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Analysis of camelid antibodies for antivenom development: Neutralisation of venom-induced pathology

Darren A.N. Cook [a], Timothy Owen [a], Simon C. Wagstaff [a], Joerg Kinne [b], Ulrich Wernery [b], Robert A. Harrison [a,*]

[a] Alistair Reid Venom Research Unit, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom
[b] Central Veterinary Research Laboratory, Dubai, United Arab Emirates

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ABSTRACT

Camelid IgG has been reported to be less immunogenic, less able to activate the complement cascade and more thermostable than IgG from other mammals, and has the ability to bind antigens that are unreactive with other mammalian IgGs. We are investigating whether these attributes of camelid IgG translate into antivenom with immunological and venom-neutralising efficacy advantages over conventional equine and ovine antivenoms. The objective of this study was to determine the preclinical venom-neutralising effectiveness of IgG from camels immunised with venoms, individually or in combination, of the saw-scaled viper, *Echis ocellatus*, the puff adder, *Bitis arietans* and the spitting cobra, *Naja nigricollis* – the most medically-important snake species in West Africa. Neutralisation of the pathological effects of venoms from *E. ocellatus*, *B. arietans* and *N. nigricollis* by IgG from the venom-immunised camels, or commercial antivenom, was compared using assays of venom lethality (ED_{50}), haemorrhage (MHD) and coagulopathy (MCD). The *E. ocellatus* venom ED_{50}, MHD and MCD results of the *E. ocellatus* monospecific camel IgG antivenom were broadly equivalent to comparable ovine (EchiTAbG®, MicroPharm Ltd, Wales) and equine (SAIMR Echis, South African Vaccine Producer, South Africa) antivenoms, although the equine antivenom required half the amount of IgG. The *B. arietans* monospecific camel IgG neutralised the lethal effects of *B. arietans* venom at one fourth the concentration of the SAIMR polyspecific antivenom (a monospecific *B. arietans* antivenom is not available). The *N. nigricollis* camel IgG antivenom was ineffective (at the maximum permitted dose, 100 μl) against the lethal effects of *N. nigricollis* venom. All the equine polyspecific antivenoms required more than 100 μl to be effective against this venom. The polyspecific camel IgG antivenom, prepared from five camels, was effective against the venom-induced effects of *E. ocellatus* but not against that of *B. arietans* and *N. nigricollis* venoms. No direct correlation was evident between either camel IgG relative avidity or titre and the effectiveness of venom neutralisation in preclinical assays.

1. Introduction

Snake envenoming is a significant cause of mortality and morbidity for the rural poor communities of sub-Saharan Africa (Kasturiratne et al., 2008; Harrison et al., 2009). However, because of a variety of complex fiscal reasons associated with commercial antivenom production and government demand, antivenom for sub-Saharan Africa has been in short supply since the 1990s (Theakston and Warrell, 2000). New strategies to ensure the long term stability of antivenom supply are therefore necessary. We are investigating whether the distinct attributes of camel IgG (see preceding paper Cook et al., 2010) can be

* Corresponding author. Fax: +44 151 705 3371.
E-mail address: robharr@liverpool.ac.uk (R.A. Harrison).

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exploited to improve the efficacy, safety and logistics of antivenom provision in many countries where snakebite is endemic. Camelid IgG can bind epitopes that are non-reactive with other mammalian IgGs (Lauwereys et al., 1998), a feature we hope can be exploited to overcome the weakly immunogenic nature of many of the most pathologenic elapid neurotoxins. Camel IgG is also less immunogenic and less capable of activating complement than equine IgG (Herrera et al., 2005) indicating that intravenous administration of a camelid antivenom might be less likely to induce the serum sickness-like and anaphylactoid adverse reactions associated with current equine and ovine antivenom treatment. In the preceding paper (Cook et al., 2010) we described the serological responses of camels immunised with venoms from the three most medically-important snake species in West Africa (the saw-scaled viper, *Echis ocellatus*; the puff adder, *Bitis arietans* and the spitting cobra, *Naja nigricollis*), to which we have developed three monospecific antivenoms (one for each snake species) and a polyspecific antivenom against all three snake species. We demonstrated that venom immunisation of camels stimulated IgG titres, antigen-specificities and relative avidities that matched that of conventional equine and ovine antivenoms currently used in West Africa.

Whilst these results are very encouraging, the 'gold standard' of preclinical assessment of antivenom efficacy is the use of venom neutralisation assays recommended by the WHO (WHO, 1981; Theakston and Reid, 1983; British Pharmacopoeia, 2002) and in particular the ED_{50} assay. The median effective dose (ED_{50}) is the least amount of antivenom required to prevent death in 50% of mice challenged with five times the venom LD_{50} dose (1 venom LD_{50} is the amount of venom that kills 50% of the injected mice). ED_{50} results provide a basis for selecting antivenoms for human clinical trials and have been used to indicate the appropriate dose range for some initial phase 1 dose-finding human clinical trials (Laing et al., 1995; Theakston et al., 1995; Abubakar et al., 2010). Although not a representation of envenoming and treatment of a patient (the assay involves incubating a mixture of venom and antivenom at 37 °C for 30 min before intravenous administration into mice), the results of ED_{50} assays usually provide a reasonably satisfactory correlation with the clinical efficacy of an antivenom (Warrell et al., 1986; Laing et al., 1992).

Envenoming by *E. ocellatus* is the predominant cause of snakebite morbidity and mortality throughout most of West Africa, including regions in Senegal (Trape et al., 2001), Cameroon (Einterz and Bates, 2003) and particularly areas in Nigeria, where the number of deaths due to *E. ocellatus* envenoming has been reported as high as 60 per 100,000 population per year (Pugh and Theakston, 1980). The potency of *E. ocellatus* venom and a typically low incidence (less than 10%) of 'dry bites' (victims being bitten without being envenomed) leads to an untreated mortality rate in envenomed victims of 10–20% (Warrell et al., 1977). The pathology subsequent to *E. ocellatus* envenoming is characterised by haemorrhage, oedema, blistering and necrosis close to the bite site (local effects) and incoagulable blood and spontaneous bleeding from areas distant from the site of envenoming (systemic effects) (Warrell et al., 1977). The systemic effects can lead to death by intra-abdominal or intracranial bleeding (Warrell and Arnett, 1976). Snake venom metalloproteinases (SVMPs) are primarily responsible for both localised and systemic haemorrhage (Kamiguti et al., 1998) whilst venom prothrombin activators cause consumptive coagulopathy (Warrell et al., 1977). The local manifestations of *B. arietans* envenoming include oedema, blistering, haemorrhage and necrosis and systemic effects include haemorrhage and hypotension but, unlike *E. ocellatus* envenoming, no effects on blood clotting (Warrell et al., 1975). Unusually for African elapid species, human envenoming by *N. nigricollis* is not associated with neurotoxicity but with extensive tissue necrosis, and occasionally with systemic haemorrhage and complement depletion (Warrell et al., 1976).

To assess the performance of the experimental camel IgG antivenoms we used assays that mimic, as closely as possible, the clinical effects of *E. ocellatus*, *B. arietans* and *N. nigricollis* venom. Therefore, in addition to the ED_{50} assays, we examined the ability of the camel monospecific and polyspecific IgG antivenoms to reverse (i) *E. ocellatus* and *B. arietans* venom-induced haemorrhage by determining the minimum amount of antivenom required to neutralise the minimum haemorrhagic dose (MHD) (Theakston and Reid, 1983) and (ii) *E. ocellatus* venom-induced consumptive coagulopathy by finding the minimum dose of antivenom that prevents the minimum coagulant dose (MCD) of venom from inducing coagulation of a standard solution of citrated human plasma (Theakston and Reid, 1983). The efficacy of the camel IgG antivenoms to reverse the effects of *N. nigricollis* envenoming was performed using the venom lethality ED_{50} assay, and not a specific assay of neurotoxicity, because human *N. nigricollis* envenoming is not associated with neurotoxicity. The results from the camel IgG antivenom assays are compared with that of equine and ovine antivenoms currently used in Africa.

2. Materials and methods

2.1. Animals

2.1.1. Snakes and snake venom

Specimens of *E. ocellatus*, *B. arietans* and *N. nigricollis* of mixed age and sex collected from Nigeria were maintained in the herpetarium of the Alistair Reid Venom Research Unit at the Liverpool School of Tropical Medicine. Venom was extracted from the snakes, frozen, lyophilised and stored at 4 °C as a powder.

2.1.2. Dromedary camels

Animals of mixed age and sex with no previous history of disease or immunisation were maintained in the Central Veterinary Research Laboratory, Dubai for this project. The health of the camels was monitored daily and haematology as well as blood chemistry analyses performed as a weekly routine. Blood taken from the jugular vein was left at room temperature to clot and sera collected by centrifugation and stored at −20 °C.

The five camels immunised with venom from all three snake species to generate the polyspecific antivenom are identified as PS 1–5. The camel immunised with only *E.*