

Snake venom as therapeutic agents: From toxin to drug development

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Snake bite injuries and death are socio-medical problems of considerable magnitude. In India a large number of people suffer and die every year due to snake venom poisoning. Snake venom, though greatly feared, is a natural biological resource, containing several components that could be of potential therapeutic value. Use of snake venom in different patho-physiological conditions has been mentioned in Ayurveda, homeopathy and folk medicine. It is well known that snake venom is complex mixture of enzymes, peptides and proteins of low molecular mass with specific chemical and biological activities. Snake venom contains several neurotoxic, cardiotoxic, cytotoxic, nerve growth factor, lectins, disintegrins, haemorrhagins and many other different enzymes. These proteins not only inflict death to animals and humans, but can also be used for the treatment of thrombosis, arthritis, cancer and many other diseases. An overview of various snake venom components that have prospects in health and diseases are discussed in this review.

Venomous and poisonous animals are a significant cause of global morbidity and mortality. In snakes, venom, used for defense, is an evolutionary adaptation to immobilize the prey. Venoms are the secretory substances of the venomous animals, which are synthesized and stored in specific areas of their body. Snake venoms are a unique physiological product of nature as they are mixtures of different substances, which are highly specific and have great affinity for different crucial and essential functional organization of cells and tissues. Efforts are already on for the use of these natural resources as powerful probes for elucidating complex biological processes of vital importance. Several isolated snake venom proteins with a known mode of action have found practical application as pharmaceutical agents, diagnostic reagents or preparative tools in haemostaseology, neurobiology and complement research¹. Varieties of toxins are of interest in drug design, because the toxins provide three-dimensional templates for creating small molecule mimicking interesting pharmacological properties².

The use of snake venom as medicine was known to man for centuries. It is over sixty years since it was first realized that the physiologically active components of snake venoms might have therapeutic potential^{3,4}. In *Charak Samhita* cobra venom has been said to be useful in *Dushyodara* and *Jalodara* (ascities). *Sushruta* and *Vagbhata* also mentioned similar use. *Saranghara Samita* mentioned the use of cobra venom in '*Sannipatik Jwara*'. In the Unani system of medicine cobra venom has been used as a tonic, aphrodisiac, hepatic stimulant and for revival in collapsed conditions⁵. Venoms of *Viper*, *Crotalus*, *Cobra* and *Lacasis* are also routinely used in homeopathic medicine. Chinese physicians use snake venom products routinely to treat stroke and view them as effective and relatively safe⁶. Natural protease inhibitors to haemorrhagins in snake venoms and their potential use in medicine have also been reported⁷.

Fibrinogenolytic and fibrinolytic activity of snake venom

The ability of some snake venom enzymes to clot fibrinogen has resulted in great therapeutic importance. These enzymes remove fibrinogen from the circulation without converting it to fibrin, or causing platelet aggregation. Snake venom proteinases that cleave peptide bonds in the fibrinogen molecule can be divided in to three groups (i) Thrombin like enzymes (thrombin proteases), (ii) fibrinogenolytic enzymes, (iii) enzymes activating plasminogen⁸. The venoms from three snakes have been shown to induce defibrinogenation, accord from the venom of *Calloselasma rhodostoma*,

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batroxobin from the venom of *Bothrops atox moojeni* and crotalase from *Crotalus adamanteus*. Ancord and batroxobin have been investigated in patients with stroke, deep vein thrombosis and cerebral infarction⁹, myocardial infarction, peripheral atrial thrombosis, priapism and sickle cell crisis. Gasmi *et al.*¹⁰ reported the thrombolytic activity of *Vipera lebetina* fibrinogenase (VIF) in a rat model of venous thrombosis. Thrombus was produced in the posterior vena cava by injecting human fibrinogen and thrombin. Injection of VIF induces flow restoration after one hour and measurement of the fibrinogen level decreases by about 30% after 3 hr. VIF action is not dependent on plasminogen activators and may act synergistically with them, thereby providing an intriguing potential clinical application for dissolution of blood clot. Further, that several fibrinogenolytic enzymes have been purified by different laboratories, like atroxase from *Crotalus atrox*¹¹, fibrolase from *Agkistrodon contortrix*¹², and lebetase from *Vipera lebetina*¹³. At present there is growing interest in fibrinolytic or fibrinolytic agents because of their possible application in thrombosis episodes^{14,15}, and in experimental tumor metastasis studies¹⁶. The *in vitro* activity of fibrolase^{17,18} and atroxase¹⁹ were investigated in a venous thrombosis model system. These studies demonstrate the potentiality of the venom fibrinolytic enzymes as clinically useful thrombolytic agents. Zhu *et al.*²⁰ worked out the fibrinogenolytic properties of natrahagin, a proteinase from cobra venom and its effects in human platelet aggregation. Natrahagin is α - γ fibrinogenase with an inhibitory effect on platelet membrane glycoprotein dependent platelet aggregation. The effects of green pit viper *Trimeresurus albolabris* and *Trimeresurus macrops* venom on the fibrinolytic system in humans were also studied. It was found that venoms from both the vipers possess a thrombin like effect *in vitro* but cause a defibrination syndrome *in vivo*²¹. Proteins found in venoms, especially of the snake family Viperidae, often exert with a narrow specificity, activating, inactivating or other converting effects on different components of the homeostatic and fibrinolytic system respectively. Siigur *et al.*²² confirmed the existence of both coagulants and anticoagulants of the hemostatic system in the venom of *Vipera lebetina*. This venom contains both factor X activator and factor V activator fibrinolytic enzymes. Wang *et al.*²³ reported defibrinogenating effects of batroxobin (Defibrase) in rats and inhibition of migration of human vascular smooth muscle cells by the plasma of batroxobin treated rats *in vitro*. Gomes and De²⁴ have

identified a peptide toxin from the Indian King Cobra *Ophiophagus hannah* venom by TLC followed by HPLC. The peptide named as "Hannahpep" possessed fibrinolytic activity, with a possibility of use in thrombosis.

Cardiotonic and antiarrhythmic activity of snake venom

Significant contribution comes from the work of Sherman *et al.*²⁵. He observed that Malayan pit Viper venom has blood thinning properties and could be effective in treating stroke patients. From a study of 500 stroke patients, 42% who were given the snake venom drug (Ancord) within 3 hr of stroke. Mayberg & Fulan²⁶ reported that onset regained significant functioning compared to 34% who got a placebo. The role of ancord in patients with heparin induced thrombocytopenia thrombosis has been clearly established²⁷. Use of ancord has also been reported successive in cardiac catheterization and coronary artery bypass grafting²⁸. The antithrombotic and thrombolytic activities of agkisacutacin (Agk), a component isolated from *Agkistrodon acutus* venom, were determined *in vitro* and *in vivo*. Agk can significantly inhibit thrombus formation and accelerates thrombolysis of pulmonary emboli in rats²⁹. Recently, Gomes *et al.*³⁰ identifies a non protein micromolecular toxin (mol.wt 260) from the Indian King Cobra venom. This toxin possesses antiarrhythmic properties at microgram level.

Antineoplastic activity of snake venom

Snake venom has been used to develop newer drugs to combat various diseases including cancer. Calmette *et al.*³ investigated the use of cobra venom in the treatment of cancer in mice. Match^{31,32} showed that cobra venom, in extremely minute doses produced analgesic effects. This led to the possibility of therapeutic use of the cobra venom in arthritis and cancer. Phospholipase A2 was isolated from *Bothrops newweidii* venom which produced cytotoxic activity on B16 F10 melanoma cell³³. Basavarjappa *et al.*³⁴ and Rudrammaji *et al.*³⁵ showed that Indian cobra *Naja naja* venom was cytotoxic to Ehrlich ascites tumour cells. Cytotoxic P4 was isolated from *Naja nigricollis* venom, which produced cytotoxic effect on Wehi-B leukemia cell³⁶. VRCTC-310, an animal derived anticancer agent, from snake venom a combination product of crotoxin and cardiotoxin, possessing antitumour activity *in vivo*. A phase I study was performed to evaluate the maximum tolerated dose (MTD), safety profile and pharmacokinetic data of

VRTC-310 with PLA2 activity and inhibitory effects against human and murine tumour cell lines³⁷⁻³⁹. Two purified venom toxins have been combined to produce this unique product^{40, 41}. Beside this, another factor was isolated from *Naja naja atra* venom which possessed a cytotoxin without phospholipase activity and had a selective cytotoxic action on human cancer cell lines⁴². Cobra venom factor treatment was introduced in latent feline leukemic virus immune cats. After one to three weeks an increase in viral antigen in marrow myelomonocytic cells, a circulating immune complex was noted by Kraut *et al.*⁴³. The interaction between tumour cells and microvasculature including the adhesion of tumour cell to endothelium and extra cellular matrix as well as their migratory activity are prerequisites for metastasis to occur. In this connection, it has been observed that thrombin is capable of enhancing *in vitro* tumour metastasis potential in terms of adhesive properties and migratory response. Rhodopsin an arg-gly-asp containing anti-platelet snake venom peptide served as an inhibitory agent in the prevention of thrombin enhanced metastasis⁴⁴. Hevnandez *et al.*⁴⁵ reported that fraction of *Crotalus durissus terrificus* venom, crotoxin and crotoxin complex A and B when treated for spontaneous sarcoma cells of female rats, induced both tumour regression and increase in animals survival time. In contrast to these findings Shian-Lin *et al.*⁴⁶ failed to obtain satisfactory results in tumour growth control with the venom of *Naja naja*. Iwaguchi *et al.*⁴⁷ studied *in vivo* and *in vitro* effects of cytotoxin I and II from *Naja naja* venom on normal and tumour cell lines. No antitumour effects were observed *in vivo*. However, *in vitro*, they noticed higher cytolytic effects on tumour cells than on normal cells. *In vitro* and *in vivo* experiment carried out to evaluate the effects of *Naja naja siamensis* venom and that of crotoxin complex A and B from *Crotalus durissus terrificus* venom on tumour cell viz. human metastatic breast adenocarcinoma, murine sarcoma180, Ehrlich ascites tumour and breast carcinoma, failed to demonstrate any antitumour effects⁴⁸. Another family of snake venom anticoagulants known as disintegrins are currently being tested as antitumour agents because they also interfere with the function of integrin on tumour cells. Markland *et al.*⁴⁹ has tested a disintegrin from southern copperhead venom for activity against human mammary tumour in a mouse model. Disintegrin inhibits tumour growth and also angiogenesis and metastasis, probably because they prevent the normal function of integrin on endothelium⁶. Salmosin is a snake venom

derived novel disintegrin antagonized platelet aggregation. Both the metastatic tumour growth and solids tumour growth that developed in mice were effectively suppressed by salmosin⁵⁰. The anticancer activity of Indian krait *Bungurus caeruleus* venom has been evaluated against Ehrlich ascites carcinoma (EAC) in Balb C mice. This venom, at various dose levels inhibited tumour cell growth, both *in vivo* and *in vitro*. This cytotoxic activity of venom was also observed by MTT assay and confirmed by ³H thymidine incorporation. Superoxide dismutase was also influenced by venom⁵¹. Recently Markland *et al.*⁵² isolated a novel snake venom disintegrin that inhibits human ovarian cancer dissemination in ovarcar 5 cell line and angiogenesis in an orthopedic nude mouse model.

Antiparalytic activity of snake venom

Venoms of several snakes are known to cause muscular paralysis. Subsequently several neurotoxic components that inhibit neuromuscular transmission by attacking different targets have been isolated. Neurotoxins from snake venoms have been utilised in different pharmacological and biochemical studies of nicotinic acetylcholine receptors (nAChRs) in the neurone and neuromuscular junction. The use of ¹²⁵I labelled snake venom toxin to identify AChR and their antibodies ultimately permitted both the purification and characterisation of AChR and an understanding of the pathological mechanism impairing neuromuscular transmission in myasthenia gravis (MG). Anti-AChR antibodies have been demonstrated to be the principle agents in the pathogenesis of MG, and their determination allowed definite diagnosis of MG⁵³. Clinical trials are now considered to evaluate the effects of Notexin, a snake venom phospholipase that attacks motor nerve terminals and muscular cells, in the treatment of ptosis. It was found that the satellite cells between the muscle cells contain mostly normal mitochondria. When skeletal muscle is damaged by notexin, satellite cells divide to form replacement muscle cells and because cell regeneration is accompanied by mitochondria regeneration, the mitochondria in new muscle cell are in much better shape than in the old cells. As the ptosis is a result of muscle weakness in the small easily accessible levator muscle of the eyelid, it is an ideal candidate for notexin therapy⁶.

Anti arthritic activity of snake venom

Just as proteases control the activation and destruction of hormones and other biologically important

proteins, they are also regulated by another class of molecules known as the protease inhibitors. These substances, many of which are themselves proteins, bind to protease enzymes in such way as to prevent them from reacting with and clearing the bonds of hormones and other proteins when they are no longer needed. Interest in venoms as potential source of anti-inflammatory substances gained much attention after the discovery of a purified protein component from cobra venom, which temporarily depleted the terminal C3-C9 component of complement cascade. It was found that cobra venom factor pretreatment of rabbits prevented the induction of experimental immune complex arthritis⁵⁴. Snake venom has been used to elucidate the pathophysiology of several experimental model of arthritis. Because fibrin is commonly found in large amount in arthritic joint and because fibrinolysis induction with phenformin has ameliorated signs and symptoms in human patients with rheumatoid arthritis, it was postulated that fibrin plays a role in the acute and chronic arthritis process. Intraperitoneal injection of aqueous suspension of peptidoglycan polysaccharide complex induced chronic arthritis in rat. Pretreatment of rat with cobra venom factor delayed the acute inflammation observed in this model for 3 days, but had no effects on the subsequent development of chronic arthritis⁵⁵. It has been found that small dose of cobra venom factor depleted rat C3 for about 3 days and delayed the onset of adjuvant arthritis about 3 days if given within 9 days of adjuvant administration. It is also inhibited the maximum inflammatory response if given 14 days after adjuvant. These observations suggest that the third component of complement played an essential role in adjuvant arthritis⁵⁶. Ford *et al.*⁵⁷ defibrinated rabbit with arvin, an extract from the venom of Malaysian pit viper *Calloselasma rhodostoma* in an attempt to suppress the acute and chronic inflammatory response in experimental immune arthritis. No inhibitory effect on the arthritic process was observed. Later, Stanzler and Miller⁵⁸ observed that systemic decompensation with cobra venom factor had no anti-inflammatory effect in an immune complex model of rabbit arthritis.

Other possible therapeutic action of snake venom

The use of snake venom as an analgesic agent is well documented⁵⁹. The antinociceptive activity of the venom was dose and time dependent and persisted after neutralization with a specific antivenin. Morphine enhanced the analgesic effect of *Crotallus durissus terrificus* venom and naloxone antagonized

this effect, suggesting an endorphin-like activity for the factors⁶⁰. Chen and Robinson⁶¹ demonstrated an antinociceptive effect in mice with cobrotoxin, a fraction obtained from the venom of *Naja naja atra* and a similar effect has also been reported for snake venom neurotoxins administered orally or by injection into mice⁶². A novel analgesic toxin (hannalgesic) was isolated from the venom of King Cobra *Ophiophagus hannah*. The toxin did not increase the convulsion threshold in the dose range of 8-64ng/g in the maximal electroshock seizure tests in mice. The neurotoxin produced analgesia in the dose range of 16- 32ng/g (ip) without causing any neurological or muscular deficit⁶³. Two venom based medications, cobroxin and nyloxin were marketed for the treatment of pain, arthritis and other disorders but were banned by the US Food and Drug administration in 1970 because of infectiveness⁶⁴. The Indian King Cobra *Ophiophagus hannah* venom induces immunomodulatory and haemopoietic stimulant activity⁶⁵. Recently from the venom of *Vipera russelli*, a heat stable compound has been identified which was found to produce cardio-respiratory modulation in animal models⁶⁶ with a possible application in cardiorespiratory related pathophysiological condition.

Conclusion

It may be concluded that information is now available to establish that snake venom toxin may serve as a starting material for drug design to combat several pathophysiological problems such as cardiovascular disorders, neurological problems and cancer therapy. Very few clinical studies are available and there is a need for extensive research programs to clinically work out the above-mentioned areas, with a view to develop newer drugs to combat human suffering and death globally.

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