Efficacy of Trypsin in Treating Coral Snake Envenomation in the Porcine Model

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Abstract Antivenom is the definitive treatment for venomous snakebites. Alternative treatments warrant investigation because antivenom is sometimes unavailable, expensive, and can have deleterious side effects. This study assesses the efficacy of trypsin to treat coral snake envenomation in an in vivo porcine model. A randomized, blinded study was conducted. Subjects were 13 pigs injected subcutaneously with 1 mL of eastern coral snake venom (10 mg/mL) in the right distal hind limb. After 1 min, subjects were randomized to have the envenomation site injected with either 1 mL of saline or 1 mL of trypsin (100 mg/mL) by a blinded investigator. Clinical endpoint was survival for 72 h or respiratory depression defined as respiratory rate <15 breaths per minute, falling pulse oximetry, or agonal respirations. Fisher's exact *t* test was used for between group comparisons. Average time to toxicity for the saline control was 263 min (191–305 min). The development of respiratory depression occurred more frequently in control pigs than treated pigs ($p=0.009$). Four of the six pigs that received trypsin survived to the end of the 3-day study. No control pigs survived. Two of the trypsin treatment pigs died with times to toxicity of 718 and 971 min. Survival to 12 and 24 h was significantly greater in the trypsin treatment group ($p=0.002$, $p=0.009$, respectively). Local injection of trypsin, a proteolytic enzyme, at the site of envenomation decreased the toxicity of eastern coral snake venom and increased

survival significantly. Further investigation is required before these results can be extended to human snakebites.

Keywords Snakebite · Coral snake · Trypsin · Envenomation

Introduction

Antivenom is an effective treatment for venomous snakebites. In regions of the world where antivenom is readily available, mortality from snakebites is rare. In parts of the world where morbidity and mortality from snakebites are a significant problem [1], antivenom and transport to a hospital may not be readily available. It is expensive, and complete immunoglobulin antivenom has significant potential for anaphylactic reactions and serum sickness [2]. Inexpensive alternatives would be desirable. If the major venom toxins are proteins, injection of a proteolytic enzyme into the site immediately after envenomation is a potential treatment that could be potentially self-administered by a bite victim in the field. Since the major toxins in coral snake venom are proteins [3, 4], a proteolytic enzyme such as trypsin injected in the site of a coral snake bite could reasonably be expected to neutralize the venom. The current study investigates the efficacy of trypsin in an in vivo porcine model by injection of trypsin into the site of eastern coral snake (*Micrurus fulvius*) venom injection.

Methods

The study was a randomized blinded controlled trial. Subjects were 6-week-old female domestic pigs weighing 10 to 15 kg. The setting was a university animal care facility accredited by the Association for Assessment and Accreditation of Laboratory Care International. Pigs were fed with 5P94 Prolab Mini-

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Pig Diet, vegetables, and fruits and provided with water ad libitum. The Institutional Animal Care and Use Committee approved the experimental protocol. Thirteen pigs were divided into two experimental groups: venom plus saline treatment (controls; $n=7$) and venom plus trypsin treatment (experimental; $n=6$). The number of pigs was determined based on a power analysis of a previous study of *in vitro* incubation of coral snake venom with trypsin [5]. This analysis determined that for the observed difference in survival time of 200 min and standard deviation of 90 min, six animals/group would be needed to reach 90 % power at $p=0.05$.

All pigs were fasted a day prior to the experiment. They were sedated with Telazol (5 mg/kg) plus xylazine (2 mg/kg) and intubated but not ventilated. Anesthesia was obtained with 2 % isoflurane and 20 % oxygen. Analgesia was provided with buprenorphine (0.02 mg/kg) pre-operatively and meloxicam (0.4 mg/kg) post-operatively. Freeze-dried eastern coral snake venom (MedToxin, Deland, FL) was dissolved in sterile water at 10 mg/mL. The dose of 10 mg was utilized based on a prior study of snake envenomation in the porcine model in which all non-treated pigs expired before 8 h [6]. Chromatographically purified, diafiltered, lyophilized trypsin powder from bovine pancreas (Worthington Biochemical Corporation, Lakewood, NJ) was dissolved in normal saline for a final concentration of 100 mg/mL. All animals received a 1 mL (10 mg) subcutaneous injection of eastern coral snake venom in the right distal hind limb (27-gauge needle, depth 3 mm). Animals were randomized into saline control or trypsin treatment group by a forced randomization. One minute after the injection of venom, 1 mL of saline or trypsin dissolved in saline was injected in the same site with a 27-gauge needle, at a depth of 3 mm. Method of randomization was to draw a card labeled treatment or control after the injection of venom. An odd number of animals were available for experimentation, so the control group was assigned seven animals prior to randomization. One minute post injection, a blinded investigator injected subjects at the site of envenomation with either 1 mL of sterile normal saline or 1 mL of trypsin dissolved in sterile normal saline at a concentration of 100 mg/mL.

Pulse oximetry and cardiac rhythm were monitored continuously for 4 h by investigators blinded to treatment assignment. Animals surviving to 4 h were extubated then monitored by blinded investigators every 8 h for the next 3 days. Clinical endpoint was time to toxicity (defined as respiratory rate less than 15 breaths per minute, apnea, falling pulse oximetry, or agonal respirations). If animals displayed signs of respiratory distress and self-mutilation or became moribund during the survival period, they were euthanized with a pentobarbital injection. Pain was assessed by veterinarians experienced in assessing pain in pigs, with monitoring for vocalization, immobility, lack of interest in food, and lack of socialization. Analgesia was administered as needed.

Results

There were no significant differences before treatment between the female domestic pigs obtained from the same vendor, with the same housing and diet. Pigs randomized to the control group weighed 12.8 ± 1.7 kg. Pigs randomized to the treatment group weighed 14.1 ± 1.9 kg. The difference in weight was not significantly different with a p value of 0.22. All pigs received a similar, single dose of buprenorphine analgesia prior to randomization and receipt of study drug, and this dose did not produce respiratory depression in any animal.

All pigs in the control group expired before the end of the 3-day study period with average time to toxicity of 263.4 ± 36.5 min. Four pigs in the treatment group survived to the end of the study period. The two pigs in the treatment group died at 718 and 971 min. Survival curves are presented in Fig. 1. Using Fisher's exact test, control pigs were more likely to develop signs of toxicity ($p=0.009$) and less likely to survive to 12 h ($p=0.002$) or 24 h ($p=0.009$) than treated pigs. Trypsin injection produced inflammation evolving to ulceration at the site of injection in all treated pigs that survived the 3-day study period, but these animals were walking on the extremity without evidence of significant pain. No qualitative measurements were made of the ulcers, which are estimated to be less than 2 to 3 cm.

Discussion

Antivenom is an effective treatment for venomous snakebites and is recommended for significant snakebites when available [2]. In the case of snakebites with neurotoxic venoms that cause respiratory paralysis, antivenom use if efficacious might prevent the need for mechanical ventilation. Globally, the morbidity and mortality of snakebites are significant [1], particularly in locales where access to antivenom and advanced emergency medical services is limited. Low cost-effective

Fig. 1 Survival curves for pigs injected with 10 mg eastern coral snake venom, followed by injection with 100 mg trypsin in 1 mL saline, or 1 mL of saline without trypsin

treatments are needed in these areas. The cost of trypsin varies with the purity, supplier, and amount purchased. The cost of trypsin obtained from Worthington Biochemical Corporation as a dialyzed lyophilized powder purchased in a 10-g container to treat one pig with 100 mg is US\$9.15, though bulk samples may be less expensive [7]. This study found that the immediate injection of trypsin in the site of eastern coral snake envenomation in pigs significantly reduced toxicity, in agreement with two previous studies [5, 8]. A prior study demonstrated the efficacy of trypsin, a protease, to treat cobra envenomation in dogs and mice [8]. All mice survived if injected with trypsin locally and promptly (up to 15 min) after envenomation, while 50–90 % survived if trypsin was administered 20–50 min later [8]. In vitro incubation of trypsin with eastern coral snake (*M. fulvius*) venom before intra-peritoneal injection into mice prolonged survival compared to venom controls [5].

A study of the effects of trypsin in a mouse model of tiger snake (*Notechis scutatus*) venom, brown snake (*Pseudonaja textilis*) venom, Indian cobra (*Naja naja*) venom, and king cobra (*Ophiophagus hannah*) venom found mixed results, depending on the dose of venom and time to administration [9]. It was found that trypsin was inferior to intravenous antivenom for experimental tiger snake venoms and had to be administered immediately after envenomation to have any efficacy. King cobra venom mixed with trypsin immediately before injection resulted in 100 % survival, which the authors opined is of no practical value because to be efficacious, the trypsin had to be injected immediately.

The predominant mechanism of toxicity from *M. fulvius* venom is the neurotoxicity produced by blocking the postsynaptic motor endplate by a protein, alpha neurotoxin [10, 11]. Current antivenom treatment for *M. fulvius* envenomation is equine-derived immunoglobulins. This treatment carries the risks of anaphylaxis, anaphylactoid reaction, and delayed serum sickness [2]. Adverse reactions to eastern coral snake antivenom have been reported as high as 18.25 % [12]. The only US Food and Drug Administration-approved antivenom for *M. fulvius* envenomation is no longer in production, but expired product is still approved for administration [13]. A coral snake antivenom, Coralmyx[®], is available in other countries but currently is not approved by the FDA for use in the USA.

A limitation of this study is efficacy of trypsin was only demonstrated for one venom in one species. Generalization of our results to humans and other venoms is not possible at this time. Trypsin was injected 1 min after venom, which would not be practical in most real-world situations. However, if treatment administered immediately was not efficacious, further study would not be a consideration. A limitation is that the effects of trypsin administered without antivenom was not studied. Further, since venom levels were not measured, it has not been proven that degradation of venom by trypsin was the mechanism of efficacy.

The adverse effects of trypsin at the injection site were not studied quantitatively and only observed for 3 days. Long-

term effects of injecting trypsin into an extremity must be studied before trypsin injections can be considered as a treatment.

Conclusion

Trypsin injected within 1 min into the site of *M. fulvius* envenomation increased survival in this porcine model as compared to controls.

Conflict of Interest There are no conflicts of interest to disclose.

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