Advancing drug discovery with reptile and amphibian venom peptides

Venom-based medicines

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Amphibian skin has long been known by humans to possess intriguing biological properties and scientific examination of these secretions has revealed that their components possess a range of medical properties. In Japan and China, toad venom has been used as an expectorant, anti-haemorrhagic, diuretic and cardiac stimulant, and some peptides in the secretions of South American frogs and toads have antibiotic and anticancer properties. In fact, the pharmaceutical industry was built upon the realization of the healing and therapeutic potential of many lead compounds used in ethnic or traditional medicines (e.g. morphine, quinine and aspirin).

Previous research has found that amphibian venoms contain hundreds of peptides and that some of these peptides are, in terms of activity, identical with peptides found in the human brain and gut. Therefore, through the identification and analysis of the peptides found in amphibian venoms, novel drug leads can be developed. With many thousands of amphibious species, all potentially containing hundreds of different peptides, the prospective library of natural samples is sizeable.

The importance of venom

The realization that amphibians may hold the key to drug discovery is attributable to the Italian pharmacologist Vittorio Erspamer, who discovered the brain chemical serotonin (5-hydroxytryptamine). Erspamer first researched the active principles in toad venom after his dog attacked a toad in his garden and became very sick. Intrigued, Erspamer began studying the venom secreted by glands in the skin of toads. He discovered that they contained an abundance of chemicals that have marked affects on animal tissue. He then spent most of his career studying the peptides that most frogs produce in their skin glands in abundant complex mixtures and established that these peptides had nearly exact counterparts in the nervous system of mammals.

Venoms are highly evolved and naturally selected cocktails of biologically active peptides/proteins and other molecules, each of which plays a defined role through interaction with highly specific molecular targets. It is this ‘cruise missile’ type of precision and optimization of structure for function that renders these molecules intriguing models for the drug discovery scientist, in terms of both new lead discovery and new target identification. Many of the component peptides either may represent novel site-substituted analogues of known actives or may be entirely novel structures to science. In each instance, with the insight of creative pharmacologists and cell biologists focused on an array of discrete research end-points, a plethora of new information can be generated, some of which may be crucial in the development of novel therapeutic approaches.

Venom analysis

In order to increase functional genomic understanding of peptides, researchers utilize high-throughput molecular technologies involving de novo peptide sequencing, cDNA cloning and pharmacological screening. This method allows for the rapid acquisition of structural data and the generation of peptide molecular libraries for novel drug leads. Samples are largely collected under field conditions in a manner that preserves the chemical and biological information and as such techniques have been developed accordingly. Researchers extract the venom by electrically stimulating the tiny muscles in the venom glands. This causes them to extrude the toxin on to the surface of the skin. It can then be washed off, recovered and freeze-dried ready for analysis. The stimulating current (provided by the equivalent of a personal stereo battery) is small and does no harm and is simple enough to use in the field.

One of the most common methods of venom analysis is proteomics which requires samples of the skin secretions. This method is often supported by transcriptome analysis, which used to require dissection of the skin for cDNA library construction. However, in 2003, scientists found that the skin secretions contain polyadenylated mRNAs that encode dermal gland peptides, so both proteome and transcriptome analysis could be carried out humanely, while preserving the species. As such, the techniques that researchers are adapting and developing for the acquisition of venom samples are totally non-invasive and non-lethal to the donor animals, a factor that is of particular importance as many of the donor species are examples of threatened biodiversity. Once samples have been obtained by such techniques and freeze-dried, researchers can use them for parallel peptidomic and
genomic studies almost indefinitely.

An integrated functional genomic approach has been developed for the study of venom-derived peptides that initially involves fractionation of crude material and subsequent isolation and structural characterization of individual components of interest. Another starting point involves the construction of a cDNA library from freeze-dried venom, a method discovered and pioneered by the Experimental Therapeutics Research Group at the School of Pharmacy, Queen’s University Belfast. By means of degenerate primer-directed PCR, researchers can ‘shotgun’ clone bioactive peptide precursor-encoding cDNAs, from which the mature primary structures of novel peptides can often be deduced. These can then be located by means of computed molecular mass in fractions of the corresponding venoms. Primary structures can then be confirmed by tandem mass spectrometry (MS/MS) fragmentation sequencing. Alternatively, reverse-phase HPLC fractions of venoms can be screened for a bioactivity using discrete bioassays. All of these techniques, when combined in an integrated approach, produce holistic data sets that can aid in the evaluation of individual molecules as novel drug leads.

**Analysis challenges**

The biggest obstacle to overcome is in *de novo* sequencing of peptides that are not represented in any online databases. When this research was initiated some 12 years ago, researchers relied exclusively on micro sequencing of highly purified peptides by Edman degradation. This provided a lot of absolutely unequivocal data, but was very expensive and time-consuming to perform. The use of MS/MS fragmentation technology in peptide identification was a *sine qua non* for the rapid development and employment of proteomics. However, software relied on trawling fragmentation spectra against a database of translated protein amino acid sequences derived largely from genome sequencing projects, many of which have now produced complete proteome datasets. Frog proteins are not well represented in these, apart from *Xenopus*, which is not regarded as particularly typical.

**Application example**

In collaboration with Thermo Fisher Scientific, the Experimental Therapeutics Research Group at the School of Pharmacy, Queen’s University Belfast is currently using a high-performance mass spectrometer to analyse peptides within amphibian venom. The team needed an instrument that would deliver molecular characterization of peptides identical with the natural compound. It is imperative that the research team can be
The Thermo Scientific instrumentation has enabled the research team at Queen's University Belfast to characterize the structure of small molecules which would not have been possible without the use of the LTQ Orbitrap XL. The revolutionary Orbitrap technology has enabled the team to determine that the synthetic replicate produced in analysis is state-of-the-art in terms of molecular characterization identical with the natural compound, meaning researchers can now be 100% confident that they have got the correct molecule. This is the first time such research has been possible using proteomics instrumentation and means that researchers can avoid years of fruitless research.

Venom-based medicines

Angiotensin-converting enzyme (ACE) inhibitors are perhaps the best examples of a drug class that has been developed from a peptide lead derived from venom. These represent the major therapeutics used in the treatment of hypertension for many years – this being a major and increasing cause of morbidity and ultimately cardiovascular mortality in the developed world. More recently, exenatide, a synthetic analogue of exendin-4, which is a venom peptide of a venomous lizard, the Gila Monster, from the deserts of the Southwest USA and Mexico, has passed clinical trials and is now available as a prescription medicine for the treatment of Type 2 diabetes. Additionally, chlorotoxin, a peptide derived from the venom of a dangerous desert scorpion, has now been proven to be an effective treatment for malignant glioma – a type of brain tumour. This list, which is by no means exhaustive, reflects the small degree of effort put into drug discovery from venom sources rather than the enormous untapped potential that remains to be realized. The Experimental Therapeutics Research Laboratory now has several novel lead compounds at international patent stage that could have fundamental therapeutic applications in cancer, vision science and wound healing.

The future

Any pharmaceutical scientist who is involved in contemporary natural product research has to become involved in, or at the very least...
become familiar with the global issues of species conservation and/or biodiversity. It is quite remarkable just how many pharmaceuticals originate from nature rather than in the chemist’s test tube. These are by no means niche drugs or those for the treatment of rare conditions. Analgesics based on morphine or aspirin, the majority of antibiotics, anti-hypertensives, cardioactive agents, insulin, statins, antimalarials and key anticancer drugs are a few that come to mind. In addition, there are many new and emerging ‘biologics’ based on monoclonal antibodies and recombinant proteins.

Nature will remain the major source of therapeutic leads provided preservation continues in terms of species and habitats. Every species extinction event is paralleled by the extinction of its genes and proteins – a potentially new concept in bioscience. Improvements in technologies devoted to protein and gene characterization, sequencing and warehousing/interrogation of data will continue to be fundamental in our efforts to develop new drugs for currently intractable diseases, as will the design and nurturing of scientists prepared to take on established thinkers and their thoughts. The pioneering aspect of this research, the intellectual challenges that have to be faced and the beauty and mystery of the donor animals all help to keep the butterflies fluttering in the stomach and the adrenaline pumping through the veins.

Conclusions

The Experimental Therapeutics Research Laboratory at Queen’s University Belfast is an example of a productive collaboration between academic scientists and commercial instrument providers to find innovative uses for existing technologies and instrumentation. The Molecular Therapeutics Research Group is currently focusing its efforts on the isolation, structural characterization and functional evaluation of novel peptides extracted from amphibian, snake and arthropod venoms. Previous research in this area by other scientists has unearthed a number of groundbreaking drug discoveries including potential treatment for high blood pressure and possible cures for deep vein thrombosis and heart disease.

Both deduced primary structures and molecular masses of novel peptides can be confirmed by mass spectrometric hardware to provide unequivocal peptide identification. The LTQ Orbitrap XL provides a solution which delivers the excellent mass accuracy required for complex peptide identification and the reliability of data needed for the de novo sequencing of peptides. The instrument provides unparalleled sensitivity in MS/MS, superior mass accuracy and high resolution leading to confident de novo sequencing results. Recognizing the huge potential for drug discovery from the analysis of natural peptides, the team now hopes that the research facility at Queen’s University Belfast will prove pivotal in the future of drug discovery and that these trials will mark the start of significant advancement in this research area and enable novel peptide drug discovery leads in the future.

For more information about the Thermo Scientific LTQ Orbitrap XL high performance mass spectrometer, please call +1 800-532-4752, email analyze@thermo.com or visit www.thermo.com/orbitrap.

Chris Shaw received his BSc (Hons) in Biological Sciences from the University of Ulster in 1980 and his PhD in Molecular Endocrinology from Queen’s University Belfast in 1984. He spent 1985–1986 as a visiting senior research fellow in the Max Planck Institute for Gastrointestinal Endocrinology, Gottingen, Germany, in the laboratory of Dr Mike Conlon. He has held positions of lecturer, reader and professor in Queen’s University, Faculty of Medicine, and of Professor of Biotechnology in the University of Ulster. He is currently Professor of Drug Discovery in the School of Pharmacy, Queen’s University, Belfast. His research interest is in all aspects of bioactive peptides and currently his research focus is directed towards the discovery of novel peptides from venoms, especially those of amphibians, which may have clinically important effects or may serve as leads for drug development. He has written over 300 peer-reviewed scientific papers and has delivered numerous invited international lectures. email: chris.shaw@qub.ac.uk

References