A SYSTEMATIC REVIEW ON SNAKE VENOM AND ITS THERAPEUTIC IMPLICATION

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ABSTRACT

Nature endowed snakes with a lethal secretion known as venom, which has been fine-tuned over millions of years of evolution. Snakes utilize venom to subdue their prey and to survive in their natural habitat. Venom is known to be a very poisonous mixture, consisting of a variety of molecules, such as carbohydrates, nucleosides, amino acids, lipids, proteins and peptides. Proteins and peptides are the major constituents of the dry weight of snake venoms and are of main interest for scientific investigations as well as for various pharmacological applications. These enzymes include metalloproteases (MP), disintegrins, L-amino acid oxidases (LAAOs), C-type lectins, and phospholipases A_2 (PLA_2s). Their mechanisms of action include direct toxic action (PLA_2s), free radical generation (LAAOs), apoptosis induction (PLA_2s, MP, and LAAOs), and antiangiogenesis (disintegrins and lectins). The functional specificity of peptides belonging to the same family can be attributed to subtle variations in their amino acid sequences. Currently, complementary tools and techniques are utilized to isolate and characterize the peptides, and study their potential applications as molecular probes, and possible templates for drug discovery and design investigations.

Keywords: Arterio-venous shunt; Bleeding risk; GPIIb/IIIa receptor; Platelet aggregation; Thrombosis.

INTRODUCTION

Nature has always been an intriguing source for drug design investigations. From among the animal kingdom, insects and reptilians are interesting and valuable sources for identifying molecules with potential pharmaceutical applications. To date, various bioactive proteins and peptides have been reported from the venom of different species of snakes, conus, scorpions, centipedes, lizards, spiders, sea anemones, bees and octopus. In this context, venomous snakes are a focus of investigations, and a large number of pharmacologically useful molecules have already been isolated and characterized from snake venoms. Among the approximately 3000 known snake species, only a relatively small number are venomous, mainly belonging to Viperidae and Elapidae families. Some members of Colubridae and Atractaspididae are also reported to be venomous.

Snake venom is a glandular secretion which snakes use to immobilize and digest their prey. It is also used as a defensive and a survival tool. This lethal mixture is composed of amino acids, nucleic acids, carbohydrates, lipids, proteins and peptides (adapted from our own reference) illustrates the composition of snake venom.

The composition and evolutionary histories of animal venoms have fascinated the scientific community for centuries. Venoms have evolved over millions of years to facilitate prey capture and/or defense from predators and rivals. Snake venoms, in particular, likely originated in the Cenozoic Era and they are amongst the most well-characterized of animal venoms, comprising a complex mixture of toxic, and pharmacologically-active proteins and peptides. Tragically, snake envenomation is a significant health and economic burden worldwide. It is estimated 1.8–2.7 million snakebites and 81,410–137,880 deaths occur annually worldwide a problem which is mostly associated with agricultural work, especially in South and Southeast Asia, sub-Saharan Africa, and Central and South America. In 2018, the World Health Organization (WHO) finally recognized the snakebite as a priority neglected tropical disease in which morbidity and mortality affects mostly individuals under 30 years old, who are often the most economically productive members of a community. In the present review, we focus our discussion on snake venom peptides, their classification and bioactivities. We define snake venom peptides as the group of non-enzymatic polypeptides in the venom which fold into monomeric domains and are smaller than 80–100 residues. However, variations can be found. For example, three-finger toxins and disintegrins can exist as dimers. Among three-finger toxins, haditoxin [Protein Data Bank (PDB) code: 3HH7] and κ-bungarotoxins (PDB code: 2ABX) are non-covalent dimers, and irditoxin (PDB code: 2H7Z) and α-cobratoxin (PDB code: 2CTX) are covalent dimers Nonetheless, we shall not classify these dimers separately, because they bear structural and functional similarities to their monomeric counter parts. These peptides are of great value, due to their diversified and distinct pharmacological activity, and high affinity and selectivity towards their receptors. For example, snake venom toxins have proved to be invaluable tools in determining the structure and functions of receptors. An excellent example in this regard are α-neurotoxins. Snake venom peptides are mostly stable molecules, able to survive the harsh proteolytic environment of the venom gland itself. Also, it is the inherent stability of these peptides that helps them reach their target receptors, inside their prey (upon envenomation). The stability of these molecules is attained when they are recruited into the venom gland through disulfide bonds formation and/or post-translational modifications. In these animal venoms, the pharmacological effects are primarily caused by disulfide bridged peptides, whilst snake venoms consist of a more diverse array of larger proteins and peptides which results in a wider variety of pharmacological and toxicological effects.

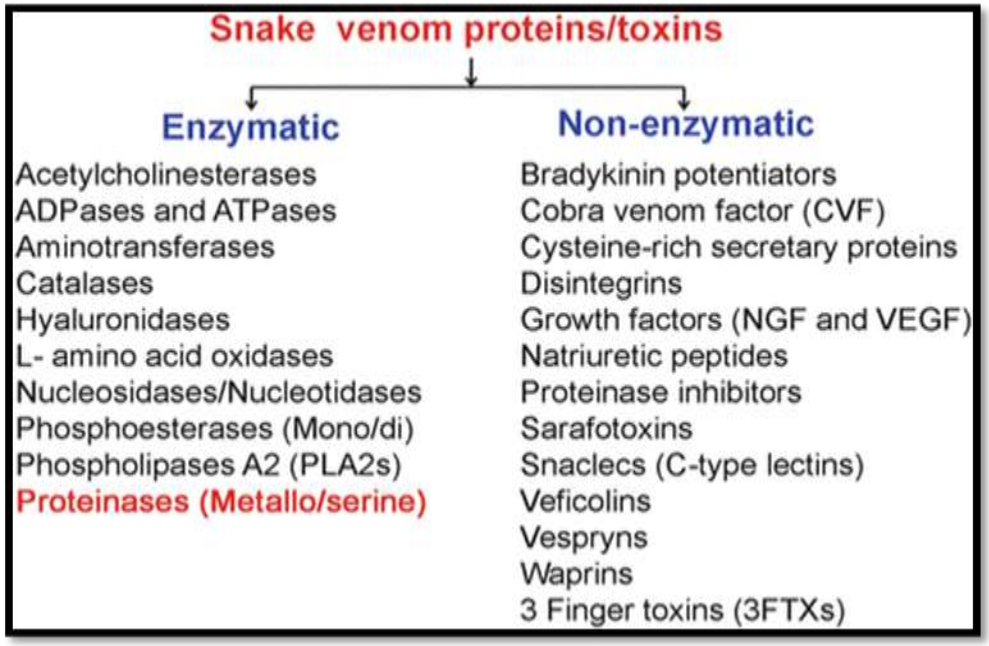

FIG 1: SNAKE VENOM PROTEIN

These venoms comprise 50–200 components distributed in dominant and secondary families which can be presented in multiple proteins and peptides isoforms. The dominant families are secreted phospholipases A_2 (PLA2s), snake venom metalloproteinases (SVMP), snake venom serine proteases (SVSP), and three-finger peptides (3FTX), while the secondary families comprise cysteine-rich secretory proteins, L-amino acid oxidases, kunitz peptides, C-type lectins, disintegrins, and natriuretic peptides. Interestingly, snake venom composition varies interspecifically, as well as intraspecifically, with many factors influencing this diversity including age gender location (diet and season This variability phenomenon underpins toxin diversity and multifunctionality, and is of great importance to be considered in antivenom production and envenomation treatment .

The pharmacological effects of snake venoms are classified into three main types, hemotoxic, neurotoxic, and cytotoxic. The major toxins involved in these effects are the PLA2s, SVMPs, SVSPs, and 3FTXs, that alone or in combination, are responsible for the multiple pharmacological effects occurring in snakebite victims. For example, some PLA2s and 3FTX are able to act on pre- or post-synaptic junctions as antagonist of ion channels and nicotinic or muscarinic receptors to induce severe neurotoxicity such as paralysis and respiratory failure. In addition, other PLA2s and 3FTXs, along with SVMPs, cause local tissue damage resulting in swelling, blistering, bruising, and necrosis, and systemic effects such as hypovolemic shock. Furthermore, SVSPs and SVMPs induce hemostatic and cardiovascular effects as coagulopathy, hypotension and hemorrhage. Interestingly, some PLA2s, SVSPs, and SVMPs are also capable of triggering severe pain by modulating pain pathways through activation of ion channels, such as transient receptor potential vanilloid type 1 (TRPV1) and acid-sensing ion channel (ASIC) and/or by pain sensitization through inflammatory mediators The inflammation induced by the elapid and viper venoms is widely reported to produce pain or hyperalgesia in human and in experimental models Unfortunately, these are not completely reversed by antivenom and anti-inflammatory therapies The toxicological effects induced by snakebite are currently treated with intravenous administration of antivenom in combination to analgesics, fluid therapy, hemodialysis and/or antibiotics Although sufficient in most cases, snakebite treatments have been challenged by the continuous high numbers of clinical illness and mortality associated with snakebites worldwide. Furthermore, chronic morbidity following snakebites have been underestimated, with many victims reporting chronic symptoms in the bitten region, including complex regional pain syndrome (CRPA). Available snakebite treatments face challenges associated with limited para-specificity, poor antibody specificity, high incidences of adverse reactions, low availability and poor affordability to those who need them, along with poor efficacy against local tissue effects Therefore, current research efforts are directed to the development of more effective snakebite therapies able to generically fully inhibit the major toxic components of snake venoms in order to better overcome severe acute and chronic effects caused by snakebite.

In light of the public health importance and the complexity of snake venoms, in this review we highlight the multifunctionality, structure-activity relationships and evolution of proteins and peptides in snake venoms. We aim to provide a better understanding of their action mechanisms and effects, and to bring attention to their undetermined targets and a host of potential novel therapeutic targets that might have implications for improving the treatments of snakebites.

PHOSPHOLIPASE OF SNAKE VENOM

Phospholipases A_2 (PLA_2s) are enzymes of high medical-scientific interest due to their involvement in several inflammatory human diseases and in envenomation by snake and bee venoms. PLA_2s also play an important role in diet lipid catabolism and in the general metabolism of lipid membranes. In addition,

arachidonic acid, one of their hydrolysis products, is the precursor of important eicosanoids displaying prominent biological activities, namely, prostaglandins, prostacyclins, thromboxanes, and leucotrienes. PLA_2s constitute a super-family of different enzymes belonging to four groups based on their source, amino acid sequences, and biochemical characteristics.

Altered lipid biosynthesis and deregulated lipogenesis are typical features of cancer. Consequently, these pathways have been investigated as novel therapeutic targets. Lipolytic phospholipase A_2 (PLA_2) enzymes have been explored as novel anticancer agents .

Different types of phospholipases have been shown to possess antitumor and antiangiogenic properties, such as acidic and basic PLA_2s, and synthetic peptides derived from PLA_2 homologues [97–100]. Recently, two phospholipases A_2 from *Cerastes cerastes* venom, CC-PLA_2-1 and CC-PLA_2-2, were purified and characterized. They were able to inhibit cancerous cell adhesion and migration, along with angiogenesis, both *in vitro* and *in vivo*. Phospholipase A_2 from *Macrovipera lebetina transmediterranea* venom (MVL-PLA_2) inhibited tumor cell adhesion and migration, as well as angiogenesis. This process occurs through an increase in microtubule dynamics and disorganization of focal adhesions.

Some PLA_2s isolated from Viperidae venoms are capable of inducing antitumoral activity, suggesting that these molecules may be a new class of anticancer agents and provide new molecular and biological insights into cancer drug development.

PLA_2 activity is related to the metabolism of cell membranes. In 1989, Chwetzoff et al. reported that a *Naja nigricollis* PLA_2, called nigexin, displays important cytotoxicity upon cell cultures of several tumors, such as epithelial, neuroblastoma, and leukemia tumors. Most PLA_2s do not show this profile and the authors suggest that the enzymatic activity is not responsible for the cytotoxic effect and other mechanisms must be involved.

INFLAMATION AND DISINTEGRINS

Disintegrins are strong candidates to be exploited in the development of anti-inflammatory and antiangiogenic therapies for chronic inflammatory processes. Immune cell movement, intercellular adhesion and binding to microenvironments are mediated by integrins, which may be antagonized by snake venom disintegrin . In animal models of inflammation, alternagin-C, a disintegrin obtained from the venom of *Bothrops alternatus*, can modulate cellular behaviors such as adhesion, migration and proliferation, as well as the production of various growth factors via α2β1 integrin, important processes during inflammation and angiogenesis, which, although they appear as distinct events, act concomitantly in several chronic inflammatory diseases. Similar effects have been reported with jararhagin, from *B. jararaca* snake venom in a murine sponge model. Jararhagin also regulated the release of proinflammatory cytokines (IL-1beta, IL-6 and TNF-alpha) from murine peritoneal adherent cells after treatment with LPS (lipopolysaccharide). Rhodostomin from *Calloselasma rhodostoma* snake venom interacts with αvβ3 integrin on monocytes/macrophages, leading to interference with the activation of phagocytes triggered by LPS, suggesting a protective function of this disintegrin in LPS-induced endotoxemia due to its anti-inflammatory activity in vivo.
Trimucrin from *Trimeresurus mucrosquamatus* suppresses LPS-induced activation of phagocytes primarily through blockade of NF-κB and MAPK activation.

APPLICATION OF SNAKE VENOM AND MECHANISM OF ACTION

3-FINGER TOXINS

The 3FTxs family of polypeptides is comprised of 60–74 amino acid residues. These peptides show diverse functionalities, however, having a conserved structure. A distinct structural feature of 3FTxs is the unique fold, consisting of three loops (β-stranded), emerging from a hydrophobic globular core, FIG.2. Four to five disulfide bonds are present in these 3FTxs, thereby stabilizing the three-dimensional structure. Subtle variations in the length of their loops, conformations and amino acid residues are responsible for their distinct biological functions. Evolutionary studies on 3FTxs showed that these peptides evolved from genes encoding non-toxic ancestral proteins, as the three-finger scaffold can be found in other non-venom proteins. It was reported by Fry and colleagues that three-finger neurotoxins are evolving under positive selection in line with the receptors of prey species, while the evolution of three-finger cytotoxins is limited by negative selection, as they interact nonspecifically with the cell membranes.

FIG:2. DIAGRAM OF 3FTXs

KUNITZ TYPE SERINE PROTEASE

Kunitz-type inhibitors are a family of serine protease inhibitors found in the venoms of Elapidae and Viperidae snakes. It has been suggested that they play a role in disturbing the prey's homeostasis, mainly by interfering with the blood coagulation cascade. Proteome analysis of different snake venoms has shown the relative abundance of snake venom Kunitz-type inhibitors. The Kunitz-type inhibitors represent approximately 28% of the venom proteome of the snake *Daboia russelii russelii*, found also in Pakistan, and approximately 16% of *Dendroaspis angusticeps*. Furthermore, it is known that more than one type of Kunitz-type inhibitor can be found in the venom of a single snake species.

Evolutionary studies explain the diversification of snake venom Kunitz-type inhibitors by a positive Darwinian selection, and it was suggested that the driving force for the variability of the inhibiting loop was caused by selective pressure, driven by the presence of diverse prey proteases. With respect to functional activities, snake venom Kunitz-type inhibitors are divided into two major groups: non-neurotoxin (trypsin and chymotrypsin inhibitors) and neurotoxin (potassium and calcium blockers) snake venom Kunitz-type inhibitors.

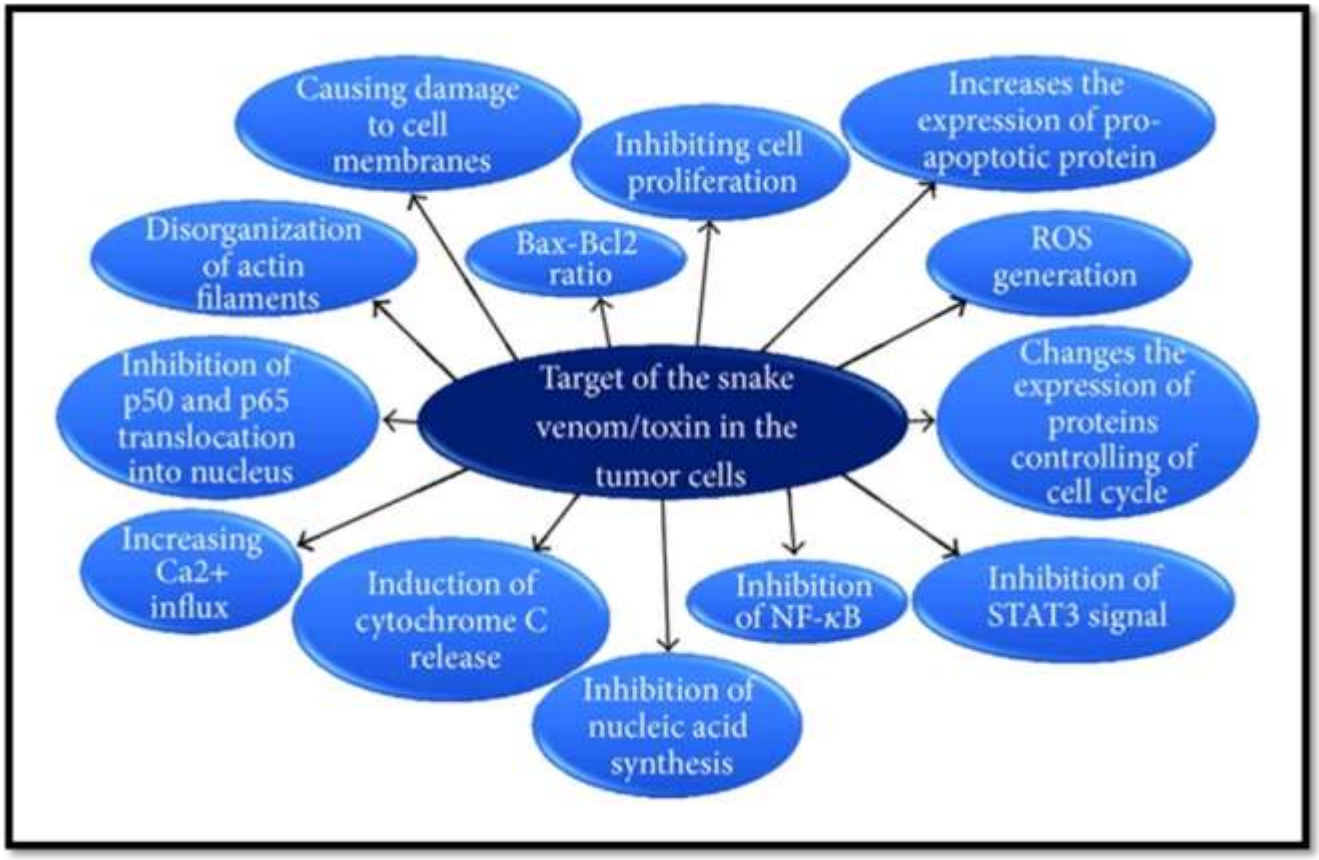

FIG:3. SNAKE VENOM WORK AS TOXIN IN TUMER CELL

Snake venom Kunitz-type inhibitors bear a structural similarity to aprotinin and consist of approximately 60 amino acids. They have three conserved disulfide bridges responsible for the stability of the molecule and two antiparallel β-strands, which are linked by a β-hairpin in the central part of the molecule. There are two helical regions, one 3_{10}-helix near the N-terminus and an α-helix near the C-terminus. Kunitz-type inhibitors interact with some serine proteases through an exposed loop in a canonical confirmation. The P1 amino acid residue is considered to be the primary reactive site, determining the specificity and extent of reactivity of the Kunitz-type inhibitor towards the serine protease. X-ray structures of Kunitz-type inhibitors in complex with serine protease revealed both the canonical binding (P3-P3′) and secondary binding loops, which represent important regions of the peptide interacting with the protease.

SAROFOTOXINS

Snake venom SRTXs are structurally and functionally related to vertebrate endothelin (ETs). Both families of peptides are potent vasoconstrictors and interact with endothelin receptors (ET_A and ET_B), which can modulate the contraction of cardiac and smooth muscles in different tissues. SRTXs are highly toxic venomous peptides, whereas ETs are hormones produced by the mammalian vascular system. SRTXs are exclusively and highly expressed in various isoforms in the venom gland of *Atractaspis* genus. These peptides range in length from 15 to 30 amino acids as established by mass spectrometric experiments. The most abundant isoform has 25 amino acids, while all other isoforms of these peptides contain a common core of 21 amino acids and adopt a highly conserved three-dimensional scaffold stabilized by an α-helical structure. The amino acid Trp^{21} is invariably present and plays a crucial role in endothelin receptor binding. It was also described in a previous study that the amino acids at positions 4–7 present a variable region of these peptides.

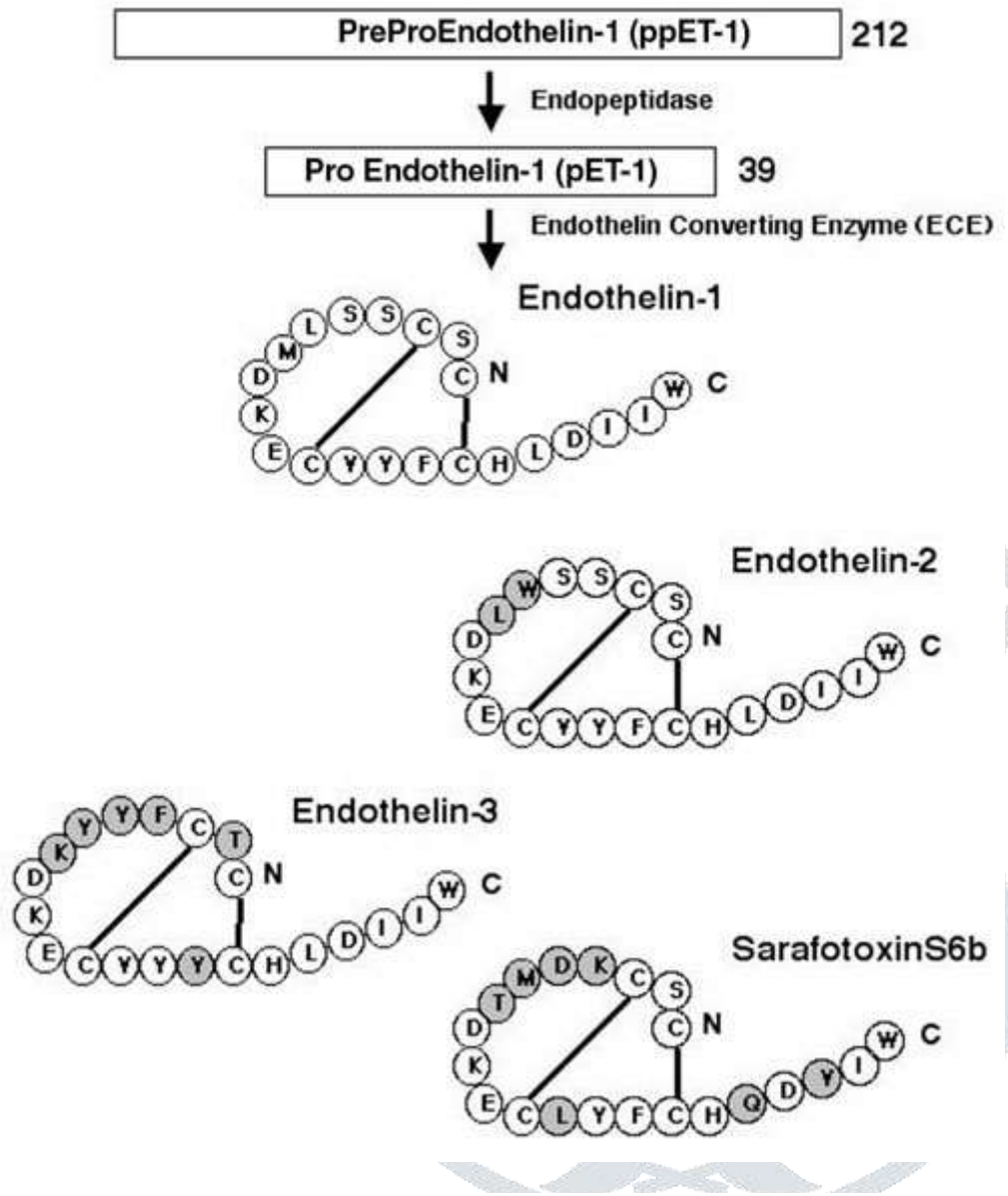

FIG:4. PATHWAY OF BIOSYNTHESIS AND AMINO ACID SEQUENCE

On the other hand, C-terminal extension determines the affinity and selectivity towards endothelin receptors. It was demonstrated experimentally by an in vitro study applying cloned human ET receptors that the C-terminus extension reduces the binding affinity of SRTXs to both ET_A and ET_B receptors. Furthermore, it was also suggested that steric hindrance associated with this extension was responsible for a reduced interaction between SRTX and ET receptor. The respiratory effects of sarafotoxin-b (short isomer) and sarafotoxin-m (long isoform) were studied in vivo, revealing that both peptides acted differently on the respiratory mechanics. This difference of action of both peptides was also attributed to a C-terminus extension for the long isoforms. C-terminal extension can result in different spatial conformation of the two peptides. SRTXs can serve to better understand the endothelin system and related diseases, such as pulmonary hypertension, asthma, and/or heart failure. SRTX was used to uncover the communication pathways between the peptide and ET receptor. It was suggested that phosphoinositide second messenger system and Ca^{2+} signal-transduction mechanisms are involved in this communication

CONCLUSION

Nowadays, some works have been published with emphasis on the evaluation of specific points of tumor metabolism, like its immunological aspects and the induction of apoptosis. Cellular proliferation is not the only event to be fought in cancer treatment; the capacity of the tumor to invade adjacent tissues and to create new blood vessels can also be used as targets for new treatments. Disintegrins interact with integrins via glycoprotein receptors located on cellular surfaces, which are related to cell-cell and cell-matrix interactions. These complex mechanisms have led to several studies on the elucidation of events or factors that may affect focal adhesion in cancer. Snake venoms have, until now, been considered a mostly untapped resource of unique peptides and proteins. Snake venom peptides have been identified to be mostly very stable, as they have to reach the site of action before the internal mechanisms of their prey degrades, neutralizes or excretes them. Nature has met this requirement through the recruitment of highly stable molecular scaffolds, which are mostly resistant to protease degradation and can bypass other immune mechanisms of prey. Several investigations have shown that these venom peptides influence important physiological systems, such as blood pressure regulation, homeostasis, the nervous system, etc. These peptides can serve as molecular probes. Also, they can be used directly or as lead compounds for drug discovery and drug design investigations, because they are easy to synthesize and less prone to inducing an immune response.

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