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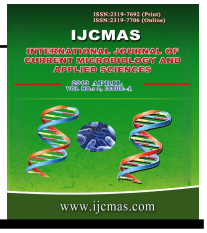

Original Research Article

Raising Antibody against Snake Venom (*Naja naja*) in *Gallus domesticus* - Drug Discovery Approach

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K e y w o r d s

Snake Venom; *Naja naja; Gallus domesticus.*

A B S T R A C T

Snake (*Naja naja*) venom was introduced in different experimental groups in different doses in *Gallus domesticus*. After the experimentation duration the blood serum was collected from the animals. The synthesized proteins (antibodies) in the serum samples were identified by SDS – PAGE electrophoresis. Different protein bands such as 66, 97, 116 KDa proteins were identified in all experimental and control groups. 18.4 KDa and 19 KDa proteins were synthesized as antibodies by the experimental animals against snake venom.

Introduction

Venomous snake bite is an important public health hazard in tropical and subtropical countries. Snake bite is a severe life threatening medical emergency often faced by farmers, farm labourers and villagers. In India 35,000 – 50,000 lives are lost per year due to venomous snake bite. First commercial antivenom production begun in Australia (1930) against tiger snake venom. This was followed by other antivenoms for other important species such as taipan, brown snake, death adder, Papuan black snake, seasnake in the world. More recent activity has focused on veterinary antivenoms and production of new generation human antivenoms for export (CroFab and ViperaTAB). In the present investigation is aimed to raise antibody against snake venom in fowls a drug discovery approach.

Poultry - *Gallus domesticus*

Chickens are often favored because of their phylogenetic distance from mammals (Igor *et al*., 2010). Chickens transfer high quantities of immunoglobulin (IgY), into the egg yolk, which eliminates the need for invasive bleeding procedures. However, as the antibody is contained in egg yolk, purification must be undertaken before the antibody can be used in assays (Polson *et al*., 1980).

Indian Cobra and Venom

Naja naja is a species of venomous snake found in the Indian subcontinent. It is one of the big four, species which are responsible for causing the most snakebite cases in India. The Indian cobra's venom has a powerful post-synaptic neurotoxin. The venom acts on the synaptic gaps of the nerves, thereby paralyzing muscles, and possibly leading to respiratory failure or cardiac arrest (Achyuthan and Ramachandran, 1981). The venom components contain enzymes such as hyaluronidase that cause lysis and increase the spread of the venom (Theakston *et al*., 2003).

Materials and Methods

Collection of snake venom

The freeze-Snake venom powders of *Naja naja* were obtained from Irula's Snake Catchers Industrial Co-operative Society Limited, Perur, Tamil Nadu and it was stored at 4^{o}c.

Experimental animals

Adult *Gallus domesticus* breed chickens were procured from Pioneer Pvt. Ltd. Kumanan chavadi, Chennai - 77 and were maintained at Biomedical Research Unit and Lab Animal Centre (BRULAC), Saveetha University, Chennai. All the animals were maintained in standard cages and food and water were provided.

Antigen preparation

The antigen must be non-toxic and it must be prepared aseptically, or otherwise rendered sterile and free of toxins and pyrogens. The pH must be adjusted within physiological limits. The most commonly used adjuvants include: Freund's Incomplete Adjuvant (FIA) or Freund's Complete Adjuvant (FCA) Mineral-based adjuvants (Leskowitz *et al*., 1966).

Complete Freund's Adjuvant

FA, a water-in-oil emulsion having heat-killed mycobacteria or mycobacterial cell wall components, is an effective means of potentiating cellular and humoral antibody response to injected immunogens. Adjuvant activity is a result of sustained release of antigen from the oily deposit and stimulation of a local innate immune response resulting in enhanced adaptive immunity. This response is an intense inflammatory reaction at the site of antigen deposition resulting from an influx of leukocytes and their interaction with antigen. This adjuvant may result in local inflammation and granulomatous reactions at the injection site. The mycobacteria in CFA is resuspended by vortexing the vial.

Immunization

Injection site selection and preparation

Chicken may need sedation with 1 mg/kg of Acepromazine IM approximately 15 minutes prior to immunization. Sterile syringes and needles are used to minimize microbial contamination of injected tissues (Tables 1-4). Injection sites should be sufficiently separated (at least an inch apart) to avoid overlapping of inflammatory lesions if they develop. Booster injection sites are distanced from previous injection sites.

Blood Collection

To obtain red blood cells, the blood is collected into anticoagulant vials. The cells are washed and used to test for the

presence of virus in the heam agglutination test. They are also used in the heam agglutination inhibition test for the presence of antibodies.

Double immunodiffusion

A total of 0.5 g agar was boiled in 100 ml water by using microwave oven. The agar solution was allowed to cool to 50˚C and then 2 ml of it was taken onto each microscope slide placed on a leveling table. The agar was then allowed to solidify and then left overnight at room temperature or for four to six hours at 40˚C until completely dry. Preparation of microscope slides Agar (1g) was boiled in 100 ml water using microwave. The 1% agar solution was then allowed to cool to 45˚C and then 3.5 ml of it was pipetted onto a precoated slide. The slides were left to solidify at room temperature after which four holes were punched out by using a gel puncher. The plugs of agar were removed from each well by using a Pasteur pipette attached to a vacuum line. The test was conducted by pipetting 16 µl *Naja naja* venom (10 mg/ml) and 16 µl blood serum (100 mg/ml) into wells 1 and 2 respectively followed by incubation overnight at room temperature in a humid chamber. For its fraction MP188ECT3 at a concentration of 10 mg/ml was used. Slides were examined for immunoprecipitin lines.

SDS-PAGE analysis

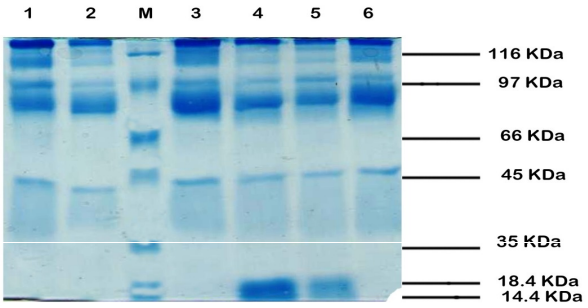
The total cell and outer membrane proteins from *Pseudomonas aeruginosa* were analyzed bysodiumdodecyl sulfate polyacrylamide gel electrophoresis. The reagents used for SDS-PAGE analysis were from LifeTeck SDS-PAGE kit.

Results and Discussion

Antibodies are typically produced by immunization of a suitable mammal, such as a mouse, rabbit or goat. Larger mammals are often preferred as the amount of serum that can be collected larger. An antigen is injected into an animal, it induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This polyclonal IgG is purified from the mammal's serum.

The identified protein bands through SDS-PAGE electrophoresis were compared with marker proteins and molecular weight of the new proteins were identified. Different protein bands such as 66, 97, 116 KDa proteins were identified in all experimental and control groups. It reveals that these proteins were present in normal blood serum. In the present study, no band was observed in control group as well as experimental control group. In snake venom introduced experimental group, two protein bands (18.4 and 19 KDa were observed. It clearly indicates that the protein 18.4 and 19 KDa were synthesized as antibodies by the experimental animals against snake venom (Figure 1).

Figure.1 SDS-PAGE analysis of Venom



Lane 1 : Control
Lane 2: Exp. control
Lane M: Protein Marker (14.4 - 116 KDa)
Lane 3,4,5 &6: Samples 1, 2, 3 &4

Table.1 Injection site selection and preparation in first term

First term						
Group	**Chicken no**	**Inject period**	**Cobra venom concentration**	**Dose level**	**Adjuvant**	**Test bleed**
1	Control	0-day	-	-	-	-
2	Experimental Control	0-day	Only PBS+adjuvant	0.25ml	Freunds complete	-
3	1 & 2	0-day	200µg	0.25ml	Freunds complete	-
4	3 & 4	0-day	200µg	0.5ml	Freunds complete	-
5	5 & 6	0-day	200µg	0.4ml	Freunds complete	-
6	7 & 8	0-day	200µg	0.1ml	Freunds complete	-

PBS - Phosphate buffer saline

Table.2 Injection site selection and preparation in second term

Second term						
Group	**Chicken no**	**Inject period**	**Cobravenom concentration**	**Dose level**	**Adjuvant**	**Test bleed**
1	Control	0-day	-	-	-	BC
2	Experimental Control	0-day	Only PBS+adjuvant	0.25ml	Freunds complete	-
3	1 & 2	14-day (Boosted)	400µg	0.2ml	Freunds incomplete	BC
4	3 & 4	14-day (Boosted)	400µg	0.2ml	Freunds incomplete	BC
5	5 & 6	14-day (Boosted)	400µg	0.2ml	Freunds incomplete	-
6	7 & 8	14-day (Boosted)	400µg	0.2ml	Freunds incomplete	-

PBS-phosphate buffer saline; **BC**-blood collect

Table.3 Injection site selection and preparation in third term

Third term						
Group	**Chicken no**	**Inject period**	**Cobravenom concentration**	**Dose level**	**Adjuvant**	**Test bleed**
1	Control	0-day	-	-	-	-
2	Experimental Control	0-day	Only PBS+adjuvant	0.25ml	Freunds complete	-
3	1 & 2	$24,25^{th}$ –day (Boosted)	1mg/ml	0.1ml	Without adjuvant	-
4	3 & 4	$24,25^{th}$ –day (Boosted)	1mg/ml	0.1ml	Without adjuvant	-
5	5 & 6	$24,25^{th}$ –day (Boosted)	1mg/ml	0.1ml	Without adjuvant	BC
6	7 & 8	$24,25^{th}$ –day (Boosted)	1mg/ml	0.1ml	Without adjuvant	BC

PBS-phosphate buffer saline; **BC**-blood collect

Table.4 Injection site selection and preparation in fourth term

Fourth term			
Group	**Chicken no**	**Sample Collection**	**Test bleed**
1	Control	34^{th} day	BC
2	Experimental Control	34^{th} day	BC
3	1 & 2	34^{th} day	BC
4	3 & 4	34^{th} day	BC
5	5 & 6	34^{th} day	BC
6	7 & 8	34^{th} day	BC

BC - blood collect

One of the important aspects of assessing the cause of death in snake-bite victim is by detection of the snake venom antigens in the specimens collected from the victim. Most frequently, specimens which are sent to the Forensic Laboratory for analysis include kidneys, liver, viscera, blood, skin, lungs, heart, brain, spleen, bladder, bone and ear. Previous reports have established that the order of distribution of snake venom in different organs of the body such as, site of injection or skin, heart, liver, kidneys, lungs, spleen and brain. Post-mortem cardiac blood has been suggested to be the most suitable specimen for venom detection. Thus, skin (tissue at the bite area) and blood are the suitable source of biological specimen for the analysis of snake venom (Brunda and Sashidhar, 2007).

In the fields of medicine, biotechnology and pharmacology, drug discovery is the process by which drugs are discovered and/or designed. Most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. The process of drug discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Once a compound has shown its value in these tests like the synthesized antibodies against antigenic substance, it will begin the process of drug development prior to clinical trials (Feher and Schmidt., 2003). Polyclonal antibodies (IgY) extracted from egg yolk after inoculation of chickens with cobra venom revealed that IgY is functionally equivalent to IgG of equines in both yield and potency of venom neutralization (Rajendra Prabhu, *et al*., 2009).

Drug design, also sometimes referred to as rational drug design, is the inventive process of finding new medications based on the knowledge of the biological target (Madesen *et al*., 2002). The present investigation clearly indicates that the two proteins were synthesized by the animals as antibodies against the antigenic substance snake venom. Hence these proteins may be used against snake bite as drug.

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