ELSEVIER

Toxicon 49 (2007) 57–67

TOXICON

www.elsevier.com/locate/toxicon

Efficacy of antivenom against the procoagulant effect of Australian brown snake (*Pseudonaja* sp.) venom: In vivo and in vitro studies

Geoffrey K. Isbister[a,b,*], Margaret A. O'Leary[b], Jennifer J. Schneider[c], Simon G.A. Brown[d], Bart J. Currie[a], on behalf of the ASP Investigators

[a] *Tropical Toxinology Unit, Menzies School of Health Research, Charles Darwin University, Darwin*
[b] *Department of Clinical Toxicology and Pharmacology, Newcastle Mater Hospital, Newcastle, NSW, Australia*
[c] *School of Biomedical Science, University of Newcastle, Newcastle, NSW, Australia*
[d] *Discipline of Emergency Medicine, The University of Western Australia and Emergency Physician, Fremantle Hospital, Perth, Western Australia, Australia*

Received 8 June 2006; accepted 12 September 2006
Available online 17 September 2006

Abstract

Snake venom induced consumption coagulopathy (VICC) is a common complication of snake bite due to prothrombin activators or thrombin-like enzymes in the venom. This study aimed to determine the efficacy and dose of antivenom for treating VICC in patients envenomed by brown snakes (*Pseudonaja* spp.), including in vitro coagulation studies. In serial blood samples from patients with brown snake envenoming, venom and antivenom concentrations were measured using enzyme immunoassays. In vitro mixtures of brown snake venom and antivenom were used to investigate antivenom binding, neutralisation of prothrombin activity, prevention of venom-mediated clotting and effect on thrombin generation parameters using a thrombinoscope. In 27 envenomed patients the median venom concentration was 20 ng/mL (Interquartile range[IQR]:12–44 ng/mL) prior to antivenom and was not detected after antivenom administration, including 9 patients given one vial. In vitro, 200 μg/mL of antivenom bound all free venom at venom concentrations seen in patients. In vitro prothrombinase activity of the venom (using a chromogenic substrate) was not neutralised by antivenom. However, for venom concentrations seen in humans, 100 μg/mL of antivenom prevented venom clotting activity in human plasma and 479 μg/mL neutralised procoagulant venom activity measured by triggering thrombin generation. One vial of antivenom appears to be sufficient to bind and neutralise all venom in patients with severe brown snake envenoming.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: *Pseudonaja* sp.; Snake envenoming; Antivenom; Procoagulant; Brown snake; Coagulopathy

1. Introduction

Worldwide, there are in the order of 2.5 million snake envenomings each year and about 100,000 deaths in healthy and productive individuals (White et al., 2003). This is a particular problem in the rural

*Corresponding author. C/o Newcastle Mater Hospital, Edith St, Waratah, New South Wales 2298, Australia.
Tel.: +612 4921 1211; fax: +612 4921 1870.
E-mail address: geoffrey.isbister@menzies.edu.au (G.K. Isbister).

0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.09.007

tropics and coagulopathy from snake bite occurs in many cases. Although often not recognised, snake venom induced consumption coagulopathy (VIC-C)(Isbister et al., 2002) is probably the commonest cause worldwide of acquired afibrinogenaemia due to prothrombin activators (Warrell and Arnett, 1976) or thrombin-like enzymes in the venom. (Milani et al., 1997). Although snakebite is an uncommon cause of death in Australia (1–4 per annum) (Sutherland and Leonard, 1995) it continues to be a significant treatment issue in regional and rural hospitals.

Brown snake (*Pseudonaja* sp.) envenoming is arguably the most important in Australia. Envenoming causes a rapid consumption of coagulation factors or VICC due to a potent prothrombin activator in the venom (Masci et al., 1988; Filippovich et al., 2005; Birrell et al., 2006). This results in a defibrination coagulopathy and the potential for life-threatening haemorrhage (Yeung et al., 2004; Currie, 2004b). Despite the presence of neurotoxins in the venom, neurotoxicity is rare (Currie, 2004b, 2000). Other effects including thrombotic microangiopathy and renal failure are also uncommon. Brown snake envenoming continues to cause deaths each year, most commonly from haemorrhage or multi-organ failure (Jelinek et al., 2005; White, 2000). The medically important toxin is a prothrombin activator complex that makes up a significant proportion of the venom (Masci et al., 1988; Birrell et al., 2006). The toxin and thus whole venom is procoagulant in vitro where it causes whole blood and plasma to rapidly clot, but in vivo it causes a coagulopathy due to consumption of essential clotting factors.

An antivenom has been available since 1956 for brown snake envenoming and has been used for the treatment of thousands of cases since that time. Over the last decade there has been increasing concern that larger doses of brown snake antivenom are required than the dose initially recommended by the manufacturer, CSL Ltd. (Yeung et al., 2004; Sprivulis et al., 1996; Tibballs and Sutherland, 1991). This has changed recommendations for the initial dose of antivenom and the manufacturer has doubled the antivenom content in each vial since its introduction. Most guidelines now recommend five vials (equivalent to 10 times the initial dose recommended when antivenom was introduced), (White, 2001; Isbister, 2004). Despite numerous anecdotes, case reports and animal studies, there are fundamental questions regarding the efficacy of this antivenom, the dose of antivenom and end-points for treatment.

Animal studies, (Tibballs and Sutherland, 1991) in vitro human studies (Sprivulis et al., 1996) and clinical studies (Yeung et al., 2004) have all suggested that larger doses are required to neutralise the procoagulant activity of brown snake venom. The usual explanation for this has been that polyclonal $F(ab')_2$ directed at the prothrombin activator has low binding affinity and so larger amounts are required to bind all of the prothrombin activator and neutralise its activity (Madaras et al., 2005; Sprivulis et al., 1996). However, no binding studies have been undertaken and studies investigating the effectiveness of the antivenom in neutralising the venom procoagulant activity provide contradictory results (Madaras et al., 2005; Tibballs and Sutherland, 1991; Sprivulis et al., 1996).

Previous in vitro studies that were unable to demonstrate neutralisation of the procoagulant activity of the venom used very high venom concentrations (Madaras et al., 2005; Sprivulis et al., 1996). This has been based on the assumption that large amounts of venom enter the systemic circulation and are required to cause envenoming. We have recently shown in a small series that the concentration of brown snake venom in serum in envenomed cases ranges from 8 to 95 ng/mL (O'Leary et al., 2006) which is much lower than the concentrations of up to 50 μg/mL used in previous neutralisation studies (Madaras et al., 2005; Sprivulis et al., 1996).

We investigated the effect of antivenom on the venom, using four techniques: Enzyme immunoassay (EIA), prothrombinase activity, clotting times and thrombinoscopy. The aim of the study was to determine how much antivenom was required to bind and neutralise venom and to establish the dose of antivenom that is likely to neutralise the effects of brown snake venom in envenomed patients.

2. Methods

2.1. Materials

Brown snake (*Pseudonaja textilis*) venom was purchased from Venom Supplies, South Australia and Brown Snake Antivenom was produced by CSL Ltd. Hen anti-brown snake venom affinity-purified IgY was kindly donated by Frank Madaras (Madaras et al., 2005). Tetramethylbenzidine (TMB), horseradish peroxidase (HPO) and HPO-

Download English Version:

https://daneshyari.com/en/article/2065486

Download Persian Version:

https://daneshyari.com/article/2065486

Daneshyari.com