Transactions of the Royal Society of Tropical Medicine and Hygiene 106 (2012) 731–737

Contents lists available at SciVerse ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Cross neutralisation of Southeast Asian cobra and krait venoms by Indian polyvalent antivenoms

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ARTICLE INFO

Article history:
Received 5 July 2011
Received in revised form 16 July 2012
Accepted 16 July 2012
Available online 11 October 2012

Keywords:
Snake venoms
Antivenoms
Bungarus
Naja

ABSTRACT

Cross neutralisation of venoms by antivenom raised against closely-related species has been well documented. The spectrum of paraspecific protection of antivenom raised against Asiatic *Naja* and *Bungarus* (krait) venoms, however, has not been fully investigated. In this study, we examined the cross neutralisation of venoms from common Southeast Asian cobras and kraits by two widely used polyvalent antivenoms produced in India: Vins Polyvalent Antivenom (VPAV) and Bharat Polyvalent Antivenom (BPAV), using both in vitro and in vivo mouse protection assays. BPAV was only moderately effective against venoms of *N. kaouthia* (Thailand) and *N. sumatrana*, and either very weakly effective or totally ineffective against the other cobra and krait venoms. VPAV, on the other hand, neutralised effectively all the Southeast Asian *Naja* venoms tested, as well as *N. naja*, *B. candidus* and *Ophiophagus hannah* venoms, but the potency ranges from effective to weakly effective. In an in vivo rodent model, VPAV also neutralised the lethality of venoms from Asiatic *Naja* and *B. candidus*. In anesthetised rat studies, both antivenoms effectively protected against the *N. kaouthia* venom-induced cardio-respiratory depressant and neuromuscular blocking effects. Overall, our results suggest that VPAV could be used as alternative antivenom for the treatment of elapid envenomation in Southeast Asian regions including Malaysia, Thailand and certain regions of Indonesia.

1. Introduction

Snake envenomations have been a serious yet often overlooked public health threat especially in tropical and subtropical countries, including Southeast Asia.[1,2] The medically important venomous land snakes in Southeast Asia include snakes from the Elapidae and Crotalidae families. Among the elapids, there are only 11 species that are considered of medical importance, represented by the Asiatic cobras (including *Naja kaouthia*, *N. sumatrana*, *N. siamensis*, *N. sputatrix*, *N. philippinensis* and *N. atra*), the king cobra (*Ophiophagus hannah*) and the kraits (*Bungarus candidus*, *B. fasciatus*, *B. flaviceps* and *B. multicinctus*).[3]

Antivenom is the only proven effective treatment for snake envenomation.[2] Many nations in Southeast Asia (with the possible exception of Thailand) however, either do not produce or do not have the supply of monovalent antivenoms against all the medically important venomous snakes found in the country. It will not be economically practical for each nation in Southeast Asia to produce its own monospecific antivenoms against all the relevant medically important venomous snakes. Furthermore, differential diagnosis of biting species is often impractical in Southeast Asia because of cost, and therefore polyvalent antivenoms are preferred over monovalent antivenoms in snakebite treatment. In view of this, the Global Snakebite Initiative (2011) has recently proposed the development

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http://dx.doi.org/10.1016/j.trstmh.2012.07.009

of a new Pan-Asian Polyvalent Antivenom.[4] However, the practicality of developing a polyvalent antivenom that can neutralise venoms from all the medically important venomous snakes in Asia is debatable. Casasola et al., for example, reported that an experimental antiserum raised against the African *N. melanoleuca* venom could neutralise most, but not all, African *Naja* venoms.[5] Petras et al. reported that the polyvalent antivenom EchiTAb-Plus-ICP could neutralise the lethality of venoms of the African spitting cobras *N. nigricolis*, *N. mossambica* and *N. pallida*, but not venoms of *N. katiensis* and *N. nubiae*.[6] In fact, our knowledge on the paraspecific protection of the Asiatic antivenom is far from complete. It is not known, for example, to what extent an antivenom raised against a certain Asiatic cobra venom can effectively cross neutralise venoms from other Asiatic cobra. Asiatic cobra venoms generally contain similar lethal toxins including three-finger toxins and phospholipases A_2. Cobra venoms from different geographical areas, however, have been reported to cause significantly different clinical effects.[7,8] A better and more complete understanding of the paraspecific neutralisation capacity of cobra antivenom raised against Asiatic cobras is required for the design of new broad-spectrum antivenoms against Asiatic cobras or elapids.

In this study, we investigated the cross neutralisation of venoms from various common Southeast Asia cobras and kraits by two widely used polyvalent antivenoms produced in India. The two polyvalent antivenoms examined were: Snake Venom Antiserum manufactured by Vins Bioproudcts Ltd (Andhra Pradesh, India). and Snake Venom Antiserum (Asian) from Bharat Serums and Vaccines Ltd (Mumbai, India). To date (2012), there is no scientific report regarding the cross neutralisation of Southeasst Asian venoms by these Indian polyvalent antivenoms, though the failure of the Bharat Snake Venom Antiserum to neutralise the African viper *Echis ocellatus* has been noted.[9] The findings of this study can be used to assess the therapeutic potential of these Indian antivenoms in snakebite treatment in Southeast Asia.

2. Methods and materials

2.1. Venoms and antivenoms

Venoms of *N. naja* (Sri Lanka, sample 1), *N. naja* (India, sample 1 and 2), *N. sputatrix*, *N. kaouthia* (Thailand), *N. siamensis*, *N. philippinensis*, *O. hannah*, *B. candidus* and *B. fasciatus* venoms were purchased from Latoxan (Valence, France). Venoms of *N. sumatrana* and *N. kaouthia* (Malaysia) were pooled samples obtained from the milking of adult snakes captured in Malaysia. *Naja naja* (Sri Lanka sample 2) venom was a pooled sample obtained from the milking of several adult cobras captured in Sri Lanka.

Vins Polyvalent Antivenom (VPAV) (full name: Snake Venom Antiserum I.P. Lyophilised, Polyvalent, Equine immunoglobulin; Batch no. 01082/10-11, exp. Date Nov 1st, 2014) was purchased from Vins Bioproduct Limited, Hyderabad, India. The antivenom is freeze-dried $F(ab')_2$, obtained from hyperimmunised horses immunised against venoms of Indian cobra (*N. naja*), common krait (*B. caeruleus*), Russell's viper (*Daboia russelli*) and saw-scaled viper (*Echis carinatus*). Bharat Polyvalent Antivenom (BPAV) (full name: Polyvalent Antisnake Venom Serum, or ASVS- Asian, Batch no. A5309049, exp. date 3/2013), was a gift from Bharat Serums and Vaccines Limited, Mumbai, India. BPAV is also $F(ab')_2$ obtained from hyperimmunised horses immunised against the same four Indian snake venoms mentioned above. For neutralisation studies, both antivenoms were reconstituted in the same manner: 10 ml of sterile water/normal saline was added to one vial of the freeze-dried antivenom. According to the attached fact sheet, 1 ml of both the reconstituted antivenom can neutralise approximately 0.6 mg of *N. naja* venoms.

2.2. Reagents and animals

All reagents and chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA) and were of analytical grade. Albino mice (ICR strain, 20-25 g) and male Sprague Dawley rats (220-300 g) were supplied by the Laboratory Animal Centre, Faculty of Medicine, University of Malaya. The animals were handled according to the guidelines given by CIOMS on animal experimentation.[10]

2.3. General methods

Protein was determined by Bradford method.[11] The median lethal dose, LD_{50}, of the venom was determined by intravenous and intramuscular injection of the venoms into four ICR mice (20–25 g). The survival ratio was recorded after 48 h.

2.4. In vitro neutralisation of lethality by the antivenoms

In vitro neutralisation of lethality was conducted as described by Ramos-Cerrillo et al.[12] Briefly, challenge dose of the venom in 50 μL saline was pre-incubated at 37 °C for 30 min with various dilutions of the reconstituted antivenom (VPAV or BPAV) in normal saline, to give a total volume of 250 μL. The mixture was subsequently centrifuged at 10 000 g before injected intravenously into the caudal vein of the mice. The number of survivors after 48 h was recorded. Generally, the challenge dose used was 5 $LD_{50.}$ However, if 200 μL of the reconstituted antivenom (maximum amount of antivenom that can be used in mice) failed to give full protection of the mice, a lower venom challenge dose of 2.5 LD_{50} was used instead. The antivenom was considered ineffective when none of the animals injected with the pre-incubated mixture survived. Neutralising potency of the antivenom was expressed as ED_{50} (the amount of reconstituted antivenom in μl or the ratio of mg venom/mL reconstituted antivenom that gives 50% survival of the animals tested) or 'potency' (the amount of venom that is completely neutralised by unit volume of antivenom) calculated according to Morais et al.[13]

2.5. In vivo neutralisation of venom lethality by Vins polyvalent antivenom in a rodent model

This was carried out by intramuscular injection of 5 LD_{50} (i.m.) or 2.5 LD_{50} (i.m.) of the venom into four mice followed by intravenous injection of 200 μL of appropriately diluted