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

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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 $A_2$ inhibitor
- Phospholipase $A_2$ 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
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• Pre-incubation of the venom with trypsin (V +Tryp) significantly increased the survival time compared to control (venom alone) mice (319.7 ± 201.0 vs. 120.3 ± 64.4 min; p=0.007).

• RA provided a non-significant increase in survival time compared to controls (238.1 ± 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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References