Phytotoxins

PATB 4140/5140

Why Plants?

- Common source of human exposure
  - 60-80% of poison control center calls
- Major source of pharmaceuticals
- Food safety
- Mechanisms
  - Substituting for homeostatic signals
  - Damaging cellular components
  - Aberrant metabolic pathways

Ricin (Castor Bean)

- *Ricinus communis*
- East African native
- Naturalized in virtually all sub-tropics
- Woody shrub/tree
- Ornamental
- Oil crop

In North America

Castor Bean

Castor Oil

- Lubricant
  - Viscosity, lubricity
- Synthetic feedstock
- $10^6$ Tonnes processed annually for castor oil
- Waste mash 3-5% ricin
Ricin

- Stillmark, 1888
  - Tested beans' extract on red blood cells and saw them agglutinate. "lectin"
  - Protein
  - Agglutination actually due to RCA (Ricinus communis agglutinin).
  - Ricin is a potent cytotoxin but a weak hemagglutinin, whereas RCA is a weak cytotoxin and a powerful hemagglutinin.

Ricin - Mechanism

- 66 kd heterodimer = "Ribosome Inactivating Protein"
  - "A" chain (type 1 RIP) - cleaves specific adenine residue near 3' end of 28S ribosomal subunit
  - "B" chain (type 2 RIP) - binds cell membrane, triggers endocytosis

Ricin - Medical Uses

- Used to demonstrate presence & function of circulating antibodies
- Chemotherapy
  - Low doses successful against solid tumors
  - Conjugate A subunit to antibody or nutrient selective for target cell

Ricin – CBW

- Compound "W" - U.S. & Britain 1919 - 1950's
- Terrorist
  - Low tech preparation
  - Abundant raw material
  - Administration less of a problem than with military use

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Clinical Ricin Toxicity

- 100X variation in toxicity to various species
  - Horse > dog, mouse > chicken > frog
- Route of exposure
  - LD_{50 inh} = 3-5 ug/kg, 60 hours
  - LD_{50 ip} = 22 ug/kg, 100 hours
  - LD_{50 po} 20 ug/kg, 80 hours
- Considerable variation between route of exposure. Due to lectin properties.
Ricin, Oral Exposure

- Rapid onset cramps, nausea, vomiting
- Sore throat, bloody diarrhea
- Mydriasis, headache
- Anuria, fever, thirst
- Vascular collapse, shock; death in 2-3 days

Ricin, Parenteral Exposure

- Low dose (clinical trial), 18-20 µg/m² i.v. (x40) "well tolerated"
  - "Flu-like symptoms": fatigue, myalgia, arthralgia, nausea
- Lethal dose (~500 µg)
  - Local pain, then weakness within about 5 hrs
  - 15 - 24 hrs: fever, nausea, vomiting
  - 36 hrs: ↑HR, swollen lymph nodes, induration and inflammation at the injection site
  - Terminal: ↓HR/BP, ↑↑WBC, renal failure, arrhythmias

Ricin, Inhalation

- Diffuse, necrotizing pneumonitis
  - 8-12 hrs: inflammation and ↑ protein in bronchioles, alveoli
  - by 18 hrs: ↑extravascular lung fluid
  - 30 hrs: hypoxemia, acidosis

Annual Ryegrass Toxicity

- Lolium rigidum
- Nematode
- Corynebacter
- Unidentified phage
- Environmental
Corynetoxin

- Causative agent of Annual Ryegrass Toxicity, Flood Plain Staggers
- Produced by complex grass-nematode-bacterial interaction
- Similar to tunicamycins
  - Structural - differ by length of FA chain
  - Inhibit N-glycosylation of proteins
    - (uridine diphospho-N-acetylglucosamine:dolichol-P N-acetylglucosamine-1-phosphate transferase)

ARGT

- Stair-climbing gait, head high
- Incoordination, weakness hind limbs
  - Sawhorse stance
- Collapse $\rightarrow$ paddling seizures
- May recover temporarily
- Die within 24 hours of first signs

Corynetoxin & Food Safety

- Japanese cattle poisoned by imported hay
- Potential for human exposure
  - ARG common contaminant of grain crops
  - Poisoning in animals associated with contaminated grains
  - Colonization of other grasses by Anguina and Clavibacter
- Potency - LD$_{50}$ in ug/kg range

Maternal Transfer

- Model
  - Long Evans rats
  - Injected with 150 ug/kg (sub-lethal dose) on day 15
- Endpoints
  - Pup liver enzyme activity
  - Corynetoxin ELISA

ARGT

- Post mortem
  - “Snow angel” pattern from paddling
  - Fatty necrosis liver
  - Diffuse hemorrhages in multiple organs
  - Hemorrhage & discoloration in cerebellum
Histology
- Hepatic vacuolization
- Uterus
  - Severe hydropic degeneration myocytes
  - Single cell necrosis
  - +/- Fibrosis, edema
- Fetus
  - Widespread vacuolation of skeletal and cardiac muscle

Phalaris aquatica
- Eurasian import
  - Forage
  - Reclamation
  - Weed
- Sudden death
  - Tryptamine (serotonin analogs) and β-carboline alkaloids
- Staggers
  - β-carbolines
- Polioencephalomalacia
  - Type II thiaminase
  - Vasoactive agent

Cardiac Syndrome
- Cardiac arrhythmia
  - ↓ Cardiac output, ventricular fib
  - Respiratory distress
  - Death in minutes - hours
- Precipitated by stress
**Tryptamines**

- Serotonin
- Dimethyltryptamine

**Tyramines**

- Hordenine
- Gramine
- Tyramine
- Methoxytyramine
- N-methyltryamine

_Release norepinephrine from stores_

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**Phalaris Staggers**

- Stressed grass, monoculture
  - Water, continuous grazing
- Signs days - months after exposure
  - Head bobbing, stiff gait, tremors, incoordination, fall when try to move quickly
  - Temporary recovery, very aggressive
  - Incoordination of tongue
  - Collapse & death

**Phalaris Toxicity**

- Sudden death
  - NMT 10X higher hypertensive than hordenine
  - Monoamine oxidase inhibitors also present

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**Poison Hemlock (Conium)**

- Umbelliferae
  - Resembles wild carrot
- Winter biennial
- Used for execution in pre-Christian Greece
- Seeds → birds → people

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**Conine (1)**

**Nicotine (2)**
### Clinical - Neurotoxic
- Nicotinic ACh receptors
  - Transient stimulation
    - Tremors, hyperesthesia
  - Blockade
    - Flacid paralysis, weakness
- Autonomic
- Mortality
  - Respiratory paralysis
  - Myoglobinuria from muscle damage

### Clinical - Teratogenic
- Cattle, pigs. Other spp. Resistant
- Nicotinic ACh receptors
- Skeletal
  - Arthrogryposis, valgus
- Cleft palate
- Long susceptible period
- Results from impaired movement

### Water Hemlock (*Cicuta*)
- Umbell w/ lanceolate leaves
- Chambered taproot
  - Wet, marshy areas
  - Isolated or colonies

### Mechanism
- GABA antagonist
  - Blocks chloride channels
- Blocks Na channels *in vitro*
- Potent convulsant, autonomic poison

### cicutoxin

![Chemical Structure of Cicutoxin](image)

### source
- Hungry livestock
- Ground into prepared feeds
- Human – foraging for natural foods
- Low morbidity, high mortality
Clinical

- Potent – 1 root lethal to cow
- Salivation, vomiting w/i 30 min
- Grand mal seizures
- Death from respiratory arrest
- Survivors don’t remember anything

Digitalis (Foxglove)

- Perennial shrub (Eurasia)
- Medical – 1785
  - “Dropsey”
  - “Digitalis USP”
- ~20 cardenolides
  - Commercial source of digoxin, digitalis

Digitalis Glycosides

Cardenolide = Cardiac Glycoside = aglycone + sugar(s)

Other Sources

- Asclepias (milkweeds)
- Dogbane
- Oleander

Mechanism

- Cytosolic Ca\(^{2+}\) required for contraction
- Removed by Ca-Na exchange (plasma membrane, Ca-ATPase (SR membrane)
- Na\(^+\) gradient maintained by Na-K ATPase
- ↓ATPase => ↓[Na\(^+\)] => ↑[Ca\(^{2+}\)]
  - ↑ HR, strength
  - arrhythmias
**Mechanism**

- Gastrointestinal
  - Vomiting, diarrhea, colic
- Cardiac
  - Weakness, stupor
  - Pulmonary edema => respiratory distress
  - Cyanosis
  - "Loud" irregular heart beat

**Clinical**

**Therapy**

- Anti-digoxin Fab

**St. Johnswort (Hypericum)**

- Morphologically diverse genus 200 sp.
- Occasionally used as ground cover
  - Usually weeds

**Hypericin**

- Protein kinase C inhibitor
- Anti-retroviral
- Serotonin, etc. uptake inhibitor
- Astringent, antibacterial, diuretic, sedative
- Photosensitizer
Astragalus (Locos)

- Astragali = ankle bones
- Legume
- Approximately 7500 species
  - Include non-toxic species
- Used for soil reclamation
- Folk medicine - American Indian
Toxic Principle(s)

- Swainsonine
  - Common with *Swainsonia canescens*
- Miserotoxin (3-nitropropanoic acid)
  - "Cracker heels"
- Selenium

Mechanism

\[
\begin{align*}
\text{Swainsonine cation} & \quad \text{Mannosyl cation} \\
\text{Inhibits } \alpha-D\text{-Mannosidase}
\end{align*}
\]

Kinetics

- Water soluble
  - Rapid absorb, excretion via urine, milk
  - Rapid equilibration
- 1st order kinetics
  - \( T_{1/2} = 16 \text{ hrs} \)
Clinical

- Usually late Winter, early Spring
  - Habituation
- CNS
- Cardiovascular
- Reproductive
  - Infertility
  - Teratogenesis
- Immune ??

Clinical-CNS

- “Depression”, lethargy, weight loss
- Ataxia - awkward stiff-legged gait
  - Hypermetria, loss motor control
- Over-response to stimuli
  - “otherwise docile animals may become quite violent, even maniacal …”
  - Refractory to conventional tranquilizers
  - Apparently recovered animals

Clinical

- Reproductive
  - Early term abortion, dystocia
    - Luteal cell damage → ↓ progesterone
  - Infertility
    - Libido
    - Spermatogenesis, sperm motility
  - Terata
    - Small, weak neonates
    - Skeletal deformities

Clinical

- Cardiac
  - Potentiates congestive heart failure
    (“high mountain disease”)
- Immune
  - Vacuolation peripheral blood lymphocytes
  - Augments NK activity

“3-Nitro” glycosides

- Miserotoxin
- 3-nitropropanolic acid
- 3-nitropropanol

Kinetics

- Ruminal N reduction to beta alanine and aminopropanol
- 3NPOH more readily absorbed

- Inhibits mitochondrial succinate dehydrogenase
  - Opens K+ channels → depolarization
  - ↑ SOD & ↑ GSH peroxidase => lipid peroxidation
Pathophysiology

- Neuronal & myelin degeneration
  globus pallidus and substantia nigra
- Selective inhibition TCA in GABA neurons
- Excitotoxicity due to stim NMDA receptors

Clinical

- Acute: sl. ataxia, recumbency, death
- Chronic
  - "Cracker heels" - proprioceptive def.
  - Incontinence, posterior paresis
  - stertorius

Sources

- (Europe) livestock forage/hay
- "Fiddlenecks"
Pathophysiology

- Thiaminase
  - Monogastric herbivores
- "Radiomimetic Factor"
  - Bone marrow suppression
- Cancinogen

Thiaminase

- Monogastrics (that eat enough)
  - Horses, swine
- Rapid reversal of signs with thiamin
- Type I thiaminase
  - Produces thiamin antagonist
  - Polyneuropathy

Thiaminase

- Originally in Britain, Pacific NW
- 20-25% BW 3+ weeks
  - Hay or green foliage
- Transketolase - key enzyme in TCA
  - Increased blood pyruvate
- Decreased blood thiamin

Clinical

- Depression
- Constipation
- Ataxia, incoordination, twitching

Cancer

- Long history of use in humans
  - Flour
  - Imported into Japan as delicacy
  - Relay toxicity via milk (Azores)
- Urinary bladder neoplasia in cattle

Cancer

- Cyclopropane ring
- Covalently binds, cleaves DNA
- Highest in young fronds
Radiomimetic Factor

- Thrombocytopenia
  - Epistaxis, melena, dyspnea, anemia
- Leukopenia
  - Respiratory disease
- Ptaquiloside??

Solanum (Nightshades)

Pyrrolizidines??