

***In vitro* neutralization with trypsin  
or rosmarinic acid reduces toxicity  
of *Micrurus Fulvius* venom**

**Jennifer Parker-Cote, Kori L Brewer, Dorcas  
O'Rourke, Matthew Rosenbaum, Susan N Miller,  
William J Meggs**

**Brody School of Medicine at East Carolina University**

# Background

- Antivenom is the definitive treatment for snakebites
- Antivenom is expensive, difficult to manufacture, & not available in many parts of the world
- More economical and readily available treatments for toxic bites and stings could be helpful

# trypsin

- Inexpensive, readily available proteolytic enzyme.
- Many venom toxins are proteins.
- Prior studies are contradictory.
  - Effective, mouse & dog, cobra venom (Ching-yen)
  - No efficacy, tiger snake (Broad)

# Rosmarinic Acid

- plant derivative
- phospholipase A<sub>2</sub> inhibitor
- Phospholipase A<sub>2</sub> component of coral snake venom
- Potentiates antivenom, *Bothrops* (Ticli)

# Objective

- **To evaluate the effectiveness of trypsin & rosmarinic acid, in neutralizing the toxic effects of coral snake venom after incubation prior to injection with coral snake venom in the murine model.**
- **Useful approach in anti-venom studies to screen for efficacy.**

# Materials

- Coral snake venom was obtained from Medtoxin Venom Lab (Delland, FL)
- Trypsin & rosemarinic acid were obtained from Sigma Aldrich (St. Louis, MO).

# Gel electrophoresis

- Preliminary investigation
- To determine the doses of trypsin that successfully degraded the venom protein.

# subjects

- Fifty CD-1 mice
- 20-30 g
- premedicated with buprenorphine (0.1mg, s.c.) to limit pain and distress of injections



# Study groups

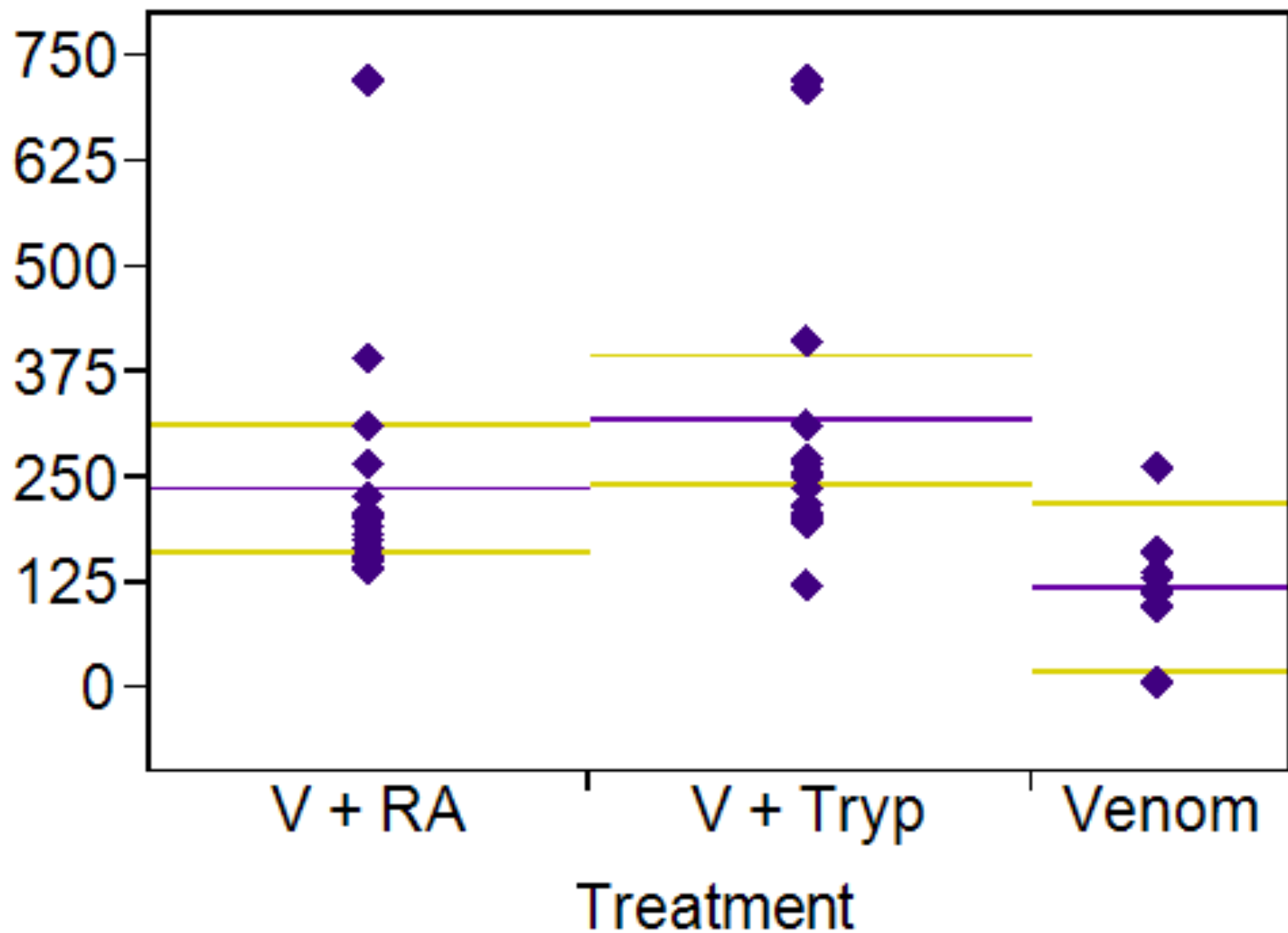
- **Venom alone (2mg/kg; n =10)**
- **Trypsin-venom mixture (n=17)**
  - 2 mg/kg of a 0.2 mg/mL incubated *in vitro* for 1 hour at room temperature (22° Celsius) with 1 mg trypsin in 0.1 mL prior to intraperitoneal (IP) injection
- **RA-venom mixture(n=17)**
  - 2 mg/mL of rosmarinic acid was incubated *in vitro* for one hour with 2 mg/kg of venom at a 1:10 ratio of venom:rosmarinic acid
- **RA alone (n = 3)**
- **Trypsin alone (n=3)**

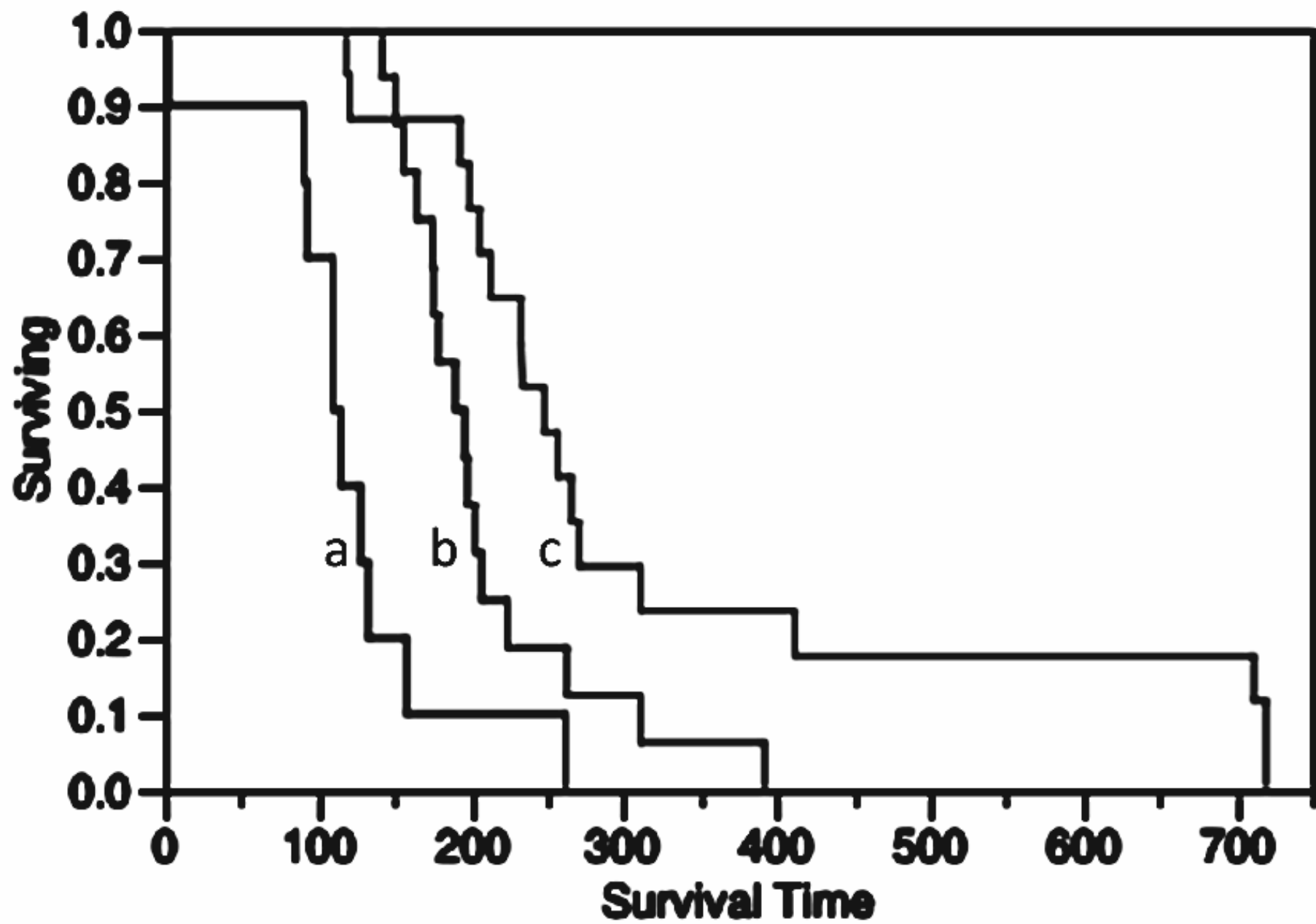
# Study Endpoint

- **Time to toxicity** [respiratory depression ( $< 25$  breaths/min), loss of spontaneous locomotor activity, and/or inability to upright self]
- Measured by an observer blinded to study group
- Animals either spontaneously expired or were euthanized per animal use protocol once signs of toxicity developed.
- Observation period 12 hours.

# Results

Time to Sacrifice  
(minutes)





# Results

- Pre-incubation of the venom with trypsin (V +Tryp) significantly increased the survival time compared to control (venom alone) mice ( $319.7 \pm 201.0$  vs.  $120.3 \pm 64.4$  min;  $p=0.007$ ).
- RA provided a non-significant increase in survival time compared to controls ( $238.1 \pm 139.2$  min;  $p=0.15$ ).

# Conclusion

- ***In vitro* pre-incubation of trypsin with *Micrurus fulvius* venom significantly increased time to toxicity in mice.**
- ***In vitro* pre-incubation of rosmarinic acid with *Micrurus fulvius* venom increased time to toxicity in mice (Not significant).**
- **This preliminary study justifies progressing to an *in vivo* model of trypsin and *Micrurus fulvius* in an *in vivo* model.**

# Further Study: *In vivo* Treatment

- Wyman Cabaniss: trypsin, brown recluse spider venom, guinea pigs
  - Completed, December 2013
  - Presentation at SAEM
- Jennifer Parker-Cote: trypsin, in vivo model, swine, coral snake
  - Completed March 2014
  - 2013-2014 EMF/ACMT resident research grant
  - Toxicology fellow, UVA next year
  - Submission for fall presentation



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