Drugs of the Future 2011, 36(1): 25-32

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.1.1561066

REVIEW ARTICLE

CONOPEPTIDES AS NOVEL OPTIONS FOR PAIN MANAGEMENT

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SUMMARY

Conopeptides are the peptidic components of the venoms of marine cone snails from the Conus genus. Aside from their natural function in pharmacologically immobilizing prey, they have attracted the attention of drug designers in recent years because of their potency and selectivity at a range of pharmaceutically important targets in mammals, including ion channels and neurotransmitter transporters. One conopeptide, MVIIA (ziconotide, Prialt®), is on the market for the treatment of intractable neuropathic pain and others are in preclinical or clinical development. This article provides an overview of recent developments in the conopeptide field, with particular reference to their potential applications as drugs for the treatment of pain.

INTRODUCTION

Conopeptides are bioactive peptides found in the venoms of marine cone snails of the genus *Conus*. These carnivorous snails, which comprise more than 500 species, prey on marine worms, snails or fish. Although their primary purpose is to assist in prey capture, these 10- to 40-amino-acid conopeptides serendipitously interact with a number of pharmaceutically relevant receptors in mammals and thus have attracted great attention as potential leads in drug design and development (1-4). Conopeptides have been extensively reviewed from the perspective of their discovery (5), evolution (6), structures (7), pharmacology (8), structure–activity relationships (9-12) and applications (13, 14), but our focus here is exclusively on the applications of conopeptides as drugs, and in particular on their potential use for the treatment of pain.

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"Conopeptide" is an all-encompassing term that includes the majority of the peptidic components contained in cone snail venoms. The term conotoxin has been more specifically used to refer to those conopeptides that are disulfide-rich (6). Figure 1 outlines a generic classification scheme for the various types of conotoxins/conopeptides, which are divided into pharmacological families (denoted using Greek characters) and superfamilies. Conotoxins are systematically named starting with a letter (or two letters in the case of redundancies) based on their species name (e.g., M for *Conus magus*, Mr for *Conus marmoreous*), followed by a Roman numeral denoting their Cys framework (which is defined by the number and spacing of Cys residues) and the letters A, B, C, etc., based on the order of discovery of the particular peptide with a given framework from a given species (15). For example, ω-conotoxin MVIIA is the first peptide discovered from *C. magus* with Cys framework VII. Some researchers no longer prefer to use the term conotoxin, so as to remove the connotation "toxin", as now much of the interest in these peptides is on their therapeutic uses, but for historical reasons the term conotoxin is still widely used. In this article we will use the term conopeptide to include both disulfide-rich and disulfide-poor peptidic venom components. Recently, a database (Conserver, http://www.conosoerver.org) has been developed to catalogue and curate conopeptide sequences, and readers are referred there for a more comprehensive listing of conopeptide sequences and further information on nomenclature and classification schemes (15, 16).

Table I lists a selection of conopeptides that are, or have been, in clinical or preclinical trials for the treatment of pain (17-24). Given their peptidic nature and pain targets, it is not surprising that the majority of delivery routes involve intrathecal administration (i.e., direct infusion into the spinal cord). Only one conopeptide, MVIIA, is on the market so far (generic name ziconotide and trade name Prialt®) and is approved for the treatment of severe neuropathic pain. Several other conopeptides show promising activities in clinical or preclinical trials and in the remainder of this article we provide a brief overview of progress in the development of these various conopeptides.

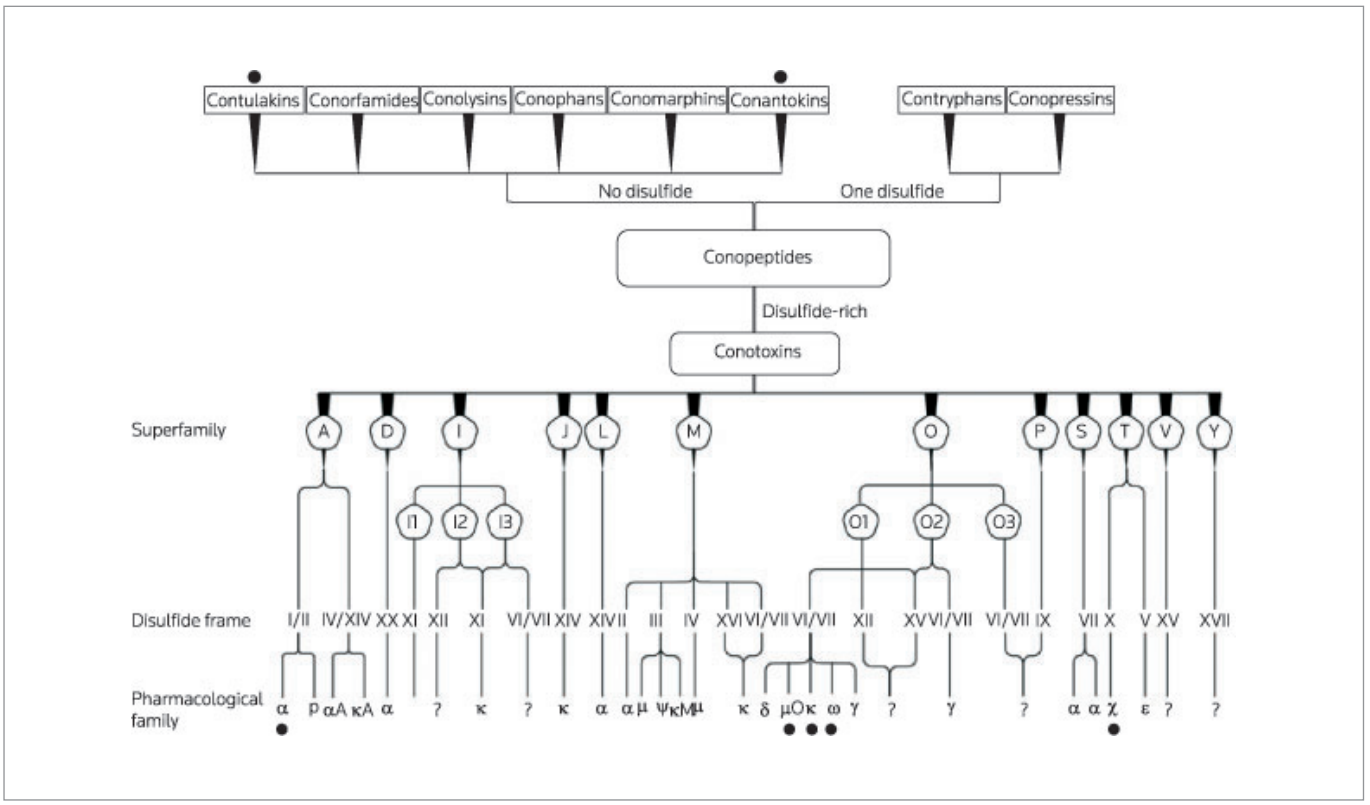

Figure 1. Overview of conopeptide classification. In general, conopeptides are divided into disulfide-rich (lower panel) and disulfide-poor (upper panel). The latter contain either none or one disulfide bond. Conantokins are drawn on the boundary between these two categories, as some members of the family contain one and others contain no disulfide bonds. Black circles denote families that have been implicated in therapeutic uses. Disulfide-rich peptides are divided into superfamilies (A-Y), which are in some case divided into subgroups, e.g., the I superfamily is divided into I1, I2 or I3, and the O superfamily is divided into O1, O2 or O3. Disulfide frameworks refer to the spacing and arrangement of the cysteine residues within the peptide sequence and are numbered using Roman numerals. Pharmacological families refer to the biological activity of the conotoxin and are denoted with Greek letters. For example, the ω-conotoxins target calcium channels, the κ-conotoxins target potassium channels and the α-conotoxins target nicotinic acetylcholine receptors. As is apparent from the figure, there is some crossover between pharmacological families, frameworks and superfamilies.

Table I. *Conotoxins in the drug development pipeline.*

Name	FDA status	Delivery	Comment	Ref.
MVIIA (SNX-111/ziconotide/Prialt®)	Approved	I.T.	FDA approved in 2004; 2009 sales of US $20 million Developed by Neurex/Elan; currently sold by Azur Pharma	17
CVID (AM-336) (Leconotide/CNSB-004)	Abandoned	I.T.	Phase II I.T. studies abandoned by Amrad; rights subsequently taken up by other companies (see below)	71
	Phase I	S.A.	Taken up by CNSBio (now Relevare Pharmaceuticals); placebo-controlled phase IIa systemically administered clinical trial in cancer patients for treatment of intractable pain planned (www.relevare.com)	18
MrVIB	Preclinical	I.T.	Reduces hyperalgesia in animal models	22, 23
MrIA derivative (XEN-2174)	Phase II	I.T.	Currently in phase II clinical trials for cancer pain and postoperative pain; development by Xenome, Ltd. (www.xenome.com).	19
Vc1.1 (ACV-1)	Abandoned	I.M.	Metabolic Pharmaceuticals (www.metabolic.com.au)	20
cVc1.1 (IMB-007)	Preclinical	Oral	The University of Queensland	21
Contulakin-G (CGX-1160)	Phase I	I.T.	Cognetix, Inc.; current status unknown	24

I.T., intrathecal; I.M., intramuscular; S.A., systemic administration.

Table II. *Structural and pharmacological data on pharmaceutically interesting conotoxins.*

Name	Sequence	Size (aa)	S-S bonds	Pharmacological target	Ref.
MVIIA	CKGKGAKCSRLMYDCCTGSCRSGKC-NH_2	25	3	N-type Ca_v channel	25
CVID	CKSKGAKCSKLMYDCCSGSCSGTVGRC-NH_2	27	3	N-type Ca_v channel	9
MrVIB	ACSKKWEYCIVPILGFVYCCPGLICGPFVCV	31	3	Sodium channels	26
XEN-2174	ZGVCCGYKLCHOC	13	2	Neuronal norepinephrine transporter	19
Vc1.1	GCCSDPRCNYDHPEIC-NH_2	16	2	nAChR/$GABA_B$	20
cVc1.1	cyclo-GCCSDPRCNYDHPEICGGAAGG	22	2		20
Contulakin-G	ZSEEGGSNA(gTr)KKPYIL	16	0	Neurotensin receptor	27
Conantokin-G	GEγγLQγNQγLIRγKSN-NH_2	17	0	NMDA receptors	28
Conantokin-T	GEγγYQKMLγNLRγAEVKKNA-NH_2	21	0	NMDA receptors	29

Z, pyroglutamate; γ, γ-carboxyglutamic acid; gTr, β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→)-threonine; O, 4-*trans*-hydroxyproline; nAChR, nicotinic acetylcholine receptor.

Table II summarizes the sequences of a selection of conopeptides with potential for pain therapy (9, 19, 20, 25-29) and Figure 2 shows selected structures of these peptides. The diversity of sequences and structures apparent from these representations demonstrates one of the exciting strengths of the conopeptide field, i.e., the powerful and diverse armory of peptidic molecules available to drug developers and the variety of targets that they can potentially interact with.

MVIIA

MVIIA is a 25-amino-acid peptide originally isolated (25) from the venom of the cone snail *C. magus* (or Magician's cone). Its structure has been determined by several groups (30-32) and comprises a compact fold with three disulfide bonds in a cystine knot arrangement (33, 34), as shown in Figure 2. Cystine knot structures are typically very stable, and this is the case with MVIIA. Pharmacologically, it is a member of the ω-conotoxin family that targets N-type voltage-gated calcium channels. The history and development of this molecule have been reviewed extensively (17), and, as ziconotide, it has been on the market since 2004 in the U.S. and since 2005 in the E.U. Ziconotide is a synthetic peptide identical in sequence to the native MVIIA conopeptide sequence and in clinical use is delivered intrathecally via a surgically implanted pump.

The efficacy of ziconotide in providing pain relief has been demonstrated in at least four clinical trials in cancer and AIDS patients, and in these trials ziconotide was associated with statistically significant pain relief, as measured by the percent reduction in Visual Analogue Scale of Pain Intensity scores. It has been recommended as a viable alternative therapy for patients with severe, refractory pain who cannot tolerate i.v. administration of morphine or hydromorphine (35-37). The name Prialt® is in fact a condensation of the expression "***Pri***mary ***alt***ernative therapy for morphine".

Side effects reported for ziconotide treatment include memory impairment, dizziness, nystagmus, speech disorders, nervousness, somnolence and abnormal gait (38). Given the patient cohort being treated, these can be regarded as relatively minor compared to the consequences of nontreatment for patients suffering severe chronic pain. Significant clinical experience is beginning to emerge on the use of ziconotide in combination with a variety of other drugs and few adverse combination effects have been reported. Furthermore, Ilias and Todoroff noted that several accidental overdoses of ziconotide have occurred (39), but no significant adverse reactions have been reported. In addition, intrathecal ziconotide can be suddenly discontinued without major withdrawal symptoms for patients who do not tolerate the drug because of adverse effects (38).

Given the widespread application of intrathecal therapy for currently developed conopeptide drug leads and its established clinical use for ziconotide, it is useful to discuss the advantages and disadvantages of this delivery approach. In general, intrathecal therapy is regarded as an important option for patients who do not experience sufficient analgesia using other treatments (40). On the one hand, it offers the potential for greater pain relief with lower doses of drugs, and hence the possibility of a lower incidence of side effects (38), but on the other hand, there are some intrinsic disadvantages, including the need for a surgical procedure and the risk of infection. Burton et al. (40) recently examined spinal analgesia trialing methods for ziconotide therapy. They noted that long-term intrathecal therapy involves implantation of a pump and catheter, and hence, to improve patient outcomes preliminary trials are generally performed to determine patient responses to analgesia before pump implementation. Analgesia was associated with all three forms of trialing that were compared (continuous, limited-duration infusion and bolus), indicating that all three methods are viable for trialing ziconotide. However, they concluded that small sample sizes prevented a thorough investigation of the safety of these different methods and more studies are required to compare the different trialing procedures.

CVID

CVID (also known as leconotide, or CNSB-004, or previously as AM-336) is a 27-amino-acid ω-conotoxin isolated from *Conus catus* (9) that is very similar in structure to MVIIA (Fig. 2). It shares 18 common residues with MVIIA and the most significant sequence differences occur in loop 4. (Note that the term "loop" is commonly used in conotoxin nomenclature to refer to regions in the backbone between successive Cys residues, which are considered to make up the molecular framework.) Despite the relatively small

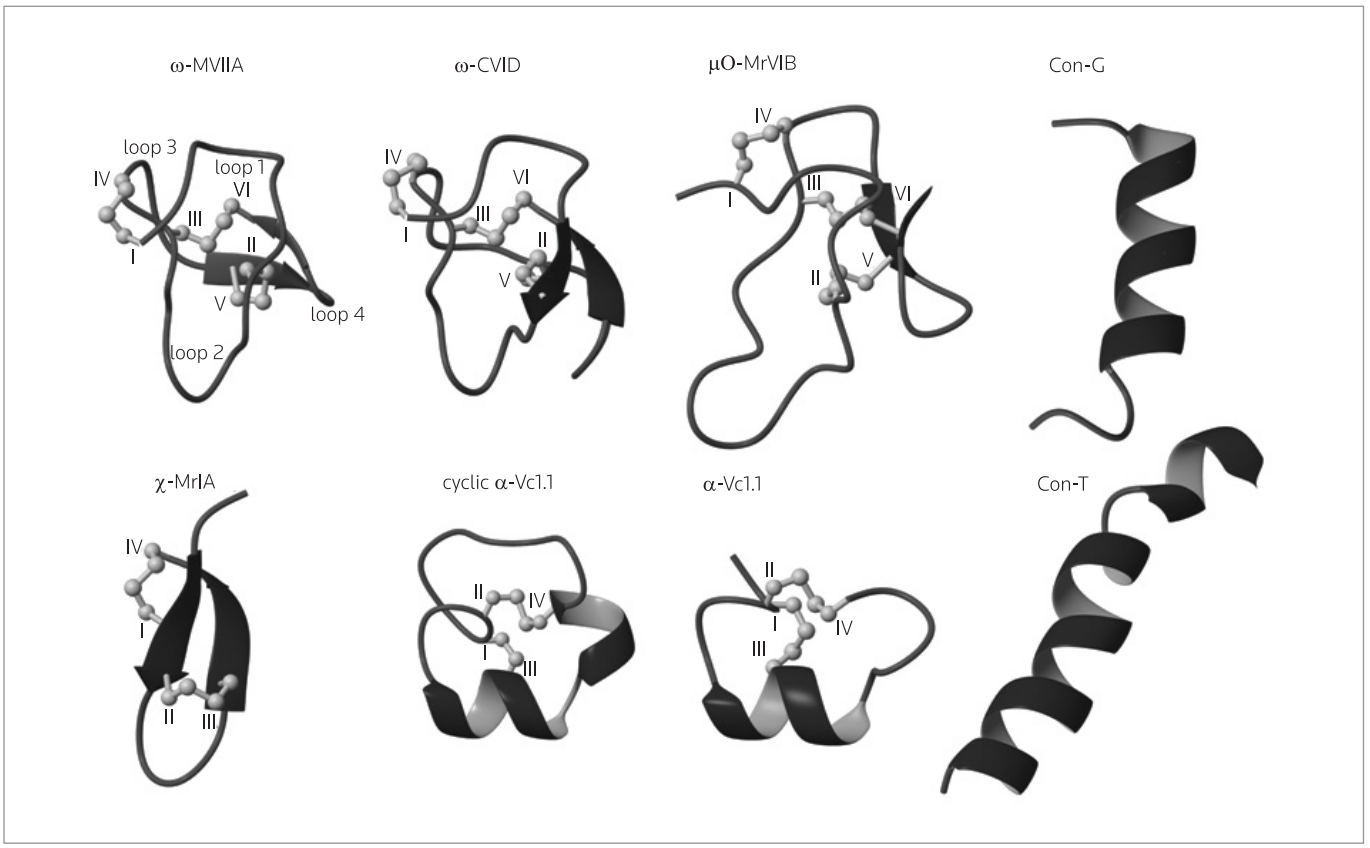

Figure 2. Three-dimensional structures of conotoxins with therapeutic potential. MVIIA (PDB ID code 1ttr), CVID (9), MrVIB (PDB ID code 1rmk), MrIA (PDB ID code 2ew4), cyclic Vc1.1 (21), Vc1.1 (PDB ID code 2h8s), Con-G (PDB ID code 1onu) and Con-T (PDB ID code 1ont). The disulfide bonds are shown in ball-and-stick format. The diagram was made with MOLMOL (72).

differences in sequence, CVID has greater selectivity for N-type over P/Q-type voltage-gated calcium channels (9) compared to MVIIA. Using a spinal nerve ligation model of neuropathic pain in rats, Scott et al. (41) showed that intrathecal administration of CVID had a similar level of potency to MVIIA, but less toxicity. Studies in rabbits with i.v. administration of CVID indicated that a nonspinal method of delivery might be possible without severe cardiovascular effects (42). Recently, this hypothesis has been supported by data from a rat model of neuropathic pain, which showed that CVID has potent antihyperalgesia, without side effects, after i.v. administration (18).

Conus species are a rich source of ω-conotoxins and there is every chance that the discovery of new ω-conotoxins with selectivity profiles that produce fewer side effects might lead to the development of better N-type voltage-gated calcium channel analgesics. This potential is highlighted by the two novel peptides, CVIE and CVIF, which have recently been described (43). These two peptides differ from one another by one residue. They have similar loops 1 and 3 to CVID but significant differences in the other loops. They significantly reduced allodynic behavior in a rat partial sciatic nerve ligation model of neuropathic pain. However, it remains to be determined if these peptides will have more favorable therapeutic properties than MVIIA or CVID.

MrVIB

MrVIB is a 31-residue conopeptide isolated from *Conus marmoreus* that contains three disulfide bonds, which form a cystine knot motif, as illustrated in Figure 2. Despite its similarity in overall fold to MVIIA, MrVIB has a large disordered loop 2 and a very different pharmacological profile. MrVIB was discovered in 1995 and reported to target both sodium and molluscan calcium channels (26). Although it is not currently in clinical trials, it has analgesic activity in animal models that occurs via a different mechanism to MVIIA, and is of much current interest.

Since the initial study on MrVIB (26), it has been shown to inhibit the transient tetrodotoxin-resistant sodium current in rat dorsal root ganglion (44). Specifically, it selectively blocks the voltage-gated sodium $Na_v1.8$ channel (22, 23). Voltage-gated sodium channels have been validated as a target for pain, with examples of local anesthetics acting via these channels. In particular, $Na_v1.8$ has received significant attention as a candidate for development of subtype-selective voltage-gated sodium channel therapeutics for per-

sistent pain (22). This interest is a result of $Na_v1.8$ being expressed exclusively by primary afferent neurons, and > 85% of neurons expressing $Na_v1.8$ are nociceptors. Consistent with the interest in antagonists of $Na_v1.8$, MrVIB has analgesic activity in local anesthetic and postincision allodynia assays, with effects lasting up to 24 h (23). It also appears to have no motor side effects when injected at high doses. Given that the nonselective voltage-gated sodium channel blocker lignocaine displays no selectivity for allodynia and hyperalgesia versus motor side effects, it appears that selective voltage-gated sodium channel antagonists have a greater therapeutic index than nonselective antagonists (22). Overall, the selectivity and animal data suggest that MrVIB is a very interesting drug lead.

XEN-2174

XEN-2174 is a slightly modified analogue of the naturally occurring χ-conotoxin MrIA, originally isolated from *C. marmoreus*. MrIA is a 13-residue peptide that is a noncompetitive inhibitor of the neuronal norepinephrine transporter (NET) (45). The three-dimensional structure consists of a β-hairpin that is cross-braced by two disulfide bonds in a "ribbon" connectivity (Cys1-CysIV, CysII-CysIII). Native MrIA has an *N*-terminal asparagine residue, and such residues are well known to be unstable, as they can undergo cyclization involving the β-carboxamide group. In an extensive structure–activity relationship (SAR) study (19), a series of analogues were synthesized with various *N*-terminal mutations, including the introduction of non-native residues and D-amino acids. In addition to an analysis of their chemical stability and binding to NET, selected peptides were tested in a chronic constriction injury rat model of neuropathic pain. The peptides were administered as a bolus injection via a chronically implanted intrathecal cannula. From these studies, XEN-2174 was chosen as the development candidate with the best mix of chemical stability and therapeutic index.

XEN-2174 has also been shown to have anti-allodynia activity in an L5/L6 spinal nerve injury pain model in rats, but produces little antinociception against mechanical and thermal hyperalgesia in rat models. Overall, XEN-2174 is a promising candidate for development as a novel therapeutic for intrathecal administration to patients with persistent neuropathic pain. It is currently in phase II clinical trials (19).

Vc1.1 AND CYCLIC DERIVATIVES

Vc1.1 is a 16-residue α-conotoxin first isolated from a cDNA library of *Conus victoriae* (20). A synthetic peptide corresponding to the deduced peptide sequence was subsequently shown to alleviate neuropathic pain in rat models (46). However, after several preclinical and clinical trials, development was halted by Metabolic Pharmaceuticals. This molecule has nevertheless turned out to be an interesting substrate for efforts at improving the biopharmaceutical properties of conopeptide-based drug leads, as described below.

Studies in our laboratory have focused on the use of head-to-tail cyclization to provide extra stabilization to conopeptides. The impetus for this work came from discoveries of a range of naturally occurring macrocyclic peptides in bacteria, plants and animals (47). These macrocyclic peptides range in size from ~14 to 70 amino acids and are distinguished from conventional proteins by their exceptional stability. For example, the cyclotides (48), ~30-amino-acid peptides from plants, which contain a head-to-tail cyclic backbone and cystine knot motif (34), are exceptionally resistant to enzymes, heat treatment and chemical chaotropes (49), and have been proposed as valuable frameworks for peptide-based drug design applications (50-52). Similarly, bacteriocins, which lack disulfide bonds but have a cyclic backbone, have very high unfolding temperatures and are exceptionally stable (53). Thus, noting that cyclization produced exceptional stability in a range of peptides, we undertook studies to cyclize model conopeptides.

The first studies were on the prototypic α-conotoxin MII, a 16-amino-acid peptide from *C. magus* that has been implicated as being of potential use for the treatment of Parkinson's disease and nicotine addiction (54). Computer modeling studies suggested that linkers of five or six amino acid residues would be sufficient to bridge the *N* and *C* termini to incorporate a cyclic backbone that would not perturb the three-dimensional structure of the remainder of the molecule. In our initial synthesis of cyclic α-MII analogues, we found that this was almost the case, but there was a slight distortion to the characteristic α-conotoxin helix, and as a result, cyclo-5-α-MII lost activity relative to the native peptide. Cyclic α-MII peptides comprising six or seven amino acid linkers, by contrast, maintained the structure of native α-MII (55, 56), were of high stability in human serum and maintained biological activity (57). The linkers chosen to achieve cyclization included combinations of Gly and Ala residues, e.g., GGAAG, GGAAGG and GAGAAGG.

These initial studies led to further work on pharmaceutically more relevant conopeptides, including Vc1.1, which had been reported to have analgesic activity in an animal model of neuropathic pain when injected i.m. (20). We synthesized a number of versions of cyclic Vc1.1 and recently reported studies on one containing a 6-amino-acid linker (21). As was the case for α-MII, we chose linkers comprising Gly and Ala residues having no more than three residues of the same type consecutively, mainly for logistical reasons in simplifying interpretation of NMR spectra that were to be used to assess the folding. The cyclic derivatives folded reproducibly into the native conformation, which is characterized by a small helix in a wide range of α-conotoxins (58-62).

Analysis of cyclo-6-Vc1.1, also known as IMB-007, in the chronic constriction injury animal pain model (63) demonstrated that the cyclized version had efficacy comparable to the clinically used treatment for neuropathic pain, gabapentin, but achieved this efficacy at a much lower dose. We also found that the cyclized version was more stable than the linear version (21). Most importantly, IMB-007 exhibited analgesic activity when administered orally to rats. The achievement of oral activity for a peptide-based therapeutic represents a potentially very significant milestone. It remains to be seen whether this efficacy will be seen in human clinical trials.

CONANTOKINS AND CONTULAKINS

The conantokins and contulakins are distinct classes of conopeptides that generally lack disulfide bonds and thus are structurally different from the peptides mentioned so far here. Furthermore, the mechanisms involved in their analgesic activities differ from those for disulfide-rich peptides. Conantokins are rich in γ-carboxyglutamic acid residues and were originally referred to as

"sleeper" peptides because they induce sleep in young mice (28). Conantokins form helical structures (Fig. 2), particularly in the presence of divalent cations, and the γ-carboxyglutamic acid residues appear to be a stabilizing feature in these peptides, perhaps compensating for their lack of disulfide bonds (10, 64-66). The γ-carboxyglutamic acid residues are also important for activity, as decarboxylation leads to a loss in sleep-inducing activity (28). Con-G and Con-T are 17- and 21-residue peptides, respectively, that exhibit potent antinociceptive effects in mouse models of pain induced by tissue injury, nerve injury or inflammation (67). Con-G has also been shown to be neuroprotective in rat models of ischemia. Conantokins are the only known peptide ligands of *N*-methyl-D-aspartate (NMDA) receptors and studies indicate that the analgesic activity of conantokins might be related to their NR2B selectivity (a particular subunit). Analgesic effects of Con-G are higher than Con-R[1-17], which is a nonselective inhibitor of NMDA receptors (68). Con-G and Con-T reached human clinical trials, but the current status of their development is unknown.

Contulakin-G was originally isolated from *Conus geographus* (27) and showed potent antinociceptive properties after intrathecal delivery in both rat and dog models (69, 70). It is a 16-residue glycopeptide without disulfide bonds. Glycosylation is important, as the peptide lacking the glycan has significantly less activity in vivo than the glycosylated peptide (27). Contulakin-G interacts with the neurotensin NTS_1 receptor, but is 100-fold more potent as an analgesic than neurotensin, suggesting that activity occurs via a mode of action distinct from neurotensin binding. Contulakin-G has demonstrated efficacy in a broad range of preclinical models of acute and chronic pain and reached phase I clinical trials.

SUMMARY AND OUTLOOK

With one conopeptide on the market now for more than 6 years and several others in clinical or preclinical evaluation, the future for conopeptide-based therapeutics seems promising, although this optimism must be balanced with the realization that sales of ziconotide so far have been relatively modest and the delivery route (intrathecal) is not widely applicable to the majority of pain sufferers. However, the very recent promising findings of oral activity of a conopeptide (cyclized Vc1.1, IMB-007) in an animal model of pain offer hope that other conopeptides will be found that have a similar favorable delivery route.

ACKNOWLEDGMENTS

Work in our laboratory on conopeptides is supported by grants from the National Health & Medical Research Council (NHMRC) (Australia) (Grant ID: 631457) and the Australian Research Council (Grant ID: DP0986281). DJC is an NHMRC Professorial Fellow (Grant ID: 569603). NLD is a Queensland Smart State Fellow. We thank David Wilson for help with figures and tables and our colleagues listed in the references for their valuable contributions to conopeptide research.

DISCLOSURES

The authors are inventors on patents associated with therapeutic applications of conotoxins, and in particular on cyclized conotoxins.

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