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Review

SNAKE VENOMS AND THEIR THERAPEUTIC POTENTIAL

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ABSTRACT

Snake venoms are complex heterogeneous mixtures containing different bioactive molecules with multivalent nature. Most of these components are proteins displaying enzymatic or non-enzymatic biological and pharmacological activities and toxicological potential. Their functional activities on various tissues and organs lead to the manifestation of diverse effects such as neurotoxicity, myotoxicity, cytotoxicity, inflammation, influence on hemostasis, apoptosis, necrosis and other, causing simultaneous damage to various physiological systems. At the same time, the unique molecular features of snake venom compounds make them valuable scientific tools for understanding different physiological processes, and allows them to fulfil their great potential in medicine and pharmacology as therapeutic agents and diagnostic tools. Many snake venom toxins have been found to exert analgesic, antiplatelet, hypotensive, antitumor, anti/pro-inflammatory or other potencies, as some of them have already been used as a base for design of commercially available pharmaceuticals.

Key words: Snake venoms, enzymes, neurotoxins, therapeutic applications, drugs

INTRODUCTION

Snake venoms are glandular secretions, designed to cause immobilisation, death and initial digestion of the prey. They represent highly complex chemical cocktails consisting predominantly of bioactive proteins, peptides, toxins and non-protein compounds such as lipids, carbohydrates, nucleic acids and biogenic amines. The majority of these mixtures are peptides and protein molecules accounting for about 90-95% of the venom's dry weight (1). Most of the proteins are enzymatically active molecules such as: L-amino acid oxidases, metalloproteases, phospholipases, serine/thrombin-like proteases, 5'-nucleotidases, while others lack catalytic activity and usually interfere with cellular receptors to induce diverse physiological responses. Chemical composition of snake venom varies interspecifically and intraspecifically, and depends on factors such as age, environmental conditions, habitation and diet (2, 3). The components of venom secretions exert broad spectrum of bioactivities including cytotoxicity, myotoxicity, neurotoxicity, cardiotoxicity, inflammatory processes, necrosis, etc. (4). Due to their numerous pharmacological activities and multifunctionality they have been used in basic science as research tools to understand various biological processes. For example, venom derived neurotoxins (and other toxic components) have the ability to target with great selectivity and high affinity specific cell surface receptors (affecting vital functions in different tissues) and have been used in determining their structure and functions (5). Despite their harmful nature, snake venoms have been applied in medicine for developing toxin-based diagnostic methods and even drugs (6-8). This makes it possible for structurally engineered or recombinant forms mimicking their functions to be applied as drugs. The first venom-derived therapeutic agent (Captopril) approved by U.S. Food and Drug Administration (FDA) was discovered in 1970s, and since then a tremendous scientific efforts have been made to reveal the potential use of snake venoms for medical and pharmacological purposes (9).

ENZYMATIC COMPOUNDS IN SNAKE VENOM COMPOSITION

Secretory phospholipases A_2 ($sPLA_2s$, EC 3.1.1.4) are among the most widely distributed

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enzymes in snake venoms, found in almost every family. They represent a subclass of the phospholipase A_2 superfamily, catalyzing the hydrolysis of the ester bond at the sn-2 position of membrane glycerophospholipids (10). Secretory PLA_2s are a heterogeneous group of enzymes with low molecular mass *(*Mr 13–19 kDa*)* and 5 to 8 disulfide bridges, stabilizing the tertiary structure. Their classification includes 16 subgroups of which groups IA and IIA are the most abundant in the members of *Elapidae* and Viperidae families (11, 12). $sPLA_2$ enzymes are major contributors to the toxic manifestations upon envenomation. The hydrolytic reaction induces changes in cell membrane permeability and organization by releasing free fatty acids and lysophospholipids as products, which possess their own activity as signalling molecules and can act as proinflammatory mediators producing widespread cellular pathology (13). Some of these enzymes are known to exert toxicity in a non-catalytic manner by interacting with specific membrane receptors through functional regions distinct from the catalytic site and designated as specific pharmacological sites (14). This structural multifunctionality of phospholipases allows them to induce a wide variety of pharmacological effects in victims such as neurotoxicity, myotoxicity, cytotoxicity, anticoagulant and hemolytic effects, cardiotoxicity, antihemorrhagic activity and edema-inducing activity (15). Venom $sPLA_2$s can be present either as monomers, or in the form of homo- or heterodimers.

Snake venom serine proteases (SVSPs) are considered responsible for one of the most dramatic manifestations of envenomation, affecting the haemostatic system. These enzymes act by activation or proteolytic degradation of specific components, involved in blood coagulation cascade, platelet aggregation, complement system and fibrinolysis, causing dysregulation of haemostasis (16, 17). They are typically present in venoms with hemotoxic and coagulopathic potencies essential for the members of *Viperidae, Elapidae, Crotalinae* and *Colubridae* families. SVSPs are usually glycoproteins with high variations of molecular mass ranging from 26 to 67 kDa. Their structure is stabilized by six disulphide bridges and catalytic mechanism involves Ser195–His57–Asp102 catalytic triad. SVSPs are highly homologous enzymes, sharing up to 85% of sequence identity, nevertheless their substrate specificity and physiological activities differs significantly (18). Because of their multiple haemostatic activities, substrate specificity along with their high resistance to endogenous serine protease inhibitors, SVSPs have been extensively studied for therapeutic potentials in the cardiovascular area. Greatest success has been achieved with batroxobin (Defibrase®), a serine protease isolated from *Bothrops moojeni* venom which is widely used in clinical practice for the management of thrombotic diseases (19). Ancrod is an another fibrinolytic serine protease derived from the venom of *Calloselasma rhodostoma* that has been extensively investigated for potential uses in treating patients with myocardial infarction and strokes. However, its efficacy has been found insufficient for reaching marketing approval (20, 21).

Snake venom metalloproteinases (SVMPs) are zinc-dependent endopeptidases varying in size from 20 to 100 kDa. They are present in the venom of many species, and are considered the most abundant constituent in the composition of most viperid venoms (~30% of total protein) (22). Structurally, SVMPs are classified into three main groups (P-I, P-II, and P-III), according to their domain organisation. The enzymes from P–I class has the simplest composition, containing only a metalloproteinase domain. In P-II group there is an additional disintegrin domain linked to the C-terminus of the metalloproteinase domain and P-III are the largest and most complex, comprising metalloproteinase, disintegrin-like and a cysteine rich domains (23). SVMPs are potent toxins, responsible for inducing severe clinical manifestations such as haemorrhage and local tissue damage, mainly through hydrolysis of extracellular matrix proteins. Some of the SVMPs have been shown to interfere with hemostasis by mediating pro- or anticoagulant effects, inhibition of platelet aggregation, activation of complement, induction of apoptosis and inflammation (24).

L-Amino acid oxidases (LAAOs) are a class of flavoenzymes found in a variety of snake venoms including members of *Viperidae, Elapidae* and especially *Crotalidae* family. They perform a number of biological and pharmacological activities with high relevance for the development of the pathological symptoms of envenomation. Their action includes cytotoxicity, myotoxicity, induction of apoptosis, hemolysis, hemorrhage and edema formation (25). Mechanistically, LAAOs exert

their activity by catalyzing the stereospecific oxidative deamination of L-amino acids which are further nonenzymatically hydrolyzed to yield the corresponding α-oxoacids (26). The reaction mechanism involves hydrogen peroxide generation which is a primary factor for the pronounced cytotoxic potency of these enzymes. Based on this, LAAOs have elicited interest in biomedical research for the development of antimicrobial, antitumor and antiparasitic agents.

The distribution of these four major classes of snake venom enzymes between different snake families, genera and species can vary drastically, but they always act synergistically in the complex venom **(Figure 1).**

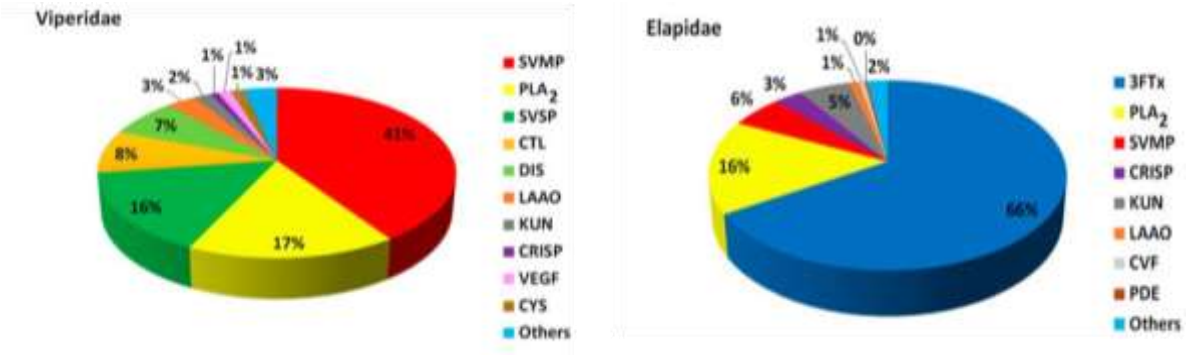

Figure 1. Graphical representation of snake venom composition *of Viperidae and Elapidae* families. Abbreviations: PLA_2 - phospholipase A_2; SVSP - snake venom serine protease; SVMP - snake venom metalloproteinase; LAAO - L-amino acid oxidase; 3FTxs - three-finger toxins; DIS - disintegrin;KUN - Kunitz-type peptides; CRISP - cysteine-rich secretary protein; CTL - C-type lectin; VEGF - vascular endothelial growth factor; CYS - cystatin; CVF - cobra venom factor; PDE - endonucleases/phosphodiesterases (27).

SNAKE VENOM NEUROTOXINS AND THEIR THERAPEUTIC PROPERTIES

Neurotoxins are the major component of snake venoms with diverse structural variations whose main function is to affect the neuromuscular transmission, primarily in the skeletal muscles. They can act either presynaptically by damaging motor nerve endings and inhibiting the neurotransmitter release (β-neurotoxins), or postsynaptically by competitively binding to acetylcholine receptors (α-neurotoxins) (28). Beside its neurotoxic action, some of the studied neurotoxins have demonstrated valuable therapeutic properties, such as analgesic, antitumoral, antibacterial, anti-inflammatory.

Crotoxin (CTX) is the *predominant compound in the* venom of the south American rattlesnake *Crotalus durissus terrificus* comprising ~60% of venom's total dry weight. It is a heterodimeric complex (Mr 24–26 kDa) formed by two non-covalently associated subunits, basic weakly toxic enzymatic subunit with PLA_2 activity (CB) and an acidic non-toxic and enzymatically inactive subunit (CA) named crotapotin (29). Several different isoforms have been described for both CB and CA subunits which can form up to sixteen crotoxin variations within an individual venom (30). CTX functions primarily as a presynaptic neurotoxin causing peripheral neuromuscular paralysis and asphyxia. These biological actions are ascribed to the enzymatic CB subunit while CA is believed to be pharmacologically inactive. However, crotapotin has been shown to increase the potency of the toxic subunit by directing and modulating its activity (31). In addition to neurotoxicity, CTX can exert other pharmacological activities such as myotoxicity, nephrotoxicity and cardiotoxicity (32).

Aside from its role in pathophysiology, the neurotoxin has been studied for potential therapeutic uses: CTX has long-lasting anti-inflammatory activity and is able to reduce bacillus Calmette-Guérin and carrageenan-induced mice paw edema (33, 34); the nontoxic crotapotin has been shown to inhibit the edematogenic effect of three different $sPLA_2$s isolated from *Bothrops* snake venoms in mice model (35); CTX has also immunomodulating activity and is capable of inhibiting lymphocyte activity and the spreading and phagocytic activities of macrophages (36, 37); Crotoxin exert strong analgesic activity and can alleviate acute phasic and tonic pain by acting on the central nervous system (38). Long term anti-nociceptive effects have been observed against chronic neuropathic pain induced by rat sciatic nerve transection (39); Crotoxin has anti-

tumoral activity and it has been tested as a potential treatment in patients with advanced tumor progressions refractory to conventional therapy (40).

Crotamine is another neurotoxic protein isolated from *Crotalus durissus terrificus* venom. It consists of a single polypeptide chain containing 42 amino acids and 3 disulfide bonds stabilizing the structure (41). Crotamine has biochemical properties that gives it a promising prospect in pharmacology. Due to its highly positively charged residues and high penetration rate it has been proposed as a drug delivery agent. It has been shown to possess both antitumor and antibacterial activities (42). The most pronounced characteristic of crotamine is its analgesic effect with potency over 30-fold higher than that of morphine (43).

Cobrotoxin, a subtype of the non-enzymatic three finger toxins, is the main active component in the venom from Taiwan cobra *Naja naja atra*. It is a short-chain polypeptide (Mr 7 kD) composed of 62 amino acids forming four disulfide bridges. Cobrotoxin is a postsynaptic α-neurotoxin inducing neuromuscular blockage by serving as a high affinity ligand for nicotinic acetylcholine receptors (nAChRs) on the motor end plate (44). Cobra venom has long been recognized in Asian traditional folk medicine, so research of cobrotoxin's possible therapeutic applications is of utmost importance. It turns out that the toxin has a potent analgesic action which has led to its incorporation in a commercially available drug (Keluoqu) used for the treatment of chronic cancer pain (45). Cobrotoxin also has anti-inflammatory and immunosuppressive properties (46, 47), as well as beneficial effects on rheumatoid arthritis as indicated by its ability to downregulate proinflammatory cytokines and the number of inflammatory cells in peripheral blood in rats with adjuvant-induced arthritis (48). Because of its immunoprotective, anti-inflammatory and antiviral activities, cobrotoxin has been proposed as an alternative therapy for patients with COVID-19 (49).

Cobratoxin is another α-neurotoxin with postsynaptic activity, part of the three-finger protein family. It is a long-chain polypeptide isolated from the Thailand cobra *Naja naja kaouthia*. Cobratoxin inhibits neurotransmission by targeting specifically alpha 7 subtypes of nicotinic receptors which are known to conduct calcium ions (50). Its pain relief effects are most probably mediated by activation of the cholinergic system (51). Chemically modified cobratoxin has been investigated for therapeutic use in adrenomyeloneuropathy, however, no significant improvements with treatment were observed (52).

Vipoxin is a postsynaptic neurotoxin isolated from the Bulgarian *long-nosed* viper *Vipera ammodytes meridionalis*. It is a heterodimeric complex (27.747 kDa) comprised of two non-covalently associated subunits: basic and toxic secretory GIIA PLA_2 enzyme (Mr 13.828 kDa and pI 10.4) also known as vipoxin basic component - VBC-$sPLA_2$, and an acidic, non-enzymatic and non-toxic subunit designed as vipoxin acidic component - VAC (*Mr 13.639 kDa, pI 4.6*) (53). Both of the subunits are composed of 122 amino acid residues forming 7 disulfide bonds and sharing similar structure with 62% of sequence identity (54). Vipoxin inhibits neuromuscular transmission in skeletal muscles through postsynaptic mechanism that prevents the binding of acetylcholine to its receptor. In contrast to the complex, the monomeric VBC-$sPLA_2$ exert presynaptic activity. The acidic subunit of the complex - VAC is considered to have chaperone-like function by preventing the unspecific binding and directing the enzyme subunit to its target site (55).

Aside from its neurotoxic activity, vipoxin and its separated PLA_2 has been proved to induce hemolysis of the red blood cells and this effect is attributed to the enzymatic $sPLA_2$ activity (56, 57). *The catalytic subunit of* vipoxin has been reported to manifests weakly anticoagulant activity due to enzymatic hydrolysis of procoagulant phospholipids, but such effect has not been observed for the whole complex (56).

The pharmacological effects of vipoxin and its individual components were examined on *HepG2* human hepatoma cell line (58). It was established that the monomeric PLA_2, but not the whole complex, induced cytotoxic effect against the cell line which is the basis for further studies on potential anti-cancer activities of vipoxin toxic component.

SNAKE VENOMS IN MEDICINE

Captopril is the first animal derived drug approved officially for medical use in 1981. It is an antihypertensive agent developed by

chemical modification of the bradykinin potentiating peptide (BPP) isolated from the Brazilian pit viper *Bothrops jararaca* venom. BPPs are small proline-rich peptides which can affect blood pressure by inhibiting the angiotensin-converting enzyme (ACE) responsible for the generation of angiotensin II, which is a vasoconstrictor and hypertension mediator therefore blocking its production results in lowering the blood pressure (59, 60). Since their first discovery, BPPs have been isolated from many other sources and ACE inhibitors have become widely used as a class of hypotensive drugs (61, 62). Enalapril is another drug formulated on the same basis with few additional modifications designed in order to eliminate the adverse effects reported from Captopril (63).

Tirofiban is a thrombolytic drug approved by the FDA in 1998 for treatment of acute coronary syndrome. Its structure is derived from a specific RGD motif of the disintegrin echistatin isolated from the saw-scaled viper *Echis carinatus* venom (64). Disintegrins are small non-enzymatic snake venom proteins containing functionally active arginine-glycine-aspartic acid (RGD) sequence. They are known to cause hemorrhage by inhibiting platelet aggregation through competition with fibrinogen for its platelet receptor αIIBβ3 integrin (65). Precisely, this molecular mechanism is imitated by Tirofiban to induce an antithrombotic effect.

Eptifibatide is another antiplatelet medication approved for management of ischemic cardiac events. It is designed on the molecular basis of a disintegrin protein barbourin isolated from the Southeastern pygmy rattlesnake *Sistrurus miliarius barbouri* (66). The function of barbourin is similar to that of echistatin and other disintegrins but is endowed with higher ligand binding specificity due to the presence of unique KGD (Lys–Gly–Asp) motif (67).

Batroxobin is thrombin-like serine protease from the pit viper *Bothrops moojeni* venom clinically used for treatment of thrombotic conditions such as deep vein thrombosis and ischemic stroke. It promotes defibrinogenation by specific cleavage of fibrinogen A chain releasing fibrin monomers (68).

Table 1. *Approved snake venom-based drugs.*

Drug	Protein/peptide	Venom source (species)	Mechanism of action	Therapeutic application
Captopril	Bradykinin potentiating peptide	*Bothrops jararaca*	Inhibition of Angiotensin-converting enzyme (ACE)	Hypertension
Enalapril	Bradykinin potentiating peptide	*Bothrops jararaca*	Inhibition of Angiotensin-converting enzyme (ACE)	Hypertension
Tirofiban	Disintegrin (echistatin)	*Echis carinatus*	Inhibition of glycoprotein IIb/IIIa receptors	Acute coronary syndrome and antithrombotic therapy
Eptifibatide	Disintegrin (barbourin)	*Sistrurus barbouri*	Inhibition of glycoprotein IIb/IIIa receptors	Acute coronary syndrome and antithrombotic therapy
Batroxobin (Defibrase)	*Thrombin-like serine protease*	*Bothrops moojeni*	Converts fibrinogen into fibrin	*Thrombotic diseases; Stroke; Myocardial infarction*

CONCLUSION

The diversity of snake venom components and their complex nature offers abundant source for the development of new drugs and diagnostic tools. Numerous studies have revealed their curative potential and with right technological strategies they can be transformed into therapeutic agents targeting different pathophysiological conditions. The biomedical relevance of snake venoms is determined primarily by their chemical composition. Giving that there are over 3000 snake species, comparatively small portion of them have been studied in detail in terms of venom content and functional mechanisms. Studies of the molecular mechanisms and cell activities of numerous potent bioactive compounds, isolated from snake venoms, as well as from other venomous animals, are extremely important for further evaluation and design of new strategies for improving their medicinal effectiveness and increase their applications as therapeutic tools.

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