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Full Length Article

Differential procoagulant effects of saw-scaled viper (Serpentes: Viperidae: *Echis*) snake venoms on human plasma and the narrow taxonomic ranges of antivenom efficacies

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ABSTRACT

Saw-scaled vipers (genus *Echis*) are one of the leading causes of snakebite morbidity and mortality in parts of Sub-Saharan Africa, the Middle East, and vast regions of Asia, constituting a public health burden exceeding that of almost any other snake genus globally. Venom-induced consumption coagulopathy, owing to the action of potent procoagulant toxins, is one of the most relevant clinical manifestations of envenomings by *Echis* spp. Clinical experience and prior studies examining a limited range of venoms and restricted antivenoms have demonstrated for some antivenoms an extreme lack of antivenom cross-reactivity between different species of this genus, sometimes resulting in catastrophic treatment failure. This study undertook the most comprehensive testing of *Echis* venom effects upon the coagulation of human plasma, and also the broadest examination of antivenom potency and cross-reactivity, to-date. 10 *Echis* species/populations and four antivenoms (two African, two Asian) were studied. The results indicate that the venoms are, in general, potently procoagulant but that the relative dependence on calcium or phospholipid cofactors is highly variable. Additionally, three out of the four antivenoms tested demonstrated only a very narrow taxonomic range of effectiveness in preventing coagulopathy, with only the SAIMR antivenom displaying significant levels of cross-reactivity. These results were in conflict with previous studies using prolonged preincubation of antivenom with venom to suggest effective cross-reactivity levels for the ICP Echi-Tab antivenom. These findings both inform upon potential clinical effects of envenomation in humans and highlight the extreme limitations of available treatment. It is hoped that this will spur efforts into the development of antivenoms with more comprehensive coverage for bites not only from wild snakes but also from specimens widely kept in zoological collections.

1. Introduction

Envenoming and deaths resulting from snakebite represent an important public health concern, particularly throughout rural areas of South Asia and Sub-Saharan Africa in which access to sufficient medical facilities and antivenoms can be limited (Gutiérrez et al., 2006; Kasturiratne et al., 2008; Maduwage and Isbister, 2014). It is estimated that snakebite affects around 5 million people and accounts for more than 100,000 deaths annually (Chippaux, 1998; White, 2005; Kasturiratne et al., 2008). However these numbers are dramatic underestimations due to poor or entirely absent epidemiological data in many regions. Most severe cases of envenoming are attributed to species belonging to the Elapidae and Viperidae

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families (Gutiérrez et al., 2006). Typically, venoms from elapid snakes induce neuromuscular paralysis and are thus classed as neurotoxic, whereas viperid snake venoms most commonly target haemostasis (e.g., coagulation dysfunction, fibrinolysis, thrombosis) and induce local tissue damage, and are thus broadly categorised as cytotoxic and haemotoxic (Boyer et al., 2015; Markland, 1998; Sajevic et al., 2011; Warrell, 2010; White, 2005). Coagulopathy, which contributes to sustained bleeding and consequent haemodynamic disturbances (Warrell, 2010), is considered to be one of the most common serious systemic clinical pathologies induced by snake envenoming (Isbister, 2010). Accordingly, the Viperidae family contains some of the most medically significant snake genera worldwide (Gutiérrez et al., 2006; Warrell, 2010). Among them, saw-scaled or carpet vipers (Viperidae: *Echis*) are thought to be responsible for causing more snakebite deaths annually than any other genus (Warrell and Arnett, 1976).

Currently, the genus *Echis* is thought to comprise at least nine species, distributed across four main clades: *E. carinatus, E. coloratus, E. ocellatus,* and *E. pyramidum* (Pook et al., 2009; Alencar et al., 2016). They can be found across the semi-arid and seasonal climate regions of Sub-Saharan Africa north of the equator, Arabia, Iran, Afghanistan and Uzbekistan, and in Pakistan, India and Sri Lanka, often in relative abundance. These areas are typically remote, rural, with absent or inadequate medical facilities, and with inhabitants of low socio-economic status. This, in conjunction with yielding highly toxic venom, renders *Echis* a common cause of injurious or fatal snakebite in the regions they occupy (Habib and Warrell, 2013). Though accurate statistics are sometimes difficult to ascertain due to poor documentation of bite cases in such developing nations, *Echis ocellatus* is responsible for as many as 95% of snake bites in northern Nigeria, for example (Meyer et al., 1997; WHO 2010a). Victims are most commonly young males, a result of higher encounter rates arising from land cultivation or walking (Warrell and Arnett, 1976). In the absence of effective antivenom, case fatality rates following envenomation can be as high as 20% even with supportive medical treatment (Warrell and Arnett, 1976; Pugh and Theakston, 1980; Visser et al., 2008), and those who survive are often left with permanent disability and disfiguring sequelae (I.S. Abubakar et al., 2010; S.B. Abubakar et al., 2010). Local effects of *Echis* viper envenoming typically include pain, swelling, blistering, and haemorrhage, which, in severe cases, can lead to necrosis and amputation. In such cases, the long-term socio-economic impact upon both bite victims and their families can be severe and is an often-overlooked consequence of snakebite (Vaiyapuri et al., 2013).

In addition to localised effects, *Echis* toxins also induce dysfunctions of haemostasis. Mortality following envenomation is typically a result of systemic haemostatic disruption, which often leads to systemic haemorrhage (Warrell et al., 1977; Boyer et al., 2015). Of the three main processes involved in haemostasis (vasoconstriction, platelet plug formation and coagulation) (Jin and Gopinath, 2016), the majority of snake venoms affecting haemostasis, including those of *Echis*, target the coagulation cascade. The coagulation cascade, whereby blood forms a clot following the stepwise activation of multiple clotting factors, requires the presence of Ca^{2+} ions and platelet-phospholipids. These molecules act as cofactors to clotting proteins present in plasma, ultimately inducing the proteolytic cleavage of prothrombin to thrombin (Berny et al., 2010; Davie et al., 1991; Jackson and Nemerson 1980; Munnix et al., 2007). Soluble fibrin monomers are formed by the cleavage of fibrinogen by the activated thrombin, and these monomers subsequently form an insoluble fibrin meshwork, resulting in a clot (Jin and Gopinath, 2016).

The snake venom components responsible for perturbing haemostasis are variable, although the majority can be classified into four categories according to the part of the coagulation pathway upon which they act: factor V activators, factor X activators, prothrombin activators, and thrombin-like enzymes (TLEs) or fibrinogenases (Lu et al., 2005; Alencar et al., 2016; Rosing and Tans, 1992; Slagboom et al., 2017). Within the genus *Echis*, the presence of prothrombin activators in species from each of the four main clades have been demonstrated (Gillissen et al., 1994; Mann, 1978; Mion et al., 2013; Porath et al., 1992; Warrell et al., 1977; Yamada et al., 1996). Prothrombin activators found within the genus are categorised into two subgroups according to their calcium dependence: 'ecarin-like' (Ca^{2+}-independent) and 'carinactivase-like' activators (Ca^{2+}-dependent), classified into groups A and B of snake venom prothrombin activators respectively (Kini, 2015). Those belonging to the 'ecarin-like' group are named so following the discovery of a prothrombin activator (ecarin) with an unprecedented Ca^{2+}-independent activity, isolated from *Echis carinatus* venom (Morita and Iwanaga, 1978). In contrast, 'CA-like' activators exhibit Ca^{2+}-dependent activity, e.g., the prothrombin activator carinactivase, isolated from *Echis leucogaster* venom (Yamada et al., 1996). These toxins are responsible for the catalysis of prothrombin into thrombin. In natural prey items, this results in rapid subjugation through intravascular coagulation leading to cardiovascular collapse. However, in humans, the dilution of the venom into a much larger blood volume results in the formation of millions of microthrombi. In and of themselves, microthrombi are not lethal, but their excessive formation consumes the majority of available essential clotting factors. Clinically, this leads to low or undetectable concentrations of fibrinogen (Isbister, 2010) and to multiple blood factor deficiencies, a potentially lethal condition known as venom-induced consumption coagulopathy (VICC) (Gillissen et al., 1994; Mann, 1978; Mion et al., 2013; Porath et al., 1992; Warrell et al., 1977). Coagulopathy also contributes to the generation of additional systemic pathologies in human bite victims, including internal haemorrhage, such as cerebrovascular accident (Boyer et al., 2015; Warrell et al., 1977).

Despite envenomation by all *Echis* species manifesting similar clinical symptoms, previous studies have documented considerable inter- and intraspecific variations in their venom composition (Barlow et al., 2009; Casewell et al., 2009, 2014; Schaeffer, 1987), apparently driven at least in part by natural selection for different diet spectra between the clades (Barlow et al., 2009; Richards et al., 2012; Savanur et al., 2014). This suggests that variation in the molecular mechanisms inducing these pathologies also exists. Such interspecific variation in toxin expression, and therefore in venom antigenicity, can greatly affect the ability of an antivenom to neutralise a given venom (Bénard-Valle et al., 2015; Fry et al., 2003; Harrison et al., 2003). Antivenoms consist of polyclonal antibodies purified from the serum or plasma of animals hyperimmunised with the target species' venom (Bénard-Valle et al., 2015; Heard et al., 1999; Theakston and Warrell, 1991). Due to their polyclonal nature, antivenoms are able to neutralise multiple venom components (Gutiérrez et al., 2003, 2014); however, the antibodies are specific to the venoms from which they were developed (Bénard-Valle et al., 2015). Consequently, while antivenoms are typically marketed as a therapeutic treatment for envenomation by a given species, intraspecific venom variation can reduce antivenom efficacy, dependent upon the difference between the venom composition of the individuals which were used for antivenom manufacture and the individual which delivered the bite (Bénard-Valle et al., 2015; Boyer et al., 2015). It is well documented that the success rates achieved by different antivenoms in treating *Echis* snakebites can vary significantly depending on the geographical location of the bite due to regional variation of the species' venom (I.S. Abubakar et al., 2010; S.B. Abubakar et al., 2010; Calvete et al., 2016; Casewell et al., 2010), which can translate into catastrophic treatment failure and case fatality rates increased by an order of magnitude in a tropical clinical setting (Alirol et al., 2015; Visser et al., 2008; Warrell and Arnett, 1976; Warrell et al., 1980).

In light of the complications associated with *Echis* envenomings, from a clinical perspective, there is a distinct need to characterise the coagulant activity of venoms of this genus, with particular reference to geographical variation, and to explore the implications this may have for antivenom cross-reactivity. In this study, a comparative analysis of the venoms of six representative species belonging to the four main clades of *Echis* was conducted. Coagulation assays were used to explore the interspecific and intraspecific variations of