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An examination of cardiovascular collapse induced by eastern brown snake (*Pseudonaja textilis*) venom

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HIGHLIGHTS

- Brown snake-induced early cardiovascular collapse is a cause of mortality following snake envenoming in Australia.
- Brown snake venom produces cardiovascular collapse in anaesthetized rats.
- This is prevented by prior administration of small priming doses of venom from snakes from a different family or genus.
- A commercial polyvalent antivenom inhibits this effect.

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ABSTRACT

The *Pseudonaja* genus (Brown snakes) is widely distributed across Australia and bites account for significant mortality. Venom-induced consumption coagulopathy (VICC) and, less often, early cardiovascular collapse occur following envenoming by these snakes. We have previously examined possible mechanism(s) behind the early cardiovascular collapse following Papuan taipan (*Oxyuranus scutellatus*) envenoming. In the present study, we investigate early cardiovascular collapse in anaesthetized rats following administration of eastern brown snake (*Pseudonaja textilis*) venom, and prevention of this effect with prior administration of 'priming' doses (i.e. doses of venom which caused a transient hypotensive response) of venom. *P. textilis* venom (5–10 μg/kg, i.v.) induced cardiovascular collapse in anaesthetized rats, characterized by a rapid decrease in systolic blood pressure until non recordable. Prior administration of 'priming' doses of *P. textilis* venom (2 and 3 μg/kg) or, at least, 4–5 doses of *O. scutellatus* (2 μg/kg, i.v.) or *Daboia russelii limitis* (20 μg/kg, i.v.) venoms prevented cardiovascular collapse induced by *P. textilis* venom. Moreover, early collapse was also inhibited by prior administration of 2 discrete doses of *Acanthophis rugosus* venom. Prior administration of commercial polyvalent snake antivenom (500–3000 units/kg, i.v.) or heparin (300 units/kg, i.v.) also inhibited *P. textilis* venom-induced cardiovascular collapse. Our results indicate that *P. textilis* venom-induced cardiovascular collapse can be prevented by prior administration of sub-lethal doses of venom from *P. textilis*, *O. scutellatus*, *A. rugosus* and *D. russelii limitis*. This suggests that sudden cardiovascular collapse following envenoming is likely to involve a common mechanism/pathway activated by different snake venoms.

1. Introduction

Envenoming by snakes of genus *Pseudonaja* (brown snakes) is a significant cause of morbidity and mortality in Australia (Allen et al., 2012). Clinical symptoms following envenoming by *Pseudonaja* spp. include venom-induced consumption coagulopathy (VICC) and sudden cardiovascular collapse, with neurotoxicity being rare (Allen et al., 2012; Barber et al., 2012) despite the presence of a pre-synaptic neurotoxin, textilotoxin (Barber et al., 2012). This peculiarity of brown snake envenoming, often referred to as the 'Brown snake paradox', appears to be due to the small amount of

Abbreviations: ANOVA, analysis of variance; i.v., intravenous administration; i.p., intraperitoneal administration; MAP, mean arterial pressure; PVDF, polyvinylidene difluoride; HRP, horseradish peroxidase; TBST, Tris-buffered saline and Tween 20.

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pre-synaptic neurotoxin in the whole venom and the relative low potency of this toxin (Barber et al., 2012).

A recent study has shown that about one quarter of patients with brown snake envenoming have an early collapse or hypotension, with cardiac arrest and death occurring in 6% of cases (Allen et al., 2012). The early cardiovascular collapse often occurs in the pre-hospital setting and appears to respond to basic and advanced life support (Allen et al., 2012; Johnston et al., 2002).

A number of toxins have been isolated and characterized from *Pseudonaja* spp. venoms. These include pre- (Su et al., 1983) and post- (Tyler et al., 1987) synaptic neurotoxins, phospholipase A_2 (PLA_2) components, and prothrombin activators (Rao and Kini, 2002; Stocker et al., 1994). Prothrombin activators promote the conversion of prothrombin to thrombin leading to consumption of clotting factors (fibrinogen, factor V and factor VIII) which can result in serious hemorrhage including life-threatening intracranial hemorrhage (Isbister et al., 2010).

Administration of brown snake or tiger snake (*Notechis* sp.) prothrombin activators, to anaesthetized dogs, caused severe depression of systolic blood pressure and cardiac output which was suggested to be due to thrombotic obstruction of the pulmonary vasculature (Tibballs, 1998; Tibballs et al., 1992). Recently, we have shown that administration of Papuan taipan (*O. scutellatus*) venom caused cardiovascular collapse and prior administration of small priming (i.e. hypotensive) doses of venom 'protected' rats from the effects of a subsequent larger dose which would otherwise cause cardiovascular collapse if given alone (Chaisakul et al., 2012).

Antivenom is the major treatment for systemically envenomed patients although studies have questioned the *in vivo* effectiveness (Isbister et al., 2009) and *in vitro* (Judge et al., 2006) efficacy of brown snake antivenom. Large quantities and multiple administrations of antivenom have been recommended to neutralize cardiovascular depression and coagulopathy following brown snake envenoming (Johnston et al., 2002; Tibballs and Sutherland, 1991). However, recent studies indicate that much lower quantities of antivenom (i.e. one vial) can be used in all cases (Allen et al., 2012; Kulawickrama et al., 2010).

Although sudden cardiovascular collapse following envenoming by brown snakes has been studied (Tibballs and Sutherland, 1991; Tibballs et al., 1989), the mechanism of the collapse and potential therapeutic agents that prevent this collapse have not been defined. In this study, we determined whether administration of small priming doses of *P. textilis* venom could confer protection against sudden cardiovascular collapse. We also examined cross genus and cross family neutralization, as well as the efficacy of the commercially available snake antivenom to prevent sudden cardiovascular collapse in brown snake (*Pseudonaja* spp.) envenoming.

2. Materials and methods

2.1. Venom preparation and storage

Freeze-dried eastern brown snake (*Pseudonaja textilis*), Papuan taipan (*Oxyuranus scutellatus*), Irian Jayan death adder (*Acanthophis rugosus*) and Russell's viper (*Daboia russelii limitis*) venoms were from Venom Supplies (Tanunda, South Australia). Venom was dissolved in MilliQ water and stored at −20 °C until required. Thawed solutions were kept on ice during experiments. Venom protein content was determined via a BCA Protein Assay Kit obtained (Pierce biotechnology; Illinois, USA) as per manufacturer's instructions.

2.2. Anaesthetized rat preparation

All procedures were approved by the School of Biomedical Science (SOBS)-B Animal Ethics Committee, Monash University. Male Sprague-Dawley rats (250–350 g) were anaesthetized with pentobarbitone sodium (60–100 mg/kg, i.p., supplemented as required). Cannulae were inserted into the trachea, jugular vein and carotid artery, for artificial respiration (if required), administration of drugs/venom and measurement of blood pressure, respectively. Arterial blood pressure was recorded using a Gould Statham P23 pressure transducer connected to a PowerLab system. Venoms were administered via the jugular vein. At the conclusion of the experiment animals were killed by an overdose of pentobarbitone (i.v.).

As indicated, CSL polyvalent antivenom or inhibitors were administered 10 min prior to the administration of *P. textilis* venom. Priming doses of snake venoms were administered at 5–10 min intervals.

2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method described by Laemmli (Laemmli, 1970). *P. textilis* venom (20 μg) was resolved on a 12.5% SDS-PAGE under reducing condition (5% β-mercaptoethanol in Laemmli's sample buffer (62.5 mM trisma–hydrochloride (trisma–HCl), 25% glycerol, 2% SDS, 0.01% bromophenol blue). Venom was heated for 5 min at 95 °C prior to resolving on SDS-PAGE. Protein bands were visualized by staining with BioSafe Coomassie G-250 solution (BioRad Laboratories; Hercules, CA USA), followed by de-staining in distilled water. Gel image was captured utilizing Typhoon Trio scanner (GE Healthcare; Uppsala, Sweden).

2.4. Western blot

P. textilis venom (20 μg) was resolved on a 12.5% SDS-PAGE and transferred onto a PVDF (polyvinylidene difluoride) membrane. The membrane was incubated

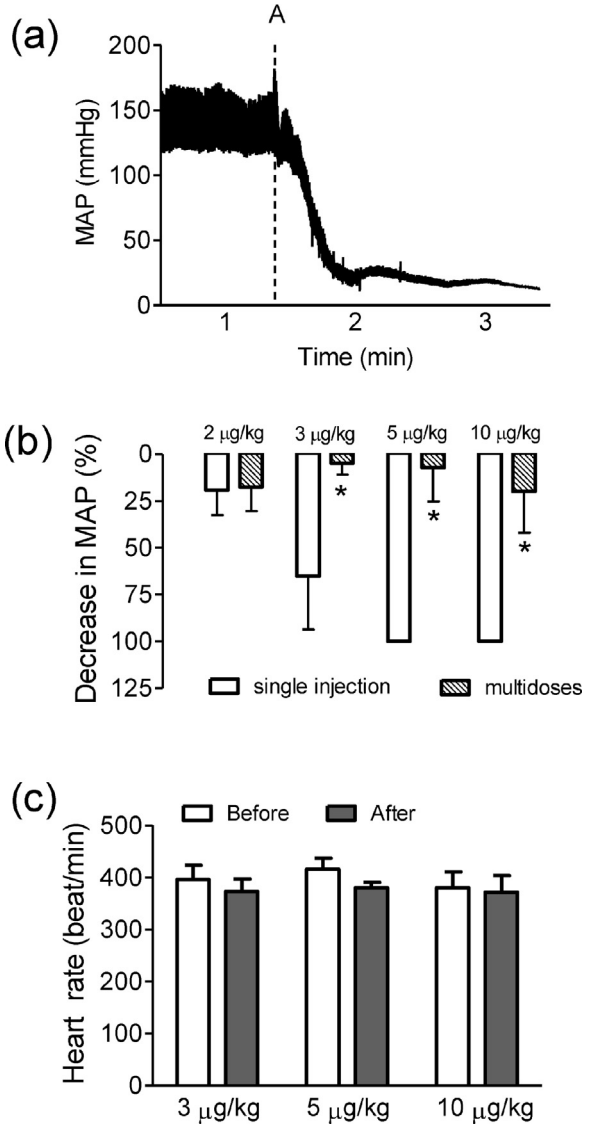

Fig. 1. (a) Effect of *P. textilis* venom (10 μg/kg, i.v.) on MAP in an anaesthetized rat. (b) Effect of *P. textilis* venom after a single injection (2, 3, 5 or 10 μg/kg, i.v., $n = 5$) in different rats or sequential addition of venom (2, 3, 5 and 10 μg/kg, i.v., $n = 7$) in the same rat on MAP. $^*P < 0.05$, significantly different from single injection, Student's unpaired *t*-test. (c) A comparison of heart rate before and after single injection of venom (3, 5 and 10 μg/kg, i.v., $n = 3$) (measured at the point when MAP had decreased by 50%).