CHARACTERIZATION OF SOUTHERN PACIFIC RATTLESNAKE VENOMS (*CROTALUS OREGANUS HELLERI*): EXAMINATION OF TOXICITY SCORE

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Abstract

Envenomation by the Southern Pacific Rattlesnake are the most common snakebite accidents in Southern California and although there are two commercially available antivenoms (CroFab® and Anavip®) for rattlesnakes that help neutralize snake venom, intraspecies venom variation may lead to unresponsive antivenom therapy. More importantly, some Southern Pacific Rattlesnake venom can have a neurotoxin, which can quickly attack the nervous system leading to paralysis or death. C. *helleri* 677's fractions were tested on lethality. The results show the most toxic fraction were 1,7,9, and 10 respectively. These fractions had a toxicity score of 2.80,2.70,26.26,26.18 With further research, the components of these fractions can be identified and provide a model in which priority can be given for antidote targets.

Introduction

According to the World Health Organization statistics, it was estimated that in 2018 there were 81,000-138,000 new deaths world-wide due to snake envenomation. Snake venoms are made up of numerous diverse components which include enzymatic molecules.

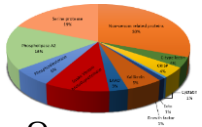


Figure 1: C. *helleri* 677 venom composition was analyzed by mass spectrophotometry.

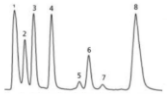
One new approach that was used to study snake venom is the use of a Toxicity Score defined by the % of protein fraction abundance divided by the LD50 value. By giving individual anion exchange fractions a Toxicity Score and identifying individual proteins within the fractions, it can provide a more precise account of venom toxicity and determine the most toxic components to be targeted for neutralization

Methods

1. Collect snake venom

Lyophilized Southern Pacific Rattlesnake (*C. helleri*), venom was obtained from the National Natural Toxins Research Center serpentarium

2. Fractionate venom



Anion Exchange Chromatography DEAE

3. Gelatinase assay

Test for proteolytic activity

4. Lethality assay

Injected 100ul of crude venom and fractions 1,7,9, and 10 over a 48-hour period to determine the LD50 for each fraction.

Results

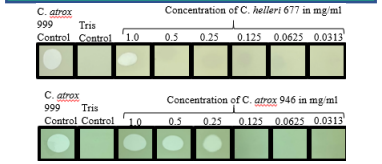


Figure 2: Gelatinase assay for C. *helleri* 677.
Kodak X-OMAT scientific imaging film with a gelatin coating, weas soaked with Milli-Q water for 30 min in 37 °C incubator prior to use. 20 µL of each sample was added to the film and incubated at 37 ˚C for 4 h and then washed with distilled water. A clear spot on the film represented hydrolysis of the gelatin and the results were quantified by determining the minimal gelatinase dose (MGD) or the minimal amount of sample used to cause hydrolysis

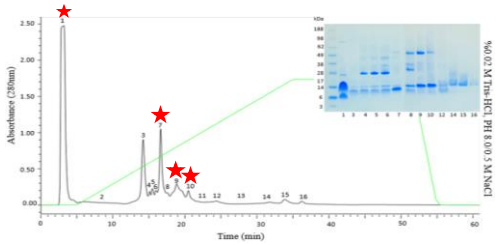


Figure 3: Anion Exchange Chromatography 200ul (30 mg/mL) of venom was injected into a DEAE column. The column was equilibrated with 0.02 M Tris-HCl buffer, pH 8.0, and the fractions were eluted using 0.02 M Tris-HCl buffer containing 0.5 M NaCl, pH 8.0, over a period of 60 min with a flow rate of 1ml/min. Breeze 2 computer software system was used to generate the chromatogram.

Table 1: Toxicity score for the crude venom and the four most toxic components of C. *helleri* 677 were calculated

Fraction	% Area[1]	LD50 (mg/kg)	Toxicity Score[2] % Area/LD50
Crude venom	100	0.06	166.67
1	42.05	15.04	2.80
7	12.72	4.71	2.70
9	9.19	0.35	26.26
10	4.45	0.17	26.18

Conclusion

- After determining the LD50 for each toxic fraction, the toxicity score was calculated. From highest to lowest, the Toxicity Scores were, 26.26 (fraction 9), 26.18 (fraction 10), 2.80 (fraction 1), 2.70 (fraction 7).
- We suspect, based on the molecular weight, that these fractions contain Mohave toxins and isoforms

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References

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