

Snake venom as Anticancer agent- Current Perspective

Aarti C¹ and Khusro A^{2*}

¹Department of Biotechnology, M.S.Ramaiah College of Arts, Science & Commerce, Bengaluru, India

²Department of Plant Biology & Biotechnology, (PG. Biotechnology), Loyola College, Nungambakkam, Chennai, India

*Corresponding Author E-mail: armankhan0301@gmail.com

ABSTRACT

Cancer is one of the leading cause of death worldwide. There is urgent need to search for new drugs from the natural products. In recent years remarkable progress has been made for the treatment of cancer. The major drawback of the current methods of cancer treatment is the resistant to the therapies after initial treatments. It has led to the increased use of anticancer drug developed from natural sources. The biodiversity of snake venom is a unique source from which novel therapeutics can be developed. Snake venoms toxins contributed significantly to the treatment of cancer. Some of the compounds found in snake venom present a great potential as antitumor agent. In view of this, we presented the recent years of research involving the active compounds of snake venoms that have anticancer activity.

Keywords- Anticancer agents, Apoptosis inducer, Cancer, Snake venom.

INTRODUCTION

Snake venoms are the secretion of venomous snakes, which are synthesized in venom glands. Snake venoms are complex mixture of enzyme, toxins, nucleotides, proteinaceous and peptidyl toxins^{1,2}. About 90% of venom's dry weight is proteinaceous. These proteins may be toxic or non-toxic in nature. Snake venoms contain a number of bioactive substance which have pharmacological importance. It is a natural source of neurotoxic, cardiotoxic, cytotoxic and many other different active compounds³. Snake venoms are produced in venom glands throughout life of the snake which is collected with scientific approaches and techniques⁴. Different species of snake have different types of venom depending upon its location, habitat, age etc. The concentration of secreted venom from glands depends upon the climate and season also⁵. Snake venom is harmless when it is ingested but it produces toxicity when it is injected into the blood. It is a viscous and transparent liquid, which can be dried in the form of crystals⁶. Snake venoms have been classified as 3 groups (Cytotoxin, Neurotoxin and Hemotoxin) according to its mode of action and effects⁷. Neurotoxins target central nervous system causing heart failure and breathing problems. They have the ability to inhibit ion movement across the cell membrane and blocks the communication between neurons⁸. Hemotoxins are the toxins that cause destruction of RBC. It affects cardiovascular system and blood functions. It also targets the muscle tissue of the host. Cytotoxic venoms target specific cellular sites or muscles. Cobra, Kraits, Sea snakes contain neurotoxic venom whereas Rattle snake and Copper heads have hemotoxic venoms. Snake bite results in subcutaneous injection of venom into the prey which results in local and systemic effects. The local effects include hemorrhage and dermonecrosis. Systemic toxicity includes myotoxicity, anticoagulant activities and hypotensive^{9,10}. Enzymes present in snake venom hydrolyse proteins and membrane components leading to blood clotting and tissue necrosis. Cancer is one of the major public burden all over the world. It is a complex disease which may occur due to genetics, environmental factors, radiation, carcinogens etc. it is a multigenic and multi-cellular disease with a multi-factorial etiology.¹¹ Various therapies like chemotherapy, radiotherapy, immunotherapy and gene therapy have been used for the treatment of cancer. The major drawback of chemotherapy is that

patients eventually gets resistant after some times¹². Radiation therapy being an important part of cancer treatment, there is less rate of successful treatment because of the exposure of radiation on both the cancer cells and surrounding normal cells which results in late radiation toxicity¹³. Immunotherapy do not play a significant role in the death of the cancer cells because it serves only to direct immune effectors to the tumor cells¹⁴. It only enhances the immune system to overcome cancer cells rather than killing the tumor cells. Now a days cancer treatment is a major challenge worldwide. Surgery, chemotherapy and radiotherapy are very costly and have numerous side effects. Present methods of treatment affects normal cells as well as tumor cells. It leads to discovery of cancer cure from natural products. Some compounds of snake venoms have the capability to retard the growth of cancerous cells. The mode of action of snake venom is similar to the medicines. This property of the venom made it a potential product as a therapeutic agents. Keeping in view about the future use of venom in pharmaceutical fields, it will generate a new era for the treatment of cancer. The purpose of this article is to review recent findings regarding use of snake venom as an anticancer agent.

BASIC COMPOSITION OF SNAKE VENOM

Snake venoms are complex mixture of peptides, proteins, chemicals and inorganic cations (Ca, K, Mg, Na etc.). Snake venoms also contain carbohydrates, lipids and free amino acids.¹⁵ Snake venoms contain at least 25 enzymes in varying combinations and concentration, but more of them contain all of them. Higher catalytic efficiency, thermal stability and resistance to proteolysis make these enzymes attractive models for every research¹⁶.

1) Hyaluronidase

It is an endoglycosidase that degrades the beta- N- acetyl- glucosaminidic linkages in HA polymer¹⁷. It is commonly known as “Spreading factor” in venom. It is not only involved as spreading agent but also it is required as a therapeutic agent for inhibiting the systemic distribution of venom and also for minimizing local tissue destruction at the site of bite¹⁸. The major function of this enzyme is to damage the extracellular matrix at the site of bite leading to severe morbidity. Hyaluronidase isolated from snakes and other venoms are proteinaceous in nature. These are neutral enzymes as they exhibit measurable activity at around pH 8. At pH 7, the testicular enzyme exhibits *in vitro* transglycosylase activity¹⁹. Venom hyaluronidase, apart from causing tissue damage and spreading, it also leads to systemic effects.

2) L-amino acid oxidase (LAAO)

It represents 1-9% of total venom protein²⁰. This enzyme is also called as ophio- amino-acid oxidase. LAAO are the flavoenzymes that catalyze the stereospecific oxidative deamination of an L-amino acid substrate to corresponding a ketoacid with hydrogen peroxide and ammonia production²¹. This enzyme belongs to oxidoreductase family and it has affinity for hydrophobic amino acids²². This enzyme is responsible for the yellow colour in snake venoms. LAAO are widely present in Viperidae, Crotalidae and Elapidae family²³.

3) Phospholipase A2

Snake venoms are the richest sources of PLA2. Snake venoms are complex mixture of active proteins or peptides belonging to calcium ion dependent secretory PLA2, which serve as digestive enzyme as well as defense weapon by immobilizing the prey²⁴. It is of two types- 1 PLA2 and 2 PLA2. IPLA2 is found in Cobras, Kraits and Sea snakes whereas 2PLA2 is present in Viper and Pit viper²⁵. Phospholipase A2 constitute major components of snake venoms which display a variety of relevant toxic actions such as neurotoxicity, cytotoxicity, cardiotoxicity, hypotensive and proinflammatory effects^{26,27}.

4) Cholinesterase

Cholinesterase is one of the enzyme present in snake venom which targets the nervous system. Its high reactivity towards organophosphorus compound suggests that exogenous cholinesterase can serve as an effective therapeutic agent in the treatment of prophylaxis and organophosphorus poisoning²⁸.

5) Collagenase

Collagenase is a proteinase enzyme which is specific for digesting the collagen. Collagen present in snake venom digests mesenteric collagen fibers²⁹.

6) Thrombin- like enzymes

These enzymes are glycoprotein in nature which acts as anticoagulants *in vivo* and *in vitro* they clot plasma and purified the fibrinogen. These enzymes have got more attention due to its action as defibrinating agent. Some examples of thrombin- like enzymes are crotalase, ancrod and batroxobin which can be purified from snake venoms. Carotalase play a role in the formation of fibrin in burns in the animals. Ancrod and Batroxobin have been employed to remove the fibrinogen³⁰.

7) Other enzymes

Proteolytic enzymes, RNase, DNase, Polypeptides and Lactate dehydrogenase have also been found in different snake venoms.

ROLE OF SNAKE VENOM AS AN ANTICANCER AGENT

Khunsap *et al*³¹ reported that *C. albolabris* and *C. rhodostoma* venoms showed similar cytotoxic effects on KATO-III, SW620, BT474, ChaGO and Hep-G2 cancer cells. Both venoms showed higher potency on KATO-III and BT474 cells than the anti-cancer drug. *C. albolabris* venom had high percent Sub-G1 to Hep-G2 and BT474 cells. *C. rhodostoma* venom had potent on SW620 and KATO-III. Zhang *et al*³² reported that ACTX-6 could induce cell apoptosis. The main factor responsible for apoptosis is reactive oxygen species (ROS). Naumann *et al*³³ isolated and purified L-amino acid oxidases (LAAOs) from *Bothrops leucurus* (BI-LAAO) and reported its effect on platelet function and cytotoxicity. Cytotoxicity of BI-LAAO was determined in the stomach cancer MKN-45, adenocarcinoma HUTU, colorectal RKO and human fibroblast LL-24 cell lines. Kim *et al*³⁴ purified the venom of *Ophiophagus hannah* and determined the cytotoxic componenets in purified venom. The cytotoxic activity of L-amino acid oxidase was obtained in stomach cancers, murine melanoma, fibrosarcoma, colorectal cancer and Chinese hamster ovary cell lines. *In vitro* and *in vivo* antitumor activity of p-bromophenacyl bromide modified bothropstoxin-I (BPB-BthTx-I) isolated from *Bothrops jararacussu* venom was reported by Gebrim *et al*³⁵ who determined the susceptibility of tumor cell lines against BPB-BthTx-I. Song *et al*³⁶ reported the antitumor activity of snake venom toxin from *Vipera lebetina tunica* against ovarian cancer cells through the inhibition of NF-KB and STAT 3 signal. This toxin increases the expression of pro-apoptotic protein Bax and Caspase-3 but down-regulates the anti-apoptotic protein Bcl-2. Debnath *et al*³⁷ demonstrated the cytotoxic effect of venom on human leukemia cells (U937/K562). According to Chien *et al*³⁸ apoptosis was induced by activation of both ER pathway and mitochondrial death pathway which results in increased level of Ca²⁺ and glucose-related protein 78 (GRP78). Disintegrin has the ability to inhibit the tumors. Salmosin (a disintegrin) isolated from Korean snake venom had the capability to suppress growth of metastatic tumor and solid tumor in mice.³⁹ A homodimeric disintegrin, Contortrostatin (CN) present in Southern Copperhead snake venom, was studied on OVCAR-5 (human epithelial carcinoma cell line of ovary) cells for its anticancer activity. Contortrostatin blocks the adhesion of OVCAR-5 cells to several extracellular matrix proteins and inhibit tumor cell invasion through an artificial basement membrane⁴⁰. Gomes *et al*⁴¹ purified the heat stable protein toxin called drCT-1 from *Daboia russelli russelli* venom. drCT-1 had anticancer activity against EAC cells *in vivo* and human leukemia cells (U937, K562) *in vitro*. EAC cell count was decreased by the effect of drCT-1. drCT-1 was responsible for induction of apoptosis by G₁ phase arrest of the cell cycle. Karthikeyan *et al*⁴² reported antitumor activity of sea snake venom (*Lapemis curtus*) against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice and HeLa and Hep2 tumor cell cultures. The finding resulted in decrease in tumor volume and viable tumor cell count. Muhammad Alla and Oman⁴³ had observed that snake venom had influenced on the growth of breast cancer cell lines (T470 and MRDMB-468 cells). They found the effect of venom on the cell lines at lower dose. This is consistence with the observation of Omran *et al*⁴⁴. Sheikh and Jokhio⁴⁵ reported that crude Cobra snake venom significantly decreases the production of RNA and DNA in

breast cancerous tissue. So it can be a better substitute of antitumor drug, for therapeutic use in breast cancer. Recent study determined the effectiveness of snake venom on breast carcinoma cell lines. The snake venom extracted from *Walterinnesia aegyptia* (WEV), alone or in combination with silica nanoparticles can decrease the proliferation of human breast carcinoma cell lines (MDA-MB-231). This study reported the activation of Caspase-3. But this was not the case with non-cancerous cell lines⁴⁶. Nolte *et al*⁴⁷ purified Bjc1, a lectin from *Bothrops jararacussu* venom by chromatographic techniques and showed its effect on gastric carcinoma cells MKN45 and AGS.

CONCLUSION

Snake venom is a complex mixture of several proteins, peptides, enzymes, organic and inorganic molecules. Snake venom is used as an anticancer agent. The cytotoxic effects of snake venom have potential to destroy tumor cancer cells. Many articles reviewed that snake venom acts by inhibiting cell proliferation and causing cell death. The therapeutic use of snake venom provides an overview of different use of snake venom for different therapies. Above description clearly indicates that different components of the snake venom are being used for clinical trial and they can be used as a natural therapeutic agent against cancer. In view of this snake venom may open the doors for new era of medicines and research for treatment of cancer.

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