VENOMOUS ALCHEMY: UNLEASHING THE MEDICINAL WONDERS OF SNAKE VENOM

Ms. Ambika D. Nagarbhadiya[1] , Mr. Minhaj M. Shaikh[2] , Mr. Yash R. Kawale[3], Mr. Somesh S. Bhandare[4] ,Mr. Prasad R. Giram[5]

Students [1234]

Ishwar Deshmukh Institute of Pharmacy, Digras, Mahaashtra, India.

ABSTRACT:

Venomous snakes belonging to the family Viperidae, Elapidae, Colubridae and Hydrophidae , produces snake venom in order to facilitate immobilization and digestion of prey, act as defence mechanism against threats. Owing to their lethality, snakes have often been associated with images of perfidy, treachery and death. However, snakes did not always have such negative connotations. Venom contains zootoxins which is a highly modified saliva that is either injected via fangs during a bite or spitted. The modified parotid gland, encapsulated in a muscular sheath, present on each side of the head, below and behind the eye, have large alveoli which temporarily stores the secreted venom and later conveyed by a duct to tubular fangs through which venom is injected. Snake venoms are complex mixtures of small molecules and peptides/proteins, and most of them display certain kinds of bioactivities. They include neurotoxic, cytotoxic, cardiotoxic, myotoxic , and many different enzymatic activities. This review is mainly focused on the chemistry of the snake venom and the potential for venom to be exploited for medicinal purposes in the development of drugs and derive new therapeutic drug.

Introduction:

Venomous creatures, constituting a captivating subset of the animal kingdom, play a significant role in the intricate web of Earth's biodiversity. With more than 220,000 species, these organisms contribute to approximately 15% of the planet's animal diversity. Among them, snakes emerge as prominent figures, boasting a global presence encompassing around 2500 species. In India, a nation abundant in biodiversity, 250 snake species thrive, with 50 of them being venomous, including the iconic Cobra, King Cobra, Krait, Banded Krait, Russel's Viper, Saw-scaled Viper, and Sea Snakes, notably the Krait. Despite their ubiquity, certain regions such as Greenland, Ireland, Iceland, Jamaica, and New Zealand remain devoid of snakes, with vipers notably absent in America.

The anatomy of snakes presents a distinctive adaptation to their environment. Their bodies are elongated, with a short tail and a limbless structure, accompanied by fused eyelids. The head features two eyes, two nostrils, an absence of external ears, and a distensible mouth. A forked tongue serves as a sensory organ, aiding in environmental navigation. The teeth are thin and directed backward, with long, canalized upper marginal fangs that can be replaced within 3-6 weeks if broken. Venomous snakes possess

specialized fangs connected to venom glands through ducts, originating from modified parotid salivary glands. Reproductive methods vary, with colubrine snakes being oviparous and vipers viviparous.

Venom, a potent biochemical cocktail, primarily comprises proteins and peptides. Cone snails, spiders, scorpions, and snakes serve as well-explored sources of venom. This intricate mixture consists of over 20 compounds, predominantly proteins and polypeptides. Cone snail, spider, and scorpion venoms stand out for their short disulfide-rich peptides with the inhibitor cysteine knot (ICK) motif. In contrast, snake venoms, exhibiting a diverse range of 20 to >100 components, consist of over 90% peptides and proteins, inducing neurotoxicity, haemotoxicity, and cytotoxicity. The variability in venom composition is influenced by environmental factors, age, and sex, adding complexity to understanding these potent biological cocktails.

A deeper exploration of venom delves into the sophisticated interplay of bioactive components, where proteins and peptides take centre stage. Cone snails, spiders, scorpions, and snakes contribute significantly to the knowledge in this field. Venoms, comprised of over 20 different compounds, showcase prominent toxic and lethal properties. Cone snail, spider, and scorpion venoms, marked by short, disulfide-rich peptides with the ICK motif, contrast with snake venoms, characterized by a multitude of peptides and proteins that contribute to various bioactivities.

The complexity of snake venoms, arising from their diverse mixtures of peptides and proteins, plays a pivotal role in the manifestation of neurotoxic, haemotoxic, and cytotoxic properties. These properties, varying across snake species, add an intricate layer to understanding the venomous world. Beyond the differences at the species level, individual factors such as environment, age, and sex further influence venom composition, rendering each venom unique. The unraveling of secrets within these potent biological cocktails goes beyond satiating curiosity, holding promising applications in fields like medicine and drug development.

In conclusion, the vast and intricate world of venomous creatures and their venoms forms a captivating tapestry of biological diversity. Snakes, as key contributors to this diversity, showcase a complex array of peptides and proteins within their venoms. The exploration of venoms extends beyond mere curiosity, offering potential applications in medicine and drug development. As we continue to decode the mysteries of these potent substances, we not only gain insights into the natural world but also open doors to innovative possibilities for enhancing human well-being. The journey through the intricate realm of venoms promises continuous discoveries and advancements, contributing to both scientific knowledge and practical applications in various domains. This journey underscores the importance of preserving biodiversity and understanding the delicate balance that sustains life on our planet.

❖ Snake venom

Snake venom, a captivating and intricate symphony of biochemical complexity, emerges as a heterogeneous concoction known as toxalbumin, meticulously produced and harboured within the specialized confines of salivary glands. This enigmatic elixir, constituting a staggering 90% water, unfolds a rich tapestry of enzymatic and nonenzymatic proteins, lipids, carbohydrates, and biogenic amines. Within this potent elixir, the very essence of lethality resides in a category denoted as venins, whilst the entirety encapsulates the mystique of venom.

Upon its initial secretion, snake venom manifests as a diaphanous and ethereal liquid, gradually maturing into a yellowish, opaque, granular powder upon desiccation. A transformative alchemy transpires in this process, endowing the venom with a remarkable longevity that persists over the expanse of numerous years. It is in the proteins dwelling within this venomous elixir, assuming the nuanced forms of enzymes, peptides, and polypeptides, where the narrative of predation and potent physiological effects unfolds.

The enzymatic ballet within the venom encapsulates a diverse ensemble of proteinases, hydrolases, transaminase, hyaluronidase, phospholipases A, B, C & D, ribonucleases, deoxyribonuclease, phosphomonoesterase, phosphodiesterases, 5-nucleotidase, ATPase, alkaline phosphatase, acid phosphatase, cholinesterases, coagulases, agglutinins, fibrinolysin, haemolysin, and a myriad of others, each contributing to the venom's symphony of lethality. Hyaluronidase, ubiquitous in most snake venoms, orchestrates the catalytic cleavage of internal glycoside bonds and mucopolysaccharides, bestowing a synergistic enhancement upon the lethal ensemble.

Among these enzymatic virtuosos, phospholipase A assumes a prominent role, precipitating the hydrolytic disintegration of membrane phospholipids—a recurring motif within the pharmacopeia of snake venoms. Collagenase, a luminary within this venomous opera, choreographs the digestion of collagen and the consequential breakdown of connective tissue.

The taxonomy of venomous snakes unravels further nuances, offering a diptych between elapids and vipers. Elapids, the purveyors of potent neurotoxins, bestow upon their venom a composition rich in polypeptides. These neurotoxins, ardent conductors orchestrating a concerto of physiological disruption, act upon neuromuscular junctions, diminishing acetylcholine output. Their profound toxicity resonates across the central nervous system, respiration, and the heart, rendering an indelible mark on their victims.

Viperine venom, on the other hand, emerges as a triad of haemolytic, haemorrhagic, and necrotic effects. This venom orchestrates a macabre ballet within the circulatory realm, inducing intravascular haemolysis and casting a pall of depression upon the intricate dance of coagulation mechanisms. The sea snake, ensconced within its aqueous realm, weaves a myotoxic narrative—ushering forth manifestations of muscle pain, myoglobinuria, and the orchestration of hyperkalaemia.

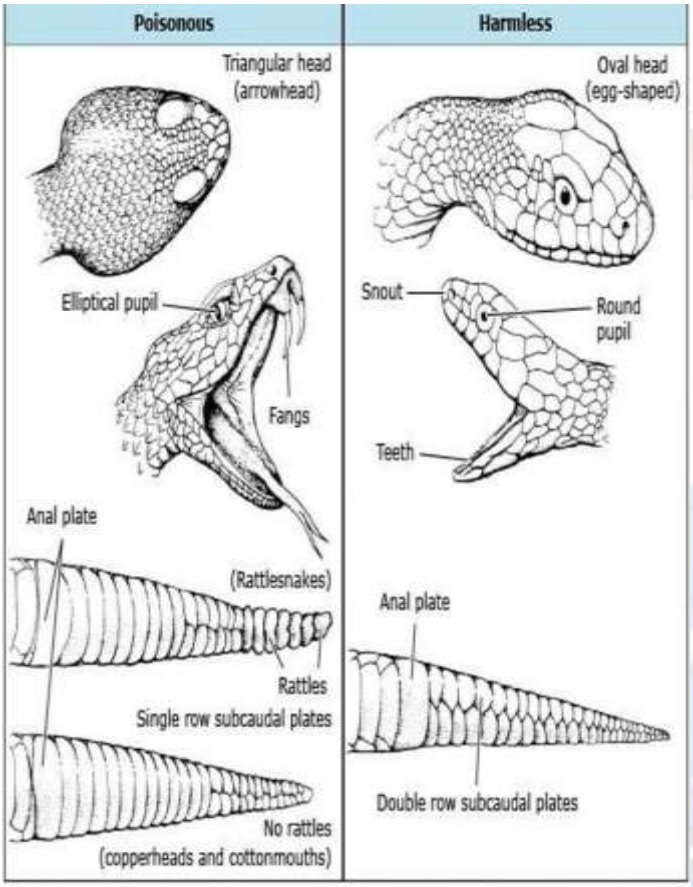
Within the realm of snake venoms, a pantheon of proteins emerges, each holding sway over distinct biological functions. These proteins, arbiters of blood coagulation, regulators of blood pressure, and conductors of nerve or muscle impulse transmission, weave an intricate tapestry of influence. Their nuanced effects beckon researchers into a realm of inquiry that transcends mere curiosity—delving into the realm of pharmacology and diagnostics.

As the intricacies of snake venom continue to unfurl, the potential applications of these biochemical orchestrations echo across the vast expanse of medical and diagnostic landscapes. The serpentine symphony of venomous creatures, once regarded with trepidation, metamorphoses into a realm of promise — a canvas upon which therapeutic interventions and innovative diagnostic tools may find their genesis.

In the exploration of snake venoms, the journey undertaken extends beyond the boundaries of biological inquiry. It is a voyage into the very essence of life's intricate design a testament to the multifaceted

intricacies that govern the interplay between predator and prey, and an ode to the potentialities that lie dormant within nature's most potent elixirs.

The difference between poisonous and non-poisonous snakes:



❖ **Chemistry of snake venom and its Pharmacology**

➢ **STRUCTURE AND FUNCTION OF COBRA NEUROTOXIN**

Within the intricate realm of venomous creatures, the enigmatic narrative of snake neurotoxins unfolds as a captivating tale, marked by both lethal precision and structural elegance. These neurotoxins reign supreme as the principal toxic proteins within the venoms of cobra, krait, tiger snakes, and sea snakes, orchestrating a symphony of biochemical warfare that culminates in the demise of their prey through respiratory paralysis.

The protagonists in this venomous saga, snake neurotoxins, find their place in a taxonomy defined by their distinct actions at the neuromuscular junction. Classified into two archetypal types – postsynaptic and presynaptic neurotoxins – their narrative is intricately woven into the mechanisms that underlie their potent effects on living organisms. The neuromuscular junction, the stage upon which this drama unfolds, serves as the battleground where these neurotoxins exert their influence.

Postsynaptic neurotoxins emerge as formidable actors, their role defined by a specific affinity for the nicotinic acetylcholine receptor (AChR) situated at the motor endplate. With precision akin to an artist's brushstroke, they bind to this receptor, initiating a nondepolarizing block of neuromuscular transmission. This disruption in communication between nerves and muscles manifests as a devastating impact on the victim, illustrating the potency of these neurotoxins in orchestrating paralysis.

In stark contrast, presynaptic neurotoxins adopt a different strategy, intervening at the presynaptic motor nerve terminals. Their mission is to impede the release of acetylcholine, the crucial neurotransmitter responsible for signalling. By disrupting this fundamental step in neuromuscular communication, presynaptic neurotoxins contribute to the overall incapacitation of the prey, sealing their fate within the intricate dance of predator and prey.

A crystalline character within this narrative is Cobrotoxin, an emblematic neurotoxin isolated from the venom of Naja naja atra, the Chinese cobra. Its isolation, once an elaborate process involving ammonium sulfate fractionation and repeated chromatography on CM-cellulose, has evolved into a streamlined procedure with a single-step chromatography on an SP-Sephadex C-25 column.

The structural elegance of Cobrotoxin lies not only in its journey from venomous milieu to crystalline form but also in its lethal efficacy, surpassing the venom's toxicity by a factor of seven. This unequivocally establishes Cobrotoxin as the principal toxic protein in cobra venom.

The architectural blueprint of Cobrotoxin further enriches this tale of biochemical intricacies. As a small basic protein, it unveils itself as a single peptide chain, an intricate sequence of 62 amino acid residues, intricately crosslinked by four disulfide bonds. This structural finesse not only accentuates the lethal efficacy of Cobrotoxin but also underscores the sophisticated design within the venomous arsenal of snakes.

In the broader context, this narrative of snake neurotoxins beckons towards a profound understanding of the natural world, where the delicate balance of predator and prey hinges on the biochemical weapons wielded by these creatures. The detailed unravelling of their actions at the neuromuscular junction not only satisfies scientific curiosity but also opens doors to potential applications in medicine and drug development. Each revelation, each structural intricacy, contributes to a nuanced comprehension of the intricate dance between organisms, their venoms, and the profound interplay of life and death in the natural world.

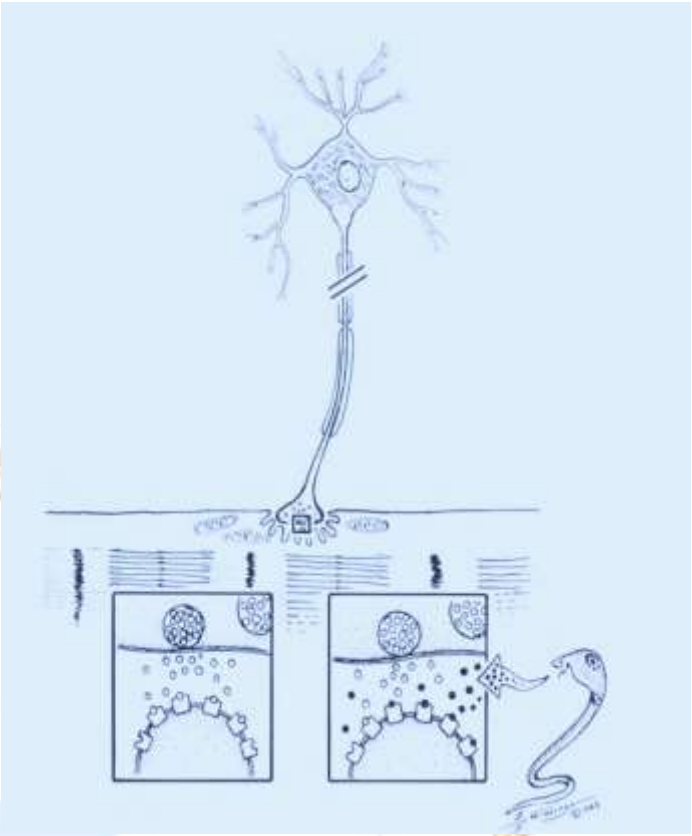


Figure: A diagram showing nerve transmission across the neuromuscular junction. Left: Normal transmission by acetylcholine (open circle). Right: Blockage of acetylcholine receptor by postsynaptic neurotoxin (solid circle)

- **STRUCTURE OF POSTSYNAPTIC NEUROTOXIN**

To date, more than 120 toxins with neurotoxic activity have been isolated in pure state from elapid and hydrophid (sea-snake) venoms. Over 100 highly homologous postsynaptic neurotoxins belonging to two distinct size groups, short and long neurotoxins, have been sequenced. Short neurotoxins contain 60-62 amino acid residues with four disulfide bonds, and long neurotoxins comprise The pairing of four disulfide bonds is similar in both short and long neurotoxins. The "extra" disulfide bond in the long neurotoxins, at 29-33, pinches off a short pentapeptide section in the second disulfide loop, thereby shortening the loop to about the same length as in the short neurotoxins. The extra disulfide bond is exposed at the surface of the toxin molecule and can

be selectively reduced without affecting binding affinity. Long neurotoxins extend seven amino acid residues beyond the carboxyl terminus of the short toxins . The removal of the C-terminal four or five residues of alpha-bungarotoxin by mild tryptic digestion (Wu et al., 1983) or carboxypeptidase P induces no global conformational change in the molecule but affects only a limited region. Therefore, the C-terminal tail of long neurotoxin appears to be unimportant in maintaining the specific polypeptide chain folding.

All postsynaptic neurotoxins are similar in their overall folding,but differ in details such as the extent of secondary structure and the position of an invariant side-chain. Recently, the NMR three dimensional structure of cobrotoxin in solution has been determined (Yu et al., 1993). The mean solution structure was compared with the X-ray crystal structure of homologous protein erabutoxin b which has been solved to a resolution of 1.4 A.

➢ STRUCTURE AND FUNCTION OF COBROTOXIN.

Cobrotoxin, a venomous protein boasting a mere 62 amino acid residues, distinguishes itself with four disulfide bonds and an absence of free sulfhydryl groups. To unravel the interplay between disulfide bonds and biological activity, an 8 M urea solution dissolved cobrotoxin, and the reducing agent p-mercaptoethanol was introduced to cleave disulfide bonds. Remarkably, this reduction led to the emergence of eight sulfhydryl groups, resulting in the loss of lethal toxicity and antigenic specificity, accompanied by a conformational shift. Intriguingly, reoxidation of reduced cobrotoxin reinstated a biologically active product mirroring the native toxin's IR spectra, underscoring the indispensability of intact disulfide bonds for cobrotoxin's biological functions.

With two tyrosine residues at positions 25 and 35, cobrotoxin's spectrophotometric titration unveiled one tyrosyl group readily titrated at a normal apparent pK of 9.65. In contrast, the other exhibited slow ionization post irreversible conformational changes beyond pH 11.3. Nitration experiments targeting Tyr-35 showcased selective modification without compromising biological activity or conformation. However, modification of the invariant Tyr-25 in the presence of 5 M guanidine-HCl led to a loss of biological activity and significant changes in the CD spectrum. Notably, the commonality of Tyr-25 in all snake neurotoxins suggests its pivotal role in maintaining the toxin's active conformation.

Distinguishing between structurally and functionally important groups becomes imperative. Disulfide bonds and the tyrosine residue at position 25 emerge as structurally crucial for maintaining the toxin's active conformation. On the other hand, functionally important groups, such as those directly involved in binding toxins to an AChR of the muscle motor endplate, play a pivotal role in preventing transmission across the cholinergic synapse for postsynaptic neurotoxins. This intricate web of molecular interactions underscores the delicate balance required for cobrotoxin's biological functionality.

➢ NEUROTOXIN-AChR INTERACTIONS

Cobrotoxin strategically targets the AChR on the postsynaptic membrane, engaging in competitive interactions with cholinergic agonists to effectively thwart neuromuscular transmissions. The AChR, a complex assembly of transmembrane proteins composed of five homologous subunits in a 2α~3βγ stoichiometry, sees cobrotoxin binding reversibly to acetylcholine-binding sites within two α-subunits.

To unravel the intricate structure of the ACh-binding sites on the native, membrane-bound AChR, Dennis et al. (1988) employed a photoaffinity reagent, [3H] DDF, illuminating labeled amino acids within three distinct regions of the large amino-terminal hydrophilic domain of the α-subunit. Atassi et al. (1991) expanded on this, mapping toxin-binding regions with synthetic peptides. Surprisingly, the region a182-198 showed minimal binding to cobrotoxin, contrasting with its significance for the long neurotoxin, a-bungarotoxin.

Further insights from quantitative radiometric titrations, synthetic peptide affinities, and high-resolution NMR studies construct a three-dimensional model of cobrotoxin. Notably, the a-subunit residues 126-136 align with the central loop of cobrotoxin, offering a harmonious fit based on energy minimization. Ruan et al. (1990) extended this understanding, assessing the relative binding affinities of peptide-peptide interactions and constructing a 3D model of the binding-site cavity for a-bungarotoxin on human AChR.

Short and long neurotoxins share similar dissociation constants and LD50 values but differ in their rates of association and dissociation from the receptor. Chemical modifications targeting individual amino acid residues highlight the collaborative nature of neurotoxin binding to AChR, relying on multiple attachment points. The central core, featuring four disulfide bonds and the buried Tyr-25, maintains the toxin's active conformation, while extended loops, endowed with functional residues, facilitate tight binding.

These loops, forming two antiparallel sheets, exhibit dynamic structures. Notably, a segment in loop 3, exposed to solvent, displays higher conformational freedom, potentially influenced by cationic reactive groups (Arg-33 and Lys-47) responsible for the protein's neuromuscular blocking activity. In this intricate dance of molecular interactions, cobrotoxin emerges as a sophisticated player in disrupting neuromuscular communications.

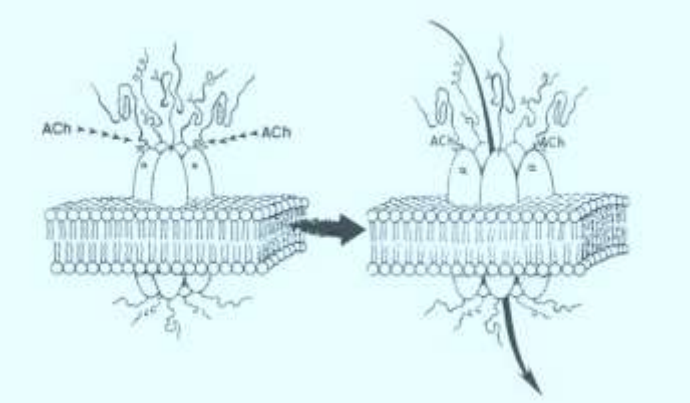


Figure: As two moles of acetylcholine (ACh) attach to two a-subunits of acetylcholine receptor (AChR), the pore opens to form an ion channel in the membrane that allows ions to pass through the channel. This is the role of AChR in muscle depolarization.

- **Enzymatic toxins from snake venom**

Snake venoms, intricate cocktails of enzymes and non-enzymatic proteins, serve a dual purpose: immobilizing and digesting prey. Within this venomous symphony, acetylcholinesterases, L-amino acid oxidases, serine proteinases, metalloproteinases, and phospholipases A2 stand as the most prevalent enzymatic components. The allure of these enzymes lies in their higher catalytic efficiency, thermal stability, and resistance to proteolysis, making them captivating subjects for biochemists, enzymologists, and structural biologists seeking to unravel nature's biochemical intricacies.

Exploring the structures of these snake venom enzymes unveils a tapestry of molecular intricacies. Acetylcholinesterases, crucial for cholinergic neurotransmission disruption, exhibit a structural elegance that transcends their venomous origins. L-amino acid oxidases, with their capacity to oxidize amino acids, present an intriguing landscape for structural exploration. Serine proteinases, masters of hydrolyzing peptide bonds, and metalloproteinases, wielding metal ions for catalysis, each weave a distinctive structural narrative. Phospholipases A2, pivotal in membrane disruption, showcase structural motifs that underscore their role in venomous armamentarium.

The structural nuances of these enzymes are the focal point for unraveling their catalytic and inhibitory mechanisms. The intricacies of catalysis, driven by specific structural features, become a captivating avenue of exploration. Equally intriguing are the mechanisms governing inhibition, shedding light on the intricate dance of molecular interactions within the venomous milieu.

Adding a layer of complexity, some snake venom enzymes exist as protein complexes within the venomous secretion. This intertwining of proteins amplifies the intricacy of their functional interplay, creating a dynamic landscape where each component contributes to the venom's potency. The exploration of these complexes adds an additional layer of depth to our understanding of snake venom biochemistry.

In summary, snake venoms serve as reservoirs of captivating enzymatic structures, each wielding unique capabilities for prey immobilization and digestion. The structural insights into acetylcholinesterases, L-amino acid oxidases, serine proteinases, metalloproteinases, and phospholipases A2 not only illuminate the venomous arsenal but also provide inspiration for researchers delving into the realms of enzymology and structural biology. As we unravel the structures and mechanisms of these venomous enzymes, we navigate the intricate web of nature's biochemical marvels, unlocking secrets that resonate far beyond the slithering realm of snakes.

1) Snake venom metalloproteinases

Snake Venom Metalloproteinases (SVMPs) constitute a significant class of toxins primarily associated with haemorrhagic effects. These enzymes are categorized into three groups, namely P-I to P-III, based on the number of domains they possess, ranging from 1 to 3, and further differentiated into subgroups. Among these, P-III SVMPs stand out as the largest, most ancient, and complex enzymes. P-II and P-I enzymes are considered evolutionary derivatives of P-III SVMPs, having undergone domain loss.

Elapid venoms exclusively contain P-III SVMPs, while viperid venoms encompass SVMPs from all three groups, with SVMPs often emerging as a predominant and abundant toxin. P-I SVMPs consist solely of the catalytic domain, responsible for hydrolyzing a diverse range of physiologically relevant enzymes and structural proteins. This catalytic domain is shared across all three SVMP groups. Targets of hydrolysis include collagen IV, fibrinogen, and coagulation factors, leading to severe haemorrhagic consequences.

The breakdown of collagen IV by P-I SVMPs results in the weakening of capillary walls, causing their collapse under normal hemodynamic pressure. Continuous hydrolysis of fibrinogen in vivo leads to the formation of weak and inefficient fibrin clots, contributing to hypofibrinogenaemia. Additionally, the hydrolysis of blood coagulation factors disrupts the regular regulation of blood clotting processes.

P-II SVMPs possess an additional disintegrin domain, which plays a crucial role in inhibiting platelet aggregation. This inhibition occurs through specific binding to the blood platelet αIIBβ3 integrin, a vital protein that triggers fibrinogen binding and platelet aggregation. The presence of this disintegrin domain reinforces the haemorrhagic effect initiated by the hydrolysis of collagen IV.

P-III SVMPs exhibit a more intricate structure, comprising a catalytic domain, a disintegrin-like domain featuring a collagen-binding three-amino-acid Glu–Cys–Asp (ECD) motif (as opposed to the typical P-II Arg–Gly–Asp (RGD) motif), and a cysteine-rich domain. While the cysteine-rich domain is primarily associated with substrate recognition and binding, the catalytic domain also plays a role in substrate recognition through a unique conformational selection mechanism. In certain isoforms of P-III SVMPs, a C-type lectin-like domain is additionally present, adding another layer of complexity to their structure. SVMPs constitute a diverse group of toxins with distinct classifications based on their domains. The variations in their structures and functionalities contribute to their ability to induce haemorrhagic effects and disrupt vital physiological processes, making them a crucial focus in the study of snake venom and its impact on the human body..

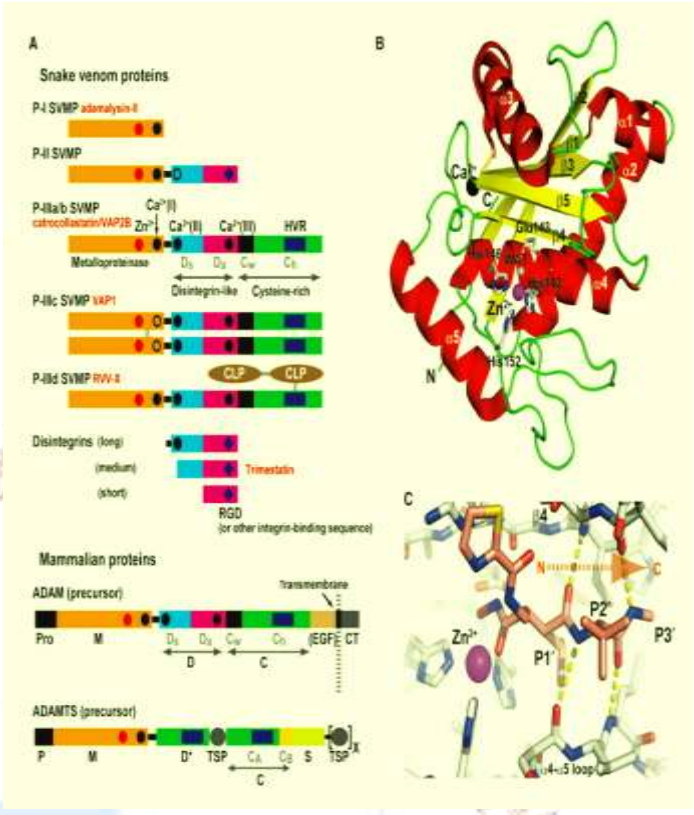


Figure: The classification and structure of Snake Venom Metalloproteinases (SVMPs) unfold through a schematic representation of their domain structure. In this illustration, different colors represent each domain or subdomain, providing a visual guide to understanding the intricate composition of SVMPs, disintegrins, and mammalian ADAM/ADAMTS family proteins. The key elements include the C-type lectin-like domain (CLP), pro domain (Pro), cytoplasmic domain (CT), thrombospondin type-1 motif (TSP), epidermal growth factor-like domain (EGF), and spacer domain (S). Notably, the D domain of ADAMTSs, while lacking a disintegrin-like structure, adopts an ADAMs' Ch-subdomain-like fold and is denoted as D* in the representation. Calcium and zinc binding sites are highlighted schematically, with specific details such as the unique positioning of the ammonium group of Lys202 in VAP1.Moving to the ribbon structure of adamalysin II, a structural prototype of P-I SVMPs (PDB ID 1IAG), the representation offers insights into the three-dimensional arrangement. Zinc and calcium ions are visually depicted as magenta and black spheres,

respectively, enhancing our understanding of the spatial orientation of these crucial elements within the protein structure. A closer examination of the catalytic site of BaP-1, bound with the peptide mimetic inhibitor WR2 (PDB ID 2W12), further elucidates the molecular interactions at play. The inhibitor, shown in light salmon, adopts an extended conformation closely mimicking the C-terminal part (P1¢ to P3¢ residues) of the enzyme-bound substrate. Noteworthy hydrogen bonds, represented by yellow dotted lines, form between WR2 and the adjacent b4 strand, as well as the loop connecting the a4 and a5 helices in BaP-1. This detailed view provides a glimpse into the specific molecular interactions critical for the catalytic activity of SVMPs, offering valuable insights for researchers in the field of toxicology.

2)Snake venom serine protease

Snake venom serine proteases (SVSPs) emerge as formidable actors in the realm of haemotoxicity, intricately influencing various facets of the circulatory system. Their multifaceted actions extend to blood coagulation, fibrinogen levels, blood pressure modulation, and platelet aggregation. Through a comprehensive exploration of these activities, SVSPs reveal their potent impact on the delicate balance that governs our cardiovascular physiology.

Among the array of activities attributed to SVSPs, a notable exception is found in an SVSP with K+-channel blocking activity. This unique feature adds a layer of complexity to their repertoire, showcasing the diversity within this venomous group. The resilience of SVSPs against endogenous serine protease inhibitors is a pivotal aspect of their toxic effects, accentuating their ability to subvert endogenous regulatory mechanisms.

Drawing parallels to the critical enzyme thrombin, a linchpin in the blood coagulation cascade, SVSPs mirror several of its activities. These shared traits contribute to their toxicity, as SVSPs act in concert with thrombin while simultaneously diverging in certain bioactivities. Crucially, it is noteworthy that no single SVSP possesses the entirety of thrombin's bioactivities. This distinctive feature underlines the toxic potential of SVSPs, leading to a deregulation of homeostasis, a departure from the finely tuned balance essential for physiological well-being. This distinct category of SVSPs, mirroring the fibrinogenolytic activities of thrombin, has aptly earned the nomenclature of "thrombin-like enzymes."

Delving into the structural realm of SVSPs, these venomous proteins reveal their monomeric glycoprotein nature. Comprising approximately 228–239 residues, they wield a molecular mass ranging from 26 to 67 kDa. This variability in mass arises from diverse patterns of N-glycosylation and O-glycosylation, unveiling the intricacies of their post-translational modifications. Structurally, SVSPs share a typical trypsin fold, a fundamental architectural feature in their molecular design. The highly conserved catalytic triad, denoted as Ser195–His57–Asp102 (chymotrypsin numbering), underscores the evolutionary significance of these venomous enzymes. The stability of their structures is further buttressed by the presence of six disulfide bonds, reinforcing the robustness of these venom components.

In navigating the expansive landscape of snake venom serine proteases, it becomes evident that their actions extend beyond mere toxicity. The interplay of shared and distinct activities with thrombin, coupled with their structural intricacies, sets the stage for a deeper understanding of their roles in envenomation

pathology. As research continues to unravel the mysteries of SVSPs, their potential implications in diagnostic and therapeutic arenas within the cardiovascular domain come to the forefront. The intricate dance of SVSPs with the intricate machinery of our circulatory system beckons further exploration, offering both challenges and opportunities for scientific inquiry and medical advancement.

While the majority of snake venom serine proteases (SVSPs) adhere to the classical reaction mechanism typical of serine proteases, there exists a fascinating subset of over 20 SVSPs exhibiting variations in the canonical catalytic triad. Unveiled through snake venom transcripts86, this diversity challenges conventional expectations. Among these distinctive SVSPs, the serine protease VaSP1 from the horned viper (Vipera ammodytes ammodytes) stands out, showcasing an unconventional Ser195–Lys57–Asp102 triad. Contrary to expectations, this rare triad configuration, with lysine in place of the usual histidine, was surprisingly found to be catalytically active.This revelation underscores an unexpected richness and complexity in the chemistry of SVSPs, providing a glimpse into the diverse enzymatic landscape these venoms harbor.

Procoagulant Snake Venom Serine Proteases (SVSPs) play a pivotal role in hemostasis by activating key factors in the blood coagulation cascade. This activation includes the initiation of FVII, FX, and prothrombin,leading to a cascade of events that result in the shortening of coagulation times. Beyond their procoagulant effects, certain SVSPs also exhibit fibrinogen-clotting activity, earning them the designation of thrombin-like enzymes.

Thrombin-like enzymes, a subset of SVSPs, have been subjects of extensive research over the last decade due to their potential therapeutic applications. Commercially available examples include ancrod, batroxobin, and reptilase, each contributing to the treatment of cardiovascular diseases.Ancrod, in particular, has found clinical utility in addressing specific conditions such as heparin-induced thrombocytopenia, thrombosis, and acute ischemic stroke. Similarly, batroxobin has been employed for the treatment of thrombotic diseases, marking its significance in managing such conditions.

Moreover, both batroxobin and ancrod are currently undergoing clinical trials for their effectiveness in treating deep vein thrombosis, reflecting the continuous exploration of these enzymes for novel therapeutic applications. The application of reptilase extends beyond treatment; it serves as a diagnostic tool for disfibrinogenemia.

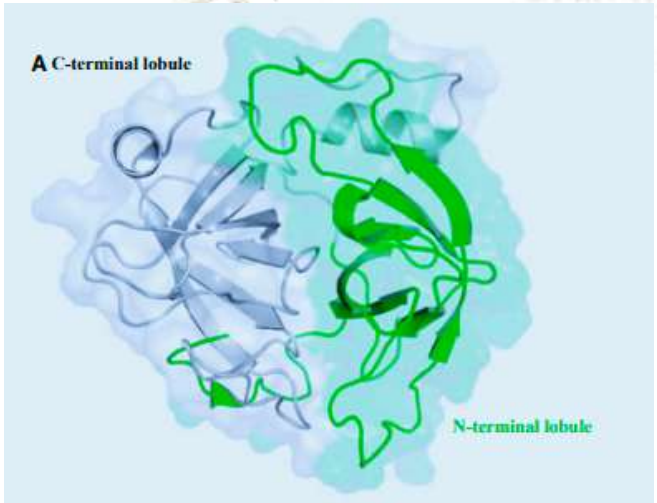
This exploration of procoagulant SVSPs and their thrombin-like activities underscores their multifaceted role in hemostasis and their potential significance in the development of therapeutic interventions for various cardiovascular conditions. The versatility of these enzymes, ranging from clinical treatments to diagnostic applications, highlights the ongoing progress in understanding and utilizing snake venom components for medical advancements.

Anticoagulant Snake Venom Serine Proteases (SVSPs) play a crucial role in activating protein C through a thrombomodulin-independent mechanism [163]. Among these enzymes, the most extensively studied is derived from Agkistrodon contortrix contortrix venom, commercially known as Protac®, which uniquely converts protein C into its activated form by hydrolyzing the Arg169–Leu170 bond. Notably, Protac® operates independently of plasmatic factors, distinguishing it from the physiological activation of protein C by thrombin, which relies on thrombomodulin [163]. Clinically, Protac® finds

application in functional assays for protein C determination, total protein S content, and other plasma protein S assays [164].

In addition to anticoagulant SVSPs, fibrinolytic SVSPs have been identified in the venoms of Trimeresurus stejnegeri, Agkistrodon blomhoffii [166], and Lachesis muta muta . These enzymes play a pivotal role in converting plasminogen to plasmin, facilitating the rapid degradation of preexisting clots. Among the fibrinolytic SVSPs, the Trimeresurus stejnegeri venom plasminogen activator (TSV-PA) stands out as the most studied. TSV-PA demonstrates high specificity by cleaving the Arg561–Val562 bond in plasminogen and exhibits resistance to inhibition.

This intricate interplay of anticoagulant and fibrinolytic SVSPs contributes to the broader understanding of snake venom's diverse enzymatic activities. The specificity and clinical applications of Protac® underscore its importance in functional assays for protein C and related determinations. Simultaneously, the fibrinolytic capabilities of TSV-PA highlight the potential therapeutic implications of snake venom components in managing clot-related disorders. The ongoing exploration of SVSPs continues to unravel their unique mechanisms and expand their potential roles in medical applications.



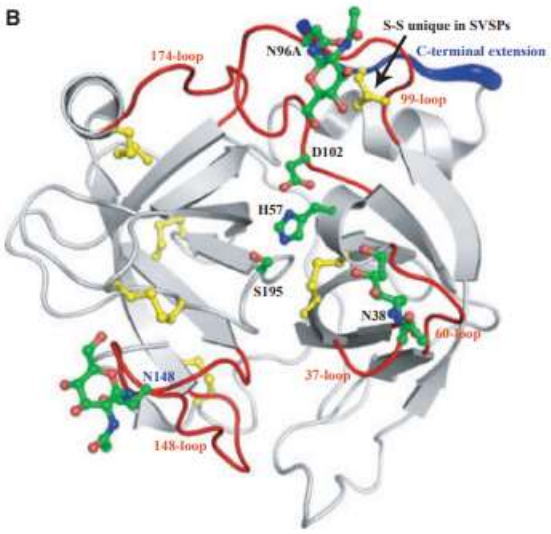


Figure: The intricate architecture of Serine Proteinases in Snake Venom (SVSPs) is elegantly depicted in both cartoon and surface representations. These visuals showcase the structural marvel of two six-stranded b-barrel lobes, artfully presented in shades of green and grey. The N-terminal domain, with its six b-strands and a gracefully solitary short a-helix, adds a symphonic touch to the ensemble.In the artistic rendering of SVSPs, the extended C-terminal tail takes center stage, adorned in a regal blue hue. This tail, embellished with an additional disulfide bridge, weaves a narrative of structural sophistication. Delicately highlighted are the atomic colors of His57, Asp102, and Ser195, casting a spotlight on the essential amino acid residues orchestrating the enzymatic symphony.As the visual journey unfolds, the canvas also captures the ethereal presence of two putative N-linked glycosylation sites, denoted by the positions N96A and N148. The choreography of intra-chain disulfide bridges, including Cys42/Cys58, Cys22/Cys157, and Cys91/Cys245E, further enhances the aesthetic panorama of SVSPs.

3) Phospholipases A2

Phospholipases A2 (PLA2s), classified as phosphatide 2-acylhydrolases with the enzyme code EC 3.1.14, form a diverse superfamily of lipolytic enzymes. Their primary function lies in the specific catalysis of the

ester bond at the sn-2 position of glycerophospholipids, leading to the production of fatty acids (notably arachidonate) and lysophospholipids. Within this expansive superfamily, encompassing approximately 15 groups further subdivided into distinct subgroups, structural and functional variations abound.

The four principal classes of PLA2s include secreted (sPLA2s), cytosolic (cPLA2s), Ca2+-independent (iPLA2s), and lipoprotein-associated (LpPLA2s) phospholipases. Among these, sPLA2s were the pioneering members discovered. Typically weighing in at 14–18 kDa, these secreted proteins are prevalent in snake, bee, scorpion, or wasp venoms, as well as mammalian tissues like the pancreas and kidneys, and arthritic synovial fluids. Characterized by their dependency on Ca2+ ions for phospholipid hydrolysis, sPLA2s often feature five to eight disulfide bonds. Noteworthy is their classification into distinct groups such as IA, IB, IIA, IIB, IIC, IID, IIE, IIF, III, V, IX, X, XIA, XIB, XII, XIII, and XIV based on their structural variations. A fascinating phenomenon displayed by many sPLA2s is interfacial activation, wherein their catalytic activity markedly increases when presented with substrates in the form of large lipid aggregates rather than monomeric structures.

Originally, snake venom PLA2s were dichotomized into two easily distinguishable groups, I and II, based on the positions of cysteine residues in their sequences. Group II PLA2s exhibit amino acid sequences with five to seven residues more than their group I counterparts, featuring deletions around residue 60 corresponding to the elapid loop found in group I PLA2s. Crystal structures of various groups I and II PLA2s have been elucidated in both unbound and ligand-bound states, revealing a shared homologous core with an invariant tertiary structure.

Given the potential significance of secretory group II PLA2s as drug targets for the development of new anti-inflammatory agents, extensive studies have focused on them. Here, our emphasis shifts towards group II secretory PLA2s and their inhibition by both natural and synthetic inhibitors. This in-depth exploration underscores the dynamic landscape of PLA2s, shedding light on their structural intricacies and opening avenues for therapeutic advancements in anti-inflammatory drug development.

- **Mechanism of action**

The mechanism of action underlying the catalytic prowess of secretory phospholipase A2 (PLA2) unfolds a narrative reminiscent of serine proteinases. At its core, the reaction mechanism follows a general base-mediated attack on the sessile bond, orchestrated through the participation of a conserved water molecule acting as a nucleophile. This orchestrated dance of molecular entities marks a crucial step in the hydrolysis of glycerophospholipids, culminating in the generation of fatty acids, prominently arachidonate, and lysophospholipids.

The catalytic network involved in this process is rich in intricacies, with residues assuming pivotal roles in catalysis. To visualize this molecular ballet, an illustration highlights the residues engaged in catalysis and their intricate hydrogen bonding network. This detailed depiction offers a glimpse into the structural choreography governing PLA2's catalytic prowess.

Delving further into the realm of interactions, the examination of PLA2's interplay with substrate analogs emerges as a valuable avenue. Such analogs serve as molecular probes, providing invaluable insights into the substrate binding site's potential recognition elements. Among these investigations, the

complex formed between PLA2 and tridecanoic acid stands as a noteworthy example.

In this complex, the intricacies of interaction unfold with precision. One of the oxygen atoms from tridecanoic acid's carboxylic group establishes a hydrogen bond with the conserved water molecule, aptly designated as OW. This interaction showcases the intimate molecular conversations governing the catalytic milieu. Moreover, the second oxygen atom from the carboxylic group engages in another hydrogen bond, finding a partner in the N atom of Gly30. This orchestrated intermolecular tango exemplifies the specificity and selectivity inherent in PLA2's substrate interactions.

The hydrocarbon chain of tridecanoic acid, the protagonist in this molecular narrative, assumes a strategic placement within the hydrophobic channel. This positioning allows for a symphony of van der Waals contacts with key residues—Leu2, Leu5, Met8, and Ile9. These contacts not only underscore the intricacies of substrate recognition but also emphasize the hydrophobic nature of the interaction landscape.

As the molecular dynamics unfold within PLA2's catalytic site, the significance of these interactions extends beyond mere biochemical nuances. The revelation of PLA2's catalytic mechanism and its interactions with substrate analogs serves as a foundation for broader implications. Understanding these intricacies lays the groundwork for potential therapeutic interventions targeting PLA2 activity, especially in the context of inflammatory responses where PLA2's role is pivotal.

The journey through PLA2's catalytic mechanism and substrate interactions, while rooted in biochemical intricacies, transcends the laboratory setting. It opens doors to possibilities in drug development, offering insights that could pave the way for designing inhibitors or modulators targeting PLA2 activity. In this unfolding narrative of molecular ballet, PLA2's role in cellular processes and potential therapeutic applications takes centre stage, showcasing the profound impact that unravelling biochemical intricacies can have on advancing our understanding and manipulating biological pathways.

- **Therapeutic uses of snake venom**

Snake venoms represent a sophisticated concoction of proteins, peptides, carbohydrates, lipids, metal ions, and organic compounds, meticulously crafted by nature to serve a dual purpose—immobilizing and digesting prey. Within this venomous arsenal, about 26 groups of enzymes have been identified, with venoms typically containing enzymes from 10 of these groups. While snake venoms have long been recognized for their lethal potential, recent research unveils a multifaceted therapeutic perspective that extends beyond their predatory role.

One intriguing avenue is the exploration of snake venom's anticarcinogenic activities. Studies on Naja kaouthia (Indian monocellate cobra) and Daboia russelli russelli venoms have showcased their efficacy against carcinoma, sarcoma, and leukemia models. Sub-lethal doses of these venoms exhibit cytotoxicity on Ehrlich ascites carcinoma (EAC) cells in vivo, extending the lifespan of EAC mice. The potent cytotoxic and apoptogenic effects on human leukemic cells add a layer of complexity to the therapeutic potential hidden within snake venoms.

Moving beyond traditional venomous suspects, hydrophidae (Lapenis curtus) venom emerges as a surprising contender with antitumor activity against EAC mice in vivo and HeLa, HepG2 tumor cell lines in vitro. The venoms of Naja naja and Bungurus

caeruleus (Banded krait) exhibit cytotoxicity against EAC tumor cells in mice, coupled with a regulatory effect on superoxide dismutase activity. The exploration extends to nanomedicine, where chitosan-encapsulated Naja naja oxiana (Indian or speckled cobra) venom is envisioned as an antigen delivery potential, presenting an alternative to conventional adjuvants.

Within the realm of clinical practices, snake venom preparations have demonstrated utility in treating drug-resistant HIV in Saudi Arabia, suggesting a potential avenue for similar applications with Indian snake venoms. This evolving narrative underscores the transformative potential of venomous alchemy in the realm of medicine.

In the realm of enzymes, two predominant classes, proteases and metalloproteases, wield influence over venom characteristics. Serine proteases dominate viperid and Colubrid venoms, playing crucial roles in regulating vertebrate hemostasis. Their involvement in testing various components of the hemostatic system, including antithrombin III, fibrinogen, and blood clotting factors, underscores their significance. For example, the green tree viper (Trimeresurus gramineus) venom houses an anticoagulant enzyme inducing fibrinolysis. Similarly, Daboia russelli russelii venom showcases a thrombin-like serine protease, grambin, with potential applications in treating venous and arterial thrombosis.

Metalloproteases from Viperidae venoms contribute to lowering plasma fibrinogen and dissolving thrombus. RVV-X from Daboia russelli, a P-IV metalloproteinase, activates factor X (FX), offering diagnostic insights into pro-factor X conversion.The smallest fibrin(ogen)olytic metalloproteinase reported, Lahirin, emerges from Naja kaouthia venom, exemplifying the diversity within this enzyme class.

Phospholipase A2 (PLA2) takes center stage, catalyzing Ca2+-dependent hydrolysis of L-phospholipids. Venoms from Asiatic viperidae, such as Daboia russelli and Echis carinatus, boast two types of PLA2s with distinct activities. The therapeutic potential of PLA2s is evident in both antibacterial properties and their role in inhibiting HIV replication. Acidic PLA2s from various snake venoms, including Naja naja, exhibit cytotoxicity against EAC tumor cells, emphasizing their multifaceted roles.

L-Amino Acid Oxidase (LAAO), another venom enzyme, stands out for its multifunctional properties, ranging from edema induction to antibacterial and anti-HIV activities. Ophiophagus hannah venom-derived LAAOs exhibit promising anti-cancer properties against various cell lines. The inclusion of acetylcholinesterase (AChE) in snake venoms, differing from vertebrate AChE, adds to the complexity. While possessing a wide range of activities, snake venom AChEs, intriguingly, are non-toxic.

Phosphodiesterase and phosphatases, found in viperid and colubrid venoms, contribute to negative cardiac reactions and remain underexplored in terms of therapeutic potential. The comprehensive exploration of these enzymes reveals a rich tapestry of functionalities, offering a promising landscape for future therapeutic interventions.

In conclusion, the intricate world of snake venoms unfolds as a captivating saga, where nature's venomous alchemy transcends its predatory purpose to offer therapeutic potentials. From anticarcinogenic activities to enzyme-mediated interventions, snake venoms harbor a treasure trove of possibilities waiting to be unlocked for the benefit of human health. The ongoing research journey promises further revelations

and applications in the ever-evolving field of venom pharmacology.

- **Non-Enzymatic Proteins**

The realm of snake venoms extends beyond enzymatic players, unveiling a rich tapestry of non-enzymatic proteins that play pivotal roles in prey immobilization. This diverse array of proteins has not only deepened our understanding of venom composition but has also paved the way for the development of potent research tools, diagnostic techniques, and potential drugs. Among these non-enzymatic proteins are various categories such as 3FTXs, Kunitz-type serine protease inhibitors, sarafotoxins, cysteine-rich secretory proteins (CRISPs), disintegrins, C-type lectins, waprins, veficolins, and vespryns. These proteins contribute to the complexity and efficacy of snake venoms, showcasing the intricate nature of nature's biochemistry.

In addition to these well-characterized proteins, certain venoms also harbor non-protein toxins like KC-MMTx from Ophiophagus hannah. KC-MMTx, a potent molecule, demonstrates a range of effects, including a significant decrease in body temperature and protection against amphetamine aggregate toxicity in mice. It exhibits promise in protecting against drug-induced convulsions in mice, showcasing its potential as a versatile candidate for drug development. Furthermore, a heat-stable 7.2 kDa protein toxin (drCT-I) from Daboia russelli russelli reveals anticancer activity against EAC cells in vivo and human leukemic cells (U937, K562) in vitro, expanding the therapeutic landscape hidden within snake venoms.

The exploration of cobra venom factor (CVF) unveils its structural and functional analogy to the mammalian serum complement factor C3. CVF, by activating and depleting the mammalian immune-complement system, has found utility in studying various aspects of the complement system. Moreover, it serves as an immunosuppressant in tissue transplantation and cancer therapy. The development of a humanized chimeric form of CVF offers novel cardioprotective functions in vivo, presenting a potential therapeutic agent for complement-mediated diseases, including myocardial infarction.

Proteins with anticancer properties have also emerged from snake venoms, exemplified by Atroporin and Kaotree isolated from the venoms of Crotalus atrox and Naja kaouthia, respectively. These proteins exhibit anticancer properties against various cancer cell lines, suggesting potential applications in chemotherapy. The combination of Atroporin and Kaotree shows enhanced anticancer effects, opening avenues for therapeutic development. Additionally, trigramin from Trimeresurus gramineus venom demonstrates the inhibition of platelet aggregation, adding another layer to the potential applications of snake venom proteins.

In the realm of neurotoxins, snake venoms house a diverse array that binds to nicotinic acetylcholine receptors (nAChRs). These neurotoxins exert reversible blocking of neural transmission, leading to death by asphyxiation. They are classified into various categories, such as short neurotoxins, long neurotoxins, -neurotoxins, and other unconventional or weak neurotoxins. Noteworthy neurotoxins include vipoxine from Daboia russelii, which targets adrenergic receptors, and novel neurotoxin annalgesic from Ophiophagus hannah, offering analgesia without causing neurological or muscular deficits. Haditoxin from Ophiophagus hannah emerges as a key molecule in developing new drugs for conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, nicotine addiction, anxiety, and depression.

Cardiotoxins, capable of causing cardiac arrest, have been characterized in Naja naja venom and offer insights into cellular processes like lipid metabolism and calcium ion regulation. A unique discovery is f-cardiotoxin from Ophiophagus hannah, causing bradycardia, offering potential as a scaffold for designing highly specific and effective beta-blocking peptides with minimal side effects. Warprin from Naja nigricollis displays similarity to elafin, a proteinase inhibitor with potent antimicrobial activity, suggesting therapeutic potential.

Snake venom lectins, divided into C-type lectins (CTLs) and C-type lectin-related proteins (CLRPs), play diverse roles in hemagglutination, platelet aggregation, anticoagulation, procoagulation, and regulation of platelet activation. While these proteins have been extensively used to study platelet physiology, their therapeutic aspects in Indian snake venoms remain to be fully ascertained. Disintegrins, originating from metalloproteases, offer diagnostic potential in cardiovascular diseases and cancer. Notably, Aggrastat (tirofiban), an antiplatelet drug developed from a compound in the venom of Echis carinatus, stands as a testament to the translational potential of snake venom components.

The identification of vascular endothelial growth factor (VEGF) from Daboia russelli adds another layer to the therapeutic landscape. These VEGFs show activity surpassing native mammalian VEGFs, inducing hypotension in rats and enhancing vascular endothelial cell proliferation. Understanding VEGFs in snake venoms contributes to unraveling physiological events under normal and diseased conditions, providing valuable insights into angiogenesis.

In conclusion, the exploration of non-enzymatic proteins in snake venoms unveils a treasure trove of potential therapeutic candidates. From anticancer properties to the modulation of cardiovascular functions, these proteins showcase the intricate and multifaceted nature of snake venoms. The ongoing research in this domain promises not only a deeper understanding of venom pharmacology but also the development of innovative drugs and therapeutic strategies for a range of medical conditions. The complex interplay between these proteins and their diverse effects on biological systems opens doors to new frontiers in pharmacology and medicine.

❖ Antivenom

Antivenom, also recognized as antivenin, venom antiserum, and antivenom immunoglobulin, stands as a specific therapeutic approach for envenomation caused by venomous bites and stings. Its formulation includes antibodies designed to counteract the toxic effects of venom, and it plays a crucial role in treating venom-induced complications. The roots of antivenom date back to the late 19th century, gaining widespread usage in the 1950s. Its administration is recommended when there is a substantial toxicity risk, and the selection of a specific antivenom hinges on the involved species. Typically, antivenom is administered through injection, and its production traditionally involves the extraction of venom from the relevant animal, followed by injecting small amounts into a domestic animal to stimulate the generation of antibodies.

For over a century, the primary response to snakebites has revolved around the administration of antivenoms. These therapeutic agents function by enhancing the immune response post a snakebite incident. Immunization of donor animals, often horses or sheep, with snake venoms leads to the production of robust

antibodies that can bind to venom components. The resulting antibodies are then harvested, purified, and transformed into antivenoms. The significance of high-quality antivenoms cannot be overstated, as they can be the difference between life and death in snakebite scenarios.

Despite the potential efficacy of antivenom treatment, its substantial impact on controlling the morbidity, disability, and mortality associated with snakebites has been curtailed by various factors:

1. Poor Regulatory Frameworks: Inadequate regulatory structures for antivenoms, coupled with the absence of suitable reference standards and a dearth of expertise within national drug control laboratories, hinder the efficient oversight of these therapeutic agents.

2. Insufficient Research and Development: Limited investment in research and development contributes to the stagnation of antivenom product safety, efficacy, and clinical effectiveness. This lack of innovation impedes progress in addressing snakebite-related issues.

3. Lack of Minimum Specifications: The absence of defined minimum specifications for neutralizing overall lethality and specific toxic activities of antivenoms creates challenges in achieving consistent clinical effectiveness definitions in specific markets.

4. Traditional Belief Systems: Cultural beliefs associating snakebite envenoming with supernatural rather than health-related events may contribute to reluctance or skepticism towards accepting antivenom treatments.

5. Erosion of Confidence: A decline in confidence in antivenom products stems from inadequate training of health workers, marketing of poor-quality or ineffective products, and other factors.

6. Health System Weaknesses: Weaknesses in health systems, insufficient infrastructure, and inefficient distribution channels for antivenoms hinder their accessibility and impact.

7. Market Dynamics: A cycle of consequences, fuelled by low investment, poor quality, and specificity of some antivenoms, leads to eroded sales, decreased production, reduced profitability, elevated prices, diminished accessibility, and ultimately, the withdrawal of manufacturers from the antivenom supply sector.

These challenges have been particularly pronounced in Sub-Saharan Africa, where local antivenom manufacturing has historically fallen short of meeting the continent's needs. Multinational antivenom producers have abandoned production in this region, citing competition from inferior products and economic factors. The resulting dearth of effective antivenom products, replaced by inadequately evaluated or poor-quality alternatives, has led to a collapse in confidence among health workers. Additionally, insufficient data on snakebite incidence and challenges in accurate forward needs assessments deter mainstream pharmaceutical manufacturers from active participation in this critical area.

- **Therapeutic uses of Antivenoms Comprehensive Exploration of Diverse Applications**

Snake venom, traditionally feared for its lethality, has become a subject of intense scientific exploration for its potential therapeutic applications. Beyond the immediate treatment of snakebites, researchers have delved into the multifaceted properties of snake venom components, leading to promising breakthroughs in various medical domains. This extensive exploration encompasses fibrinogenolytic and fibrinolytic activity, cardiotonic and

antiarrhythmic effects, antineoplastic potential, muscle depolarization, hemolysis activity, antiparalytic applications, and considerations regarding the side effects of antivenom.

1) Fibrinogenolytic and Fibrinolytic Activity:

One intriguing area of investigation revolves around the impact of snake venom enzymes on fibrinogen, a crucial blood protein. Unlike conventional blood-thinning medications that convert fibrinogen to fibrin, certain snake venoms possess anticoagulant properties that directly remove fibrinogen from circulation. The pharmaceutical potential of such venoms is exemplified by the development of Aggrastat (tirobifan), an antiplatelet drug derived from the venom of the saw-scaled viper (Echis carinatus). This medication, classified as a glycoprotein IIb/IIIa inhibitor, underscores the therapeutic promise of snake venom in addressing blood-related conditions.

2) Cardiotonic and Antiarrhythmic Activity:

Exploring the cardiovascular effects of snake venom has revealed intriguing possibilities. Researchers have observed blood-thinning properties in the venom of the Malayan pit viper, suggesting its potential efficacy in treating stroke patients. Additionally, the identification of a non-protein micro-molecular toxin from the Indian cobra has uncovered antiarrhythmic properties at microgram levels. These findings open avenues for the development of medications with cardiotonic and antiarrhythmic effects, derived from specific components of snake venom.

3) Antineoplastic Activity of Snake Venom:

The diverse components of snake venom have been harnessed in the pursuit of novel cancer therapies. Early investigations by Calmette et al. explored the potential of cobra venom in cancer treatment in mice, with minute doses demonstrating analgesic effects. Further studies isolated phospholipase A2 from Bothrops leucurus venom, showcasing cytotoxic activity against melanoma cells. Noteworthy findings from Basavarjappa et al. and Rudrammaji et al. demonstrated the cytotoxic effects of Naja naja venom on Ehrlich ascites tumor cells. The combination product VRCTC-310, derived from snake venom, displayed anticancer activity in vivo, offering a glimpse into the potential of snake venom components in cancer therapy.

4)Muscle Depolarization and Haemolysis Activity:

Certain snake venom components, such as cytotoxin or cardiotoxin, have been found to induce muscle depolarization and hemolysis. These polypeptides, approximately 60-70 amino acid residues long, are prevalent in elapid snakes. Understanding these pharmacological effects can guide the development of therapeutic agents to address conditions related to muscle dysfunction.

5)Antiparalytic Activity of Snake Venom:

The paralysis-inducing properties of snake venoms, particularly those affecting neuromuscular transmission, have prompted extensive research. Neurotoxins isolated from snake venoms have played a crucial role in pharmacological and biochemical studies related to nicotinic acetylcholine receptors (nAChRs) in neurons and neuromuscular junctions. The utilization of labeled snake venom toxin has facilitated the identification of AChRs and their antibodies, leading to advancements in understanding and treating conditions like myasthenia gravis (MG). Clinical trials are underway to evaluate the effects of Notexin, a snake venom phospholipase, in the treatment of ptosis, a condition characterized by muscle weakness in the eyelid levator muscle.

- **Side Effects of Antivenom:**

While antivenom plays a crucial role in treating envenomation, it is essential to acknowledge potential side effects. Anaphylactic reactions, characterized by difficulty in breathing, reddening of the skin, swelling of eyes and face, and fever, pose risks during antivenom administration. Pyrogen reactions, likely due to high concentrations of non-immunoglobulin proteins, and inflammation of joints along with the enlargement of lymph glands are additional side effects that need consideration in the therapeutic use of antivenom.

In conclusion, the therapeutic applications of antivenoms extend beyond the immediate treatment of snakebites, offering potential solutions for diverse medical challenges. The ongoing research in this field holds promise for the development of novel pharmaceuticals derived from snake venom components, ushering in a new era in medical therapeutics.

❖ Approved Drugs from Snake Venom: A Gateway to Therapeutic Advancements

The development of new drugs stands as a formidable challenge in the pharmaceutical industry. In the pursuit of potential therapeutic agents, toxins from various sources, including plants, animals, and microorganisms, have been explored since the mid-20th century. Snake venoms, once primarily studied for understanding envenomation pathophysiology, have emerged as a remarkable reservoir of biologically active components with substantial potential for drug development. Throughout history, snake venom components have found applications in Ayurveda, homeopathy, and traditional medicine, showcasing their versatility in treating various pathophysiological conditions.

Ancient cultures, such as the Greeks and Indians, revered snakes as symbols of medicine, emphasizing their significance in healing practices. In Ayurveda, cobra venom was utilized to address joint pain, inflammation, and arthritis. The Chinese employed cobra venoms for opium addiction treatment, combining it with opium to alleviate pain. Additionally, various snake body fluids, including blood and bile duct, played crucial roles in traditional Chinese medicine. With advancements in biotechnology, the pharmaceutical industry began recognizing animal venoms, especially snake venoms, as a valuable source of potential therapeutic compounds. In recent decades, several drugs in use or undergoing clinical trials have been isolated or derived from snake venom proteins.

1) Captopril: A Landmark Achievement

In 1975, Captopril® marked a groundbreaking milestone as the first successful drug developed from a snake venom component. Discovered by Nobel Prize winner Sir John Vane and commercialized by Squibb, Captopril mimics a bradykinin-potentiating peptide found in the venom of the Brazilian arrowhead viper Bothrops jararaca. This biomimetic compound effectively treats hypertension and cardiovascular diseases by inhibiting angiotensin-converting enzyme, responsible for converting angiotensin I to angiotensin II. FDA-approved in 1981, Captopril has been pivotal in managing high blood pressure, diabetic renal disease, and heart failure post-myocardial infarction. Its success paved the way for subsequent generations of drugs, solidifying snake venoms as a significant natural pharmacopeia for drug development.

2) Eptifibatide: Unveiling Antiplatelet Potential

Eptifibatide, an FDA-approved antiplatelet drug in 1998 (EMA approval in 1999), emerged from a disintegrin (barbourin) found in the venom of Barbour's pygmy rattlesnake (Sistrurus miliarius barbourin). Barbourin uniquely binds to the αIIBβ3 integrin through a Lys–Gly–Asp (KGD) motif, offering specificity for this integrin over others. The drug's final form, a heptapeptide cyclized through a disulfide bridge, enhances resistance to proteolysis. The structural ingenuity of eptifibatide, utilizing a 'hybrid' motif of RGD and KGD, highlights the potential for synthetic chemistry to surpass the structural constraints observed in natural protein toxins.

3) Batroxobin: Harnessing Thrombin-Like Protease

Batroxobin, a thrombin-like serine protease enzyme derived from the snake venom of Bothrops atrox and Bothrops moojeni, holds notable therapeutic applications. This enzyme robustly converts fibrinogen into fibrin by releasing fibrinopeptide A. Beyond the US, batroxobin finds use in treating various disorders like stroke, pulmonary embolism, deep vein thrombosis, myocardial infarction, and perioperative bleeding. Hemocoagulase®, derived from the venom of the Brazilian snake Bothrops atrox, has been utilized in plastic surgery, abdominal surgery, and human vitrectomy. Additionally, Exanta® (Ximelagatran), extracted from cobra venom, serves as a thrombin inhibitor anticoagulant, offering benefits as a blood thinner and thrombin inhibitor.

In summary, the journey from understanding snake venoms' impact on humans to harnessing their components for drug development represents a remarkable evolution. Snake venom proteins have transitioned from ancient medicinal practices to cutting-edge pharmaceutical advancements, proving to be a rich source of bioactive molecules with substantial therapeutic potential. As research progresses, snake venoms continue to contribute significantly to the development of innovative drugs, opening new avenues in medical therapeutics.

❖ **Conclusion:**

In conclusion, the diverse components found in snake venom, such as metalloproteinases and phospholipases A2, exhibit complex structures and potent pharmacological actions. The understanding of snake venom chemistry has led to significant advancements in therapeutic applications. Metalloproteinases, particularly P-III SVMPs, contribute to haemorrhagic effects, emphasizing their role as potential drug targets. Phospholipases A2, especially secreted variants, offer insights into inflammatory processes, making them valuable targets for anti-inflammatory agents.

The pharmacological actions of these venom components extend beyond toxicity, providing opportunities for developing therapeutic interventions. Research on snake venom has unveiled structural details and functional specificities, enabling the design of inhibitors that could mitigate their harmful effects. Targeting specific venom components, such as inhibiting group II secretory PLA2s, holds promise for developing anti-inflammatory drugs.

Furthermore, snake venom research has implications for antivenom development. Understanding the interactions between venom components and their targets aids in designing more effective antivenoms tailored to neutralize specific toxicities. This knowledge contributes to improving treatment outcomes and reducing the side effects associated with antivenom administration.

In summary, the intricate chemistry and pharmacological actions of snake venom components offer avenues for therapeutic innovation, ranging from drug development to antivenom enhancement. As research continues, further insights into venom composition and function will likely uncover additional opportunities for advancing medical interventions and improving patient outcomes.

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