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Discovery and development of the χ-conopeptide class of analgesic peptides

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ABSTRACT

Cone snail venoms continue to provide a rich source of bioactive peptides useful as research tools and leads to new therapeutics. We isolated two closely related conopeptides, MrIA and MrIB, which defined the χ-conopeptide class of bioactive peptides based on their unique ability to highly selectively and non-competitively inhibit the norepinephrine transporter (NET). An alanine scan of χ-MrIA revealed this class of peptides had a unusual cysteine-stabilised scaffold that presented a γ-turn in an optimised conformation for high affinity interactions with NET. χ-MrIA reversed the behavioural signs of mechanical allodynia in a chronic constriction injury rat model but its chemically unstable N-terminal asparagine precluded long-term use in implanted pumps. An extensive analoguing program identified Xen2174 to have improved stability and extended duration of analgaesia, without compromising efficacy *versus* side effects window observed forχ-MrIA. An open label, single IT bolus, dose-escalating study in cancer patients suffering severe chronic pain found Xen2174 relieved pain quickly over an extended period across a wide range of well-tolerated doses. Currently, Xen2174 is entering a Phase IIb double-blind study to determine safety and efficacy in bunionectomy pain.

1. Introduction

Cone snails are a large group of over 500 recently evolved species of marine molluscs that capture prey using a highly specialised envenomation machinery (Terlau and Olivera. 2004). Using a highly evolved "needle and syringe" strategy, cone snails inject a complex mixture of venom peptides through a hollow, barbed harpoon using a muscular proboscis. Fish and worm hunting Conidae use a single injection strategy that paralyses prey within seconds, ensuring efficient prey capture. In contrast, mollusc hunting species often inject prey multiple times before their prey can be easily removed from its shell. Each species of cone snail produces its own unique cocktail of mostly small, disulfided-bonded peptides. Depending on their lethality to animals, individual venom peptides are referred to as either conotoxins (lethal by injection) or conopeptides (no proven lethality).

Conotoxins are genetically encoded as propeptides which are cleaved from their precursor protein by venom endoproteases (Milne et al., 2003). Their small size (most areles than 5 kDa), relative ease of synthesis (excepting hydrophic μO- and δ-conotoxins), structural stability, and target specificity make them valuable pharmacological probes. Somewhat surprisingly, many of these conotoxins classes act on pain targets, allowing the *specific* dissection of key ion channels and receptors underlying pain, and providing new ligands with the potential to be developed into novelanalgesics (Lewis and Garcia, 2003). Recent LC/MS analyses have revealed that *Conus* spp. have evolved in excess of 50,000 conopeptides, with the venom of each species containing a unique array of over 1000 different peptides (Davis et al., 2009). To-date fewer than 0.1% of conopetides have been characterised pharmacologically, in part due to their high target and species specificity. Thus

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this broadly evolved class of venom peptides provides a mostly untapped source of bioactive peptides with potential as new research tools and leads to new therapeutics. To-date, ω-MVIIA (Prialt) has reach the market and Xen2174 is at the Phase IIb stage of clinical development, although several other classes have been recognised with clinical potential (Table 1). This review outlines the discovery, characterisation and clinical development path from χ-MrIA discovery in *Conus marmoreus* venom to Xen2174 now in the clinic as a spinal analgesic.

2. χ-Conotoxin discovery and structure

The initial discovery of the χ-conopeptides started with the isolation of two closely eluting conopeptides MrIA and MrIB that were initially suspected to be new α-conotoxins based on their small size (~1400 Da) and relatively hydrophylic nature (Fig. 1A). The cysteine spacing (CCX_4CX_2C) was reminiscent of 4/3-α-conotoxins like ImI (Fig. 1B; Table 2) although their primary sequence was unique. Despite these similarities, χ-conopeptides were shown to have a unique 3-dimensional "ribbon" structure dominated by a γ-turn stabilised by a 1–4/2–3 cysteine connectivity (1st and 2nd Cys paired with the 4th and 3rd, respectively)(see Fig. 1B). In contrast, α-conotoxins have a "globular" structure dominated by α-helices stabilised by a 1–3/2–4 cysteine connectivity. Not surprising given this sequence and structural divergences, we failed to detect any activity of χ-conopeptides at nicotinic acetylcholine receptors (Sharpe et al., 2001). To identify their mode of action, synthetic MrIA was systematically tested across a range of cell and isolated tissue preparations. The only activity detected was a profound enhancement of the second phase of electrically-induced contractile responses in the rat vas deferens that is produced by neurally released norepinephrine (NE)(Fig. 2). This effect was reminiscent of cocaine and tricyclic antidepressants, suggesting that the χ-conopeptides may inhibit the norepinephrine transporter, the main regulator of synaptic NE levels at nerves terminals innervating this tissue. This mode of action of χ-conopeptides was confirmed when we were able to show that χ-conopeptides directly inhibited the uptake of ^{3}H-NE into mammalian cells heterologously expressing NET (Sharpe et al., 2001). In contrast to cocaine and tricyclic antidepressants, this inhibition was not surmounted with increasing concentrations of NE and thus arose from a non-competive or allosteric action and not from a competitive (overlapping) interaction with NE (Sharpe et al., 2001).

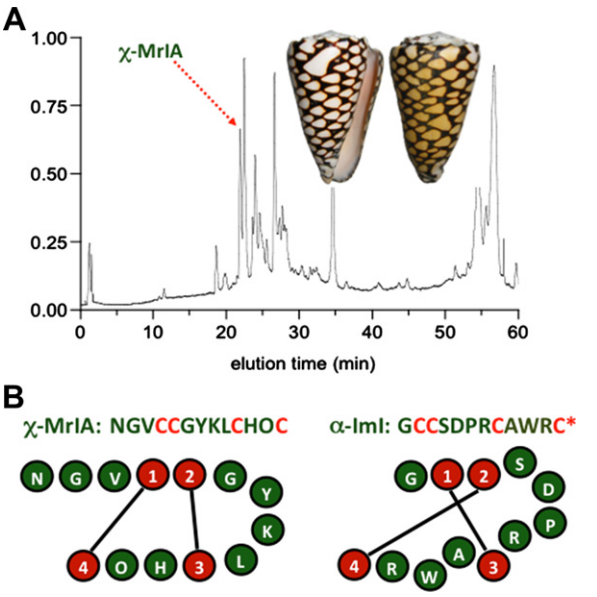

Fig. 1. (A) Reversed-phase HPLC/MS analysis of the crude venom from the venom duct of *Conus marmoreus* (see inset) collected from the Great Barrier Reef, Australia. An early eluting peak (see arrow) was identified as χ-MrIA (from Sharpe et al., 2001). (B) Sequence and disulfide bond arrangement for χ-conopeptide MrIA compared to the alternatively folded α-conotoxins such as ImI.

3. NET as a therapeutic target for peptides

The noradrenaline transporter (NET) is a member of the Na^+-dependent monoamine transporter family of proteins (Wang and Lewis, 2010). Neurotransmitter transporters are important drug targets in the CNS because they have the capacity to selectively manipulate neurotransmitter concentrations at the site of transmitter release, and thereby selectively modulate signaling through specific neuronal pathways. Their regulatory roles have been implicated in the aetiology of many neurological disease states making NET an important target of treatment of a range of neurological diseases including depression, anxiety, obsessive-compulsive disorder, and attention deficit hyperactivity disorder (Goddard et al., 2010). Antidepressants that target NET are also effective analgesics, especially for difficult to treat neuropathic pain conditions (Freeman, 2005). Xen2174 reveals that this effect can be generated spinally, presumably by enhancing the effects of descending inhibitory pathway released NE that acts on presynaptic

Table 1
Conotoxins and conopeptides at various stages of clinical development. Sequence, target, indication and current clinical status are indicated. γγ-carboxyglutamate, pEpyroglutamate, T_g glycosylated tyrosine, O hydroxyproline, and * N-terminal amidation.

Peptide	Sequence	Target	Clinical status
ω-MVIIA	CKGKGAKCSRLMYDCCTGSCRSGKC*	$Ca_V2.2$ block/pain	Marketed
Xen2174	ZGVCCGYKLCHOC*	NET block/pain	Phase IIb
ω-CVID	CKSKGAKCSKLMYDCCSGSCSGTVGC*	$Ca_V2.2$ block/pain	Stalled
Vc1.1	GCCSDPRCNYDHPEIC*	nAChR-$GABA_B$/pain	Ceased
Conantokin-G	GEγγLQγNQγLIRγKSN	NMDA-R block/pain	Ceased
Contulakin-G	pESEEGGSNAT$_g$KKPYIIL	Neurotensin agonist/pain	Ceased