Animal Venom & Poisons: Zootoxins in Our Pharmacological Toolbox

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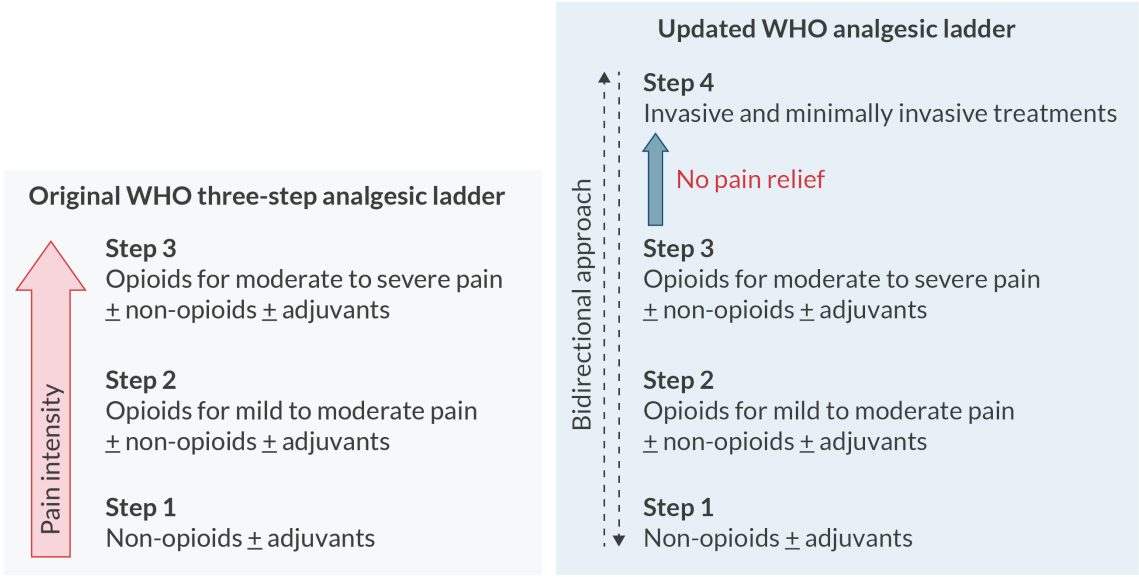
THINK OF A PATIENT you've cared for, or for that matter, a friend or family member (hopefully not you!) who suffers from severe, chronic pain. What is their experience, and what options do they have in their quest to deal with pain. Add to these factors the burden exacted on our society with the use (and overuse) of opioids.

CRNAs increasingly find themselves at the forefront of delivering care to those with severe, chronic pain. This may be incidental in nature when a sufferer presents to us as a surgical patient for care that may or may not be related to the pain. Or those of us who work in an acute or chronic pain treatment facility may be asked to provide symptomatic relief to the victim. The likely history of their pain management often includes the initial management of systemic drug delivery (usually opioids and nonsteroidal anti-inflammatory drugs) and the off-label use of antidepressants, anticonvulsants, and physical therapy.

For example, a common algorithm for treating chronic cancer pain in use today is an "analgesic ladder", developed by the World Health Organization, which was updated based on a growing literature of inadequate pain relief.[1] While designed with cancer pain in mind, it has applications beyond that. While not embraced or rigidly adhered to by all, it consists of the following:



When the first three rungs (steps) fail or are otherwise inadequate, several interventional techniques can be considered. This is especially important given that escalation of drug dosing may occur, some with grave consequences that are appreciated by us. When conventional therapeutic options are ineffective, interventional pain management such as spinal cord stimulation and intrathecal therapy (IT) may be indicated to provide relief.

IT analgesics are delivered directly to the cerebral spinal fluid using a single injection, an implanted device, or an external pump. Opioids, with or without adjunctive agents, comprise the drug class most frequently used with IT.

IT analgesia provides advantages over systemic or oral delivery in that it is directly applied to the vicinity of the spinal receptors and ion channels. It also decreases the potential for overdose associated with the more conventional therapies when carefully performed.

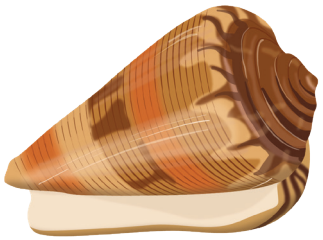
When moving to that fourth step (a big one!), clinicians with knowledge and experience in the area look at refractory pain occurring when

- multiple evidence-based biomedical therapies used in a clinically appropriate and acceptable fashion fail to reach treatment goals or have resulted in intolerable adverse effects, ***AND***
- psychiatric disorders and psychosocial factors influencing pain outcomes have been assessed and appropriately addressed.

Let's take a look at one such option, one that may surprise you, given its unusual origin.

Ziconotide©: from a poisonous sea critter to the patient's bedside

Ziconotide©, also known as SNX-111, is a novel non-opioid analgesic drug that is the synthetic version of a peptide found in the venom of the fish-eating marine snail, *Conus magus*. The Conus' body anatomy comprises five parts: the proboscis, siphon, eyestalks, mouth, and foot. The proboscis (an extensible tubular organ) contains a venomous harpoon, menacingly called 'toxoglossan radula'. This harpoon strikes its prey with astonishing speed and injects its venom.



A *Conus* shell is about 2 inches long, very beautiful, and sold in shell shops. When the snail inside is alive, don't touch it!

The sea snail's venom is composed of several 'conotoxins' specific to each type of snail; one version is known to be lethal to humans. These toxins have a number of neuromuscular effects via glutamate, adrenergic, serotonin, and cholinergic pathways. Some exert their effects on sodium, potassium, and calcium ion channels. Ziconotide© blocks N-type voltage-gated calcium channels found in the A-delta and C afferent pain fibers in the spinal cord's dorsal horn. Blockade of this calcium channel inhibits neurotransmitter release from nociceptive afferents and subsequent pain transmission, achieving its analgesic action.[3] It has no activity on opioid receptors but does act as a CNS depressant.

Due to the wide range of molecular targets and the variation in the venom of each *Conus* species, it is nearly impossible to create effective anti-venom. This marine snail's intriguing biochemical properties captured the imagination of pharmaceutical scientists who envisioned applications in treating stroke and relieving pain. Long story short, Ziconotide© was synthesized once *Conus*' biochemistry could be synthetically modeled.

Ziconotide© poorly penetrates the blood–brain barrier. To achieve optimal analgesic efficacy with reduced potential for serious side-effects, it must be administered intrathecally. This route of administration permits Ziconotide© to optimally target its site of action, encouraging a rapid onset of analgesia.[4]

Given the complexity and diversity of its biological activity, a significant rate of undesirable side effects is reported, all of which appear to be dose-related and shown to clear up fairly rapidly when the drug is discontinued. As with most drugs, contraindications exist, especially at higher dosages and occasionally even at therapeutic dosages.

The following noteworthy adverse events are associated with Ziconotide© administration:

- Cognitive impairment
- Hallucinations and related psychotic symptoms
- Suicidal thoughts surfacing
- Confusion, slurred speech, memory impairment
- Cardiovascular toxicity
- Dyskinesia
- Dizziness, rash, nausea, and vomiting
- Acute renal dysfunction with rhabdomyolysis

The use of a programmable pump can deliver small and precise amounts of Ziconotide© maintaining a desired drug level within the target compartment (the CSF in this case). The drug delivery system is designed to minimize side effects by keeping the quantity of drug delivered to a minimum.

This very novel drug possesses activity that sets it apart from all other known pain relievers, providing clinicians with another option for those suffering from chronic, severe pain.

Will future pain management providers look back on Ziconotide© as the prototypical example of a new era of venom-based analgesics? The answer to that question likely depends in part if new and easier administration routes surface.[5] All in all, the psychiatric side effects of opioids, cannabinoids, and Ziconotide© are a very real reminder of how difficult it is to interfere safely with receptors in the CNS without exacting a price. In that respect, the safety profile of Ziconotide© isn't fundamentally more worrying than that of opioids or cannabinoids, rather it is just different.

Ziconotide© is but one example of a drug modeled after animal-derived poisons and their venoms. Let's have a look at some others that you may be surprised to learn. The complexity of our CNS makes any attempt to create a single, "magic bullet" a very difficult task indeed.

> ***"All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing poison."***
>
> **—Paracelsus, Renaissance physician, scientist, astrologer; early 1500s**

There are a lot of fascinating molecules from many different animal venoms and poisons that have been studied in various trials. Consider this incomplete list of the venom sources:

- Amphibians
- Cone snails
- Sea anemones
- Snakes
- Spiders
- Bats
- Shrews
- Caterpillars
- Ticks
- Wasps, bees, hornets
- Ants

Animal poisons and venoms are comprised of many different classes of molecules displaying a bewildering range of pharmacological activities. The example of Ziconotide© described above may leave one with the impression that its evolution was simple and straightforward, but that would be greatly misleading. In fact, making these molecular poisons and venoms found in another species applicable to humans requires exacting skill and knowledge of pharmaceutical biochemistry and extensive bench and preclinical trials. Only very few applications make their way to the inevitably demanding clinical trials. The trial's purposes are to validate the molecular target(s), the mechanism(s) of action, the effective dose, and of course, discern potential adverse effects.

More than semantics: the difference between a poison and a venom

Naturally occurring poisons and venoms found in animals are luxurious sources of proteins, peptides, neurotransmitters, and chemical mimickers. Their intended purpose is to induce some form and magnitude of damage in the prey's body, providing a sole or additional mechanism by which one creature can subdue and/or kill either their prey or their predator.

If we consider the terms 'poison' and 'venom', then the delivery method becomes the discriminator. A poison is produced by an animal or is acquired in their diet, the intent of which is to cause toxicity in a predator that eats or otherwise comes into contact with the animal. In this case, it is a biological defense mechanism.

As an example, a poison dart frog has no innate defenses, and when grown in captivity has no toxic qualities. Its extremely lethal secreted toxin, even lethal to humans, is derived from its diet of eating creatures and plants that thrive in its native environment.

Venom is actively generated by an animal's tissue or even a specialized venom gland that is designed to be injected into its prey via a highly specialized "tool" of the animal. Think fang, stinger, teeth, or in the case of the *Conus* snail, a virtual harpoon. In the case of both poisons and venoms, we will use the generic term "toxin".

While great diversity of biologically active toxins is known, there is still enormous expense, resources, and time involved. Once a toxin has been identified, it can then be assessed for its potential utility in humans. It must then advance through a myriad of *in vitro* and *in vivo* tests to establish its biochemistry, pharmacology, carcinogenicity, and effects on the reproductive system, just to establish its relative safety before moving on to the clinical phases. The clinical phases offer further challenges in the effort to achieve approval for use. Let's look at a few success stories.

From animal toxin to FDA approval: not for the faint-hearted!

There are probably a dozen or more animal toxin-based drugs currently on the market, Ziconotide© being a noteworthy one, discussed at the start of this APEX Update. Others include, but aren't limited to, the following:

- Exenatide and lixisenatide from lizards
- Bivalirudin and desirudin from leeches
- Captopril, enalapril, tirofiban, eptifibatide, batroxobin†, and cobratide from snakes

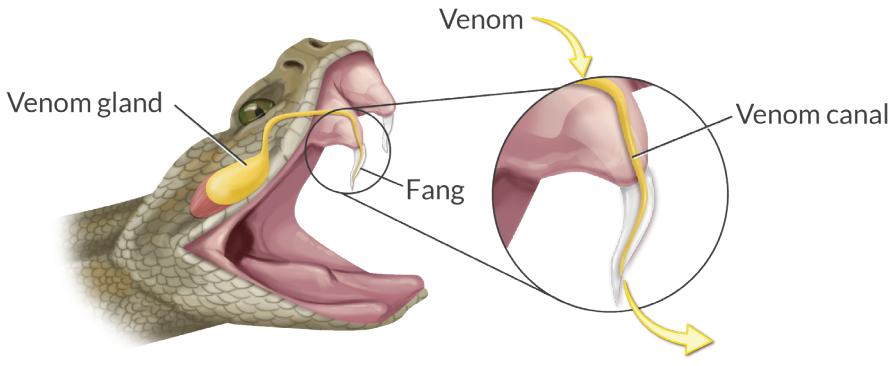
†Note: Batroxobin and cobratide are native compounds isolated from snake venoms. Desirudin is a recombinant molecule, and bivalirudin, captopril, enalapril, eptifibatide, exenatide, tirofiban, and Ziconotide are synthetic copycat molecules.

The 'abundance' of drugs from snakes (some of the most deadly in all the world) is likely due to the relatively large volume of venom produced by snakes compared to other creatures.[6] This has greatly facilitated the logistics involved in researching their constituent chemistries. Additionally, because cardiovascular disease is a major human ailment, the venom of many of the venomous snakes acts in some fashion on this system (e.g., vascular tone, hemostasis), thus making their venom a good target for drug discovery.

This is a highly evolving field for medicinal chemists and clinician-scientists, given the significant advances in instrumentation, experimental models, as well as researcher skills and knowledge. This allows for ever smaller quantities of toxins to reveal their potential.[7] For example, our understanding of the workings of ion channels, the intricacies of the human nervous system, and the genetics of protein-coding has increased exponentially over just the last few years. Given that the toxins of small venomous invertebrates (think spiders, scorpions, centipedes, caterpillars, ticks, wasps, bees, etc.) tend to target the nervous system, and as initially 'disturbing' or 'queasy' this field of study may seem to us, the potential for novel drug discovery looms large!

But for now, snake venom is where most of the action is, and as a sensitivity marker of this, consider the extent of venomous "snake farms."

A survey found 34 snake farms in 21 countries designed to maintain venomous snakes and extract their venom.[8] Facilities maintained between 50 and 1500 snakes, with some having 70 different species and extracting venom from each snake on 14- to 30-day intervals. The risk to employees varied, but snakebites were reported with at least one documented death.



Snake venom targets the hypertensive patient

Captopril (Capoten©, Bristol-Myers Squibb) was approved for human use in 1981 and mimics a peptide venom found in *Bothrops jararaca*, known in Brazil as the jararaca snake. We know this drug as a potent ACE inhibitor that obstructs the production of angiotensin II. The drug as we know it is a miniaturized version of the toxin with an added succinyl group that permits oral administration.[9] The marvels of modern chemistry!

Enalapril (Vasotec©, Merck) emerged from the same toxin and became clinically available four years later. A component of the molecule was found responsible for concerning side effects, including a skin rash and loss of the sense of taste. There was great concern involved in modifying the molecule without losing its interaction with ACE after so much money and effort had been expended to get the drug to market. The final, successful product is a prodrug that undergoes de-esterification to produce the active drug, enalaprilat.

The targeting of platelets

Tirofiban (Aggrastat©, Medicure International, Inc.) and eptifibatide (Integrilin©, Millennium Pharmaceuticals, Inc.) emerged from two different venomous snake toxins, both based on disintegrins found in each venom.[10] Disintegrins are small 40–100 amino acid, nonenzymatic polypeptides that exact a significant hemorrhage toll on the snake's prey by inhibiting the formation of a platelet plug, inhibiting the aggregation of platelets required for hemostasis, and inhibiting cellular adhesive proteins. Both were FDA approved in 1998 as potent antiplatelet agents.

Leeches to the rescue?

Hirudin is a polypeptide of 65 to 66 amino acids derived from the saliva of the leech *Hirudo medicinalis*. It is a very potent, naturally occurring thrombin inhibitor.[11] Instead of harvesting leeches, the marvels of genetic engineering provided a pathway to create recombinant forms of hirudin. Lepirudin (Refludin©) is FDA approved for anticoagulation in patients with heparin-induced thrombocytopenia and associated thromboembolic disease.

From the desert to the bedside: A poisonous lizard and diabetes

The Gila monster can grow up to two feet and weigh five pounds, making it the largest lizard native to the U.S. It is part of a rather exclusive club in that worldwide, there are only a few venomous lizards. This southwestern dweller does not envenomate as snakes do but rather grabs tightly onto its victim and chews, secreting a saliva-borne neurotoxin that moves through grooves in its teeth into the bitten animal's wound(s). Its bite is horrifically painful but has not been reported to be lethal in humans

Despite the fearsome image provoked by their appearance and chewing habits (!!), they are lethargic creatures feeding primarily on eggs it finds in nests of newborn mammals. They may spend more than 95 percent of their day underground, emerging only to feed and occasionally to bask in the desert sun. What really captured the medicinal chemists' attention was that they store fat in their plump tails and can go for months between meals.

Teeth of Gila monster lizard with grooves where venom is secreted.

Exenatide, marketed as Byetta©, was FDA approved in 2005 to treat type 2 diabetes.[12] It is marketed as a prefilled pen for subcutaneous injection and is a synthetic form of a hormone called exendin-4, which occurs naturally in Gila monster saliva. The lizard hormone is approximately 50 percent identical to our own glucagon. The hormone remains effective much longer than our glucagon, at least several hours, allowing the lizard to optimize substrate and glucose management over long periods between its feeding.

There is also an extended-release form of exenatide (Bydureon©), approved in 2012 by the FDA. It has a longer half-life of 5–6 days by virtue of its encapsulation into microspheres, which hydrate once administered and slowly degrades to release the drug over a longer period of time.

A very tiny frog might have very big potential

The golden poison dart frog of Central and South America is about two inches long and a shimmering banana yellow color. It's very beautiful to look at, but by some estimates, one frog contains enough toxin on its skin to kill 10 adults, therefore it is considered to be one of the more lethal toxins on planet Earth. The toxin, called batrachotoxin, is carefully applied by some indigenous groups in Colombia to the tips of their poison darts for hunting prey. And, oh yes, the darts do their job with astonishing speed and success.

Batrachotoxin works by disrupting the electrical signals in the heart, basically causing ventricular fibrillation. It can also disrupt electrical signals traveling through nerves and muscles throughout the body. There are very few molecules like this that are known, and there is very real potential that some version of one or more of the molecules that are in this toxin could be developed into a useful drug. And this golden dart frog is just one of over 100 different types of such poisonous amphibians.

Diagnostics and cosmeceuticals

We wish to at least give a nod to the contributions of animal toxins in a couple of other domains, one being clotting factor assays. Because many viper venoms contain enzymes that specifically degrade or impede various steps in blood coagulation, there are lab-based tests that have emerged using those very enzymes to evaluate hemostasis.

One of many examples is a thrombin-like enzyme, batroxobin, isolated from the venom of *Bothrops atrox*, commonly known as the fer-de-lance, that catalyzes the conversion of fibrinogen to fibrin (the Pefakit Reptilase Time Assay). Other assays target critical coagulation factors used in tests that have been in use in labs for some time.

And consider the realm of 'cosmeceuticals,' which has proven to be an extremely profitable enterprise. Noteworthy in this domain is the anti-wrinkling effect of the botulinum toxin (Botox), isolated from *Clostridium botulinum* bacteria, a toxic microbe for sure, accounting for global sales exceeding $3 billion per year.[13] The use of this toxin has expanded into the treatment of many conditions, including migraine headache, neck spasm, overactive bladder, and even excessive sweating. There are even applications emerging involving bee venom as a facial cosmetic to diminish wrinkles and enhance tone.

Clinical contemplations

- What type of therapeutic application is conjured up in your mind when you think of a venomous creature that you may have encountered?
- What ethical concerns do you have about the kind of research reported here?
- While this APEX Update wasn't intended to be comprehensive, are there any common animal toxin derivatives that you are aware of that should have been mentioned?

Conclusions and summary

Animal toxins are rich and varied sources of molecules with demonstrated potential in a wide range of biomedical purposing. To make the use of these molecules feasible, extensive basic science, as well as preclinical and human trials, are required, all at great expense and expenditure of resources.

Although research in animal toxins tends to be quite challenging and resource-intensive, the high selectivity of their toxins for their molecular targets makes them promising leads for the development of many different types of drugs. The major domains of interest include analgesics, modifiers of the autonomic nervous system, and hemostasis. An especially intriguing field with great potential is the use of disintegrins isolated from snake viper venom in cancer treatment.[14]

The science is still scratching the surface of identifying and understanding the complexity and the potential of animal toxins. While very few species have been extensively studied, there are thousands of unexplored organisms on land and the oceans that may hold great promise. As technology and knowledge evolve in the field, methods to produce and deliver zootoxin-based pharmaceuticals from an expanding pool of candidate venomous creatures will likely further expand what may be offered to manage the ills that afflict us.

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