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Conotoxins: Structure, therapeutic potential and pharmacological applications

Rafia Mir¹, Sajjad Karim², Mohammad Amjad Kamal³, Cornelia Wilson^{4,#}

Zeenat Mirza^{3,*}

¹University of Kansas Medical Centre, Kansas City KS – 66160, USA; ²Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia; ³King Fahd Medical Research Center, King Abdulaziz University, P.O. Box: 80216, Jeddah - 21589, Kingdom of Saudi Arabia; ⁴Faculte de Medicine Limoges, Groupe de Neurobiologie Cellulaire-EA3842 Homéostasie cellulaire et pathologies, Limoges, France.

*** Corresponding Author:**

ZEENAT MIRZA, PhD

Assistant Professor

King Fahd Medical Research Center,

PO Box-80216, King Abdulaziz University

Jeddah -21589, Saudi Arabia

Phone: +966-553017824 (Mob); +966-12-6401000 ext 72074

Fax: +966-12-6952521

Email: zmirza1@kau.edu.sa; zeenat_mirza@rediffmail.com

Co-corresponding author: CORNELIA WILSON (cornelia.wilsom@unilim.fr)

ABSTRACT

Cone snails, also known as marine gastropods, from *Conus* genus produce in their venom a diverse range of small pharmacologically active structured peptides called conotoxins. The cone snail venoms are widely unexplored arsenal of toxins with therapeutic and pharmacological potential, making them a treasure trove of ligands and peptidic drug leads. Conotoxins are small disulphide bonded peptides which act as remarkable selective inhibitors and modulators of ion channels (calcium, sodium, potassium), nicotinic acetylcholine receptors, noradrenaline transporters, N-methyl-D-aspartate receptors, and neurotensin receptors. They are highly potent and specific against several neuronal targets making them valuable as research tools, drug leads and even therapeutics. In this review we discuss their gene superfamily classification, nomenclature, post-translational modification, structural framework, pharmacology and medical applications of the active conopeptides. We aim to give an overview of their structure and therapeutic potential. Understanding these aspects of conopeptides will help in designing more specific peptidic analogues.

KEYWORDS: conotoxins, classification, structure, drugs

INTRODUCTION

Marine snails inhabit tropical coral reefs, around 700 species have been recognized and all are venomous. Deeper understanding of venom biology reveals that their toxin components have inherent exquisite selectivity which can be exploited both as magnificent pharmacological tools and as templates for rational drug design [1]. *Conus* venom is extremely rich in small, bioactive structured peptide toxins (conotoxins) which presumably evolved for rapid prey capture, defense, and competitor deterrence [2]. *Conus* species contain a large number of conopeptides and each species has venom with distinct pharmacological profile. Conotoxins are cysteine-rich-peptide based toxins containing multiple disulfide bridges. Each of these toxin serves as a highly specific ligand and has an exclusive molecular target which can be an ion channel, either voltage-gated or ion gated, and in few circumstances G-protein linked receptors. Conotoxins are used in both basic science research and therapeutic, as tools of investigations and also determining how specific receptors and ion channels work.

This review focuses on conotoxin nomenclature, classification, post-translational modification, their three-dimensional structure in native form and in complexes. The therapeutic and pharmacological applications of conotoxins have also been discussed. This compilation will help us to understand, how they display their selectivity & potency and will also hasten their progression into the clinic by supporting studies focused at designing more specific potent peptidic analogues.

Nomenclature of Conopeptides

The nomenclature of conopeptides was given by Cruz et al in 1985 [3] and was further modified by Gray et al in 1988 [4]. The name of conopeptides start with the Greek alphabet that designates its pharmacological action, followed by a one- or two-letter code that indicates the *Conus* species from which the peptide is isolated, a Roman numerical specifying the cysteine arrangement of the peptide and a capital letter to signify a specific peptide variant which designates its superfamily. Conotoxin superfamilies are defined by their signal sequence in the initial pre-peptide and the disulfide bond framework. Peptides belonging to same superfamily possess a highly conserved signal sequence and share a typical pattern of cysteine residues and a specific pharmacological function [5]. For instance, μ -Conotoxin GIIIA is a peptide which belongs to mu pharmacological family, has been isolated from *Conus geographus* and has a class III cysteine pattern grouped in superfamily A. Conopeptides which are novel and have unknown molecular target use a different convention [2]. It has species code in small letter, numerical designating the cysteine pattern and again a small alphabet indicating a particular variant. For instance, r11a is a peptide isolated from *Conus radiates* and has class XI cysteine pattern.

Conopeptides which contain one or no disulfide bonds are named differently. They are named by combing a class name, followed by one- or two-letter code that designates the species. For example, conopressin-S, conotryphan-R and contulakin-G are from *Conus striatus*, *Conus radiates* and *Conus geographus* respectively.

Classification of Conopeptides

Conopeptides are toxins, present in the venoms of cone snails, which contain one or more disulfide bonds. Peptides with none or one disulfide bonds are disulfide-poor conopeptides and those having two or more disulfides are disulfide-rich conopeptides [6], also termed as conotoxins. It is suggested that a distinction of conopeptides on the basis of cysteine content and arrangement is not phylogenetically justified [7]. Conopeptides can be classified into following three categories on the basis of their signal sequence, cysteine pattern or framework and molecular target.

Superfamilies

Conopeptides are expressed as precursor protein, which are processed into mature peptides in endoplasmic reticulum and golgi apparatus. The characteristic organization of the initial precursor consists of ER signal sequence, followed by N-terminus pro-region, the mature peptide sequence and the C-terminus pro-region. During the maturation of protein precursor, the signal sequence and the N- and C-terminal pro-regions are cleaved to produce the final venom peptide. The degrees of conservation of these three regions show great variation. The signal sequence is most conserved while the pro-regions are diverse and the mature peptide being extremely variable. Based upon the similarity between the signal sequences of the precursors, conopeptides are classified into gene superfamilies, a classification system based on precursor signal peptide identity [8, 9]. Conotoxin precursor sequences are identified by targeted-cDNA sequencing and the advanced high-throughput transcriptome sequencing.

Each superfamily has a well conserved signal sequence and is designated as capital letter, at the end, in conopeptide nomenclature. About 26 conopeptide superfamilies are known till date [10, 11]. Table 1 represents different type of conotoxin superfamilies, their mature peptide cysteine framework and the number of protein precursors for each superfamily. Several conopeptides did not cluster in any of the known superfamilies. These include contulakin and conantokin, which are cysteine-poor conopeptides and belong to superfamilies B and C respectively [7].

Structural families

The presence of one or more disulfide bonds is the characteristic of conopeptides. Depending on the pattern of cysteine residues in the mature peptide region, conopeptides are grouped into structural families [10]. For example, a mature peptide can include a variable number of cysteines (4, 6, 8 or 10) and their respective position can vary. Six cysteines can be organized as CCC-C-C-C or CC-C-C-C-C or CC-C-C-CC where ‘-’ represents a variable number of amino acids. Table 2 represents different types of cysteine pattern, number of cysteines involved in each pattern and their cystine connectivities [10]. The disulfide bonding network, in addition to particular amino acids in inter-cysteine loops, categorizes them [12].

Functional or Pharmacological families

Conopeptides are classified into functional families, also known as pharmacological families, on the basis of their molecular targets and the physiological activity. They have been shown to target broad types of receptors, ion channels and transporters [13-15]. Each *Conus* species has venom with distinct pharmacological profile. There is a large array of different peptides in venoms, and each peptide has specificity for a particular receptor target. Table 3 highlights 12 pharmacological families of conopeptides, their corresponding membrane receptor families and the peptide toxins that target these receptors [10].

Sequence of Conotoxins

About 100-200 toxins are produced in the venom of each *Conus* species and there exists a remarkable hypervariability between the peptide sequences of different cone species. This sequence diversity is found both between the species as well as within the species [16-19]. Conotoxins are small peptides, mostly 10-40 amino acid residues in length. However the shortest conotoxin, conophanmus-V [20], has 8 amino acid residues and the longest conopeptide, con-ikot-ikot, is reported to have 86 amino acid residues [21]. The molecular weight of conopeptides ranges from 847 Da - 9445 Da and their isoelectric point shows variation, from very acidic to basic. They are disulfide rich peptides, which usually contain two or three disulfide bonds, providing substantial disulfide framework variation [22]. The first conopeptide to be sequenced and confirmed by chemical synthesis was α -conotoxin-GIC. It belongs to *Conus geographus* and consists of 16 amino acid residues with two disulphide bonds [4, 23].

Post-translational modification

Conopeptides show a high rate and variability of post-translational modifications (PTMs), which also accounts to an exceptional diversity exhibited by these peptides at inter and intra-species level. PTMs are the chemical or the structural changes brought in the amino acid residues of the peptide before forming the mature product. The process involves the participation of enzymes that change the nature of specific residues. The type of PTMs present in conopeptides involves disulfide-bridge formation; γ -carboxylation of glutamic acid; hydroxylation of valine at γ -position, proline at C-4, and lysine at C-5; phosphorylation and sulfation of tyrosine; bromination of tryptophan at C-6; epimerization of L- to D-residues, like that of leucine, tryptophan, valine and phenylalanine; C-terminal amidation; N-terminal O-glycosylation and pyroglutamylation of threonine or serine [24, 25].

Important and most prevalent PTMs in conopeptides are the disulfide bonds formation. The cross-linking between the cysteine side chains to form the disulfide bridges provides great stability to the three-dimensional conformation of the peptide. Conotoxins are encoded as protein precursors and the mature peptide is produced following the excision of N- and C- terminal regions of the precursor. Cleavage of C-terminal residues may result in an amidated C-terminus and similarly cleavage of N-termini of glutamine may result in formation of pyroglutamic acid amide from glutamic acid side chain and N-terminus. Besides, conotoxins also involve glycosylation which involves the attachment of carbohydrate group to the side chain of amino acid residues. They are reported to undergo O-linked glycosylation in which a carbohydrate group is attached to a hydroxyl group of serine, threonine or tyrosine amino acid residues of the peptide back bone. The first evidence for O-glycosylation in conopeptide was found in κ A-conotoxin SIVA using electrospray-mass spectrometry [26, 27] and the glycan moiety was identified to be Hex₃HexNc₂. Another example of O-linked glycosylation is observed in contulakin-G, which contains Hex-HexNAc, SO₄(HexNAc), Hex₃ and Hex₂HexNAc₂ glycoform sequences identified using combination of MALDI-TOF-MS, liquid secondary ion mass spectrometry (LSI-MS) and electrospray ionisation mass spectrometry (ESI-MS).

The details of the glycosylation pathway for conopeptides have not been outlined. But the literature suggests that O-glycosylation initiates with the transfer of D-GalpNAc from UDP- α -D-GalpNAc to a Ser or Thr residue of the peptide backbone yielding α -D-GalpNAc-(1 \rightarrow O)-Ser/Thr [28, 29]. So far, no peptide target sequences for O-glycosylation have been suggested, but the predominance of adjacent Pro and Ala residues have been correlated with the sites of O-glycosylation (NetOGlyc 3.1 Server). Although, the role of PTMs in conopeptides remain mostly unexplored. In general, the PTMs of amino acids extends the range of biological functions of the protein; for example, glycosylation helps in providing structural components, modifying physiological properties, mediating & modulating cell-adhesion and signaling [30, 31]. In case of conotoxins, the PTMs of amino acids increase the toxin potency [24, 25, 32, 33] and stabilize the three-dimensional structure of the peptide [34-36]. Thus, glycosylation may increase lifespan of peptides by stabilizing the structural conformation and slowing down its proteolytic degradation.

Structure

Till date, 134 structures of conotoxins have been deposited in Protein Data Bank. About 123 structures are determined by NMR method while only 11 structures are determined through X-ray crystallography. About 5 three dimensional crystal structures of native conotoxins are known, which belong to α - functional family. The other six crystal structures of conotoxins are in complex with their macromolecular targets. Conotoxins exhibit high structural diversity which is attributed to their large variation between the sequence of amino acid residues and cysteine framework. With few exceptions, conotoxins contain multiple disulfide bonds and the disulfide connection pattern plays an important role in defining their structure [22]. Conotoxins with similar cysteine framework can have different disulfide connectivity and may result in vastly different structures. For example, α - and χ - conotoxins share a similar framework of cysteine residues, but exhibit large structural differences due to their different disulfide connectivity. α - conotoxins are dominated by helical structure while χ -conotoxins mainly contain β -sheet secondary structure. However, conopeptides having different number of disulfide bonds and significantly different sequences may share a common structural motif. For example, the three dimensional structure of MVIIA and RXIA contain a cysteine knot motif [37]. The cysteine knot is a protein motif containing three disulfide bridges, where two disulfide

bonds with their backbone form a loop through which a third disulfide bond passes. This motif is found in 40% of known disulfide rich proteins [38] and provides structural stability to the protein. Despite having similar overall topology, the cysteine knot toxins may show some local structural and dynamic differences. For example, MVIIA and MrVIB which target different ion channels [39, 40] possess a cysteine knot motif. Both the peptide molecules have four inter-cystein loops, but for MrVIB one of the loops is disordered, contrary to MVIIA, that has well-defined loops throughout. This disparity may be responsible for the functional variation found between the two peptide molecules.

Folding

The overall fold of conotoxins is known to be significantly influenced by their disulfide connectivity [41]. The folding of conopeptides is mainly determined by their cysteine framework and the spacing between the cysteine residues, while the final folding of the peptide involves non-covalent interactions among non-cysteine residues [33]. However, depending upon the significant variation in spacing between the cysteines, a conserved fold may or may not be formed [42]. From the three dimensional structures known so far, conotoxins can broadly have four structural backbone folds [13]. For example (as shown in Figure 1), in α -conotoxins, the formation of internal disulfide bonds bring N- and C-terminal ends of the peptide into close proximity of each other and folds the molecule to form a short helical segment. In μ -conotoxins, the peptide chain folds to form secondary structure elements comprising of a helical region and a β -hair pin, which are connected by loop structures. The structures of κ - and ω -conotoxins contain a triple stranded β -sheet and loop regions. Conantokins, which lack disulfide bridges, adopt a helical conformation [43].

Three-dimensional structure of native Conotoxins

Till date, 5 crystal structures of conotoxins, in their native state, are reported in Protein Data Bank (1HJE, 1A0M, 1AKG, 1PEN & 1NOT). All the five structures, α -conotoxin SI from *Conus striatus*, α -conotoxin EpI from *Conus episcopatus*, α -conotoxin PnIB from *Conus pennaceus*, α -conotoxin PnIA from *Conus pennaceus* and α -conotoxin GI from *Conus geographus* belong to α -conotoxin family of conopeptides.

PnIA and PnIB are 16 amino acid residue peptides which block the neuronal nicotinic acetylcholine receptor (nAChR). They have a $\alpha 4/7$ cysteine framework with two disulfide bonds formed between Cys2 - Cys8 and Cys3 - Cys16. Their sequence comparison has shown that they differ at two positions, with Leu10 and Ser11 in PnIB replacing Ala10 and Asn11 of PnIA [42]. The overall structure of the two α -conotoxins are very similar to each other, showing a well superimposed backbone and similar side chains in most cases [42]. The largest differences observed in the main chain angles between the two α -conotoxins are $\sim 20^\circ$ for Pro13 and Tyr15. Their secondary structure elements consist of the α -helical region formed by two helical turns, a 3_{10} -helical turn and type I β turn towards the C-terminus of the peptide molecule. Apart from the two disulfide linkage, the molecules are stabilized by various intramolecular, intermolecular and water associated hydrogen bonds. Besides sharing similarities between sequence and structure, they have very similar shapes and surface charge distribution which is proposed to be responsible for their functional similarities as antagonists of neuronal nAChR.

The structure of α -conotoxin GI has been elucidated by Guddat et al in 1996 [44]. It consists of 13 amino acid residues with two disulfide bonds formed between Cys2 - Cys7 and Cys3 - Cys13. It targets the muscle nAChR subtype and has a consensus sequence XCC-(H/N)PACGXX(Y/F)XC. The secondary structure elements consist of a single 3_{10} helix followed by a type I β turn. The comparison of GI with PnIA & PnIB has shown significant differences in sequence, function, structure, shape and surface charge distribution. GI has $\alpha 3/5$ cysteine framework in contrast to PnIA & PnIB which have $\alpha 4/7$ sequence framework. The two inter-cysteine loops vary in length, with

three & five residues in GI and five & seven amino acid residues in PnIA & PnIB. The comparison of their shapes has shown that GI is triangular while PnIA & PnIB are rectangular. The overall net surface charge on GI is positive which is attributed to the presence of conserved lysine and arginine residues. However, in PnIA & PnIB lysine and arginine residues are absent and the only charged regions are the negatively charged Asp14 and the positively charged N-terminus of Gly1 so a net zero charge. This difference in the net electrostatic charge on α -conotoxins could play an important role in determining their specificity for neuronal and muscle nAChR [42].

The crystal structure of α -conotoxin EpI has been reported by Hu et al in 1998 [45]. The comparison of its structure with PnIA & PnIB has shown that it has a same α 4/7 cysteine framework and loop size, and is selective to neuronal nAChR. However, EpI is known to block different mammalian neuronal subtype of nAChR, which is proposed to be due to the presence of charged residues, Asp5 & Arg7 in EpI unlike Leu5 & Pro7 in PnIA & PnIB.

Three-dimensional structure of conotoxins in complexes

Conotoxins are known to target diverse types of ion channels and receptors with strong affinity [5, 6, 46, 47]. Conotoxin subgroups, α -conotoxins, specifically bind to various subtypes of nAChRs with different affinities. α -conotoxins PnIA has substantially higher affinity for $\alpha_3\beta_2$ nAChR subtype [48] compared to α_7 -nAChR. Also, α -conotoxins ImI is unique in its selectivity towards the neuronal $\alpha_3\beta_2$ and α_7 -nAChR from fresh water snail *Lymnaea stagnalis*. The structural elucidation of conotoxins bound to their target receptor will provide the details of their binding interactions, which may potentially assist in the rational design using conotoxin scaffold leading to novel variants molecules with higher specificity and selectivity to various receptor subtypes. In general receptors are large membrane bound proteins unamenable for biophysical, structural and functional studies. Nevertheless, three-dimensional crystal structures of six α -conotoxins in complex with nAChR homolog have been determined so far. Acetylcholine binding protein (AChBP) resembles most of the α_7 subtypes of nAChR family and its ligand binding site contains all the conserved residues of nAChR receptors; making it a good model for their structural studies.

The structure of α -conotoxins complexed with nAChBP revealed that the conopeptides molecules lie deep within the ligand binding site and they do not show any significant alteration in their conformation upon binding [49]. Their binding to AChBP is mainly dominated by hydrophobic interactions. In ImI complex, Arg7 and Trp10 are critical in the interaction with the ligand binding site unlike to the structure of PnIA & TxIA in complex with AChBP. The crystal structure of PnIA - AChBP complex shows that AChBP binds in an unselective manner [49] while TxIA reveals a 20° tilt in backbone due to the formation of salt bridge between Arg5 of TxIA and Asp195 of AChBP [50].

Conotoxin: pharmacological targets and applications

Venom based peptides derived from venom of snakes, amphibians, insects, arachnids, marine cones, sea anemones and others are highly efficient in curing myriad of ailments and several are presently on the road to be developed into valuable potent drugs. Owing to their potential medical applications new peptides are identified on a regular basis. However, this is just like the tip of an iceberg where only few types of venom have been studied for potential therapeutic properties, and from these venoms few selected peptides studied in detail. Undoubtedly valuable unexplored venom peptides, in addition to previously unreported venomous animals, await discovery on land and sea.

The biological effects exerted by any cone snail venom are the combined function of the physiological targets or receptors with which the individual toxin components bind. α -conotoxin peptides are important tools for discriminating between closely related nAChRs subtypes [15], whereas five among the seven drug target sites present on vertebrate voltage-gated sodium channels are defined by animal toxins [51]. Conopeptides exhibit antinociceptive, antiepileptic, neuroprotective or cardioprotective activities and thus have pharmacological applications in cancer, neuromuscular and psychiatric disorders (Figure 2). Conotoxins can be potentially used for

pain therapeutics as they target the neuronal nAChR and thus have relevance in therapeutics of neurological diseases like Alzheimer's disease, multiple sclerosis, diabetic neuropathy, shingles, and others [52, 53]. Numerous venom-based antinociceptive agents are at various stages of development for therapeutic use. AVC1, isolated from an Australian cone species, *Conus victoriae* is highly effective in combating postsurgical and neuropathic pain, even speeding recovery post nerve injury. Conantokins have been shown to have efficacy in animal models of epilepsy [54]. Few companies commercializing conopeptides are Cognetix Inc., Metabolic Pharmaceuticals, Ltd., Elan Corp., Xenome, Ltd, and others. Several academic research centers worldwide are also participating in the preclinical development efforts. Selected therapeutic conopeptides with their commercial name and status are enlisted in Table 4.

The charm of targeting snail's venom for designing novel therapeutics lies in the exactitude and alacrity with which the various component peptides act; most of them target only a particular receptor class, excluding the rest. This indicates that they can generate a specific effect on the body's metabolic pathways with no side effects reliably and quickly; like instant reduction in heart rate or switching off the signaling of a particular class of nerve, for instance pain receptors. Presently, the known receptor targets of Conus peptides are broadly divided into following three classes: (a) ligand-gated ion channels; (b) voltage-gated ion channels; and (c) others. Hereby, we briefly discuss the therapeutic applications of the above three classes.

Ligand-gated ion channels

They are transmembrane protein channels that open to allow passage of ions in response to the binding of a chemical messenger (the ligand like neurotransmitters) to the protein, inducing a conformational transform in the protein molecule. Majority of the ligand-gated ion channel superfamily inhibitors are nicotinic acetylcholine receptor antagonists, with wide structural variations. α -conotoxins were the first characterized conopeptide which act upon nicotinic acetylcholine receptors at the neuromuscular junction as competitive antagonists. Conotoxins targeting nicotinic acetylcholine receptor are of two subtypes – neuromuscular (α -conotoxin GI, α -conotoxin MI, α -conotoxin EI, α -conotoxin PIVA, α -conotoxin EIVA, α -conotoxin OIVB, α -conotoxin PeIVB and ψ -conotoxinPIIIE), and neuronal (α -conotoxin MII, α -conotoxin PIA, α -conotoxin ImII, α -conotoxin BuIA). σ -conotoxin GVIIIA target 5HT₃ receptors. In addition, conotoxins targeting glutamate receptors (NMDA subclass: conantokin-G and conantokin-L; AMPA subclass: con-ikot-ikot) are also known [55]. α -conotoxin Vc1.1 has been shown to alleviate pain associated with neuropathy and hasten recovery of injured neurons [56].

Voltage-gated ion channels

They open or close in response to changes in the electric potential across a cell membrane. δ -conotoxins and μ -conotoxin target voltage-gated sodium channels and κ M-conotoxin peptides are voltage-gated potassium channel antagonists [55]. ω -conotoxins block specific voltage-gated calcium channels and are one of the well-studied conotoxins. Conotoxins that target sodium ion channels either hinder conductance (like the μ - and μ O-conotoxins) or block channel inactivation (the δ -conotoxins). Conotoxins with differential specificity for certain sodium channel subtypes have been recently illustrated [57]. Conotoxins targeting voltage-gated potassium channels are structurally diverse: diverse groups of fish-hunting cone snails have conotoxins belonging to different families; all targeting a single subfamily of potassium channels, the Shaker (or Kvl) channels [58].

In December 2004 the US Food and Drug Administration approved Ziconotide (Prialt®), a synthetic analog of ω -conopeptide MVIIA for intrathecal targeted infusion with morphine for the treatment of severe chronic intractable pain [59]. Ziconotide is non-addictive and is isolated from the fish-hunting marine snail *Conus magus* also known as magician cone. As it can block N-type voltage-sensitive calcium channels in mammalian pain-sensing neurons and also causes tremors in mice, it is a powerful antinociceptive in circumstances where morphine is poorly or not active, rendering it as the best intrathecal analgesic drug available. Having a superb safety profile with no reported inflammation, neurotoxicity, mutagenicity, teratogenicity, or carcinogenicity, and low immunogenicity [59], it is

being used for prevention of stroke and for refractory pain in patients suffering from AIDS or cancer who are tolerant to opioids [60].

Others

Among the miscellaneous receptor targets which have been identified, the foremost are those affecting the G-protein coupled receptors (GPCRs). Some examples which can be cited are ρ -conotoxin TIA, conopressin (-G and -S), contulakin-G and conorfamide. ρ -conotoxin TIA were isolated from the fish-hunting *Conus tulipa* venom and was found to inhibit noradrenaline transporters and acted specifically on α_1 -adrenoceptors but not at all affected α_2 -adrenoceptors, calcium or sodium channels, nicotinic acetylcholine or muscarinic receptors. ρ -conotoxin TIA has general disulfide bridge arrangement and tertiary folding pattern similar to α -conotoxins [61]. Norepinephrine transporter antagonists belonging to χ -conotoxin family (χ -conotoxin MrIA) are also reported [55]. χ -conopeptides (χ -MrIA and χ -MrIB) isolated from mollusk-hunting snail *Conus marmoreus* have been reported to bind to an allosteric site on the noradrenaline transporter and amplify the height of noradrenaline-induced contractions in rat vas deferens preparation [1, 61]. Additionally snail peptides may also target catecholamine transporters [61]. Additional classes of conopeptides were subsequently found to act at vasopressin receptors (conopressin) and neurotensin (neurotensin) receptors.

FUTURE PERSPECTIVES

The pharmacological diversity of the conopeptides have been underestimated, since the majority of the studies of the previous three decades focused on species that belong to only a few lineages, and several lineages remain largely understudied or even not studied at all [62]. Presently, detailed study of conotoxins is only in its infancy, suggesting a golden future for the discovery of new conotoxins and new therapeutic applications. Novel conotoxins based drug discovery requires concerted efforts of evolutionary biologists, taxonomists, molecular geneticists, neuroscientists, pharmacologists, structure biologists and toxicologists. The high-throughput bioassays integrated with advanced MS and next-generation sequencing methods are replacing the traditional assay-guided approach.

The venom of every species of *Conus* is probably unique [47], but unfortunately, extinction threats pose danger of a potential loss of valuable vast source medical, scientific and commercial natural treasures. It was justly stated by Nobel Laureate (Peace) Eric Chivian - Center for Health and the Global Environment, Harvard Medical School in 1985 "Cone snails may contain the largest and most clinically important pharmacopoeia of any genus in nature. To lose them would be a self-destructive act of unparalleled folly." and extinction represents of source biota. With future development in peptidic drug delivery methods assisted with nanomedicine, effective oral conopeptides as drug pills can be anticipated.

List of Abbreviations

AChBP: acetylcholine binding protein

nAChR: neuronal nicotinic acetylcholine receptor

PTMs: post-translational modifications

FIGURES

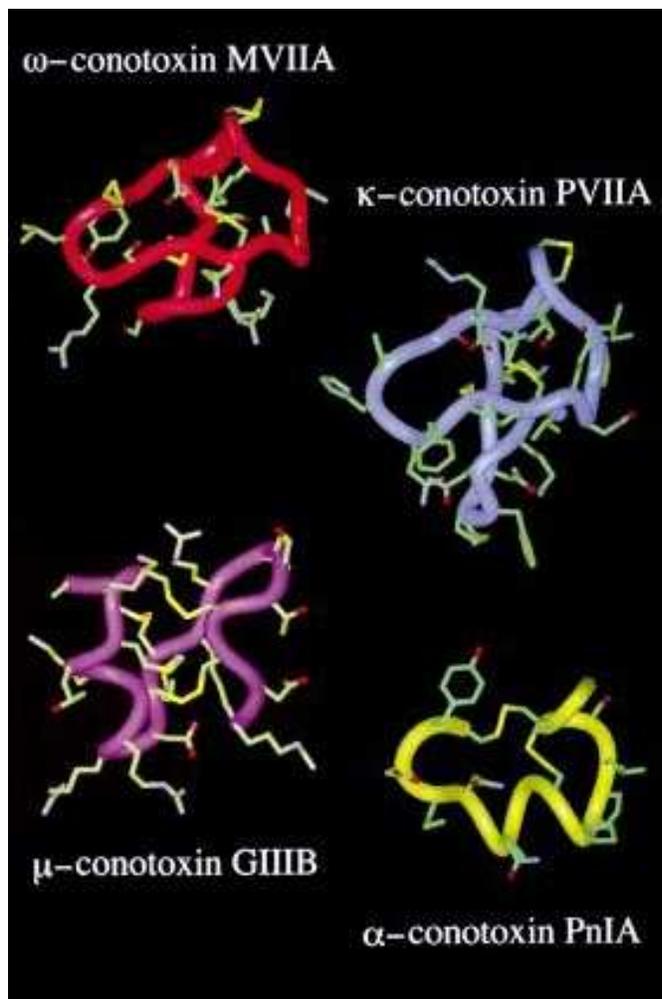


Fig 1: The three dimensional structure of Conotoxins showing four different structural backbone folds (reproduced with due permission and license from Adams et al, 1999, Drug Development Research, John Wiley and Sons)

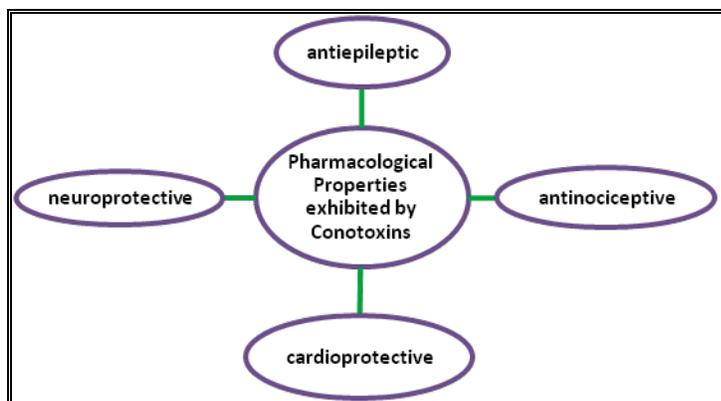


Fig 2: Main pharmacological properties of conotoxins

TABLES

Table 1. Gene Superfamilies of conotoxins.

Conotoxin Superfamily	Cysteine Framework	No. of Protein Precursors
A	I, II, IV, VI/VII, XIV, XXII	276
B1		18
B2	VIII	2
B3	XXIV	1
C		4
D	XX	28
E	XXII	1
F		2
G	XIII	1
H	VI/VII	10
I1	VI/VII, XI	26
I2	XI, XII, XIV	62
I3	VI/VII, XI	9
J	XIV	30
K	XXIII	4
L	XIV	14
M	I, II, III, IV, VI/VII, IX, XIV, XVI	443
N	XV	4
O1	I, VI/VII, IX, XII, XIV, XVI	575
O2	VI/VII, XIV, XV	133
O3	VI/VII	43
P	IX, XIV	12
S	VIII	21
T	I, V, X, XVI	234
V	XV	2
Y	XVII	1

Table 2. Cysteine framework pattern of conotoxins

Cysteine Pattern	No. of Cysteine in each Pattern	Cysteine Connectivity
CC-C-C	4	I-III, II-IV
CCC-C-C-C	6	
CC-C-C-CC	6	
CC-C-C-C-C	6	I-V, II-III, IV-VI
CC-CC	4	I-III, II-IV
C-C-CC-C-C	6	I-IV, II-V, III-VI
C-C-C-C-C-C-C-C-C-C	10	
C-C-C-C-C-C	6	I-IV, II-V, III-VI
CC-C.[PO]C	4	I-IV, II-III
C-C-CC-CC-C-C	8	I-IV, II-VI, III-VII, V-VIII
C-C-C-C-CC-C-C	8	
C-C-C-CC-C-C-C	8	
C-C-C-C	4	I-III, II-IV
C-C-CC-C-C-C-C	8	
C-C-CC	4	
C-C-C-CC-C	8	
C-C-CC-CC	6	
C-C-C-CCC-C-C-C-C	10	
C-CC-C-CC-C-C-C-C	10	
CC-C-C-C-CC-C-C-C	10	
C-C-C-C-C-C-C-C	8	
C-C-C-CC-C	6	
C-CC-C	4	
C-C-C-C-CC	6	
C-C-C-C-CC-CC	8	

Table 3: Families of conopeptides with their corresponding pharmacological target membrane receptors

Pharmacological Families	Membrane Receptor Families	Peptide Toxins
α (alpha)	Nicotinic acetylcholine receptors (nAChR)	GI
γ (gamma)	Neuronal pacemaker cation currents (inward cation current)	PnVIIA, TxVIIA
δ (delta)	Voltage-gated Na channels (agonist, delay inactivation)	TxVIA
ϵ (epsilon)	Presynaptic Ca channel or G protein-coupled presynaptic receptors	TxVA
ι (iota)	Voltage-gated Na channels (agonist, no delayed inactivation)	RXIA
κ (kappa)	Voltage-gated K channels (blocker)	PVIIA
μ (mu)	Voltage-gated Na channels (antagonist, blocker)	GIIIA
ρ (rho)	Alpha1-adrenoceptors (GPCR)	TIA
σ (sigma)	Serotonin-gated ion channels (GPCR)	GVIIIA
τ (tau)	Somatostatine receptors	CnVA
χ (chi)	Neuronal noradrenaline transporter	MrIA, CMRVIA
ω (omega)	Voltage-gated Ca channels (blocker)	GVIA

Table 4. Preclinical and Clinical Development Efforts of Conopeptides based therapeutics

Conopeptide	Commercial Name	Indication	Molecular Target / Mechanism	Clinical Stage
ω -MVIIA	Ziconotide, Prialt	Intractable pain	N-type calcium channels/blocker	Phase IV (market, FDA approved)
Contulakin-G	CGX-1160	Neuropathic pain	Neurotensin receptor/agonist	Phase Ib (SCI -sciatica patients)
ω -CVID	AM-336	Neuropathic pain	N-type calcium channels/blocker	Phase IIa (cancer patients)
Conantokin-G	CGX-1007	Intractable epilepsy	NMDA receptor/antagonist	Phase I
α -Vc1.1	ACV-1	Neuropathic pain	Nicotinic acetylcholine receptors/antagonist	Phase II
χ -MrIA	Xen2174	Neuropathic pain	Norepinephrine transporter/inhibitor	Phase IIa (cancer patients)
κ -PVIIA	CGX-1051	Acute Myocardial Infarct, Cardioprotection	K-channels/blocker	Pre-clinical
μ O-MrVIB	CGX-1002	Neuropathic pain	Sodium channels/subtype selective blocker	Pre-clinical
μ -SIIIA	PEG-SIIIA	Inflammatory pain	Sodium channels/blocker	Pre-clinical

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