Neutralization with proteolytic enzyme and a phospholipase A2 inhibitor reduces the toxicity of Mierurus fulvius venom

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ABSTRACT

Objective: Antivenom is expensive and not available in some countries. Coral snake antivenom is unavailable in the United States. Effective inexpensive treatments are needed. The effects of trypsin, a proteolytic digestive enzyme, and rosmarinic acid (RA), a plant derivative found in M. Fulvius venom were evaluated in a novel model in future studies of coral snake envenomation. (A2 with venom incubated in vitro for 1 hour prior to injection with RA at a 1:10 ratio) In vivo M. Fulvius venom incubated in vitro for 1 hour prior to injection with 1mg of trypsin (n=17); 1mg trypsin IP without venom (n=7); and 4) RA IP without venom (n=7). Animals were observed continuously for 12 hours and assessed for signs of toxicity including respiratory distress (<25 breaths/min.), loss of spontaneous locomotor activity and/or inability to upright self. Animals were euthanized at the onset of toxicity, as determined by a blinded observer. Comparison of time to euthanasia across groups was performed using Tukey-Kramer HSD. The proportion of animals surviving to 4, 6, and 12 hours was compared across groups using Chi-square analyses. Results: Time to euthanasia for controls (venom alone) was 120.3 min. Pre-incubation of the venom with RA significantly increased time to sacrifice vs. controls (238.1 min.; p=0.15). Pre-incubation with trypsin significantly increased survival time vs. controls (319.7 min.; p=0.007). Pre-incubation of venom with trypsin increased the number of animals that survived to 4 hours vs. controls (p=0.025). This effect was not seen with RA. The proportion of mice in the trypsin group and 12 hours was similar across groups (p=0.37, respectively). Two mice in the trypsin group and 1 mouse in the RA group survived to 12 hours. Conclusion: In vivo neutralization of M. Fulvius venom by trypsin justifies progressing to an in vivo model in future studies.

INTRODUCTION

Poisonous snakes are found worldwide and account for considerable mortality and morbidity. In both the US and developing countries, antivenom is not always available. Therefore, an effective inexpensive treatment is needed to prevent mortality worldwide. Due to the nature of the coral snake venom, proteolytic enzymes and phospholipase A2 inhibitors represent potential treatments for envenomation.

Rosmarinic acid (RA), a plant derived found in Cordia vereneaea and Pectranthus barbaeus, acts as a phospholipase A2 inhibitor. RA has been shown to potentiate the neutralizing and antinymotoxic effects of antivenom with Bothrops, envenomations [1,2], and to decrease dependently inhibit hemorrhage from envenomation by the Okinawa habu snake (Trimeresurus flavoviridis) [3].

Trypsin is a proteolytic enzyme that cleaves peptide chains and acts as a serine protease. There is conflicting evidence on the effectiveness of using trypsin to neutralize venom from snakes of the Elapid family [4].

RESULTS

We hypothesize that incubation of M. fulvius venom with RA or trypsin will degrade the venom protein and reduce its toxicity when administered to mice 1 hour after incubation.

MATERIALS & METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of East Carolina University.

Coral snake venom was obtained from Medtoxin Venom Lab (Delland, FL). Trypsin and RA were obtained from SigmaAldrich (St. Louis, MO).

In order to identify dosing for antidotes, gel electrophoresis was performed to determine the dose of trypsin and RA that successfully degraded the venom.

Venom (2mg/kg) was incubated in vitro for 1 hour with:

1mg of trypsin
2mg/ml of RA at a 1:10 ratio of venom:RA

Fifty CD-1 mice (20-30g) were pre-medicated with butyrophenone (0.1mg, s.c.) and randomized to receive 1 p injections of:

Venom alone (2mg/kg; n=10)
Trypsin-venom mixture (n=10)
RA-venom mixture (n=17)
RA alone (n=5)
Trypsin alone (n=3)

Time to toxicity was recorded as the primary endpoint. This was defined as respiratory distress (< 25 breaths/min.), loss of spontaneous locomotor activity, and/or inability to upright self. The proportion of animals surviving to 4, 6, and 12 hours was compared across groups using Chi-square analysis. Mean time to euthanasia was compared using ANOVA; P<0.05 was significant.

CONCLUSIONS

Both trypsin and rosmarinic acid have the potential to alter the toxic effects of M. Fulvius in vivo, potentially neutralizing the venom with efficacy to treat snakebites. In vitro neutralization of M. Fulvius venom by trypsin justifies progressing to an in vivo model in future studies of coral snake envenomation.

BIBLIOGRAPHY


Delland, FL. Trypsin and RA were obtained from SigmaAldrich (St. Louis, MO).

Trypsin alone (n=3)

Figure 1. Venom (2mg/kg) was pre-incubated with multiple doses of trypsin or RA and loaded onto pre-cast gels for electrophoresis to detect integrity of venom protein. All doses of trypsin were effective at reducing the protein structure of 10µg of venom (B) A 1:8 ratio of venom to RA almost completely degraded the venom (C).

Figure 2. Comparison of times to onset of toxicity between groups

Figure 3. Proportion of surviving mice at 4, 6 and 12 hours

Figure 4. Pre-incubation of venom with trypsin did not increase the number of animals that survived to 4 hours vs. controls (p=0.023). The proportion of animals surviving to 6 and 12 hours was similar across groups. All mice that received treatment (trypsin or RA) without venom survived to the end of the 12 hour study period.