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Chapter 9

Snake Venom Peptides: Promising Molecules with Anti-Tumor Effects

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1. Introduction

Tumorigenesis and metastasis are two processes with inter-related mechanisms. These include tumor growth and angiogenesis, detachment of tumor cells from the primary tumor, followed by migration through the local connective tissue and penetration into the circulation (intravasation). Once in the blood stream, tumor cells interact with circulating blood cells, arrest in the microvasculature of target organs, then extravasate and secondary proliferate. During each of these steps, integrin-mediated adhesion, migration, proliferation and survival of tumor cells and angiogenic endothelial cells play crucial roles [1,2].

Integrins are a family of heterodimeric transmembrane receptors that mediate cell-cell and cell-extracellular matrix (ECM) interactions. These cell adhesion molecules are composed by non covalent association of α and β subunits. Although 18 α and 8 β subunits have been described, only 24 different combinations have been identified to date [3]. Specific integrin heterodimers preferentially bind distinct ECM proteins. The repertory of integrins present on a given cell dictates the extent to which that cell will adhere to and migrate on different matrices. Several integrins, among others αv and α5β1, recognize the RGD sequence on their respective ligands. Other adhesive sequences in ECM proteins have also been observed, including the EILDV and REDV sequences that are recognized by integrin α4β1 in an alternatively spliced form of fibronectin [3]. On ligation to the ECM, integrins cluster in the plane of the membrane and recruit various signalling and adaptor proteins to form structures known as focal adhesions [4].

Integrin expression can also vary considerably between normal and tumor tissue. Most notably, integrins αvβ3, α5β1 and αvβ6 are usually expressed at low or undetectable levels in most adult epithelia but can be highly up-regulated in some tumors. Expression levels of some integrins, such as α2β1, decrease in tumor cells; potentially increasing tumor cell dissemination [5]. The integrin αvβ3 is particularly important for tumor growth and

invasiveness [6]. The receptor plays a major role in neo-vessels formation, its expression being strongly up-regulated in endothelial cells and specifically required during angiogenesis stimulated by basic fibroblast growth factor (bFGF) and tumor necrosis factor-α [7,8]. αvβ3 is functionally involved in the malignant spread of various tumor cell types such as breast carcinoma, prostate carcinoma and melanoma, and supports tumor cell adhesion and migration through endothelium [9] and matrix proteins [10,1]. Blocking αvβ3 is therefore expected to have a broad impact in cancer therapy and diagnosis. In the last decade, several clinical trials evaluating the efficacy of αvβ3 blockers have led to encouraging results. Thus, MEDI-522 (Vitaxin), a humanized antibody derived from the mouse LM609 monoclonal antibody, was recently reported to give positive results in a phase II trial enrolling patients with stage IV metastatic melanoma [11]. Cilengitide is an inhibitor of both αvβ3 and αvβ5 integrins; it is currently being tested in phase II trials in patients with lung and prostate cancers [12] and in phase II and Phase III trials studying their role against glioblastoma are currently underway.

In addition to their role in tumor cells, integrins are also important for the host cellular response against cancer. Endothelial cells, fibroblasts, pericytes, bone marrow-derived cells, inflammatory cells and platelets all use integrins for various functions, including angiogenesis, desmoplasia and immune response.

Nature has been a source of medicinal products for thousands of years among which snake venoms form a rich source of bioactive molecules such as peptides, proteins and enzymes with important pharmacological activities. International research and development in this area, based on multidisciplinary approaches including molecular screening, proteomics, genomics and pharmacological *in vitro*, *ex vivo* and *in vivo* assays, allow the identification and characterization of highly specific molecules from snake venom that can potently inhibit integrin functions. These anti-adhesive snake venom proteins belong to different families (phospholipases, disintegrins, C-type lectins and metalloproteinases). By targeting integrins, they exhibit various pharmacological activities such as anti-tumor, anti-angiogenic and/or pro-apoptotic effects.

2. Snake venom protein families

2.1. The Snake Venom Metalloproteinases (SVMP)

Metalloproteinases are among the most abundant toxins in many *Viperidae* venoms. SVMPs are monozinc endopeptidases varying in size from 20 to 100 kDa. They are phylogenetically most closely related to the mammalian disintegrin and metalloproteinase (ADAM) family of proteins. SVMPs are grouped into several subclasses according to their domain organization [13, 14, 15]. P-I SVMPs are the simplest class of enzymes that contain only a metalloproteinase (M) domain. P-II SVMPs contain a M domain followed by a disintegrin (D) domain. P-III SVMPs contain M, disintegrin-like (D) and cysteine-rich (C) domains. Formally called P-IV, the heterotrimeric class of SVMPs that contain an additional snake C-type lectin-like (snaclec) domain [16] is now included in the P-III group as a subclass (P-IIId).

Most of the functional activities of SVMPs are associated with hemorrhage or the disruption of the hemostatic system, which are primarily mediated by the proteolytic activity of the M domain. SVMPs cause hemorrhage by disturbing the interactions between endothelial cells and the basement membrane through the degradation of endothelial cell membrane proteins (e.g., integrin, cadherin) and basement membrane components (e.g., fibronectin, laminin, nidogen, type IV collagen) [17]. Blood coagulation proteins (e.g., fibrinogen, factor X, prothrombin) are also targets of their proteolytic activities.

Echis carinatus venom contains the specific prothrombin activators, ecarin [18,19] and carinactivase [20]. Adamalysin II, a non-hemorrhagic P-I SVMP isolated from *Crotalus adamantus* venom, cleaves and inactivates serum proteinase inhibitors including antithrombin III [21]. Kaouthiagin, isolated from the venom of *Naja kaouthia* specifically binds and cleaves von Willebrand factor (vWF), resulting in loss of both the ristocetin-induced platelet aggregation and collagen-binding activity of vWF [22]. Additionally, a large number of the P-III SVMPs can inhibit platelet aggregation, thus enhancing the hemorrhagic state [23]. The hemorrhagic P-III SVMP jararhagin from the venom of *Bothrops jararaca* has been shown to degrade platelet collagen receptor $\alpha_2\beta_1$ integrin in addition to fibrinogen and vWF, resulting in the inhibition of platelet aggregation [24]. Other platelet receptors are also degraded by SVMPs. GPIbα is cleaved by kistomin; mocarhagin and crotalin [25-27], and GPVI is degraded by alborhagin, crotarhagin and kistomin [28,29].

In the other side, it was reported that several SVMPs inhibited integrin-mediated adhesion of cancer cells on ECM proteins (table 1). BaG, a dimeric PIII class of SVMP from *Bothrops alternatus* with inactivated enzymatic domain but intact D/C domain, has been reported to inhibit fibronectin-mediated K562 cell adhesion *via* α5β1 integrin [30].

Proteins	Snake	Integrins	Effects	References
VAP1, VAP2	*Crotalus atrox*	α3,α6,β1	Induce apoptosis of HUVEC	[31,36]
HV1	*Trimeruserus flavoviridis*	-	Inhibits adhesion of HUVEC and induces apoptosis	[32]
Halysase	*Gloydius halys*	α1β1;α5β1	Inhibits proliferation and Induces apoptosis of HUVEC	[33]
VLAIPs	*Vipera lebetina*	-	Inhibits proliferation and Induces apoptosis of HUVEC	[34]
Graminelysin	*Trimeresurus gramineus*	α1β1;α5β1	Inhibits proliferation and Induces apoptosis of HUVEC	[35]
BaG	*Bothrops alternatus*	α5β1	Inhibits adhesion of K562 cells	[30]
TSV-DM	*Trimeresurus stejnegeri*	-	Inhibits cell proliferation and induces transient cell morphologic changes of endothelial cells.	[113]

Table 1. SVMP affecting tumor cells

Several apoptosis-inducing proteins have been purified from hemorrhagic snake venom, such as VAP1 and VAP2 (*Crotalus atrox*), HV1 (*Trimeresurus flavoviridis*), halysase (*Gloydius halys*), and VLAIPs (*Vipera lebetina*) [31-34], graminelysin [35]. They are members of the SVMP and ADAM family and induce apoptosis of human umbilical vein endothelial cells (HUVECs) [31,36]. The detachment of endothelial cells and resulting apoptosis could be an additional mechanism for the disruption of normal hemostasis by SVMPs. TSV-DM a basic metalloproteinase from *Trimeresurus stejnegeri* venom inhibits cell proliferation and induces cell morphologic changes transiently of ECV304 cells. However, DNA fragmentation and DNA content analysis demonstrated that this metalloproteinase could not induce ECV304 cells apoptosis.

2.2. The disintegrins

Disintegrins are a family of non-enzymatic and low molecular weight proteins derived from viper venom [37-39]. They are able to inhibit platelet aggregation and interact with adhesion molecules in particular integrins in a dose-dependent manner. They have a K / RTS sequence which is known as the RGD adhesive loop [37-39]. Their primary structure shows a strong conservation in the arrangement of cysteines [38]. Most disintegrins represent the C-terminal domain of metalloproteinases PIIa, d and e classes and are released into the venom by proteolytic cleavage [40,37,38]. A minority of these proteins exist as D / C domains from the class of SVMPs PIIIb.

Disintegrins can be conveniently divided into five different groups according to their length and the number of disulfide bridges [41]. The first group includes short disintegrins, single polypeptide composed of 49 - 51 amino acids with four disulfide bridges. The second group comprises medium disintegrins containing about 70 amino acids and six disulfide bridges. The third group includes long disintegrins of 83 residues linked by seven disulfide bridges. The disintegrin domains of PIII snake-venom metalloproteinases, containing approx. hundred amino acids with 16 Cysteine residues involved in the formation of eight disulfide bonds, constitute the fourth subgroup of the disintegrin family. Unlike short-, medium- and long-sized disintegrins, which are single-chain molecules**,** the fifth subgroup is composed of homo and heterodimers. The dimeric disintegrins subunits contain about 67 residues with four disulfide intra-chain bridges and two interchain bridges [42,43].

Although disintegrins are highly homologous, significant differences exist in their affinity and selectivity for integrins, which explains the multitude of effects of these molecules (Table 2).

Disintegrins were first identified as inhibitors of platelet aggregation and were subsequently shown to antagonize fibrinogen binding to platelet integrin αIIbβ3 [44,45]. After that, studies on disintegrins have revealed new uses in the diagnosis of cardiovascular diseases and the design of therapeutic agents in arterial thrombosis, osteoporosis, and angiogenesis-related tumor growth and metastasis (table 2). Triflavin from *Trimeresurus flavoviridis* venom was one of the first RGD-disintegrins shown to inhibit angiogenesis both *in vitro* and *in vivo*

[46]. Triflavin strongly inhibited cell migration toward vitronectin and fibronectin nearly thirty orders of magnitude greater than anti-αvβ3 monoclonal antibodies [46]. Triflavin was also more effective in inhibiting TNF-α-induced angiogenesis in the chicken chorioallantoic membrane (CAM) assay. Similar results were obtained with another RGD-disintegrin, rhodostomin, from *Agkistrodon rhodostoma* venom, which inhibits endothelial cell migration, invasion and tube formation induced by bFGF in Matrigel™ both *in vitro* and *in vivo* [47]. Rhodostomin effects were inhibited by anti-αvβ3 but not by anti-αvβ5 antibodies, thus supporting the hypothesis that the effects of RGD-disintegrins are mediated by blockade of the vitronectin receptor.

Proteins	Snake	Integrins	Effects	References
Triflavin	*Trimeresurus flavoviridis*	α5β1,αvβ3, α3β1	Inhibits adhesion of tumor cells to matrix proteins, cell migration and angiogenesis *in vitro* and *in vivo*	[46]
Rhodostomin	*Agikistrodon rhodostoma*	αvβ3,αvβ5	Inhibits cell migration, invasion of endothelial cells; inhibits angiogenesis *in vivo* and *in vitro*	[47]
Contortrostatin	*Agkistrodon contortrix contortrix*	αvβ3,α5β1, αvβ5, αIIββ3	Blocks adhesion, migration invasion of different type of tumor cells	[48]
Lebestatin	*Macrovipera lebetina*	α1β1	Inhibits migration and angiogenesis	[56]
Accurhagin-C	*Agkistrodon acutus*	αvβ3	Prevents migration and invasion of endothelial cells; anti-angiogenic activity *in vitro* and *in vivo*; elicites anoïkis	[58]
Eristostatin	*Eritocophis macmahoni*	α4β1,other integrin not yet determined	Inhibits cell motility; no effect on cell proliferation or angiogenesis	[59,60]
DisBa-01	*Bothrops alternatus*	αvβ3	Anti-angiogenic and anti-metastatic effect on melanoma cells	[62]
Leberagin-C	*Macrovipera lebetina*	αvβ3	Inhibits cell adhesion of melanoma tumor cells	[114]
Accutin	*Agkistrodon acutus*	αvβ3	Inhibits angiogenesis *in vitro* and *in vivo*; induces apoptosis	[115]

Table 2. Effects of disintegrins on cancerous cells

Contortrostatin, a disintegrin isolated from the venom of the southern copperhead snake, exhibits anti-cancer activity in a variety of tumor cells [48-50]. It does not display cytotoxic activity *in vitro* nor animals upon injection. Contortrostatin inhibits adhesion, migration, invasion, metastatic and angiogenesis of tumor and endothelial cells mediated by $\alpha v\beta 3$,$\alpha 5\beta 1$ and $\alpha v\beta 5$ [48,50-54]. Recently, contortrostatin showed an additive inhibitory effect in combination with docetaxel on the growth of xenograft tumors derived from prostate cancer cells [55].

Lebestatin is an example of a non toxic KTS-disintegrin isolated from *Macrovipera lebetina* that inhibits migration and VEGF-induced *in vivo* angiogenesis [56]. The presence of a WGD motif in CC8, a heterodimeric disintegrin from *Echis carinatus*, increases its inhibitory effect on $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins [57].

There are few reports regarding the effects of ECD-disintegrins on endothelial cell migration. Acurhagin-C, dose-dependently blocked HUVEC migration toward a vitronectin-coated membrane. Furthermore, acurhagin-C elicited endothelial anoïkis *via* disruption of the $\alpha v\beta 3$/FAK/PI3K survival cascade and subsequent initiation of the procaspase-3 apoptotic signaling pathway [58].

Eristostatin, an RGD-disintegrin from *Eristocophis macmahoni* was tested on individual metastasis steps such as cell arrest, extravasation and migration [59]. Eristostatin treatment did not prevent tumor cell extravasation or migration [60]. However, it was shown later that eristostatin inhibited melanoma cell motility, an effect mediated by fibronectin-binding integrins [61]. Interestingly, this disintegrin, contrary to other RGD-disintegrins, did not inhibit angiogenesis, as stated before [61]. DisBa-01, a $\alpha v\beta 3$ integrin-blocking RGD-disintegrin, inhibits not only migration of endothelial cells *in vivo* [62] but also *in vitro* migratory ability of fibroblasts and two tumor cell lines.

Since integrin receptors are also quite indiscriminate as they support cell adhesion to several substrates, it seems highly reasonable that the general RGD-disintegrin scaffold of the integrin-binding motif could be employed as a prototype for drug design for new anti-metastatic therapies *via* blocking both tumor cell adhesion and tumor angiogenesis.

2.3. The snake venom phospholipases

Snake venom is one of the most abundant sources of secretory phospholipases A2 (PLA2), which are one of the potent molecules in snake venoms [63-65].

PLA2 (EC 3.1.1.4)—are enzymes that catalyze the hydrolysis of sn-2-acyl bond of sn-3-phospholipids, generating free fatty acids and lysophospholipids as products [66]. They are currently classified in 15 groups and many subgroups that include five distinct types of enzymes, namely secreted PLA2 (sPLA2), cytosolic PLA2 (cPLA2), Ca^{2+} independent PLA2s (iPLA2), platelet-activating factor acetyl-hydrolases (PAF-AH), lysosomal PLA2, and a recently identified adipose-specific PLA2 [65,67]. PLA2 are low molecular weight proteins with molecular masses ranging from 13-19 kDa that generally require Ca^{2+} for their activities

[69,70]. Snake venom sPLA2 are secreted enzymes belonging to only two groups that are based on their primary structure and disulfide bridge pattern [68,71,72]. Those of group I are similar to pancreatic sPLA2 present in mammals, were found in venom of *Elapidae* snakes, while group II PLA2s belong to the *Viperidae* and are similar to mammals nonpancreatic, inflammatory sPLA2s [73,74]. The group II can be subdivided mainly in two subgroups, depending on the residue at position 49 in the primary structure: Aspartic acid-49 PLA2s are enzymatically active, while Lysine 49 present low or no enzymatic activity [75]. There are other subgroups, such as Asparagine-49, Serine-49, Glutamine-49 and Arginine -49 [76-83]. Studies have found that catalytic activity is reduced or even abolished when an Aspartic acid of native PLA2 is replaced by another amino acid [80,84].

Despite a high identity of their amino acid sequences, sPLA2 exhibit a wide variety of pharmacological properties such as anticoagulant, haemolytic, neurotoxic, myotoxic, oedema-inducing, hemorrhagic, cytolytic, cardiotoxic, muscarinic inhibitor and antiplatelet activities [63,85-92].

Recently, PLA2s have been shown to possess anti-tumor and anti-angiogenic properties (Table 3). CC-PLA2-1 and CC-PLA2-2 from *Cerastes cerastes* viper are non-toxic and acidic proteins. They have high inhibitory effects on platelet aggregation and coagulation. In addition, CC-PLA2-1 and CC-PLA2-2 inhibit the adhesion of the human fibrosarcoma (HT1080) and melanoma (IGR39) cells to fibrinogen and fibronectin. In the same direction, CC-PLA2-1 and CC-PLA2-2 potently reduces HT1080 cell migration to fibrinogen and fibronectin with nearly similar IC_{50} values [93]. This anti-adhesive effect was due to the inhibition of $\alpha 5\beta 1$ and αv-containing integrins [94]. A recent report demonstrated that Bth-A-I, a non-toxic PLA2 isolated from *Bothrops jararacussu* venom display an anti-tumoral effect upon breast adenocarcinoma as well as upon human leukaemia T and Erlich ascetic tumor [95].

Proteins	Snake	Integrins	Effects	References
CCPLA2-1; CCPLA2-2	*Cerastes cerastes*	$\alpha 5\beta 1, \alpha v$	Inhibits migration and adhesion of fibrosarcoma and melanoma cells	[93,94]
Bth-A-I-PLA2	*Bothrops jararacussu*	-	Anti-tumor activity on adenocarcinoma and leukaemia cells	[95]
MVL-PLA2	*Macrovipera lebetina*	$\alpha 5\beta 1, \alpha v$	Inhibits adhesion and migration of human microvascular cells and inhibits angiogenesis *in vivo* and *in vitro*.	[96]
BP II	Prothobotrops flavoviridis	-	Induces cell death in human leukaemia cells	[97]

Table 3. PLA2s targeting tumor cells

MVL-PLA2 is a snake venom phospholipase purified from Macrovipera lebetina venom that inhibited adhesion and migration of human microvascular endothelial cells (HMEC-1) without being cytotoxic. Using Matrigel™ and chick chorioallantoic membrane assays, MVL-PLA2, as well as its catalytically inactivated form, significantly inhibited angiogenesis both *in vitr*o and *in vivo*. Also, the actin cytoskeleton and the distribution of $\alpha v\beta 3$ integrin, a critical regulator of angiogenesis and a major component of focal adhesions, were disturbed after MVL-PLA2 treatment. The enhancement of microtubule dynamics of HMEC-1 cells, in consequence of treatments by MVL-PLA2, may explain the alterations in the formation of focal adhesions, leading to inhibition of cell adhesion and migration [96].

A cell death activity was discovered in Lysine 49-PLA2 called BPII. It induces caspase-independent cell death in human leukaemia cells regardless of its depressed enzymatic activity [97].

2.4. The C-type lectins

The C-type lectins are abundant components of snake venom with various function. Typically, these proteins bind calcium and sugar residues. However, the C-type lectin like proteins from snake venom (termed actually snaclec) does not contain the classic calcium/sugar binding loop and have evolved to bind a wide range of physiologically important proteins and receptors [98].

Snaclecs have a basic heterodimeric structure with two subunits, nearly always linked covalently, *via* a disulphide bond. The heterodimers are often further multimerized either non-covalently or covalently *via* additional disulphide bonds, to form larger structures [99]. The two subunits form a concave surface between them [100] thus constituting the main site of ligand binding [101,102]. The subunits have a high structural degree of homology between them and with other snaclecs [103]. Despite their highly conserved primary structure, the snaclecs are characterized by various biological activities. They were and are still considered as modulators of platelet aggregation by targeting vWF, GPIb-IX-V, GPVI and possibly other platelet receptors.

Recently, novel activities of snaclecs were highlighted. They were described for their potential anti-tumor effect by blocking adhesion, migration, proliferation and invasion of different cancer cell lines (Table 4). Among these proteins, EMS16, a heterodimer isolated from the venom of *Echis multisquamatus*, inhibits the adhesion of HUVECs cells on ECM proteins and their migration by inhibiting the binding of integrin $\alpha 2\beta 1$ to collagen [104].

Lebecetin and lebectin, purified from *Macrovipera lebetina* venom, are the only snaclecs, until today, with an evident anti-tumor effect in addition to their anti-aggregation activity on platelets. Indeed, these two non cytotoxic proteins inhibit the adhesion of various cancer cell lines: melanoma (IGR39), adenocarcinoma (HT29-D4), fibrosarcoma (HT1080) and leukemia cells (K562) on different ECM proteins. They also inhibit the proliferation, migration and invasion of HT1080 cells [105,106]. Lebectin also displays anti-angiogenic activity at very low concentrations both *in vitro* and *in vivo* [107]. Thus, lebectin presents the best anti-

angiogenic efficacy yet described for snake venom-derived peptides [108,109]. These observed effects are mediated by $\alpha 5\beta 1$ and αv integrins [107].

Extensive researches have been shown that cell adhesion activities in cancer disease are deregulated. According to this idea, it was also reported that lebectin inhibits these alterations by promoting N-cadherin/catenin complex reorganisation at cell-cell contacts, inducing a strengthening of intercellular adhesion [110].

Another snaclec, BJcuL isolated from *Bothrops jararacussa* venom, was also described for its anti-tumor, but the receptor or integrin implicated has not been determined yet. This homodimeric protein inhibits proliferation of several cell lines of renal, pancreatic, prostate and melanoma origin, but no effect was observed on colon or breast cancer cells [111]. BJcuL also affects the viability of some tumor cell lines of different origins, but has no effect on the growth of K562 and T24 cells, suggesting that these cells do not express the receptor recognized by the lectin. BJcuL induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly [112].

Proteins	Snake	Integrins	Effects	References
Lebecetin, lebectin	*Macrovipera lebetina*	$\alpha 5\beta 1$,αv	Inhibits adhesion, migration and invasion of human tumor cells; inhibits angiogenesis	[106]
BJcuL	*Bothrops jararacussu*	–	Inhibits tumor cell and endothelial cell growth; induces apoptosis of human gastric carcinoma cells; inhibits cell adhesion and actin cytoskeleton disassembly	[111,112]
EM16	*Echis multisquamatus*	$\alpha 2\beta 1$	Inhibits adhesion and migration of HUVEC cells	[104]

Table 4. Snaclecs and their effects on tumor cells

3. Potential application of snake venom compounds

Venoms are a rich source of molecules endowed with diverse pharmacological effects. Most part of these molecules act *via* the adhesion molecules. The intervention of the scientists and the clinicians in the pharmaceutical development field would employ these molecules as therapeutic agents for several pathologies such as cancer, thrombosis, diabetes....

Until now, no medicine was produced from a native molecule purified from venom. However, several peptidomimetics were designed by basing on the structure of these molecules. The benefits of these peptidomimetics compared to antibodies that can be used for the treatment of certain diseases are: a shorter half-life, reversible inhibition, easier to control a problem and very low immunogenicity. For example, the antihypertensive drug captopril, modelled from the venom of the Brazilian arrowhead viper (*Bothrops jaracusa*); the anticoagulant Integrilin (eptifibatide), a heptapeptide derived from a protein found in the

venom of the American southeastern pygmy rattlesnake (*Sistrurus miliarius barbouri*); Ancrod, a compound isolated from the venom of the Malaysian pit viper (*Agkistrodon rhodostoma*) for use in the treatment of heparin-induced thrombocytopenia and stroke and alfimeprase, a novel fibrinolytic metalloproteinase for thrombolysis derived from southern copperhead snake (*Agkistrodon contortrix contortrix*) venom (Table 5). Two venom proteins from the Australian brown snake, *Pseudonaja textilis*, are currently in development as human therapeutics (QRxPharma). The first is a single agent procoagulant that is a homolog of mammalian Factor Xa prothrombin activator, whereas the other is a plasmin inhibitor, named Textilinin-1, with antihemorrhagic properties.

Name	Snake	Target and function/treatment	Clinical stage
Capoten ® (Captropil)	*Bothrops jaracusa*	Angiotensin converted enzyme (ACE) inhibitor/ high blood pressure	Granted FDA approval
Integrilin ® (Eptifibatide)	*Sisturus miliarus barbouri*	Platelet aggregation inhibitor/acute coronary syndrome	Granted FDA approval
Aggrastat ® (tirofiban)	*Echis carinatus*	GPIIb-IIIa inhibitor/ myocardial infarct, refractory ischemia	Approved for use with heparin and aspirin for the treatment of acute coronary syndrome
Exanta	*Cobra*	Thrombin inhibitor/ arterial fibrillation and blood	Seeking FDA approval
Alfimeprase	(Agkistrodon contortrix contortrix)	Thrombolytic/ Acute ischemic stroke, acute peripheral arterial occlusion	Phase III
Ancrod ® (viprinex)	*Agkistrodon rhodostoma*	Fibrinogen inhibitor/ stroke	Phase III
hemocoagulase	*Bothrops atrox*	Thrombin-like effect and thromboplastin activity/ prevention and treatment of haemorrhage	Phase III
Protac/ Protein C activator	*Agkistrodon contortrix contortrix*	Protein C activator/clinical diagnosis of haemostatic disorder	Granted FDA approval
Reptilase	*Bothrops jaraca*	Diagnosis of blood coagulation disorder	Granted FDA approval
Ecarin	*Echis carinatus*	Prothrombin activator/ diagnostic	Granted FDA approval

Table 5. Drugs and clinical diagnostic kits from snake venom

Actually, most of the current anticancer therapies (radiotherapy, chemotherapy) are not specific and are targeting at both tumor cells and healthy cells. However, in recent years, new treatments tend to focus on the tumor microenvironment and particularly on the inhibition of tumor angiogenesis. These treatments are based on several active and non toxic proteins from snake venom, as for example contortrostatin from *Agkistrodon contortrix contortrix* and eristostatin from *Eristocophis macmahoo.* Although all these molecules are still currently in clinical trials, they could in the future open new ways of healing and could be used as drugs.

4. Conclusions

From the initial discovery of captopril, the first oral ACE inhibitor, to the recent application of disintegrins for the potential treatment of cancer, the various components of snake venoms have never failed to reveal amazing new properties. While the original native snake venom compounds are usually unsuitable as therapeutics, interventions by medicinal chemists as well as scientists and clinicians in pharmaceutical R&D have made it possible to use the snake venom proteins as potential drugs for multiple disorders or scaffolds for drug design.

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5. References

[1] Felding-Habermann, B. Integrin adhesion receptors in tumor metastasis. Clinical and Experimental Metastasis 2003;20(3) 203–213.
[2] Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Research 1975; 35(1) 218–224.
[3] Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Research 2010;339(1) 269-280.
[4] Berrier AL and Yamada KM. Cell Matrix. Journal of cellular physiology 2007;213(3) 565-573

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[5] Kren A, Baeriswyl V, Lehembre F, Wunderlin C, Strittmatter K, Antoniadis H, Fässler R, Cavallaro U, Christofori G. Increased tumor cell dissemination and cellular senescence in the absence of β1-integrin function. The EMBO Journal 2007;26(12) 2832–2842.

[6] Albelda SM, Mette SA, Elder DE, Stewart R, Damjanovich L, Herlyn M, Buck CA. Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. Cancer Research 1990;50(20) 6757–6764.

[7] Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 1994;264(5158) 569–571

[8] Enenstein J, Waleh NS, Kramer RH. Basic FGF and TGFbeta differentially modulate integrin expression of human microvascular endothelial cells. Experimental Cell Research 1992;203(2) 499–503.

[9] Voura EB, Ramjeesingh RA, Montgomery AM, Siu CH. Involvement of integrin alpha(v)beta(3) and cell adhesion molecule L1 in transendothelial migration of melanoma cells. Molecular Biology of the Cell 2001; 12(9) 2699–2710.

[10] Cooper CR, Chay CH, Pienta KJ. The role of alpha(v)beta (3) in prostate cancer progression. Neoplasia 2002;4(3) 191–194.

[11] Hersey P, Sosman J, O'Day S. A phase II, randomized, open-label study evaluating the antitumor activity of MEDI-522, a humanized monoclonal antibody directed against the human alpha v beta 3 ($\alpha v\beta 3$) integrin, ± dacarbazine (DTIC) in patients with metastatic melanoma (MM). ASCO Annual Meeting Proceedings, Journal of Clinical Oncology 2005;

[12] Beekman KW, Colevas AD, Cooney K, Dipaola R, Dunn RL, Gross M, Keller ET, Pienta KJ, Ryan CJ, Smith D, Hussain M. Phase II evaluations of cilengitide in asymptomatic patients with androgen independent prostate cancer: scientific rationale and study design. Clinical Genitourinary Cancer 2006;4(4) 299–302.

[13] Fox JW and Serrano SM. Structural considerations of the snake venom metalloproteinases, key members of the M12 reprolysin family of metalloproteinases, Toxicon 2005;45(8) 969–985.

[14] Fox JW and Serrano SM. Insights into and speculations about snake venom metalloproteinase (SVMP) synthesis, folding and disulfide bond formation and their contribution to venom complexity, FEBS Journal 2008;275 (12) 3016–3030.

[15] Takeya H, Miyata T, Nishino N, Omori-Satoh T, Iwanaga S. Snake venom hemorrhagic and non hemorrhagic metalloendopeptidases, Methods in Enzymolgy 1993;223 365–378.

[16] Clemetson KJ, Morita T, Manjunatha Kini R. Scientific and standardization committee communications: classification and nomenclature of snake venom C-type lectins and related proteins, Journal of Thrombosis Haemostasis 2009;7(2) 360.

[17] Baramova EN, Shannon JD, Bjarnason JB, Fox JW. Degradation of extracellular matrix proteins by hemorrhagic metalloproteinases. Archives of Biochemistry and Biophysics 1989;275(1) 63–71.

[18] Morita T and Iwanaga S. Purification and properties of prothrombin activator from the venom of Echis carinatus. Journal of Biochemistry 1978;83(2) 559-570.

[19] Nishida S, Fujita T, Kohno N, Atoda H, Morita T, Takeya H, Kido I, Paine MJ, Kawabata S, Iwanaga S. cDNA cloning and deduced amino acid sequence of prothrombin

activator (ecarin) from Kenyan Echis carinatus venom. Biochemistry 1995;34 (5) 1771–1778.

[20] Yamada D, Sekiya F, Morita T. Isolation and characterization of carinactivase, a novel prothrombin activator in Echis carinatus venom with a unique catalytic mechanism. Journal Biological Chemistry 1996;271(9) 5200–5207.

[21] Kress LF and Paroski EA. Enzymatic inactivation of human serum proteinase inhibitors by snake venom proteinases. Biochemical and Biophysics Research Communications 1978;83(2) 649–656.

[22] Hamako J, Matsui T, Nishida S, Nomura S, Fujimura Y, Ito M, Ozeki Y, Titani K. Purification and characterization of kaouthiagin, a von Willebrand factor-binding and –cleaving metalloproteinase from Naja kaouthia cobra venom, Thrombosis and Haemostasis 1998;80(3) 499–505.

[23] Laing GD, Moura-da-Silva AM. Jararhagin and its multiple effects on hemostasis. Toxicon 2005; 45 (8) 987–996.

[24] Kamiguti AS. Platelets as targets of snake venom metalloproteinases. Toxicon 2005;45(8) 1041–1049.

[25] Huang TF, Chang MC, Teng CM. Antiplatelet protease, kistomin, selectively cleaves human platelet glycoprotein Ib. Biochemica et Biophysica Acta 1993;1158(3) 293–299.

[26] Ward CM, Andrews RK, Smith AI, Berndt MC. Mocarhagin, a novel cobra venom metalloproteinase, cleaves the platelet von Willebrand factor receptor glycoprotein Ib alpha. Identification of the sulfated tyrosine/anionic sequence Tyr-276-Glu-282 of glycoprotein Ib alpha as a binding site for von Willebrand factor and alpha-thrombin, Biochemistry 1996;35(15) 4929–4938.

[27] Wu WB, Peng HC, Huang TF. Crotalin, a vWF and GP Ib cleaving metalloproteinase from venom of Crotalus atrox. Thrombosis and Haemostasis 2001;86(6) 1501–1511.

[28] Hsu CC, Wu WB, Huang TF. A snake venom metalloproteinase, kistomin, cleaves platelet glycoprotein VI and impairs platelet functions, Journal of Thrombosis and Haemostasis 2008;6(9) 1578–1585.

[29] Wijeyewickrema LC, Gardiner EE, Moroi M, Berndt MC, Andrews RK. Snake venom metalloproteinases, crotarhagin and alborhagin, induce ectodomain shedding of the platelet collagen receptor, glycoprotein VI. Thrombosis and Haemostasis 2007;98(6) 1285–1290.

[30] Cominetti MR, Ribeiro JU, Fox JW, Selistre-de-Araujo HS. BaG, a new dimeric metalloproteinase/disintegrin from the Bothrops alternatus snake venom that interacts with alpha5beta1 integrin. Archives of Biochemistry and. Biophysics 2003;416(2) 171–179.

[31] Masuda S, Araki S, Kaji K, Hayashi H. Purification of a vascular apoptosis-inducing factor from hemorrhagic snake venom. Biochemical and Biophysics Research Communications 1997;235(1) 59–63.

[32] Masuda S, Hayashi H, Atoda H, Morita T, Araki S. Purification, cDNA cloning and characterization of the vascular apoptosis-inducing protein, HV1, from Trimeresurus flavoviridis. European Journal of Biochemistry 2001;268(11) 3339–3345.

[33] You WK, Seo HJ, Chung KH, Kim DS. A novel metalloprotease from Gloydius halys venom induces endothelial cell apoptosis through its protease and disintegrin-like domains. Journal of Biochemistry 2003;134(5) 739–749.

[34] Trummal K, Tonismagi K, Siigur E, Aaspollu A, Lopp A, Sillat T, Saat R, Kasak L, Tammiste I, Kogerman, P, Kalkkinen, N, Siigu, J. A novel metalloprotease from Vipera lebetina venom induces human endothelial cell apoptosis. Toxicon 2005;46(1) 46–61.

[35] WU WB, Shin CC, Ming-Yi L, Tur-Fu H. Purification, molecular cloning and mechanism of action of graminelysin I, a snake-venom-derived metalloproteinase that induces apoptosis of human endothelial cells. Biochemical Journal 2001;357(Pt 3) 719-728.

[36] Masuda S, Hayashi H, Araki S. Two vascular apoptosis-inducing proteins from snake venom are members of the metalloprotease/disintegrin family. European Journal of Biochemistry 1998;253(1) 36–41.

[37] McLane MA, Sanchez EE, Wong A, Paquette-Straub C, Perez JC. Disintegrins. Current Drugs Targets. Cardiovascular & Haematological Disorders 2004; 4 327-355.

[38] Calvete JJ. Structure-function correlations of snake venom disintegrins. Current Pharmaceutical Design 2005;11(7) 829-835.

[39] Calvete JJ, Marcinkiewicz C, Monleon D, Esteve V, Celda B, Juarez P, Sanz L. Snake venom disintegrins: evolution of structure and function. Toxicon 2005;45(8) 1063-1074.

[40] Kini R M and Evans HJ. Structural domains in venom proteins: evidence that metalloproteinases and nonenzymatic platelet aggregation inhibitors (disintegrins) from snake venoms are derived by proteolysis from a common precursor. Toxicon 1992;30(3) 265-293

[41] Calvete JJ, Moreno-Murciano MP, Theakston RD, Kisiel DG, Marcinkiewicz, C. Snake venom disintegrins: novel dimeric disintegrins and structural diversification by disulphide bond engineering. Biochemical Journal 2003;372(Pt 3) 725-734.

[42] Calvete JJ, Jurgens M, Marcinkiewicz C, Romero A, Schrader M, Niewiarowski S. Disulphide-bond pattern and molecular modelling of the dimeric disintegrin EMF-10, a potent and selective integrin alpha5beta1 antagonist from Eristocophis macmahoni venom. Biochemical Journal 2000a;345 (Pt 3) 573-581.

[43] Bilgrami S, Tomar S, Yadav S, Kaur P, Kumar J, Jabeen T, Sharma S, Singh TP. Crystal structure of schistatin, a disintegrin homodimer from saw-scaled viper (Echis carinatus) at 2.5 A° resolution. Journal of Molecular Biology 2004;341(3) 829-837.

[44] Gould RJ, Polokoff MA, Friedman PA, Huang TF, Holt JC, Cook JJ, Niewiarowski S. Disintegrins: a family of integrin inhibitory proteins from viper venoms. Proceeding of the Society for Experimental Biology and Medicine 1990;195(2) 168–171.

[45] Ouyang C, Yeh HI, Huang TF. A potent platelet aggregation inhibitor purified from Agkistrodon halys (mamushi) snake venom. Toxicon 1983;21(6) 797 – 804.

[46] Sheu JR, Yen MH, Kan YC, Hung WC, Chang PT, Luk HN. Inhibition of angiogenesis in vitro and in vivo: comparison of the relative activities of triflavin, an Arg-Gly-Asp-containing peptide and anti-alpha(v)beta3 integrin monoclonal antibody. Biochemica et Biophysica Acta 1997;1336(3) 445–454.

[47] Yeh CH; Peng HC; Yang RS, Huang TF. Rhodostomin, a snake venom disintegrin, inhibits angiogenesis elicited by basic fibroblast growth factor and suppresses tumor

growth by a selective alpha(v)beta(3) blockade of endothelial cells. Molecular Pharmacology 2001;59(5) 1333–1342

[48] Swenson S, Costa F, Ernst W, Fujii G, Markland FS. Contortrostatin, a snake venom disintegrin with anti-angiogenic and anti-tumor activity. Pathophysiology of Haemostasis and Thrombosis 2005;34(4-5) 169–176.

[49] Swenson S, Costa F, Minea R, Sherwin RP, Ernst W, Fujii G, Yang D, Markland FS Jr. Intravenous liposomal delivery of the snake venom disintegrin contortrostatin limits breast cancer progression. Molecular Cancer Therapeutics 2004;3(4) 499–511.

[50] Trikha M, De Clerck YA, Markland FS. Contortrostatin, a snake venom disintegrin, inhibits beta 1 integrin-mediated human metastatic melanoma cell adhesion and blocks experimental metastasis. Cancer Research 1994;54(18) 4993–4998.

[51] Zhou Q, Sherwin RP, Parrish C, Richters V, Groshen SG, Tsao-Wei D, Markland FS. Contortrostatin, a dimeric disintegrin from Agkistrodon contortrix contortrix, inhibits breast cancer progression. Breast Cancer Research and Treatment 2000a;61(3) 249 – 260.

[52] Zhou Q, Nakada MT, Brooks PC, Swenson SD, Ritter MR, Argounova S, Arnold C, Markland FS. Contortrostatin, a homodimeric disintegrin, binds to integrin alphavbeta5. Biochemical Biophysical Research Communications 2000b;267(1) 350 – 355.

[53] Trikha M, Rote WE, Manley PJ, Lucchesi BR, Markland FS. Purification and characterization of platelet aggregation inhibitors from snake venoms. Thrombosis Research 1994;73(1) 39–52.

[54] Ritter MR, Zhou Q, Markland FS Jr. Contortrostatin, a snake venom disintegrin, induces alphavbeta3-mediated tyrosine phosphorylation of CAS and FAK in tumor cells. Journal of Cellular Biochemistry 2000;79(1) 28–37.

[55] Lin E, Wang Q, Swenson S, Jadvar H, Susan G, Ye W, Markland F, Pinski J. The disintegrin contortrostatinin combination with docetaxel is a Potent Inhibitor of Prostate cancer in vitro and in vivo. The Prostate 2010;70(12) 1359-1370

[56] Olfa KZ; Jose L; Salma D, Bazaa A, Srairi N, Nicolas A; Maxime L, Zouari R, Mabrouk K, Marvaldi J, Sabatier JM, El Ayeb M; Marrakchi N. Lebestatin, a disintegrin from Macrovipera venom, inhibits integrin-mediated cell adhesion, migration and angiogenesis. Laboratory Investigation 2005;85(12) 1507–1516.

[57] Calvete JJ; Fox JW; Agelan A; Niewiarowski S; Marcinkiewicz C. The presence of the WGD motif in CC8 heterodimeric disintegrin increases its inhibitory effect on alphaII(b)beta3, alpha(v)beta3, and alpha5beta1 integrins. Biochemistry 2002;41(6) 2014–2021.

[58] Morris VL; Schmidt EE, Koop S, MacDonald IC, Grattan M; Khokha R, McLane MA; Niewiarowski S, Chambers AF, Groom AC. Effects of the disintegrin eristostatin on individual steps of hematogenous metastasis. Experimental Cell Research 1995;219(2) 571–578.

[59] Wang WJ. Acurhagin-C, an ECD disintegrin, inhibits integrin alphavbeta3-mediated human endothelial cell functions by inducing apoptosis via caspase-3 activation. British Journal of Pharmacology 2010;160(6) 1338–1351.

[60] Sohn YD, Cho KS, Sun SA, Sung HJ, Kwak KW, Hong SY, Kim DS, Chung KH. Suppressive effect and mechanism of saxatilin, a disintegrin from Korean snake (Gloydiussaxatilis), in vascular smooth muscle cells. Toxicon 2008;52(3) 474–480.

[61] Tian J, Paquette-Straub C, Sage EH, Funk SE, Patel V, Galileo D, McLane MA. Inhibition of melanoma cell motility by the snake venom disintegrin eristostatin. Toxicon 2007; 49(7) 899–908.

[62] Ramos OH, Kauskot A, Cominetti MR, Bechyne I, Salla Pontes CL, Chareyre F, Manent J, Vassy R, Giovannini M, Legrand C, Selistre-de-Araujo HS, Crepin M, Bonnefoy AA. Novel alpha(v)beta (3)-blocking disintegrin containing the RGD motive, DisBa-01, inhibits bFGF-induced angiogenesis and melanoma metastasis. Clinical & Experimental Metastasis 2008;25(1) 53–64.

[63] Kini RM. Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. Toxicon 2003;42(8) 827–840.

[64] Burke JE and Dennis EA. Phospholipase A2 Biochemistry. Cardiovascular drugs and Therapy 2009a;23(1) 1-22.

[65] Ramar PS, Gopalakrishnakone P, Bow H, Puspharaj PN, Chow VT. Identification and characterization of a phospholipase A2 from the venom of the Saw-scaled viper: Novel bactericidal and membrane damaging activities. Biochimie 2010;92(12) 1854-1866.

[66] Ritonja A and Gubensek F. Ammodytoxin A, a highly lethal phospholipase A2 from Vipera ammodytes ammodytes venom. Biochemica et Biophysica Acta 1985; 828(3) 93 306-312.

[67] Maung-Maung T, Gopalakrishnakone P, Yuen R, Tan CH. A major lethal factor of the venom of Burmese Russell's viper (Daboia russelli siamensis): isolation, N-terminal sequencing and biological activities of daboiatoxin. Toxicon 1995;33(1) 63-76.

[68] Chakrabarty D, Datta K, Gomes A, Bhattacharyya D. Haemorrhagic protein of Russell's viper venom with fibrinolytic and esterolytic activities. Toxicon 2000; 38(11) 1475-1490.

[69] Kini RM. Phospholipase A2-a complex multifunctional protein puzzle. In: Kini, R M (ed) Enzymes: Structure, Function and Mechanism. John Wiley and Sons, Chichester, England;1997. p 1-28.

[70] Valentin E and Lambeau G. Increasing molecular diversity of secreted phospholipases A(2) and their receptors and binding proteins. Biochemica et Biophysica Acta 2000;1488(1-2) 59-70.

[71] Six DA and Dennis EA. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. Biochemica et Biophysica Acta 2000;1488(1-2) 1-19.

[72] Rouault M, Bollinger JG, Lazdunski M, Gelb MH, Lambeau G. Novel mammalian group XII secreted phospholipase A2 lacking enzymatic activity. Biochemistry 2003;42(39) 11494-11503.

[73] Lambeau G and Lazdunski M. Receptors for a growing family of secreted phospholipases A2. Trends in Pharmacological Sciences 1999;20(4) 162-170.

[74] Dennis EA. Phospholipase A2 in ecicosanoid generation. American Journal of Respiratory and Critical Care Medicine 2000 61(2 Pt 2) S32-S35.

[75] Lomonte B, Angulo Y, Calderon L. An overview of lysine-49 phospholipase A2 myotoxins from crotalid snake venoms and their structural determinants of myotoxic action. Toxicon 2003;42(8) 885-901

[76] Tsai IH, Wang YM, Chen YH, Tsai TS. Venom phospholipases A2 of bamboo viper (Trimeresurus stejnegeri): molecular characterization, geographic variations and evidence of multiple ancestries. Biochemical Journal 2004; 77(Pt 1) 215–223.

[77] Wei JF, Wei XL, Chen QY, Huang T, Qiao LY, Wang WY, Xiong YL, He SH. N49 phospholipase A2, a unique subgroup of snake venom group II phospholipase A2. Biochemica et Biophysica Acta 2006;1760(3) 462–471.

[78] Krizaj I, Bieber AL, Ritonja A, Gubensek F. The primary structure of ammodytin L, a myotoxic phospholipase A2 homologue from Vipera ammodytes venom. European Journal of Biochemistry 1991;202(3) 1165–1168.

[79] Polgar J, Magnenat EM, Peitsch MC, Wells TN, Clemetson KJ. Asp-49 is not an absolute prerequisite for the enzymic activity of low-M(r) phospholipases A2: purification, characterization and computer modelling of an enzymically active Ser- 49 phospholipase A2, ecarpholin S, from the venom of Echis carinatus sochureki (saw-scaled viper). Biochemical Journal 1996;319(Pt 3) 961–968.

[80] Bao Y, Bu P, Jin L, Wang H, Yang Q, An L. Purification, characterization and gene cloning of a novel phospholipase A2 from the venom of Agkistrodon blomhoffii ussurensis. The International Journal of Biochemistry & Cell Biology Cell Biol 2005;37(3) 558–565.

[81] Chijiwa T, Tokunaga E, Ikeda R, Terada K, Ogawa T, Oda-Ueda N, Hattori, S, Nozaki, M, Ohno M. Discovery of novel [Arg49] phospholipase A2 isozymes from Protobothrops elegans venom and regional evolution of Crotalinae snake venom phospholipase A2 isozymes in the southwestern islands of Japan and Taiwan. Toxicon 2006;48(6) 672–682.

[82] Mebs D, Kuch U, Coronas FIV, Batista CVF, Gumprecht A, Possani LD. Biochemical and biological activities of the venom of the Chinese pitviper Zhaoermia mangshanensis, with the complete amino acid sequence and phylogenetic analysis of a novel Arg49 phospholipase A2 myotoxin. Toxicon 2006;47(7) 797–811.

[83] Wei JF, Li T, Wei XL, Sun QY, Yang FM, Chen QY, Wang WY, Xiong YL, He SH. Purification, characterization and cytokine release function of a novel Arg-49 phospholipase A2 from the venom of Protobothrops mucrosquamatus. Biochimie 2006;88(10) 1331–1342.

[84] Li Y, Yu BZ, Zhu H, Jain MK, Tsai MD. Phospholipase A2 engineering. Structural and functional roles of the highly conserved active site residue aspartate-49. Biochemistry 1994;33(49)14714-14722.

[85] Kini RM and Evans HJ. Correlation between the enzymatic activity, anticoagulant and antiplatelet effects of phospholipase A2 isoenzymes from Naja nigricollis venom. Thrombosis and Haemostasis 1988;60(2) 170-173.

[86] Kasturi S and Gowda TV. Purification and characterization of a major phospholipase A2 from Russell's viper (Vipera russelli) venom. Toxicon 1989;27(2) 229-237.

[87] Stefansson S, Kini, RM, Evans HJ. The inhibition of clotting complexes of the extrinsic coagulation cascade by the phospholipase A2 isoenzymes from Naja nigricollis venom. Thrombosis Research 1989; 55(4) 481-491.

[88] Maung-Maung T, Gopalakrishnakone P, Yuen R, Tan CH. A major lethal factor of the venom of Burmese Russell's viper (Daboia russelli siamensis): isolation, N-terminal sequencing and biological activities of daboiatoxin. Toxicon 1995; 33(1) 63-76.

[89] Huang MZ, Gopalakrishnakone P, Kini RM. Role of enzymatic activity in the antiplatelet effects of a phospholipase A2 from Ophiophagus hannah snake venom. Life Sciences 1997; 61(22) 2211-2217.

[90] Kole L, Chakrabarty D, Datta K, Bhattacharyya D. Purification and characterization of an organ specific haemorrhagic toxin from Vipera russelli russelli (Russell's viper) venom. Indian Journal of Biochemistry & Biophysics 2000; 37(2) 114-120.

[91] Chakrabarty AK, Hall RH, Ghose AC. Purification and characterization of a potent hemolytic toxin with phospholipase A2 activity from the venom of Indian Russell's viper. Molecular and Cellular Biochemistry 2002; 237(1-2) 95-102.

[92] Dong M, Guda K, Nambiar PR, Rezaie A, Belinsky GS, Lambeau G, Giardina C, Rosenberg DW. Inverse association between phospholipase A2 and COX-2 expression during mouse colon tumorigenesis. Carcinogenesis 2003; 24(2) 307-315.

[93] Zouari-Kessentini R, Luis J, Karray A, Kallech-Ziri O, Srairi-Abid N, Bazaa A, Loret E, Bezzine S, El Ayeb M, Marrakchi N. Two purified and characterized phospholipases A2 from Cerastes cerastes venom that inhibits cancerous cell adhesion and migration. Toxicon 2009; 53(4) 444–453

[94] Zouari-Kessentini R, Jebali J, Taboubi S, Srairi-Abid N, Morjen M, Kallech-Ziri O, Bezzine S, Marvaldi J, El Ayeb M, Marrakchi N. CC-PLA2-1 and CC-PLA2-2, two cerastes cerastes venom derived phospholipases A2, inhibit angiogenesis both in vitro and in vivo. Laboratory Investigation 2010; 90(4) 510-519.

[95] Roberto PG, Kashima S, Marcussi S, Pereira JO, Astolfi-Filho S, Nomizo A, Giglio JR, Fontes MR, Soares AM, Fança SC. Cloning and identification of a complete cDNA coding for a bactericidal and anti-tumoral acidic phospholipase A2 from Bothrops jararacussu venom. Protein Journal 2004; 23(4) 273-285.

[96] Bazaa A, Pasquier E, Defilles C, Limam I, Kessentini-Zouari R, Kallech-Ziri O, El Battari A, Braguer D, El Ayeb M, Marrakchi N, Luis J. MVL-PLA2, a snake venom phospholipase A2, inhibits angiogenesis through an increase in microtubule dynamics and disorganization of focal adhesions. PLoS One 2010; 5(4):e10124.

[97] Murakami T, Kamikado N, Fujimoti R, Hamaguchi K, Nakamura H, Chijiwa T, Ohno M, Oda-Ueda. A [Lys49] phospholipase A2 from Protobothrops flavoviridis venom induces caspase-independent apoptotic cell death accompanied by rapid plasma-membrane rupture in human leukemia cells. Biosciences, Biotechnology and Biochemistry 2011; 75(5) 864-870.

[98] Lu Q, Navdaev A, Clemetson JM. Clemetson KJ. Snake venom C-type lectins interacting with platelet receptors: structure-function relationships and effects on haemostasis. Toxicon 2005; 45(8) 1089–1098.

[99] Eble JA, Niland S, Bracht T, Mormann M, Peter-Katalinic J, Pohlentz G, Stetefeld J.. The alpha2beta1 integrin-specific antagonist rhodocetin is a cruciform, heterotetrameric molecule. FASEB Journal 2009; 23(9) 2917-2927.

[100] Mizuno H, Fujimoto Z, Koizumi M, Kano H, Atoda H, Morita T. Crystal structure of coagulation factor IX-binding protein from habu snake venom at 2.6 A: implication of central loop swapping based on deletion in the linker region. Journal of Molecular Biology 1999; 289(1) 103-112.

[101] Horii K, Okuda D, Morita T, Mizuno H. Crystal structure of EMS16 in complex with the integrin alpha2-I domain. Journal of Molecular Biology 2004; 341(2) 519-527.

[102] Maita N, Nishio K, Nishimoto E, Matsui T, Shikamoto Y, Morita T, Sadler JE, Mizuno H. Crystal structure of von willebrand factor A1 domain complexed with snake venom, bitiscetin: insight into glycoprotein Ibα binding mechanism induced by snake venom proteins. The Journal of Biological Chemistry 2003; 278(39) 37777–37781.

[103] Runhua WR, Manjunatha K, Max CMC. Rhodocetin, a novel platelet aggregation inhibitor from the venom of Calloselasma rhodostoma (Malayan pit viper): synergistic and non covalent interaction between subunits. Biochemistry 1999; 38(23) 7584-7593.

[104] Marcinkiewicz C, Lobb RR, Marcinkiewicz MM, Daniel JL, Smith JB, Dangelmaier C, Weinreb PH., Beacham DA, Niewiarowski S. Isolation and characterization of EMS16, a C-lectin type protein from Echis multisquamatus venom, a potent and selective inhibitor of the α2β1 integrin. Biochemistry 2000; 39(32) 9859–9867.

[105] Sarray S, Berthet V, Calvete JJ, Secchi J, Marvaldi J, El Ayeb M, Marrakchi N, Luis J. Lebectin, a novel C-type lectin from Macrovipera lebetina venom, inhibits integrin mediated adhesion, migration and invasion of human tumour cells. Laboratory Investigation 2004; 84(5) 573-581.

[106] Sarray S, Delamarre E, Marvaldi J, El Ayeb M, Marrakchi N, Luis J. Lebectin and lebecetin, two C-type lectins from snake venom, inhibit alpha5 beta1 and alphaV-containing integrins. Matrix Biology 2007; 26(4) 306-313.

[107] Pilorget A, Conesa M, Sarray S, Michaud-Levesque J, Daoud S, Kim KS, Demeule M, Marvaldi J, El Ayeb M, Marrakchi N, Beliveau R, Luis J. Lebectin, a Macrovipera lebetina venom-derived C-type lectin, inhibits angiogenesis both in vitro and in vivo. Journal of Cellular Physiology 2007 211(2) 307-315.

[108] Golubkov V, Hawes D, Markland FS. Anti-angiogenic activity of contortrostatin, a disintegrin from Agkistrodon contortrix contortrix snake venom. Angiogenesis 2003; 6(3) 213–224.

[109] Marcinkiewicz C, Weinreb PH, Calvete JJ, Kisiel DG, Mousa SA, Tuszynski GP, Lobb RR. Obtustatin: A potent selective inhibitor of alpha1beta1 integrin in vitro and angiogenesis in vivo. Cancer Research 2003; 63(9) 2020–2023.

[110] Sarray S, Siret C, Lehmann M, Marrakchi N, Luis J, El Ayeb M, Andre F. Lebectin increases N-cadherin-mediated adhesion through PI3K/AKT pathway. Cancer Letters 2009; 285(2) 174–181

[111] Pereira-Bittencourt M, Carvalho DD. Gagliard AR, Collins DC. The effect of a lectin from the venom of the snake, Bothrops jararacussu, on tumor cell proliferation. Anticancer Research 1999; 19(5B) 4023-4025.

[112] Nolte S, de Castro Damasio D, Baréa AC, Gomes J, Magalhães A, Mello Zischler LF, Stuelp-Campelo PM, Elífio-Esposito SL, Roque-Barreira MC, Reis CA, Moreno-Amaral AN. BJcuL, a lectin purified from Bothrops jararacussu venom, induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly. Toxicon 2012; 59(1) 81-85.

[113] Wan SG, Jin Y, Lee WH, Zhang Y. A snake venom metalloproteinase that inhibited cell proliferation and induced morphological changes of ECV304 cells. Toxicon 2006; 47(4) 480-489.

[114] Limam I, Bazaa A, Srairi-Abid N, Taboubi S, Jebali J, Zouari-Kessentini R, Kallech-Ziri O, Mejdoub H, Hammami A, El Ayeb M, Luis J, Marrakchi N. Leberagin-C, A disintegrin-like/cysteine-rich protein from Macrovipera lebetina transmediterranea venom, inhibits alphavbeta3 integrin-mediated cell adhesion. Matrix Biology 2010; 29(2)117-126.

[115] Yeh CH, Peng HC, Yih JB, Huang TF A new short chain RGD-containing disintegrin, accutin, inhibits the common pathway of human platelet aggregation. Biochemica Biophysica Acta 1998; 1425(3) 493-504.