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

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Objective: To compare the efficacy of dapsone, diphenhydramine, colchicine, and intraleosional triamcinolone in the treatment of brown spider bites. We used a purified venom that reproducibly produces a large eschar. To mimic real-life circumstances, all agents were administered following a 2-hour delay after envenomation. The animals were evaluated for the presence of coagulopathy to determine if the incidence of systemic findings correlated with the type of treatment.

Design and Setting: In a research laboratory, 60 New Zealand white rabbits each received an intradermal injection of 20 µg of purified Loxosceles reclusa venom. The rabbits were divided into 5 groups of 12; a control group and 4 groups treated with a drug (either colchicine, triamcinolone, diphenhydramine, or dapsone). Measured end points included maximum eschar size as well as histologic grading of depth, inflammation, and thrombosis.

Interventions: Treatment with colchicine, triamcinolone, diphenhydramine, or dapsone.

Main Outcome Measures: Maximum eschar size as well as histologic grading of depth, inflammation, and thrombosis.

Results: There was no significant difference with respect to eschar size (1-way analysis of variance, \( P = .003 \)). There was no significant difference between any treatment with respect to presence or absence of ulcer, necrosis, large vessel vasculitis, or small vessel vasculitis. The only outcome of significance was that triamcinolone offered protection from thrombosis \((\chi^2 \text{ likelihood ratio}, \ P = .04)\). We also noted evidence of coagulopathy in all of the envenomated animals. The rabbits had grossly elevated partial thromboplastin time results, which were corrected with 1:1 mixing with normal rabbit plasma, suggesting an acquired factor deficiency. We did not detect an individual factor deficiency or a lupus anticoagulant.

Conclusions: In a rabbit model, none of the agents tested (dapsone, diphenhydramine, colchicine, and intraleosional triamcinolone) had an effect on eschar size. Triamcinolone appeared to offer some protection against histologic evidence of thrombosis, but this protection did not translate into a difference in clinical outcome. All animals developed evidence of coagulopathy, regardless of treatment. The coagulopathy could be corrected by fresh rabbit plasma, suggesting an acquired factor deficiency.

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Loxosceles spiders, including Loxosceles reclusa, Loxosceles laeta, Loxosceles rufescens, Loxosceles deserta, and Loxosceles arizonica, are capable of causing extensive skin necrosis. These spiders have a worldwide distribution but are particularly numerous in the South Central United States. Some studies have administered drugs prior to envenomation or immediately following envenomation. The applicability of these data to real-life circumstances is problematic. Some studies found little necrosis in the controls, making evaluation of effective therapy difficult. Further complicating the decision to treat is the observation that systemic reactions to brown recluse spider bites are commonly associated with minor-appearing eschars. Necrotic wounds may sequester the toxin, while minor wounds allow absorption of the toxin. A drug capable of preventing necrosis after envenomation could potentially cause greater absorption of toxin and increase the probability of a severe systemic reaction to the envenomation.

Various spiders are capable of producing severe dermonecrotic and systemic reactions, but many are not well characterized. In the United States, brown recluse spiders \((L. \text{ reclusa})\) appear to be the most common cause of necrotic arachnidism. The brown recluse spider is a light brown...
spider with a small oval body and long delicate legs. It is most easily recognized by a dark violin-shaped marking on the dorsum of its cephalothorax. Related spiders have a worldwide distribution. Brown spiders prefer hot, dry environments such as wood or rock piles, abandoned buildings, or boxes.

As the name implies, the brown recluse spider is shy and generally only bites when threatened. Initially, only mild symptoms are noted after a bite. Within several hours, edema, erythema, and tenderness are noted. Most bites do not result in significant necrosis, but some can produce extensive necrosis. Systemic reactions are frequently associated with minor-appearing bites. It remains to be proved how effectively thrombosis and tissue necrosis sequester the venom and prevent absorption. Even if drugs to prevent necrosis were to be proved to cause an increase in systemic toxin absorption, there may still be instances in which systemic absorption is preferable to widespread local necrosis. For instance, bites may occur on the penis. Patients with penile bites may prefer to take their chances with systemic absorption, rather than face extensive local necrosis. Unfortunately, no therapy has been definitely shown unrelated study. The study rabbits receiving triamcinolone received 2 mg/kg of colchicine by oral lavage twice a day for 7 days following envenomation. This dose was chosen because there is little published data regarding the lethal dose for 12; a control group and 4 groups treated with colchicine, triamcinolone, diphenhydramine, and dapsone. On day 1, each rabbit received an intradermal injection of approximately 20 µg of highly concentrated L. reclusa venom to an area over the dorsum of the back, on the midline, after the area had been clipped and sterilely prepped. The study rabbits receiving colchicine received 2 mg/kg of colchicine by oral lavage twice a day for 7 days following envenomation. This dose was chosen because there is little published data regarding the lethal dose of colchicine in rabbits, and this dose was well tolerated in an unrelated study. The study rabbits receiving triamcinolone received a single 4-mL injection of 10-mg/mL triamcinolone infiltrated subcutaneously in the area of envenomation, 2 hours after envenomation. The study rabbits receiving diphenhydramine received a 10-mg/kg subcutaneous injection twice daily for 7 days. The rabbits in the dapsone group received 2 mg/kg per day. We limited the dose to 2 mg/kg per day because this dose approximates the dose used in humans, and a prior brown recluse venom study at our institution demonstrated significant mortality in the test animals when a higher dose was used. All rabbits were examined daily for gross eschar size for 14 days. The lesions were recorded by digital photography while the rabbits were restrained in a standardized position by 2 blinded investigators. This was followed by measurement of the eschar area by computerized morphometry using imaging software. The animals also received 2 venipunctures at time 0 and 72 hours to obtain a small sample of blood for a disseminated intravascular coagulation panel with individual coagulation factors (VIII, IX, XI, and XII), prothrombin time, partial thromboplastin time, lupus anticoagulant, and complete blood cell count. After completion of the protocol, the lesions were excised. A sample was cut from the longest axis of each lesion, embedded in paraffin, sectioned to a thickness of 6 µm, and stained with hematoxylin-eosin. The sections were examined in random order by 2 blinded pathologists (a dermatopathologist and a veterinary pathologist). The depth of tissue necrosis, neutrophil infiltrate, vasculitis, and thrombosis were recorded.

RESULTS

The eschars spread in a gravity-dependent fashion, reaching an average size of 28.38 cm². With respect to eschar size, there were no significant differences between any of the groups, except for the diphenhydramine group, which fared worse (1-way analysis of variance, P = .003). Triamcinolone offered some protection from thrombosis (χ² likelihood ratio, P = .04) in the histologic analysis, but this protection did not correlate with a decrease in eschar size. There were no significant differences with respect to ulcer, necrosis, or vasculitis. All envenomed animals had elevations in activated partial thromboplastin time, which were corrected with 1:1 mixing with normal rabbit plasma. We were unable to detect deficiencies of the tested coagulation factors or the presence of a lupus anticoagulant. Fibrinogen levels rose from a mean of 261.7 mg/dL (7.7 µmol/L) at baseline to a mean of 1064.4 mg/dL (31.3 µmol/L) at 72 hours. Factor VIII levels rose from 580 to 1223.8 mg/dL. Factor IX levels rose from 208 to 554 mg/dL, and factor XI levels rose from 384 to 771 mg/dL at 72 hours. Factor XII levels remained essentially unchanged.

METHODS

Sixty New Zealand white rabbits were divided into 5 groups of 12; a control group and 4 groups treated with colchicine, triamcinolone, diphenhydramine, and dapsone. On day 1, each rabbit received an intradermal injection of approximately 20 µg of highly concentrated L. reclusa venom to an area over the dorsum of the back, on the midline, after the area had been clipped and sterilely prepped. The study rabbits receiving colchicine received 2 mg/kg of colchicine by oral lavage twice a day for 7 days following envenomation. This dose was chosen because there is little published data regarding the lethal dose of colchicine in rabbits, and this dose was well tolerated in an unrelated study. The study rabbits receiving triamcinolone received a single 4-mL injection of 10-mg/mL triamcinolone infiltrated subcutaneously in the area of envenomation, 2 hours after envenomation. The study rabbits receiving diphenhydramine received a 10-mg/kg subcutaneous injection twice daily for 7 days. The rabbits in the dapsone group received 2 mg/kg per day. We limited the dose to 2 mg/kg per day because this dose approximates the dose used in humans, and a prior brown recluse venom study at our institution demonstrated significant mortality in the test animals when a higher dose was used. All rabbits were examined daily for gross eschar size for 14 days. The lesions were recorded by digital photography while the rabbits were restrained in a standardized position by 2 blinded investigators. This was followed by measurement of the eschar area by computerized morphometry using imaging software. The animals also received 2 venipunctures at time 0 and 72 hours to obtain a small sample of blood for a disseminated intravascular coagulation panel with individual coagulation factors (VIII, IX, XI, and XII), prothrombin time, partial thromboplastin time, lupus anticoagulant, and complete blood cell count. After completion of the protocol, the lesions were excised. A sample was cut from the longest axis of each lesion, embedded in paraffin, sectioned to a thickness of 6 µm, and stained with hematoxylin-eosin. The sections were examined in random order by 2 blinded pathologists (a dermatopathologist and a veterinary pathologist). The depth of tissue necrosis, neutrophil infiltrate, vasculitis, and thrombosis were recorded.

COMMENT

Relatively little objective data exists regarding the efficacy of drug treatment for brown recluse spider envenomation. Dapsone has been studied more extensively than other agents, but an OVID search using the text words brown recluse, Loxosceles, spider, arachnidism, and necrosis failed to reveal any prior published studies that attempted to recreate the delay in onset of treatment that would be expected after real-life envenomation. Colchicine, like dapsone, affects neutrophils and has a rapid onset of action, a characteristic that could be of benefit in this study model. We were prompted to include diphenhydramine and triamcinolone in our study because of anecdotal reports of efficacy of these agents. One such report suggested that the tissue injected with triamcinolone was spared from necrosis, while the tissue above and below the injection site showed necrosis. This report suggested that the venom diffused through the injected area, but did not cause necrosis in the injected skin. Unfortunately, in our model, none of the agents tested had a significant positive effect on eschar size. The animals treated with diphenhydramine fared worse compared with the control animals. Although these drugs are commonly used in the setting of brown recluse envenomation, they all have potential adverse effects. Our study provided no evidence to support the efficacy of any of the tested agents. All of the envenomated animals developed signs of a coagulopathy, but because none of the drugs prevented necrosis, we were unable to test the hypothesis that prevention of necrosis could worsen the coagulopathy.
The sequence of events after a brown recluse bite is complex. Sphingomyelinase D, the demonecrotic component of L. reclusa venom, degrades sphingomyelin in cellular membranes, leading to cell lysis, while hyaluronidase promotes spreading of eschars in a gravity-dependent fashion. The extent of necrosis relates directly to diffusion of the venom, and maximal lesion severity predicts the time to healing. Serum amyloid component P appears to be involved in platelet activation following envenomation. Chemotaxis of neutrophils occurs, and their adherence to endothelial cell intercellular junctions is aided by E-selectin. Although neutrophils are not directly activated by venom, they play a role in vascular damage. Large vessel vasculitis, resembling polyarteritis nodosa may contribute to the extensive necrosis seen in some cases. Necrotizing fasciitis may occur after spider bites and may also contribute to severe necrosis. Clostridium perfringens has been isolated from the venom and fangs of Loxosceles spiders. Because the venom in our study was purified and injected with a needle under sterile conditions, it is unlikely that infection played a role in the eschars observed.

Most brown spider bites can be treated with rest, ice, and elevation. Intradermal injection of antivenin can attenuate necrosis in an animal model, when administered up to 4 hours after envenomation. Fused recombinant protein immunization has also been shown to protect rabbits and mice from Loxosceles demonecrotic reactions. These studies suggest that further work on immunotherapy is warranted. Unfortunately, antivenin is not readily available in emergency care settings. Hyperbaric oxygen therapy may be of some benefit, but is also not readily available in most locations. Because neutrophils appear to be related to the pathogenesis of necrotic arachnidism, it is tempting to speculate that blunting of the neutrophils response could lessen the degree of necrosis. Dapsone, colchicine, and corticosteroids are commonly used to treat neutrophilic dermatoses. In one study, the combination of dapsone and antivenin was superior to either agent alone, suggesting a potential role for antineutrophil agents. Unfortunately, we were not able to duplicate this success in our model.

We observed that the envenomation created a coagulopathy in our test animals, which could not be explained by a deficiency of the coagulation factors we measured. Most coagulation factors were increased at the 72-hour mark. The coagulopathy was corrected with 1:1 mixing with normal animal plasma, suggesting an acquired deficiency of another plasma component. There was no evidence of a lupus anticoagulant. Testing for this possibility was important because a lupus anticoagulant could produce elevations in partial thromboplastin time, while producing thrombosis in vivo. Because the test animals showed both thrombosis and an elevated partial thromboplastin time, we believed it was important to exclude the possibility of a lupus anticoagulant.

In our model, we failed to demonstrate a clinical benefit associated with administration of colchicine, triamcinolone, diphenhydramine, or dapsone when administered after a 2-hour delay. Animals treated with diphenhydramine fared worse than the controls. A protective effect against thrombosis was noted in the triamcinolone group on histologic examination, but the clinical eschars that occurred in this group were no different from those of the other groups. It should be emphasized that animal data do not always correlate with the clinical response in humans. Our study can, however, serve as a model for future investigation of alternative therapy. Agents that are effective in the animal model could then be tested for effectiveness in the treatment of human envenomation.

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REFERENCES