NEWS FROM THE PIT

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Venomics: Cracking the Code

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Human nature is a complex mixture of diversity, selectiveness, and potency. We enjoy puzzles, complexes, or tackling voids in knowledge. It's quite fascinating how we will tirelessly sleuth until we reach the conclusion of, "Huzzah, that's how it works!" And, if we don't... well, there are those of us who will spend a lifetime pursuing answers, buried by our own curiosity. Our obsession and quest for completion can be a simple hobby, like a crossword or jigsaw puzzle, or all the way to the other side of the spectrum: exploring the unknown in our expanding cosmos. Anywhere within this spectrum, we use scientific method to approach a deeper understanding and sometimes, a definitive answer.

As it turns out, snake venom (much like us) is a complex mixture of diversity, selectiveness, and potency. In the last 100 years, our understanding regarding the pathophysiology and consequences of envenoming have dramatically enhanced the treatment of rattlesnake envenoming (RSE), from using whiskey and electrocution to the ability of infusing specific immunoglobulins at present day. But how did we get to this point? Has our itch (for knowledge) finally been scratched? In answering these questions, I wish I could say yes with a 100% certainty, but we're getting closer every day. So, set your phasers to stun, because in this installment of NEWS FROM THE PIT, we're going to nerd-out and talk about venomics. Sidenote, I'm relieved and proud of our advances in medicine that have afforded us better treatments for RSE. It's reassuring that if I were to become a victim of a RSE, I wouldn't be handed a glass of alcohol and then be electrocuted repeatedly until the doctor deemed me to be "cured".

NEWSLETTER HIGHLIGHTS

Image 1: Western Diamond-backed Rattlesnake, Crotalus atrox; Pinal County, AZ

Image 2: Clinical Toxicology Fellow Thom Maciulewicz and herpetologist Dan Massey holding a Western Diamond-backed Rattlesnake measuring nearly 5 feet in length.

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Venomics is a multifaceted approach to understanding the encoding for venom peptides/proteins (genomics) and the expression of genes within certain cells/tissues at a specific stage or physiologic condition (transcriptomics). Additionally, proteomics is used to get a big picture look characterizing the proteins of a cell/tissue/organism to elucidate the overall structure and function of individual venom components at a specific point in time or maturation. All this information is then funneled down in a process called bioinformatics, which summarizes these scientific findings into pharmacomics (how the venom might behave in an envenomated patient). To date, more than 100 active components have been identified in snake venom! This important work takes place in those fancy and sophisticated laboratories portrayed in Hollywood productions like NCIS or Batman… but in real life, we are not quite at the point of identifying and isolating the toxins from whole venom in a matter of minutes to hours. In real life, it has taken researchers decades of dedication resulting in a sea of never-ending PhD dissertations culminating in our present-day insight regarding snake venom.

One of the most researched snakes in the world is *Crotalus atrox,* aka the Western Diamond-backed Rattlesnake (WD). If you are not exactly fresh on ancient linguistics, the genus Crotalus comes from the Greek “krotalon” meaning castanet, which references the trademark rattle at the end of the tail. The specific epithet *atrox* stems from the Proto-Indo-European of “having the appearance of fire” to include related adjectives of fierce, savage, bloody, dreadful, heinous. Putting this altogether, you’re probably visualizing a rattling tornado of doom. You have to imagine back in the day someone must have had quite the encounter or witnessed a rather severe envenoming. I can’t even imagine seeing an envenomated patient that was treated and then toxic from strychnine, mercury, or infected from a poultice of manure placed on the wound (ew, gross!).

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Apologies for the brief detour (I tend to do that quite often), but back to the WD and venomics. As an example of some venomics work by Calvete et al. in Figure 1. and Table 1., his group was able to nicely categorize the relative occurrence of venom components in the WD. From this point, researchers can perform laboratory in vitro (think in a glass petri dish) and in vivo (within a living organism like a mouse/rat) studies. These experiments can include the whole venom or even specific enzymes/proteins and observe the effect or dose relationship of toxicity. This is indeed a big step that requires a lot of pricey equipment, time, and commitment, but this is where science starts bridging observed experiences in animals to humans. Eventually, pharmacological interventions to limit/inhibit these toxicities are hypothesized and produce treatments such as our modern day antivenoms [Fab, F(ab')2]. Easy, right? (I'm looking at you Batman!)

As you might speculate from Figure 1, the larger the slice of the pie, the more pronounced effect a specific venom toxin might have on the target. Dose-dependent toxicity generally explains what we see clinically (increased likelihood of correlating clinical symptoms) from a particular species of snake with known venom components… sometimes however, it does not and makes us stop, scratch our heads and throw our hands in the air. Why is that?! Well, there are many important factors to consider that could change the composition/potency of venom. The species, age, diet, last known meal, and geographic location all play a role in influencing the character of a snake's venom… even within one country, state, or county. Additionally, the amount of venom injected (# of bites, feeding vs defensive strikes) or where the target has been struck (upper/lower limb, face/neck, vascular) are important factors to consider as well. Meaning, how fast can the venom get to where it can cause the most damage. These considerations throw a wrench in the cogs of venomics regarding the translation of nature to benchtop to bedside clinical care.

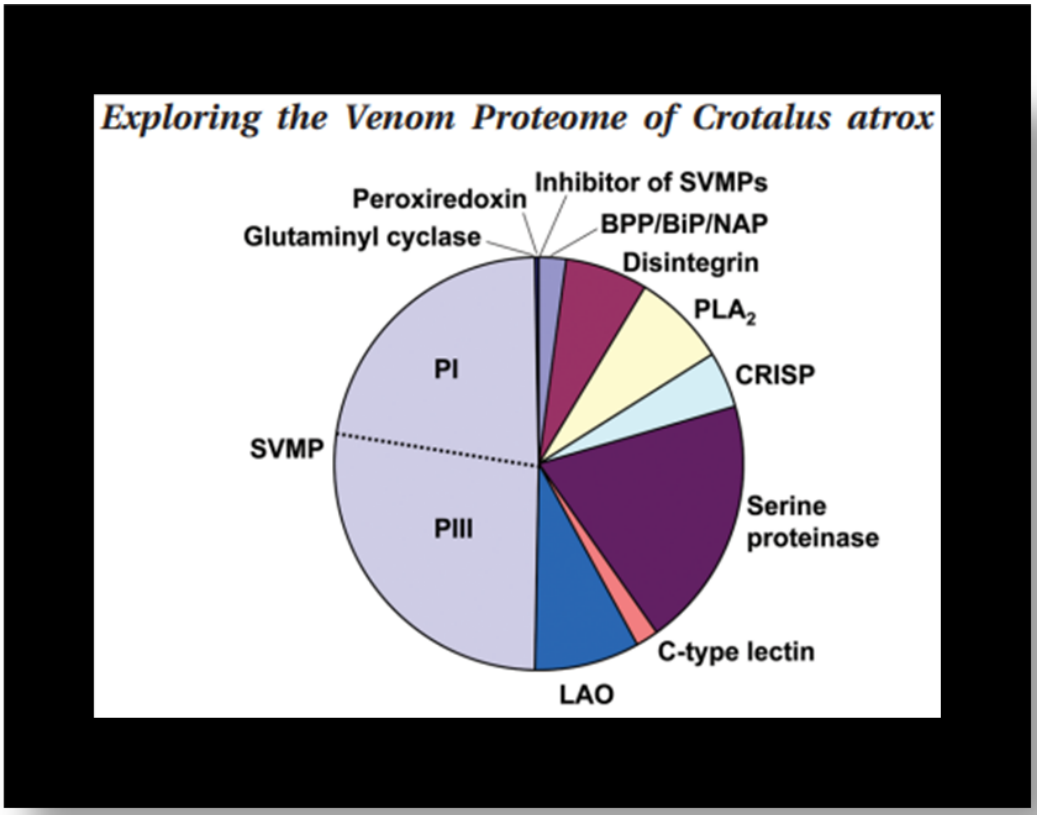

Figure 1: Venom Proteome of Crotalus atrox (Referenced and used with permission from the author)

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Protein Family		% of Total Venom Proteins	Clinical Effect*
Vasoactive peptides	Bradykinin inhibitory peptide	1.1	
	Bradykinin potentiating peptide	0.9	Hypotension
	C-type natriuretic peptide	1	Hypotension
Endogenous inhibitors of SVMPs		trace	
Medium-size disintegrin		6.2	Inhibit platelet aggregation
PLA2		7.3	Inflammation, myotoxicity, abnormal coagulation
CRISP		4.3	Paralysis, pain, convulsion
Serine protease		19.8	Fibrinolysis, platelet aggregation
C-type lectin-like	Gal-specific lectin	1.3	Increased clearance of platelets
	Other C-type lectin-like	0.1	
L-amino acid oxidase		8	Apoptosis (H2O2)
Zn2+ metalloproteinases	PI-SVMP	22.4	Vascular membrane damage: extravasate blood, proteins, fluid
	PIII-SVMP	27.3	
Other proteins	Peroxiredoxin	<0.1	Housekeeping protein
	Glutaminyl cyclase	<0.1	

Table 1: Occurrence of venom components in Western Diamond-backed Rattlesnake. *Information on clinical effects associated with protein families provided by Dr. Maciulewicz. (Table adapted, referenced, and used with permission from the author)

Antidotal therapeutics for treating envenoming have mainly focused on targeting whole venom. This approach certainly has been effective, but our puzzle-solving nature strives for better and more specific therapies (itch finally scratched). Recently, there have been studies focused on direct enzyme inhibition of secretory phospholipase A2 (sPLA2) in oral and intravenous formulations. This is pretty cool for a couple of reasons. One, it counteracts a very specific venom toxin, and two, it offers an easy oral treatment. Other discussions and advances in the scientific community have produced improvement in our current capabilities in benchtop research. We now have machines and methods that have vastly improved detection and characterization of venoms as well as producing reference libraries that help to identify and speculate activity of snake venom. We're getting much closer to pulling off the Venomics-NCIS/Batman fantasy!

Other concepts using a venomics approach include the potential ability to sample venom from the culprit (snake) or blood from the patient. Then, rapidly identifying components of the venom or patient likely responsible for clinical disease and treating it with an array of specific therapies that can be customized for that given patient. However, this requires time, resources, and specific treatments not currently available. There are laboratories with improved turnaround times that can process/characterize snake specific venom in roughly a 24-hour period, but by then you would have ideally treated your patient already. Indeed, science and medicine have come a long way, but one can only imagine what the future holds for the treatment of snake envenoming. One thing is for sure, venomics will indeed help guide us in cracking the code!

Reference: Calvete JJ, et al. "Exploring the venom proteome of the western diamondback rattlesnake, Crotalus atrox, via snake venomics and combinatorial peptide ligand library approaches." *Journal of proteome research* 8.6 (2009): 3055-3067.