Aluminum as a neurotoxin: the evidence from cell culture, in vivo, and human studies

Christopher A. Shaw
Depts. of Ophthalmology and Visual Sciences, Experimental Medicine, and Program in Neuroscience

Vaccine Safety:
Evaluating the Science
January 3-7, 2011,
Tryall Club, Jamaica
Topics to be addressed

• Conflict of interest
• Neurological disease clusters
• ALS epidemiology
• Following ALS-PDC
• Gulf War Syndrome and aluminum
• Aluminum neurotoxicity in various preparations
• Mimicking motor neuron disease with aluminum adjuvants
• Conclusions and future directions
Evaluating critical vs. non-critical events in neurological disease

- Causal events start disease process
  - essential to know
- Coincident events
  - not directly relevant, can be ignored?
- Compensatory events
  - something to increase?
- Prevention = causality
- Early treatment = timeline of critical stages
- Cure = ??? (no consensus on what this means)
Neurological disease progression: AD, PD, ALS

Causal Event(s)

Prevention

Intervention

Conventional Diagnosis

Palliative Care

?? Cure

Central Nervous System “Health”

~30

100

Neuro-degenerative Cascade

Healthy neurons

No clinical symptoms, but disease process ongoing

Symptoms of disease

Time

Symptoms of disease

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Causality: environment, genetics, and age?

**Environment**
- Low Toxin → High Low
  - Cycad toxicity
  - Rotenone/Pesticides
  - MPTP
  - Lead/Heavy metals
  - High cholesterol/Diet
  - Viral infections

**Genetics**
- High Abnormal Toxicity → Low
  - Causative Genes
    - mAPP/tau
    - PS1/PS2
    - ApoE
    - mSOD1
    - α-synuclein
    - α-synuclein
    - LKKR2

**Detoxification**
- High Low
  - CYP genes
  - ND3 (complex 1 enzyme)

**Disease Phenotype**
- Xenobiotic metabolism
- Toxic accumulation
- Previous CNS trauma
- Cerebral perfusion

**Age**
- Young
- Old
Clues from neurological disease ‘clusters’

- Defn: a large number of cases of the disease with time and geography constraints
- The Centers for Disease Control and Prevention (CDC) define a cluster investigation as, "a review of an unusual number, real or perceived, of health events (for example, reports of cancer) grouped together in time and location." Cluster investigations seek to confirm cases of the disease; establish whether the reported cases represent an unusually high occurrence of the disease; and explore potential causes when possible.

- Ex: Eastern Canada: domoic acid in mussels (example of bio-magnification)

- ALS:
  - ALS-parkinsonism dementia complex (ALS-PDC) of the Western Pacific
  - Gulf War Syndrome ALS?
Changing ASD prevalence
ALS (‘lytico’); parkinsonism with dementia (‘bodig’); combined form possible.

Lytic, bodig once 100-400x greater than ALS in N. America; leading cause of death.

Familial clustering, but no clear genetic inheritance; genetic susceptibility (e.g., apo E), not causality. Importance of gene-environment interactions.

Males > Females: 2-3:1

Strange features of ALS-PDC: olfactory disturbance; early exposure linked to delayed onset?

Strong link to dietary neurotoxin contained in cycad seeds (Cycas micronesica).
ALS-PDC

- Earliest recorded case of ALS-PDC phenotype in Chamorro population in early 1800s.
- Peak in 1950s/60s: decline in ALS-PDC coincides with change in dietary habits.
- ALS has declined to approx. N. American levels; PDC still higher.
- Saipan vs. Guam: why different?
- Single major ALS, PD, AD disease ‘cluster’.
- Water soluble BOAA, BMAA, cycasin/MAM not involved (ie., no bats involved).
- Neurological “Rosetta Stone”.

Water soluble BOAA, BMAA, cycasin/MAM not involved (ie., no bats involved).
What are the toxins in cycad?

- Evidence for:
  - cycasin/MAM: genotoxic effects, do not give ALS-PDC-like outcomes
  - BMAA: weak NMDA agonist; AMPA agonist; no ALS-PDC outcomes (…and not from bats)
  - BOAA: lathyrism not really ALS-PDC
  - Other “toxins”? Role for sterol glucosides.
  - Other environmental factors: *high aluminum*
A simple experiment:

**Cycad** (w/out BMAA, BOAA, cycasin/MAM) + Mouse = ALS-PDC

### 4 Dimensions
- Behavioural: motor, cognitive and olfactory
- Cellular
- Biochemical

Time
**Behaviour: Motor tests**

- **Rotarod** – Motor Coordination and Strength
- **Paw Print** – Gait Length Measure
- **Leg Extension** – Motor Neuron Integrity
- **Wire Hang** – Muscle Strength

![Graph of Leg Extension](image)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Cycad</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image" alt="Control Data" /></td>
<td><img src="image" alt="Cycad Data" /></td>
</tr>
</tbody>
</table>

Legend:
- * p<0.05
- + p<0.001
- # p<0.0001
Magnetic resonance microscopy
Volume measurements: MRM
MRM: some examples

![MRI images and graph showing ventral horn volume comparison between Control and Cycad-fed groups.](image-url)
Cycad feeding: measurable ALS phenotype

**Leg Extension**

![Leg Extension Graph]

- Control
- Cycad

- [* p<0.05
- + p<0.001
- # p<0.0001

**Motor Neuron Count**

![Motor Neuron Count Graph]

- Control
- Cycad-fed

- * p<0.05

**Gray Matter**

- Normalized Volume

![Gray Matter Graph]

- Control
- Cycad-fed

- ** 21%

**Ventral Horn Volume**

- Normalized Volume

![Ventral Horn Volume Graph]

- Control
- Cycad Fed

**GFAP Labelled**

- Control
- Cycad Fed

**GLT-1B Labelling**

- Control
- Cycad Fed

![MRI Volume Analysis Diagram]
### ALS-PDC vs cycad-fed mice

<table>
<thead>
<tr>
<th></th>
<th>ALS-PDC</th>
<th>Cycad-fed mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Progressive motor weakness</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gait impairment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cognitive deficits</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Olfactory dysfunction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tau</td>
<td>+</td>
<td>+/- culture</td>
</tr>
<tr>
<td>Amyloid and α-synuclein deposits</td>
<td>+new cases/20%</td>
<td>+/-? (rats +)</td>
</tr>
<tr>
<td><strong>Motor neuron loss</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal/cortical neuron loss</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SNpc DA neuron loss</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine D2r up; DAT down</td>
<td>Not determined</td>
<td>+</td>
</tr>
<tr>
<td>Reactive astrocytes/microglia</td>
<td>+</td>
<td>+/- (GFAP: mice and rats)</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Not determined</td>
<td>protein/lipid</td>
</tr>
</tbody>
</table>

**Table 1.** Comparison of ALS-PDC symptoms and cycad-induced outcomes in mice and rats
ALS-PDC and GWS ALS: similarities?

- **ALS-PDC:**
  - cycad sterol glucosides
  - **aluminum** (Gadjusek)

- **GWS ALS:**
  - cholesterol glucoside in *H. pylori* and *Mycoplasma fermentans*
  - **aluminum**: AVA and others

- Synergy of SGs and Al?
Down the neurological disease rabbit hole
H1N1

• PRODUCT INFORMATION LEAFLET
• Arepanrix™ H1N1
• AS03-Adjuvanted H1N1 Pandemic Influenza Vaccine
• Version 1 approved October 21, 2009
• Health Canada has authorized the sale of Arepanrix™ H1N1 based on limited clinical testing in humans under the provision of an Interim Order (IO) issued on October 13, 2009.
Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, acute and repeated dose toxicity, local tolerance, female fertility, embryo-fetal and postnatal toxicity up to the end of the lactation period.

Two reproductive studies were conducted with AS03-adjuvanted H5N1 antigen and evaluated the effect on embryo-fetal and peri-and post-natal development in rats, following intramuscular administration. Although no definite conclusion could be reached, regarding a possible relation to treatment with the H5N1 vaccine and/or the adjuvant AS03, and other findings were considered normal, the following observations deserve to be mentioned: In the first study, there was an increased incidence of fetal malformations with markedly medially thickened/kinked ribs and bent scapula as well as an increased incidence of dilated ureter and delayed neurobehavioral maturation. In the second study, there was an increased incidence of post-implantation loss, and the fetal variation of dilated ureter. Not all findings were observed in both studies, and hence the toxicological significance is uncertain.
Table 1. Aluminium adjuvant content in licensed vaccines. 
DTaP=Diphtheria, Tetanus, and acellular Persussis; HepA=Hepatitis A; HepB=Hepatitis B; Hib=Haemophilus influenzae type b; PCV=Pneumococcal; MenC=Meningococcal group C

<table>
<thead>
<tr>
<th>Aluminium adjuvant</th>
<th>Vaccine</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Amount (μg) per dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al hydroxide</td>
<td>DTaP</td>
<td>Infanrix</td>
<td>GlaxoSmithKline</td>
<td>625 18</td>
</tr>
<tr>
<td>HepA</td>
<td>Havrix</td>
<td>GlaxoSmithKline</td>
<td>250 18</td>
<td></td>
</tr>
<tr>
<td>HepB</td>
<td>EngerixB</td>
<td>GlaxoSmithKline</td>
<td>250 18</td>
<td></td>
</tr>
<tr>
<td>Hib</td>
<td>PedVax Hib</td>
<td>Merck and Co</td>
<td>225 18</td>
<td></td>
</tr>
<tr>
<td>Anthrax</td>
<td>Biothrax</td>
<td>Biopart Corp</td>
<td>600 43</td>
<td></td>
</tr>
<tr>
<td>Al phosphate</td>
<td>PCV</td>
<td>Prevnar</td>
<td>Wyeth</td>
<td>125 18</td>
</tr>
<tr>
<td>MenC</td>
<td>Meningitec</td>
<td>Wyeth</td>
<td>125 42</td>
<td></td>
</tr>
<tr>
<td>Al sulphate</td>
<td>HepB</td>
<td>Recombivax</td>
<td>Merck and Co</td>
<td>500 18</td>
</tr>
</tbody>
</table>
“Pervasive uncertainty”? Just how pervasive? Curiouser and curiouser...


_Vaccine_ 20 (2002) S1–S4
Conference report
Workshop summary
Aluminum in vaccines
Aluminum hydroxide induce cell death in NSC34 cells for 3 days (One way ANOVA, *p<0.05; #p<0.001)
IMR-32 cells with aluminum chloride treatment
ChAT labelled MNs

Motor Neuron Count in Lumbar SC

- Control
- Aluminum
- Squalene
- Aluminum + Squalene

Normalized number of positive labeled cell per sample area

Group

CON ALUM SQE A+S
Aluminum Toxicity: NeuN and Activated Caspase-3

Motor neuron from lumbar SC from aluminum treated mouse
Aluminum: GFAP and Morin

GFAP

Control
Aluminum

Control
Aluminum

Morin

Normalized number of positive labeled cell per sampled area

Control
Aluminum
Squalene
Aluminum+Squalene

***

*P=0.025
Iba-1 labeling in ventral horn of lumbar SC

**Control**

**Aluminum**

***
Tau labelling
Motor behavioural data 2 (Shaw and Petrik, 2009)
Still more
Water maze test as an evaluation of learning and memory. Mice injected 6× with aluminum hydroxide on average took significantly longer to complete the maze compared to saline injected mice (two-way ANOVA. *$p = 0.0389$).
Behavioural outcomes: 40mg/L dietary AlCl•6H2O male CD1 mice aged ~7-12 months
Those pesky ASD data again
Aluminum adjuvants and ASD

% increase in # of ASD cases
% increase in vaccine-administered Al

Cumulative Al burden (μg)

R² = 0.85
Conclusions/future directions

• What we’ve shown:
  • Abundant evidence for aluminum in some neurological disease states;
  • Motor neuron loss with neuroinflammatory markers;
  • Behavioural deficits of motor and cognitive function;

• What we need to do:
  • Do a lot more of same with emphasis on compound, route of administration, dose, duration, age, gender, gene-toxin interactions; key biomarkers, component quantification...
  • Translational steps leading to therapy.
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Thank you and questions?