Characterization and Profiling of *Crotalus scutulatus scutulatus* Venom Types A and B for Next Generation Antivenom Development

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ABSTRACT

To develop methods of neutralization of snake venom toxins, it is key to comprehend the components of the toxin – or combination of toxin proteins – which are causing the most lethal effects. The data on synergistic mechanisms for snake venom pathology is lacking, notwithstanding individual toxins that have been evaluated, and are necessary in development of an antidote or antivenom. Using *Crotalus scutulatus scutulatus* venom types A and B as a model, as well as for demonstration of differential toxic activity within snakes of the same species, analysis was performed to begin determining a systematic approach for the identification and quantification of the venom from Viperidae and Crotalinae snakes toxicity (toxicity score). It was ascertained that anion exchange DEAE (diethylaminoethyl) exchange was most favorable for the separation of venom in terms of number of fractions, retention of fraction activity, and venom fraction protein composition, using integrated HPLC (high performance liquid chromatography) peak analysis. Both venoms type A and AB were analyzed utilizing the fractions acquired from the integrated HPLC/DEAE anion exchange, then further subjected to SDS PAGE and gelatinase assaying. Further analysis will be pursued by finding the fifty percent lethal dose (LD_{50}) of each fraction deemed relevant, as well as the crude venom itself. This data has begun to shed new light on how toxicity scoring is an influential tool for accounting for the most toxic component(s) of a snake's venom, providing a model to determine priority of the components of which to target.

BACKGROUND AND SIGNIFICANCE

In 2019, the World Health Organization (WHO) released statistics detailing the high amount of cases of snake bites each year (approximately 5.4 million), with 1.2 to 2.7 million of these cases leading to envenomation. Annually, these cases of snake bite envenomation lead to between 81,410 and 137,880 deaths, and three times that range of amputations. While these cases of envenomation cause many detrimental effects in populations worldwide, little is known regarding the mechanism of action of the snakes intrinsic toxic activity and lethal contribution within its venom. This information is key to unlocking new knowledge and developments for this neglected tropical disease and global health issue. Rattlesnake venom is oft characterized by the presence – or lack thereof – of the neurotoxic phospholipase A_2 (such as in Mojave toxin), which increases the toxicity of venom. Populations of the Mohave rattlesnake are characterized based on the presence or absence of the Mojave toxin protein complex, which is the most lethal crotalid peptide isolated as of late, and snakes expressing the Mojave toxin (type A) have been found to exhibit a relative lack – or even total absence – of local tissue effects associated with crotalid envenomings, most notably a particular absence of ecchymosis and tissue necrosis. This 'Type A' Mohave rattlesnake venom is thus highly neurotoxic, and its 'Type B' counterpart – which lacks the key heterodimeric PLA_2 responsible for the Mojave toxin (MTX) – expresses far more snake venom metalloproteinases (SVMPs), creating a highly hemorrhagic venom containing more proteolytic and hemorrhagic venom peptides that the type A does not. 'Type A+B' Mohave rattlesnake venom possesses the phenotypes of both type A and type B venoms, and is encountered less frequently than either of the strictly type A or type B populations – though it is still highly relevant, and offers further exploration into the diversity of the venom composition within this single species.

- Most antivenoms carry a large portion of immunoglobulins that are not directed against venom components (about 70%) (Laustsen et al., 2016)
- Injection of an antivenom is known to trigger both acute and delayed allergic reactions in some people, serum sickness, anaphylactic shock
- **Each vial cost about $2,300 and an initial dose of antivenom starts with 4-6 vials ($13,800)-26-40 vials are usually given ($130,000)**

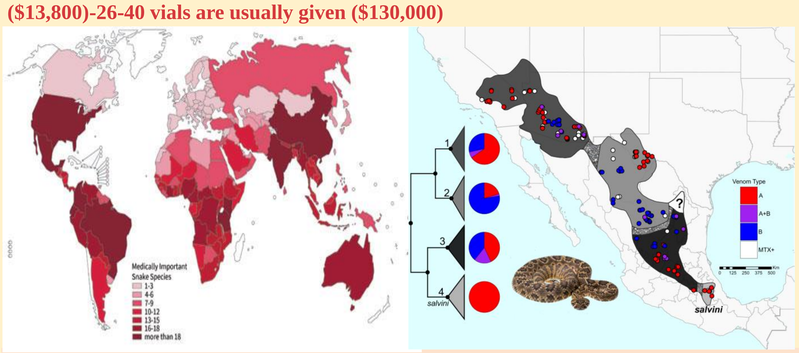

Figure 1. Maps detailing geographical range of the Mohave Rattlesnake and locations of medically significant bites. The World Health Organization (WHO) estimates that about 5 million snakebites occur each year, resulting in up to 2.7 million envenomings. Published reports suggest that between 81,000 and 138,000 deaths occur each year. Snakebite envenoming causes as many as 400,000 amputations and other permanent disabilities. Many snakebites go unreported, often because victims seek treatment from non-medical sources or do not have access to health care. As a result it is believed that many cases of snakebite go unreported.

METHODS AND RESULTS

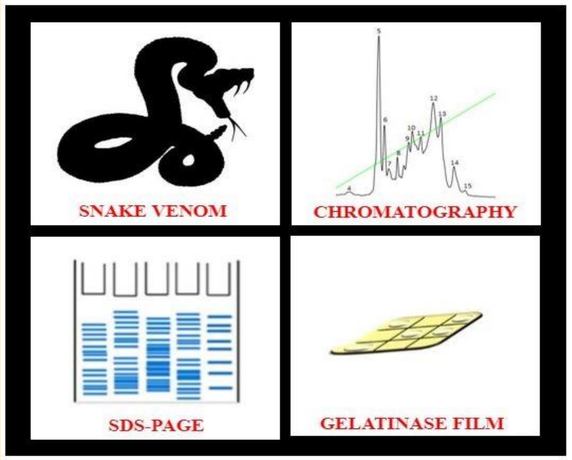

Figure 2. Schematic overview of methods used to characterize venom profile. (A) Lyophilized Mohave Rattlesnake (*C. scutulatus scutulatus*) venom was obtained from the National Natural Toxins Resource Center's John C. Perez Serpentarium, located at Texas A&M University-Kingsville, Kingsville, TX. The type A venom was designated as C. scutulatus scutulatus vial 1088 (Avid# 062-259-038) and the type AB venom was designated as *C. scutulatus scutulatus* vial 931 (Avid# 065-365-053). For both type A and B venoms, 30 mg of lyophilized venom was reconstituted in 600 µL of tris A buffer. (B) DEAE chromatography of crude *C. s. scutulatus* venom type A was performed at a concentration of 8 mg/mL and injection volume of 148 µL. (C) SDS-PAGE analysis of fractionated *C. s. scutulatus* venom type A. A total of 24 µg of samples from fractions one through fifteen through were run on a 4-12% Bis-Tris (MES) Gel (Novex®) at 100 V for 95 minutes, and the gel was stained with SimplyBlue. M: Seeblue® Plus 2 prestained standard (1X). (D) Proteolytic activity of both venom type A and type B were profiled using a gelatinase film.

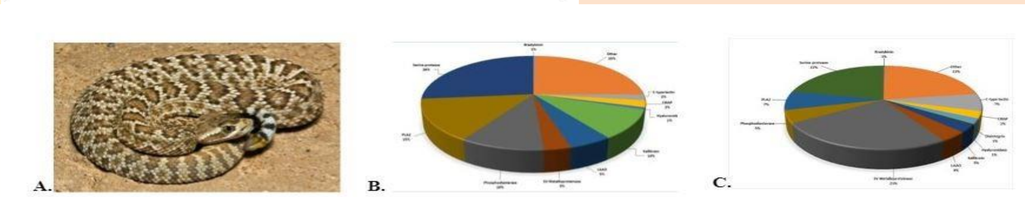

Figure 3. Identification of *C. s. scutulatus* venom components. (A) Mohave rattlesnake *(C. s. scutulatus*). *(*B) Mass spectrometry characterization of type A crude venom. (C) Mass spectrometry characterization of type B crude venom.

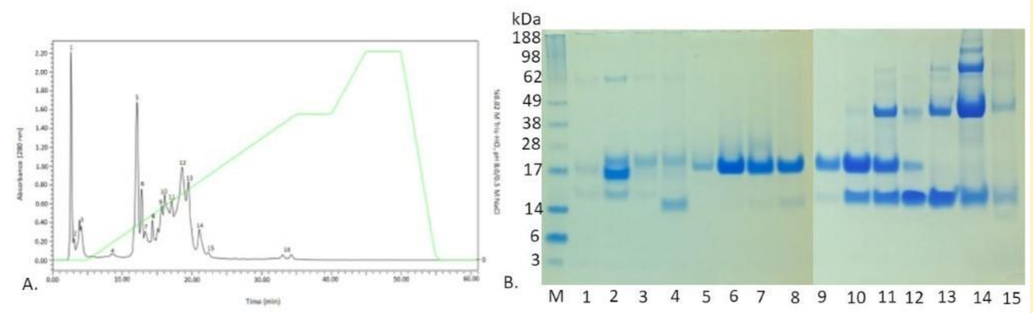

Figure 4. Fractionation and SDS-PAGE Analysis of crude *C. s. scutulatus* venom type A. (A) Integrated HPLC analysis of crude *C. s. scutulatus* venom type A was performed at a concentration of 8 mg/mL and injection volume of 148 µL. (B) SDS-PAGE analysis of fractionated *C. s. scutulatus* venom type A. A total of 24 µg of samples from fractions one through fifteen through were run on a 4-12% Bis-Tris (MES) Gel (Novex®) at 100 V for 95 minutes, and the gel was stained with SimplyBlue. M: Seeblue® Plus 2 prestained standard (1X).

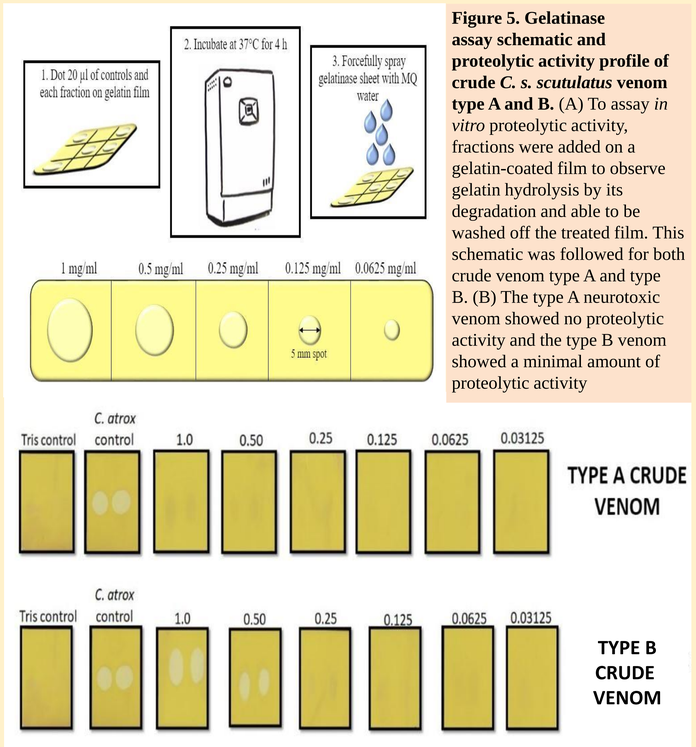

Figure 5. Gelatinase assay schematic and proteolytic activity profile of crude *C. s. scutulatus* venom type A and B. (A) To assay *in vitro* proteolytic activity, fractions were added on a gelatin-coated film to observe gelatin hydrolysis by its degradation and able to be washed off the treated film. This schematic was followed for both crude venom type A and type B. (B) The type A neurotoxic venom showed no proteolytic activity and the type B venom showed a minimal amount of proteolytic activity

DISCUSSION

- There are massive limitations regarding antivenom, including adverse side effects, cost, and effectiveness for species cross-reactivity.
- Toxicity scoring is a novel way of predicting lethality rates of snake envenoming and developing strategies of neutralization using analysis of the most lethal components.
- Venom type A contains the PLA2 MTX, causing it to be incredibly neurotoxic. MTX loans its toxicity to venom type A+B, though this is also a hemorrhagic venom. Venom type B lacks the neurotoxicity that type A and A+B contain, and is more hemorrhagic.

FUTURE PERSPECTIVES

- Toxicity scoring will be paired with artificial intelligence (AI) technology that will be used to predict the lethality rates of snake envenomings.
- More in-depth exploration into the synergistic effects of snake venom will be performed, including sonoclot and EVTRAP analysis for purification, characterization, and neutralization of the most lethal components.
- Production of antibodies and antivenoms will be developed using sharks and chickens as models.

ACKNOWLEDGEMENTS

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REFERENCES

Toxicity Score Based Characterization of *C. atrox* Venom::An Examination of Toxicity Scoring in Complex Enzyme Rich Snake Venom**, (**Sztteiter et. al., 2020).