A Randomized Controlled Trial of Trypsin to Treat Brown Recluse Spider Bites in Guinea Pigs

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Abstract Brown recluse spider bites result in necrotic skin lesions for which there is no known antidote. Since venom toxins are proteins, a proteolytic enzyme like trypsin might be effective in reducing toxicity. The aim of this study was to conduct a randomized controlled trial of trypsin to treat brown recluse spider bites in guinea pigs. Subjects were 18 female guinea pigs. Anesthesia for injections was inhaled isoflurane. Analgesia was 0.05 mg/kg of buprenorphine twice a day as needed. Intervention was intradermal injection of 30 µg of brown recluse venom (Spider Pharm, Yamell, AZ). Immediately after envenomation, subjects were randomized to two groups of nine: trypsin 10 µg in 1 mL normal saline and 1 mL of normal saline. The primary outcome was lesion area over a 10-day time period. Statistical analysis was performed with repeated measures ANOVA. Mean lesion area was smaller but not statistically different in the placebo group. Maximum lesion size occurred at day 4 in both groups, when lesion area was $76.1 \pm 108.2 \text{ mm}^2$ in the placebo group and $149.7 \pm 127.3 \text{ mm}^2$ in the treatment group. *P* value was 0.15 for placebo vs. treatment. This study did not establish a role for trypsin as a treatment for brown recluse spider bites in a guinea pig model.

Keywords *Loxosceles reclusa* · Spider bites · Trypsin

Introduction

Bites of the brown recluse spider (*Loxosceles reclusa*) produce necrotic ulcers for which there is no known antidote. Treatment is wound management. Proposed therapies include dapsone [1–3], hyperbaric oxygen [1, 4], cyproheptadine [3], colchicine [1], triamcinolone [3], diphenhydramine [3], electric shock [2], and topical nitroglycerin [5] applied to the site. None have been tested in randomized controlled studies in humans, but controlled studies in other mammalian species have been performed. In randomized controlled studies, none of these therapies prevented the development of necrotic ulcers. While some, such as hyperbaric oxygen [1] and dapsone [2], have had some success in limiting ulcer size, no therapy has prevented ulcer formation in experimental studies. Topical nitroglycerin applied directly to the site of envenomation in a rabbit model limited ulcer size, but this effect was not statistically significant, while worsening systemic toxicity [5].

Since the toxins in *Loxosceles* venoms are proteins [6, 7] and the amount of venom injected is small [8], a proteolytic enzyme injected into the site of the bite may potentially limit toxicity. Trypsin is an inexpensive and readily available proteolytic enzyme that has demonstrated efficacy against cobra venom in dogs and mice [9], but was not effective in tiger snake envenomation in mice [10]. In vitro incubation of eastern coral snake (*Micrurus fulvius*) venom with trypsin prior to injection was found to reduce toxicity in mice [11]. The current study tested the hypothesis that trypsin injected into the site of inoculation with brown recluse spider venom will reduce the size of the necrotic ulcer.

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Methods

A blinded, randomized controlled trial was performed to determine the ability of trypsin to prevent or reduce the development of necrotic lesions resulting from *Loxosceles* envenomation. All experiments were carried out in a university animal research laboratory. Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Subjects were 18 retired, female breeder guinea pigs. Guinea pigs are known to develop necrotic lesions from injection of *Loxosceles* venom [2].

Venom protein concentration in solution was determined using Coomassie Blue in Pierce Chemical commercial kits (Thermo Scientific, Pittsburgh) using the micromethod, a method that is appropriate for the small volumes and low amounts needed for this experiment. A protein standard curve was constructed using known concentrations of bovine serum albumin. Absorbance measurements were made in duplicate of both known and unknown samples at 595 nm. The protein content of *L. reclusa* venom (Spider Pharm, Yarnell, AZ) was determined. The venom was diluted in Hank's buffered saline to a protein content of 5.0 mg/mL and stored at -80°C until ready for use. Immediately prior to injection, the venom was further diluted to 30 μg in 0.1 mL of phosphate-buffered saline for injection into guinea pigs.

Animals were anesthetized with inhaled isoflurane (1 to 3 %, to effect), and 30 μg in 0.1 mL of brown recluse venom was administered intradermally on the back between the shoulder blades. Immediately after envenomation, subjects were randomized to two groups of nine subjects. The experimental (treatment) group received intradermal trypsin (Worthington Biochemical, Lakewood, NJ). Ten milligrams dissolved in 1 mL of normal saline was administered at the site of envenomation. The control (placebo) group received 1 mL of normal saline in the same manner. Forced randomization was performed by drawing cards labeled treatment or control. Injections were performed by an investigator blinded to treatment group. Animals were removed from anesthesia and returned to their home cages. Trypsin injection is painful; therefore, all guinea pigs were treated preemptively with buprenorphine (0.05 mg/kg). Subsequent administration of buprenorphine was based on behavioral indicators of pain, including vocalization, reluctance to eat and drink, isolation from cage mates, and guarding the injection site.

Envenomation sites were examined by an investigator blinded to treatment group. Measurements of the long and short axes of the lesion area were taken daily for 10 days by a single examiner blinded to interventions who traced the lesion outline onto a transparent plastic film once a day. Lesion area was calculated using the formula for the area of an ellipse. Primary outcome was lesion area development over time. Lesion areas were compared over time using repeated measures ANOVA.

Results

Mean lesion areas over time for the randomized controlled trial comparing evenomation followed by trypsin injection with evenomation with saline control are shown in Fig. 1. No significant differences in mean lesion area existed between groups at any time point. However, the area in the placebo group tended to be smaller than the treatment group at all time points. Maximum lesion area occurred at day 4 in both groups, with areas of 76.1 ± 108.2 vs. 149.7 ± 127.3 mm² in the placebo vs. treatment group, respectively. *P* value was 0.15 for placebo vs. treatment. Median areas were comparable to mean areas (70 and 127, respectively, on day 4).

An a priori power analysis was not done as there was no preliminary data available to estimate an effect size. A post hoc power analysis using mean lesion areas at day 4 (the day of the largest areas in both groups) shows that 51 animals/group would be needed to reach 90 % power at 0.05.

Discussion

The current study was based on the idea that a proteolytic enzyme injected at the site of envenomation may degrade the small amounts (approximately 30 μg) of toxic proteins that are injected by the *Loxosceles* species. This would provide a simple and inexpensive treatment option. Our data demonstrate that injection of the enzyme trypsin after envenomation tended to exacerbate the ulcer formation compared to treatment with normal saline. Hence, trypsin was worse than placebo in this randomized clinical trial, suggesting that the proteolytic activity of the trypsin produced ulcerative effects on its own.

The primary limitation to the data obtained is the large variability in mean lesion areas and the relatively small mean

Fig. 1 Mean lesion area in square millimeters vs. time after guinea pigs were injected with brown recluse spider venom followed by injection of the venom site with a 100 mg trypsin dissolved in 1 mL normal saline and b 1 mL of normal saline

lesion areas. Mean lesion areas in another study of brown recluse envenomation in guinea pigs were comparable, as was mean lesion area variability [2]. Rabbit models of brown recluse envenomation [3, 5] have larger lesion areas and less variability, suggesting that rabbit is a better animal to study *Loxosceles* envenomations than guinea pig. The treatment was administered immediately after venom injection, which is not a practical consideration in most clinical cases in humans because presentations to medical treatment usually occur after the lesion development. Immediate injection is the first step in an investigation of this type, because if a treatment given early for a toxic exposure has no efficacy, delayed administration is unlikely to be efficacious. Another limitation was that only one investigator measured the lesion dimensions with no formal evaluation of the reliability of the measurements.

Conclusion

Trypsin was not significantly different than placebo in a guinea pig model of *Loxosceles* envenomation. Mean lesion area was larger, but this effect was not statistically significant. This experiment does not support trypsin as a treatment for brown recluse spider bites.

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

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References

1. Phillips S, Kohn M, Baker D et al (1995) Therapy of brown spider envenomation: a controlled trial of hyperbaric oxygen, dapsone, and cyproheptadine. *Ann Emerg Med* 25:363–368
2. Barrett SM, Romine-Jenkins M, Fisher DE (1994) Dapsone or electric shock therapy of brown recluse spider envenomation. *Ann Emerg Med* 24:21–25
3. Elston DM, Miller SD, Young RJ 3rd, Eggers J, McGlasson D, Schmidt WH, Bush A (2005) Comparison of colchicine, dapsone, triamcinolone, and diphenhydramine therapy for the treatment of brown recluse spider envenomation: a double-blind, controlled study in a rabbit model. *Arch Dermatol* 141:595–597
4. Maynor ML, Moon RE, Klitzman B, Fracica PJ, Canada A (1997) Brown recluse spider envenomation: a prospective trial of hyperbaric oxygen therapy. *Acad Emerg Med* 4:184–192
5. Lowry BP, Bradfield JF, Carroll RG, Brewer K, Meggs WJ (2001) A controlled trial of topical nitroglycerin in a New Zealand white rabbit model of brown recluse spider envenomation. *Ann Emerg Med* 37(2):161–165
6. Machado LF, Laugesen S, Botelho ED, Ricart CA, Fontes W, Barbaro KC, Roepstorff P, Sousa MV (2005) Proteome analysis of brown spider venom: identification of loxnecrogin isoforms in *Loxosceles gaucho* venom. *Proteomics* 5:2167–2176
7. dos Santos LD, Dias NB, Roberto J, Pinto AS, Palma MS (2009) Brown recluse spider venom: proteomic analysis and proposal of a putative mechanism of action. *Protein Pept Lett* 16:933–943
8. Gomez HF, Krywko DM, Stoecker WV (2002) A new assay for the detection of *Loxosceles* species (brown recluse) spider venom. *Ann Emerg Med* 39:469–474
9. Y ü -liang H, Ju-chin T, Yi-ti H, Tz' u-ch ü an L, Hsing-liang C, Ching-yen L (1975) Experimental studies on curing elapid bite with trypsin. *Sci Sin* 18:396–405
10. Broad AJ, Sutherland SK, Lovering KE, Coulter AR (1980) Trypsin fails as Australian snake bite cure. *Med J Aust* 2:388–390
11. Parker-Cote JL, O' Rourke DP, Miller SN, Brewer KL, Rosenbaum MD, and Meggs WJ (in press) Trypsin and rosmarinic acid reduce the toxicity of *Micrurus fulvius* venom in mice. *Clin Tox*