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PRODUCTION AND PRELIMINARY CHARACTERIZATION OF ANTIBODIES AGAINST PROTEINS OF VENOM OF SRI LANKAN COBRA (*Naja naja*)

B.M.A.I.K. Bandaranayake[1], R.K.B.S. Vasanthakrisnan[1], T. Ariyadasa[1], J.G.S. Ranasinghe[1], S.B.P. Athauda[1] and N.R.K.B. Athauda[2]

[1]*Department of Biochemistry, Faculty of Medicine, University of Peradeniya,*
[2] *Base Hospital, Mawanella*

Introduction

The commonest venomous snake in Sri Lanka is cobra (*Naja naja*) and its venom is not characterized at the molecular level. The available antivenom produced against Indian snakes, Indian cobra (*Naja naja*), Common krait (*Bungarus caeruleus)*, Saw scaled viper (*Echis carinatus carinatus)* and Russell's viper (*Daboia russelli)* is a mixture of polyvalent antibodies (Theakston *et al.*, 1990). Although it has been used to treat snake bitten patients in Sri Lanka for 20 years, it is not effective against complete control of symptoms and fatality of snake bitten patients. There are many reports of severe antivenom reactions, including severe anaphylactic reactions with hypotension following treatment (Theakston *et al.*, 1990). Therefore it is very important to produce antibodies specific for major toxin proteins in venom of Sri Lankan snakes and do immunochemical characterization of them to compare the obtained results with the available antivenom imported from India.

Material and Methods

Fractionation of toxin proteins

Sephacryl S 200 column (2.7 x 36 cm) equilibrate in 0.02M phosphate buffer, 0.2 M NaCl, pH 7.5 was used to fractionate toxic proteins present in crude venom. Crude venom or standard proteins (100 μg) dissolved in 500μl of gel equilibrated buffer was applied into the column and the proteins were eluted with the same buffer and monitored by measuring the absorbance of each fraction at 280 nm. Fractions with proteins were further analyzed by SDS- PAGE.

Identification of toxin proteins

Molecular weight of each toxin protein was determined by SDS-PAGE. Different amounts (5-15 μg/ml) of crude venom proteins were separated on 17.5% SDS-PAGE under reducing and non reducing conditions. Molecular sizes of the resulting bands were determined by using standard proteins and plotting graph of the electrophoresis migration against the logarithm of molecular weight of standard proteins.

Approximate molecular weights of separated fractions were determined by calibrating the Sephacryl S-200 column with protein standards

Production of Antibodies

Cobra venom samples were collected and centrifuged at 12,000 rpm at 4^0C for 15 min. Proteins in the resulting supernatant were fractionated using Sephacryl S-200 gel filtration pooled into five fractions as described in the

gel filtration chromatogram (Figure 1 and Table 2). Rabbits were immunized with heat inactivated five separated fractions of venom of Cobra (I to V) and denatured crude venom-VI and venom-VII with Freund Adjuvant following a primary injection and two booster injection. Blood was collected after satisfactory booster injection. Antibodies were purified by Ammonium Sulphate precipitation followed by protein A-Sepharose chromatography.

Antivenom vials manufactured by VNS Bioproducts Ltd., (AV1) and Bharat Serums and Vaccines Ltd (AV2) in India were selected for the analysis.

Immunochemical characterization
Affinity of binding of produced antibodies and available antivenom (AV1 and AV2) with fractionated venom of cobra were analyzed by western blotting, dot blotting and agar gel diffusion method.

Results

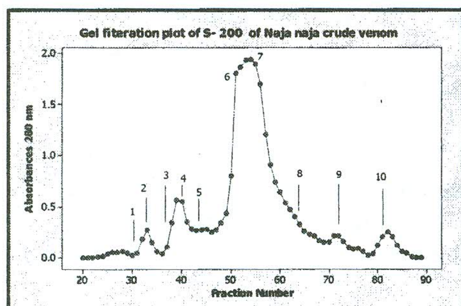

Figure 1. Sephacryl S-200 gel filtration chromatogram

Table 1. Approximate molecular weight of Sephacryl S-200 gel filtration fraction (Figure 1)

Fractions number	Molecular weight (kDa)
1	479
2	219
3	63
4	38
5	27
6	16
7	11
8	10
9	8
10	6

Table 2. Sephacryl S-200 gel filtration fraction used for immunization

Immunized Fractions	Fraction Number
I	1 & 2
II	3 & 4
III	7
IV	9
V	10

Table 3. Molecular weight of identified toxins protein in cobra venom

Molecular Weight (kDa)	Identification
99	Complement depleting factor
86	High molecular weight proteins
60	Metallo proteinases
46	Nerve growth factor
28	Cystein rich Proteins
16	Phospholipase A2
11.5	Cytotoxin

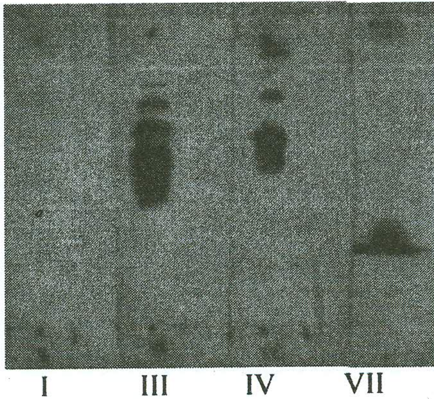

Figure 2. Western blot results of produced antibodies

Discussion

Results suggest that all produced antibodies specifically bind with respective venom proteins used to raise antibodies (Figure 2). Further Indian antivenom preparations, AV1 & AV2 bind with only low molecular weight protein fractions (Figure 3.) and not bond with other toxic venom proteins in venom.

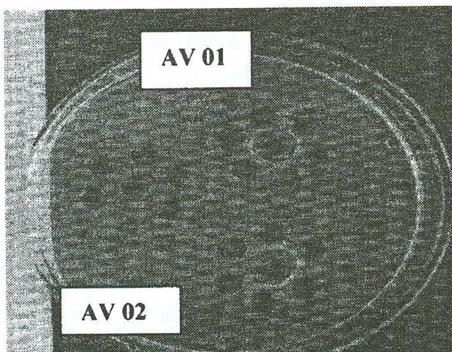

Figure 3. Affinity Binding of Indian antivenom with immunized fraction number III.

Conclusion

Immunochemical analysis results suggest that all produced antibodies against fractionated toxins specifically bind with respective venom proteins used to raise antibodies. Further binding patterns of Indian antivenom preparations AV1 and AV2 explain the less effectiveness of Indian antivenom for treatment of snake bitten patients in Sri Lanka. Further studies are in progress to clarify the above.

References

Theakston, R.D.G., Phillips, R.E., Warrell, D.A., Galadedara, Y., Abeysekara, D.T., Dissanayake P, de Silva A and Aloysius, D.J. (1990). Envenoming by the common krait (*Bungarus caeruleus)* and Sri Lankan cobra (*Naja naja naja*): Efficacy and complications of therapy with Haffkine antivenom. Transactions of the Royal society of Tropical Medicine and Hygiene, 84:301-308.