Clinical effects and antivenom dosing in Brown snake (*Pseudonaja* spp.) envenoming

Australian snakebite project (ASP-14)

GEOFF ISBISTER  
CALVARY MATER NEWCASTLE

Allen, G. O’leary M., Brown S., Buckley N., Isbister G.  
For the ASP investigators
Australia ... home of the most deadly snakes
Brown Snakes … the 2nd most deadly snake?

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-scaled snake/Fierce snake</td>
<td>Oxyuranus microlepidotus</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Common Brown Snake</strong></td>
<td><strong>Pseudonaja textilis</strong></td>
<td>0.041</td>
</tr>
<tr>
<td>Taipan</td>
<td>Oxyuranus scutellatus</td>
<td>0.064</td>
</tr>
<tr>
<td>Tiger snake</td>
<td>Notechis scutatus</td>
<td>0.118</td>
</tr>
<tr>
<td>Reevesby Island tiger snake</td>
<td>Notechis ater niger</td>
<td>0.099</td>
</tr>
<tr>
<td>Beaked sea snake</td>
<td>Enhydrina schistosa</td>
<td>0.173</td>
</tr>
<tr>
<td>Western Australian tiger snake</td>
<td>Notechis ater occidentalis</td>
<td>0.124</td>
</tr>
<tr>
<td>Chappell Island tiger snake</td>
<td>Notechis ater serventyi</td>
<td>0.271</td>
</tr>
<tr>
<td>Death Adder</td>
<td>Acanthophis antarcticus</td>
<td>0.338</td>
</tr>
<tr>
<td>Gwardar or Western Brown snake</td>
<td>Pseudonaja nuchalis</td>
<td>0.338</td>
</tr>
<tr>
<td>Australian copperhead</td>
<td>Austrelaps superbus</td>
<td>0.5</td>
</tr>
<tr>
<td>Indian Cobra*</td>
<td>Naja naja</td>
<td>0.513</td>
</tr>
<tr>
<td>Dugite</td>
<td>Pseudonaja affinis</td>
<td>0.56</td>
</tr>
<tr>
<td>Papuan black snake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow or Stephen’s banded snake</td>
<td>Hoplocephalus stephensi</td>
<td>1.44</td>
</tr>
<tr>
<td>Rough-scaled/Clarence River snake</td>
<td>Tropidechis carinatus</td>
<td>1.09</td>
</tr>
<tr>
<td>King cobra*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue-bellied or spotted black snake</td>
<td>Pseudechis guttatus</td>
<td>1.53</td>
</tr>
<tr>
<td>Collet’s snake</td>
<td>Pseudechis colletti</td>
<td>2.38 (only saline)</td>
</tr>
<tr>
<td>Mulga or King brown snake</td>
<td>Pseudechis australis</td>
<td>1.91</td>
</tr>
<tr>
<td>Red-bellied or Common black snake</td>
<td>Pseudechis porphyriacus</td>
<td>2.52</td>
</tr>
<tr>
<td>Small-eyed snake</td>
<td>Crytophis nigrescens</td>
<td>2.67 (saline)</td>
</tr>
<tr>
<td>Eastern diamond-back rattlesnake*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Australian Snakebite Project

- Dec 2001 to May 2012
- 1142 Snakebite
  - 557 envenomated
    - Brown Snake 243
    - Tiger Snake 85
    - Red-bellied black snake 73
    - Rough-scaled snake 39
    - Taipan 28
    - Mulga snake 22
    - Death Adder 19
    - *Hoplocephalus* spp. 15

  - 10 deaths = 4%
  - No deaths
Brown snake envenoming

- All 10 deaths from ASP
- Clinical impression:
  - Severe coagulopathy
  - ? major haemorrhage
- Early hypotensive collapse
  - Mainly reported with brown
  - Indicator of severity
  - Unclear mechanism
- Brown snake paradox:
  - Potent presynaptic neurotoxin BUT minor neurotoxicity?
Factor deficiencies in venom-induced consumption coagulopathy resulting from Australian elapid envenomation: Australian Snakebite Project (ASP-10)

G. K. ISBISTER, *, † † F. E. SCORGIE, § M. A. O’LEARY, † M. SELDON, § S. G. A. BROWN, † and L. F. LINZ, *, §, FOR THE ASP INVESTIGATORS
Early Collapse... rats

*Pseudonaja textilis*

20µg/kg
Solving the ‘Brown snake paradox’: In vitro characterisation of Australasian snake presynaptic neurotoxin activity

Carmel M. Barber, Geoffrey K. Isbister, Wayne C. Hodgson

Monash Venom Group, Department of Pharmacology, Monash University, Victoria, Australia

Discipline of Clinical Pharmacology, University of Newcastle, New South Wales, Australia

Figure C: Graph showing twitch height (% of initial) over time (min) for textilotoxin and taipoxin.

Figure A: Absorbance (230 nm) over time (min) for a sample with 6% activity.

Figure B: Absorbance (230 nm) over time (min) for a sample with 20% activity.
Brown snake envenoming ... antivenom

- “brown snake antivenom does not appear to be efficacious...”
- Dosing:
  - Western Australia
    - 10 vials
  - Eastern Australia
    - 4 to 6 vials
- Evidence ??
  - *in vitro* studies of antivenom
  - clinical studies of antivenom
Antivenom dosing in 35 patients with severe brown snake (*Pseudonaja*) envenoming in Western Australia over 10 years

Justin M Yeung, Mark Little, Lindsay M Murray, George A Jelinek and Frank FS Daly

There is conflicting advice about the appropriate dosing of antivenom for patients envenomed by brown snakes in Australia, with recommended initial doses ranging from one to four ampoules.\(^1\)\(^-\)\(^3\)

Determining the dose required to neutralise the venom in a given case is difficult, because there is no clear end-point against which to titrate antivenom. The two brown snakes in Western Australia — the dugite (*Pseudonaja affinis*) and the western brown snake or gwardar (*P. nuchalis*) (Box 1) — both produce defibrination coagulopathy (in severe cases, afibrinogenaemia), but few other clinical features. Unlike the common or eastern brown snake (*P. textilis*), they rarely cause neurotoxicity in humans. Patients envenomed by brown snakes in Western Australia are often asymptomatic. Even when venom is neutralised by antivenom, there is a delay before fibrinogen is produced, so determining the dose required is difficult. An objective method for determining the neutralising dose based on ELISA measurement of venom concentrations in blood has been proposed, but to date this is

**ABSTRACT**

**Objective:** To investigate the doses of antivenom administered to adult patients with severe brown snake envenoming.

**Design and setting:** Review of charts from Western Australian adult teaching hospitals, December 1991 to December 2001.

**Patients:** 35 patients with severe brown snake envenoming, defined prospectively as afibrinogenaemia (<0.3 g/L) after a bite by a brown snake (genus *Pseudonaja*).

**Main outcome measure:** The dose of antivenom required to neutralise venom, defined prospectively as the dose of antivenom given before the return of detectable fibrinogen levels.

**Results:** Of 88 patients with brown snake envenoming admitted over the 10 years, at least 35 had severe envenoming. Afibrinogenaemia persisted for 10 hours (range, 1.4–68 hours) after the first dose of antivenom; in four patients afibrinogenaemia lasted more than 24 hours. The dose of antivenom given before venom neutralisation ranged from one to 23 ampoules. In two-thirds of cases, venom was neutralised with five ampoules, and 89% had venom neutralised with 10 ampoules. Two patients died, and another had serious bleeding complications. Another patient died during the study period from intracerebral haemorrhage, but did not have fibrinogen levels measured.

**Conclusions:** Patients received initial doses of antivenom too small to neutralise circulating venom, and remained afibrinogenaemic for prolonged periods, with serious consequences. The authors now use 10 ampoules as an initial dose in severe brown snake envenoming.

*JAMA 2004; 181: 703–705*
Brown snake envenoming ... antivenom

- “brown snake antivenom does not appear to be efficacious...”
- Dosing:
  - Western Australia
    - 10 vials
  - Eastern Australia
    - 4 to 6 vials
- Evidence ??
  - *in vitro* studies of antivenom
  - clinical studies of antivenom
Efficacy of antivenom against the procoagulant effect of Australian brown snake (*Pseudonaja* sp.) venom: In vivo and in vitro studies

Geoffrey K. Isbister\textsuperscript{a,b,*}, Margaret A. O’Leary\textsuperscript{b}, Jennifer J. Schneider\textsuperscript{c}, Simon G.A. Brown\textsuperscript{d}, Bart J. Currie\textsuperscript{a}, on behalf of the ASP Investigators

- Antivenom efficacy: 1 vial sufficient to bind
- Neutralisation: 1 vial sufficient
- Patients: small study, but 1 vial sufficient in 9 patients

Conclusion: low dose (1 to 2 vials)!
We investigated the clinical and laboratory features of definite *Pseudonaja* spp. bites and antivenom treatment.
Australian Snakebite Project …ASP

- Prospective study of snake-bites
- Serial data collection with venom/antivenom levels to determine:
  - ANTIVENOM: EFFECTIVENESS, SAFETY and DOSE
  - END-POINTS for TREATMENT
- RANDOMISED CONTROLLED TRIAL of FFP in snake-bite coagulopathy

CONTACT: 1800 676944
Methods

- Patients from Australian Snakebite Project - ASP
  - 2004 to 2011
  - Possible brown snake envenoming
    - Snake identification
    - Clinical effects
    - Snake venom detection kit

- Definite brown snake bites/envenoming defined by:
  1. Snake identification – expert
  2. Confirmation of brown snake venom on venom specific enzyme immunoassay
AUSTRALIAN SNAKEBITE PROJECT

STUDY AIMS:
- We aim to determine optimum dosage of antivenom for each species of Australian Snake.
- We aim to resolve the controversy over the use of FFP/TCP precipitate for snakebite envenomation.
- We aim to determine whether this mode of first aid is effective as it is generally applied.

INCLUSION CRITERIA

Recruitment and Data Collection

- Identification of cases
  - Local hospital site investigators
  - Poison Centre/Clinical Toxicologists
  - Laboratory/Other

- National Ethics Approval

Recruitment (by chief investigators):
- Patient Info, consent, datasheets faxed by eFax
- Laboratory contacted for blood sample processing, storage and transport

SNAKEBITE STUDY

CITRATE PLASMA PROCESSING PROTOCOL

NOTE: PROCESSING (INCL. FREEZING) MUST BE COMPLETED WITHIN TWO HOURS OF COLLECTION

Blood in 3.2% Sodium Citrate (1:95)
- 2000g for 10 min in a standard bench top centrifuge
- Remaining serum (1-2 ml, aliquots into 2ml screw-cap vials if available)

Freeze within 24 hours and transport in dry ice to 80°C storage until able to transport in dry ice to 80°C storage until available

Please keep samples (citrate and serum) from each time point separate in a single specimen bag, along with a copy of the corresponding request form.

LAB INVESTIGATION RESULTS:
Please attach copies of all investigation results reported by your hospital laboratory.
Recruitment and Data Collection

- Follow-up
  - Re-contact ED and/or ICU to get information faxed back (dedicated secure fax to pdf/email)
  - Confirmation of samples with laboratory
- Data-entry:
  - Purpose built relational database
  - Data collation by CIs + RAs
  - Data entry by RA and checked by CI
Venom assay: Enzyme Immunoassay
Sandwich ELISA

Microplate reader – 405nm

Streptavidin Horseradish Peroxidase conjugate

Biotinylated Antibody
Anti-snake venom IgG

Venom

Antibody
Anti-snake venom IgG

Development of a sensitive enzyme immunoassay for measuring taipan venom in serum

S. Kulawickrama a, M.A. O’Leary b, W.C. Hodgson c, S.G.A. Brown d,e, T. Jacoby d,f, K. Davern f, G.K. Isbister b,g,h,*

Toxicon 2010
Results

Cases recruited to ASP
1109 snake bites

Possible brown snake bites/envenoming
227 cases

Definite brown snake envenoming
136

Definite brown snake bites
13

No pre-AV blood
Incorrect ID
- 4 tigers on EIA
# Demographics

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [yr] (median, range)</td>
<td>42; (2-81)</td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>99</td>
<td>73%</td>
</tr>
<tr>
<td>Lower Limb</td>
<td>81</td>
<td>60%</td>
</tr>
<tr>
<td>PBI</td>
<td>121</td>
<td>89%</td>
</tr>
<tr>
<td>Antivenom</td>
<td>126</td>
<td>93%</td>
</tr>
<tr>
<td>Snake Handler</td>
<td>10</td>
<td>7%</td>
</tr>
<tr>
<td>Alcohol involved</td>
<td>8</td>
<td>6%</td>
</tr>
<tr>
<td>Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gardening/outdoor work</td>
<td>40</td>
<td>29%</td>
</tr>
<tr>
<td>Intentionally interacting with snake</td>
<td>41</td>
<td>30%</td>
</tr>
<tr>
<td>Bush/walking/playing</td>
<td>45</td>
<td>33%</td>
</tr>
<tr>
<td>Indoors</td>
<td>8</td>
<td>6%</td>
</tr>
</tbody>
</table>
Distribution

Legend
- Envenomed
- Non-envenomed
# Clinical Effects

- **Local:** Minimal; often no mark and no pain
- **Venom induced consumption coagulopathy:** 100%
  - partial in 20%
- **Systemic symptoms (N,H,V):** 45%
- **Neurotoxicity:** 1%
- **Myotoxicity:** Nil
- **Haemorrhage (major):** 4%
- **Hypotensive collapse:** 27%
- **Thrombotic microangiopathy:** 9%
  - Renal failure 8%
# Clinical effects: Deaths

<table>
<thead>
<tr>
<th>Sex/Age</th>
<th>Collapse Onset (min)</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>M43</td>
<td>Few minutes</td>
<td>Bitten at desk. Early cardiac arrest, CPR by family.</td>
</tr>
<tr>
<td>M20</td>
<td>????</td>
<td>Found unconscious after running; very hot day; hyperthermic</td>
</tr>
<tr>
<td>F61</td>
<td>40 min</td>
<td>Bitten while gardening, chest pain, seizure and asystolic arrest</td>
</tr>
<tr>
<td>M16</td>
<td>????</td>
<td>Picked up snake in bush. Found collapsed, asystolic, no CPR by bystanders.</td>
</tr>
<tr>
<td>F10</td>
<td>Few minutes</td>
<td>Walking in garden, collapsed, CPR by family</td>
</tr>
<tr>
<td>F69</td>
<td>None</td>
<td>Headache, severe uncontrolled hypertension, drowsy 17hrs post bite, CT: large cerebral haemorrhage</td>
</tr>
</tbody>
</table>
## Coagulopathy and Bleeding

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VICC</strong></td>
<td>Complete</td>
<td>109</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>Partial</td>
<td>27</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Bleeding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major haemorrhage</td>
<td></td>
<td>6</td>
<td>4%</td>
</tr>
<tr>
<td>Intracranial Haemorrhage</td>
<td></td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>Gastrointestinal Haemorrhage</td>
<td></td>
<td>4</td>
<td>3%</td>
</tr>
<tr>
<td>Minor Haemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bite site</td>
<td></td>
<td>32</td>
<td>24%</td>
</tr>
<tr>
<td>Intravenous cannula site</td>
<td></td>
<td>43</td>
<td>32%</td>
</tr>
<tr>
<td>Gum bleeding</td>
<td></td>
<td>19</td>
<td>14%</td>
</tr>
</tbody>
</table>
Cardiovascular effects

- **Hypotensive collapse** 37 (27%)
  - Onset in 19 patients; median 30 min (2 to 90)

- **Associated Effects**
  - **Cardiac arrest** 7 (5%)
    - 4 fatal asystolic cardiac arrests
  - **Seizures** 8 (6%)
    - Hypotensive prior to seizure in 7 cases
  - **Abnormal troponin**
    - median 0.7 (0.1 to 8) n=12
sVDK results

- 117 bite sites tested
  - 98 positive (84%)
  - 18 negative (10 positive urine)
  - 1 conclusive
  - No other snakes

- 19 not tested:
  - Washed bite site
  - Urine done
Venom concentrations

- 131 pre-AV samples available
  - Median venom concentration 1.6 ng/mL
    (IQR: 0.6 to 5; 0.15 to 200)
Antivenom treatment

- 114 received AV (median dose 2 vials, IQR 2-4)
- 22 received 1 vial BSAV:
  - No difference in outcome
  - No deaths
  - 29% collapse
  - Similar recovery in VICC
- No severe AV reactions
  - 4 moderate reactions
  - 15 mild reactions
Venom concentrations

- 131 pre-AV samples available
  - Median venom concentration 1.6 ng/mL
    (IQR: 0.6 to 5; 0.15 to 200)
Recovery of VICC

Hours until INR < 2 vs. antivenom dose (vials)

Proportion recovered vs. Hours after envenomation

Lines represent different doses of antivenom:
- 3+ Vials
- 2 Vials
- 1 Vial
Antivenom – venom concentrations

- 115 patients with post-antivenom samples
  - 112 no venom detected
    - 20 given 1 vial of antivenom
  - 3 with low venom concentrations (0.4 to 0.9 ng/mL)
    - Low antivenom concentrations
    - 2 given 1 vial
    - 1 given 2 vials
    - ? Bound venom

Neutralisation

Increased Elimination
Conclusions

- Brown snake envenoming more severe
- Characterised by:
  - VICC, mainly complete
    - Major haemorrhage occurs, but rare
  - Hypotensive collapse common
    - Associated with cardiac arrest and death
  - Neurotoxicity, mild and rare
- Treatment:
  - 1 vial sufficient to treat brown snake envenoming
- Venom concentrations: very low
**ASP investigators**

Geoff Isbister  Simon Brown
Nick Buckley  Bart Currie
Julian White  Colin Page
David Spain  Alan Tankel
Ovidu Pascui  Helen Mead
Richard Whitaker  Peter Garrett
Mark Miller  Anna Holdgate
Randall Greenberg  Yusuf Nagree
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**Acknowledgements**

Photographs: Gunther Schmida

http\:\:\:\: star.ferntree.com
## Clinical Effects

<table>
<thead>
<tr>
<th>Clinical Syndrome/Effects</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>VICC</td>
<td>136</td>
<td>100%</td>
</tr>
<tr>
<td>Major haemorrhage</td>
<td>6</td>
<td>4%</td>
</tr>
<tr>
<td>Neurotoxicity (mild)</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>Myotoxicity</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Systemic Symptoms</td>
<td>61</td>
<td>45%</td>
</tr>
<tr>
<td>Local Effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early collapse/hypotension</td>
<td>37</td>
<td>27%</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>6</td>
<td>4%</td>
</tr>
<tr>
<td>Thrombotic microangiopathy</td>
<td>12</td>
<td>9%</td>
</tr>
</tbody>
</table>
Distribution

Legend
- Envenomed
- Non-envenomed

*P. textilis*
*P. nuchalis*
*P. affinis*
*P. mengdeni*
*P. modesta*