Identification and Characterization of C-type Lectin Isolated from the Venoms of *Crotalus scutulatus scutulatus* and *Crotalus atrox* and Its Effects on Endothelial Permeability

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TEXAS A&M UNIVERSITY KINGSVILLE® McNAIR SCHOLARS PROGRAM

NATIONAL NATURAL TOXINS RESEARCH CENTER (NNTRC)

Abstract

Snake venoms are composed of an array of toxins that serve multiple functions to ultimately aid in prey capture and digestion. The venoms of North American vipers contain ubiquitous toxins that can cause rapid local effects (e.g., tissue death, excessive bleeding, and swelling) that graduate in severity over time. If treatment is not received promptly after envenomation occurs, this cascade of events can result in the surgical removal of damaged tissue, paralysis, amputation, or death. Limited research has been conducted on the acute endothelial dysfunction caused by non-enzymatic toxins in the venoms of North American snakes. Nonenzymatic C-type lectins (CTLs) have been found to activate the integrin receptors on endothelial cells, leading to the release of proinflammatory cytokines. This, in turn, can result in cytoskeletal remodeling and increased vascular permeability. **The purpose of the present study is to identify and characterize the nonenzymatic components of *Crotalus scutulatus scutulatus* and *Crotalus atrox* venoms, specifically CTL, that could majorly contribute to the acute local effects of viper venoms.** We attempted to isolate CTLs from the venoms of *C. s. scutulatus* and *C. atrox* using immobilized D-galactose gel. While CTL from *C. s. scutulatus* has yet to be found, preliminary results showed that purified CTL from *C. atrox* venom increases HDLEC permeability. We will further isolate CTL from *C. s. scutulatus* and identify it using N-terminal sequencing. *C. s. scutulatus* CTL will then be tested for its effect on cell permeability of endothelial cells.

Introduction

Snake bite envenomation is considered a neglected tropical disease by the WHO[1]. It claims between 80,000 and 140,000 lives every year[1]. The potency of snake venoms is largely due to complex proteins and peptides that have a wide variety of effects and targets[2].

This study focused on toxins' effects on endothelium, as endothelial cells serve as a barrier between our tissues and bloodstream, and regulate the exchange of substances between them[3,4]. The permeability of endothelium will increase naturally for several reasons, including angiogenesis and response to injury (edema)[3]. Previous research has shown that the toxins which disrupt endothelial function serve as a key to the introduction of foreign substances into the blood stream[5].

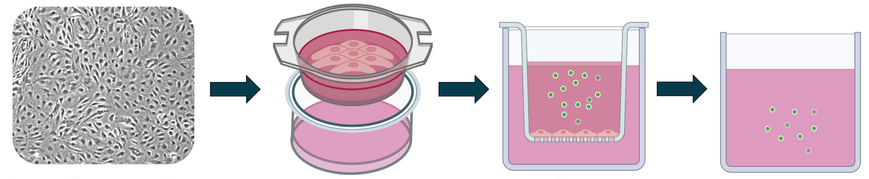
The venom of North American Vipers is highly variable but contains 10-20 ubiquitous protein families that may be enzymatic or nonenzymatic[6]. While the enzymatic toxins in snake venom have been widely characterized regarding barrier disrupting effects[5,7,8], nonenzymatic toxins are understudied. This led to the selection of CTLs, nonenzymatic ubiquitous proteins in the venom of Crotalus species[6], as the topic of this research.

Methods

Purification and Identification of CTLs

D-Galactose Affinity Column | SDS-PAGE | N-terminal Sequencing

Endothelial Permeability Assay



Culture Endothelial Cells | Seed | Treat | Measure

Results

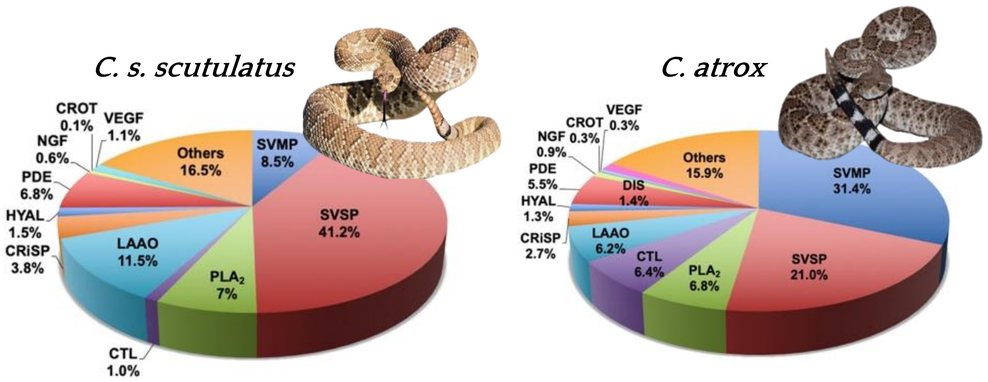
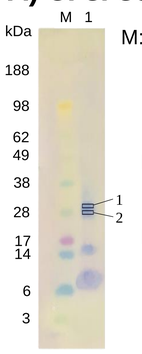


Figure 1. Venomic profiles of CTLs from the venom of two crotaline snakes.[10]

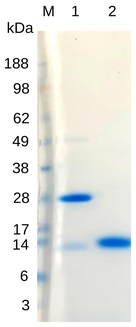
A) *C. s. scutulatus venom* elution



M: marker

	Identification
Band 1: 29 kDa (non-reducing)	Serine protease
Band 2: 28 kDa (non-reducing)	Serine protease

B) *C. atrox* venom elution



	Identification
Lane 1: 28 kDa (non-reducing)	Dimeric CTL
Lane 2: 14 kDa (reducing)	Dimeric CTL

Figure 2. SDS-PAGE of elution fraction of the venoms of *C. s. scutulatus* (A) and *C. atrox* (B) fractionated from D-galactose affinity column.

Preliminary results show that the venoms of *C. atrox* (blue) and its purified CTL (green) increase endothelial permeability.

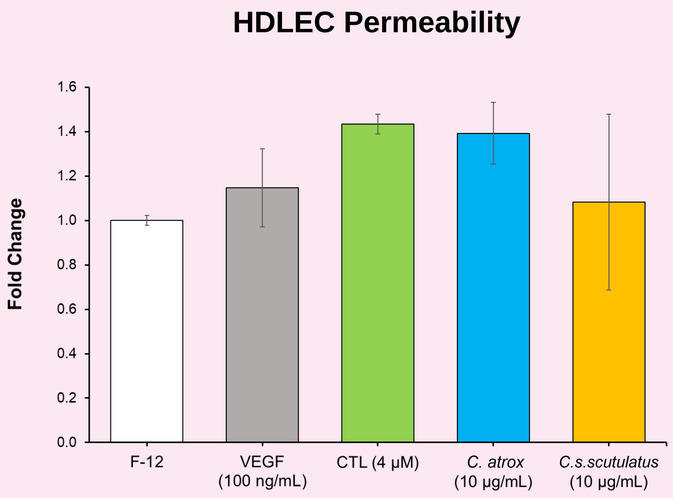


Figure 3. Preliminary permeability assay results. From left to right: F-12 culture media (negative control); vascular endothelial growth factor (VEGF; 100 ng/Ml; positive control); CTL from *C. atrox* (4 µM); *C. atrox* crude venom (10 µg/mL); *C. s. scutulatus* crude venom (10 µg/mL).

Discussion

- CTL will be further isolated and identified from *C. s. scutulatus* venom.
- We successfully isolated CTL from the venom of *C. atrox*.
- Limitations: Human error due to inexperience, not enough time to test with both cell types as human endothelial cells have a low growth rate

Future research

- Perform endothelial permeability of purified CTLs on human dermal blood endothelial cells (HDBECs).
- Determine the extent to which barrier-disrupting toxins play a role in greater injury from snake bite
- Characterize the signaling pathways by which toxins affect endothelial permeability

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Acknowledgements

Funding for this project was made possible by the Texas A&M University – Kingsville Ronald E. McNair Scholars Program, the NIH/AREA, NIH/NHLBI grant #2R15HL137134-02 (Texas A&M University-Kingsville, Dr. M. Suntravat), NIH/ORIP, Viper Resource Grant #5P40OD010960-19 (NNTRC, Texas A&M University-Kingsville, Dr. E.E. Sánchez), the Spring 2023 Research Support, the Dick and Mary Lewis Kleberg College of Agriculture and Natural Resources, and the Robert A. Welch Foundation Department, grant# AC-0006 (TAMUK-Department of Chemistry). We would also like to thank Nora Diaz DeLeon, Mark Hockmuller, Juan J. Salinas, and all the NNTRC personnel.