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 pathological conditions has been mentioned in Ayurveda, homoeopathy 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 potential3,4. In Charak Samhita cobra venom has been said to be useful in Dashyodara and Jalodara (ascities). Sushruta and Vagbhata also mentioned similar use. Saranghara Samita mentioned the use of cobra venom in 'Samapatik 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 Lacasius 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, anecord from the venom of Calloselasma rhodostoma,
batroxobin from the venom of Bothrops atrax moojeni and crotalase from Crotalus adamanteus. Ancord and batroxobin have been investigated in patients with stroke, deep vein thrombosis and cerebral infarction, myocardial infarction, peripheral arterial 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 atrox11, fibrolase from Agkistrodon contortrix12, and lebetase from Vipera lebetina13. At present there is growing interest in fibrinolytic or fibrinolitic agents because of their possible application in thrombosis episodes14,15, and in experimental tumor metastasis studies16. The in vitro activity of fibrolase17,18 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 natrakin, a proteinase from cobra venom and its effects in human platelet aggregation. Natrakin 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 vivo21. 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. Sigur 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 agkisactacin (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.31 investigated the use of cobra venom in the treatment of cancer in mice. Match31,32 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 neuwiedii venom which produced cytotoxic activity on B16 F10 melanoma cell33. Basavarjappa et al.34 and Rudramma 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 crototoxin and cardotoxin, possessing antitumour activity in vivo. A phase I study was performed to evaluate the maximum tolerated dose (MTD), safety profile and pharmacokinetic data of
purified venom to x in s h ave b ee n combined to produ ce and h a d c e ll produced in la t e nt feline le uk e mi c v iru s VRTC-310 with PLA2 activity and inhibitory effects against human and murine tumour cell lines\textsuperscript{5,39}. Two purified venom toxins have been combined to produce this unique product\textsuperscript{30, 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 lines\textsuperscript{42}. 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.\textsuperscript{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 \textit{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 metastasis\textsuperscript{44}. Hevnandez et al.\textsuperscript{35} reported that fraction of Crotalus durissus terrificus venom, crotamine 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.\textsuperscript{46} failed to obtain satisfactory results in tumour growth control with the venom of Naja naja. Iwaguchi et al.\textsuperscript{47} studied \textit{in vivo} and \textit{in vitro} effects of cytotoxin 1 and II from Naja naja venom on normal and tumour cell lines. No antitumour effects were observed \textit{in vivo}. However, \textit{in vitro}, they noticed higher cytolytic effects on tumour cells than on normal cells. \textit{In vitro} and \textit{in vivo} experiment carried out to evaluate the effects of Naja naja sianensis venom and that of crotoxin complex A and B from Crotalus durissus terrificus venom on tumour cell viz. human metastatic breast adenocarcinoma, murine sarcoma180, Ehlich ascites tumour and breast carcinoma, failed to demonstrate any antitumour effects\textsuperscript{48}. Another family of snake venom anticoagulants known as disintegrins are currently being tested as antitumour agents because they also interfere with the function of intregin on tumour cells. Markland et al.\textsuperscript{49} has tested a disintregin 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 intregin on endothelium\textsuperscript{6}. Salmosin is a snake venom derived novel disintregin antagonized platelet aggregation. Both the metastatic tumour growth and solids tumour growth that developed in mice were effectively suppressed by slamosin\textsuperscript{50}. The anticancer activity of Indian krait Bungarus caeruleus venom has been evaluated against Ehlich ascites carcinoma (EAC) in Balb C mice. This venom, at various dose levels inhibited tumour cell growth, both \textit{in vivo} and \textit{in vitro}. This cytotoxic activity of venom was also observed by MTT assay and confirmed by \textsuperscript{39} thymidine incorporation. Superoxide dismutase was also influenced by venom\textsuperscript{51}. Recently Markland et al.\textsuperscript{52} isolated a novel snake venom disintegrin that inhibits human ovarian cancer dissemination in ovacar 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 neuromuscular junction. The use of $^{125}$I 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 definitive diagnosis of MG\textsuperscript{53}. 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 therapy\textsuperscript{54}.

**Anti arthritis 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 arthritis. 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 arthritis. 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 arthritis. Ford et al. 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 Miller observed that systemic decomplementation 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 documented. 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 Crotalus durissus terrificus venom and naloxone antagonized this effect, suggesting an endorphin-like activity for the factors. Chen and Robinson 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 mice. 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 deficit. Two venom based medications, cobroxin and nylozin 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 infectiveness. The Indian King Cobra Ophiophagus hannah venom induces immunomodulatory and haemopoietic stimulant activity. Recently from the venom of Vipera russelli, a heat stable compound has been identified which was found to produce cardiorespiratory modulation in animal models 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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References


