Send Orders for Reprints to reprints@benthamscience.ae

582 *Current Pharmaceutical Design,* 2016, *22,* 582-589

Conotoxins: Structure, Therapeutic Potential and Pharmacological Applications

Rafia Mir[1], Sajjad Karim[2], Mohammad Amjad Kamal[3], Cornelia M. Wilson[4,*] and Zeenat Mirza[3,*]

[1]*University of Kansas Medical Centre, Kansas City KS – 66160, USA;* [2]*Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia;* [3]*King Fahd Medical Research Center, King Abdulaziz University, P.O. Box: 80216, Jeddah - 21589, Kingdom of Saudi Arabia;* [4]*University of Liverpool, Institute of Translational Medicine, Department of Molecular & Clinical Cancer Medicine, Liverpool, UK*

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 disulfide 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 therapeutics, as tools of investigations and also determining how specific receptors and ion channels work.

This review focuses on conotoxin nomenclature, classification, post-translational modification, and their three-dimensional (3D) 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 and 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- propeptide 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 combining 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 bond 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 the 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 the protein precursor, the signal sequence and the N- and C-terminal pro-regions are cleaved to produce the

*Address correspondence to these authors at the King Fahd Medical Research Center, P.O. Box-80216, King Abdulaziz University, Jeddah -21589, Saudi Arabia; Tel: +966-553017824; Mob: +966-12-6401000; Ext: 72074; Fax: +966-12-6952521; E-mails: zmirza1@kau.edu.sa; zeenat_mirza@rediffmail.com; cornelia.wilson@liverpool.ac.uk

1873-4286/16 $58.00+.00

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 advanced high-throughput transcriptome sequencing.

Each superfamily has a well-conserved signal sequence and is designated as a capital letter, at the end, in conopeptide nomenclature. About 26 conopeptide superfamilies are known to 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].

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

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 connectivity's [10-12]. The disulfide-bonding network, in addition to particular amino acids in inter-cysteine loops, categorizes them [13].

Functional or Pharmacological Families

Conopeptides are classified into functional families, also known as pharmacological families, on the basis of their molecular targets and physiological activity. They have been shown to target broad types of receptors, ion channels and transporters [14-16]. 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 [17-20]. Conotoxins are small peptides, mostly 10-40 amino acid residues in length. However the shortest conotoxin, conophanmus-V [21], has 8 amino acid residues and the longest conopeptide, conikot-ikot, is reported to have 86 amino acid residues [22]. 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 [23]. 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 disulfide bonds [4, 24].

POST-TRANSLATIONAL MODIFICATION

Conopeptides show a high rate and variability of post-translational modifications (PTMs), which also accounts for 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 [25, 26].

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

Table 2. Cysteine framework pattern of conotoxins (Ref: Kaas *et al*, 2008, 2010, 2012; http://www.conoserver.org/?page=classification&type=cysteineframeworks)

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	

'-' represents variable number of amino acid residues inbetween

the 3D 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. Proteolytic cleavage of C-terminal residues may result in an amidated C-terminus and similarly cleavage of the N-termini of glutamine may result in the formation of pyroglutamic acid amide from glutamic acid side chain at the N-terminus. Besides proteolytic cleavage, conotoxins can also be glycosylated which involves the attachment of a carbohydrate group to the side chain of amino acid residues. 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 is reported [27]. The first evidence for O-glycosylation of a conopeptide was found in κA-conotoxin SIVA

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)	Somatostatin receptors	CnVA
χ (chi)	Neuronal noradrenaline transporter	MrIA, CMRVIA
ω (omega)	Voltage-gated Ca channels (blocker)	GVIA

using electrospray-mass spectrometry [28, 29] and the glycan moiety was identified to be Hex_3HexNc_2. Another example of O-linked glycosylation is observed in contulakin-G, which contains HexHexNAc, 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-Gal*p*NAc from UDP-α-D-Gal*p*NAc to a Ser or Thr residue of the peptide backbone yielding α-D-Gal*p*NAc-(1→O)-Ser/Thr [30, 31]. 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 [32, 33]. In case of conotoxins, the PTMs of amino acids increase the toxin potency [25, 26, 34, 35] and stabilize the 3D structure of the peptide [36-38]. Thus, glycosylation may increase lifespan of peptides by stabilizing the structural conformation and slowing down its proteolytic degradation [39].

STRUCTURE

Till date, 134 structures of conotoxins have been deposited in the Protein Data Bank. One hundred and twenty three structures are

determined by NMR method while only 11 structures are determined through X-ray crystallography. Five 3D 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 that is attributed to their large variation between the sequence of amino acid residues and cysteine framework. With a few exceptions, conotoxins contain multiple disulfide bonds and the disulfide connection pattern plays an important role in defining their structure [23]. 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 3D structure of MVIIA and RXIA contain a cysteine knot motif [40]. 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 [41] 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 target different ion channels [42, 43] and possess a cysteine knot motif. Both the peptide molecules have four inter-cysteine 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.

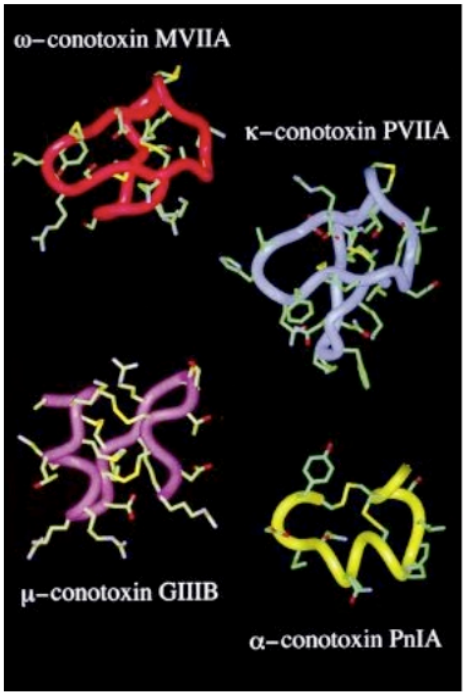

Fig. (1). The 3D structure of Conotoxins showing four different structural backbone folds (reproduced with due permission and license from Adams *et al*, 1999, Drug Development Research, John Wiley and Sons).

FOLDING

The overall fold of conotoxins is known to be significantly influenced by their disulfide connectivity [44]. 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 [35]. However, depending upon the significant variation in spacing between the cysteines, a conserved fold may or may not be formed [45]. From the 3D structures known so far, conotoxins can broadly have four structural backbone folds [14]. For example, 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 (Fig. **1**). 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 [46].

THREE-DIMENSIONAL STRUCTURE OF NATIVE CONOTOXINS

To date, 5 crystal structures of conotoxins, in their native state, are reported in the 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 that block the neuronal nicotinic acetylcholine receptor (nAChR). They have a α4/7 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 [45]. 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 [45]. The largest differences observed in the main chain angles between the two α-conotoxins are ~20° 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 [47]. 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 α3/5 cysteine framework in contrast to PnIA & PnIB which have α4/7 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 G1 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 with 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 [45].

The crystal structure of α-conotoxin EpI has been reported by Hu *et al.* in 1998 [48]. The comparison of its structure with PnIA &

PnIB has shown that it has the same α4/7 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, 49, 50]. 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 [51] 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, 3D crystal structures of six α-conotoxins in complex with nAChR homolog have been determined so far [52, 53]. 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 [54, 55].

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 [52]. 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 binding in an unselective manner [52] while TxIA reveals a 20° tilt in the backbone due to the formation of salt bridge between Arg5 of TxIA and Asp195 of AChBP [54].

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 [16], whereas five among the seven drug target sites present on vertebrate voltage-gated sodium channels are defined by animal toxins [56]. Conopeptides exhibit antinociceptive, antiepileptic, neuroprotective or cardioprotective activities and thus have pharmacological applications in cancer, neuromuscular and psychiatric disorders (Fig. **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 [57, 58]. 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 [59]. 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**.

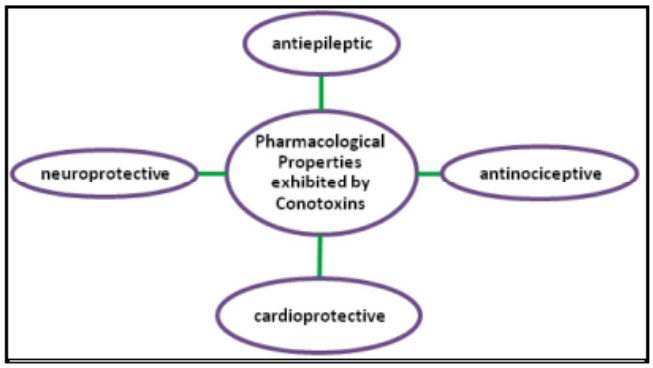

Fig. (2). Main pharmacological properties of conotoxins.

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 nil 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 the 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 change 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 conopeptides that 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 PelVB and ψ-conotoxin PIIIE), and neuronal (α-conotoxin MII, α-conotoxin PIA, α-conotoxin ImII, α-conotoxin BuIA). σ-conotoxin GVIIIA targets $5HT_3$ receptors. In addition, conotoxins targeting glutamate receptors (NMDA subclass: conantokin-G and conantokin-L; AMPA subclass: con-ikot-ikot) are also known [60]. α-conotoxin Vc1.1 has been shown to alleviate pain associated with neuropathy and hastens recovery of injured neurons [61].

Voltage-Gated Ion Channels

They can 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 κM-conotoxin peptides are voltage-gated potassium channel antagonists [60]. ω-conotoxins

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	Niconitic 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
μO-MrVIB	CGX-1002	Neuropathic pain	Sodium channels/subtype selective blocker	Pre-clinical
μ-SIIIA	PEG-SIIIA	Inflammatory pain	Sodium channels/blocker	Pre-clinical

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 μO-conotoxins) or block channel inactivation (the δ-conotoxins). Conotoxins with differential specificity for certain sodium channel subtypes have been recently illustrated [62]. 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 [63].

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 [64]. 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 anti-nociceptive 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 [64], it is being used for prevention of stroke and for refractory pain in patients suffering from AIDS or cancer who are tolerant to opioids [65].

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 [66]. Norepinephrine transporter antagonists belonging to χ-conotoxin family (χ-conotoxin MrIA) are also reported [60]. 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, 66]. Additionally snail peptides may also target catecholamine transporters [66]. 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 [67]. 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 [50], but unfortunately, extinction threats pose danger of a potential loss of valuable vast source of 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 a source of threat to 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

3D = three-dimensional

nAChR = neuronal nicotinic acetylcholine receptor

PTMs = post-translational modifications

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Harvey AL. Toxins 'R' Us: more pharmacological tools from nature's superstore. Trend Pharmacol Sci 2002; 23 (5): 201-3.
[2] Olivera BM, Cruz LJ. Conotoxins, in retrospect. Toxicon J Int Soc Toxinol 2001; 39 (1): 7-14.
[3] Cruz LJ, Gray WR, Yoshikami D, Olivera BM. Conus venoms: a rich source of neuroactive peptides. Toxin Rev 1985; 4 (2): 107-32.
[4] Gray WR, Olivera BM, Cruz LJ. Peptide toxins from venomous Conus snails. Annl Rev Biochem 1988; 57 665-700.
[5] McIntosh JM, Santos AD, Olivera BM. Conus peptides targeted to specific nicotinic acetylcholine receptor subtypes. Ann Rev Biochem 1999; 68 59-88.
[6] Terlau H, Olivera BM. Conus venoms: a rich source of novel ion channel-targeted peptides. Physiol Rev 2004; 84 (1): 41-68.
[7] Puillandre N, Koua D, Favreau P, Olivera BM, Stocklin R. Molecular phylogeny, classification and evolution of conopeptides. J Mol Evol 2012; 74 (5-6): 297-309.
[8] Olivera BM. CONUS VENOM PEPTIDES: Reflections from the Biology of Clades and Species. Ann Rev Ecol Syst 2002; 33 (1): 25-47.
[9] Woodward SR, Cruz LJ, Olivera BM, Hillyard DR. Constant and hypervariable regions in conotoxin propeptides. EMBO J 1990; 9 (4): 1015-20.
[10] Kaas Q, Westermann JC, Craik DJ. Conopeptide characterization and classifications: an analysis using ConoServer. Toxicon J Int Soc Toxinol 2010; 55 (8): 1491-509.
[11] Kaas Q, Yu R, Jin AH, Dutertre S, Craik DJ. ConoServer: updated content, knowledge, and discovery tools in the conopeptide database. Nucleic Acids Res 2012; 40 (Database issue): D325-30.
[12] Kaas Q, Westermann JC, Halai R, Wang CK, Craik DJ. ConoServer, a database for conopeptide sequences and structures. Bioinformatics (Oxford, England) 2008; 24 (3): 445-6.
[13] Balaji RA, Ohtake A, Sato K, *et al*. lambda-conotoxins, a new family of conotoxins with unique disulfide pattern and protein folding. Isolation and characterization from the venom of Conus marmoreus. J Biol Chem 2000; 275 (50): 39516-22.
[14] Adams DJ, Alewood PF, Craik DJ, Drinkwater RD, Lewis RJ. Conotoxins and their potential pharmaceutical applications. Drug Develop Res 1999; 46 (3-4): 219-34.
[15] Lewis RJ. Conotoxins: molecular and therapeutic targets. Prog Mol Subcell Biol 2009; 46: 45-65.
[16] Lewis RJ, Dutertre S, Vetter I, Christie MJ. Conus venom peptide pharmacology. Pharmacol Rev 2012; 64 (2): 259-98.
[17] Davis J, Jones A, Lewis RJ. Remarkable inter- and intra-species complexity of conotoxins revealed by LC/MS. Peptides 2009; 30 (7): 1222-7.
[18] Dutertre S, Biass D, Stocklin R, Favreau P. Dramatic intraspecimen variations within the injected venom of Conus consors: an unsuspected contribution to venom diversity. Toxicon 2010; 55 (8): 1453-62.
[19] Romeo C, Di Francesco L, Oliverio M, *et al*. Conus ventricosus venom peptides profiling by HPLC-MS: a new insight in the intraspecific variation. J Separation Sci 2008; 31 (3): 488-98.
[20] Rivera-Ortiz JA, Cano H, Mari F. Intraspecies variability and conopeptide profiling of the injected venom of Conus ermineus. Peptides 2011; 32 (2): 306-16.
[21] Pisarewicz K, Mora D, Pflueger FC, Fields GB, Mari F. Polypeptide chains containing D-gamma-hydroxyvaline. J Am Chem Soc 2005; 127 (17): 6207-15.
[22] Walker CS, Jensen S, Ellison M, *et al*. A novel Conus snail polypeptide causes excitotoxicity by blocking desensitization of AMPA receptors. Curr Biol 2009; 19 (11): 900-8.
[23] Daly NL, Craik DJ. Structural studies of conotoxins. IUBMB Life 2009; 61 (2): 144-50.
[24] McIntosh JM, Dowell C, Watkins M, *et al*. α-Conotoxin GIC from Conus geographus, a novel peptide antagonist of nicotinic acetylcholine receptors. J Biol Chem 2002; 277 (37): 33610-5.
[25] Craig AG, Bandyopadhyay P, Olivera BM. Post-translationally modified neuropeptides from Conus venoms. Eur J Biochem 1999; 264 (2): 271-5.
[26] Buczek O, Bulaj G, Olivera BM. Conotoxins and the posttranslational modification of secreted gene products. Cellular Mol Life Sci 2005; 62 (24): 3067-79.
[27] Peter-Katalinić J, Methods in Enzymology: O-Glycosylation of Proteins. In: Burlingame AL, Ed. Methods in Enzymology. USA: Academic Press 2005; Vol. 405: pp. 139-71.
[28] Craig AG, Zafaralla G, Cruz LJ, *et al*. An O-glycosylated neuroexcitatory conus peptide. Biochemistry 1998; 37 (46): 16019-25.
[29] Craig AG, Norberg T, Griffin D, *et al*. Contulakin-G, an O-glycosylated invertebrate neurotensin. J Biol Chem 1999; 274 (20): 13752-9.
[30] Brockhausen I SH, Stanley P. O-GalNAc Glycans. Chapter 9. Cold Spring Harbor New York, USA: Cold Spring Harbor Laboratory Press 2009.
[31] Brockhausen I. Pathways of O-glycan biosynthesis in cancer cells. Biochim Biophys Acta 1999; 1473 (1): 67-95.
[32] Varki A LJ, Chapter 6, Biological Roles of Glycans. New York, USA: Cold Spring Harbor 2009.
[33] Taylor MED, K. , Introduction to Glycobiology, 3rd ed. Oxford, UK: Oxford University Press 2011.
[34] Craig AG. The characterization of conotoxins. Toxin Rev 2000; 19 (1): 53-93.
[35] Bulaj G, Olivera BM. Folding of conotoxins: formation of the native disulfide bridges during chemical synthesis and biosynthesis of Conus peptides. Antioxidants Redox Signal 2008; 10 (1): 141-55.
[36] Kindahl L, Sandström C, Craig AG, Norberg T, Kenne L. 1H NMR studies on the solution conformation of contulakin-G and analogues. Canadian J Chem 2002; 80 (8): 1022-31.
[37] Kang J, Low W, Norberg T, *et al*. Total chemical synthesis and NMR characterization of the glycopeptide tx5a, a heavily post-translationally modified conotoxin, reveals that the glycan structure is α-d-Gal-(1→3)-α-d-GalNAc. Eur J Biochem 2004; 271 (23-24): 4939-49.
[38] Marx UC, Daly NL, Craik DJ. NMR of conotoxins: structural features and an analysis of chemical shifts of post-translationally modified amino acids. Magnet Reson Chem 2006; 44: S41-50.
[39] Gerwig G, Hocking H, Stöcklin R, Kamerling J, Boelens R. Glycosylation of Conotoxins. Marine Drug 2013; 11 (3): 623.
[40] Craik DJ, Daly NL, Waine C. The cystine knot motif in toxins and implications for drug design. Toxicon 2001; 39 (1): 43-60.
[41] Cheek S, Krishna SS, Grishin NV. Structural classification of small, disulfide-rich protein domains. J Mol Biol 2006; 359 (1): 215-37.
[42] Olivera BM, Cruz LJ, de Santos V, *et al*. Neuronal calcium channel antagonists. Discrimination between calcium channel subtypes using omega-conotoxin from Conus magus venom. Biochemistry 1987; 26 (8): 2086-90.
[43] McIntosh JM, Hasson A, Spira ME, *et al*. A new family of conotoxins that blocks voltage-gated sodium channels. J Biol Chem 1995; 270 (28): 16796-802.
[44] Gehrmann J, Alewood PF, Craik DJ. Structure determination of the three disulfide bond isomers of alpha-conotoxin GI: a model for the role of disulfide bonds in structural stability. J Mol Biol 1998; 278 (2): 401-15.
[45] Hu SH, Gehrmann J, Alewood PF, Craik DJ, Martin JL. Crystal structure at 1.1 A resolution of alpha-conotoxin PnIB: comparison with alpha-conotoxins PnIA and GI. Biochemistry 1997; 36 (38): 11323-30.
[46] Skjaerbaek N, Nielsen KJ, Lewis RJ, Alewood P, Craik DJ. Determination of the solution structures of conantokin-G and conantokin-T by CD and NMR spectroscopy. J Bio Chem 1997; 272 (4): 2291-9.
[47] Guddat LW, Martin JA, Shan L, Edmundson AB, Gray WR. Three-dimensional structure of the alpha-conotoxin GI at 1.2 A resolution. Biochemistry 1996; 35 (35): 11329-35.
[48] Hu SH, Loughnan M, Miller R, *et al*. The 1.1 A resolution crystal structure of [Tyr15]EpI, a novel alpha-conotoxin from Conus episcopatus, solved by direct methods. Biochemistry 1998; 37 (33): 11425-33.
[49] Lewis RJ, Garcia ML. Therapeutic potential of venom peptides. Nature reviews. Drug Discov 2003; 2 (10): 790-802.
[50] Olivera BM, Cruz LJ, Yoshikami D. Effects of Conus peptides on the behavior of mice. Curr Opin Neurobiol 1999; 9 (6): 772-7.

[51] Tsetlin VI, Hucho F. Snake and snail toxins acting on nicotinic acetylcholine receptors: fundamental aspects and medical applications. FEBS Lett 2004; 557 (1-3): 9-13.

[52] Celie PH, Kasheverov IE, Mordvintsev DY, *et al*. Crystal structure of nicotinic acetylcholine receptor homolog AChBP in complex with an alpha-conotoxin PnIA variant. Nat Struct Mol Biol 2005; 12 (7): 582-8.

[53] Ulens C, Hogg RC, Celie PH, *et al*. Structural determinants of selective α-conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP. Proc Natl Acad Sci USA 2006; 103 (10): 3615-20.

[54] Dutertre S, Ulens C, Buttner R, *et al*. AChBP-targeted alpha-conotoxin correlates distinct binding orientations with nAChR subtype selectivity. EMBO J 2007; 26 (16): 3858-67.

[55] Azam L, McIntosh JM. Alpha-conotoxins as pharmacological probes of nicotinic acetylcholine receptors. Acta Pharmacol Sin 2009; 30 (6): 771-783.

[56] Klint JK, Senff S, Rupasinghe DB, *et al*. Spider-venom peptides that target voltage-gated sodium channels: pharmacological tools and potential therapeutic leads. Toxicon 2012; 60 (4): 478-91.

[57] Livett BG, Gayler KR, Khalil Z. Drugs from the sea: conopeptides as potential therapeutics. Curr Med Chem 2004; 11 (13): 1715-23.

[58] Buckingham SD, Jones AK, Brown LA, Sattelle DB. Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. Pharmacol Rev 2009; 61 (1): 39-61.

[59] White HS, McCabe RT, Armstrong H, *et al*. *In vitro* and *in vivo* characterization of conantokin-R, a selective NMDA receptor antagonist isolated from the venom of the fish-hunting snail Conus radiatus. J Pharmacol exp ther 2000; 292 (1): 425-32.

[60] Olivera BM, Imperial JS, Concepcion GP, - Snail Peptides. Chapter 61. In: Kastin AJ, Ed. Handbook of Biologically Active Peptides (Second Edition), Boston: Academic Press 2013; pp 437-50.

[61] Satkunanathan N, Livett B, Gayler K, *et al*. Alpha-conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurones. Brain Res 2005; 1059 (2): 149-58.

[62] Wilson MJ, Yoshikami D, Azam L, *et al*. mu-Conotoxins that differentially block sodium channels NaV1.1 through 1.8 identify those responsible for action potentials in sciatic nerve. Proc Natl Acad Sci USA 2011; 108 (25): 10302-7.

[63] Imperial JS, Silverton N, Olivera BM, *et al*. Using chemistry to reconstruct evolution: on the origins of fish-hunting in venomous cone snails. Proc Am Philos Soc 2007; 151 (2): 185-200.

[64] Kress HG, Simpson KH, Marchettini P, Ver Donck A, Varrassi G. Intrathecal therapy: what has changed with the introduction of ziconotide. Pain Pract 2009; 9 (5): 338-47.

[65] Staats PS, Yearwood T, Charapata SG, *et al*. Intrathecal ziconotide in the treatment of refractory pain in patients with cancer or AIDS: a randomized controlled trial. JAMA 2004; 291 (1): 63-70.

[66] Sharpe IA, Gehrmann J, Loughnan ML, *et al*. Two new classes of conopeptides inhibit the alpha1-adrenoceptor and noradrenaline transporter. Nat Neurosci 2001; 4 (9): 902-7.

[67] Puillandre N, Bouchet P, Duda TF, Jr., *et al*. Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea). Mol phylogenet Evol 2014; 78 290-303.

Received: October 10, 2015 **Accepted: November 24, 2015**