PALYTOXIN & OTHER MARINE TOXINS

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OBJECTIVES

• Discuss two common emerging aquatic toxins:
  – Palytoxin
  – Domoic acid

• Explain the reported mechanism of toxicity

• Review clinical manifestations
DISCLOSURES

- No financial, litigational, or other conflicts of interest to disclose
CASE

- Family of 6 (5 children, ages 10-17 years) and father all present to ED with:
  - Weakness/malaise
  - Chest pain with SOB
  - Sore throat
  - 3 also with fever, mild nausea
• On exam, all with varying degrees of
  – Tachypnea
  – Tachycardia
  – Hypoxia

• One child also has infiltrates on chest radiograph
CASE (CONT.)

- Dog found dead at home
- Hazmat tested for:
  - CO, CO$_2$
  - Mercury
  - Radiation
  - Hydrogen sulfide
  - “Nerve gas”
  - VOCs
IN THE HOUSE...
PALYTOXIN

- 1838 David Malo described a “poisonous moss” used to apply to spears
- 1961: University of Hawaii researchers found the poisonous moss
- 1971: Species Palythoa toxica named; a zoanthid coral which contains palytoxin
OUTBREAKS

• Numerous sporadic cases throughout US
• Published outbreaks described in Georgia, Alaska
• Numerous anecdotal cases described on internet in “underground” coral community
PALYTOXIN

- C$_{129}$H$_{223}$N$_3$O$_{54}$
- MW: 2680 Daltons
- One of most complex non polymeric natural molecules
PALLYTOXIN

- No definitive melting point; only chars when heated to 300°C

- Can be neutralized by soaking coral for 30 minutes in a $\geq 0.1\%$ household bleach solution
  - 1 part 5-6% sodium hypochlorite to 10 parts water
Also numerous analogues
- Also isolated from soft coral
- Most not well studied; few that are studied have similar activity
TOXICITY

- Maitotoxin, produced from the dinoflagellate Gambierdiscus
  - Highest IP mouse lethality (50 ng/mL); 1g can kill 1 billion mice
- Palytoxin with 2nd highest
  - IP mouse lethality of 300 ng/kg
Palytoxin Organisms

- Palytoxin identified in organisms in tropical, subtropical, and temperate regions
- Found in phylogenetically-different species – bacterial production.
MECHANISM OF ACTION

- Binds to Na\(^+\)/K\(^+\)/ATPase
- Induces conformational change of pump
- Uncouples ion transport
- Changes transmembrane pump into nonspecific monovalent cation channel
MECHANISM (CONT.)

- Both gates on the Na\(^+\)/K\(^+\) pump are open simultaneously
  - Inability to maintain ion gradients

- Prolonged opening of Na\(^+\)/K\(^+\)/ATPase

- Na\(^+\) overload is the first step in palytoxin-induced cellular damage
MECHANISM (CONT.)

• $\uparrow$ in $[\text{Ca}^{2+}]$ via $\text{Na}^+$ overload triggers $\text{Ca}^{2+}$ dependent cytotoxic effects

• Some data on $\text{H}^+/\text{K}^+$ pump inhibition

• $\uparrow$ in $[\text{H}^+]$ is the driving force for $\text{O}_2^-$ production via reversal of mitochondrial ETC, leading to necrotic cell death
**PALYTOXIN VS. CARDIAC GLYCOSIDE**

- In vitro studies suggest different binding site from ouabain.

- Different structure from cardiac glycosides; not a typical steroid.
HUMAN TOXICITY

• Human toxicity described via
  – Inhalational exposure
  – Dermal exposure
  – Oral consumption of contaminated seafood
  – Ocular exposure

• Constellation of symptoms somewhat route-dependent
ORAL EXPOSURE

• Documented through consumption of
  – Crabs (Demania reynaudii)
  – Goldspot herring (Herklotsichthys quadrimaculatus)
  – Parrotfish (Scarus ovifrons)
  – Serrand fish (Epinephelus species)

• GI symptoms in addition to other toxicities
INHALATIONAL EXPOSURE

• Typically associated with inhalation of vapors from home marine aquaria
  – Mostly from Cnidarian

• Primarily associated with soft corals
  – Coral often cleaned by pouring boiling water onto coral and then brushing the rocks

• Systemic symptoms plus respiratory symptoms
CUTANEOUS EXPOSURE

- Associated with collecting zoanthid colonies with bare hands
- Risk increased with cutaneous abrasions
- Systemic manifestations plus local irritation and burning sensation
OCCULAR EXPOSURE

• Associated with contaminated water splashed into the eyes
• In addition to systemic manifestations, can see
  – Eye pain
  – Keratoconjunctivitis
  – Photophobia
  – Epithelial erosions
SYSTEMIC EFFECTS: CONSTITUTIONAL

- Generalized malaise, weakness
- Bitter, metallic taste
- Rhinorrhea, low grade fevers
- Headache
<table>
<thead>
<tr>
<th>SYSTEMIC EFFECTS: GASTROINTESTINAL</th>
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<tbody>
<tr>
<td>• Nausea, vomiting, diarrhea</td>
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<td>• Abdominal pain</td>
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<td>• Dry mouth, odynophagia</td>
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<td>• Anorexia</td>
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SYSTEMIC EFFECTS:
RESPIRATORY

- Observed with both inhalational exposures and oral ingestions
- Cough
- Bronchospasm/wheezing
- Respiratory failure
SYSTEMIC EFFECTS: NEUROLOGIC

- Weakness
- Tremor
- Ataxia
- Dizziness
- Paresthesias
SYSTEMIC EFFECTS: MUSCULAR

- Myalgias
- Muscle spasms
- Rhabdomyolysis
DIAGNOSTIC TESTING

• Not routinely available
  – Testing through FDA or research labs

• Hemolysis neutralization assay

• LCMS

• HPLC with LCMS
DIAGNOSTIC TESTING

• Can be performed on blood
• Sand/soil
• Coral
• Water
TREATMENT

• Largely supportive

• No antidote

• Common treatment modalities in published cases
  – Beta agonists +/- corticosteroids
  – Fluid resuscitate
  – NSAIDS
**RED TIDE**

- Discoloration of seawater due to blooms of planktonic algae

- 5000 marine algal species in ocean
  - 300 with high proliferation rate resulting in high density thus contributing to red tide

- Toxin production by plankton consumed by larger fish and enter food chain
Several different shellfish poisonings (SP) identified:

- Paralytic SP: Saxotoxin
- Diarrheic SP: Okadaic acid
- Neurotoxic SP: Brevetoxin
- Azaspiracid SP: Azaspiracid
- Amnestic SP: Domoic acid
DOMOIC ACID

[Chemical structures of domoic acid]
HISTORY

• 1959: Domoic acid isolated in C. armata

• 1966: Final structure of determined

• Closely intertwined with kainic acid
  – Both excitatory amino acid analogues of glutamate
HISTORY

• Implicated in amnesic shellfish poisoning
• First reported outbreak was in Prince Edward Island in 1987
  – 200 cases of mussel related illness
  – 107 met case definition of amnesic shellfish poisoning
  – Additional 38 “probable” cases
  – 4 deaths
Toxic algae bloom might be largest ever

Originally published June 15, 2015 at 9:05 pm | Updated June 16, 2015 at 11:41 am

WASHINGTON DEPARTMENT OF FISH & WILDLIFE
FISHING & SHELLFISHING

Razor Clams

DOMOIC ACID - A major concern to washington state’s shellfish lovers

Toxin from algae bloom off West Coast found in wildlife

5:45 p.m. PST November 3, 2015
HISTORY

- 2015-2016 large outbreak extending from California to Alaska
DOMOIC ACID

- Hydrophilic with low molecular weight
  - Primarily eliminated by kidney
- Diatom *pseudo-nitzschia pungens* is most abundant producer of domoic acid
- Synergistic effects with endogenous glutamate and NMDA receptor agonists
  - Partial agonist at the KA subtypes of inotropic glutamate receptor
EXPOSURE

• Associated with consumption of molluscan shellfish from NE and NW coasts

• Domoic acid found in viscera of Dungeness, tanner, and red rock crab
  – Also other fish and squid
DISTRIBUTION OF ASP IN US

Events from 1997-2006
- Red circle: 6-10 times
- Orange circle: 2-5 times
- Yellow circle: 1 time
- White circle: sampled but not detected
TARGETS

• Domoic acid primarily targets hippocampus
  – Affects processing memory and visceral functions
• Also affects:
  – Amygdaloid nucleus
  – Claustrum
  – Secondary olfactory areas
  – Nucleus accumbens
DOMOIC ACID: MOA

- Glutamate receptors act as mediators of inflammation and cellular injury
  1) Tissue swelling
  2) Increased Ca^{2+} influx
  3) Misc. messenger pathways
  4) Degenerative changes in astrocytes
Tissue Swelling

- Inotropic glutamate receptors (iGluR) are ion-gated channels, sensitive to Na\(^+\), K\(^+\), Ca\(^{2+}\).

- Neuronal damage due to influx of extracellular Na\(^+\), water, and cell volume expansion.

- Focal swellings along dendrites called varicosities are a hallmark of excitotoxic neuronal injuries.
CALCIUM INFLUX

- Glutamate opens voltage-dependent Ca\(^{2+}\) channels (via NMDA and non NMDA glutamate receptors)
  - Activation of phospholipases, PKC
  - Damage oxidative phosphorylation
  - Free radical formation

- Activation of phospholipases results in activation of arachidonic cascade $\rightarrow$ PG production
CALCIUM INFLUX

- Activation of phospholipase A$_2$ generates platelet activating factors $\rightarrow$ ↑ release of glutamate

- Positive feedback cycle created with ↑ phospholipase A$_2$ production

- Ca$^{2+}$ influx increases NOS $\rightarrow$ NO $\rightarrow$ ↑ free radical formation
NEURONAL DAMAGE

• Elevated Ca$^{2+}$ also creates:
  – Mitochondrial dysfunction
  – Activate endonucleases $\rightarrow$ DNA fragmentation and apoptosis

• Neuronal degeneration results in dose-dependent necrotic degeneration
  – $[\text{DOM}] \leq 0.1 \, \mu\text{M} \rightarrow$ apoptosis dominates
  – $[\text{DOM}] > 0.1 \, \mu\text{M} \rightarrow$ necrosis dominates
ASTROCYTES

- Failure of astrocytes to remove excess extracellular glutamate is key component in domoic acid neurotoxicity
- Domoic acid can trigger microglia to release TNF alpha
### CLINICAL DATA

- Most experience from 1987 Canadian outbreak
- Involved organ systems:
  - GI
  - Neurologic
  - Cardiovascular
GI SYMPTOMS

- Onset within 24 hours
  - Nausea/vomiting
  - Abdominal cramps
NEUROLOGIC SYMPTOMS

- Onset within 48 hours
  - Confusion/disorientation 1.5-48 hours post exposure
  - Maximal deficits 4-72 hours
  - Most symptoms with improvement between 24 hours-12 weeks
- Some deficits persist
- Elderly more susceptible to neurotoxicity
NEUROLOGIC SYMPTOMS

- Range of symptoms
  - Seizures
  - Myoclonus
  - Memory deficits
  - Ataxia
  - Spastic hemiparesis with opthalmoplegia

- Hallmark: Anterograde amnesia with preservation of other cognitive functions
NEUROLOGIC SYMPTOMS

• Moderate deficits: Encode information but delayed recall
• Severe: difficulty learning verbal and visuospatial material with impaired recall
• Ability to form concepts generally maintained
CARDIOVASCULAR SYMPTOMS

- Hypotension and arrhythmias observed
- Some evidence of direct cardiac effects, rather than CNS control centers
  - Cardiac NMDA receptors implicated
• Extremity weakness and distal atrophy
• Non-progressive neuronopathy involving anterior horn cells or diffuse axonopathy affecting motor axons
TREATMENT

• No specific treatment
• Supportive care
  – Phenytoin relatively ineffective for treating seizures
  – Benzos/barbiturates appear more effective
• Melatonin?
TESTING

- Primarily done via FDA and local health departments
  - FDA Action level: 20 ppm except in viscera of Dungeness crab (30 ppm)

- Numerous testing methods:
  - LC with UV detection (DA in shellfish)
  - HPLC, LCMS, etc.
SUMMARY: PALLYTOXIN

- Coral (household and ocean)
- Discussed role of Na⁺/K⁺/ATPase
- Numerous routes of exposure (inhalational, oral, dermal, ocular)
- Symptoms include constitutional, respiratory, GI, and musculoskeletal
- Treatment supportive
SUMMARY: DOMOIC ACID

- Associated with red tide
- Multiple mechanisms implicated, including neuronal edema, astrocyte injury, and Ca$^{2+}$ influx
- Hippocampus disproportionately affected
- GI symptoms within 24 hours, neuro symptoms within 48 hours
THANK YOU!