CROSS-REACTIVITY OF VENOMS AND ANTIVENOMS DETERMINED *IN VITRO* USING SIZE-EXCLUSION CHROMATOGRAPHY

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Abstract

Background: Size-exclusion chromatography (SEC) can be used to characterize venom-antivenom immune complex formation. Complex formation would be expected between antivenom and venom used in the antivenom production process. Binding of antivenom with venom not used in the production process might be predicted due to similarities of venom composition, but would typically be confirmed by standard assays, such as inhibition of venom lethality or neutralization of venom toxicity. Evaluation of complex formation is relevant since venom-antivenom binding is required for neutralization of venom lethality or toxicity, though binding does not guarantee protection. SEC may be a useful tool in comparing the binding of different antivenoms to a particular venom, as well as binding of different venoms to a particular antivenom. The data presented in this study demonstrates the use of SEC to evaluate the cross-reactivity of two distinctly different antivenoms with venoms used in antivenom production.

Materials:

Antivenoms:

Ovine Fab (FabAV);
Crotalidae Polyvalent Immune Fab (Ovine); (CroFAB™, Protherics Inc., Brentwood, TN, USA); $M_r \approx 50$ kDa; one binding site/molecule.

Equine F(ab')$_2$ [F(ab')2AV];
Bothropic antivenom (Butantan Institute, Sao Paulo, SP, Brazil); $M_r \approx 100$ kDa; two binding sites/molecule.

Venoms:

Crotalus atrox (western diamondback rattlesnake, Sigma, St. Louis, MO, USA); one of four venoms used in FabAV production.

Bothrops jararaca (*via* Rita Collaço, State University of Campinas, Campinas, SP, Brazil); one of five venoms used in F(ab')2AV production.

Reaction mixtures:

A. FabAV: *C. atrox* venom (Binding - expected)	**B.** F(ab')2AV: *B. jararaca* venom (Binding - expected)
C. F(ab')2AV: *C. atrox* venom (Binding - expected?)	**D.** FabAV: *B. jararaca* venom (Binding - expected?)

Crotalus atrox
(western diamondback rattlesnake)

C. adamanteus
(eastern diamondback rattlesnake)

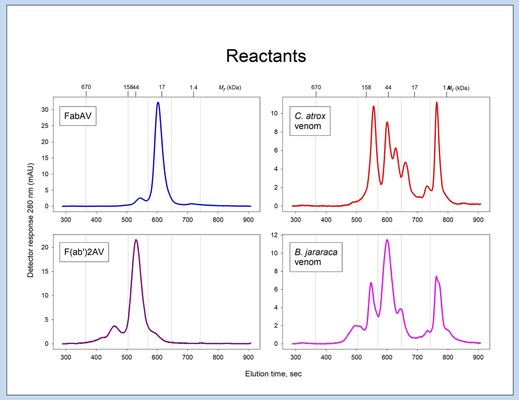
C. scutulatus scutulatus
(Mohave rattlesnake, venom A)

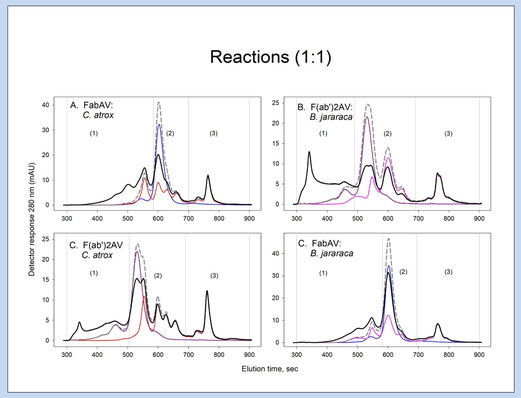
Agkistrodon piscivorus piscivorus
(eastern cottonmouth)

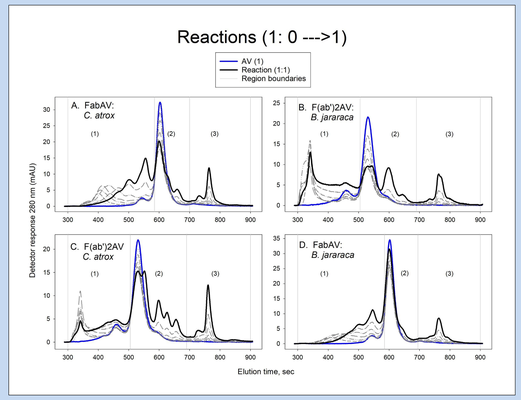
Bothrops jararaca (50%)
B. alternatus (12.5%)
B. neuwiedi (12.5%)
B. jararacussu (12.5%)
B. moojent (12.5%)

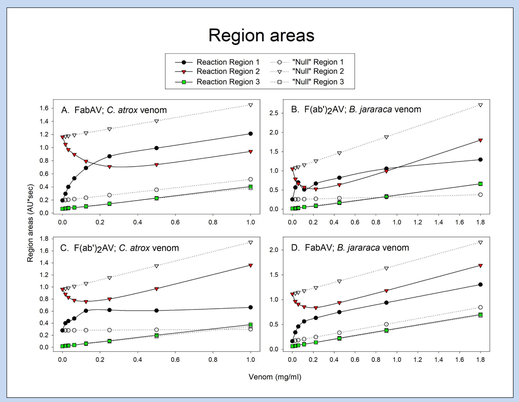
F_{ab} antivenom (FabAV)

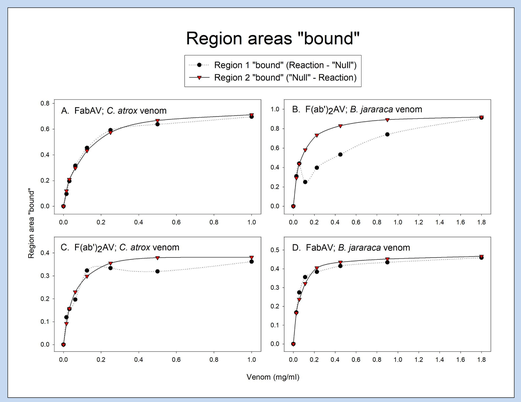
$F(ab')_2$ antivenom [F(ab')2AV]

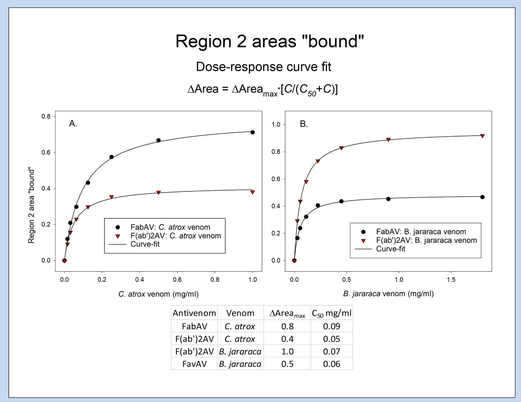












Antivenom	Venom	$\Delta Area_{max}$	C_{50} mg/ml
FabAV	*C. atrox*	0.8	0.09
F(ab')2AV	*C. atrox*	0.4	0.05
F(ab')2AV	*B. jararaca*	1.0	0.07
FavAV	*B. jararaca*	0.5	0.06

Methods: Venom, antivenom, and venom-antivenom mixtures were prepared at 4ºC in 50 mM sodium phosphate, pH 7.0, containing 0.15 M NaCl (column elution buffer) and incubated for 30 minutes at 37ºC. (Samples were stored at 4ºC prior to SEC.) Samples (20 μL) were injected into the SEC column (TSKgel G3000SWxl 7.8 mm ID x 30 cm, 5 mm, TOSOH Bioscience) at a constant flow rate of 1 mL/min. Elution profiles were monitored using a photodiode array detector (Waters). Three regions within the elution profiles were chosen for integration based on comparison of control and venom-antivenom mixture profiles. Venom-antivenom binding was estimated from differences between control and venom-antivenom mixture region areas (i.e. ΔArea). Concentration-dependent changes in ΔArea were fit to a hyperbolic dose-response function (Eq.1) to estimate $\Delta Area_{max}$ (maximum binding) and C_{50} [effective concentration of reactants (C) at one-half $\Delta Area_{max}$)].

$$\Delta Area = \Delta Area_{max} \cdot [C/(C_{50}+C)] \qquad \text{Eq. 1}$$

Venom and antivenom reactants and venom-antivenom mixtures were comprised of: (1) *C. atrox* venom, FabAV; (2) *C. atrox* venom, F(ab')2AV; (3) *B. jararaca* venom, F(ab')2AV; and (4) *B. jararaca* venom, FabAV.

Results: Complex formation was apparent in all four combinations of venoms and antivenoms. *C. atrox* venom binding to FabAV was greater than to F(ab')2AV. *B. jararaca* venom, however, bound to F(ab')2AV greater than to FabAV. Venoms used in antivenom production tended to bind preferentially to the respective antivenom product. Concentrations of venoms at one-half $\Delta Area_{max}$ (C_{50}) were similar for all four combinations of venom and antivenom.

Conclusion: The data presented in this study demonstrate the use of SEC to evaluate the binding of different antivenoms to a particular venom, as well as binding of different venoms to a particular antivenom. SEC may have application in evaluating cross-reactivity of different types of antivenoms and venoms.

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