Oklahoma State University – Center for Health Sciences / Biochemistry

ANAVIP® INTERACTION WITH WESTERN PYGMY RATTLESNAKE VENOM: IN VITRO ASSESSMENT OF REACTIVITY USING SE-HPLC

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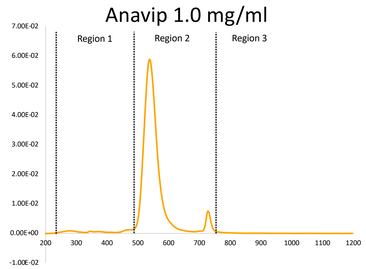
INTRODUCTION

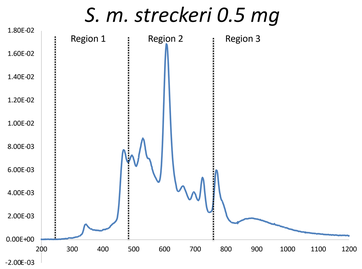
Every year there are a large number of venomous snake bites that occur around the world and especially in tropical areas. This is a problem that is faced worldwide with the World Health Organization classifying venomous snake bites as one of their highest priority neglected tropical diseases. One of the reasons for this classification is the short supply of antivenom compared to the number of snake envenomations that occurs each year. The standard of care for snake envenomation is administration of antivenom. Many antivenoms are polyvalent in that they are produced using venoms from multiple species of snakes. These polyvalent antivenoms can treat envenomation from the snake venoms that are used in the production, but also show cross-reactivity against snake venoms that share similar components. Determining the cross reactivities of antivenoms could help improve the quality of treatment, and provide a better understanding of venom-antivenom binding. Until recently there has only been one antivenom available for treatment of North American Crotaline envenomation. With the introduction of an $F(ab')_2$ antivenom (Anavip®) into the United States, we look at the cross-reactivity of the western pygmy rattlesnake, *Sistrurus miliarius streckeri (S. m. streckeri),* against Anavip.

METHODS

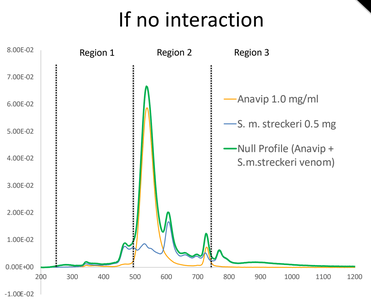
SE-HPLC was used to assess cross-reactivity. SE-HPLC is a viable method to analyze antivenom-venom reactivity based on separation of higher molecular weight complexes that form vs unreacted components. Estimates of venom-antivenom reactivity was measured in reaction mixtures based on the increase in the elution profile area where higher molecular weight complexes are observed (region 1) and on the decrease in the elution profile area where reactants are observed (region 2). Reaction mixtures contained Anavip (1.0 mg/ml) and *S. m. streckeri* venom (0.125, 0.25, 0.5, or 1.0 mg/ml). Controls were Anavip and *S. m. streckeri* (1.0 & 0.5mg/ml). Mixtures were incubated at 37° C for 30 minutes, then stored at 4° C prior to SE-HPLC.

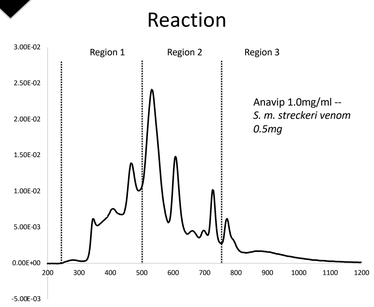
RESULTS



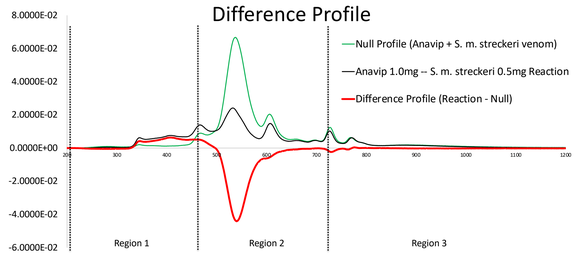


Profile of control reactants taken at AU 214nm shown on the left. Elution profiles divided into Regions 1, 2, & 3 to show changes in changes in composition between reactants and products.



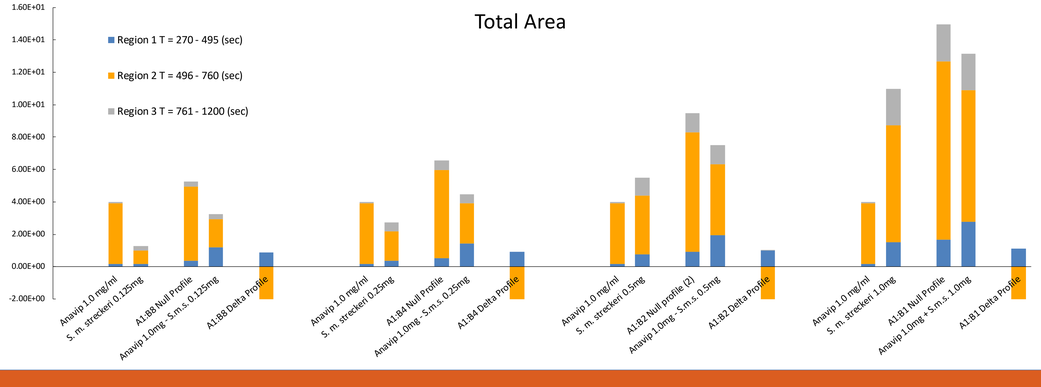


The graphs on the Left show elution profiles if no reaction had occurred and the elution profile of the reaction between Anavip and *S. m. streckeri* venom. Changes in the elution profile are seen in the reaction compared to the null, showing cross-reactivity of Anavip to the venom.



The difference elution profile shows the increase in Region 1 (immune complexes) and the decrease in the Region 2 (reactants). These changes were observed at all venom-antivenom concentration.

The maximum venom-antivenom binding was calculated, base on changes in the profile region areas, to be approximately 67% relative to the total area of the antivenom profile.



CONCLUSION

Apparent saturation of reactive antivenom was observed at all venom concentrations. Estimates of Anavip reactivity with *S. m. streckeri* venom are seen in the changes of the elution profile region areas, showing the formation of larger molecular weight complexes and decrease in reactants. This shows that Anavip could provide protective effects against *S. m. streckeri* envenomation. Further studies are needed to determine binding within a broader range of venom concentrations, as well as the composition of reactive and unreacted components. Results suggest that binding of Anavip to *S. m. streckeri* venom does occur, which is consistent with protective effects that are observed clinically.

Discussion

Decreases in the total profile areas are seen between the null profiles and reaction profiles. This is consistent with previous studies with $F(ab')_2$ antivenoms. We suspect this decrease in total profile area is due to the antivenom binding with multiple venom components creating high molecular weight immune complexes that do not enter the column (i.e. insoluble in elution buffer). Despite the decrease in the total profile areas we are still able to determine relative binding of Anavip and observe reactivity between Anavip and *S. m. streckeri* venom.

ACKNOWLEDGEMENTS

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- *S. mliarius streckeri* venom from Texas A&M serpentarium.