Toxicologic Pathology of the Adrenal Glands
Proliferative and Non-Proliferative Lesions

Sundeep Chandra, BVSc, PhD, DACVP, FIATP
Outline
– Non-Proliferative/Background lesions of the adrenal gland
– Proliferative lesions of the adrenal gland
– Adrenal gland and Gene therapy
Commonly Observed Microscopic Adrenal Gland Findings

**Macroscopic**
- Changes to size/weight
- discoloration

**Microscopic**
- Hypertrophy/hyperplasia
- Atrophy
- Vacuolation – increased/decreased
- Degeneration/necrosis/hemorrhage
- Inflammation

Chronic toxicity studies
- atrophy, proliferative lesions/neoplasia

- Not all compounds with adrenal gland macroscopic or microscopic findings lead to altered hormone production
Mechanism of Toxicity

- Impaired steroidogenesis (inhibition of cholesterol synthesis/metabolism)
  - \(^{↑}\) lipid (neutral lipids) in cells
- Activation by CYP-450 enzymes (ROS and amphophilic compounds)
  - phospholipidosis
- Exogenous steroids (disrupt negative feedback)
  - atrophy or hyperplasia (sex steroids)
Cortical Hypertrophy

Adrenal cortex hypertrophy
(Thymic atrophy/decreased cellularity (stress)
Hypertrophy: Diffuse and Focal

Enlarged cortical cells with eosinophilic finely granular or compact cytoplasm, decreased vacuolation is a concomitant feature

"vacuolation is not a dominant feature"

Increased vacuolation: Diffuse & Focal

Small, foamy and optically clear vesicles (microvesicular) or large vacuoles (macroversicular)

The cells may be enlarged and/or the number of cortical cells may be increased.

Cellular enlargement caused primarily by the accumulation of lipid is diagnosed as vacuolation and not hypertrophy.

Increased vacuolation can represent lipid vacuoles, swollen mitochondria, or phospholipidosis.

Decreased Vacuolation: Diffuse & Focal

The cytoplasm is more dense and homogeneous eosinophilic than usual with a reduction in lipid vacuoles.

The cortical cells are normal in size, or decreased in size

Decreased Vacuolation (Focal):

Occurs in adult to aged rats.

It represents a focal (or multifocal) change in metabolic status rather than a proliferative lesion
Hypertrophy vs Increased vacuolation vs Decreased Vacuolation

Hypertrophy, cortical, focal

Vacuolation, cortical, increased, focal

Vacuolation, cortical, decreased, focal
Synonyms: Basophilic focus; eosinophilic focus; focus of cytoplasmic alteration.
Vacuolation, increased
(impaired steroidogenesis)

Accumulation of either cholesterol and/or steroid precursors

E.g. Aminoglutethimide.
Mutsuga et al., Exp Toxicol Pathol. 2017 Sep 5;69(7):424-429.
Vacuolation, increased.
Adrenal glands dog (impaired steroidogenesis- inhibition of cholesterol synthesis/metabolism)

↑lipid (neutral lipids) in cells

Mouse, necrosis

Nitriles, thiols, amines (acrylonitrile, 2-propanethiol, allylamine)
Hemorrhage and necrosis
Rats administered VEGFR antagonist

Hemorrhage is an uncommon lesion and may overlap with angiectasis, thrombosis and cortical necrosis. Hemorrhage often occurs with cystic degeneration in rats.
## Potential Effects of Increased GC Production or GC Agonists

### Morphology
- Adrenal cortical atrophy (GC agonists)
- Adrenal cortical hypertrophy/hyperplasia (stress)*
- Thymus: weight decrease/atrophy
- Liver weight ↑
- Glycogen content in liver ↑
- Male sex accessory organ weight ↓
- Decrease in corpora lutea and ovulations

* Cortical hypertrophy will not be evident with GC agonists

### Hematology
- Neutrophilia
- Lymphopenia
- Eosinopenia

### Clinical Chemistry
- ↑ Alkaline phosphatase (esp. in dog)
- ↑ Blood glucose levels
- ↑ Free fatty acids
Potential Effects of Mineralocorticoids

Potential Effects of Increased MC Production or MC Agonists

- Elevated serum Na and low K
- Hypokalemia may lead to muscle weakness
- Increased blood pressure
- Pressure diuresis (Increased urination leading to lowering of blood pressure)

Potential Effects of Impaired MC Production or MC Antagonists

- Elevated K and low Na in serum may suggest low aldosterone levels (salt wasting)
- Hyperkalemia may lead to arrhythmia
- Dehydration (Increased BUN)
- Hypotension
- Hemoconcentration
Cortical atrophy - Dogs
(zona fasciculata and reticularis)
GC treatment for one year
Examples of drugs that cause atrophy.
ZG – ACE inhibitors, ANF
ZF – Excess corticosteroids
Proliferative Lesions of the adrenal gland
Tumor types in carcinogenicity studies – Pharmaceutical Manufacturers Association Survey

1. Liver
2. Mammary gland
3. Thyroid
4. Pituitary
5. Adrenal glands

Chemically induced lesions in the endocrine organs – NTP Review

1. Liver = 176
2. Lung = 70
3. Kidney = 69
4. Stomach = 41
5. Mammary gland = 39
6. Hematopoietic system = 39
7. Thyroid Gland = 37
8. Skin = 30
9. Adrenal Gland = 28
10. Zymbal gland = 22
   Pituitary Gland = 7
   Parathyroid = 0
Proliferative Lesions of the adrenal cortex

- Hyperplasia, Cortical, Focal or diffuse
- Subcapsular hyperplasia (mouse)
- Cortical adenoma (rare in mice)
- Cortical carcinoma (rare in mice)
Focal Hyperplasia

- smaller or larger than surrounding cortical cells.
- Focal or multifocal.
- No or minimal compression of the adjacent cortical tissue.
- The cytoplasm of hyperplastic cortical cells may be comparable to the surrounding cells or may exhibit tinctorial differences (eosinophilic, basophilic, or amphophilic).
- Not larger than the normal width of the cortex.
Diagnosis?
Adrenal Gland Tumors – National Toxicology Program

Number of Test Articles Associated with Site-Specific Neoplasia that produced positive, clear or some evidence of carcinogenicity.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Male Rats</th>
<th>Female Rats</th>
<th>Male Mice</th>
<th>Female Mice</th>
<th>Total</th>
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<tr>
<td>Adrenal Cortex</td>
<td>0</td>
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<td>0</td>
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<td>Adrenal Medulla</td>
<td>17</td>
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<table>
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<tr>
<th>Publication No.</th>
<th>CASRN</th>
<th>Test Article Name</th>
<th>Species</th>
<th>Sex</th>
<th>Route</th>
<th>Organ</th>
<th>Level of Evidence</th>
<th>Tumor Type/Incidence</th>
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<tr>
<td>TR-033</td>
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<td>Tetrachlorvinphos</td>
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<td>Dosed-Feed</td>
<td>Adrenal Gland Cortex</td>
<td>Positive</td>
<td>ADENOMA</td>
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<td>TR-206</td>
<td>96-12-8</td>
<td>1,2-Dibromo-3-chloropropane</td>
<td>Rats</td>
<td>Female</td>
<td>Inhalation</td>
<td>Adrenal Gland Cortex</td>
<td>Positive</td>
<td>ADENOMA 0/50 7/50 5/48</td>
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</tbody>
</table>
Cortical adenoma

- Compression of the adjacent cortical tissue.
- May be larger than the normal width of the adrenal cortex.
- cords, trabeculae or solid clusters of cells.
- Cytoplasmic vacuolation (lipid droplets), angiectasis, hemorrhage, or thrombosis may be present.

**Differential Diagnoses**

- Hyperplasia, Cortical, Focal:
- Vacuolation, Cortical, Increased, Focal
Cortical carcinoma

• Invasive growth into surrounding adrenal tissue
• Neoplastic cells are organized in thickened trabeculae, sheets or solid clusters with disruption of normal architecture.
• Vacuolation, cystic degeneration, necrosis, angiectasis or hemorrhage may be present.

Differential Diagnoses
• Adenoma, Cortical:
• Carcinoma, Subcapsular (Mouse)

Cortical carcinomas are rare in mice.
## Adenoma vs Carcinoma

### ADENOMA
- Well-circumscribed nodule or mass with distinct compression of the adjacent cortical tissue.
- May be larger than the normal width of the adrenal cortex.
- Thin connective tissue capsule may be present.
- Architecture (radial arrangement of the cortical cords) is not maintained.
- Cellular pattern may be cords, trabeculae or solid clusters of cells.
- Cells may be smaller or enlarged and may exhibit tinctorial differences (rat).
- Usually composed of enlarged, eosinophilic or amphophilic cells (mouse).
- Cytoplasmic vacuolation (lipid droplets), angiectasis, hemorrhage, or thrombosis may be present.
- Cellular atypia may be present.
- Mitotic figures may be present.

### CARCINOMA
- Invasive growth into surrounding adrenal tissue, beyond the adrenal capsule and/or distant metastasis.
- Neoplastic cells are organized in thickened trabeculae, sheets or solid clusters with disruption of normal architecture.
- Cytoplasm is usually eosinophilic or amphophilic.
- Cellular atypia and pleomorphism are usually present.
- Mitotic figures may be numerous.
- Vacuolation, cystic degeneration, necrosis, angiectasis or hemorrhage may be present.

INHAND: Non-proliferative and Proliferative Lesions of the Rat and Mouse Endocrine System
Subcapsular cell hyperplasia, mouse

In both sexes, the most common proliferative lesions is subcapsular cell hyperplasia followed by focal cortical hyperplasia, and subcapsular cell tumor.

Composed of large polygonal cells with clear cytoplasm (type B), mixed with fusiform, basophilic cells (type A).

Housing, stress, gonadectomy – increase incidence

Mouse – Cortical hyperplasia less common than subcapsular cell hyperplasia

Subcapsular cell adenoma, mouse
Compression of the adjacent cortex, differentiate from hyperplasia
Proliferative Adrenal Medullary Lesions
Adrenal Medulla

- Less common site for direct toxicity
- Rats (males) have higher incidence of medullary tumors than mice

Proliferative lesions of the adrenal medulla
- Hyperplasia
- Pheochromocytoma, benign
- Pheochromocytoma, malignant
- Pheochromocytoma, complex
- Ganglioneuroma
- Neuroblastoma
### Organ Sites with Neoplasia
#### NTP data base

**Number of Test Articles Associated with Site-Specific Neoplasia that produced positive, clear or some evidence of carcinogenicity.**

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benign or malignant pheochromocytoma
Pheochromocytoma

Rat
High Incidence (M>F. 3-16% in males; 3-18% in females)
Increased by increase food intake
Increased incidence due to autonomic nervous system stimulation – E.g. Reserpine
Role of Calcium: Altered Ca homeostasis
  CPN
  sugars and sugar alcohols
  retinoids & Vit D
Often multiple
30% malignant in some studies
Clinical effects may be seen due to catecholamine secretion
Cells can synthesize noradrenaline

• Human
Rare (0.3% in autopsy cases)
Solitary (unless there is a genetic predisposition)
Cause hypertension (secrete noradrenaline (and adrenaline)
Hyperplasia is rare in the mouse.
Benign pheochromocytoma: The most common tumor of the adrenal gland.

- Incidence is much higher in SD and F344 rats than in Wistar strains.
- Males are more affected than females (related to a higher incidence and severity of chronic progressive nephropathy in male rats- Nyska et al. 1999).
Pheochromocytoma - Rats
- In male rats usually lack PNMT (produce noradrenaline only)
- The ones in female rats produce adrenaline
Malignant pheochromocytoma

Infiltration of the capsule
Metastasis is rare, found in only 2% of rat tumors
Hyperplasia vs. Benign & Malignant Pheochromocytoma

Hyperplasia
- Focal increase in number of chromaffin cells.
  - Usually solitary, infrequently multifocal.
  - No compression or minimal compression of medulla or cortex.
  - Cellular architecture is maintained.
  - Cells may be smaller or larger and have tinctorial differences of the cytoplasm.
  - Nuclear-cytoplasmic ratio may be increased.
  - Mitotic figures are rare.
  - Lesion size is usually less than 50% of normal size of the medulla when the entire medulla is in the plane of sectioning.

Benign
- Mass located in the adrenal medulla, may extend into the cortex.
  - Single or multiple, unilateral or bilateral in rats. Usually unilateral in the mouse.
  - May replace the entire medulla and cortex.
  - Compression of the normal cortex or medulla at the tumor periphery.
    - Cells organized in nests, rows, and cords.
    - Often contains dilated blood vessels.
    - Cells are large or small and the smaller cells have greater cytoplasmic basophilia.
    - Cellular atypia may be present.
    - Hemorrhage and necrosis may be present.
    - Mitotic figures are absent or infrequent.
    - Usually larger than 50% of the normal medulla.

Malignant
- Invasive growth into the adrenal cortex or through the adrenal capsule, vascular invasion, or distant metastasis to the lungs, liver, lymph nodes, bone marrow.
  - Cells organized in nests, rows and cords often with distended blood vessels.
  - Cells are large or small and the smaller cells have greater cytoplasmic basophilia.
  - Cellular atypia may be present.
  - Hemorrhage and necrosis may be present.
  - Mitotic figures may be numerous.
Benign Pheochromocytoma, IHC – mouse
marker to distinguish adrenal pheochromocytomas

Both normal medulla and pheochromocytoma are strongly immunopositive for tyrosine hydroxylase.

Phenylethanolamine-N-methyltransferase (PNMT) – adrenaline synthesizing enzyme shows minimal staining of tumor with strong signal in the normal medulla [adrenaline the major adrenal catecholamine].

Tyrosine ↓
Tyrosine hydroxylase

DOPA ↓
DOPA decarboxylase (stain noradrenaline and adrenaline secreting cells)

Dopamine ↓ Dopamine beta hydroxylase

Noradrenaline (norepinephrine) ↓ PNMT (stain adrenaline secreting cells)

Adrenaline (epinephrine)

Adrenaline cells (66 - 75%); Noradrenaline cells (25 - 33%)
Ratio of E:NE varies among species

Complex pheochromocytoma
Ganglioneuroma
Neuroblastoma
Adrenal gland tumors – rat strains differences

Table 11. Comparison of Selected Adrenal Gland Neoplasms from Different Rat Strains Including F344 (National Toxicology Program 2010), Crl: Wl(Han) (Charles River 2011), Crl: CD(SD) (Charles River 2004), Hsd: SD (Weber, Kaiser, and Klein 2012), and RccHan™: WIST Rats from Studies Performed at Harlan Laboratories Ltd., Switzerland, during 1981 to 2012 (Weber, Razerger, et al. 2011; Envigo 2016)—Total Incidence (%).

<table>
<thead>
<tr>
<th>Strain/ gender</th>
<th>F344 M</th>
<th>F344 F</th>
<th>Hsd: SD M</th>
<th>Hsd: SD F</th>
<th>Crl: CD(SD) M</th>
<th>Crl: CD(SD) F</th>
<th>Crl: Wl(Han) M</th>
<th>Crl: Wl(Han) F</th>
<th>RccHan™: WIST M</th>
<th>RccHan™: WIST F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>1,298</td>
<td>1,245</td>
<td>120</td>
<td>120</td>
<td>2,144</td>
<td>2,344</td>
<td>1,217</td>
<td>1,217</td>
<td>3,226</td>
<td>3,205</td>
</tr>
<tr>
<td>Pheochromocytoma, benign</td>
<td>14.13</td>
<td>2.58</td>
<td>20.33</td>
<td>2.54</td>
<td>10.81</td>
<td>1.92</td>
<td>1.31</td>
<td>0.41</td>
<td>3.23</td>
<td>1.29</td>
</tr>
<tr>
<td>Pheochromocytoma, malignant</td>
<td>1.85</td>
<td>0.57</td>
<td>7.63</td>
<td>0</td>
<td>1.40</td>
<td>0.55</td>
<td>0.33</td>
<td>0</td>
<td>1.09</td>
<td>0.38</td>
</tr>
<tr>
<td>Cortical adenoma</td>
<td>1.16</td>
<td>1.93</td>
<td>0.84</td>
<td>7.62</td>
<td>2.05</td>
<td>3.24</td>
<td>1.73</td>
<td>1.15</td>
<td>1.98</td>
<td>1.59</td>
</tr>
<tr>
<td>Cortical carcinoma</td>
<td>0.15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.51</td>
<td>0.73</td>
<td>0.16</td>
<td>0.08</td>
<td>0.12</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Note. Crl: CD(SD) = Charles River, CD rat, Sprague-Dawley derived; F344 = Fisher 344; Hsd: SD = Harlan Sprague-Dawley strain; RccHan™: WIST = Wistar Hannover (Han)—derived strain, continued breeding by RCC Ltd., Switzerland, thereafter continued breeding by Harlan; M = male; F = Female.

Weber et al., Toxicol Pathol. 2017;45(1): 64-75.
Adrenal Gland Pathology – key points

Adrenal gland weight

- Review zone specific histologic changes microscopically
- Decreased/increased weight – atrophy or hypertrophy/hyperplasia (ZF/ZR)
- Increased weight with cortical hypertrophy is common in toxicity studies (role of ACTH/Stress)
- ZG – aldosterone pathway (ACE inhibitors, ANP), renin-angiotensin pathway

No weight change

- Vacuolation (increased) – either due to impaired steroidogenesis (lipid accumulation) or direct cytotoxicity.
- Differentiate from phospholipidosis

Proliferative lesions

- Subcapsular cell hyperplasia – fairly common in mice
- Adrenal medullary tumors are common in rats.
Comparative Pathophysiology
Beyond morphology – Role of the pathologist
Rare inherited autosomal recessive disorders

- Cortisol
- Aldosterone
- Testosterone

Classical CAH
- Low cortisol and aldosterone
- Overproduction of testosterone
  - Female: Ambiguous genitalia
  - Male: Enlarged external genitalia. Early puberty

Non-classical CAH (20–60% of normal activity)

Current Treatments:
- Life-long glucocorticoid & mineralocorticoid

Gene therapy – Replace the missing enzyme

Congenital Adrenal Hyperplasia
mutation in the gene encoding for 21-hydroxylase

Lack of negative feedback from cortisol leads to overproduction of ACTH, resulting in adrenal hyperplasia
• AAV serotype rh.10 gene transfer vector to 21-hydroxylase deficient mice
• Duration of therapeutic efficacy lasted for only 8 weeks, accompanied by loss of 21OH-HA expression (Markmann, et al., Hum Gene Ther. 2018 Apr;29(4):403-412.)
Adrenas Therapeutics, BridgeBio, Raleigh, NC, USA
The adult adrenal cortex is subject to extensive cell renewal
- distinct pools of stem & progenitor cells in the capsule, subcapsule,
- In mice, all cortical cells are replaced within 200 days.
- Tissue turnover of the adrenal cortex in females is 3 times higher than in males (mice)
  - Female mice: Capsular stem cells contribute to adult cortex replenishment
  - Male mice: homeostasis relies on proliferation of cells within the steroidogenic zone
Acknowledgements

NTP and NTP colleagues
EPL Inc
INHAND Team Members