The Effect of Snake Venom CRiSPs on the Production of Cytokines in Human Dermal Blood and Lymphatic Endothelial Cells

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Introduction

Snake venoms are very complex mixtures composed of several proteins each playing a different role in envenomation. Snake venom cysteine-rich secretory proteins (svCRiSPs) are widespread in snake venoms. svCRiSPs are known to inhibit ion-channels, smooth muscle contraction, and the growth of new blood vessels. They also induce pro-inflammatory responses. Yet, little is known of the contribution that they make to the local pathophysiology of snakebite. We have recently demonstrated that svCRiSP from the Southern Pacific rattlesnake, *Crotalus oreganus helleri* increase vascular permeability *in vivo* and cell permeability *in vitro*. However, the mechanism of action is still unknown. A svCRiSP isolated from the venom of *Bothrops jararaca*, Bj-CRP has been shown to induce inflammatory responses in mice with an increase of neutrophils and the production of IL-6. In addition, pro-inflammatory cytokines (such as IL-6 and TNF-α) are secreted by cells, including endothelial cells, and mediate endothelial activation and increased endothelial cell permeability.

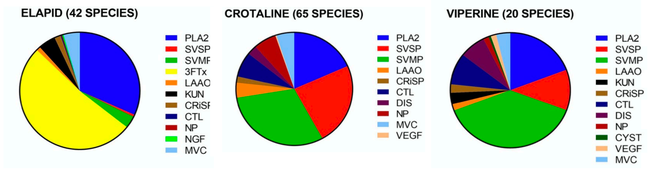


Figure 1. Snake venom is a highly complex and diverse cocktail of different protein. (Tasoulis et al., 2017)

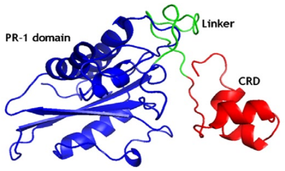


Figure 2. *Naja atra* CRiPS molecule consists of three parts: PR-1 domain (blue), CRD (red), and a linker (green). (Wang et al., 2006)

The objective of this study is to investigate the biological activities of svCRiSPs isolated from North American snakes (*Crotalus scutulatus scutulatus* and *Agkistrodon piscivorus piscivorus*) on the function of the blood and lymphatic vessels and in the induction of cytokine production (IL-6 and IL-8) in human dermal blood lymphatic cells (HDLECs) and human dermal blood endothelia cells (HDBECs). These studies will provide insight and a better understanding of the mechanism of action svCRiSPs on pro-inflammatory responses and the role they play in the pathophysiology of snake bites.

Vascular Permeability & Cytokine

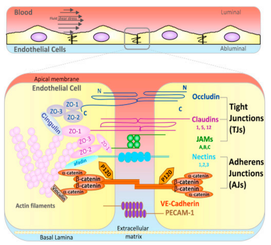


Figure 3. The endothelium is a semi-permeable barrier that lines the vasculature and regulates fluid and solute exchange between the blood and interstitial space. (Cerutti et al., 2017)

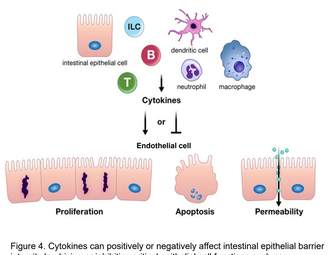
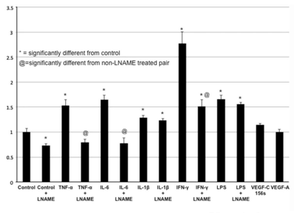


Figure 4. Cytokines can positively or negatively affect intestinal epithelial barrier integrity by driving or inhibiting critical epithelial cell functions such as proliferation, apoptosis, and appropriate epithelial barrier permeability. (Andrews et al., 2018)



All listed cytokines (TNF-α, IL-6, IL-1β, INF-γ, and LPS) increased permeability on the Lymphatic endothelial cell monolayers.

Figure 5. An increase in cell permeability on the LEC monolayer in response to several cytokines. (Cromer et al., 2013)

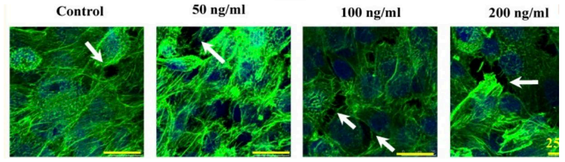
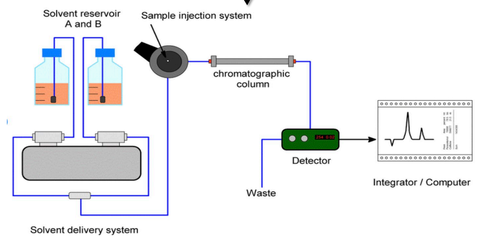


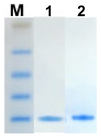
Figure 6. Cultured cells treated with 0 (as controls), 50, 100 and 200 ng/ml IL-8 for 4h were stained with F-actin, and examined by laser confocal microscopy. The white arrows in figures showed visible cell-cell gaps formation. Scale bar in all images = 25 μm. (Yu et al., 2013)

Methods

Snake Venom Extraction

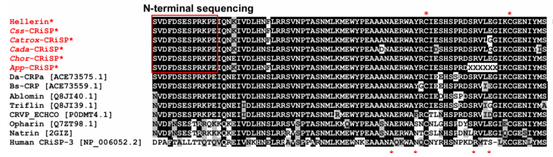


Purification and Identification



Lane	CRiSPs	MW
M	Marker	3
1	Css-CRiSP	24.8
2	App-CriSP	26.6

SDS–PAGE



N-Terminal Sequences

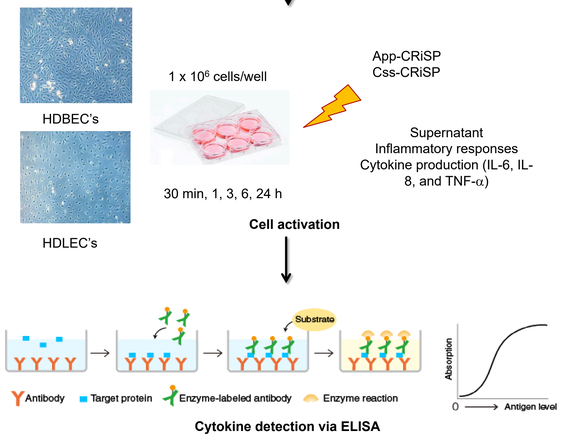


Figure 7. Purification steps following SDS-PAGE, cell activating and Cytokine production via ELISA

Results

Css-CRiSP induced production of IL-6 at 6 and 24 h in HDLECs (A) and App-CRiSP increased production of IL-6 at 6 and 24 h in HDBEC (B) in comparison to the control. Css-CRiSP and App-CRiSP increased production of IL-8 at 3 and 24h in HDLEC (C) and Css-CRiSP and App-CRiSP increased the production of IL-8 at 6h in HDBEC (D).

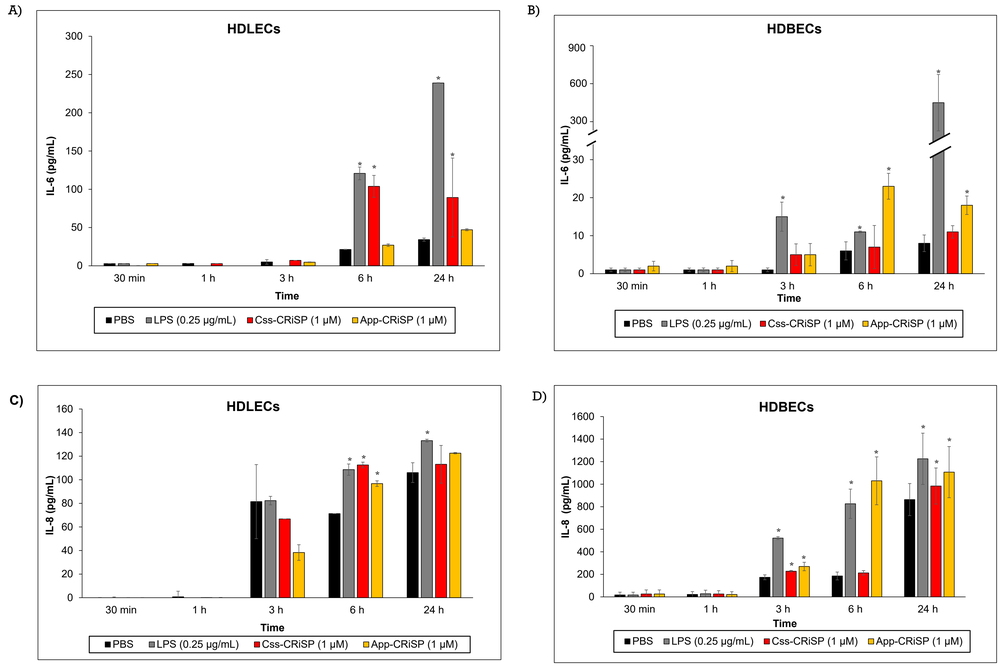


Figure 8. The effect of Css-CRiSP and App-CRiSP in the production of IL-6 and IL-8 in HDLECs (A and C) and HBECs (B and D) using ELISA. Cells were stimulated with Css-CRiSP and App-CRiSP (1 μM) and the culture supernatants were collected at various incubation times. Cytokines in the supernatants were measured by a sandwich ELISA, according to the manufacturer's suggested protocols. Data expressed as mean ± SD of two individual experiments (n = 2). *p < 0.05, compared with untreated control.

Conclusion

- Our results suggest that Css-CRiSP and App-CRiSP has an effect on cytokine production as early as 6 h for IL-6 in HDBECs and HDLECs and IL-8 as early as 3 h for both HDBECs and HDLECS.
- The production of TNF-a showed no increase at the various incubation times.
- The effects of svCRiSPs on the production of cytokine release may not be the cause of vascular permeability at 1h.
- svCRiSPs can activate the release of IL-6 and IL-8 in endothelial cells, which could enhance the pathophysiology of snake bites.

Acknowledgements

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