

Producing Anti-venom for Cobra Toxin using Embryonal Carcinoma Cells

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Anti-venoms are very important requirement in the medicine world in the countries like India which is full of a number of poisonous snakes like Cobras. At present anti-venoms are produced by milking the snake to obtain its venom and injecting this venom into other animals to raise the antibody against it, which is very low in quantity, and eventually this antibody is marketed at very high price as the resources are very limited. In order to address this problem, the proposal is to express the protein (venom) in human Embryonal Carcinoma Cells (hECCs) and after isolation of this venom from hECCS and to raise the antibody *in vitro*.

Introduction

α -cobra toxin is a venom of Cobras, a species of snake of *Naja* genus, which is highly toxic. It is a Nicotinic Acetylcholine Receptor Antagonist which attacks the central nervous system (CNS) and causes paralysis in the victim. The venom produced by these snakes is a mixture of proteins, carbohydrate, and other substances.

In order to survive the fang of this deadly venomous snake, Anti-venom is required. Anti-venom is antibody against the poison which is raised in the body of horse, rabbit, donkey, cats, chickens, rodents, mice etc. Venom is milked by the Cobra and processed and injected in the body of the other animal for antibody production. The antibody is extracted and used as an Anti-venom in the market. All of this process including milking the snake, immunising other animals against the venom, causes a lot of health problem to both the snake and the host animal. While this milking process venom gland is pressed from outside and sometimes electrical signal is also given. Injecting other animals with the venom causes harm to the body as this is injected to various site in the body. Anti-venom produced in this mentioned way is very few in amount. Hereby it is proposed that instead of using venom of cobra, amplify the genetic sequence coding this venom and allowing it to express in Embryonal Carcinoma Cell (ESCs) [1] and raising the antibody against this venom *in vitro*. Embryonal carcinoma cells are the subpopulation of stem cells with the properties of cancerous cells. It has a capacity of dividing and proliferating for a longer number of times as compared to other differentiated cells. As these cells possess the cancer cell like properties so it is quite probable that these cells will sustain the toxicity produced by the venom. Using these cells, no organism will be exploited and moreover the amount of antigen produced will be much higher in amount. Eventually it may reduce the cost of anti-venom also which is available in the market [2].

Materials and method

Cells will be taken from snake and the chromosomal DNA will be isolated. The particular gene cobra venom has been sequenced already [3]. Specific primers will be designed and using PCR this

gene will be amplified. Using a potent promoter like actin in a mammalian expression vector, this gene will be ligated. Now this ligated product which is vector and our cobra toxin gene needs to be transfected in a Human Embryonal Carcinoma Cell (hECCs). hECC need to be cultured in Stem cell medium [3]. Allow these cells to proliferate. Since the cells are dividing the cobra toxin gene express its product which is the venom of our interest. After some days isolation of the protein of interest (cobra toxin) using some protein isolation techniques will be done [4]. Using mass-spectrometry, the obtain product will be compared with the database already present like (SPDB). Now this protein which is cobratoxin will be used as our antigen to raise antibody *in vitro*. To raise antibody *in vitro*, a technique called *in vitro* immunization will be used [2]. Purification of antibody will be done using affinity chromatography. Ag-Ab interactions will also be checked using various assays like double diffusion method etc. of the charge distribution.

Controls and limitations

Transfecting hECCs with cobratoxin may have two probabilities. First is the hECCs will sustain the toxin and in that case we can proceed to next step or in second case hECCs will not survive and in that case we will have to optimise the toxin concentration by studying the extent of toxicity. Above all, it may also happen that hECCs will not express the cobratoxin, and in that case we will have to use more efficient expression vector.

Conclusions and recommendations

Snake bites constitute 0.5% of the total deaths in India and as previously discussed this number is drastically deviates from reality. Snake bite deaths are underestimated and unacknowledged. This menace can be curbed by creation of co-operative societies on a large scale like the Irula society in Tamil Nadu. This will ensure safe and supervised large scale collection of venom. There is also a desperate need for superior techniques for making anti-venoms. It is recommended that ASV should be made available in the public as well as private sector, clinics/hospitals particularly in the rural areas where snake bites

are prevalent.

Although anti-venom is being produced using other techniques in the country which makes the anti venom for common people very expensive as it requires many resources. Using this proposal in

5 which hECCs are being used as a system, the amount of anti-venom produced will be much higher and hence the cost will be less expensive using lesser sources and without exploiting any wild animals.

Notes and References

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