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REVIEW

Snake Venom Proteins Isolated from Tunisian Vipers: Pharmacological and Therapeutic Overview

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Abstract

The venoms of Tunisian wildlife snakes are complex mixtures containing proteins/peptides and non-protein molecules. Proteins and peptides are the most abundant compounds responsible for the biological effects of venoms. Snake venoms proteins have enzymatic or non-enzymatic activities, which are grouped into different families including C-type lectin proteins, disintegrins (long, medium and short disintegrins), Kunitz-type serine protease inhibitors, natriuretic-like peptides, vascular endothelial growth factor-related proteins, L-amino acid oxidases, phospholipases A2 and serine proteinases. With technological advancements, the toxic effects of venoms were turned into potential benefits for clinical diagnosis, basic research and development of new research tools and drugs of potential clinical use. Our research team has shown that *Macrovipera lebetina* and *Cerastes cerastes* venom components of Tunisian wildlife snakes had great potential for the development of new drugs for the treatment of cancer, angiogenesis disorders or cardiovascular diseases. This review is an overview on snake venom proteins from *Macrovipera lebetina* and *Cerastes cerastes* and their biochemical, pharmacological and molecular characterization and their importance as protein resources with therapeutic potential.

Keywords: Cancer, Angiogenesis, Cardiovascular disease, Disintegrin, Phospholipase A2, C-type lectin protein, Kunitz-type inhibitor, L-amino acid oxidase

1. INTRODUCTION

Snake venoms are used as tools for defense or to immobilize and digest prey, but venom gland is also a factory for human valuable medicines as it produces a large variety of chemicals with pharmacological properties. Snake venoms are a crude mixture of proteins, peptides and other organic compounds of low molecular weight, and inorganic compounds [1]. Proteins, with or without catalytic activity, constitute the major portion of the total dry mass of crude snake venom. This include neurotoxins, cardiotoxins, C-type lectins, proteinases, hyaluronidases, acetylcholinesterases, L-amino acid oxidases, three-finger toxins, phospholipases A2 and nucleases [2,3]. All these constituents of snake venom exhibit a wide range of pharmacological activities including platelet aggregation, hemorrhage, hemostasis, edema, anti-coagulation, anti-tumor, apoptosis induction, anti-inflammation, anti-parasitic and anti-microbial activities [4]. More specifically, research on snake venom compounds has led to the fruitful discovery of potential therapeutic agents.

Proteins isolated from snake venoms target mainly cell membrane receptors, ions channels and components of the hemostatic system with high selectivity and affinity. Most of the pharmacological and toxic effects of snake venoms are the result of the interaction of venom derived-molecules with cell surface ligands such as selectins, collagen receptors, GPIb and integrins. These latter are α and β heterodimeric transmembrane receptors that play a vital role in mediating cell–matrix and cell–cell interactions. They have central functions in platelet aggregation, adhesion, spreading, retraction, migration, angiogenesis, inflammatory reactions and other biological processes [5].

Due to the richness, heterogeneity and synergistic or antagonistic action of different molecules, snake venoms are considered as a rich bio-resource of biologically active compounds. These biomolecules present diverse pharmacological activities which could also be used as tools mainly for medical research or diagnosis [6]. However, less than 0.01% of snake venom derived-molecules have been identified and characterized. For instance, Capoten® (Captopril), Integrilin® (Eptifibatide) and Aggrastat® (Tirofiban) are drugs based on snake venoms, which have been approved by the Food and Drug Administration (FDA). In addition to these approved drugs, many other snake venom components are now involved in preclinical or clinical trials for a variety of therapeutic applications [1]. These examples show that snake venoms can be a valuable source of new principle components in drug discovery.

Our research team has initially established a proteomic profile as exhaustive as possible of venoms from the most represented vipers in Tunisia, namely *Macrovipera lebetina* and *Cerastes cerastes*. These species represent the most important poisonous snakes in Tunisia. However, little is known about their venom protein composition and potential therapeutic proteins. The proteomic study of Tunisian vipers venom provides a catalogue of proteins that are further used to understand the venom biological effects and to carry out function-structure relationship studies [7]. The comparative analysis of the venom proteomes from *Macrovipera lebetina* and *Cerastes cerastes* snake species showed a great complexity in the protein composition, which are divided into enzymatic or non enzymatic proteins/peptides.

This review summarizes the biochemical properties, structural characteristics and various biological functions of Tunisian snake venom molecules identified by our research team. This work might provide useful inputs for future studies on snake venom molecules.

2. NON ENZYMATIC PROTEINS/PEPTIDES

2.1. C-Type Lectin Proteins

C-lectin proteins are a large family of non enzymatic glycoproteins that can specifically bind non-covalently and reversibly to certain carbohydrates and glycoconjugates [8]. C-lectin proteins are widely distributed in snake venoms. They were found in the Viperidae and Elapidae snake families, including the genera *Bothrops, Crotalus, Bitis, Agkistrodon, Lachesis, Dendroaspis and Trimeresurus* [1]. C-type lectins proteins display various biological activities and are known to affect especially platelet aggregation. In addition, a few of them have been reported to be endowed with anti-tumor activities. Our research team has previously purified C-type lectin proteins from the venom of Tunisian viper *Macrovipera lebetina*. Furthermore, the phylogenetic study and amino acid sequence alignment confirmed that this viper produces a panoply of C-type lectin protein isoforms [9] (Figure 1).

The first one, called lebectin, was shown to inhibit integrin-mediated adhesion, migration and invasion of human tumor cells [10]. It acted by blocking the adhesive function of both the α5β1 and αv integrins [11]. We also demonstrated that lebectin promoted N-cadherin/catenin complex reorganization at cell-cell contacts, inducing a strengthening of intercellular adhesion. This reorganization is associated with phosphorylation of beta-catenin on the tyrosine 142 residue [12]. Interestingly, lebectin acted on N-cadherin-mediated cell-cell contacts through the PI3K/Akt pathway. This effect could contribute to the blockage of tumor cell migration previously observed [10]. In addition, lebectin strongly inhibited both human brain microvascular endothelial cells (HBMEC) *in vitro* tubulogenesis on Matrigel™ and proliferation assays. Interestingly, using both embryo chick chorioallantoic membrane (CAM) and Matrigel™ plug assays in nude mice, lebectin showed a potent anti-angiogenic activity *in vivo* [13]. Lebectin thus represents a new anti-angiogenic C-type lectin with great potential for the treatment of angiogenesis-related diseases.

The second C-type lectin from *Macrovipera lebetina,* called lebecin, was shown to inhibit proliferation, adhesion and migration of human breast cancer cells (MDA-MB231) [14]. The third one, named lebecetin, showed a potent inhibitory effect on platelet aggregation induced by thrombin in a concentration dependent manner [15], as well as anti-tumor actions [11]. In addition, we found that lebecetin had beneficial effects in neovascular ocular diseases. We initially showed that lebecetin interacted with α5β1 and αv-containing integrins which regulate endothelial cell proliferation and stabilization [11]. Lebecetin inhibited HBMEC adhesion, migration, and proliferation as well as tubulogenesis [13]. Furthermore, it was found to have inhibitory effect on angiogenesis in cultured aortic and choroidal explants [16]. Lebecetin is thus the first C-type lectin, interacting with α5β1 and αv-type integrins, that demonstrates efficacy in mouse *in vivo* choroidal and retinopathy neovascularization models, with a single injection, whereas other tested molecules required repeated injections [16]. Interestingly, lebecetin was active with local administrations that minimize the risk of systemic effect. Lebecetin seems to be highly specific for actively proliferating

vascular endothelial cells with no significant effect on mature blood vessels, which predicts a good safety profile. Thus, lebecetin is a strong candidate for the treatment of age-related macular degeneration (AMD) and ischemic retinopathies, in particular in patients who are resistant to anti-VEGF treatments. Lebecetin is currently patented as an innovative agent against deleterious ocular neovascularisation (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018108945).

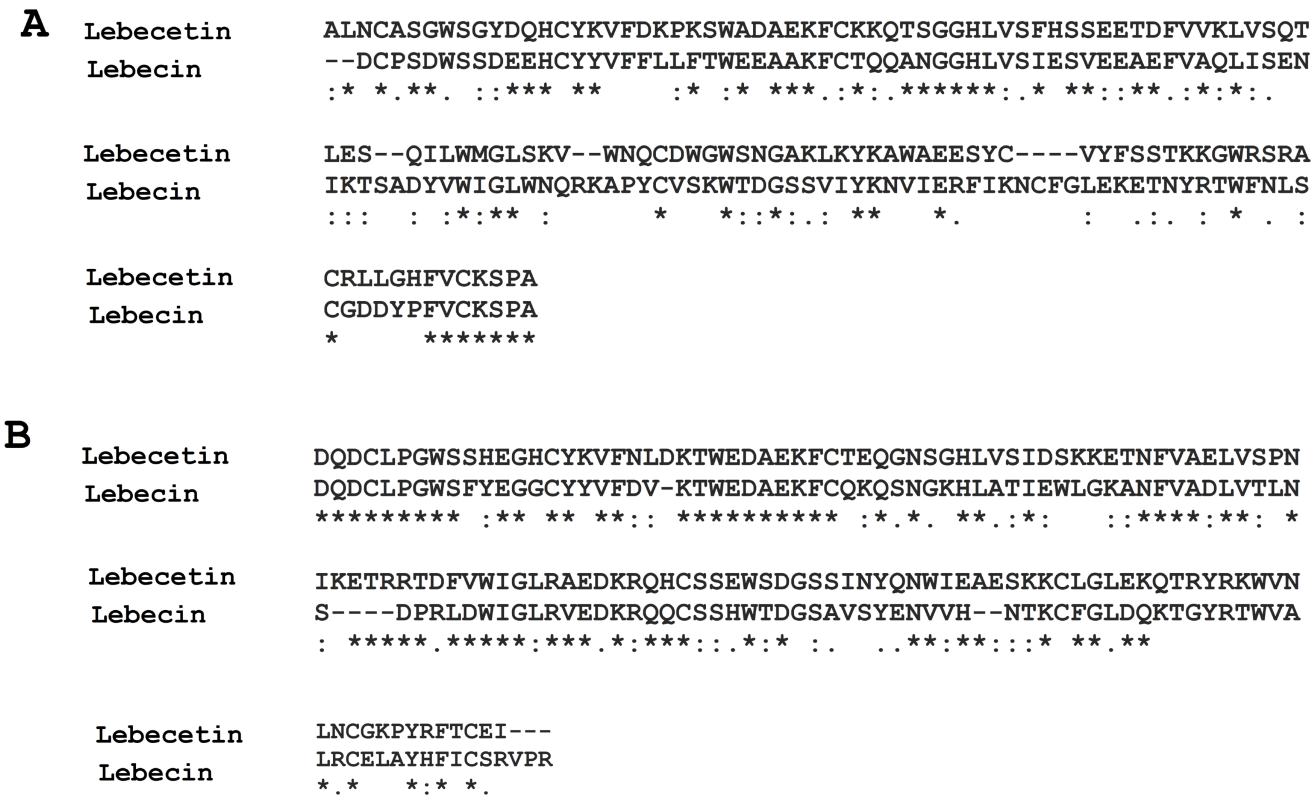

Figure 1. Alignment of the amino acid sequences of alpha (**A**) and beta subunits (**B**) of lebecetin and lebecin. ‘‘*’’ means that the residues in that column are identical in all sequences in the alignment. ‘‘:’’ means conserved substitutions. ‘‘.’’ means semi-conserved substitutions.

2.2. Disintegrins

Disintegrins from snake venoms play a role as antagonists of cell adhesion and migration by binding integrins and blocking their function. We characterized various disintegrins from Tunisian *Macrovipera lebetina* and *Cerastes cerastes* snake venoms. In particular, lebestatin, a short disintegrin with a single-chain polypeptide of 41 amino acids, displays a pattern of cysteines similar to other short disintegrins, but contains the sequence KTS rather than RGD in its integrin-binding loop [17]. Lebestatin presents a high similarity with obtustatin and viperistatin [18]. It inhibited both adhesion and migration of pheochromocytoma cells (PC12) by interacting specifically with the α1β1 integrin. This disintegrin also affected adhesion and migration of endothelial cells and exhibited an anti-angiogenic effect *ex vivo* when using the CAM model [17].

CC5 and CC8, two highly homologous dimeric disintegrins from the *Cerastes cerastes* viper venom, were firstly described with antiplatelet activity by Calvete et al. [19]. Later, our team demonstrated that these disintegrins were able to inhibit angiogenesis [20]. These effects appear to

require the RGD and/or WGD disintegrin loops. CC5 and CC8 also inhibited tube formation on Matrigel™ and displayed potent anti-angiogenic activities *ex vivo* using the CAM model [20]. Both disintegrins also displayed pro-apoptotic potential in human endothelial cells (HMEC-1) by down regulating FAK/AKT/PI3K axis and caspase activation [20]. Moreover, both CC5 and CC8 inhibited platelet aggregation and the adhesion of cells expressing integrins αIIbβ3, αvβ3 and α5β1 to their appropriate ligands. This effect is likely mediated by the WGD motif, which enhances the inhibitory activity of disintegrins toward the integrins [19]. These disintegrins may thus be relevant agents for the treatment of thrombotic diseases (involving platelet aggregation), such as myocardial infarction (heart attack) and strokes, which are the most common cause of death. The available antithrombotic drugs, even if they are effective at reducing thrombosis in patients with cardiovascular disease, have deleterious side effects such as bleeding, which limits their use [21]. In this setting, venom-derived disintegrins could lead to the generation of safe and effective antithrombotic drugs with larger therapeutic windows to treat thrombotic occlusion.

A cysteine-rich disintegrin, leberagin-C, from the *Macrovipera lebetina transmediterranea* venom was also identified [22]. It is a new monomeric member of the disintegrin-like/cysteine-rich (D/C) family, with a molecular mass of 25,787 Da. Leberagin-C share many conserved aminoacids with other known D/C proteins, like the SECD binding site and a pattern of 28 cysteines. Leberagin-C was able to inhibit platelet aggregation induced by thrombin and arachidonic acid [22]. It also inhibited the adhesion of melanoma tumor cells on fibrinogen and fibronectin by interfering with the function of αvβ3, αvβ6 and α5β1 integrins. To our knowledge, this is the first D/C disintegrin abolishing tumour cell adhesion *via* these integrins.

2.3. Kunitz-Type Serine Protease Inhibitors

Snake venoms of Viperidae and Elapidae contain serine protease inhibitors such as the Kunitz-type inhibitors [23]. The Kunitz-type motif was first discovered in the bovine pancreatic trypsin inhibitor (BPTI)-like proteinase inhibitors, which inhibit a spectrum of serine proteinases including trypsin, chymotrypsin, plasmin and plasma kallikrein [24, 25]. However, some snake neurotoxins, such as dendrotoxins, calcicludine and B chain of β-bungarotoxin, possess a Kunitz/BPTI-like domain although they are unable to inhibit protease activity [24, 26]. The snake Kunitz-inhibitors are considered to act on coagulation, fibrinolysis, inflammation and cancer [26].

A novel Kunitz-type serine proteinase inhibitor, termed PIVL, was purified to homogeneity from the venom of the Tunisian snake *Macrovipera lebetina transmediterranea*. It is a monomeric polypeptide chain cross-linked by three disulfide bridges with molecular mass of 7691.7 Da. The 67-residue full-length PIVL sequence was deduced from a venom gland cDNA clone. Structurally, PIVL is built by a single Kunitz/BPTI-like domain. Functionally, it was able to specifically inhibit trypsin activity. Interestingly, PIVL exhibited an anti-tumor effect and displayed integrin inhibitory activity without being cytotoxic [27]. PIVL was able to dose-dependently inhibit adhesion, migration and invasion of human glioblastoma U87 cells. Our results also showed that PIVL impaired the function of αvβ3 integrin and to a lesser extent, the activity of αvβ6, αvβ5, α1β1 and α5β1 integrins. Interestingly, molecular docking of PIVL with αvβ3 integrin, showed that the peptide represents a steric obstruction preventing the fibrinogen from enforcing the interaction with

the integrin receptor (Figure 2). We demonstrated that the (41)RGN(43) motif of PIVL is in part responsible for its anti-cancer effect. By using time-lapse videomicroscopy, we found that PIVL significantly reduced U87 cells motility and affected cell directionality persistence by 68%. These findings reveal novel pharmacological effects for a Kunitz-type serine proteinase inhibitor [27]. Furthermore, we showed that PIVL increased microtubule dynamic instability in HMEC-1 transfected with EGFP-tagged α-tubulin. Using Matrigel™ and CAM assays, we also demonstrate that PIVL exhibited a strong anti-angiogenic effect both *in vitro* and *in vivo* [28].

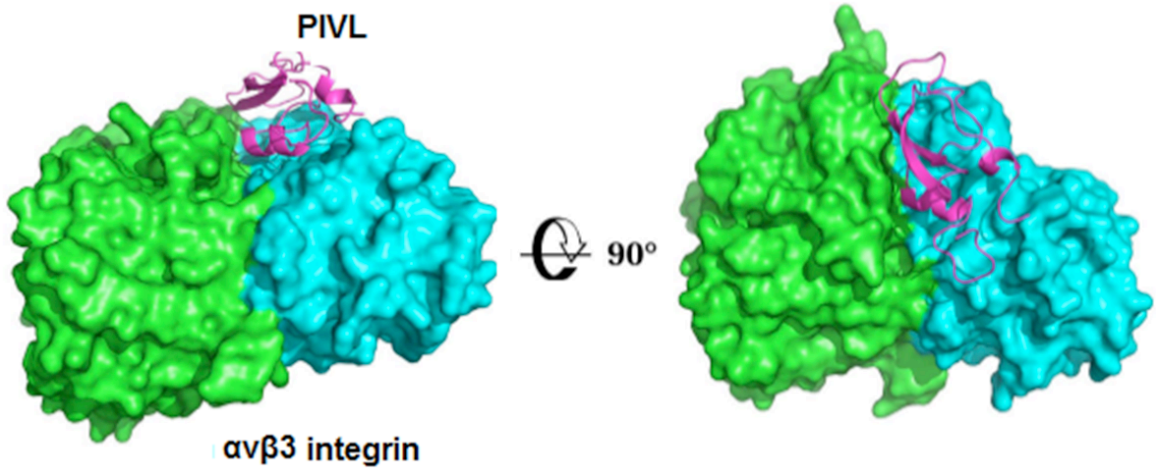

Figure 2. Molecular docking and recognition process of PIVL with αvβ3 integrin.

2.4. Natriuretic-like Peptides

Mammalian natriuretic peptides (NPs) are hormones that are mainly secreted by heart and have important natriuretic properties. A-type (ANP) and B-type (BNP) were first used for diagnosis of heart failure (HF) severity [29] and then found to exert protective effects on renal and cardiovascular systems [30]. Snake have produced some of the more interesting NPs, such as Dendroaspis natriuretic peptide (DNP), isolated from the green mamba (*Dendroaspis angusticeps*) venom [31]. A new chimeric compound CD-NP (cenderitide) developed by fusing the 15-residue *C*-terminal extension of DNP with the *C*-terminus of full-length human C-type natriuretic peptide (CNP) is now in phase II clinical trials for the treatment of heart failure [32]. Our research team had identified, from *Macrovipera lebetina transmediterranea* venom, a low molecular weight natriuretic-like peptide called lebetin 2 [33]. *N*-terminal sequencing of purified native lebetin 2 revealed two isoforms representing 80% (lebetin 2α) and 20% (lebetin 2β) of the total yield. They contain one disulfide bond each and differ by a single Gly residue at the *N*-terminus of lebetin 2-α, which explains why they could not be separated. Their molecular weights determined by ES-MS were 3943.74 and 3886.96 Da, respectively. According to amino acid sequence alignment, lebetin 2 has structural similarity with NP family members [34] (**Figure 3**). This molecule showed a potent inhibitory effect on platelet aggregation *in vitro* and *in vivo* [33].

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L2(38aa)   GDNKPPKKGPPNCFGGHKIDRIGSHSGLGCNKVDDNKG
BNP(32aa)  ----NSKMAHSSCFGGQKIDRIGAVSRLGCDGLRLF--
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Figure 3. Amino acid sequences alignment of lebetin 2 (L2) and B-type natriuretic peptide (BNP). Identical amino acid positions are shaded in black box while conserved or similar residues are in gray. Lebetin 2 and BNP display 54% similarity. Alignment was performed using GeneDoc, version 2.7.000.

Recently, we reported that lebetin 2 displayed potent cardioprotective properties by stimulating NP receptors in myocardial ischemia-reperfusion injury murine models, on isolated perfused rat hearts and *in vivo,* either after acute or prolonged ischemia-reperfusion [35,36]. In these models, lebetin 2 was found more potent than BNP in improving post-ischemic cardiodynamics and promoting resolving inflammatory processes. The cardioprotective effects of lebetin 2 are mediated by activation of a 3',5'-cyclic guanosine monophosphate (cGMP) pathway, mitochondrial ATP-dependent K^+ channel opening and subsequent mitochondrial permeability transition pore (mPTP) inhibition. Investigations are currently extensively conducted by our research team to explain how lebetin 2 exerts post-ischemic additional actions compared to BNP.

In addition to their use as therapeutic agents for the treatment of cardiovascular diseases, in the last years, a new property was ascribed to ANP as an attractive candidate for anticancer therapy [37]. Several studies demonstrated the *in vitro* and *in vivo* efficiency of ANP on inhibiting tumor growth [38]. ANP eliminated 80% of human pancreatic carcinoma [39] and 43% of human small cell lung carcinoma growing in mice [40]. Moreover, CNP decreased the growth of human renal carcinoma cells but only at concentrations 100-fold higher than ANP, while BNP has no significant anticancer effect [41]. Moreover, *Dendroaspis angusticeps peptide* (DNP), which has similar amino acids to ANP [31], also had anticancer effects [42]. Nevertheless, ANP decreased the growth of human glioblastoma cells 4-fold more efficiently than DNP [38].

Recently, we also demonstrated that lebetin 2 possess an anti-tumor activity by targeting the integrin receptor function. It was thus able to impair both adhesion and migration of pheochromocytoma cells (PC12) and α1β1 integrin-expressing CHO cells (CHO-α1) to type I and IV collagens. Moreover, this peptide affected proliferation of PC12 cells by modulating AKT phosphorylation. Furthermore, lebetin 2 exhibited a potent anti-angiogenic effect as assessed *in vitro* and *ex vivo*, using both the embryo CAM model and the rat aortic ring assay. Interestingly, the interaction mode of lebetin 2 with the integrin α1β1, assessed *in silico*, showed that the peptide represents a steric obstruction preventing the collagen from enforcing the interactions with the integrin [43].

2.5. Vascular Endothelial Growth Factor-related Proteins

Research on vascular endothelial growth factor (VEGF)-related proteins has led to the identification of a new class of VEGF, VEGF-F, which is a new family of non-enzymatic snake venom proteins acting *via* the receptor tyrosine kinase VEGF-R2 with a high selectivity [44]. VEGF-like snake venom molecules, such as vammin and VR-1, have demonstrated more potent pro-angiogenic activity than mammalian VEGFs [44]. The recently discovered *Macrovipera lebetina* viper venom protein, called Increasing Capillary Permeability Protein (ICPP, 27 kDa) [45, 46], has structural homology to VEGF, displaying a *N*-terminal amino acid sequence with a considerable similarity to that of VEGF and platelet-derived growth factor (PDGF). ICPP has been shown to exert a potent pro-angiogenic VEGF-like action through VEGFR2 receptor stimulation in mouse embryonic stem cells or human umbilical vein endothelial cells [45, 46]. Thereafter, we showed that ICPP, initially described as a vascular permeability factor, exerted a powerful protective effect against acute cardiac ischemia by improving ischemia-induced mitochondrial

dysfunction, after a single intravenous injection [47]. The effects against cardiac ischemia injury were mediated through stimulation of VEGFR2 receptors, ERK pathway activation and subsequent inhibition of mitochondria permeability transition pore opening and improvement of oxidative phosphorylation at the time of reperfusion [47]. Thus, ICPP is a potent myocardial protective agent in the setting of cardiac ischemia in the mouse. Its application would be also of interest in the treatment of other pathologies including arterial hypertension, coronary artery disease and limb ischemia.

3. ENZYMATIC PROTEINS

3.1. Phospholipases A2

Secreted phospholipase A_2 enzymes, especially from *Viperidae* snake venom, exhibit a wide variety of pharmacological effects despite their structure similarity. From the *Cerastes cerastes* snake venom, two acidic PLA2, namely CC-PLA2-1 and CC-PLA2-2 with a molecular weight of 13,737.52 and 13,705.63 Da, respectively, were purified (**Figure 4**). These enzymes strongly inhibited coagulation and exhibited a marked inhibitory effect on platelet aggregation induced by ADP and arachidonic acid [48]. Interestingly, CC-PLA2-1 and CC-PLA2-2 impaired in a dose-dependent manner adhesion and migration of HT1080 fibrosarcoma and IGR39 melanoma cells to fibrinogen and fibronectin [48]. These enzymes also inhibited HBMEC cell adhesion and migration to fibrinogen and fibronectin. This anti-adhesive effect was mediated by α5β1 and αv-containing integrins and led to impairment of tubulogenesis on Matrigel™ and to anti-angiogenic activity *in vivo* in CAM assay [49].

An acidic Asp 49 phospholipase A2, named MVL-PLA2, with a molecular mass of 13,626.64 Da was purified from *Macrovipera lebetina* venom. Structural analysis and amino acid sequence alignment of MVL-PLA2 showed a high similarity with both CC-PLA2-1 and CC-PLA2-2 from *Cerastes cerastes* venom (**Figure 4**). This enzyme was not cytotoxic up to 2 μM and completely abolished cell adhesion and migration of various human tumor cells [50]. MVL-PLA2 also displayed potent anti-angiogenic properties. Indeed, this protein inhibited adhesion and migration of HMEC-1 cells without being cytotoxic. Besides, MVL-PLA2, as well as its catalytically inactivated form, significantly inhibited angiogenesis both *in vitro* and *ex vivo* as assessed by Matrigel™ and CAM assays. Furthermore, MVL-PLA2 disturbed the actin cytoskeleton and the distribution of αvβ3 integrin and significantly increased microtubule dynamicity in HMEC-1 cells [51].

The kinetic and interfacial properties of Tunisian secreted PLA2 viper venoms showed that these enzymes have great different abilities to bind and hydrolyse phospholipids. CC-PLA2-1 and 2 are thermoactive enzymes, which exhibited a higher hydrolytic activity than MVL-PLA2 and showed a preference to negatively charged head group phospholipids. These characteristics seem to be related to an interesting dipole moment created by a marked anisotropy of electrostatic charge which was determinant for the enzymes selectivity and activity [52]. These nontoxic secreted phospholipase A_2 could be new tools to disrupt different steps of tumor progression and angiogenesis through integrins blockage.

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MVL-PLA2     MRTLWIVAVCLMGVEGHLTQFGDMINKKTGTFGLLSYVYYGCYCGLGGKGKPQDATDRCC 60
CC-PLA2.1    MRTLWIVAVWLMGVEGNLYQFGKMIKHKTGKSALLSYSAYGCYCGWGGQGKPQDATDHCC 60
CC-PLA2.2    MRTLWIVAVWLMGVEGNLFQFGKMIKHKTGKSALLSYSGNPCYCGWGGQGPPQDATDHCC 60
             ********* ******:* ***.**::***. .****    **** **:* ******:**

MVL-PLA2     FVHDCCYGTVNGCDPKLSTYSYSFQNGDIVCGDDDPCLRAVCECDRVAAICFGENMNTYD 120
CC-PLA2.1    FVHDCCYGEVSGCYPKT-AFTLKFENQDIICGDEDPCNRAVCECDRVAAICFGENVNTSD 119
CC-PLA2.2    FVHDCCYGEENACYPKT-AFTLKFENQIIIC-DEDPCNYAVCMCDRVAAICGGENVATSD 118
             ********  ..* **  ::: .*:*  *:* *:***  *** ******** ***: * *

MVL-PLA2     KKYMLYSLFDCMEESEKC 138
CC-PLA2.1    KKYLFYSSSYCEEESEQC 137
CC-PLA2.2    AKYLFYRSMGCEEESVQC 136
             **::*    * *** :*
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Fig. (4). Sequence alignment of Tunisian Viperidae sPLA2: CC-PLA2-1 (ACO92622), CC-PLA2-2 (ACO92623) and MV-PLA2 (CAR40186). Gaps (-) have been introduced to optimize alignment.

3.2. L-Amino Acid Oxidases

Snake venom L-amino acid oxidases (SV-LAAO) are multifunctional enzymes that exhibit a wide range of pharmacological activities. A new L-amino acid oxidase, named CC-LAAO, was purified from *Cerastes cerastes* snake venom. It is a homodimeric glycosylated flavoprotein with a molecular mass of 115 kDa in its native form. This enzyme displayed a Michaelis Menten behavior with an optimal pH at 7.8 [53]. Interestingly, unlike known SV-LAAOs which display their maximum activity at 37°C, CC-LAAO has an optimal temperature at 50°C. Kinetic studies showed that the enzyme displayed high specificity towards hydrophobic L-amino acids. CC-LAAO sequence and its tertiary model shared high similarity with other SV-LAAOs [53]. This protein did not exhibit cytotoxic activities against erythrocytes and peripheral blood mononuclear cells (PBMC). However, CC-LAAO caused cytotoxicity on several cancer cell lines and induced platelet aggregation. Furthermore, the enzyme showed remarkable effect against Gram-positive and Gram-negative bacteria. These activities were inhibited by the addition of catalase or substrate analogs, suggesting that H_2O_2 liberation is required for these effects. Binding studies revealed that CC-LAAO binds to the cell surface and enables the production of highly localized concentration of H_2O_2 in or near the binding interfaces. The interaction of CC-LAAO with a mimetic phospholipid film indicated that phospholipid/CC-LAAO interactions are not involved in their binding to membrane and in the pharmacological activities [54].

3.3. Serine Proteinases

The venoms of Viperidae snakes contain numerous serine proteinases that possess one or more of the essential activities of thrombin on fibrinogen and platelets. Cerastocytin, a thrombin-like enzyme from the venom of the sand viper, *Cerastes cerastes*, has been identified [55]. It is a basic protein (isoelectric point higher than 9) made of a single polypeptide chain of 38 kDa. Its N-terminal sequence shows strong similarities with other thrombin-like enzymes from snake venoms. Nanomolar concentrations of cerastocytin induced aggregation of blood platelets. On the other hand, both amidolytic activity and platelet aggregating activity of cerastocytin were unaffected by hirudin or by antithrombin III in the presence of heparin. High concentrations of cerastocytin (1-10 μM) also cleaved prothrombin and Factor X.

Table 1. Biological activities and targets of snake venom peptides and proteins isolated from Tunisian viper

Molecule	Protein family	Biological activity	Target	Reference
Lebecetin	C-type lectin protein	Antiplatelet aggregation Anti-tumor Anti-angiogenic	$\alpha_5\beta_1$ and α_v-containing integrins	[11,15,16]
Lebectin		Anti-tumor	$\alpha_2\beta_1$ and $\alpha_5\beta_1$ integrins	[10, 11, 12, 13]
Lebecin		Anti-tumor	$\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins	[14]
Lebestatin	Disintegrin	Anti-tumor Anti-angiogenic	$\alpha_1\beta_1$ integrin	[17]
CC5		Antiplatelet aggregation Anti-tumor Anti-angiogenic	$\alpha_v\beta_3$ and $\alpha_5\beta_1$integrins	[20]
CC8				[19, 20]
Leberagin-C	Disintegrin-like	Antiplatelet aggregation Anti-tumor	αvβ3, αvβ6 and α5β1 integrins	[22]
PIVL	Serine- proteinase inhibitor (Kunitz)	Anti-tumor Anti-angiogenic	$\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins	[27, 28]
Lebetin	Natriuretic peptide	Antiplatelet aggregation Anti-tumor Anti-angiogenic Cardioprotective properties	$\alpha_1\beta_1$ integrin	[33, 35, 36, 43]
CC-PLA2-1	Phospholipase A_2	Antiplatelet aggregation Anti-tumor Anti-angiogenic	$\alpha_5\beta_1$ and α_v-containing integrins	[48, 49]
CC-PLA2-2				[48, 49]
MV-PLA2				[50, 51]
CC-LAAO	L-amino acid oxidase	Aggregation activator Apoptosis	ROS/ Glycosylated site?	[54]
Cerastocytin	Serine proteinase	Procoagulant Platelet Aggregation activator	GPIIbIIIa ($\alpha_{IIb}\beta_3$ integrin)	[55]
Cerastotin		Agglutinating properties	GPIb	[56]
Cerastatin		platelet aggregation inhibitor	fibrinogen receptors	[57]

Cerastotin, another thrombin-like enzyme purified from *Cerastes cerastes* venom is a neutral glycoprotein with a single polypeptide chain of 40 kDa. Cerastotin possesses esterase and amidolytic activities. This enzyme efficiently clotted human plasma and cleaved preferentially the alpha chain of fibrinogen. Cerastotin did not induce aggregation of washed normal platelets, but did aggregate platelets in the presence of exogenous fibrinogen. Cerastotin also agglutinated formalin-

fixed and washed platelets, only in the simultaneous presence of fibrinogen and of Von Willebrand factor [56].

Further, Cerastatin, a potent platelet aggregation inhibitor, was purified from *Cerastes cerastes* venom. It is devoid of phospholipase A2, esterase, fibrinogenolytic and amidolytic activities. It inhibits aggregation of washed platelets, induced by either collagen, PAF acether or thrombin. Cerastatin also inhibits the thrombin-induced clot retraction of platelet-rich plasma. Pretreatment of platelets with cerastatin irreversibly inhibits the aggregation induced by thrombin. Cerastatin therefore inhibits platelet aggregation by interfering with the interaction of fibrinogen with fibrinogen receptors [57].

The venom-derived thrombin-like enzymes may have growing interest in the use of targeted therapies with specific pro-coagulant hemostatic factor concentrates in place of plasma in the treatment of patients with major bleeding with or without hemophilia.

4. CONCLUSION

With recent technological advancements using combinations of complex tools such as genomics, transcriptomics, proteomics and bioinformatics, snake venom compounds have gained attention as potential biotools for developing new types of therapeutic agents for the treatment of many diseases due to their effective biological activities and unique secret reaction mechanisms. Thus, the deleterious effects of snake venom compounds were turned into more effective molecules with less toxicity and fewer side effects. Actually, different snake venom proteins / peptides-derived drugs are in clinical use or in developmental stages. They found applications in diagnosis of hemostatic disorders and serve as scaffolds from which new drugs could be developed [58]. In this review, we have focused on pharmacologically characterized peptides derived from the venoms of *Macrovipera lebetina* and *Cerastes cerastes* the most important poisonous vipers of Tunisia (**Table 1**).

The proteomic study has led to the classification of the various molecules derived from the two viper venoms according to well-known pharmacological classes. Extensive studies conducted *in vitro*, *ex vivo* or *in vivo*, have shown that some of these compounds can interfere with hemostasis, or display anti- or pro-aggregating platelet properties [15, 33, 56, 57]. Some others have anti-tumor effect *via* several pathways [12, 20, 27, 48, 51]. Other molecules can even, by acting on transmembrane receptors, prevent ischemic or neovascular disorders in a broad range of diseases [16, 35, 36].

Since these proteins have different molecular masses, those with low molecular masses have been chemically synthesized, such as lebestatin [18] and lebetin 2 peptides [35,36], but the others, like lebecetin protein, have been expressed by molecular biology [59]. Furthermore, structure-activity relationship studies combined with *in silico* advances made recently in the field of bioinformatics and molecular docking, allow the targeting of protein regions responsible for the pharmacological activity, thus leading to the design of chimeric compounds or peptide mimetics displaying more effectiveness and less adverse effects. Today, snake venom continue to be an interesting source of material for researchers to better understand many biological mechanisms.

Thus, applied science on snake venoms can be perceived as a promising source of discovery of new pharmaceutical drugs.

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