases renin to regulate the peripheral renin-angiotensin-aldosterone system (juxtaglomerular apparatus)
Objectives

• Review of normal structure and function
• Understanding of why kidney is a target organ of toxicity
• Review of renal pathological terms
• Understanding mechanism of toxicity in some examples
• Integration of pathology data in determining the mechanism of nephrotoxicity
• Use of animal models in toxicology studies
Normal mouse kidney

Papilla is vulnerable to aspirin toxicity
Be able to draw this

Textbook of Veterinary Histology

PDG
Renal Function

- Filtration and excretion
- Homeostasis of water-soluble molecules
- Electrolyte homeostasis/acid-base balance
- Metabolism/detoxification
- Hormone production
Urinary system

- Functions of the kidney is achieved by selected filtration of plasma (glomerulus)
- With selective reabsorption of filtrate (tubules)
- The two together make up the functional unit of the kidney, the nephron
Urinary system

• Proper urinary system function requires:

• Adequate perfusion with blood- kidney is very metabolically active using 25% of cardiac output yet only weighing 0.5% of body weight

• Adequate functional renal tissue: kidney has large reserve, but loss of 60-70% of nephrons induces renal insufficiency

• Adequate outflow: lower urinary tract must be open (patent) for urine to be removed from body
Structure and Function

- The tubule resorbs greater than 99% of the glomerular filtrate
- The proximal tubule has extensive resorption and selective secretion (convoluted - S1 and S2, straight - S3). S2 is primary site for low MW protein resorption and S3 is primary site for P-450 metabolizing enzymes.
- Thin loop of Henle - resorption of fluids
- Distal tubule - resorption of fluids and acid-base balance
- Collecting duct - resorption of fluids, antidiuretic hormone and acid-base balance
Hormonal Activity

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Renal Proximal Tubules

1. Function in resorption, secretion and transport of proteins, ions, or other organic molecules.

2. Differentiated into P1, P2 and P3 (aka, S1, S2, S3) segments based upon histochemical and ultrastructural characteristics. See EM figures

3. Sex differences exist, in some species, in the metabolic capabilities of proximal tubule cells leading to toxicity
Renal system clinical pathology

- Renal failure:
- Disturbance of electrolyte and water balance: dehydration, hyperkalemia (elevated serum potassium), acidosis, generalized edema
- Accumulation of toxic wastes, blood urea nitrogen (BUN), creatinine
- Anemia, calcium metabolism disturbance
Urinalysis- helps to ID site of injury

- Proteinuria – (protein in the urine) indicates glomerular damage
- Glycosuria – (glucose in the urine) indicates tubular damage
- Urine volume and osmolarity (increase quantity of dilute urine)
- Elevated renal enzymes - indicates tubular damage
- Microscopic examination - casts, crystals, bacteria, etc.
Kidney as a Target Organ of Toxicity

- Is a frequent site of toxic injury in rodent toxicity studies (second to the liver).

- Role in filtration, metabolism, and excretion of xenobiotics and their metabolites.

- In renal disease - increased blood levels of drugs that are cleared by kidneys.

- Toxins can concentrate in the glomerular filtrate or within tubule cells.

- Can metabolically activate xenobiotics (drugs etc)
Metabolism

The kidney conducts both Phase I and Phase II metabolism.

Phase I (P450 metabolism) Phase II (conjugating -to make compound water soluble and excreted)

Renal cytochrome P-450 levels are only about 20% of liver and are unevenly distributed among the different cell types within the proximal tubule (S3 has the highest, rat).

Activity may be different between genders (i.e. male mice have more P-450 activity than female mice).
Kidney (Renal Cortex) 40x

- glomeruli
- Bowman's capsule
- Bowman's space
- capillary
- mesangium
- renal tubules
Normal Kidney (Medulla) 40x
Kidney - Vasculature

Glomeruli contains tiny tufts of coiled capillaries (afferent arteriole) enters the glomerulus structure > becomes capillary > and as it exits it is called the efferent arteriole.
Normal Glomerulus

Filtration: from blood to urine
1. Through fenestrations in endothelium >
2. Basement membrane >
3. Between foot processes into urinary space >
4. then to tubule

Be able to trace the path from blood to tubule
Pedicle = Foot process

Basal lamina = basement membrane
Diseased Glomerulus

- Disease of the glomerulus-antigen antibody deposition
- 1. Fusion of the foot processes
- 2. Subepithelial deposits in the glomerular basement membrane induces thickened basement membrane
- = Impairment of filter function and loss of protein into the filtrate
EM- antigen-antibody deposits in the glomerular basement membrane
Membranous glomerulopathy

- Immune complexes (antigen-antibody complexes) are deposited in the glomerulus filter of the kidney
- Subsequent reduction of glomerular function as a selective filter inducing renal disease
- Toxins that induce this include: penicillamine, gold, captopril, mercury
Glomerular Disease: Toxicities due to Alteration of Anionic Charge (negative charge = normal for the filter)

- Hexadimethrine - polycationic molecule reduces anionic charge, which permits escape of anionic molecules such as albumin and IgG
Tubular disease - background anatomy

Textbook of Veterinary Histology
P.D. Garrett
Normal Morphology of different sections of the renal tubule
brush border

blood vessel

EM of PCT
Acute renal failure

attenuated brush border of tubule
Renal toxin case

- 6 week old male Sprague Dawley rat
- Acute Tox study IP 100mg/kg/day
- Sacrificed and necropsied on day 9
- Kidneys mildly bilaterally enlarged with surface cortical pallor
- 4 fold increase in serum creatinine day 9
- Urinary glucose, GGT, LDH elevated day 1 dosing only
Renal tubular epithelial necrosis

- Renal tubule in the center contains pink protein and sloughed renal tubular epithelial cells
- This necrosis is due to the antibiotic gentamycin toxicity
- If the basement membrane under the lost epithelial cells is still intact - Regeneration will occur and remaining epithelial cells will divide and repopulate the lining of the tubule
Kidney, tubular degeneration and necrosis, acute to subacute, multifocal, hyaline droplets, with tubular regeneration

- Sloughed lightly eosinophilic and granular tubular luminal debris
- Less affected cells varying degree of swelling, increased granularity, vacuolation and hyalin droplets (seen of EM)
- Proximal tubules attenuation of epithelium
- Necrotic cells adjacent to less affected cells and within tubules, eosinophilic casts
- Adjacent regenerative tubules have large, plump nuclei, prominent nucleoli, basophilic cytoplasm and frequent mitotic figures
- Minimal infiltrate lymphocytes, plasma cells, macrophages and neutrophils
Gentamicin toxicity-pathogenesis

- Induces phospholipidosis, a storage disease characterized by cellular accumulation of excess phospholipids
- Cationic amphophilic drugs (CAD) with hydrophobic ring and a hydrophilic side chain with a positively charged cationic amine group (antibacterial, antifungals, antimalarials) induce condition

- CAD are reabsorbed by endocytosis, bind to phospholipids in brush border of proximal tubule and stored in secondary lysosomes - leads to cell death
- EM = myelin figures in phagolysosomes, concentric multilaminated membranous whorls
Alterations of immunohistochemical localizations of GM and HSP73 in proximal tubular epithelial cells in gentamicin-treated rat kidneys on electron microscopy.

Damage to the Renal Tubule - examples

- Halogenated hydrocarbons - chloroform, hexachlorobutadiene, trichloroethylene, dibromochloropropane, & bromobenzene
- Heavy metals - cadmium, mercury & lead
- Antibiotics - cephalosporins & aminoglycosides
- Mycotoxins - ochratoxin A & citrinin
- Ethylene glycol, melamine
- Antineoplastic drugs - cisplatinum
Mercuric chloride - tubular necrosis

- Two tubules here are filled with bright red granular proteinaceous material due to epithelial necrosis
Renal tubular necrosis—certain regions of the tubule are more sensitive to different types of injuries

- The proximal tubule is more sensitive to toxins because it contains cytochrome P450 enzymes that can metabolize compounds - make toxic metabolites - reactive chemically to next secondarily damage the cell.
Why is nitrogen important?

Triazines - Melamine

3 N’s

6 N’s

Thanks to Renate Reimschuessel (FDA)
Contaminated Pet Food/Animal Feed Incident – 2007
Sequence of Events

3/19/07  4/11/07  4/12/07  4/13/07  4/18/07

LARGEST RECALL – 18,700 calls in 11 weeks

Slide courtesy of Tracy Forfa
Needle like crystals arranged in a radial sphere
2 year rat melamine cancer study

Bladder stones: **Melamine:** **Uric acid** – 1:1

**Microcrystals** in renal pelvis

Urinary bladder carcinogenesis induced by melamine in F344 male rats:
Bacteria can metabolize Melamine to Cyanuric acid

all triazines

Bottom line – all have high nitrogen
Investigating effects of Melamine and Triazine analogues in rats

April 13 – contacted FDA CVM to discuss their ideas and research.

They had used MEL+3 analogues at 10:1:1:1 ratio.

Discussed and shared our ideas:

- uric acid nephropathy mechanism suggested
- DON’t fix in FORMALIN
- try using 1:1 ratio of MEL:CYA

Collaboration on further rat work and with the cat case.
Response to Formalin

Kidney unfixed
Friday PM

Dirt on slide

Formalin fixed
Monday AM

Dirt on slide

60 hours later no crystals

Same field – photo taken unfixed, then flooded with formalin, left on microscope over weekend. Crystals not visible on Monday (same field)
Cat crystals consistent with Melamine:Cyanurate spectrum

Fourier Transform Infrared Spectroscopy
Melamine and Cyanuric Acid

Crystal Lattice Structure

Renal crystals

Low Toxicity

Melamine

Cyanuric Acid

Hydrogen bonding - Melamine-cyanurate
What was happening?

• Melamine was being added to watered down milk by dairy farmers

  (arrests with melamine in possession)

• Melamine was being added to watered down milk by producers/distributers.

  (arrests with melamine in possession)
Sept 11, 2008
News - Melamine In Formula

- Children on formula a long time
- Bloody urine
- Sandy urine
- Stones – urinary obstruction
- Renal Failure (Obstruction)
- Formula contained MELAMINE

300,000 infants diagnosed
What do stones look like?
Renal Calculus = stone (made of uric acid): 2.5 yr old SF Shitzu Canine
Adult Male Baboon: Ureter with Calculi (stone)
Review of the urinary and reproductive tract anatomy

- Case: Adult Male Baboon: Urethra obstructed with stone or calculus
Adult Male Baboon: Penile Urethra, Calculus

This could induce a dilation of the kidney = hydronephrosis
Drug-induced interstitial nephritis

- Sulfonamides
- Penicillins
- Diuretics (thiazides)
- NSAID phenybutazone
- Usually occurs after 2 or more weeks for exposure to drug – fever eosinophilia, skin rash and renal abnormalities like hematuria, mild proteinuria, rising serum creatinine and in severe cases, oliguria
- Grossly the kidneys are enlarged
- Histologically, interstitium has edema and mixed inflammatory cells eosinophils (type I hypersensitivity), neutrophils, plasma cells, lymphocytes and giant cells (type IV hypersensitivity).
- Variable degree of tubular necrosis
- Pathogenesis- type of hypersensitivity the drug acts as hapten (IV)
Kidney - interstitial nephritis

- Inflammation
- Fibrosis
- Tubular dilation
Renal infarct- example of necrosis of large section of tissue

This is a renal infarction (1-2 weeks old?). Note the wedge shape of this zone of coagulative necrosis resulting from loss of blood supply with resultant tissue ischemia that produces the pale infarct. The small amount of blood supply from the capsule supplies the immediate subcortical zone. The remaining cortex is congested, as is the medulla.
Renal infarct - example of necrosis of large section of tissue

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Papillary necrosis

- Analgesic abuse
- Aspirin
- Inhibits the vasodilation effects of certain prostaglandins results in ischemia
- Induces papillary necrosis through ischemia induced injury
How does pathology contribute to understanding renal toxicity?

- **Site specificity** to lesion within the tubule often gives clue to mechanisms i.e., S3 tubule involved = p450 metabolism may be involved

- Is pathology caused by:
  - Pre-renal cause = (vascular problem with blood flow to kidney)
  - renal cause
  - post-renal cause = (block in ureter, stone)

- Pathology information allows compounds to be classed with regard to type of injury - mechanism
Pathology Evaluation

• Routine histopathology
• Immunohistochemistry
• Cell Proliferation
• Morphometry
• Ultrastructural Studies
• Molecular pathology assessment
Necropsy and Macroscopic Evaluation

• Only **one chance** to conduct necropsy
• Organ weights can be important
• Consider tissue sampling scheme, fix in formalin, freeze etc

[Image: Note pallor and lumpy surface]
Quantitation of Lesions

- Multiplicity
- Tissue sampling schemes
- Step and serial sections to increase sensitivity
Measurement of Cell Proliferation in the Kidney

- Sensitive means to determine cellular origin to tubular toxicity
- Pulse BRDU = proliferation markers
- Also PCNA - a proliferation markers
- Quantitative
- Easy to conduct
Hydroquinone (HQ) → Hepatic Metabolism → tris-GHQ (2, 3, 5-tris-glutathione-S-yl) → Renal Metabolism → Proximate Carcinogen

**Parent Compound**
- Generally non-mutagenic in short term *in vitro* assays
- Positive in NTP Bioassay
  - 2yr. chronic exposure
  - High doses
  - Associated with nephrotoxicity
- Positive in oxidant sensitive *Salmonella* tester strains

**Penultimate Carcinogen**
- Metabolite of HQ
- Mediates HQ nephrotoxicity
- Generation of ROS and oxidative DNA damage
Normal Rat Kidney versus Treated with tris-GHQ Necrosis

Control

Selective necrosis of S3 in treated rat
Immunostain for PCNA in tris-GHQ Treated Rat

Control

Treated

PCNA marks proliferating cells, injury is followed by cell division
Proliferation helps you ID the sensitive cells
Proliferative Lesions in tris-GHQ Treated Rats

Atypical Tubule Adenoma

Damage to tubule lead to excessive proliferation, if mutations accumulate, cancer develops
Renal Neoplasms

- Cortical Tubular Cell: Adenoma, Carcinoma
- Collecting duct cell: Oncocytoma
- Transitional Cell: Papilloma, Carcinoma, Squamous Cell Carcinoma
- Mesenchymal Tumors (Rat)

- Important in rodent bioassay
- Can be related to earlier nephrotoxicity

- Necrosis
- Proliferation
- cancer
Use of Genetically-modified Rodent Models in Toxicology Studies

“Alternative” carcinogenesis bioassay models

Rodent models of human disease to study the interactions of environment and genetics

Cytochrome P450 2E1 knock-out mouse example
Chloroform-induced toxicity
Chloroform-induced toxicity
Liver and renal wildtype
cytochrome P-450 2E1 “knockout” mice

Metabolism of chloroform by cytochrome P450 2E1 is required for induction of toxicity in the liver, kidney, and nose of male mice.

Constan AA, Sprankle CS, Peters JM, Kedderis GL, Everitt JI, Wong BA, Gonzalez FL, Butterworth BE.
Chloroform-induced toxicity
Liver and renal
wildtype
cytochrome P-450 2E1 “knockout” mice

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Explain the finding
Urinary Bladder 4x

- **serosa**
- **muscularis**
- **transitional epithelium**
- **lamina propria**
Macaque: Bladder Transitional Cell Carcinoma

Cyclophosphamide causes a ulcerative hemorrhagic cystitis to chronic fibrosis and mineralization.

Chronic treatment in the dog induces transitional cell carcinoma.

Other agents that induce bladder cancer include bracken fern (plant) and 2-naphthylamine.

Theme ?
Parasite induced bladder cancer and Artificial sugar was blamed

- The use of the rat (*Rattus rattus*) as an animal model for tumors in the urinary bladder has increased (Cohen & Lawson, 1995; Wakui et al., 1999) and *T. crassicauda* can be a serious problem for carcinogenesis assays (Baker et al., 1979). The parasite induces an increase in the mitotic index in the epithelium of infected bladders, which makes the epithelium more susceptible to the effects of the carcinogenic substances (Hicks et al., 1976).
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