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Clinical report

Analysis of camelid IgG for antivenom development: Immunoreactivity and preclinical neutralisation of venom-induced pathology by IgG subclasses, and the effect of heat treatment

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ABSTRACT

Antivenom is the most effective treatment of snake envenoming and is manufactured from the IgG of venom-immunised horses and sheep. Camelids have a unique IgG structure which may account for the report that camel IgG is less immunogenic and less likely to activate complement than equine or ovine IgG. Camelid IgG therefore offers potential safety advantages over conventional IgGs used for antivenom manufacture. The reported thermostability of camelid IgG also holds promise in the inclusion of a relatively inexpensive anti-microbial heat step in antivenom manufacture. However, these potential benefits of camelid IgG would be much reduced if any one of the three camel IgG subclasses dominated, or under-performed, the serological response of camels to venom immunisation because of the prohibitive manufacturing costs of having to purify, or exclude, one or more IgG subclasses. This study compared the titre, antigen-specificity, relative avidity and ability to neutralise the haemorrhagic and coagulopathic effects of *Echis ocellatus* venom of each IgG subclass from the venom-immunised camels. The results demonstrated that no one IgG subclass consistently out-performed or under-performed the others in their immunoreactivity to venom proteins and ability to neutralise venom-induced pathologies. We concluded therefore that IgG taken from a pool of immunised camels could be processed into antivenom without requiring the implementation of expensive chromatographic separations to select, or indeed to exclude, a specific IgG subclass. The immunoreactivity of the heavy and light chain, IgG1 subclass, was markedly more vulnerable to extreme heat treatment than the heavy chain-only IgG2 and IgG3 subclasses.

1. Introduction

Snakebite is a serious, often life-threatening, daily hazard in large tracts of Africa and Asia (Kasturiratne et al., 2008), which primarily affects the rural poor (Harrison et al., 2009). Antivenom therapy represents the most effective treatment for snake envenoming but because of the complex interplay between diverse fiscal, epidemiological and clinical issues, including (i) high costs of manufacture that are often passed on to governments and patients, (ii) a general paucity on burden data for specific regions, (iii) a lack of political recognition of regional snakebite problems and (iv) inadequate advocacy and funding, some regions, particularly in Africa, suffer from a severe shortage of life-saving antivenom (Theakston and Warrell, 2000). The toxinological community has responded to this situation with publications highlighting

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the problem (Gutierrez et al., 2006; Williams et al., 2010) and, in Latin America, by the formation of an 'alliance' (CYTED – Ciencia y Technologia para el Desarrollo) to coordinate disparate activities to improve antivenom delivery and effectiveness in the region (Gutierrez et al., 2007, 2009) and by the inclusion of snakebite in the World Health Organisation list of Neglected Tropical Diseases (WHO, 2009). One of the common recommendations is that research is needed to make antivenoms more effective, safer and less expensive (Gutierrez et al., 2006; Harrison et al., 2009; Williams et al., 2010). With that objective we have embarked on a research project assessing the potential benefits of developing antivenom using camel IgG (Harrison et al., 2006; Harrison and Wernery, 2007; Cook et al., 2010a,b).

Camelid IgG is unique amongst mammals in that over 50% of the IgG lack light chains (Hamers-Casterman et al., 1993), and perhaps because of its unique heavy chain-only IgG subclasses, offers exciting possibilities to improve antivenom in five main areas: (a) The report that camelid IgG proved less immunogenic and less likely to activate complement than equine IgG (Herrera et al., 2005) suggests the possibility that camel IgG antivenom might induce fewer incidences of anaphylactic and serum sickness adverse reactions noted in patients treated with many equine antivenoms. (b) Reports that camelid IgG binds some epitopes that are not bound by IgG of other mammalian species (Lauwereys et al., 1998; De Genst et al., 2006) suggested that camelid IgG antivenom might show an improved binding to the low molecular weight elapid neurotoxins that are typically weakly immunogenic in venom-immunised horses (Chinonavanig et al., 1988; Pratanaphon et al., 1997). (c) The reported greater thermostability of camelid heavy chain IgG subclasses and, in particular, the antigen-binding domain (VHH) (Omidfar et al., 2007) indicated that a cost–benefit anti-microbial heat step could perhaps be introduced into the manufacturing process, and that a liquid camel IgG antivenom could be stored at ambient temperature without the expense required to maintain a cold chain. (d) Camels might be very attractive to the manufacture of antivenom in countries where the climatic conditions are not favourable for the maintenance of horses or sheep. (e) The small size (15 kDa) of the VHH of the heavy chain-only IgG subclasses (Lauwereys et al., 1998; van der Linden et al., 1999) could perhaps be exploited to develop, for the first time, a treatment of the tissue-destructive effects of snake envenoming (Harrison and Wernery, 2007).

The studies performed in Tunisia describing the experimental venom-neutralising effectiveness of conventional heavy and light chain IgG or heavy chain-only IgG (Meddeb-Mouelhi et al., 2003) indicate that camels hold promise in the development of antivenoms to treat scorpion envenoming. We demonstrated that camels and llamas immunised with *Echis ocellatus* venom responded with virtually indistinguishable IgG reactivities and preclinical venom-neutralising effectiveness (Harrison et al., 2006), indicating that either camelid species could be used for antivenom production. Encouraged by these results, we immunised dromedary camels with venoms from the most medically important snakes of West Africa (the saw-scaled viper, *E. ocellatus*; the puff adder, *Bitis arietans*; the spitting cobra, *Naja nigricollis*), either with a single venom to prepare monospecific antivenoms to each snake species, or with the three venoms combined to prepare a polyspecific antivenom for West Africa. The monospecific and polyspecific camel antisera (Cook et al., 2010a) responded to venom immunisation with similar venom species-specific IgG titres (ELISA), specificity (immunoblotting) and antigen-binding strength (relative avidity ELISA) as conventional equine and ovine antivenom controls. We next demonstrated that the *E. ocellatus*- and *B. arietans*-monospecific antivenoms (but not the *N. nigricollis*-monospecific antivenom) were as effective in preclinical assays of venom-induced lethality, haemorrhage and coagulopathy, as comparable antivenoms in current clinical use in Africa (Cook et al., 2010b). These results, and those from a study of venom-immunised llamas (Fernandez et al., 2010) confirmed the therapeutic potential of camelid IgG antivenom.

Camel IgG consists of three subclasses; IgG1, a conventional heavy and light chain molecule and IgG2 and IgG3 that lack light chains. A recent report showing that the serological response of nematode-infected llamas was dominated by the IgG1 subclass (Daley et al., 2005) caused some concern because it suggested the possibility that the immunological reactivity and preclinical efficacy of the camel IgG antivenoms might similarly be dominated by one IgG subclass. We felt it was important to ascertain whether there were strong immunological and/or venom-neutralising reasons to select, or exclude, a specific IgG subclass for the development of camelid IgG antivenoms – an expensive additional step in antivenom manufacture, which might risk commercial interest in the development of camel antivenoms, irrespective of the potential clinical gains. The objective of this study was therefore to compare the immunoreactivity profiles and venom-neutralising capabilities of IgG1, IgG2 and IgG3 subclasses isolated from venom-immunised camels. In consideration of the potential fiscal benefits of introducing an inexpensive anti-microbial heat step into the manufacture of camelid antivenoms, we also assessed the comparative immunoreactivity of the IgG subclasses after treatment under pasteurisation conditions (10 h, 60 °C).

2. Materials and methods

2.1. Venom

Specimens of *E. ocellatus* 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.

2.2. Preparation of IgG

One camel (Eo-MS) was immunised with *E. ocellatus* venom, to generate monospecific antibodies and 2 other camels (PS1 and PS2) were immunised with *E. ocellatus*, *B. arietans* and *N. nigricollis* venom to produce polyspecific antibodies (see Cook et al., 2010a for details of the venom immunisation protocol). Sera collected at 7 time-points was used in this study and IgG extracted using 5% caprylic