The next generation of venom-based drugs

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Some venom-derived drugs, such as captopril and exenatide, are already used therapeutically, and researchers are continuing to develop new venom-based treatments for a wide range of conditions, including autoimmune and cardiovascular diseases, chronic pain and cancer. This article explores the potential for new venom-based drugs and some of the problems researchers need to overcome.

By the pricking of my thumbs, the next generation of venom-based drugs this way comes. You might think that science has come a long way since Macbeth’s witches hubbled and bubbled fillet of a fenny snake, a toad that swelter’d venom and adder’s fork. Yet if you manage heart disease or diabetes, some of your patients will, almost certainly, take venom-based drugs. Meanwhile, researchers are developing new venom-based drugs for, among other conditions, autoimmune and cardiovascular diseases, chronic pain and cancer. “Animal-based venoms are hugely promising as an avenue for new drugs,” says Ray Norton, Professor of Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Australia.

Some venom-based drugs are already therapeutic mainstays. Captopril, the first ACE inhibitor, derives from studies of bradykinin-potentiating peptides isolated from the venom of Bothrops jararaca, a South American pit viper.1 The venom of the Gila monster (Heloderma suspectum) – found in New Mexico and Arizona and one of the few venomous lizards – contains a group of peptides called exendins. These are structurally similar to glucagon-like peptide-1 (GLP-1), a human incretin hormone that reduces blood glucose. Exenatide, a synthetic version of exendin-4, and other GLP-1 mimetics, are widely used type 2 diabetes treatments.1

Specialists have a wider choice of venom-based drugs (see Table 1). In order to feed, a leech needs to keep the blood flowing. So, leeches evolved a protein called hirudin, which potently inhibits thrombin. A synthetic version of hirudin is available as an anticoagulant. Other venom-based drugs alleviate chronic pain and reduce the risk of death and myocardial infarction in certain patients with acute coronary syndromes.1 This article...
briefly explores the potential for new venom-based drugs and some of the hurdles facing researchers.

**An evolving idea**

Venom seems to have evolved about 90 times across the animal kingdom and almost 15% of animal species seem to be venomous. Ron Jenner, Research Leader in the Division of Invertebrates at London’s Natural History Museum, notes that many venoms disrupt the nervous system or blood-vascular system to quickly disable a prey or cause pain that deters further attack. So, a venom can give an animal a marked survival advantage. “A non-venomous reptile may be able to catch and overcome prey with claws and teeth alone, but if it is able to inject a venom at the same time to help disable prey more quickly, it may be selected for in evolution,” he says. “The adaptive value is illustrated by venoms having evolved dozens of times in the animal kingdom, and by the fact that once venom has evolved it is rarely or never lost.”

Indeed, venoms emerged early in evolution of life on earth. The Burgess Shale, which is about 505 million years old, probably contains fossilised remains of early venomous animals. “The earliest venomous animals are probably ancient relatives of jellyfish and sea anemones, a group known as Cnidaria,” remarks Dr Jenner, co-author of *Venom: The Secrets of Nature’s Deadliest Weapon*.

An acces-sible introduction to this fascinating area is the Burgess Shale contains fossils of ancient cnidarian relatives. Although we don’t know if they were venomous, they could very well have been.”

Over the millennia, some animals developed exquisitely toxic venoms. A single bite of a coastal taipan (*Oxyuranus scutellatus*) could kill 3.4 million mice – or 1000 adult humans. Being venomous means more than just being toxic. The animal must make the toxin in special-ised cells and have a specific delivery mechanism. The skin of the pufferfish (*Tetraodontidae* sp.) contains tetrodo-toxin, which is some thousand times more toxic to humans than cyanide (see Figure 1). So, predators spit the pufferfish out.

### Toxic themes

The earliest centipedes seem to have hunted using venoms containing four toxins. Over the last 400 million years, centipedes seem to have evolved 93 distinct families of peptides and proteins as toxins that show “astonishing structural diversity.”

Table 1. Currently available venom-based drugs, the species they were originally sourced from and their indications

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Species</th>
<th>Examples of indication</th>
</tr>
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<tbody>
<tr>
<td>Bivalirudin</td>
<td>Medicinal leech (<em>Hirudo medicinalis</em>)</td>
<td>Anticoagulant for adults undergoing percutaneous coronary intervention (PCI); adults with unstable angina/ non-ST segment elevation myocardial infarction (MI) planned for urgent or early intervention</td>
</tr>
<tr>
<td>Captopril</td>
<td>Jararaca pit viper (<em>Bothrops jararaca</em>)</td>
<td>Hypertension, congestive heart failure, MI, type 1 diabetic nephropathy</td>
</tr>
<tr>
<td>Eptifibatide</td>
<td>Pigmy rattlesnake (<em>Sistrurus miliarius barbouri</em>)</td>
<td>Prevention of early MI in adults presenting with unstable angina or non-Q-wave MI</td>
</tr>
<tr>
<td>Exenatide</td>
<td>Gila monster (<em>Heloderma suspectum</em>)</td>
<td>Adults with type 2 diabetes mellitus</td>
</tr>
<tr>
<td>Tirofiban</td>
<td>Saw-scaled viper (<em>Echis carinatus</em>)</td>
<td>Prevention of early MI in adults presenting with acute coronary syndromes without ST elevation; reduction of major cardiovascular events in patients with acute ST elevation MI (STEMI) intended for primary PCI</td>
</tr>
<tr>
<td>Ziconotide</td>
<td>Cone snail (<em>Conus magus</em>)</td>
<td>Severe, chronic pain in adults who require intrathecal analgesia</td>
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</tbody>
</table>

Venomous animals actively deliver toxins. The Gila monster produces venom in glands in its lower jaw, which travels along grooved teeth and into the victim as the lizard chews. Mouthparts like teeth have evolved into sophisticated venom delivery structures in reptiles and some groups of mammals, which have grooved or tube-like teeth to deliver venom more effectively,” Dr Jenner comments. “Pointy spines in fish, which were probably already used as defence structures, have, in some cases, evolved into sophisticated syringe-like venom injection machinery.”

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that alpha-bungarotoxin, isolated from the venom of the Taiwanese banded krait snake (*Bungarus multicinctus*), blocks postsynaptic neuromuscular transmission in higher vertebrates. Alpha-bungarotoxin allowed researchers to discriminate between and physically separate nicotinic receptors and acetylcholinesterase, both of which bind acetylcholine.6 “We still need pure venom-derived molecules as molecular probes,” says Professor Norton.

Displacement studies of known ligands for a particular target can be an efficient route to novel venom-derived ligands (peptides or otherwise) for that target.”

For example, a species of European bloodworm (*Glycera tridactyla*) causes massive, chronic, but reversible, acetylcholine release into the neuromuscular junction. This allows researchers to explore cholinergic transmission.2

Indeed, some toxins are remarkably specific. Over 400 peptide toxins target sodium channels, including more than 182 from scorpions, 116 from spiders and 55 from sea anemones.2 Tetrodotoxin also blocks sodium channels,4 which is why *fugu* (pufferfish) can, if poorly prepared, kill adventurous gourmets. “Many neurotoxic peptides show great affinity for particular types of ion channels,” Dr Jenner says. “By binding to these specific ion channels, neurotoxins can stimulate or block nerve impulses, which can lead to whole body paralysis.”

Ion channels are ubiquitous in the animal kingdom. But that's no guarantee that a venom will work in humans. “Neurotoxins present in venoms from snakes in the cobra family (Elapidae) have specific molecular targets in nerves and at the neuromuscular junction. So they can paralyse a wide range of animals, including humans,” Dr Jenner says. “But ion channels can vary considerably between animal groups. A neurotoxin in the venom of a bird-eating snake may have much more powerful effects on bird ion channels than mammal ion channels.”

Other venom components have non-specific effects. The sea wasp jellyfish (*Chironex fleckeri*) delivers a venom that triggers inflammation and is toxic to nerves, blood, cells, the heart, muscle and skin.2 “Many animal venoms, including fish and snake venoms, contain the enzyme hyaluronidase,” Dr Jenner says. “Hyaluronidase breaks down the extracellular matrix, and can thereby act as a spreading factor that allows venom toxins to better penetrate the bodies of envenomated victims.” Incidentally, hyaluronidase may increase the penetration of chemotherapy to some malignancies, including certain pancreatic cancers.7

“Other venom components, like the so-called pore-forming toxins, are also relatively non-specific,” Dr Jenner says. Indeed, pore-forming peptides from the venom of some species of scorpion, such as the African yellow leg scorpion (*Opistophthalmus carinatus*), or the burrowing thick tail scorpion (*Parabuthus schlechteri*), seem effective against several Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* (see Figure 2).8

New venom-based drugs

The toxin's effect can offer a clue to possible clinical applications. If you're male, in Brazil and unfortunate enough to be bitten by the wandering spider (*Phoneutria nigriventer*) or yellow scorpion (*Tityus serrulatus*), you'll probably suffer tachycardia, sweating, excessive salivation and vomiting. If that wasn't enough, you could experience spontaneous erections. So, these species' venoms are being investigated as a possible treatment for erectile dysfunction that doesn't respond to sildenafil or other existing drugs.2,9

The cone snail (*Conus geographus*) – the world's deadliest snail – induces hypoglycaemia to sedate fish before capture. *C. geographus* also kills humans by causing paralysis with, Professor Norton says, “a cocktail of neuro- and myotoxins”.2 “Our work on cone snail-derived insulins also offers hope for new insulin analogues to treat diabetes,” Professor Norton adds.

Indeed, the seas seem to be rich sources of pharmaceutically active toxins. The Caribbean sun anemone (*Stichodactyla helianthus*), for instance, produces a toxin that strongly blocks several potassium channels on T lymphocytes, the brain and cardiac tissues.

Figure 2. Pore-forming peptides from the venom of some species of scorpion (such as *Opistophthalmus carinatus*, shown) may be effective against several Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa* and *Haemophilus influenzae*
Modifying this toxin produced dalazatide, which is 100 times more selective for potassium channels on T lymphocytes than those on brain and cardiac cells. Dalazatide is showing promise in several autoimmune diseases including multiple sclerosis, rheumatoid arthritis and psoriasis.1,10,11

Professor Norton’s laboratory is developing HsTX1[R14A], isolated from the giant forest scorpion (Heterometrus spinifer), which blocks voltage-gated potassium channels, for autoimmune diseases and other conditions. In a rat model of rheumatoid arthritis, HsTX1[R14A] was effective when administered weekly. PEGylation may further increase the duration of action, perhaps allowing fortnightly administration.12,13

Venom-based drugs could also lead to a new generation of cancer treatments. A toxin from one of the few venomous mammals, the northern Short-tailed shrew (Blarina brevicauda), inhibits growth of ovarian and breast cancer in animal models as well as showing promising activity and tolerability in preliminary studies of advanced epithelial tumours.1

Chlorotoxin, isolated from the venom of the deathstalker scorpion (Leiurus quinquestriatus hebraeus), blocks chloride channels. Chlorotoxin also inhibits and lowers expression of a certain subtype (MMP-2) of the enzyme matrix metalloprotease. Gliomas and some other cancer cells express high levels of MMP-2, which is not normally expressed by brain cells.1,8 MMP-2 degrades and remodels extracellular matrix, which contributes to the growth and spread of the cancer.8 Chlorotoxin could target radiochemicals and imaging agents to the tumour to treat or diagnose certain cancers. One approach attaches a fluorescent dye to chlorotoxin. So, the surgeon can see the cancer in the operating room and remove as much of the tumour as possible.1

**Antivenoms and pesticides**

Snakes envenomate 1.8 million people a year worldwide. About 100,000 people die. And, as many snake venoms cause massive tissue destruction, some 400,000 people need amputations. Scorpions and spiders kill another 5000 people.2 Antivenoms are antibodies that neutralise the toxin. However, antivenoms are highly species-specific and can induce severe immunological reactions and other side-effects in between 43% and 81% of patients.3 The cost also puts anti-venoms out of reach of many people in the poorest parts of the world, who are the most likely to be envenomated.2

The growing understanding of the pharmacological actions of venoms offers new treatments for this major public health problem. The enzyme sphingomyelinase D is largely responsible for severe tissue necrosis and systemic effects than can follow a bite from a recluse spider (Loxosceles sp.). Researchers developed small molecules that inhibit sphingomyelinase D. Some of these inhibited the enzyme’s binding to human keratinocytes and reduced venom-induced cell death and the development of necrotic lesions in rabbits.14 “This is an interesting area of development,” Professor Norton says. “For example, if the harmful effects of a particular venom can be attributed to a particular enzyme – such as phospholipase, which is common in snake venoms – an inhibitor could be a useful treatment for envenomation. It’s important that the inhibitor be specific for the venom, as opposed to the endogenous, enzyme.”

“The more we understand about the composition of a venom and the actions of its toxins individually and in combination, the better chances we have of developing treatments for envenomation,” Dr Jenner adds. “Many animals are resistant in varying degrees to snake venoms, including venomous snakes themselves, their prey, and their predators. For instance, many vipers have haemorrhagic metalloprotease enzymes in their venom. Some predators of rattlesnakes, such as opossums, have serum proteins that neutralise the enzymes.”

Many animals produce toxins to subdue insect prey. Thus, venom-based insecticides could help tackle the 1000 or so insect pests that reduce the world’s crop production by an estimated 10–14%. One peptide-based insecticide derives from the venom of the Australian Blue Mountains funnel web spider (Hadronyche versuta), which is about 10,000-fold more selective for voltage-dependent calcium channels in insects than vertebrates.1 “I believe this is a very promising avenue of development for venom-derived peptides,” comments Professor Norton.

**Untangling toxins**

Despite the success of captopril and the promise of sphingomyelinase D inhibitors, developing orally active venom-based drugs (peptidomimetics) is difficult. So, venom-based drugs will, initially at least, probably reach the market as peptides. “Peptides are becoming cheaper and easier to make, and there are several options for delivering peptides via non-oral routes, including inhaled, slow-release and transdermal formulations,” Professor Norton notes. “Small drug-like compounds generally cannot match the potency and target specificity of peptides, which typically have a much larger contact surface area with their receptors. We and others have tried designing peptidomimetics, but with limited success. Nonetheless, molecular design continues to advance, and, combined with screening of chemical libraries, offers the prospect of small-molecule mimetics of venom peptides in the future.”

To complicate matters further, venoms are, typically, complex chemical cocktails. The venom of a cone snail or spider can contain hundreds or thousands of different proteins.2 And the components often act in concert. For instance, the venom of the Chinese red-headed centipede (Scolopendra subspinipes) is 3500 to 50,000 times more insecticidal than the neurotoxins tested alone.2

“There are examples where activity-driven fractionation of a venom falls off a cliff because the activity declines or disappears as purification proceeds,” Professor Norton says. “ Venom toxins work together to disrupt normal physiological functioning,” Dr Jenner adds. “In some cases, we understand exactly how venom components interact. But because venoms can comprise hundreds of distinct chemicals, there is still much to be learned about the toxic synergy of
venom components.”

Researchers face other challenges, getting enough venom to study. “Isolating active peptides is straightforward with modern chromatographic methods,” Professor Norton comments. “A bigger challenge is getting sufficient venom to begin fractionation.” Venom may account for 0.5% of a snake’s or scorpion’s body weight. But refilling the venom glands can mean raising the metabolic rate by 20% in a snake and up to 40% in some scorpions. As manufacturing venoms is metabolically expensive, most animal produce small amounts. An approach called ‘venomics’ – which integrates proteomics, genomics and transcriptomics (study of RNA derived from genes) – allows comprehensive analyses of venoms and should help identify potential leads for new drugs, despite the limited quantities of venom.

Indeed, researchers have barely scratched the surface of venoms’ pharmaceutical potential. There are, for example, more than 3100 described species of centipedes across five orders. But most of the research, including that for new drugs, focuses on a few species from a single family (Scolopendridae). More than 2900 fish species use venom as a defence, but Dr Jenner points out most are poorly studied.

“There are probably more than 200,000 species of living venomous animals, the vast majority of which have not yet had their venoms profiled,” Dr Jenner says. “Each is chock-full with bioactive compounds, and a number of them have already been used to develop pharmaceuticals. However, the route from a promising venom toxin to a drug is long, arduous, expensive, and frequently a dead end.”

“Genomic, transcriptomic and proteomic studies of venom are identifying thousands of, mainly, new peptides, some of which can be turned into valuable therapeutic leads,” Professor Norton concludes. “Our challenge is to screen these candidates efficiently in order to kiss as few frogs as possible before finding the princes.”

References

Declaration of interests
Mark Greener is a full-time medical writer and journalist and, as such, regularly provides editorial and consultancy services to numerous pharmaceutical, biotechnology and device companies and their agencies. He has no shares or financial interests relevant to this article.

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