Small Ruminant Research 141 (2016) 63–69

Contents lists available at ScienceDirect

Small Ruminant Research

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

Antivenom production in the alpaca (*Vicugna pacos*): physiological and antibody responses to monovalent and polyvalent immunisation with Australian elapid venoms

Andrew M. Padula[a,b,*], Kenneth D. Winkel[b]

[a] 26 Howitt Ave, Bairnsdale, Victoria, 3875, Australia
[b] Australian Venom Research Unit, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Melbourne, Parkville, Australia

ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 9 July 2016
Accepted 10 July 2016
Available online 15 July 2016

Keywords:
Venom
Antivenom
Snakebite
Tiger snake
Notechis
Brown snake
Pseudonaja
Taipan
Oxyuranus
Black snake
Pseudechis
Common death adder
Acanthophis
Alpaca
Antibodies
Camelid
ELISA

ABSTRACT

Alpaca (*Vicugna pacos*) are domesticated members of the camelid family that have been shown to produce an IgG subclass that is heavy chain only IgG. This camelid-type IgG has been postulated to have improved neutralising properties and reduced immunogenicity, compared with conventional mammalian IgG. This study was undertaken to describe the physiological and antibody responses of alpaca (*Vicugna pacos*) used for the production of an experimental camelid antivenom. Various antivenom products were developed by immunisation of alpaca with combinations of five Australian elapid snake venoms: common tiger snake (*Notechis scutatus*), eastern brown snake (*Pseudonaja textilis*), Papuan taipan (*Oxyuranus scutellatus canni*), mulga snake (*Pseudechis australis*) and common death adder (*Acanthophis antarcticus*) were emulsified with Freund's Adjuvant and injected monthly using a low dose multi-site approach. Venom specific immune responses were monitored by ELISA. Physiological effects upon alpaca were monitored by clinical examination combined with serial haematology and biochemical profiles. Serum was harvested once immune responses had peaked and the IgG fraction concentrated by ammonium sulphate precipitate of non-IgG proteins. Total serum IgG and total serum protein concentrations increased and decreased in synchrony with boosting specific antibodies. The difference in serum creatinine kinase concentration measured at 24 h after each of the first three immunisations decreased as the alpaca mounted an antibody response to the venom antigens measured by ELISA. In conclusion, alpaca responded to repeated immunisation with various Australian elapid venoms by mounting an increasing antibody response that persisted following prolonged rest with minimal physiological impact on the animal.

1. Introduction

Horses have been largely favoured as serum donors for antivenom production, however a wide range of animal species have been successfully used for experimental antivenom production (Russell, 1988). Nevertheless, of nearly 200 different commercial antivenom products available worldwide in 2003, only six were not produced in horses (Landon and Smith, 2003). As one alternative to the equine standard, camels and llamas has recently been examined for their potential as antivenom production animals (Cook et al., 2010a,b,c; Darvish et al., 2016; Harrison et al., 2006; Harrison and Wernery, 2007). Potential benefits of camels for this purpose are their hardiness, adaptation to hot climates, and distinctive immunoglobulin (IgG) profile that may provide some unique advantages over equine antivenoms (Hamers-Casterman et al., 1993). All members of the camelid family have been found to possess a unique IgG that is devoid of light chains to varying degrees (Hamers-Casterman et al., 1993).

Whilst these studies have stimulated interest in the use of camels for experimental antisera production, this has not translated into any commercial camelid antivenoms. One reason for this difficulty in successfully utilising the advantages of camelid antivenoms into commercial products might relate to the practical challenges of camel husbandry. Alpaca (*Vicugna pacos*) are more easily domesticated members of the camelid family and, similarly to camels, have been shown to produce an IgG subclass that is heavy chain only IgG (Hamers-Casterman et al., 1993); although in lesser proportion of total IgG than in camels (Maass et al., 2007).

* Corresponding author: 26 Howitt Ave, Bairnsdale, Victoria, 3875, Australia.
E-mail address: andrew.padula@unimelb.edu.au (K.D. Winkel).

http://dx.doi.org/10.1016/j.smallrumres.2016.07.007
0921-4488/

Table 1
Treatment groups used for alpaca immunisation showing number of animals, snake common name and species scientific name.

Treatment Group	N	Common Name	Scientific Name
Monovalent Papuan Taipan	4	Papuan taipan	*Oxyuranus scutellatus canni*
Monovalent brown snake	4	Eastern brown snake	*Pseudonaja textilis*
Monovalent tiger snake	4	Tiger snake	*Notechis scutatus*
Polyvalent	4	Papuan taipan	*Oxyuranus scutellatus canni*
		Eastern brown snake	*Pseudonaja textilis*
		Tiger snake	*Notechis scutatus*
		Common death adder	*Acanthophis antarcticus*
		Mulga snake	*Pseudechis australis*
Bivalent (tiger/brown)	4	Tiger snake	*Notechis scutatus*
		Eastern brown snake	*Pseudonaja textilis*

This camelid type IgG has been postulated to have improved neutralising properties and reduced immunogenicity compared with conventional mammalian IgG (Harrison et al., 2006).

Australia has a large and expanding population of alpaca, currently estimated at over 150,000 animals (AAA, 2016). The commercial market opportunities for alpaca products in Australia are presently focused on fibre production with a much smaller and newly emerging market for meat products. Australia is also a low risk for many of the major infectious disease of livestock including bovine tuberculosis, bovine brucellosis, foot and mouth disease and spongiform encephalopathies. Hence alpaca based antivenoms, by offering the potential for large volume production of safe and effective antibodies, might offer one solution to the challenges of the global antivenom crisis (Williams et al., 2010). Specifically, this work was conducted as a proof-of-concept study to evaluate the immune and physiological responses of alpaca to repeated immunisation with snake venom from the five major families of venomous elapids in Australia and Papua New Guinea (PNG). The use of alpaca derived anti-snake venom antibodies for both therapeutic and diagnostic purposes has been described (Padula and Winkel, 2016a,c).

2. Materials and methods

2.1. Animals

The experimental animals [n = 20] consisted of mature adult alpaca aged 2–8 years old, predominantly male castrates or infertile females were located in the East Gippsland region of Victoria, Australia. Alpaca grazed pasture and were supplemented with lucerne hay, oats, lupins and alpaca pellets. Alpaca were monitored for internal parasites by regular faecal egg counts and treated for internal parasites using injectable ivermectin anthelmintic (Bomectin™, Boma Products, Australia). Alpaca were supplemented in early winter with a single injection of Vitamin A, D & E (Vitamec ADE Injection, AgVantage Pty Ltd, Australia). Alpaca are gentle animals by nature and only minimal facilities were used for handling and restraint. Approval for all experimental work involving animals (alpaca and mouse bioassay studies) was obtained from the Wildlife and Small Institutions Animal Ethics Committee, Bureau of Animal Welfare, Victorian Department of Primary Industries, Mickleham Road, Attwood.

2.2. Blood collection

Blood samples (Vacutainer™, BD, USA) were generally collected from the right jugular vein of each alpaca at the level of the fifth cervical vertebra. Depending upon the assay requirements a single 10 mL plain tube and a 2 mL EDTA tube were collected at each time point. On some occasions the left side of the neck was used but due to the close anatomical proximity of the oesophagus the left side was generally avoided (Amsel et al., 1987). Harvesting of larger quantities of serum was performed in a similar manner. Whole blood was collected into sterile 800 mL PVC blood fluid collection bags (Haemologic, Sydney, Australia). A 1.5 mL subcutaneous injection of lignocaine (Lignomav 2%, Ilium, Australia) was given over the jugular vein to anaesthetise the skin area. A bulk bleed was performed on each alpaca on Day 126. Blood bags were allowed to clot at room temperature for 4 h before centrifugation and aspiration of the serum. Serum was stored frozen at −20 °C until required for use. To further purify the IgG fraction a saturated solution of ammonium sulphate (AS) was added to an equal volume of alpaca serum at room temperature, mixed on a magnetic stirrer for 30 min, and then refrigerated at 4 °C overnight. The mixture was then centrifuged, the supernatant discarded and the pellet resuspended 0.9% sterile saline. This procedure was repeated once more before final filtration through a 0.22 μm sterilising filter. The IgG fraction was then stored in glass vials at 4 °C.

2.3. Monitoring of alpaca health post-venom immunisation

To ensure that the animal health implications of immunising alpaca with snake venom were fully investigated, a comprehensive health monitoring program was used for the first three venom immunisations. It was considered that the first three immunisations would be most likely to elicit adverse signs in the animals. To detect biochemical and haematological abnormalities, blood samples were collected immediately prior to immunisation and again 24 h after each of the first three immunisations. Animals were monitored clinically and had rectal temperatures recorded. Blood samples were submitted to a commercial veterinary pathology laboratory (Gribbles Veterinary Pathology, Clayton, Australia) for clinical biochemistry and haematology profiles including fibrinogen levels. Parameters of particular interest included, amongst others, the muscle enzymes creatinine kinase (CK) and aspartate transaminase (AST).

2.4. Venoms and treatment groups

Alpaca were randomly allocated to one of five treatment groups (Table 1). The groups were chosen to parallel current commercial antivenom products used in Australia and PNG. All venoms were obtained from a commercial venom supplier (Venom Supplies Pty Ltd, Tanunda, South Australia). Venoms were stored frozen at −20 °C as lyophilised powder until required for use. Tiger snake (*Notechis scutatus*) and eastern brown snake (*Pseudonaja textilis*) venoms were blended from multiple snakes to form a geographically representative pool as regional differences in venom have been observed (Flight et al., 2006). Papuan taipan (*Oxyuranus scutellatus canni*) venom originated from a collector in Merauke, Bali (supplied by Venom Supplies Pty Ltd). The Papuan taipan was chosen over the Australian coastal taipan because of its reportedly higher toxicity and greater neurotoxin content (Kornhauser et al., 2010).