Venoms - a natural source for mini-protein drugs

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Abstract
Venom bioactives, isolated from natural product libraries, constitute a rich source of highly structured peptides, so called mini-proteins. This category of compounds represents an important alternative to synthetic libraries and peptides obtained from phage display methods for the identification of actives in the lead discovery process. This contribution highlights the potential of venomous peptides as drugs focusing on toxins from cone snails, the conopeptides.

Background to peptide toxins

Nowadays, more than 100,000 toxic animal species are known. Since the venom from a particular species contains a complex mixture of hundreds of compounds, this class of natural library is considered to be huge, comprising tens of millions of molecules. Interestingly, these compounds were designed by nature to exert their function in a highly potent and selective fashion. Furthermore, the stability of these peptide toxins represents an important feature. As a consequence, the problem of fast degradation, often encountered during the development of human peptide hormones, has already been perfectly solved by natural selection for the category of venom peptides.

The cone snail holds the key

The family of venomous marine cone snails of the genus *Conus* comprises about 700-800 representatives. These carnivorous animals feed on fishes, molluscs, or worms. Therefore, they have developed a highly sophisticated venom apparatus. Harpoons, a few millimetres in length and designed like a micro hypodermic needle, are used to spear their prey, followed by injection of a few microliters of the venom cocktail (Figure 1).

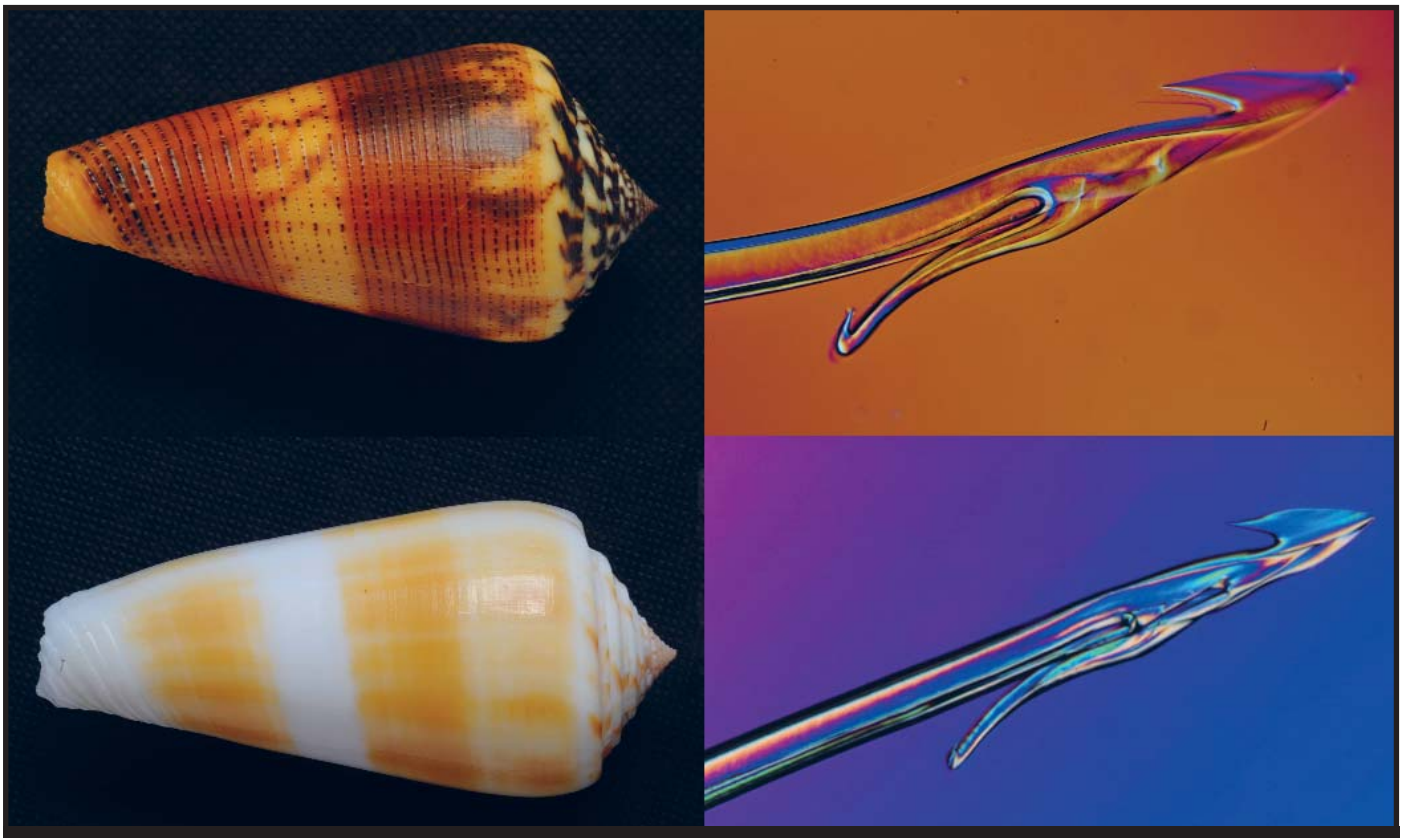

Figure 1: Shells (photos by Thierry Parel) and harpoons (photos by Jean-Jacques Soin) of *Conus magus* (the magician's cone - the origin of the drug Prialt) and *Conus consors* (bottom), two closely related fish-hunting species.

Both species are currently investigated in the framework of an EU-project "CONCO – the cone snail genome project for health" (www.conco.eu). As a result of an attack by the snail, the prey is usually paralysed within less than a second. The victim is then swallowed immediately. Impressive videos, both live and fully digital, are available at www.toxinomics.org.

Properties of venomous mini-proteins

Venoms have been poorly studied in the past. A few hundred species of venomous animals were investigated, and these studies permitted only partial characterization of around a thousand molecules. Venom components are typically classified according to their structural scaffold and/or pharmacological target. Besides small organic molecules, linear peptides and large proteins, the so-called mini-proteins are without doubt amongst the most fascinating and promising venom bioactives. Mini-proteins typically range from 10-70 amino acids and exhibit one to five disulfide bonds. They are extremely resistant to proteases, and by contrast to therapeutic proteins and antibodies, they are poorly immunogenic. Interestingly, compounds isolated from venoms are usually very water-soluble. The dried venom typically represents 30-50% of the wet weight. In addition, these mini-proteins offer high selectivity towards important pharmaceutical targets, *e.g.* ion channel sub-types, receptors, transporters, enzymes or microorganisms. The potency usually is in the low nM or pM range and therefore only a limited development is needed to arrive at a viable drug candidate.

Venomics for lead discovery

Recent developments in the quest for the discovery of innovative leads have focused on the exploration of natural libraries. These activities include the large-scale preparation of pre-fractionated venoms ready-made for high-throughput screening (www.melusine.com). The complex composition of venoms is exemplified by the HPLC chromatogram of the compound mixture obtained from *Conus consors* (Figure 2). In the course of lead identification and structure-activity relationship studies, results can be coupled to bioactivity-guided, structure-driven (genomics, transcriptomics and peptidomics), and biocomputing-assisted processes to speed up the discovery process. Furthermore, efficient lead selection and optimisation is supported by data mining, enabling the identification of analogous compounds in related venoms.

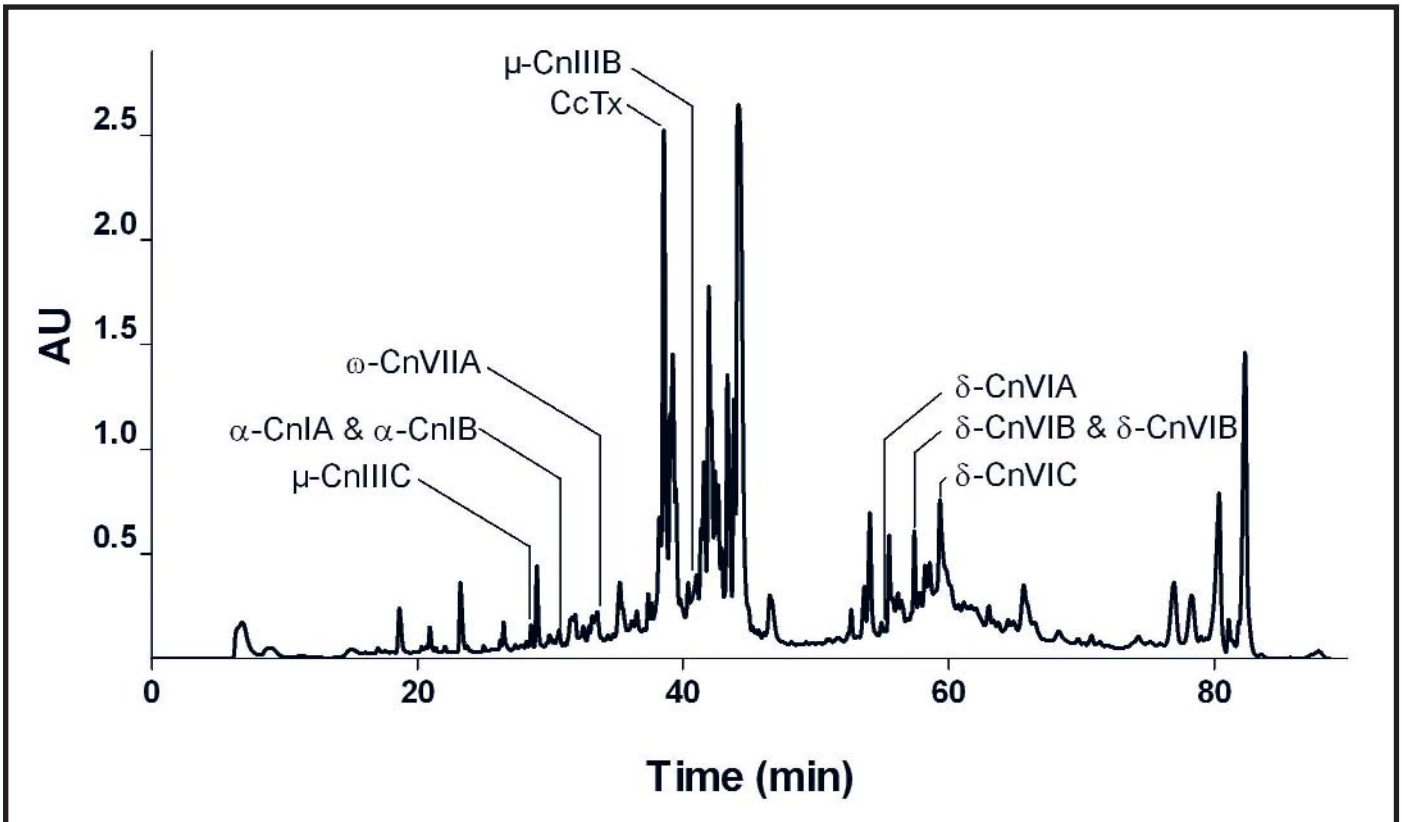

Figure 2: RP-HPLC profile of the venom of *Conus consors*. More than 1,700 molecules were identified in this venom by mass spectrometry.

Venom-derived drugs

While the cocktail can kill, individual venom components can cure. Five venom-derived peptide drugs have already reached the market (Table 1), and many more are currently undergoing pre-clinical and clinical development, *e.g.* to treat cancer, pain, multiple sclerosis, stroke, allergies, diabetes or microbial infections.

Table 1: Examples for successful drug development starting from venoms.

Company	Drug	Molecule	Species	Target	Disease
Amylin & Eli Lilly	Exenatide (Byetta)	Peptide (natural)	*Heloderma suspectum* (Gila monster)	GLP-1 receptor	Type-2 diabetes
Bristol-Myers-Squibb	Capoten (Captopril)	Peptidomimetic	*Bothrops jararaca* (Brazilian lancehead)	Angiotensin converting enzyme	Hypertension
COR Therapeutics	Integrilin (Eptifibatide)	Cyclic peptide	*Sistrurus miliarus barbouri* (Southeastern pygmy rattlesnake)	Platelet aggregation	Ischemic stroke
Elan Corporation	Ziconotide (Prialt)	Mini-protein (natural)	*Conus magus* (Magician's cone)	N-type voltage-gated Ca^{2+} channel	Severe chronic pain
Medicure Pharma & Merck	Tirofiban (Aggrastat)	Peptidomimetic	*Echis carinatus* (Saw-scaled viper)	Platelet aggregation	Angina & infarction

Captopril was the first peptidomimetic developed from a toxin of a *Bothrops* snake species. The NDA was approved in 1981, and the compound became generic in 1996. Integrilin, a cyclic heptapeptide, based on the RGD sequence of the 73-mer Barbourin, is approaching blockbuster status. Tirofiban is a small molecule originating from the KGD sequence found in the protein disintegrin of *Echis carinatus*. Both peptides are marketed since 1998 and reversibly inhibit platelet aggregation. Despite many attempts to improve on the natural molecule, Prialt (Figure 3) reached the market in its native form in 2004 (omega conopeptide MVIIA: a 25-mer containing three disulfide bonds). The sequence of the type II diabetes drug Exenatide also relates to its native structure, a linear 39 residue-peptide analogue to GLP-1

Figure 3: 3D-structure (stereo display) of the omega-conopeptide MVIIA (Prialt).

Outlook

Animal venoms constitute an inexhaustible source of well-structured mini-proteins. They have not been extensively investigated and can be considered as almost unexplored. The deconvolution nowadays is straight-forward to unambiguously identify the active principle of an isolated molecule. In addition, chemical peptide synthesis has progressed to enable efficient large-scale manufacturing of mini-proteins. With dozens of these compounds currently undergoing pre-clinical and clinical development, this category of mini-proteins will constitute a new class of innovative therapeutics.

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