Effect of Chemical Agents on Venom of Bungaruscaeruleus / Indian Common Krait

Anju Dhir

Lecturer of Microbiology
SIN, Shimla
Pursuing PhD, Department of Biotechnology, MVGU, Jaipur

***Abstract*- Many workers have worked on venom of Naja, Russel's viper, Agkistrodom, Crotalus and Echis from time to time. But the venom of common krait has not been studied at large. In the present study common krait venom has been treated with various physical and chemical agents so as to see their effect on the toxicity and immunogenicity of the venom and to see whether the toxins present in the krait venom are converted into toxoids or the venom component are simply denatured. Venom was found to be denatured by treatment with glutaraldehyde. Action of Iodine is less chaotic and still retained immunogenic components. Formaldehyde showed lesser action and fair amount of immunogenicity has been retained by venom treated with formaldehyde. The action of H_2O_2 has been best so far as far as the immunogenicity of the treated venom is concerned.**

***Keywords* -Bungaruscaeruleus, Formaldehyde, glutaraldehyde, Iodine, hydrogen oeroxide**

INTRODUCTION

Snakes are limbless vertebrates and belong to the class of living creatures known as reptiles. They are scattered all over the world. Snakes like heat, it means life to them therefore the snakes are most abundant in the tropical and semi tropical regions of the globe. More than 2,000 different types of snakes exist and nearly 400 are known to be venomous. More than 40,000 people die every year from snake bite in India. The annual mortality rate from snake bite is more in India i.e. 46,000, out of this about 12,000 die because of snake bite [1].

The common four species responsible for these casualties belong to two families of snakes: elapidae and viperidae. The venom of elapidae is neurotoxic and that of viperidae is cadiotoxic. The common krait and cobra belong to elapidae family, whereas Russel's viper and *Echiscarinatus*belong to viperidae family.The venom of elapids is neurotoxic and that of viperidae is cardiotoxic. The Indian common krait and cobrabelong to elapidae family. The venom of *Bungaruscaeruleus*has been analysed to lesser degree whereas the venom of allied group namely, the common cobra has been analysed up to the level of amino acids. Burma DMR working group in 1986 used formalin to make toxoid of Russel's viper venom and obtained good results when tested by intramuscular inoculation in monkeys [2].

In 1980, a coordination committee on venoms and antivenom sera was convinced by WHO and the progress on the characterisation of venoms has been published in the form of an offset [3]. In this publication also, not much has been discussed on the characterisation of common krait venom. This venom had not been includedin the list of the international preparationsof reference pools of snake venoms for characterisation. Therefore this study was undertaken to characterise the venom of this potentially dangerous snake.

The proteins are the main components of snake venom and can be grouped as-
1. Proteins with toxic principles.
2. Proteins with enzymatic activities.
3. Proteins with no known biological activities.

The need for prophylaxis with snake toxoids was felt as people observed hypersensitivity reactions with horse serum anti venom. For the first time Sewaidetoxified the venom of *B.multicinctus* by using dihydrothiotic acid (DHTA) at 37°C [4]. Rawalalso showed that when snake venomis subjected to physical or chemical agents, some of the native protein components of venom are lost like venom loses its toxicity and hemolytic activity and iodine destroys the proteolytic clotting and hemolytic activity of the venom [5].

In the previous study the effect of pH on the toxicity of venom was studied [6]. It was observed that the venom loses its toxicity at high pH like 9 or when kept at pH 5. Another attempt was to observe the effect of temperature by author [7]. The high temperaturemakes the venom lose most of the toxic and immunogenic components. In the present study, the author has tried to observe the effects of chemical agents on Indian common krait venom.

AIMS AND OBJECTIVE

- This venom had not been includedin the list of the international preparationsof reference pools of snake venoms for characterisation. Therefore this study was undertaken to characterise the venom of potentially dangerous snake. The venom is neurotoxic in nature.
- An attempt to observe the effect ofchemical agents on the toxicity and immunogenicity of the krait venom in order to make it less toxic for inoculating in laboratory animals in which antisera is raised against the venom while retaining immunogenic components.

MATERIAL AND METHOD

Materials

1. Venom: The common krait /*B.caeruleus*venom used in this study was obtained from Central Research Institute Kasauli& the normal toxicity of this venom were checked before starting these experiments.

2.Chemicals: Formaldehyde solution, glutaraldehyde solution, iodine solution, hydrogen peroxide solution- each 10mM concentration were prepared in sterile distilled water according to Cruickshank [8].
4.Slides- Glass slides of 20 ×5 cm were used for immunodiffusion tests.

Methods

Experimental method was used throughout experiments. Toxicity was checked by inoculating intravenously in mice and LD_{50} was calculated.
For observing immunogenic components, immunodiffusion test was used, followed by staining of slides.

In all the above mentioned experiments the toxicity tests were observed after every 7 days interval for 28 days and immunological components were observed by immunodiffusion tests.

The venom was mixed with equal quantity of different chemicals like formaldehyde, glutaraldehyde,iodine, hydrogen peroxide.The vials were kept at 4°C for 9 days. The aliquots were takenafter1 day, 3 days, 5 days, 7 days and 9 days. The toxicity and any change in immunological components were noted.

RESULTS

Effect of different chemicals showed that glutaraldehyde has a chaotic action on toxicity as well as immunogenicity of krait venom. Venom was found to be denatured by treatment with glutaraldehyde. Action of Iodine is less chaotic and still retained immunogenic components. Formaldehyde showed lesser action and fair amount of immunogenicity has been retained by venom treated with formaldehyde. The action of H_2O_2 has been best so far as far as the immunogenicity of the treated venom is concerned.

Figure - Effect of chemicals on immunogenicity of *Bungaruscaeruleus* Venom

1- Untreated venom 2--- Venom kept in hydrogen peroxide

3-- Venom kept in formaldehyde 4--- Venom kept in iodine

5-- Venom kept in glutaraldehyde 6--- Anti krait venom Serum

Table1: The effect of chemical agents on toxicity and immunogenicity onvenomof *B. caeruleus*

(Indian common krait)

Interval after start (days)	Visible change if any	Solutions of venom treated with				
		Normal venom solution control	Formaldeh -yde	Hydrogen peroxide	Iodine	Glutaralde-hyde
0	No change	1.6384	1.781	1.781	1.781	1.781
1	no change	1.6384	1.992	2.707	3.478	195.66
3	no change	1.6384	2.473	13.40	14.69	128.00
5	turbidity	1.7660	14.90	14.02	168.30	184.10
7	turbidity	1.0781	125.00	179.1	176.8	1000
9	turbidity	1.781	173.6	181.3	195.66	1000
immunogenicity after 9 days	Number of components	7	6	5	2	0

Table2.Effect of chemical agents on toxicity and immunogenicity onvenomof *B. caeruleus*

(Indian common krait)

Property		Venom Exposed to				
		Normal venom control	Formaldehyde	Hydrogen peroxide	Iodine	Glutaraldehyde
Toxicity %	R	100	1.03	0.98	0.91	0.18
	L	0	98.97	99.02	99.08	0.18
Immunogenicity %	R	100	85.71	71.42	28.57	0
	L	0	14.29	28.58	71.43	100

Abbreviations: R- retained, L- lost

DISCUSSION

A number of properties of protein change when they are subjected to physical or chemical agents. When the venom was exposed to variouschemical agents like formaldehyde, hydrogen peroxide, glutaraldehyde and iodine, they reduced its toxicity whereas immunogenicity was retained. Table 2, shows effect of group specific reagents.

The effect of chemical agents on the toxicity and immunogenicity was studied in this work to see whether the toxins present in the Indian common krait venom are converted into toxoids or the venom component are simply denatured? Forthis purpose gel diffusion test was used to observe the immunogenicity of venom. Lethal toxicity of Indian common krait venom was studied in mice by inoculating intravenously. Hydrogen peroxide is found to modify the protein structures. The venom is made up of protein so it is affected by the action of chemicals. The chemicals reduce toxicity of venom.

Iodine is an oxidising agent as well as electrophilic agent. It is not selective in its action. It oxidises the peptide chain in the venom. This reduces the antigenicity when exposed to prolonged treatment.

Aldehydes are good reducing agents. Formaldehyde has turned venom into toxoid/venoid and toxicity is reduced by 99% yet retaining 86% immunogenicity. Hydrogen peroxide reduced the toxicity to 99% but immunogenicity is lost up to 29%. Iodine has reduced toxicity upto 99% but loss of immunogenicity is 71.43%.

Glutaraldehyde is not good for this purpose as it has practically denatured the venom and toxicity as well as immunogenicity is destroyed. Formaldehyde turned venom into toxoid as it reduced toxicity but retained immunogenicity. So it can be used for converting venom into toxoid/venoid. Further research is required.

According to the experts committee WHO,it seems that this species of snakes is considered as less important as it has not been included in the paper. There are not many results available on the characterisation of venom from *B. caeruleus*. The present study can contribute in this field too.

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