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Pathophysiological and Pharmacological Effects of Snake Venom Components: Molecular Targets

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Abstract

Snake venoms are a mixture of hydrolases which produce complex pathogenesis such as bleeding, dermo/myonecrosis, inflammation and coagulation disorders. The toxicity of venoms cannot be attributed to only one component. It is well known that venom components present antagonist activities, while some of them work synergistically. Binding to their intra- and extra-cellular or molecular targets, leads these components to generate severe disturbances which might concern several systems through complex mechanisms. Some of these mechanisms are still not yet elucidated. Thus, some of these components can act at different steps of blood coagulation by activating or inhibiting several molecular or cellular targets thereby inducing blood disorders. Despite their effects, it is well established that some of components from snake venoms present beneficial effects when acting alone as purified entity. Appropriate treatments of snakebite victims need a complete understanding of the pharmacological roles of the different venom components. Thus, this review emphasizes the toxicological relevance of snake venoms mainly those of Viperidae and their components as pharmacological bioactive tools.

Keywords: Snake venoms; Toxicology; Bleeding; Myotoxicity; Hemostasis; Biomolecules; Immunotherapy

Introduction

Snakes are the most feared venomous animals in the world due to their induced morbidity and mortality worldwide which represent 5,400,000 bites over 2,500,000 fatalities followed by about 125,000 deaths [1]. However, some retrospective studies reported that the incidence, mortality and long term disability due to snakebites were shown to be much higher [2-4]. They are poikilothermic and carnivorous reptiles, they are very abundant in hot regions of the world [5]. Snakes belong to the phylum of vertebrates and class of reptiles; they form with the Saurians, the order of Squamates which are divided into four families: Elapidae located in southeast of Asia, in central and southern America and Australia. Hydrophidae distributed in Asia [6]. Crotalidae are found in North America as well as in South Asia. Viperidae are more abundant in Europe, Southeast of Asia and very common in Africa. This family was represented by more than 250 species which are adapted to environmental conditions; it appeared 25 million years before. Snakes represent the most venomous animals, their venoms are complex mixtures of molecules that induce diverse effects on the human systems (hemorrhage, edema, myonecrosis and bleeding disorders) (Figure 1) [7,8].

Due to the richness, heterogeneity and synergistic or antagonistic action of different components, the mechanisms of all these effects are not yet all fully understood. Envenoming induced by snakebite is characterized by local tissue damage involving hemorrhage, blistering, myonecrosis and inflammation. The inflammatory response has relevance in the evolution of tissue damage; it is associated with edema, pain, leukocyte infiltration and release of several mediators. Pathogenesis induced by snake venoms is multi-factorial and complex; it is characterized by local and systemic alterations.

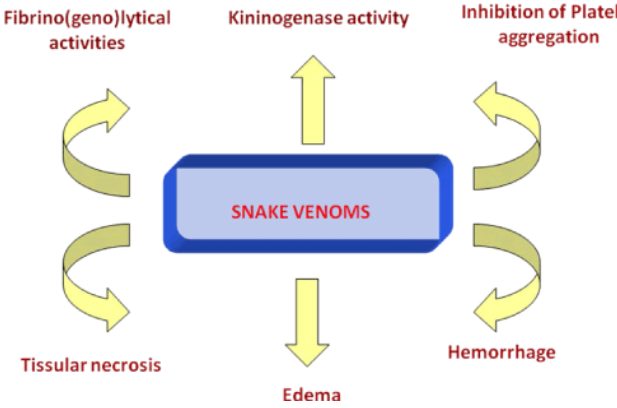

Figure 1: Pathophysiological effects induced by snake venoms.

The induced symptoms after bites vary in humans, depending on the amount of inoculated venom, bite site, age, weight and response of each bitten patient. Several studies reported clinical symptoms in relationship with the biochemical variability of venom composition which leads to tissue damage causing failure of various vital organs. Death can occur few days or several weeks after snake bite.

Snake Venoms and their Relevant Components

Venoms

Snake venoms are rich bio-resource of biologically active compounds, but only one percent of these molecules have been characterized; therefore, many of the bioactive components remain to be explored. These biomolecules present diverse activities which could

also be used as tools mainly for medical research or diagnosis [9-11]. Identification and characterization of toxic compounds present in snake venoms are the main step not only to understand the pathophysiological changes observed after bites, but they can also be useful to improve the treatment after snake bites.

Components

Secreted proteins represent the major components of snake venoms, they are encoded by poly-adenylated mRNA of venomous glands (12S and 20S) [12]. These proteins have diverse biological activities, some of them are hydrolytic enzymes which help snake in its digestion, and some others are able to induce metabolic dysfunctions of the prey and/or to kill it [13]. In addition to secreted proteins, other components are also found in the venoms, such as lipids, polysaccharides, nucleotides, nucleosides, free amino acids, riboflavin, serotonin and histamine. Pharmacological active substances of the venoms are enzymes and low molecular weight peptides. Some of these enzymes may contribute to the toxic activity of the venoms [14]. Main targets of isolated enzymes from snake venoms are cell membranes, vascular wall and blood coagulation cascade [15,16]. Snake venoms and mainly those of Viperidae contain also molecules that act on the four interconnected blood systems as producers of inflammatory mediators, (i) coagulation system, (ii) fibrinolysis, (iii) complement and (iiii) kinin system (Figure 2) [17].

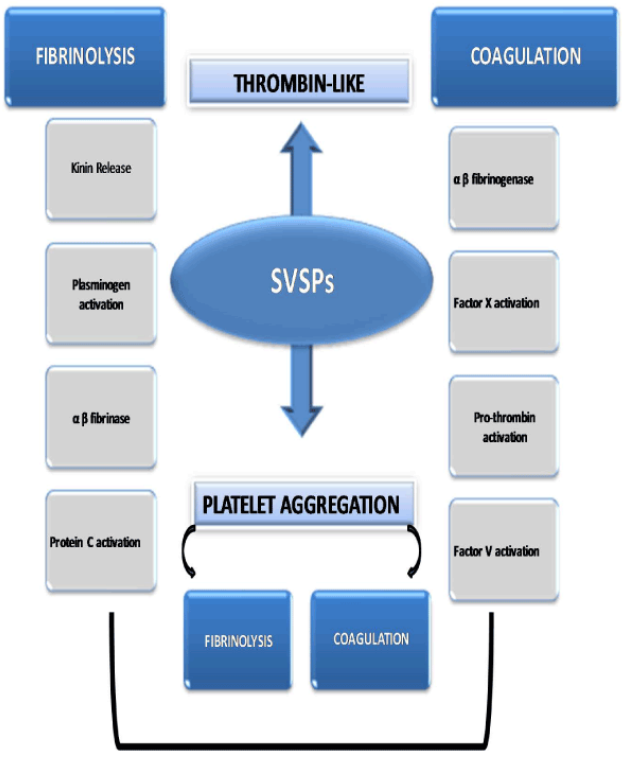

Figure 2: Involvement of SVSPs and SVMPs in hemostatic disturbances [9-11,20,21,24,25].

Proteinases

Knowledge of the combined effects of different components of venom leads to the better understanding of the observed symptoms in snake envenomation [18]. Proteolytic enzymes are particularly involved in the pathogenesis of tissue necrosis, hemorrhage and bleeding disorders. Proteinases isolated from viper venoms represent a heterogeneous group of enzymatic proteins of 15 and 100 kDa [19]. Some of these proteinases act on blood coagulation factors and can be pro-coagulant or anti-coagulant since they may exert activating or inhibiting effects of plasma factors. They are also endowed with fibrino(geno) lytic activities as well as thrombin and plasmin. Venom proteases are divided into two broad classes of enzymes: Snake Venom Serine Proteases (SVSPs) and Snake Venom Metalloproteinases (SVMPs) [20-23]. The structure of these enzymes is stabilized by disulfide bridges [20-22,24]. These enzymes are able to hydrolyze natural substrates such as casein, hemoglobin and fibrinogen as well as synthetic substrates [25].

Snake Venom Serine proteases (SVSPs): Serine proteases are abundant in snake venoms, they have been identified in venoms mainly from the subfamily of Crotalinae (Agkistrodon, Crotalus, Lachesis, Trimeresurus), Viperinae (*Cerastes cerastes*, *Cerastes vipera* and *Bitis gabonica*) and Colubrinae (*Dipholidus typus*) [25]. According to their effects on the hemostatic system, they can be classified as kallikrein-like which lead to the release of bradykinin or as fibrinogenases [26,27]. Most of these proteases affect several targets of hemostasis, platelets, plasma coagulation and fibrinolytic system [25]. They are called "Thrombinic enzymes from snake venom" or Snake Venom Thrombin-Like Enzymes (SVTLEs). SVSPs (20 to 100 kDa) present preserved a common domain which consists in a catalytic triad of three basic amino acid histidine (His 57), serine (Ser 195), hence the name "serine protease" and aspartic acid (Asp 102), each of these amino acids play a key role in the catalytic activity. SVSPs target mainly the coagulation cascade and act as potent platelet aggregating molecules and/or as exogenous plasma factors (Figure 2) [28]. Several proteolytic enzymes are purified from snake venoms; among them those purified from *Cerastes cerastes* venom as α, β-fibrinogenases based on their ability to hydrolyze the fibrinogen such as the procoagulant proteinases and fibrinogenases (RP34, afaâcytin and CC3-SPase). Afaâcytin, RP34 and CC3-SPase displayed, respectively, α,ß-fibrinogenase and α-fibrinogenase activity [26,29].

Snake Venom Metalloproteinases (SVMPs): Several SVMPs (22 to 100 kDa) isolated from snake venoms have been characterized as Zn^{2+}- metalloproteinases which have components of the basement membrane of the endothelial cells as main targets [30,31]. Hemostatic system disorders are the most studied biological effect induced by SVMPs [14,32]. In addition to their procoagulant and anti-coagulant activities, SVMPs are also involved in the pathogenesis of edema, inflammation, myonecrosis, skin damage and the development of cardiovascular shock [32-37]. They are able to inhibit platelet aggregation which potentiates the effect of bleeding [38]. SVMPs are also able to degrade the extracellular matrix components (collagen, proteoglycans, laminin and fibronectin) inducing massive extravasation of blood [36,39]. These metalloproteinases are responsible of the induced local and systemic bleeding after bites; affecting various organs (heart, liver, lungs, intestines and brain), so they are called hemorragins. They can also cause swelling, blisters and necrosis. These enzymes are, therefore, widely involved in the pathogenesis of tissue necrosis [40]. SVMPs are synthesized in vivo as inactive zymogens, organized into domains containing a signal peptide, a pro-domain and a conserved catalytic domain with a binding site for zinc, disintegrin-type or lectin-domain can be associated at these domains [15,32,41-43]. SVMPs, Zn^{2+}- or both Zn^{2+}/Ca^{2+}-dependent, are characterized by a required sequence

(HEXXHXXGXXH) for the Zn^{2+} binding, which is essential for their proteolytic activity [44-47]. Histidyl residues are involved in zinc binding, while the glutamate residue allows the polarization of water molecule by zinc. Based on their structures and molecular masses, SVMPs were previously classified into four classes [47-50]. However, further investigations on SVMPs structure updated this classification into only three classes. The mature P-I class contains only a metalloproteinase domain. This domain is followed by disintegrin domain in class P-II while in the P-III class these two domains are linked to disintegrin-like and cysteine-rich domains [51-53]. Isolated metalloproteinases from snake venoms are able also to disrupt hemostatic system since they exhibit α or β-fibrinogenase activities or inhibit platelet aggregation enhancing bleeding effect [15,32,38].

Disintegrins and C-type Lectins

Protein components of snake venoms belong to families of disintegrins and C-type lectins are, increasingly, used in biomedical research, in diagnostic or/and therapeutic purposes. Due to their effects on various platelet receptors (GPIb, GPIIb/IIIa, GPVI, α2β1 ….), disintegrins and C-type lectin domains have been considered as modulators of the platelet aggregation (Figure 3).

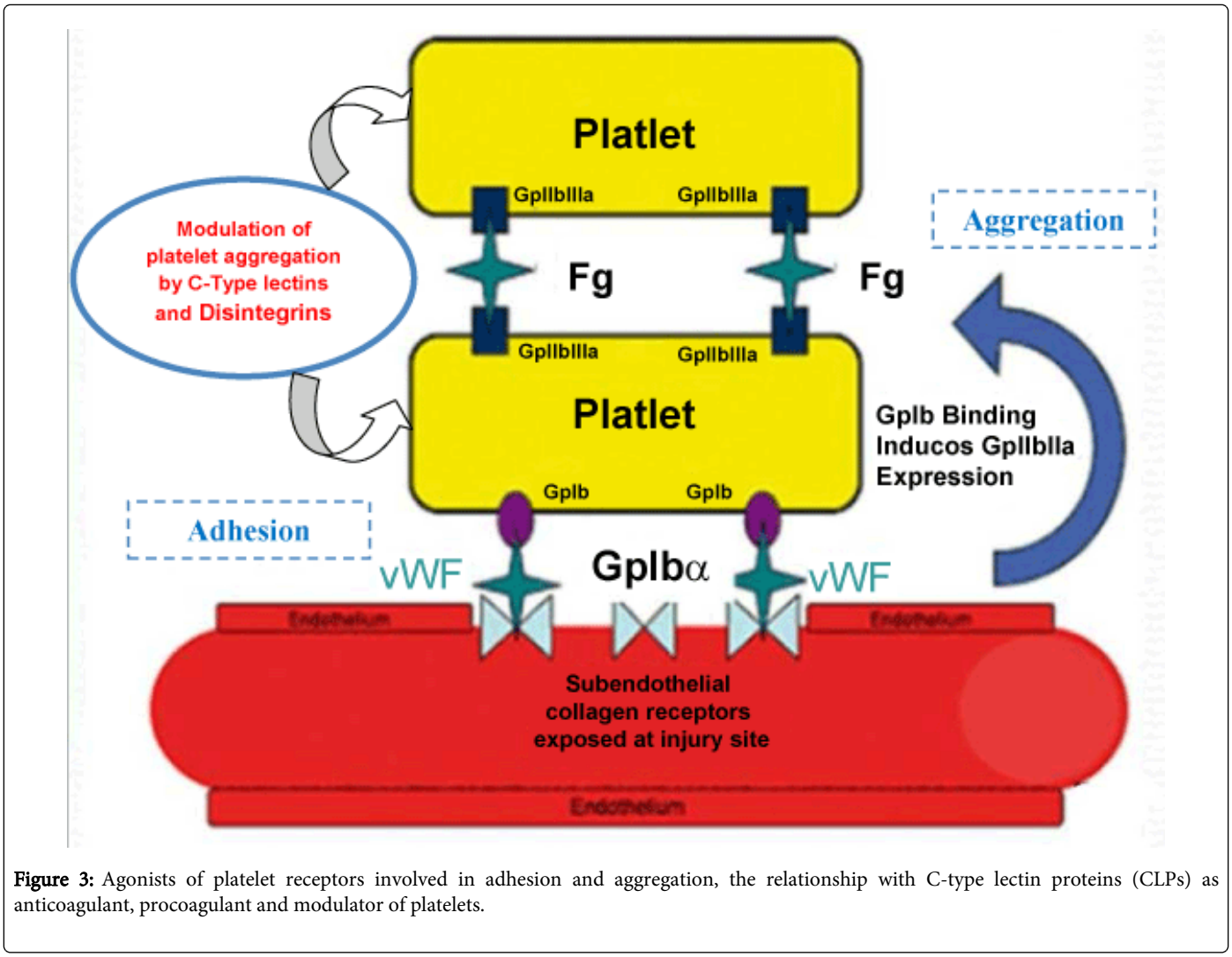

Figure 3: Agonists of platelet receptors involved in adhesion and aggregation, the relationship with C-type lectin proteins (CLPs) as anticoagulant, procoagulant and modulator of platelets.

By binding to αIIbβ3 integrin on activated platelets, and thus preventing its interaction with fibrinogen, RGD disintegrins inhibit platelet aggregation induced by a wide range of agonists, e.g. ADP, α-thrombin, collagen and arachidonic acid. Based on their ability to inhibit adhesion, migration, proliferation and invasion of different cancer cell lines some of these disintegrins and C-type lectins have been described as anti-tumoral potential effect. C-type lectins have also in vivo and in vitro an anti-angiogenic powerful by interacting with integrins of endothelial cells [54,55]. Several disintegrins have been identified in vitro and in vivo as potent inhibitors of platelet aggregation, their structures served also as a model in drug design. Some of them are clinically used as anti-platelets for coronary artery diseases, such as Eptifibatide (Integrilin) and Tirofiban. Eptifibatide is a cycle peptide derivative of the disintegrin barbourin isolated from dusky pigmy rattlesnake (*Sistrusus barbouri*), while, Tirofiban, is a synthetic molecule mimicking the disintegrin Echistatin from *Echis carinatus* [56,57]. The possibility to identify lectins using new receptors (other than GPIb, GPVI and α2β1) could be for help to develop and provide new opportunities in the diagnosis and treatment of hemostasis. Meanwhile, new anti-tumor and anti-angiogenic activities of some lectins (lebecetin, lebectin and BJcuL) open new therapeutic perspectives in the field of cancer treatment.

Phospholipases A2 (PLA2s)

Snake venoms are one of the richest sources of PLA2s. They often contain a large number of iso-enzymes such as in Naja naja, Vipera russelli, Trimeresurus flavoviridis venoms [58]. Snake venom PLA2s are divided into two groups according to their primary sequence and the position of their disulfide bridges [59,60]. PLA2s of Elapidae and Hydrophidae venoms belong to the Group I, while those of Viperinae and Crotalinae belong to the Group II. The structure of these two groups is very similar, it consists of 120-125 amino acid residues, stabilized by seven disulfide bridges [59,61,62]. SV-PLA2s induced various biological effects such as neurotoxic, myotoxic, cytolytic, edematic, cardiotoxic and anticoagulant effects [63-67].

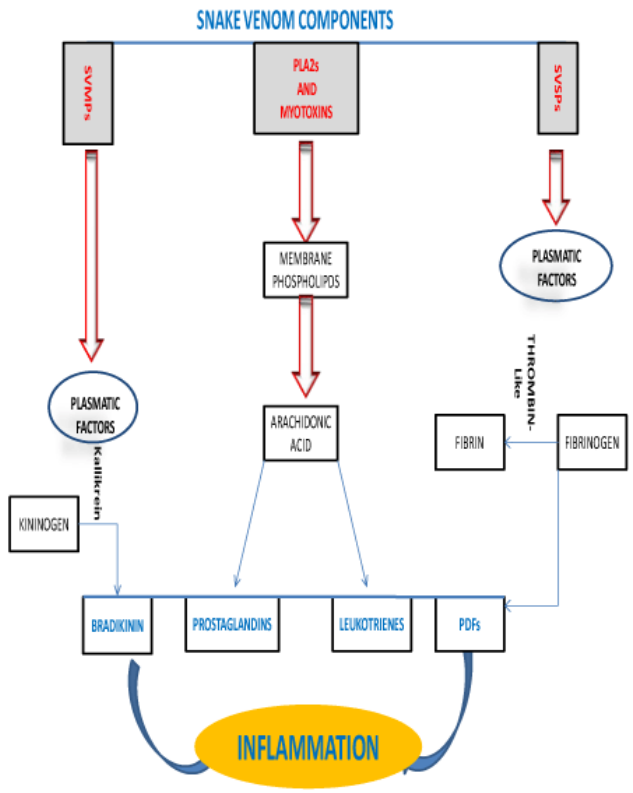

Figure 4: Probable mechanism of myotoxin.

Myotoxic PLA2s and myotoxic peptides: PLA2s are the most abundant myotoxic components of snake venoms; they induce similar degenerative events in muscle cells. The myotoxic PLA2s may be endowed with a phospholipase activity or not [68-70]. The active site of myotoxic PLA2s is highly conserved (His 48, Asp 49, Tyr 52 and Asp 99). The residue Asp 49 binds to calcium, which is essential to the PLA2 activity [66]. Myotoxins devoid in phospholipase activity, have the same active site residues (His 48, Tyr 52 and Asp 99). The residue Asp 49 could be substituted by Lysine or Serine. Otherwise, the substitution of aspartate 49 could prevent the binding of calcium, resulting in a loss of phospholipase activity. This type of molecule nevertheless retains myotoxicity [66,71]. The region (115-129 in C-terminus) of the Lys49 PLA2s is responsible for their ability to alter the integrity of the bilayer membrane. The onset of muscle damage even in the absence of phospholipase activity showed that the catalytic domain of PLA2s differ of their biological activities, including their myotoxic activity (Figure 4).

However, Ser49 or Lys49 PLA2s altered muscle tissue by an independent mechanism of enzymatic hydrolysis of membrane phospholipids [72-74].

Myotoxins without PLA2 activity are peptides of 42 to 52 amino acid residues, stabilized by three disulfide bonds. This activity is related to their two successive sequences at their C-terminus (Figure 4). The first cationic sequence is rich in positive charged residues, while the second sequence is rich in hydrophobic residues; both are bound by three to four disulfide bridges.

Toxicological Relevance of Snake Venoms: Envenomation And Therapy

Envenomation

Biodistribution of snake venoms and their components: Many studies on biodistribution of snake venoms were performed using sandwich ELISA, the biodistribution could explain their induced clinical symptoms [75]. These studies showed that snake venoms are rapidly absorbed from the injection site and diffused to the tissue and the vascular compartment; however, their elimination is very slow. Conducted study on Vipera aspis venom radio-labeled with Iodine125 and injected by i.v. route (260 mg/kg of rabbit weight) showed a rapid distribution with an estimated time of 15 ± 3.6 minutes determined by radio-labeling and 31 ± 8.4 minutes determined by sandwich ELISA [75]. Further study on Walterinnesia aegyptia venom showed a rapid absorption with 20 ± 2.1 minutes and a wide distribution of venom in vascular and tissular compartments of rabbit [76]. This biodistribution was correlated to that of Vipera aspis venom injected by i.v. route which [76]. Parallaly, after envenomation with Vipera aspis, observed symptoms appeared slowly and sustainably develop [76]. Furthermore, the toxicokinetic study of the venom injected subcutaneous is widely distributed throughout the body, indicating that the components of venom widely diffuse out of the vascular compartment; this would result in very long half-life time elimination. Vipera aspis venom injected intramuscularly revealed large volume of distribution of the venom (Vd = 2 L.kg) and low amount of venom (less than 4%) was excreted through the kidneys [77]. In another hand, biodistribution of Cerastes cerastes and Vipera lebetina venoms carried out by sandwich ELISA in the different compartments of rats revealed that rapid diffusion of venoms from the serum to the tissues. Maximum serum concentration is reached after 3 hours of envenomation. The kidneys, liver, lungs, heart and pancreas, are the main target organs for Cerastes cerastes and Vipera lebetina venoms. The absence of the venoms in the brain is attributed to the presence of the blood- lymphatic barrier that prevents the passage of toxins in the venom of the brain [76].

Induced pathophysiology by venom components and involved mechanisms II.1.2.1. Inflammatory response: Involvement of inflammatory process in the pathogenesis of snake envenomation was reported since 90s [50,77,78]. It is well known that snake venoms contain various activities able to activate several pathways (Figure 5).

Increase of capillary permeability was reported after snake envenomation leading to the release of several mediators. Many components of snake venoms (PLA2s, bioamines and proteinases) contribute to the induced inflammatory response which is initiated by an increase of vascular permeability followed by cell infiltration [79]. Several studies reported that SVMPs such as BaP1 from *Bothrops*

asper and Jararhagin from *Bothrops jararaca* are involved in the inflammatory pathogenesis leading to an increase of pro-inflammatory cytokine production [80,81]. The induced inflammatory response by snake venoms particularly those of *Viperidae* is amplified by the presence of metalloproteinases, serine proteases, phospholipases A2 and other non-enzymatic proteins such as disintegrins and C-type lectins, which alter the vessel walls and trigger tissue damage. SVMPs play a relevant role in the complex multifactorial inflammatory response induced by snakebite. High levels of IL-6 and IL-1β were observed in muscular tissue of envenomed animals by *Bothrops asper* venom [82]. However, a rapid increase of IL-6 levels versus a late onset of IL-1 and TNF-α was observed after injection of *Bothrops asper* venom [78]. It was reported that Promutoxin (R49sPLA2) isolated from the venom of *Probothrops muscrosquamatus* induced a release of IL-12, TNF-α, IL-6 and IL-1β from a cell culture of human monocytes and IL-2 cytokines, TNF and IL-6 from human T lymphocytes. Some SV-PLA2s hydrolyze membrane phospholipids of platelets, leading to the release of agonists, mainly arachidonic acid a precursor of several inflammatory substances such as prostaglandins and leukotrienes [9,11]. Activation of the complement system results in the formation of many additional degradation products that serve as important mediators of inflammation. Snake venoms stimulate the activation of mast cells which lead to histamine release, inducing vascular permeability and vasodilatation leading to extravasation. Furthermore, the kinin system can also be activated directly by the proteinases of snake venoms that activate the release of bradykinin [83,84]. This system is initiated by activation of Hageman factor (FXII) following tissue injury. This plasmatic factor activates in turn, the pre-kalikrein into kalikrein, in presence of kininogen, leading to vasoactive peptides causing fever and pain. Bradykinin is a nano-peptide responsible for the increased vascular permeability due to its binding with specific receptors on sensory neurons; it, therefore, activates the alternative complement pathway which amplifies the inflammatory response. SVMPs such as fibrolase isolated from Agkistrodon contortrix contortrix venom, is described to be involved in the biosynthesis/degradation pathways of bradykinin [85].

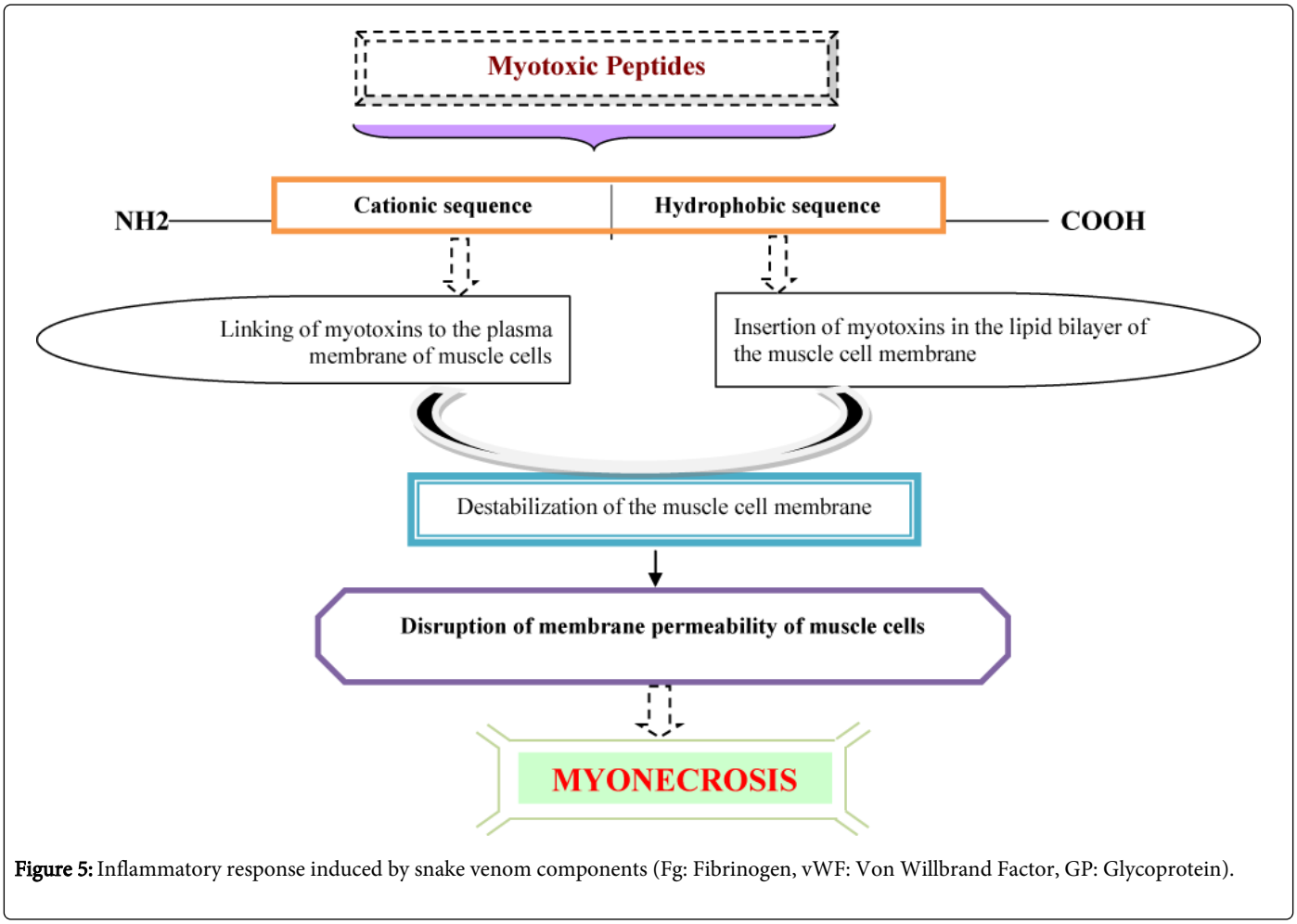

Figure 5: Inflammatory response induced by snake venom components (Fg: Fibrinogen, vWF: Von Willbrand Factor, GP: Glycoprotein).

Blood disorders

Coagulation and fibrinolysis: Proteolytic enzymes isolated from snake venoms were identified as α, β or γ fibrinogenases depending on their ability to hydrolyze the fibrinogen *in vitro* [10].

Several thrombin-like molecules isolated from snake venoms induce the release of either fibrinopeptides A or B or both of them. These fibrinopeptides act as mediators of inflammation and induce vascular permeability and neutrophil chemotaxis [15,11]. The formed thrombus is considered as a potent chemotactic agent for neutrophils and in vascular permeability acting on the kinin system. Viperidae and Crotalidae venoms are also able to induce fibrinolysis. Purification and characterization of three procoagulant proteinases (RP34, Afaâcytin and CC3-SPase proteinase) from Cerastes cerastes venom, showed fibrinogenolytic activities when analyzed by SDS-PAGE, afaâcytin and RP34 displayed, respectively, α,ß-fibrinogenase and α-fibrinogenase

activity [9,10,25]. Like afaâcytin, CC3-SPase is also characterized as an α,β-fibrinogenase due to the release of both A and B fibrinopeptides. TSV-PA, a serine proteinase purified from the venom of *Trimeresurus stejnegeri* plays a role of plasminogen activator. It shares about 70% of homology with other serine proteases and has significant structural similarities with the t-PA, it converts plasminogen to plasmin in the same way that the latter by the cleavage of bond Arg561-Val562 [86,87].

Hemorrhage: Spontaneous systemic bleeding is caused by hemorrhagins which damage vascular endothelium. Additional effects caused by snake envenomations (coagulopathies, hemorrhage, impaired and few platelets, and vessel wall damage) can result in severe bleeding, a common cause of death after bites by *Viperidae, Elapidae* and *Colubridae*. SVMPs through their disintegrin domain may have multiple roles; it would allow to the amplification of hemorragin action affecting platelet aggregation [9,50]. Disintegrins interact and block platelets integrins via their integrin-binding tri-peptide motifs; RGD (Arginine-Glycine-Aspartate) is the most common motif between many disintegrins, while other variants of motifs can also be found in some disintegrins e.g., KGD, WGD, MGD, KTS, RTS and MLD [88]. These motifs can act and inhibit platelet aggregation through their selective binding to integrins such as GPIIb/IIIa or αIIBβ3. SVMPs degrade also platelet membrane glycoproteins and their ligands such as collagen, GPIb and Von Willbrand factor [89,90].

Myotoxicity

Snake envenomation induces prominent local tissue damage that often results in permanent disability and systemic alterations associated with haemorrhage, coagulopathies, cardiovascular shock and renal failure. Clinical reports indicate that, in humans, the main invalidating effect is the irreversible disruption of muscle tissue [91]. Tissue necrosis is a relevant local effect caused after snakebites, it is considered as a serious consequence in severe cases of envenomation. When myonecrosis appears tissues are altered leading to the gangrene and infections. This type of complication can be the cause of amputation. Indeed, myotoxins of snake venoms affect mainly the plasma membrane of muscle cells to which they bind through their cationic sequence [73,92]. Molecular mechanism by which they caused the muscle tissue damage is not yet fully elucidated. Myonecrosis is due to the myotoxins that induce irreversible damage of skeletal muscle fibers. These molecules bind to the plasma membrane of muscle cells and alter its permeability and integrity (Figure 4). The induced muscle tissue damage could be due to the penetration of myotoxins into muscle cells by endocytosis, probably through membrane receptors onto the surface of muscle cells or following hydrolysis of phospholipids causing membrane disruption. These molecules enter into the cytosol, reach and alter the membrane of mitochondria and sarcoplasmic reticulum of muscle cells. The intracellular effect of these toxins occurs only after their initial action on the plasma membrane, which marks the onset of degenerative events [93,94].

Treatment

The complexity of the snake venoms and their induced effects after envenomation makes difficult their treatment. However, more attention was given to loco-regional disorders that sometimes lead to amputations and permanent disabilities. Human suffering attributable to snake bites remains a public health problem in many countries of the world, several people over the world are known to be envenomed and some them are killed or maimed by snakes every year. Preventive efforts should be aimed towards education of regions at-risk to reduce contact with snakes and to understand snakes' behavior [88]. Whatever the therapy used, it should include not only the neutralization of toxicity but also the other effects induced by venoms (hemorrhage and necrosis…). To treat snake envenoming, the production and clinical use of antivenom must be improved. Although antivenom was effective in the neutralization of systemic complications. It has limited effectiveness against the development of local damage, metabolic dysfunctions and tissue damage caused by venom components that are responsible for its health hazards due to their fast distribution and effects such as hemorrhage, myotoxicity and edema-forming [93]. Collaboration between physicians, epidemiologists and toxinologists should enhance the understanding and treatment of envenoming [88].

Beneficial Effects of Isolated Components from Snake Venoms

Most of venom compounds acquire interesting properties increasingly used in biomedical research and as tools in diagnosis and/or therapies. Indeed, the specific nature of coagulant or anti-coagulant properties of venoms makes them useful to better understand the haemostatic mechanisms [10,11]. Some of these components act synergistically at different stages of the coagulation cascade [95]. SVSPs and SVMPs present an interest as biomedicines and may be used as diagnostic tools; they act on this system as pro-coagulants, anticoagulants, and on platelet aggregation as pro- or anti-platelet [10]. Some of these molecules are used in the diagnosis and treatment of thrombotic and heart diseases. Components of snake venoms contain two categories of components that act antagonistically through activation or inhibition of coagulation factors and platelet aggregation. These compounds, able to hydrolyze the coagulation factors with high specificity, are divided into serine proteinases and metalloproteinases. Plasma defibrinogenation induced by snake venom components is of interest. Indeed, Arvin "ancrod", isolated from *Agkistrodon rhodostoma* venom, is one of the molecules used in patients treated with anti-coagulants. It is also used to lower the levels of fibrinogen in the treatment of peripheral vascular disorders. Botroxobin, another molecule isolated from the venom of *Bothrops atrox* and *Bothrops moojeni*, converts fibrinogen into fibrin by releasing only fibrinopeptide A. It is used for its defibrinogenating effect in the treatment of thrombotic diseases. The coagulating properties of Afaâcytin (*Bothrops asper*), insensitive to specific plasma thrombin inhibitors could be useful as hemostatic agent in some cases of bleeding and thrombocytopenia such as observed in post-operative situations [4,28]. The fibrinogenase RP34 could serve as defibrinogenating agent in the case of certain diseases [24,28,96]. All these defibrinogenating biomolecules share their properties, may be used as tools in clinical applications or in basic research. More in-depth studies in pharmacology, toxicology of these biomolecules are to be undertaken to determine their mode of action *in vivo*

Conclusion

Snake venoms, considered to be one of the most important bio-resources, include pharmacologically active molecules. To better understand the diversity of biological actions of snake venoms and propose new treatment for some pathologies, many studies focused on purification and characterization of new bioactive compounds from

venoms. Currently, biomolecules of snake venoms are of great fundamental diagnostic and therapeutic interest. Characterization, biological properties establishment of these bioactive molecules and investigation on their mechanisms, may lead to their eventual use for therapeutic purposes. Therapeutically, proteinases, disintegrins and C-type lectins from snake venoms are widely used as anticoagulants or/and anti-platelets. Furthermore, they are valuable tools for understanding the different mechanisms of hemostasis and are also used in the diagnosis of dysfunctions related to coagulation factors such as enzyme activity in thrombin-like venoms that are used for the fibrinogenopathy screening. Venom compounds are also used for diagnostic analysis of various coagulation factors (factors V, VII, X, platelet factor III, protein C and factor of Willbrand).

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