Drugs From the Sea: Conotoxins as Drug Leads for Neuropathic Pain and Other Neurological Conditions

D. Alonso[*,a], Z. Khalil[b], N. Satkunanathan[b] and B.G. Livett[c]

[a]*NeuroPharma, S.A.; Avda. de la Industria 52, 28760 Tres Cantos, Madrid Spain*

[b]*National Aging Research Institute and,* [c]*Department of Biochemistry and Molecular Biology, University of Melbourne, Victoria 3010, Australia*

Abstract: The oceans are a source of a large group of structurally unique natural products that are mainly found in invertebrates such as sponges, tunicates, bryozoans, and molluscs. It is interesting to note that the majority of marine compounds currently in clinical trials or under preclinical evaluation are produced by these species rather than as secondary metabolites from algae [1]. Through the combined efforts of marine natural products chemists and pharmacologists a number of promising compounds have been identified that are either already at advanced stages of clinical trials such as the new anti-cancer drug marine alkaloid ecteinascidin 743 [2], or have been selected as promising candidates for extended preclinical evaluation [3]. This is the case for conotoxins, (Table 1) where a number of conopeptides are currently being developed as analgesics for the treatment of neuropathic pain.

CONOTOXINS

Conotoxins are obtained from the venom ducts of predatory snails of the genus *Conus* [4, 5], found mainly in tropical waters. It has been estimated that the venom of each *Conus* species has between 50 and 200 peptide components. These highly constrained sulphur rich components or conotoxins [6-8] represent a unique arsenal of neuropharmacologically active peptides [9, 10] that can also be used as research tools to target voltage-gated and ligand-gated ion channels [11].

This complex group of peptides, with over two thousand members known, can be classified into six structurally different classes. Individual members can be highly specific for receptor subtypes of the target molecule. The toxicity of the venom derives not so much from the considerable toxicity of a limited number of peptides as from the additive or synergistic effects of several toxins acting at different sites.

Depending on the site of action we can classify **Conotoxins** into:

α-CONOTOXINS

Alpha Conotoxins [12-16] were the first toxins isolated from *Conus* venom and were designated *alpha* because they had the same action as the *alpha* neurotoxins from snake venoms (e. g. *alpha* bungarotoxin) which inhibit the muscle-type nicotinic receptor. The alpha-conotoxins GI, GIA, and GII (from *Conus geographus*) and MI (from *Conus magus*) are homologous peptides of 13 and 15 amino acids respectively. Each contains two disulphide bridges in 3:5 loop configuration and has a highly basic region of primary sequence. These alpha conotoxins cause

postsynaptic inhibition at the neuromuscular junction resulting in paralysis and death. The symptoms resemble those of curare poisoning with the eventual respiratory failure. The mechanism by which paralysis is brought about is thought to involve alpha conotoxins binding to the alpha subunit of the nicotinic acetylcholine ligand-gated ion channel and blocking the binding of acetylcholine and of agonists such as nicotine. By preventing the agonist-induced conformational change in the receptor ion channel required for the influx of sodium essential for membrane depolarisation, the alpha conotoxins inhibit neurotransmitter action and induce paralysis. Another goup of alpha-conotoxins have their two disulphide bridges linked in 4:7 loop configuration. These "neuronal-type" α-conotoxins do not target nicotinic receptors at the neuromuscular junction and do not cause paralysis. Instead they target neuronal-type nicotinic receptors in the brain and on peripheral sensory neurons. In figure **1** we can see the structure of α-conotoxin Vc1.1 [17], having 16 amino acids with a C-terminus amide and two disulfide bonds in 4:7 linkage; this is the first α-conotoxin being developed as a potential analgesic for the treatment of a range of painful neuropathic conditions in humans.
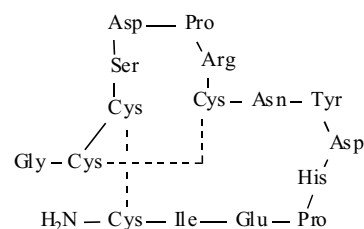

Fig. (1). Structure of Vc1.1.

ω-CONOTOXINS

ω-conotoxins [18, 19] are peptides with 24-30 amino acids and three disulfide bonds. The best defined are omega-conotoxin GVIA from *Conus geographus* and omega conotoxins MVIIA, MVIIC and MVIID from *Conus magus*

*Address correspondence to this author at the NeuroPharma, S.A.; Avda. de la Industria 52, 28760 Tres Cantos, Madrid, Spain; E-mail: dalonso@neuropharma.es
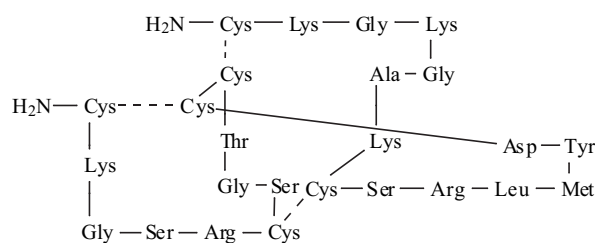
Fig. (2). Structure of the ω-conotoxin, Ziconotide (or Prialt[TM]) .

venom. They are known as 'shaker peptides' as they induce persistent tremors in mice when injected intracerebrally. GVIA , GVIB and GVIC all block the neuromuscular junction of skeletal muscle, and act by blocking calcium channels without interfering with cellular action potentials. Differential effects are observed upon the application of GVIA. It appears that of the three types of calcium channel (L, N and P), the toxin will block L and N type on neurons, but only has effect on the N type in muscle. This channel subtype specific response may be useful in the identification and manipulation of calcium channels. In figure **2** we can see the structure of Ziconotide [20] (now termed Prialt[TM] a 25 amino acid) ω-conotoxin, with three disulfide bonds. It has successfully completed phase III clinical trials for two therapeutic applications: to alleviate pain associated with malignant disease and as an analgesic for non-malignant neuropathic pain.

μ-CONOTOXINS

μ-Conotoxins [21], GIIIA , GIIIB and GIIIC, are hydroxyproline-rich basic peptides of 22 amino acids. The peptides contain 3 hydroxyprolines and 6 cysteines. The presence of several Lys and Arg residues confers a high positive charge of +6 on GIIIA and +7 on GIIIB and GIIIC. The μ-conotoxins act upon sodium channels in muscle, and also, to a very limited extent, in neurons, where they bind to a site designated 'binding site one' at the mouth of the sodium channel, and thereby inhibit the influx of sodium into the cell which renders the organism paralysed.

δ-CONOTOXINS

δ-Conotoxins [22] are one of the most intriguing families of peptides derived from snail-hunting cones. They target Na$^+$ channels but do not compete with the well-known agents such as tetrodotoxin and μ-conotoxins. Chemically they are very unusual, with a core of disulfides that leaves no room for the usual burying of hydrophobic amino acids in the interior, but are instead forced into the surrounding

Table 1.   Conopeptides Being Developed for Treatment of Neuropathic Pain Conditions

Conopeptide	*Conus* Species (*)	Target	Stage	Company **	Other name(s)	Comment	Reference
α-Conotoxin Vc1.1	*Conus victoriae (m)*	Competitive blocker of selected *neuronal-type* nicotinic ACh receptors	Preclinical	Metabolic Pharmaceuticals, Melbourne, Australia	*ACV1*	Effective against peripheral neuropathic pain in animal models and accelerates functional recovery of injured neurons	[16, 29]
rho- Conotoxin TIA	*Conus tulipa (p)*	Acts as reversible noncompetitive inhibitor of α-1 adrenergic receptors	Preclinical	Xenome, Ltd., Brisbane, Qld., Australia		Acts as a reversible noncompetitive inhibitor of alpha-1 adrenergic receptors	[30])
ω-conotoxin CVID	*Conus catus (p)*	Blocks N-type calcium channel specific sub-type.	Stage II	AMRAD Operations under licence from Univ. of Queensland	AM336	Reported to have a better therapeutic index than Prialt[TM]	[31]
ω-conotoxin MVIIA	*C. magus (p)*	N-type calcium channels	Stage III	Elan Corporation (Elan Pharmaceuticals), CA, USA	SNX-III, C1002, Ziconotide, Prialt[TM]	Significant pain relief to patients in clinical trials. Side effects in some patients. Hence call for repeat of Stage III clinical trials for cancer pain.	[32-35]
χ-conopeptides (χ-CTX MrIA/B)	*Conus marmoreus (m)*	Acts as reversible noncompetitive inhibitor of the neuronal noradrenaline transporter	Preclinical	Xenome, Ltd., Brisbane, Qld., Australia		Being developed to "treat certain types of pain, for which there is currently a lack of effective treatment" neuropathic pain.	[30]
Contulakin-G	*Conus geographus (p)*	Binds to neurotensin receptor	Stage II	Cognetix Inc, Salt Lake City, USA	CGX-1160	Short term management of post-operative pain	[36]
Conantokin-G	*Conus geographus (p)*	Selective inhibitor of the NMDA receptor (NR2B subtype)	Stage II	Cognetix Inc, Salt Lake City, USA	CGX-1007	Potent antinociceptive effects in several models of injury-induced pain. Also, control of seizures in intractable epilepsy	[36]
Conantokin-T	*Conus tulipa (p)*	Selective inhibitor of the NMDA receptor (NR2A and NR2B) subtypes	Stage II	Cognetix Inc, Salt Lake City, USA	CGX-100	Potent antinociceptive effects in several models of injury-induced pain	([36]

* Prey preference for *Conus* species: *p = piscivorous* (fish-hunting); *m = molluscivorous* (mollusk hunting); *v = vermivorous* (worm-hunting)

** Websites of commercial developers: Elan Corporation http://www.elan.com ; Cognetix Inc. http://www.cognetix.com ; Xenome Pty Ltd: http://www.xenome.com ; Amrad Operations http://www.amrad.com.au ; Univ. of Melbourne: Cone Shell and Conotoxin HomePage http://grimwade.biochem.unimelb.edu.au/cone ; Metabolic Pharmaceuticals, http://www.metabolic.com.au

solvent. It is suspected that the peptides may dissolve in the lipid membrane and interfere with opening and closing of Na^+ channels by binding laterally from within the lipid membrane, rather than from the aqueous environment.

κ-CONOTOXINS

κ-Conotoxin, PVIIA (κ-PVIIA), a 27-amino acid toxin from *Conus purpurascens* venom inhibits the Shaker potassium channel [23,24], and has been chemically synthesized in a biologically active form. The disulfide connectivity of the peptide was determined. Kappa-Conotoxin PVIIA is the first *Conus* peptide identified to target K^+ channels.

CONANTOKINS

The conantokins [25-27], target the major excitatory receptors in the vertebrate central nervous system, the glutamate receptors. The conantokins [28] selectively inhibit a subtype of glutamate receptor, the NMDA (N-methyl-D-aspartate) receptors, which are ligand-gated Ca^{2+} channels involved in seizures in intractable epilepsy. Unlike the conotoxins, the conantokins have no disulphide bonds but derive their structural stability from post-translationally modified glutamic residues present as γ-carboxy glutamate (Gla).

CONTULAKINS, CONTRYPHANS AND OTHER CONOPEPTIDES

A number of other conopeptides are being developed as pro-drugs for the treatment of a range of neurological conditions (see Table 1). These include contulakin-G a glycosylated 15 amino acid conopeptide from the venom of *Conus geographus* [36] that is being developed for short-term management of post-operative pain; *rho*-conotoxin TIA, a non-competitive inhibitor of α-1 adrenergic receptors [30]; *chi*-conopeptides (Mr IA/B) from *Conus marmoreus*, that act as reversible non-competitive inhibitors of the neuronal noradrenaline transporter, being developed for treatment of neuropathic pain [30]; and the contryphans from *Conus ventricosus* and *Conus regius* [see 37 for review], the smallest bioactive conopeptides, 8 or 9 amino acids and just one disulphide bond, which are distinguished by their numerous post-translational modifications (including proline hydroxylation, C-terminus amidation, leucine and tryptophan isomerization and tryptophan bromination). Although the disease target for the contryphans is not yet known the peptides have been shown to elicit body tremor and mucous secretion when injected into fish, suggesting they may have biological activity related to endocrine or neuronal function.

CONCLUSION

In conclusion, the conotoxins are the large family of peptides that have received increasing attention because of their highly specific actions on key elements of excitable membranes and chemical synapses. Nowadays, they represent invaluable pharmacological tools for revealing the complexity of voltage-gated and ligand-gated ion channels and in the near future will provide a new armory of neurologically active drugs. Some of these new drug candidates have entered clinical trials, several of them targeted to the treatment of intractable neuropathic pain.

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