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Commentary

Conotoxins and their regulatory considerations

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ABSTRACT

Venom derived peptides from marine cone snails, conotoxins, have demonstrated unique pharmacological targeting properties that have been pivotal in advancing medical research. The awareness of their true toxic origins and potent pharmacological nature is emphasized by their 'select agent' classification by the US Centers for Disease Control and Prevention. We briefly introduce the biochemical and pharmacological aspects of conotoxins, highlighting current advancements into their biological engineering, and provide details to the present regulations that govern their use in research.

1. Introduction to conotoxins

The venom of *Conus* sea snails has evolved as an efficient means of prey incapacitation and as an effective defense mechanism (Armishaw and Alewood, 2005). The deadly effects of this venomous cocktail are a direct result of conotoxins, small disulfide-rich peptides that are potent antagonists of a range neuronal receptors and ion channels (Table 1). These bioactive peptides express selectivity towards their target receptors and are even able to discriminate between receptor subtypes. These properties are being utilized to design receptor-modulating ligands with potential therapeutic applications.

Conotoxins are typically about 10–40 amino acids in length, but exhibit many motifs that are normally found in larger proteins. These structural features are stabilized by disulfide bonds generated from the abundance of cysteine moieties in its primary structure (Armishaw and Alewood, 2005; Bingham et al., 2005). Interestingly, these cysteine residues are found in predictable locations within a conotoxin's sequence, giving rise to loops of known amino acid lengths, which influence their bioactivity (Bingham et al., 2005; Espiritu et al., 2014).

Although disulfide bonds play a major role in determining conotoxin structure activity relationships, assessing how they influence bioactivity of conotoxins should be handled on a case-by-case basis as they stabilize peptide structure in varying degrees.

The number of disulfide isomers possible within a conotoxin is directly correlated with an increasing number of cysteines within a peptide sequence (Kaas et al., 2010). Notably, when the disulfide bond connectivity of α-conotoxin AuIB was rearranged, the resulting ribbon isomer exhibited greater bioactivity than the globular native toxin (Dutton et al., 2002). This finding is valuable to conotoxin bioengineering as it expands the repertoire of modifiable conotoxins to include non-native isomers.

Despite their great sequence diversity, the cysteine frameworks of these bioactive peptides are quite predictable. For majority of conotoxins containing four cysteines, their cysteine framework is given by the formula $(X)_{n1}C^{i}C^{ii}(X)_{n2}C^{iii}(X)_{n3}C^{iv}(X)_{n4}$ (Bingham et al., 2005). Interestingly, the number of amino acids in $n2$ and $n3$ of these conotoxin's cysteine frameworks can have profound consequences in their selectivity toward a specific receptor class. Generally, α-conotoxins with a 3/5 cysteine framework ($n2 = 3$ and $n3 = 5$) will target muscle nAChRs (Gray et al., 1981) while conotoxins with a 4/7 framework ($n2 = 4$ and $n3 = 7$) will be specific for neuronal nAChRs (Armishaw and Alewood, 2005; Bingham et al., 2010, 2012; Gray et al., 1981). This has important implications regarding conotoxin classification.

Conotoxins can be systematically ordered into superfamilies and further organized into families (sometimes referred to as classes). Superfamilies are based on disulfide bond framework patterns, while families are founded on their pharmacological target (Armishaw and Alewood, 2005; Terlau and Olivera, 2004). Currently, there are twenty main conotoxin superfamilies and within these inclusive categories lie their respective families, often denoted as a single Greek letter prefix. These categories are rapidly expanding with the discovery of new conotoxins (refer to Table 1).

Along with conotoxins, cyclotides are another category of natural products that are garnering immense interest due to their therapeutic potential. Cyclotides are disulfide rich plant peptide characterized by head to tail cyclization and six conserved cysteine

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Table 1
The classification of conotoxins based on their pharmacological families (adapted from Kaas et al., 2012).

Conotoxin family	Definition	Gene superfamily	Cysteine framework	References
α (ALPHA)	Nicotinic acetylcholine receptor (nAChR)	A, B3, D, J, L, M, S	I, II, III, IV, VIII, XIV, XX, XXV	Corpuz et al. (2005), Gray et al. (1981), Imperial et al. (2006), Liu et al. (2008), Loughnan et al. (2009), Luo et al. (2013), Peng et al. (2006), Santos et al. (2004)
γ (GAMMA)	Neuronal pacemaker cation currents (inward cation current)	O1, O2	VI/VII	Fainzilber et al. (1998), McIntosh et al. (1995), Zhangsun et al. (2006)
δ (DELTA)	Voltage gated Na Channel (agonist, delay inactivation)	O1	VI/VII	Fainzilber et al. (1991), McIntosh et al. (1995)
ε (EPISILON)	Presynaptic calcium channels or G protein coupled presynaptic receptor	T	V	Rigby et al. (1999), Walker et al. (1999)
I (IOTA)	Voltage gated Na Channel (agonist, no delayed inactivation)	I1, M	III, XI	Buczek et al. (2007), Corpuz et al. (2005), Jimenez et al. (2003)
κ (KAPPA)	Voltage gated K Channel (blocker)	A, I2, J, M, O1	III, IV, VI/VII, XI, XIV	Buczek et al. (2005b), Imperial et al. (2006), McIntosh et al. (1995), Santos et al. (2004), Terlau et al. (1996a)
μ (MU)	Voltage gated Na Channel (antagonist, blocker)	M, O1, T	III, IV, V, VI, VII	Corpuz et al. (2005), Cruz et al. (1985), McIntosh et al. (1995), Walker et al. (1999)
ρ (RHO)	Alpha 1 adrenoreceptor (GPCR)	A	I	Santos et al. (2004), Sharpe et al. (2001)
σ (SIGMA)	Serotonin gated ion channels (GPCR)	S	VIII	England et al. (1998), Liu et al. (2008)
T (TAU)	Somatostatine receptor	T	V	Petrel et al. (2013), Walker et al. (1999)
χ (CHI)	Neuronal noradrenaline transporter	T	X	Sharpe et al. (2001), Walker et al. (1999)
ω (OMEGA)	Voltage gated calcium channel	O1, O2	VI/VII, XVI, XXVI	Kerr and Yoshikami (1984), McIntosh et al. (1995), Zhangsun et al. (2006)

residues arranged in a knotted topology (Craik et al., 2012; Daly et al., 2009). Cyclotides have insecticidal properties and function in plants as host-defense agents (Craik, 2012). In addition to their natural function cyclotides have been reported to posses anti-HIV, anticancer and hemolytic activities (Daly et al., 2009; Henriques and Craik, 2010). Like conotoxins, cyclotides contain a high frequency of cysteine residues in their sequence. The presence of disulfide bonds confers considerable stability to these natural products thereby making them worthwhile research tools for structural studies and as scaffolds for stable delivery of drugs (Poth et al., 2013; Schroeder et al., 2013). The idea of merging the notable pharmacological properties of cyclotides with the specificity of conotoxins has drawn much attention to the bioengineering of cyclic forms of conotoxins (Bingham et al., 2012).

2. Biological spectrum of conotoxins

2.1. Phyla selectivity

One of the most important and well known aspects of conotoxins is their ability to selectively target ion channel subtypes (Terlau and Olivera, 2004). This dynamic receptor–ligand interaction can be utilized to understand subtle differences in the normal physiology of various ion channel subtypes. As such, conotoxins are viewed as valuable resources for phyla selective receptor probes. Phyla selectivity is commonly seen amongst individual conotoxins, representing their native predatory preferences (Terlau and Olivera, 2004). Many peptides isolated from a piscivorous (fish eating) cone snail such as *Conus magus*, will only display activity in receptor subtypes related to higher order organisms, whereas molluscivorous (mollusk eating) cone snails tend to produce conotoxins which target mollusk receptor subtypes (Bergeron et al., 2013). This type of selectivity can be greatly utilized in terms of creating safe and effective pesticides. Potential pesticides produced from the venoms of molluscivorous cone snails may provide compounds that could be naturally degraded by microorganisms without producing harmful byproducts or causing environmental damage (Bruce et al., 2011).

2.2. General and clinical pharmacological activity of conotoxins

A third feature of conotoxins is their nature to exhibit a high amount of biological activity (see Table 2). To date, ω-Conotoxin MVIIA (SNX-111) is the only United States Food and Drug Administration (FDA) approved conotoxin for use as a therapeutic and is known for being extremely potent, displaying bioactivity in the low nanomolar range (7.6 nM IC_{50}) (Lee et al., 2010; Olivera et al., 1987). However, this type of activity is not unique to this single peptide, as many conotoxins appear to be exceedingly potent. Examples of nanomolar potency among conotoxins include but are not limited to: the μO-conotoxin MrVIA (IC_{50} = 345 nM) (Safo et al., 2000; Terlau et al., 1996b) and the α-conotoxin MIC (K_i = 248.7 nM) (Kapono et al., 2013).

ω-Conotoxin MVIIA, otherwise known as Ziconotide, has undergone extensive clinical testing (FDA, 2006). Pharmacokinetics on Intravenous (IV) and Intrathecal (IT) injections of this peptide has been established. Classical distribution, metabolism, and excretion studies were undertaken before Ziconotide was entered into clinical trials. Ziconotide is the first non-opioid IT treatment for the management of chronic refractory pain and is known by its clinical name Prialt® (PRImary ALTernative to morphine). The therapeutic is designed to be delivered intrathecally and should not be administered via any other Route of Administration (RoA). Several publications describing the safety and efficacy of Prialt® have been published and can be studied for more comprehensive information.

2.3. Oral bioavailability

A second feature of conotoxins is their poor oral bioavailability. Many peptides and biologic based compounds are degraded easily by the digestive tract due to their susceptibility to peptidases, making them virtually ineffective in terms of oral delivery. A second reason for the poor oral bioavailability of conotoxins (along with most peptides) is their large size. According to Lipinski's rule of five compounds over 500 Daltons are typically seen as ineffective orally due to poor membrane and paracellular permeability. These properties have provided a justifiable means for the current