Toxicon 56 (2010) 75–85

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Endogenous thrombin potential as a novel method for the characterization of procoagulant snake venoms and the efficacy of antivenom

Geoffrey K. Isbister [a,b,c,*], David Woods [a], Steven Alley [a], Margaret A. O'Leary [b], Michael Seldon [d], Lisa F. Lincz [a,d]

[a] Faculty of Health, University of Newcastle, Newcastle, New South Wales, Australia
[b] Department of Clinical Toxicology and Pharmacology, Calvary Mater Newcastle, New South Wales, Australia
[c] Tropical Toxinology Unit, Menzies School of Health Research, Charles Darwin University, Darwin, Australia
[d] Hunter Haematology Research Group, Calvary Mater Newcastle, New South Wales, Australia

ARTICLE INFO

Article history:
Received 7 January 2010
Received in revised form 9 March 2010
Accepted 17 March 2010
Available online 23 March 2010

Keywords:
Thrombin potential
Antivenom
Snake
Procoagulant venom
Prothrombin activator

ABSTRACT

Venom-induced consumption coagulopathy occurs in snake envenoming worldwide but the interaction between procoagulant snake venoms and human coagulation remains poorly understood. We aimed to evaluate an assay using endogenous thrombin potential (ETP) to investigate the procoagulant properties of a range of Australian whole venoms in human plasma and compared this to traditional clotting and prothrombinase activity studies. We developed a novel modification of ETP using procoagulant snake venoms to trigger thrombin production. This was used to characterise the relative potency, calcium and clotting factor requirements of five important Australian snake venoms and efficacy of commercial antivenom, and compared this to prothrombinase activity and clotting assays. All five venoms initiated thrombin generation in the absence and presence of calcium. *Pseudonaja textilis* (Brown snake; $p < 0.0001$), *Hoplocephalus stephensii* (Stephen's-banded snake; $p < 0.0001$) and *Notechis scutatus* (tiger snake; $p = 0.0073$) all had statistically significant increases in ETP with calcium. Venom potency varied between assays, with ETP ranging from least potent with *Oxyuranus scutellatus* (Taipan) venom to intermediate with *N. scutatus* and *H. stephensii* venoms to most potent with *P. textilis* and *Tropidechis carinatus* (Rough-scale snake) venoms. ETPs for *N. scutatus*, *T. carinatus* and *H. stephensii* venoms were severely reduced with factor V deficient plasma. Antivenom neutralized the thrombin generating capacity but not prothrombin substrate cleaving ability of the venoms. Contrary to previous studies using clotting tests and factor Xa substrates, these venoms differ in calcium requirement. ETP is a useful assay to investigate mechanisms of other procoagulant venoms and is a robust method of assessing antivenom efficacy.
Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.

1. Introduction

Snake venom-induced consumption coagulopathy (VICC) is a common manifestation of snake envenoming worldwide (White, 2005). Despite isolation and characterization of numerous prothrombin activators and thrombin-like enzymes and more recently the cloning and sequencing of Australian elapid prothrombin activators (St Pierre et al.,

* Corresponding author. c/o Calvary Mater Newcastle Hospital, Edith St, Waratah NSW 2298, Australia. Tel.: +61 2 4921 1211; fax: +61 2 4921 1870.
E-mail address: geoff.isbister@gmail.com (G.K. Isbister).

0041-0101/$ – see front matter Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2010.03.013

2005), our understanding of the procoagulant process in human envenoming remains limited (Isbister, 2009). In a number of instances this has hampered our understanding, development and clinical application of antivenom therapy for snakebite coagulopathy.

VICC is the most common manifestation of severe snake envenoming in Australia and results from three major groups of snakes: *Pseudonaja* spp. (Brown snakes), *Oxyuranus* spp. (Taipans) and the tiger snake group (*Notechis* spp., *Tropidechis carinatus* and *Hoplocephalus* spp.) (Isbister et al., 2008). However, there has been significant controversy about the potency and dose of antivenom over the last two decades (Isbister, 2004; Yeung et al., 2004), much of which has arisen from a number of in vitro studies of the clotting effects of these snake venoms (Madaras et al., 2005; Sprivulis et al., 1996; Tibballs and Sutherland, 1991). It is a concern that despite the existence of antivenom in Australia for decades, the appropriate dose has not been established (Isbister, 2004). Recent work suggests that this may be in part due to the lack of a standard approach to in vitro testing of snake venom clotting effects to determine the efficacy and dose of antivenom (Isbister et al., 2007).

The production of thrombin from prothrombin is a crucial step in the human coagulation cascade. This is achieved through the actions of factor Xa complexed with Va (the prothrombinase complex, XaVa) in the presence of Ca^{2+} and phospholipids. Venoms from Australian elapids and some African vipers, including species from the genus *Echis* spp (Carpet vipers), contain prothrombinase-like toxins (Rosing and Tans, 1992). The prothrombin activators in Australian elapid venoms are classified as group C and D prothrombin activators because they cleave the two peptide bonds necessary to convert prothrombin to functional thrombin [Arg 273-Thr 274 and Arg 322-Ile 323] (Joseph and Kini, 2001). Venoms with group C prothrombin activators [*Oxyuranus scutellatus* (Coastal Taipan) and *Pseudonaja textilis* (Common brown snake)] can catalyse this reaction without human factor V, whereas venoms with group D prothrombin activators [*Notechis scutatus* (Common tiger snake), *T. carinatus* (Rough-scale snake) and *Hoplocephalus stephensii* (Stephen's-banded snake)] depend on the presence of plasma factor V. This requirement has been demonstrated in clotting studies of most of these venoms and isolated toxins, and recently confirmed by sequence comparison of recombinant forms of the toxins to human factor X and V (St Pierre et al., 2005). It is also assumed that the prothrombin activators both within and across groups C and D have similar requirements for calcium and phospholipid (St Pierre et al., 2005).

Although the structure of these prothrombin activators is well defined there are limited studies on their exact mechanism of action, degree of dependence on factor V and Ca^{2+}, and efficacy of the relevant antivenom to prevent their action. Traditionally, the function of procoagulant toxins has been assessed directly, by simple clotting studies (Marshall and Herrmann, 1983; Masci et al., 1988; Sprivulis et al., 1996), a technique which gives a single parameter for a complex process (Isbister, 2009). Alternatively, the use of prothrombin-like peptides as chromogenic substrates specific for Factor Xa [FXa] (such as S-2222 or S-2765) can provide the rate as well as extent of the prothrombin conversion reaction. Despite having FXa-like components, the venoms of *P. textilis* (Rao and Kini, 2002), *N. scutatus* (Tans et al., 1985), *O. scutellatus* (Speijer et al., 1986) and *T. carinatus* (Joseph et al., 1999), have much poorer ability to cleave FXa-specific substrates, such as S-2222, than does human FXa itself. In addition, peptide cleavage by FXa-like venom components does not necessarily correlate with procoagulant activity (Isbister, 2009). For example, sodium thiocyanate destroys the coagulating action of Pseutarin C (prothrombin activator in *P. textilis* venom) but does not affect its ability to generate chromogenic activity using S-2222 as a substrate (Rao and Kini, 2002). Conversely, antivenom appears to enhance the chromogenic reaction produced by *P. textilis* venom (using S-2765), but neutralises the coagulation effect (Isbister et al., 2007).

A substrate for thrombin, for example S-2238, provides a common measure of thrombin generation and is a functional assay that can be used to provide an indication of the thrombin-forming capacity of the haemostatic coagulation system in response to a given stimulus. The standard thrombin generation test can be modified to use snake venom, rather than thromboplastin as the trigger for coagulation, and thus measure the thrombin generating effect of procoagulant toxins or venoms. The area under the thrombin generation curve (the Endogenous Thrombin Potential; ETP) is then a quantitative measure of venom potency. We have previously reported this approach for assessing venom–antivenom mixtures in plasma for *P. textilis* venom, using a fluoroscopic method (Thrombinoscope™ instrument) (Isbister et al., 2007). However the fluoroscopic method has some limitations in terms of modifying the assay technique, particularly when a different triggering agent is used. We now extend the study in order to comprehensively evaluate the use of an ETP assay to investigate the procoagulant properties of a range of whole snake venoms in human plasma and compare these results to traditional clotting and prothrombinase activity studies. We include five representative snakes from Australia because of their clinical importance and the relevance of determining the potency and dose of antivenom for each of these snake types.

2. Materials and methods

P. textilis (common brown snake), *N. scutatus* (common tiger snake), *T. carinatus* (rough-scale snake), *H. stephensii* (Stephen's-banded snake), *O. scutellatus* (taipan) and *Acanthophis antarcticus* (death adder) venoms were purchased from Venom Supplies, South Australia. All venoms used are pooled from more than one snake milked on multiple occasions. Ancrod® and Unitrate TG® (H-β-Ala-Gly-Arg-pNA) were obtained from Unicorn diagnostics LTD, UK. Innovin® and factor-deficient plasmas were purchased from Dade Behring, Marburg, Germany. Normal Plasma 5® was from Hyphen biomed biophen for the ETP experiments. Ancrod®, Unitrate TG®, Innovin®, Normal Plasma 5® and the factor-deficient plasmas were all reconstituted with distilled water as directed by the manufacturer. $CaCl_2$ solution was purchased commercially and diluted with distilled water to a concentration of 0.2 M. ETP buffer

Download English Version:

https://daneshyari.com/en/article/2066382

Download Persian Version:

https://daneshyari.com/article/2066382

Daneshyari.com