The pathophysiological mechanisms of ciguatera

DR IRINA VETTER
UNIVERSITY OF QUEENSLAND
INSTITUTE FOR MOLECULAR BIOSCIENCE & SCHOOL OF PHARMACY

I.VETTER@UQ.EDU.AU

CENTRE FOR PAIN RESEARCH
Institute for Molecular Bioscience, University of Queensland

- Brisbane, Queensland
Sensory Neuropharmacology Group

- Peripheral mechanisms of pain
- Neuropharmacology
- Analgesic drug discovery
What is ciguatera?

- The most commonly reported marine toxin disease in the world
  - Associated with consumption of reef fish contaminated with ciguatoxin
  - 50,000 people/year (?)
  - Under-recognised in non-endemic areas
    - Mis-diagnosed as multiple sclerosis, chronic fatigue syndrome, bacterial/viral food poisoning
  - Under-reported in endemic areas
What are ciguatoxins?

- Lipid soluble polyethers
- Resistant to heat, freezing, stomach acid
- Produced by benthic dinoflagellates
  - *Gambierdiscus* spp.
- Named according to origin:
  - Pacific ciguatoxin
  - Caribbean ciguatoxin
  - Indian ciguatoxin
Bioaccumulation of ciguatoxins

1. **Gambierdiscus spp blooms**
   - G. toxicus
   - G. pacificus
   - G. polynesiensis
   - G. australes

2. **Herbivores**
   - Feed off algae contaminated with *Gambierdiscus* spp

3. **Carnivores**
   - Feed on herbivores contaminated with ciguatoxins

Ciguatera

(> 0.1 micrograms CTX)
## Ciguatera symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Onset</th>
<th>Frequency</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td>30 min – 48 h</td>
<td>50-77%</td>
<td>Up to 1 week</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td></td>
<td>26-82%</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td></td>
<td>43-75%</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow heart beat</td>
<td>12%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low blood pressure</td>
<td>16%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neurological</strong></td>
<td>Delayed (&lt; 3 days)</td>
<td>64-100%</td>
<td>Weeks-months</td>
</tr>
<tr>
<td>Numbness/tingling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse/localized pain</td>
<td></td>
<td>56-83%</td>
<td></td>
</tr>
</tbody>
</table>

*Mattei et al; Toxicon. 2014 Dec;91:76-83.*
Mechanism of action of ciguatoxin

- Ciguatoxin is the most potent known activator of voltage-gated sodium channels
  - Proteins in neurons that are crucial for electrical transmission of signals
  - Nine human isoforms ($NaV_{1.1}$-$NaV_{1.9}$)
    - **blocked** by local anaesthetics
  - Crucial for normal function of pain pathways
Mechanism of action of ciguatoxin

- Ciguatoxin is the most potent known activator of voltage-gated sodium channels
  - Proteins in neurons that are crucial for electrical transmission of signals
  - Nine human isoforms (Na\textsubscript{v}1.1-Na\textsubscript{v}1.9)
    - **blocked** by local anaesthetics
  - Crucial for normal function of pain pathways
Ciguatoxin directly acts at peripheral nerve endings

- Intradermal injection of CTX (~50 picograms) in humans
  - Burning pain
  - “flare” – similar to bee sting

Intradermal ciguatoxin causes cold allodynia

- Local injection of ciguatoxin in the skin causes hypersensitivity to cooling
- “cool” becomes “painful”
- Provides evidence that effects at peripheral nerve endings are responsible for ciguatera symptoms

Which types of neurons mediate effect of ciguatoxin?

- **Sensory neurons are very heterogeneous**
  - Different sizes
  - Respond to different stimuli (hot, cold, mechanical)
  - Different functions

- **Ciguatera has very unique symptoms**
  - Probably mediated by specific subset of sensory neurons

  - Assessed responses of different sensory neurons to ciguatoxin
Which types of neurons are activated by ciguatoxin?

Grow sensory neurons in dish → Measure response to ciguatoxin → Identify neuronal subpopulations
Neuronal populations activated by ciguatoxin

- P-CTX-1 activates a subset of neurons
  - Varying sizes
  - Nearly all neurons expressing TRPA1 responded to ciguatoxin

Ciguatoxin-induced cold pain involves TRPA1

- **TRPA1 (transient receptor potential ankyrin 1)**
  - Protein expressed in sensory neurons
  - Involved in sensing noxious chemicals & noxious cold

- clove
- horseradish
- wasabi
- garlic
- mustard
- cinnamon
- ginger
- cold
Ciguatoxin does not cause cold sensitivity in sensory neurons without TRPA1

Mechanism of ciguatoxin-induced cold pain

- **Ciguatoxin-induced cold pain**
  - Increased excitability of sensory neurons
    - Voltage-gated sodium channels
  - Activation of cold-sensitive TRPA1 channels

- **Blockers of voltage-gated sodium channels or TRPA1 channels might be beneficial for treatment of ciguatera**
Which sodium channels need to be blocked?

- **9 isoforms:**
  - Na\(_v\)1.1 – brain
  - Na\(_v\)1.2 – brain
  - Na\(_v\)1.3 – brain
  - Na\(_v\)1.4 – skeletal muscle
  - Na\(_v\)1.5 – heart
  - Na\(_v\)1.6 – sensory/motor neurons, brain
  - Na\(_v\)1.7 – sensory neurons
  - Na\(_v\)1.8 – sensory neurons
  - Na\(_v\)1.9 – sensory neurons

- **Need selective blockers:**
  - Venoms and toxins!
Venoms as sources of subtype selective sodium channel blockers

- Venoms have evolved to rapidly incapacitate prey/predators
- Highly complex mixtures of bioactive compounds
  - small molecule, peptide, protein
  - 100s or 1000s of individual components
  - Highly potent & highly selective
  - Thousands of venomous species

Lewis et al Nature Reviews Drug Discovery 2, 790-802 (October 2003)
### Which sodium channels need to be blocked?

<table>
<thead>
<tr>
<th></th>
<th>Tetrodotoxin (TTX)</th>
<th>μ-conotoxin GIIIA</th>
<th>μ-theraphotoxin Pn3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na\textsubscript{v}1.6</td>
<td>✓</td>
<td>✓</td>
<td>❌</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.7</td>
<td>✓</td>
<td>❌</td>
<td>✓</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.8</td>
<td>❌</td>
<td>❌</td>
<td>❌</td>
</tr>
<tr>
<td>Na\textsubscript{v}1.9</td>
<td>❌</td>
<td>❌</td>
<td>❌</td>
</tr>
</tbody>
</table>
Multiple sodium channel isoforms mediate the symptoms of ciguatera

Different sensory neurons & sodium channels mediate different symptoms

- **Na\textsubscript{v}1.8/TRPA1**
  - Cold pain

- **Na\textsubscript{v}1.6**
  - Cold pain

- **Na\textsubscript{v}1.7/Na\textsubscript{v}1.6**
  - Non-thermal pain

- **Na\textsubscript{v}1.8**
  - Gastrointestinal pain

**Ciguatera**
Treatment approaches for ciguatera

- No validated treatments available
  - Intravenous mannitol (to reduce neuronal swelling)
    - Within 48 h – no proven efficacy in clinical trials
  - Cholestyramine (bile-acid binding resin)
    - No clinical trials

- Which sodium channel blockers might be useful?
Repurposing existing drugs

- Several drugs with the required channel blocking activity already exist:

  - amitriptyline
  - carbamazepine
  - flupirtine
  - lamotrigine
  - mexiletine
  - phenytoin
  - topiramate
Repurposing existing drugs

- Several drugs with sodium channel blocking activity already exist:
  - Flupirtine, lamotrigine and phenytoin may be useful to treat ciguatera
    - Clinical studies need to be carried out to validate therapeutic effect
Acknowledgements

University of Queensland
Richard J Lewis
Jennifer Deuis
Marco Inserra
Mathilde Israel
Paul F Alewood
Thomas Durek
Zoltan Dekan

Collaborators
Katharina Zimmermann (Uni Erlangen)
John Wood (UC London)
Andrej Romanovsky (Barrow Neurological Institute Arizona)
Stuart Brierley (Uni Adelaide)
Barbara Namer (Uni Erlangen)
Angelika Lampert (Uni Erlangen)

Funding
ARC
NHMRC
Thank you!

- HYDRO vzw and the Flanders Marine Institute vzw (VLIZ)
  - Jan Mees, Heidi Coussens, Tina Mertens
  - Dr Edouard Delcroix & selection committee
Summary

- Specific subtypes of sensory neurons mediate diverse symptoms of ciguatera
- Ciguatoxin acts through different mechanisms in each subtype
- Treatment with existing drugs (eg. Flupirtine, lamotrigine) may be useful for ciguatera