Species-Dependent Variations in the in Vitro Myotoxicity of Death Adder (Acanthophis) Venoms

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Based on early studies on Acanthophis antarcticus (common death adder) venom, it has long been thought that death adder snake venoms are devoid of myotoxicity. However, a recent clinical study reported rhabdomyolysis in patients following death adder envenomations, in Papua New Guinea, by a species thought to be different to A. antarcticus. Subsequently, a myotoxic phospholipase A2 component was isolated from A. rugosus (Irian Jayan death adder) venom. The present study examined the venoms of A. praelongus (northern), A. pyrrhus (desert), A. hawkei (Barkly Tableland), A. wellsi (black head), A. rugosus, A. sp. Seram and the regional variants of A. antarcticus for in vitro myotoxicity. Venoms (10–50 μg/ml) were examined for myotoxicity using the chick directly (0.1 Hz, 2 ms, supramaximal V) stimulated biventer cervicis nerve-muscle preparation. A significant contracture of skeletal muscle and/or inhibition of direct twitches were considered signs of myotoxicity. This was confirmed by histological examination. All venoms displayed high phospholipase A2 activity. The venoms (10–50 μg/ml) of A. sp. Seram, A. praelongus, A. rugosus, and A. wellsi caused a significant inhibition of direct twitches and an increase in baseline tension compared to the vehicle (n = 4–6; two-way ANOVA, p < 0.05). Furthermore, these venoms caused dose-dependent morphological changes in skeletal muscle. In contrast, the venoms (10–50 μg/ml; n = 3–6) of A. hawkei, A. pyrrhus, and regional variants of A. antarcticus were devoid of myotoxicity. Prior incubation (10 min) of CSL death adder antivenom (5 U/ml) prevented the myotoxicity caused by A. sp. Seram, A. praelongus, A. rugosus, and A. wellsi venoms (50 μg/ml; n = 4–7). In conclusion, clinicians may need to be mindful of possible myotoxicity following envenomations by A. praelongus, A. rugosus, A. sp. Seram, and A. wellsi species.

Key Words: Acanthophis; A. antarcticus; antivenom; death adder; myotoxic; phospholipase A2; A. praelongus; rhabdomyolysis; A. rugosus; venom.

Death adders (genus Acanthophis) are unique among Australian snakes in both morphology and behavior. Although classified into the Elapidae family of snakes they are viper-like in appearance and habit (Campbell, 1966; Cogger, 2000). Death adders are the widest ranging of the Australian elapids being found not only in continental Australia, but north throughout the Torres Straight Islands, Papua New Guinea, Irian Jaya, and the Indonesian islands of Seram, Halmahera, Obi, and Timor. Although up to 12 species and 3 subspecies of death adders have been described thus far (Hoser, 1998), considerable debate remains about species identification (Wuster et al., 1999). Of these, only the venom of the common (A. antarcticus) death adder has been studied in detail.

Acanthophis antarcticus venom has previously been examined for lethality, neurotoxicity, myotoxicity, and its effects on blood coagulation, both experimentally and clinically (Broad et al., 1979; Campbell, 1966; Kellaway, 1929a,b; Mebs and Samejima, 1980; Sutherland et al., 1981; Wickramaratna and Hodgson, 2001). In addition, five postsynaptic neurotoxins and four phospholipase A2 (PLA2) components have been isolated and sequenced from A. antarcticus venom (Chow et al., 1998; Kim and Tamiya, 1981a,b; Sheumack et al., 1979, 1990; Tyler et al., 1997; van der Weyden et al., 1997). However, no myotoxic components have been isolated from this venom.

Previously, using the chick isolated biventer cervicis nerve-muscle (CBCNM) preparation, we studied the venoms of the northern (A. praelongus), desert (A. pyrrhus), Barkly Tableland (A. hawkei), black head (A. wellsi), Irian Jaya (A. rugosus), and A. sp. Seram for in vitro neurotoxicity (Fry et al., 2001). All venoms (1–10 μg/ml) caused dose-dependent neurotoxicity, which was postsynaptic in nature. In the same study, CSL death adder antivenom (1 U/ml), which is raised against A. antarcticus venom, prevented the neurotoxic effects of A. pyrrhus, A. praelongus, and A. hawkei venoms. However, it was markedly less effective against the venoms of A. rugosus, A. wellsi, and A. sp. Seram (Fry et al., 2001). At a higher concentration, antivenom (5 U/ml) was effective against all venoms. In another study, the venoms of major species and regional variants of death adders were investigated by liquid chromatography/mass spectrometry (Fry et al., 2002). This study revealed a great diversity in venom composition.

Based on early studies on A. antarcticus venom it was thought that death adder venoms were devoid of myotoxic
activity (Sutherland et al., 1981). A. antarcticus venom displayed no myotoxic activity in vivo in Rhesus monkeys (Macaca fascicularis; Sutherland et al., 1981). In another study, Mebs and Samejima (1980) fractionated A. antarcticus venom by ion-exchange chromatography. None of the isolated fractions caused myoglobinuria in mice after sc injection. However, a clinical study reported myotoxic activity following injections caused myoglobinuria in mice after sc injection. How-

by ion-exchange chromatography. None of the isolated frac-

tions (Sutherland et al., 1994) using a Grass S88 stimulator. After a 30-min equilibration period, to
ensure selective stimulation of muscle, d-tubocurarine (10 μM) was added and left in the organ bath for the duration of the experiment. Death adder venoms (10–50 μg/ml), 4-BPB modified A. rugosus, A. sp. Serum, and A. praelongus venoms (50 μg/ml) or relevant controls were left in contact with the preparations for a 3 h period. A significant contracture of skeletal muscle (i.e., a rise in baseline) and/or inhibition of direct twitches were considered signs of myotoxicity (Harvey et al., 1994). Where indicated, CSL death adder antivenom, which has been raised against A. antarcticus venom, in neutralizing the myotoxic activity of A. rugosus venom.

The first aim of this study was to examine the venoms of A. praelongus, A. pyrrhus, A. hawkei, A. wellsi, A. sp. Serum, and the regional variants of A. antarcticus for in vitro myotoxic activity. The second was to determine the effectiveness of CSL death adder antivenom in neutralizing the myotoxic activity of death adder venoms.

MATERIALS AND METHODS

Venom preparation and storage. A. antarcticus venoms were obtained from populations in New South Wales (NSW), Queensland (Qld), South Australia (SA), and Western Australia (WA). A. praelongus venom was from populations in Cairns, Queensland; A. pyrrhus venom from Alice Springs, Northern Territory; A. wellsi venom from the Pilbarra region of Western Australia; A. hawkei venom from the Barkly Tableland region of Northern Territory; A. rugosus venom from Irian Jaya (West Papua), and A. sp. Serum from the island of Seram, Indonesia. Venoms were either purchased from Venom Supplies Pty. Ltd., South Australia, or milked from specimens caught by Dr. Bryan Fry. For each geographic variant or species, venoms were collected and pooled to minimize the effects of individual variations (Chippaux et al., 1991). Freeze-dried venoms and stock solutions of venoms prepared in 0.1% bovine serum albumin (BSA) in 0.9% saline were stored at −20°C until required.

Determination of phospholipase A, activity. The PLA₂ activity of death adder venoms was determined using a secretory PLA₂ colorimetric assay kit (Cayman Chemical, Ann Arbor, MI). The assay uses the 1,2-dithio analogue of diheptanoyl phosphatidylcholine as a substrate. Free thiols generated upon hydrolysis of the thio ester bond at the C-4 position by PLA₂ are detected by DTNB (5,5'-dithiobis(2-nitrobenzoic acid)). Color changes were moni-
tored by the CERES9000 microplate reader (Bio-Tek Instruments, Winooski, VT) at 405 nm, sampling every min for a 5 min period. PLA₂ activity was expressed as micromoles of phosphatidylcholine hydrolysed per min per mg of enzyme.

Inactivation of PLA₂ activity with 4-bromophenacyl bromide. The PLA₂ activity of A. rugosus, A. sp. Serum, and A. praelongus venoms were inhibited by alkylation with 4-bromophenacyl bromide (4-BPB). A. rugosus, A. sp. Serum, and A. praelongus venoms (10,000 μg/ml) were made up in sodium cacodylate-HCl buffer (25 μl, 0.1 M, pH 6.0), and 4-BPB made up in acetone was added to give a final concentration of 1.8 mM (Abe et al., 1977; Bell et al., 1998; Crachi et al., 1999b). Each vial containing the above solution was then incubated at 30°C for 16 h. As a positive control, A. rugosus, A. sp. Serum, and A. praelongus venoms (10,000 μg/ml) made up in sodium cacodylate-HCl buffer were incubated with acetone. As a negative control, sodium cacodylate-HCl buffer was incubated with 1.8 mM 4-BPB in acetone.

Chick isolated biventer cervicis nerve-muscle preparation. Male White leghorn chicks aged between 9 and 11 days were killed with CO₂ and both biventer cervicis nerve-muscle preparations were removed. These were mounted under 1 g resting tension in organ baths (5 ml) containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25; and glucose, 11.1. The Krebs solution was bubbled with carbogen (95% O₂ and 5% CO₂) and maintained at 34°C. Direct twitches were evoked by stimulating the muscle directly every 10 s with pulses of 2 ms duration at a supramaximal voltage (Harvey et al., 1994) using a Grass S88 stimulator. After a 30-min equilibration period, to ensure selective stimulation of muscle, d-tubocurarine (10 μM) was added and left in the organ bath for the duration of the experiment. Death adder venoms (10–50 μg/ml), 4-BPB modified A. rugosus, A. sp. Serum, and A. praelongus venoms (50 μg/ml) or relevant controls were left in contact with the preparations for a 3 h period. A significant contracture of skeletal muscle (i.e., a rise in baseline) and/or inhibition of direct twitches were considered signs of myotoxicity (Harvey et al., 1994). Where indicated, CSL death adder antivenom, which has been raised against A. antarcticus venom, in neutralizing the myotoxic activity of death adder venoms.

Morphological studies. After the conclusion of the functional myotoxic experiments, the tissues were quickly placed in Tissue Tek and frozen with liquid nitrogen. The tissues were stored at −80°C until required. Using a Leica CM1800 cryostat, tissues were cut into transverse sections (14 μm) and placed onto gelatin-coated slides. Tissue sections were post fixed for 15 min in a solution containing 4% paraformaldehyde in phosphate buffered saline (PBS; [mol/l] NaCl, 0.137; KH₂PO₄, 0.002; and Na₂HPO₄, 0.008). Tissue sections were routinely stained with haematoxylin and eosin, and examined under a light microscope (Olympus BX 51, Olympus Optical Co., Japan). Areas exhibiting typical pathological changes were photographed using an Olympus C-4040ZOOM (Olympus Optical Co., Japan) digital camera.

Chemicals and drugs. The following drugs and chemicals were used: 4-bromophenacyl bromide (4-BPB), BSA, cacodylic acid (sodium cacodylate), d-tubocurarine chloride, eosin, Mayer’s Hematoxylin solution (Sigma Chemical Co., St. Louis, MO). Except where indicated, stock solutions were made up in distilled water. 4-BPB was made up in acetic acid. Death adder antivenom, which is raised against A. antarcticus venom in horses, was obtained from CSL Ltd. (Melbourne, Australia).

Analysis of results and statistics. For isolated tissue experiments, re-
sponses were measured via a Grass force displacement transducer (FT03) and recorded on a MacLab System. The twitch height was expressed as a percent-
age of the initial twitch height (i.e., prior to the addition of venom or vehicle). Full data (i.e., response curves over a 3 h period) are shown for twitch height and baseline tension at 50 μg/ml venoms. However, for brevity full data are not shown at venom concentrations of 10 μg/ml and 30 μg/ml. Instead, data for all venom concentrations are summarized in Figures 2a and 2b using only the twitch height and baseline tension values at the 180-min time point. Statistical difference was determined by a two-way ANOVA on the twitch heights, at the 180-min time point, at different concentrations of venoms. Likewise, a two-way ANOVA was performed on the contractile responses induced by the venoms at different concentrations at the 180-min time point (i.e., only the data at the 180-min time point have been statistically analyzed). For 4-BPB modified venom studies, statistical difference was determined by a two-way ANOVA on the data at the 180-min time point. Statistical difference between the PLA₂ activity of 4-BPB treated and untreated venom was deter-
mained by a Student’s unpaired t-test. All ANOVAs were followed by a
Bonferroni-corrected multiple t-test. Statistical significance was indicated when $p < 0.05$. All statistical tests were carried out using the SigmaStat (ver. 1.0) software package.

**RESULTS**

**Phospholipase A$_2$ Activity**

High PLA$_2$ activity was detected in all death adder venoms (Table 1). While there was a large variation in the PLA$_2$ activity of death adder venoms, *A. pyrrhus* venom had the highest specific activity, 476.4 ± 12.4 μmol/min/mg ($n = 12$). The positive control, bee venom PLA$_2$, had a specific activity of 287.5 ± 17.5 μmol/min/mg ($n = 4$). 4-BPB treated *A. rugosus*, *A. sp. Seram*, and *A. praelongus* venoms had significantly reduced PLA$_2$ activities of 2.0 ± 0.9, 1.7 ± 1.1, and 1.8 ± 1.0 μmol/min/mg ($n = 8$) compared to their untreated venoms with specific activities of 140.2 ± 10.4, 420.4 ± 10.8, and 255.0 ± 8.6 μmol/min/mg, respectively ($n = 6–8$; Student’s unpaired t-test, $p < 0.05$).

**Chick Isolated Directly-Stimulated Biventer Cervicis Nerve-Muscle Preparation**

**Myotoxic studies.** The venoms (10–50 μg/ml) of *A. antarcticus* (NSW, Qld, SA, WA), *A. hawkei*, *A. pyrrhus*, and *A. wellsi* had no significant inhibitory effect on the direct twitches compared to the vehicle ($n = 3–6$; two-way ANOVA, $p < 0.0001$; Figs. 1a and 2a). In contrast, *A. sp. Seram* venom (10–50 μg/ml) caused a significant inhibition of direct twitches compared to the vehicle ($n = 4–6$; Figs. 1a and 2a). However, this effect was not concentration-dependent as there was no significant difference in the twitch inhibition caused by *A. sp. Seram* venom at 10 μg/ml and 50 μg/ml ($n = 4$; Fig. 2a). Both *A. praelongus* venom (30–50 μg/ml) and *A. rugosus* venom (30–50 μg/ml) caused a significant inhibition of direct twitches compared to the vehicle ($n = 4–6$; Figs. 1a and 2a). This effect was concentration-dependent with *A. praelongus* (50 μg/ml) and *A. rugosus* (50 μg/ml) venoms causing a significantly greater inhibition of direct twitches compared to *A. praelongus* and *A. rugosus* venom at 10 μg/ml, respectively ($n = 5–6$; Fig. 2a). When taking all concentrations into consideration *A. sp. Seram* venom was significantly more potent in causing direct twitch inhibition than either *A. praelongus* venom or *A. rugosus* venom ($n = 4–6$). In contrast, there was no significant difference between *A. praelongus* venom and *A. rugosus* venom ($n = 5–6$; Fig. 2a).

The venoms (10–50 μg/ml) of *A. antarcticus* (NSW, Qld, SA, WA), *A. hawkei*, and *A. pyrrhus* had no significant effect on the baseline tension compared to the vehicle ($n = 3–6$; two-way ANOVA, $p < 0.0001$; Figs. 1b and 2b). While *A. wellsi* venom (10–30 μg/ml) had no significant effect on the baseline tension, *A. wellsi* venom (50 μg/ml) induced a significant increase in baseline tension compared to the vehicle ($n = 4–6$). The venoms (10–50 μg/ml) of *A. sp. Seram*, *A. praelongus*

![FIG. 1. The effect of Acanthophis venoms (10–50 μg/ml; $n = 3–6$) or vehicle ($n = 6$) on (a) direct twitches or (b) baseline tension of the CBCNM preparation at the 180-min time point. *$p < 0.05$, significantly different from vehicle, two-way ANOVA.](https://academic.oup.com/toxsci/article-abstract/74/2/352/1716334/2)
gus, and A. rugosus induced a significant increase in baseline tension compared to the vehicle (n = 4 – 6). However, there was no significant difference in the baseline contraction caused by A. sp. Seram venom at 10 μg/ml and 50 μg/ml (n = 4; Fig. 2b). This was also the case with A. praelongus and A. rugosus venoms.

**Antivenom studies.** Prior incubation (10 min) of CSL death adder antivenom (5 U/ml) prevented the inhibition of direct twitches and the increase in baseline tension caused by A. sp. Seram, A. praelongus, A. rugosus, and A. wellsi venoms (50 μg/ml; n = 4 – 7; Figs. 3a,b). A. sp. Seram, A. praelongus, A. rugosus, and A. wellsi venoms (50 μg/ml) in the presence of antivenom (5 U/ml) had no significant inhibitory effect on the direct twitches compared to the antivenom control (n = 4 – 7; Fig. 3a; one-way ANOVA, p = 0.49). Furthermore, A. sp. Seram, A. praelongus, A. rugosus, and A. wellsi venoms (50 μg/ml) in the presence of antivenom (5 U/ml) had no significant effect on the baseline tension compared to the antivenom control (n = 4 – 7; Fig. 3b; one-way ANOVA, p = 0.40).

**4-BPB modified venom studies.** A. rugosus, A. sp. Seram, and A. praelongus venoms (50 μg/ml) incubated with 4-BPB had no significant inhibitory effect on direct twitches compared to 4-BPB plus vehicle (n = 4 – 6; two-way ANOVA, p < 0.0001; Fig. 4a). However, A. rugosus A. sp. Seram, and A. praelongus venoms (50 μg/ml) incubated with vehicle (ace-
and cellular infiltrate. In contrast, tissues exposed to \textit{A. antarcticus} (NSW, Qld, SA, WA), \textit{A. hawkei}, and \textit{A. pyrrhus} venoms (10–50 µg/ml) were similar in morphology to the vehicle control tissues (Fig. 5f; data not shown for other venoms). Prior incubation of CSL death adder antivenom (5 U/ml) prevented most of the morphological changes from occurring due to \textit{A. sp. Seram}, \textit{A. praelongus}, \textit{A. rugosus}, and \textit{A. wellsi} venoms. In the case of \textit{A. rugosus} venom (50 µg/ml) a few vacuoles were evident in some tissues even in the presence of antivenom (5 U/ml; Fig. 5g). There were no detectable morphological changes in tissues equilibrated with antivenom alone (data not shown). \textit{A. rugosus}, \textit{A. sp. Seram}, and \textit{A. praelongus} venoms (50 µg/ml) incubated with vehicle (i.e., acetone) induced morphological changes similar to the corresponding venom (50 µg/ml). However, no detectable morphological changes were seen in tissues exposed to \textit{A. rugosus} (Fig. 5h). \textit{A. sp. Seram} and \textit{A. praelongus} venoms (50 µg/ml) incubated with 4-BPB or vehicle incubated with 4-BPB (data not shown).

**DISCUSSION**

Based on earlier studies on \textit{A. antarcticus} venom it was thought that death adder venoms were devoid of myotoxic activity (Mebs and Samejima, 1980; Sutherland et al., 1981). However, a recent clinical study reported evidence of rhabdomyolysis in patients following death adder envenomations, in Papua New Guinea, by a species different to \textit{A. antarcticus} (Lalloo et al., 1996). More recently, a myotoxic PLA$_2$ from \textit{A. rugosus} venom was isolated (Wickramaratna et al., 2003). Consequently, the present study examined the venoms of \textit{A. praelongus}, \textit{A. pyrrhus}, \textit{A. hawkei}, \textit{A. wellsi}, \textit{A. sp. Seram}, and the regional variants of \textit{A. antarcticus} for \textit{in vitro} myotoxic activity. In addition, this study examined the effectiveness of CSL death adder antivenom in neutralizing the myotoxic activity of death adder venoms.

Death adder venoms were examined for \textit{in vitro} myotoxicity using the directly stimulated CBCNM preparation. \textit{A. antarcticus} (NSW, Qld, SA, WA), \textit{A. hawkei}, and \textit{A. pyrrhus} venoms did not cause a significant inhibition of the direct twitch height or an increase in the baseline tension. Furthermore, light microscopy studies indicated that tissues treated with these venoms had morphology similar to vehicle control tissues. Thus, these studies have shown that \textit{A. antarcticus} (NSW, Qld, SA, WA), \textit{A. hawkei}, and \textit{A. pyrrhus} venoms are devoid of \textit{in vitro} myotoxic activity. While several previous studies have shown that \textit{A. antarcticus} venom is devoid of myotoxic activity none have examined the regional variations of this venom (Mebs and Samejima, 1980; Sutherland et al., 1981; Wickramaratna and Hodgson, 2001). Liquid chromatography–mass spectrometry studies have shown variations in venom composition among the venoms of \textit{A. antarcticus} regional variants (Fry et al., 2001, 2002). Furthermore, functional studies have shown variations in neurotoxicity among the venoms of \textit{A. antarcticus} regional variants (Fry et al., 2001).
FIG. 5. Transverse sections of CBCNM preparations exposed to (a) vehicle (BSA); (b) *A. rugosus* venom (10 µg/ml); (c) *A. rugosus* venom (50 µg/ml); (d) *A. sp. Seram* venom (50 µg/ml); (e) *A. wellsi* venom (50 µg/ml); (f) *A. antarcticus* venom (WA; 50 µg/ml); (g) *A. rugosus* venom (50 µg/ml) in the presence of antivenom (5 U/ml); (h) *A. rugosus* venom (50 µg/ml) incubated with 4-BPB (1.8 mM). Scale bars, 100 µm in all micrographs. Arrows indicate prominent vacuoles; arrowheads indicate necrotic cells; double arrows indicate edema; double arrowheads indicate cellular infiltrate.
Although *A. wellsi* venom had no effect on the direct twitch height it induced a dose-dependent increase in baseline tension. At the higher concentration, *A. wellsi* venom also caused morphological changes in skeletal muscle. Thus, suggesting that at higher concentrations this venom causes in *vitro* myotoxic activity. Both *A. praelongus* and *A. rugosus* venoms caused concentration-dependent inhibition of direct twitches, and an increase in baseline tension. Inhibition of direct twitches and a rise in baseline tension have been postulated to be indicative of myotoxic activity (Harvey et al., 1994). Light microscopy studies showed that tissues exposed to *A. praelongus* and *A. rugosus* venoms caused dose-dependent morphological changes. Although we have previously shown that *A. rugosus* venom causes in *vitro* myotoxic activity (Wickramaratna et al., 2003), this venom was included in the present study to allow for a comparison between venoms. In contrast to this study, a previous study showed that *A. praelongus* venom at 30 µg/ml did not cause a significant inhibition of direct twitches compared to the vehicle control (Wickramaratna and Hodgson, 2001). However, in that study the venom did cause a significant increase in baseline tension (Wickramaratna and Hodgson, 2001). This previous study neither examined a higher concentration of *A. praelongus* venom nor the morphology of exposed tissues. The use of younger chicks in the previous study may have contributed to this variability between the two studies (Harris, 1991).

At all concentrations tested, *A. sp.* Serum venom caused a significant inhibition of direct twitches and an increase in baseline tension. However, the twitch inhibition and the increase in baseline tension were not dose-dependent. Perhaps, had lower concentrations been tested, a dose-dependent effect may have been observed. Morphological studies however, showed dose-dependent skeletal muscle changes in tissues exposed to *A. sp.* Serum venom. Clearly, of all death adder venoms tested, *A. sp.* Serum venom was the most myotoxic.

While death adder envenomations have been uncommon in Australia in recent times due to habitat destruction and consequent decimation of populations, they are still significant health problem in Papua New Guinea and Irian Jaya (Currie, 2000; Currie et al., 2001; Laloo et al., 1995, 1996; Sutherland, 1992). CSL death adder antivenom is the principal therapy for envenomation by any death adder species (AMH, 2003; White, 1998). Since *A. antarcticus* venom lacks myotoxic activity, and given that death adder antivenom has been raised against *A. antarcticus* venom, it was of clinical relevance to examine the efficacy of death adder antivenom against the *in vitro* myotoxicity of *A. praelongus*, *A. rugosus*, *A. sp.* Serum, and *A. wellsi* venoms. Prior incubation of antivenom totally prevented the inhibition of direct twitches and the increase in baseline tension caused by *A. praelongus*, *A. rugosus*, *A. sp.* Serum, and *A. wellsi* venoms. In addition, antivenom prevented most of the morphological changes from occurring due to these venoms. Therefore, CSL death adder antivenom is effective in neutralizing the *in vitro* myotoxic activity of death adder venoms. Previously, we have shown that death adder antivenom was effective in neutralizing the *in vitro* myotoxic activity of acanmyotoxin-1 (Wickramaratna et al., 2003).

Since the most important clinical symptoms of death adder envenomations are due to postsynaptic neurotoxicity, anticholinesterase therapy has been suggested to supplement death adder antivenom (Currie et al., 1988). Indeed, several clinicians have used anticholinesterases successfully to reduce the amount of antivenom administered (Currie et al., 1988; Laloo et al., 1996; Little and Pereira, 2000). Anticholinesterase therapy has proven especially useful in Papua New Guinea and Irian Jaya to reduce the high costs associated with the use of death adder antivenom (Currie, 2000). However, given the results of the present study, clinicians may need to be mindful of possible myotoxicity following envenomations from *A. praelongus*, *A. rugosus*, *A. sp.* Serum, and *A. wellsi* species. With concomitant anticholinesterase therapy the neurotoxicity of death adder envenomations may resolve, however, unchecked myotoxicity could cause myoglobinuria and then renal failure.

Previously it was shown that acanmyotoxin-1, a myotoxic component from *A. rugosus* venom, contained high PLA2 activity (Wickramaratna et al., 2003). Studies have also shown that myotoxic fractions from other Australian elapid venoms contain PLA2 activity (Harris and MacDonell, 1981; Mebs and Samejima, 1980). Liquid chromatography–mass spectrometry studies have shown that death adder venoms contain numerous components with molecular weights representative of PLA2s (Hodgson et al., 2001). Therefore, death adder venoms were examined for PLA2 activity. While high PLA2 activity was detected in all death adder venoms, *A. pyrrhus* venom had the highest specific activity. In order to examine whether the PLA2 activity of *A. rugosus*, *A. sp.* Serum, and *A. praelongus* venoms is necessary for the myotoxic action, these venoms were subjected to 4-BPB modification. Although a myotoxic PLA2 component has previously been isolated from *A. rugosus* venom this venom was subjected to 4-BPB modification to determine the presence of other components that may cause myotoxicity but are not mediated by PLA2 activity. Studies have shown that PLA2 activity can be inhibited by acylation using 4-BPB (Abe et al., 1977; Volwerk et al., 1974). 4-BPB treated *A. rugosus*, *A. sp.* Serum, and *A. praelongus* venoms had significantly reduced PLA2 activity and no myotoxic activity. Thus, suggesting that PLA2 activity is necessary for the myotoxic activity of these death adder venoms. However, no direct relationship was found between the degree of PLA2 activity and the myotoxic activity of death adder venoms. For example, while *A. pyrrhus* venom had the highest PLA2 activity it was devoid of myotoxic activity. This suggests the presence of other non-myotoxic PLA2 components in those non-myotoxic death adder venoms. In fact, several PLA2 components with antiplatelet activity have been isolated from *A. antarcticus* and *A. praelongus* venoms (Chow et al., 1998; Sim, 1998). Similarly, it is possible that other non-myotoxic...
PLA₂ components may also contribute to the PLA₂ activity of myotoxic death adder venoms.

In conclusion, A. sp. Serum, A. praelongus, A. rugosus, and A. wellsi venoms caused in vitro myotoxicity in the CBCNM preparation. In contrast, A. antarcticus (NSW, Qld, SA, WA), A. hawkei, and A. pyrhhias venoms were devoid of myotoxic activity. Although CSL death adder antivenom has been raised against A. antarcticus venom it is effective in neutralizing the myotoxic activity of A. praelongus, A. rugosus, A. sp. Serum, and A. wellsi venoms. Given the results of this study clinicians need to be mindful of possible myotoxicity following envenomations by A. praelongus, A. rugosus, A. sp. Serum, and A. wellsi death adder species.

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