DISCLOSURES: NO NE
OBJECTIVES

1. Identify plant species known to cause mitochondrial disruption resulting in human toxicity.
2. Become familiar with the distribution of these plants.
3. Become familiar with the toxins of these plants.
4. Become familiar with human outbreaks involving mitochondrial dysfunction secondary to plant ingestions.
5. Understand risks and identify at risk populations for these exposures.
6. Explain potential treatment plans for patients who suffer from these exposures.
7. Think of preventive measures to limit further exposures.
CONCEPT: STRENGTH OF TOXIN AND DURATION OF EXPOSURE

Acute, life-threatening mitochondrial dysfunction

Subacute, prolonged, mitochondrial dysfunction

Chronic, prolonged, mitochondrial dysfunction
• Acute, severe dysfunction of the mitochondria frequently produces a rapidly progressive illness:
  • Agitation
  • Encephalopathy
  • Seizures
  • Hypoglycemia
  • Metabolic Acidosis
  • Respiratory Failure
  • Cardiac Failure
  • Hepatic Failure
  • Renal Failure
CONCEPT: STRENGTH OF TOXIN AND DURATION OF EXPOSURE

Subacute, prolonged dysfunction of the mitochondria frequently produces progressive illness that may appear to have a sudden onset:

- Upper motor neuron
- ALS-like disease

- weakness
  - inability to walk
    - postural instability
    - spastic paraparesis (LEs > UEs)
Chronic, prolonged dysfunction of the mitochondria frequently produces slowly progressive illness:
- Atypical Parkinson’s disease
- Dementia
MITOCHONDRIA = THE ENERGY BANK:
ENERGY (CURRENCY) EXCHANGE
STORE ENERGY
TRANSFER ENERGY TO DISTANT SITES
Fatty Acids → Acyl-CoA → Acylcarnitine

CAT

Acylcarnitine

Acyl-CoA

Beta-oxidation of Fatty Acids

Acetyl-CoA

Krebs Cycle

FADH₂

NADH

H⁺

ECT-I

ECT-II

ECT-III

ECT-IV

Q

O₂

H₂O

ADP + P

ATP

H⁺

Matrix

Intermembrane Space

ATP synthase

ADP

ATP-ADP Trans

uncoupler

ADP

ATP
MITOCHONDRIA = THE DUMP
• Dump extra calcium, an important second messenger and neurotoxin, into mitochondria to protect the rest of the cell

• Protect rest of cell from ROS, the toxic wastes of energy production
CLASSIFICATION BY SITE OF ACTION

- **Neuroexcitation (glutamate agonists: toxins that produce Ca$^{++}$ influx into mitochondria)**
  - Grass pea (neurolathyrism from BOAA or β-ODAP)
  - Cycads with symbiotic cyanobacteria (ALS/PDC from BMAA)

- **Beta-oxidation of Fatty Acids Inhibitors**
  - Ackee (hypoglycin A and B)
  - Lychee (methylene cyclopropylglycine)

- **Krebs Cycle Inhibitors (block one or more steps in Krebs cycle; also interferes with complex II)**
  - Gifblaar (monofluoracetate)
  - Willow (methyl salicylate)
CLASSIFICATION BY SITE OF ACTION

• **Electron Transport (Respiratory) Chain Inhibitors (toxins that interfere with Complex 1-IV)**
  - Tuba plant (rotenone; complex I)
  - Soursop (annonicin; complex I)
  - Grass pea (neurolathyrism from BOAA or β-ODAP; complex I)
  - Cyanogenic plants (cyanide; complex IV)

• **Oxidative Phosphorylation/Transport Inhibitors (bind ADP-ATP translocase carrier sites)**
  - Cocklebur (carboxyatractyloside)
  - Blue lime or blue thistle (atracutyloside)

• **Uncouplers (abolish linkage of electron transport chain and phosphorylation at the mitochondrial inner membrane)**
  - Willow (methyl salicylate)
CASE 1

• 22 yo Ethiopian teenage male farmer had a 10 year history of BLE spasticity which had developed acutely and progressed over several days.

• His weakness improved slightly over weeks, but he developed bilateral lower limb spasms and rigidity.

• He had no sensory symptoms.

• He had consumed guaya-kitta, guaya-shiro, and guaya-kollo for his meals.

• Exam: Gait – spastic and scissored with toe walking. He had sustained clonus and brisk DTRs, with positive Babinski sign. He had LE weakness. CNs intact. UEs normal.
SUBACUTE NEURO EXCITATION:
GLUTAMATE AGONISTS

• Neurolathyrism – from Grass Pea or Chickling Pea (Lathyrus sativus, L. cicero, Vicia sativa, V. ervilia)
• Epidemics: Ethiopia, Bangladesh, India, Nepal
SUBACUTE NEUROEXCITATION:
GLUTAMATE AGONISTS

- **Diet of Grass Pea:**
  - 300-400 g/day for 2-4 months, with little else to eat
- **Toxin:**
  - BOAA (beta-N-oxalylamino-L-alanine) or beta-ODAP (beta-N-oxalyl-alpha,beta-diaminopropionic acid)
    - a non-protein amino acid that acts as an AMPA receptor agonist and indirect metabotropic (mGluR1) receptor agonist
    - Results in ROS that inhibit mitochondrial complex I
    - upper motor neuron disease
    - selectively in the motor cortex and lumbar spinal cord
- **Treatment:**
  - Tolperisone and other muscle relaxers (expensive)
  - Prevention: autoclaving the seed with lime removes the toxin; moist heat; famine relief
ROLE OF GSH

• Neuroexcitation decreases formation of glutathione and increases oxidative stress.
• ? NAC
CHRONIC NEUROEXCITATION:
GLUTAMATE AGONISTS
OTHER OUTBREAKS

ALS-Parkinsonism-Dementia Complex of Guam
CHRONIC NEUROEXCITATION: GLUTAMATE AGONISTS

- Endemic ALS-Parkinsonism-Dementia Complex – from cyanobacteria in Cycas micronesica (Cycads)
  - BMAA (beta-methylamino-L-alanine) is an AMPA and KA receptor agonist
    - ALS
    - Parkinson’s
    - Dementia
  - Long latency
  - Autopsy: elevated brain BMAA levels, neurofibrillary tangles, linear retinal pigmentary epitheliopathy
NEUROLATHYRISM AND ALS-PDC OF GUAM

- Months of exposure:
  - Subacute mitochondrial failure
  - Permanent, devastating neurological damage

- Years of exposure:
  - Chronic mitochondrial failure
  - Permanent, devastating neurological damage

The Dump
Excessive calcium
Excessive ROS
CASE 2: AN OUTBREAK

• India (Bihar), June 2013
  • Outbreak of acute neurologic illness in 133 young children (most 5 yrs and younger)
    • Encephalopathy
    • Cerebral edema
    • Seizures
    • Hypoglycemia
    • Afebrile
    • Upper motor neuron findings (hypertonia; Babinski's sign)
  • Tended to be chronically undernourished
  • High fatality rate (44%)

Shrivastava A, 2015
OUTBREAK: LYCHEE FRUIT (LITCHI SINENSIS)

- Epidemiology studies: lychee orchards
- Since 1990s, unexplained outbreaks of acute encephalitis in Vietnam

LYCHEE CULTIVATORS AND OTHER AREAS OF CONCERN AROUND THE WORLD

- Taiwan*
- Thailand*
- India*
- Bangladesh
- Vietnam* (limb paralysis in 13% in VN outbreak)
- China*
- Madagascar
- South Africa
- Mauritius
- Australia
- USA (Hawaii, Florida)

*Main producers
LYCHEE (LITCHI CHINENSIS)

- Lychee fruit contains methylenecyclopropylglycine (MCPG):
  - MCPG is found in high concentrations in the seed and semi-ripe pulp
  - MCPG forms compounds with carnitine and coenzyme A
- Lychee: A member of the soapberry family (Sapindaceae), like Ackee
TREATMENT FOR ACKEE (BLIGHIA SAPIDA) AND LYCHEE (LITCHI CHINENSIS)

• Treatment:
  • supportive
    • close monitoring and replacement of glucose
    • benzos and barbs for seizures
  • L-carnitine
  • Possibly: methylene blue
LYCHEE AND ACKEE FRUITS

- Acute mitochondrial failure*
- Acute metabolic and neurological failure
- Acute multisystem organ failure

* occasional subacute findings with Lychee exposure
ATYPICAL PARKINSONISM IN THE CARIBBEAN
CASE 3 (PART OF AN OUTBREAK)

- An African-Caribbean man from Marie-Galante (Guadeloupe archipelago) developed mental and physical slowing at age 53.
- He developed a frontal lobe syndrome (dementia with euphoria, echolalia, impaired verbal fluency, disorientation, memory loss, frontal lobe type bladder incontinence) and visual hallucinations and delusions.
- Later, he had postural instability with falls.
- Parkinsonism with vertical supranuclear palsy involving up-gaze and down-gaze.
- Eventually, he developed dysarthria and dysphagia.
- Exposure history: He drank teas from a local plant as an aphrodisiac.
GUADALOUPEAN PARKINSONISM: SOURSOP (GRAVIOLA)

Annonacin

Also found in: Cuba, Hispaniola, Puerto Rico, Mexico, Central and South America (Colombia, Venezuela, Brazil, Peru, Ecuador), Africa (Eastern Nigeria, The Plateau, The Democratic Republic of Congo), Southeast Asia and the Pacific.
ANNONACIN: MECHANISM OF ACTION
How do sporadic and familial Parkinson’s disease compare:

- Post-mortem brain tissue show reduced activity of complex I in the substantia nigra.
- Mitochondrial dysfunction likely underlies motor neuron disorders (Parkinson’s disease, ALS, Friedreich’s ataxia, Huntington’s disease, Alzheimer’s disease).
Fatty Acids → Acyl-CoA → CAT → Acylcarnitine → Acyl-CoA

Acyl-CoA → NADH → Krebs Cycle

NADH → FADH₂ → β-oxidation of Fatty Acids

ECT-I → ECT-III → ECT-IV → ATP synthase

ADP + P → ATP

O₂ → H₂O

H+ → Matrix

= annonacin
CASE 4

• A 54 y.o Chinese woman presents to the ED:
  • At home, c/o acute onset of GI distress and lethargy after eating 40 pieces of yam bean seeds (Pachyrhizus erosus or Pachyrhizus tuberosus) for breakfast 4 hours earlier.
  • She became dyspneic and drowsy.
  • Exam: HR 34 bpm, BP - not measurable (both responded to atropine).
  • GCS = 4, resulting in intubation.
  • AG = 31, BE = -11.5 (metabolic acidosis)
  • Head CT normal at 24 hours.
  • Negative cyanide testing.
CASE

• After 2 weeks, patient remained altered.
• EEG suggested generalized cerebral dysfunction.
• Head MRI (3 weeks after exposure):
• In conjunction with her neurobehavioral deficits, a diagnosis of leukoencephalopathy from toxin-induced brain insult was made.
ROTENONE: DERRIS ELIPTICA

- Used by primitive peoples as a fish poison since prehistoric times.
- Suicidal agent in the Philippines and Netherland East Indies
  - Drink a juice from the tuba plant (Derris eliptica)
- In 1930s, derris root (known as a “bun,” a “tuba” or an “akar-tuba”), was the most common method of suicide in New Ireland a mandated territory of New Guinea (East Indies)
  - Piece of fresh root 3 inches long can kill an adult
  - Post-mortem examinations: acute congestive heart failure

Sources: Holland EA, 1983, Redaniel MT, 2011, MacDonald CJH, 2003, Simmons, JS in Global Epidemiology, 1944
• Blocks NADH dehydrogenase (Complex 1) in the respiratory chain.

• Acute signs and symptoms: N/V, incoordination, seizures, encephalopathy, metabolic acidosis, respiratory distress, bradycardia, and dysrhythmias (high fatality rate)

• Interestingly, chronic low-dose rotenone exposure is suspected to increase the risk for PD in humans.

• Experimental treatment: methylene blue
CASE 5 (SERIES)

• At a bamboo shoot pickling factory in Thailand:
  • Patient 1 dropped 20 kg fresh bamboo shoots into shoot pickling well and jumped in to retrieve the bag. He immediately lost consciousness in the well.
  • Then Patient 2, followed by Patients 3, 4 and 5 and then Patients 6, 7, and 8.
  • Finally, other workers wore cloth masks and tied themselves with ropes and went down to lift the 8 patients out of the well.
  • Rescue workers arrived 30 minutes later.

<table>
<thead>
<tr>
<th>Patient (Age)</th>
<th>Initial Findings (ED and day 1)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (29 yrs)</td>
<td>Follows commands; dyspnea; agitated; tachycardic</td>
<td>Survived; discharged day 3</td>
</tr>
<tr>
<td>2 (20 yrs)</td>
<td>Agitated; unreactive pupils; tonic seizures; VDRF (pink frothy sputum)</td>
<td>Survived; discharged day 5</td>
</tr>
<tr>
<td>3 (26 yrs)</td>
<td>Conscious, but dizzy</td>
<td>Survived; discharged day 3</td>
</tr>
<tr>
<td>4 (47 yrs)</td>
<td>Unconscious (GCS 3); unreactive pupils; hypotension; cardiac arrest with CPR</td>
<td>Died 13 hours after admission</td>
</tr>
<tr>
<td>5 (35 yrs)</td>
<td>Drowsy (GCS 10); progressive coma and dyspnea; VDRF; agitation</td>
<td>Survived; discharged day 3</td>
</tr>
<tr>
<td>6 (57 yrs)</td>
<td>Unconscious; dyspnea; VDRF</td>
<td>Survived; discharge day 3</td>
</tr>
<tr>
<td>7 (30 yrs)</td>
<td>Unconscious; cardiac arrest</td>
<td>Died 30 hours after admission</td>
</tr>
<tr>
<td>8 (34 yrs)</td>
<td>Conscious with agitation; palpitations; CP; tachycardia; VDRF</td>
<td>Survived; discharged day 3</td>
</tr>
</tbody>
</table>
• A 41 yo woman ingested apricot kernels purchased from a health food store and became weak and dyspneic within 20 minutes. She was comatose and hypothermic upon presentation.

• Acute cyanide poisoning = acute mitochondrial failure

Suchard J R, 1998
• Uniform clinical findings were found in 39 cases.
• Abrupt onset of difficulty walking, with progressive paraparesis over 2-3 days.
• Complained of generalized weakness (inability to rise from supine to sitting).
• No fever or symptoms of infection.
• Lumbar pain radiating down legs and leg numbness.
• Typically, awoke with symptoms or symptoms developed after a long walk.
• Hyperreflexia and ankle clonus.
• Extensor planter responses.
• Increased tone with spasm in flexion of LEs.
• UE relatively spared, but some had weakness.
• Sensation remained intact.
• Eventual contractures.
• Thoracolumbar kyphoscoliosis.
CASSAVA AND DROUGHT

• Konzo:
• Cassava (*Manihot esculenta*)
  • Linamarin
  • Broken down by linase
# CYANOGeneric GLYCosides

<table>
<thead>
<tr>
<th>Cyanogenic Glycoside</th>
<th>Plant</th>
<th>Clinical Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdalin</td>
<td>Bitter almonds (Prunus amygdalus) and Prunus spp.</td>
<td>Acute cyanide toxicity</td>
</tr>
<tr>
<td>Prunasin</td>
<td>Stone fruits (Prunus spp.)</td>
<td>Acute cyanide toxicity</td>
</tr>
<tr>
<td>Linamarin</td>
<td>Cassava (Manihot esculenta) Lima beans</td>
<td>Konzo: subacute cyanide toxicity</td>
</tr>
<tr>
<td>Lotaustralin</td>
<td>Cassava Lima beans</td>
<td>Konzo: subacute cyanide toxicity</td>
</tr>
<tr>
<td>Dhurrin</td>
<td>Sorghum (Sorghum album)</td>
<td></td>
</tr>
<tr>
<td>Taxiphyllin</td>
<td>Bamboo shoots (Bambusa vulgaris)</td>
<td>Acute cyanide toxicity</td>
</tr>
<tr>
<td>Konzo from Cassava (<em>Manihot esculenta</em>)</td>
<td>Neurolathyism from Grass Pea (<em>Lathyrus sativus</em>)</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Vulnerable populations:</strong> poor and malnourished, during environmental stress (droughts, famine and war)</td>
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<td></td>
</tr>
<tr>
<td><strong>Diet deficiencies:</strong> methionine, cysteine</td>
<td><strong>Diet deficiencies:</strong> methionine, cysteine</td>
<td></td>
</tr>
<tr>
<td><strong>Most affected:</strong> young children and women of child-bearing age</td>
<td><strong>Most affected:</strong> young men</td>
<td></td>
</tr>
<tr>
<td><strong>Location:</strong> Central and southern Africa</td>
<td><strong>Location:</strong> Ethiopia and Indian sub-continent</td>
<td></td>
</tr>
<tr>
<td><strong>Toxin:</strong> cyanide from cyanogenic glycosides (linamarin, lotaustralin)</td>
<td><strong>Toxin:</strong> BOAA or β-ODAP (AMPA agonist)</td>
<td></td>
</tr>
<tr>
<td><strong>Target:</strong> cytochrome oxidase (electron transport chain)</td>
<td><strong>Target:</strong> glutamate receptor (overwhelm mitochondria with Ca++)</td>
<td></td>
</tr>
<tr>
<td><strong>End-result:</strong> mitochondrial dysfunction and irreversible loss of motor neurons</td>
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<td></td>
</tr>
<tr>
<td><strong>Clinical outcome:</strong> spastic paraparesis of LEs</td>
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<td></td>
</tr>
</tbody>
</table>

Ngudi DD, 2012
CYANOGENIC GLYCOSIDES

Acute mitochondrial failure

Subacute mitochondrial failure
CASE 8 (OUTBREAK)

- Bangladesh, November 2007
- Flooding ruined rice crops and prices rose. Poultry died.
- A woman and her child presented unconscious to the hospital
  - antecedent vomiting and restlessness prior to LOC
  - They died within hours (another one of her 2 remaining children died within hours)
- In the following days, 18 patients presented with similar symptoms
- Ultimately, 76 patients were identified (many died before seeking care):
  - Vomiting (100%)
  - Fever (61%)
  - Encephalopathy (59%; 38% unconscious)
  - Elevated LFTs
  - Fatality (25%) - children accounted for the majority of deaths, with a mortality closer to 35%
  - Consumed “ghagra shak” a few hours prior to illness onset

Gurley ES, 2010
OUTBREAK: XANTHIUM STRUMARIUM

• Fatal outbreak:
  • Ghagra shak = common cocklebur = Xanthium strumarium
  • Population had previously consumed small amounts of this plant previously
  • Recent dependence on X. strumarium resulted in increased toxicity

Gurley ES, 2010
XANTHIUM STRUMARIUM

- Cocklebur (*Xanthium strumarium* and *X. spinosum*)
  - worldwide distribution
  - seeds taste like sunflower seeds
  - contain carboxyatractyloside
    - inhibits translocation of ADP and ATP across mitochondrial membranes
  - abdominal pain, nausea, vomiting, diaphoresis, respiratory depression
  - seizures, coma and death
  - seizures difficult to treat
COCKLEBUR

- metabolic acidosis
- hepatomegaly
- rhabdomyolysis
- consumption coagulopathy
- EKG - ST segment abnormality
- Autopsy: centrilobular hepatic necrosis, renal proximal tubular necrosis, microvascular hemorrhage of CNS, leukocytic infiltrates of muscle, pancreas, lungs and myocardium
ACUTE MITOCHONDRIAL FAILURE

- Ox-eye daisy (*Callilepis laureola*) and bird-lime or blue-thistle (*Atractylis gummifera*):
  - Found in Africa and Mediterranean, respectively
  - Contain atractyloside and carboxyatractyloside
- Transport inhibitor:
  - Blocks the adenine nucleotide porter by binding to the outward-facing conformation blocks transport of ADP at mitochondrial membrane
    - Blocks conversion of ADP to ATP
  - Inhibits p450 and b5 cytochromes
  - Clinically, similar to Cocklebur, which contains the same toxin

ADP

ATP
Fatty Acids

Acyl-CoA

Acyl-CoA

Acylcarnitine

CAT

Acyl-CoA

Acylcarnitine

Fatty Acids

Acyl-CoA

Acylcarnitine

Acylcarnitine

Acyl-CoA

Beta-oxidation of Fatty Acids

Acetyl-CoA

NADH

FADH₂

Krebs Cycle

ect-I

ect-II

ect-III

ect-IV

H⁺

H⁺

H⁺

H⁺

O₂

H₂O

ADP + P

ATP

ATP synthase

uncoupler

ATP

ADP

ATP-ADP Trans

Intermembrane Space

Matrix

= atracyloside and carboxyatracyloside
SPECTRUM OF ILLNESS

Acute mitochondrial failure

Rapid onset of:
- Encephalopathy
- Seizures
- Metabolic acidosis
- Multiple system organ failure

Subacute mitochondrial failure

After accumulation of toxin (months), quick, progressive onset of:
- Upper motor neuron disease
- ALS phenotype

Chronic mitochondrial failure

After accumulation of toxin (years), progressive illness of:
- Parkinson’s disease
- Dementia
# Plant Mitochondrial Toxins

## Acute
- Ackee (hypoglycin A and B)
- Lychee (MCPG)
- Tuba Plant (rotenone)
- Cocklebur (carboxyatractyloside)
- Blue-lime/Blue thistle (attractyloside)
- Cyanogenic glycosides

## Subacute
- Grass pea (BOAA or β-ODAP)
- Cycads (BMAA)
- Cyanogenic glycosides

## Chronic
- Cycads (BMAA)
- Soursop (annonacin)