

## Animal, Plant and Microbial Toxins – 15th World Congress

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### Introduction

The Animal, Plant and Microbial Toxins 15th World Congress is the major meeting of the International Society on Toxinology, an organization comprising more than 400 members that was formed to promote research into toxins, their receptors and their applications. The international congress is held every three years, with smaller, separate meetings of the European, Pan-American and Asia-Pacific sections of the society held in intervening years.

### Disulfide-rich microproteins as scaffolds in pharmaceutical design

William 'Pim' Stemmer, CEO and founder of US-based Annunx Inc, provided the opening plenary lecture of the meeting, describing research into the use of small disulfide-rich proteins as potential therapeutics. Dr Stemmer noted that such protein scaffolds offer important advantages for the creation of antibody-like drugs. Scaffolds of particular interest include those based on natural injectable microproteins, such as ones from leeches, snakes, snails, spiders, scorpions and anemones; many disulfide-rich microproteins are derived from the toxic venom peptides of these animals. Dr Stemmer also described the potential of a family of plant-based proteins, called cyclotides, which contain a knotted disulfide arrangement and a circular peptide backbone; the prototypic cyclotide molecule kalata B1 is used as a uterotonic agent in native medicine applications. The advantages of microprotein antibody mimics include the presence of only a single protein chain, the ability to be manufactured microbially, high stability, solubility, potency and specificity; long half-life; low immunogenicity; and protease resistance. Dr Stemmer also highlighted the vast variety of cystine frameworks among conotoxins, which were discussed in several presentations at the meeting.

### Developments with conotoxins

#### Ziconotide: The first conotoxin drug to reach the market

In the opening session of a symposium on Toxins and Drug Discovery, George Miljanich (AIRMID LLC, USA) presented an overview on the development of the analgesic ziconotide (Figure 1), the first conopeptide to receive approval for marketing by the FDA and EMA. The product is approved

for the treatment of severe chronic pain in patients for whom intrathecal therapy is warranted and who are intolerant or refractory to other treatments, such as adjunctive therapy, systemic analgesics, or intrathecal opiates. Following approval in December 2004, Elan Pharmaceuticals Inc had launched the drug in the US by April 2005. In February 2005, the drug was approved in Europe and, following acquisition of the European rights in March 2006, Eisai Co Ltd had launched the drug in the UK and Germany by July 2006 and was preparing to launch the product in other European markets. Ziconotide is a synthetic peptide corresponding to  $\omega$ -conotoxin MVIIa, which was originally isolated from the venom of *Conus magus*. The compound is the first in a new class of therapeutics that are based on blockade of N-type calcium channels.

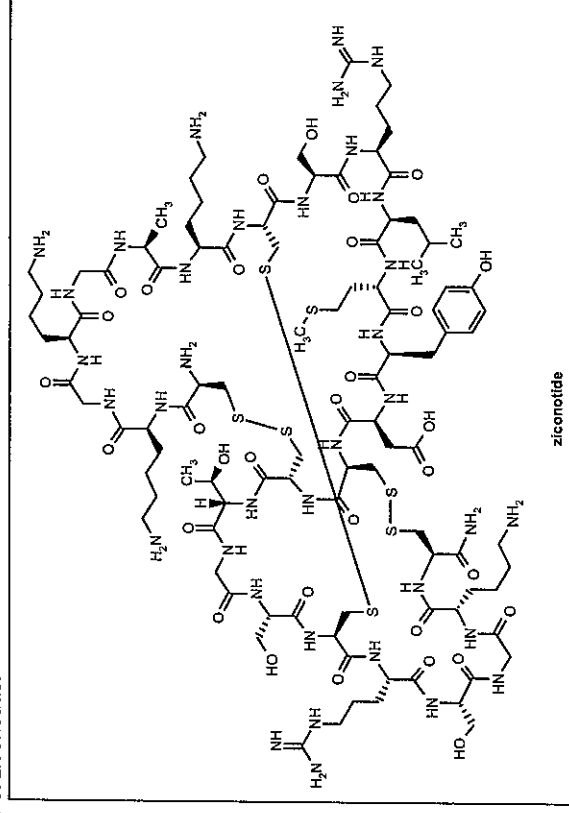
Approval for ziconotide from the regulatory authorities in the US and EU was based in part on safety data from approximately 1250 patients with pain, and on analgesic efficacy demonstrated in a placebo-controlled, double-blind clinical trial of 220 patients suffering severe chronic pain. Dr Miljanich noted that larger numbers of patients have since been administered ziconotide: over 170 patients have received the drug for more than one year and total exposure to the drug is now more than 660 patient years. The clinical trials for ziconotide indicated that the drug is an effective analgesic in patients who have failed to obtain adequate pain relief from oral and/or intrathecal opiate therapy, as well as in patients who are receiving oral opiates concomitantly. Ziconotide has not been observed to elicit respiratory depression, analgesic tolerance, or withdrawal syndrome upon discontinuation, and does not appear to pose a risk of drug dependency. Adverse effects attributed to the drug include dizziness, ataxia, abnormal gait, confusion, memory impairment, nausea and fatigue; psychiatric symptoms may also occur with ziconotide treatment. Dr Miljanich indicated that the clinical profile for ziconotide is consistent with its unique mechanism of action, as determined by extensive nonclinical studies revealing that ziconotide diminishes pain behaviors by blocking N-type calcium channels on sensory nerves, thereby inhibiting transmission of noxious signals to the brain. In summary, ziconotide is a non-opioid alternative for treating severe chronic pain. The nonclinical pharmacology of ziconotide and its therapeutic profile demonstrate that N-type calcium channels play a key role in pain perception, and are a valid target for analgesic therapy. Dr Miljanich noted that several companies are currently attempting to develop nonpeptide mimics to target N-type calcium channels.

#### Update on the development of the analgesic

##### conotoxin ACV-1

Several other presentations at the meeting described preclinical and clinical studies on conopeptides, revealing the apparent importance of these promising disulfide-rich molecules as drug leads. Bruce Livett (Bio21 Molecular

Figure 1. The structure of ziconotide.



Science and Biotechnology Institute, University of Melbourne, Australia) presented two posters that updated progress on the analgesic peptide ACV-1 (Metabolic Pharmaceuticals Ltd) and related conotoxins derived from the venom of *Conus victoriae*. ACV-1 is a synthetic peptide corresponding to the non-post-translationally modified venom peptide Vc1.1a. The conotoxin compound is currently undergoing phase II clinical trials for neuropathic pain. In the first poster, Dr Livett and colleagues described a chemical analysis of the *C. victoriae* venom in combination with the specific chemical synthesis of some post-translationally modified analogs of ACV-1. In several animal models of human neuropathic pain, previous research had established that the 16-amino acid peptide itself is active, but that Vc1.1a, which contains a hydroxyproline at position 6 and a  $\gamma$ -carboxyglutamic acid at position 14, is inactive. The new research detected the presence of a previously unreported dimeric version of the post-translationally modified ACV-1 peptide. An isolation of this fraction resulted in a peptide that exhibited 36% of the activity of the monomeric peptide in a neuronal nicotinic receptor assay. The second poster provided the first report that ACV-1 is effective at alleviating neuropathic pain in an animal model of diabetic neuropathy. In the streptozotocin-induced diabetic rat model of peripheral neuropathy, an anti-allodynic effect was observed at doses of 30 and 300  $\mu\text{g}/\text{kg}$  within 1 h of dosing. ACV-1 attenuated allodynia for up to 1 week following cessation of treatment, and also reduced oxidative stress markers such as lipid hydroperoxidases and nitrotyrosine. These findings broaden the indications for the development of ACV-1 to treat neuropathic pain and diabetic neuropathy.

#### **Xen-2174 for the treatment of neuropathic pain**

Richard Lewis (Xenome Ltd, Brisbane, Australia) provided an overview of the development of Xen-2174 (Xenome Ltd), a derivative of conotoxin MrlA (from *Conus marmoratus*) that is being developed as a treatment for neuropathic pain.

Dr Lewis noted that the native conotoxin sequence itself was not suitable as a drug because of stability problems, but that the synthesis of an extensive series of derivatives had led to the identification of Xen-2174 as a development candidate. Dr Lewis revealed that Xen-2174 contains a pyroglutamic acid at the N-terminus, a substitution that ameliorates the chemical stability problems associated with the native Asn residue at this position. Xen-2174 is currently undergoing a phase II clinical trials for pain.

#### **Defining structure-activity relationships of conopeptides**

Several other presentations described pharmacological, structural or genetic studies on conotoxins. David Adams (University of Queensland, Australia) presented a talk on the pharmacological and structural aspects of Vc1.1, the post-translationally modified version of Vc1.1a. The cysteine spacing of this peptide places it in the 4/7 class of conotoxins (four amino acids between Cys residues in the first loop and seven in the second loop). Vc1.1 has been demonstrated to reversibly inhibit nicotine-evoked membrane currents in isolated bovine chromaffin cells in a concentration-dependent manner, and to also inhibit acetylcholine (ACh)-evoked membrane currents of recombinant ACh receptors expressed in *Xenopus* oocytes, preferentially targeting peripheral receptor subtypes over central subtypes. In contrast, the native venom peptide Vc1.1a had no effect on ion channel currents. The 3-D structure of Vc1.1 comprises a small  $\alpha$ -helix-spanning residues Pro<sup>6</sup> to Asp<sup>11</sup>, braced by two disulfide bonds in a classic  $\alpha$ -conotoxin arrangement. The combined structure-activity data for Vc1.1 should provide new opportunities for further development of the peptide or its analogs as analgesic agents.

In another lecture on conotoxins, Norelle Daly (University of Queensland, Australia) described novel structural findings for a new conotoxin, Bu1A, which has a unique

4/4 spacing within the cystine framework and is the only  $\alpha$ -conotoxin reported to date to contain four residues in the second loop. Two synthetic forms of the peptide that contained different disulfide connectivities, so-called globular and ribbon forms, were synthesized. The globular connectivity, which is the native form in all other  $\alpha$ -conotoxins, displayed structural heterogeneity, but was active at inhibiting ion currents in the  $\alpha_6$  subtype of the nicotinic ACh receptor; in contrast, the structurally better-defined ribbon isomer was inactive. This research provided an example of the diversity in cystine frameworks that was described by Dr Stenmer in the opening plenary lecture, and highlighted the various biological activities that can be achieved for conotoxins with different frameworks and disulfide connectivities.

Frank Mari (Florida Atlantic University, USA) emphasized the theme of novel conotoxin frameworks in a presentation describing novel sequences from several *Conus* species that had been collected from water sources off the US coastline, including some deep sea species that had been collected with the aid of a robotic submarine. The scaffolds observed from these species expand the structural diversity that is known for conopeptides, and potentially enhances the neuropharmacological applications of these molecules.

Baldomero Olivera (University of Utah, USA), the founder of the field of conotoxins, presented a lecture on the evolutionary and molecular biology aspects of these peptides. Dr Olivera indicated that most *Conus* peptides are encoded by a small number of gene superfamilies that are initially translated as prepropeptide precursors and are then post-translationally modified. The range and diversity of post-translational modifications that has been observed in conotoxins is particularly large and, as noted previously, can have substantial impacts on biological activities. A characteristic of the conotoxin gene superfamilies is an ability to diversify rapidly. While such diversification is presumably a biological advantage for an organism that is in a continuously competitive environment for prey capture, the net result is an exceptionally diverse and large library of bioactive lead molecules for drug developers.

#### **Exenatide: From concept to launch**

Michael Hanley (Amylin Pharmaceuticals Inc, USA) described the development of exenatide (Byetta), a 39-amino acid incretin mimetic peptide from the salivary gland of the Gila monster lizard (*Heliobolus suspectorum*). As with the conotoxins, this peptide provides an example of the diverse range of pharmacological resources that are present in venom or salivary glands. The peptide exhibited superior pharmaceutical properties to related glucagon-like peptides (GLPs) from mammals that have generated substantial interest in recent years as potential treatments for endocrine disorders, such as type 2 diabetes. However, the clinical application of mammalian GLPs has been limited by their classic peptidic properties, resulting in a need for delivery via injection or infusion and in a short circulating half-life. Yet only a few sequence differences between the mammalian peptides and the reptile salivary peptide result in remarkably improved pharmacokinetic (PK) properties. Exendin-4, for example, exhibits an *in vivo* half-life of 7 to 8 h, compared to a half-life of a few minutes for most GLPs. Dr Hanley described the progression of exenatide from concept to clinic to commercialization, and suggested that the compound provides several lessons regarding the drug development of naturally occurring peptides, including solutions for immunogenicity, optimal solubility, low aggregation potential, patient-friendly drug delivery, beneficial PK, enhanced chemical stability and reverse engineering of favorable drug properties.

Exenatide was launched by Amylin Pharmaceuticals Inc and Eli Lilly & Co in the US in June 2005 as an adjunctive therapy for type 2 diabetes in patients who had not achieved adequate glycemic control on metformin and/or a sulfonylurea. In April 2005, the FDA indicated that the drug was also approvable as a monotherapy, and that data submitted to support approval for this usage would receive a 6-month review. By January 2006, Eli Lilly had submitted the drug for regulatory approval for type 2 diabetes in the EU. The drug is also being developed for type 1 diabetes; by July 2003, a phase II clinical trial had been initiated in collaboration with the National Institute of Diabetes and Digestive and Kidney Diseases for this indication.

