Loxoscelism and negative pressure wound therapy (vacuum-assisted closure): an experimental study

S Lindsey Wong, Andrew M Schneider, Louis C Argenta, Michael J Morykwas

ABSTRACT
Brown recluse spider (Loxosceles) bites cause lesions ranging from chronic necrotic ulcers to acute life-threatening sepsis. Based on our experience in treating acute and chronic wounds with negative pressure, we postulated that vacuum-assisted closure (VAC) would be valuable in this application. Chester pigs were procured and injected with purified brown recluse spider venom, 1 μl of venom in two anterior sites and 0·1 μl of venom in two posterior sites on their dorsum. For each concentration of venom, treatment consisted of either VAC or dry, non-adherent dressings (control group). Each day, the wounds were inspected and measured. For wounds receiving 1·0 μl of venom, the control wounds decreased in surface area to 50% of initial size after 7 days and none had healed, whereas VAC-treated wounds were less than 50% after 48 hours and completely healed and reepithelialised after 8 days. Wounds with 0·1 μl of venom had 50% reduction after 5 days with no complete healing for control wounds, and the VAC wounds were 50% after 48 hours and all had closed and reepithelialised after 5 days. Our experimental study showed an accelerated healing time in the animals treated with the VAC as compared with controls.

Key words: Brown recluse spider bite • Loxoscelism • Negative pressure wound therapy • Vacuum-assisted closure

INTRODUCTION
Envenomation by Loxosceles species brown recluse spider (Loxosceles) results in wounds that range from areas of skin necrosis to life-threatening systemic sequelae. Venom from the bite, as well as a local toxic milieu, is thought to contribute to the difficulty in healing these wounds. Because no definitive treatment of these injuries has been offered, we attempted to develop a laboratory model to test the efficacy of negative pressure wound therapy (NPWT) in these injuries.
formation, while it decreases edema and bacterial levels (1,2).

Previous studies have showed that injuries from chemotherapeutic drug extravasation that usually result in large ulcers can be ameliorated with the VAC (3). We postulated that similar mechanisms would be useful in envenomation injuries such as the brown recluse spider bite. Negative pressure applied to the wound would produce a pressure gradient across the wound, facilitating removal of the venom before it could damage adjacent cells within the zone of stasis. This would protect adjacent cells by removing the toxin and by decreasing bacterial count and increasing blood flow into the area of injury, thus lessening the chance of secondary infection. In this paper, we present a novel application for the use of this therapy in noxious spider bites using a swine model treated with the VAC.

METHODS
A controlled, experimental pilot study using VAC therapy was initially performed to confirm efficacy and ratify safety issues. Approval for the study was obtained from the Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals.

Eight 20 kg Chester pigs were procured and allowed to acclimate to their new housing conditions for 7 days prior to commencing the study. On the day of envenomation, the animals were sedated with a cocktail of ketamine/xylazine/acepromazine (20/2/2 mg/kg IM). Anaesthesia was maintained using 1% halothane by inhalation. Intra-muscular analgesia of butorphanol (0·1 mg/kg IM) was also given. The back of each animal was shaved prior to injection. Four sites were injected on each animal, two on each side of the spine, 3 cm lateral to the spine with 8 cm between each site on each side. Using a 25-gauge needle attached to a microsyringe, the anterior sites were injected with 1 μl of venom (Spider Pharm, Inc., Feasterville, PA), whereas the posterior sites were injected with 0·1 μl of venom. These doses were selected on the advice of the supplier to conform to what is thought to be the range of venom inoculation of spider bites.

Treatment was then randomised to one of each of the two wounds containing a different concentration of venom: (i) NPWT or (ii) dry, non adherent dressings (control group). Treatment was initiated 1 hour after injection of the venom to allow the site where the needle penetrated the skin to close. NPWT was applied using foam dressings (Granufloam, KCI, San Antonio, TX) that were trimmed to approximately 2 cm larger than the wound and covered with an adhesive film (Ioban, 3M, St Paul, MN) to create an airtight seal. The remaining wounds were dressed with a non adherent gauze dressing. All wound dressings were then protected using a saddle created from a perforated thermoplastic sheet (Aquaplast; WFR/AquaPlast, Wyckoff, NJ) and covered with a tubular bandage (Stockinette; McKesson Corp., Richmond, VA), which was held in place with 2 inch wide elastic tape (Elastikon, Johnson & Johnson, Skillman, NJ). The tubing for the NPWT dressing required winding around the tape at the neckband several times to minimise interference and prevent removal of the dressing by the animal. The tubing was then suspended from the top of the cage and connected to a VAC pump. Pressures were maintained in a continuous fashion at 125 mm Hg, and wounds were treated for a total of 8 days.

Each day the wounds were inspected and measured. The wound was approximated as an ellipse and the area in square millimetres was calculated using the following: length/2 × width/2 × π. The data were analysed using SigmaPlot (Systat Software, Inc., San Jose, CA) and significance accepted at P < 0·05.

RESULTS
Five of the pigs died within the first 36 hours of injection. Veterinarians examining the carcasses believed that death was from anaphylactic shock, although it did not seem to be related to the quantity of venom. For the remaining three animals, the observed control lesions appeared identical to an actual clinical spider bite scenario. A central area of compromised tissue appeared within 12 hours of inoculation followed by necrosis and ulceration of this area over the next 48 hours, despite the treatment applied. A zone of firm erythema surrounded the area.

For the wounds that received 1·0 μl of venom, the control wounds gradually decreased
There is also evidence that untreated injections resulted in ulcer formation, whereas injections treated with the VAC resulted in no ulceration. None of the wounds had healed. This is in contrast to the VAC-treated wounds, where the surface area decreased to less than 50% maximal size after 48 hours of treatment (65.5 mm² versus 23.8 mm²). The size of the VAC-treated wounds at day 3 were significantly ($P < 0.05$) smaller than the control wounds at day 3. All of the VAC-treated wounds were healed and had completely reepithelialised after 8 days. For those wounds injected with 0.1 µl of venom, the maximal size of all wounds was approximately half the maximum surface of the wounds resulting from injection of 1 µl of venom (65.5 mm² (1 µl) versus 27.0 mm² (0.1 µl) for VAC treated and 62.0 mm² (1 µl) versus 33.5 mm² (0.1 µl) for control wounds, Figure 2). A reduction in surface area to less than 50% of maximum with 0.1 µl of venom occurred after 5 days of treatment for control wounds (33.5 mm² versus 17.3 mm²). None of the control wounds had healed completely after 7 days. In the VAC-treated group, the size of the lesion had decreased to half the maximum size after 48 hours (27 mm² versus 12 mm²). The size of the VAC-treated wounds at day 3 were significantly ($P < 0.05$) smaller than the control wounds at day 3. In the VAC-treated group, two of the three wounds had closed and epithelialised after 7 days.

The fluid suctioned out by the VAC was reinjected into the dorsum of the pigs after completion of the study and similar small ulcers were formed. Despite the small number of animals studied, statistical significance was achieved in comparing the wounds treated with VAC therapy versus control therapy, and the study ended once clinical significance was met in accordance with the Institutional Review Board-approved protocol.

**DISCUSSION**

Brown recluse spider bites are not uncommon, and they notoriously result in the formation of slow-healing necrotic wounds (4). NPWT with the VAC offers an effective novel non-pharmacological approach in treating these difficult wounds. Experimental studies have showed significant increases in granulation tissue formation in wounds treated with NPWT compared with control wounds treated with wet-to-dry saline dressings (5). Use of NPWT prevented ulcer formation in a swine model that received intra-dermal injections of doxorubicin hydrochloride, a chemotherapeutic agent, which usually results chronic ulcers (3). Untreated injections resulted in ulcer formation, whereas injections treated with the VAC resulted in no ulceration. There is also evidence that NPWT may salvage compromised or ischemic tissue adjacent to the necrotic area.

Treatment of random pattern flaps with NPWT significantly increased flap survival (5). In an experimental-burn model, NPWT attenuated burn wound progression as signified by a reduction in cell necrosis and improvement in dermal perfusion (6). In a rabbit model, the application of NPWT to an affected body part following a prolonged crush and ischemic injury led to lower serum myoglobin levels than in control animals, thus averting systemic distribution of myoglobin and potential myoglobin-induced renal failure (7). Von Gossler and Horch (8) and Miller et al. (9) have described initial reports of successful applications of NPWT for insect bites.

**Key Points**

- Use of NPWT prevented ulcer formation in a swine model that received intra-dermal injections of doxorubicin hydrochloride, a chemotherapeutic agent, which usually results chronic ulcers.
- Untreated injections resulted in ulcer formation, whereas injections treated with the VAC resulted in no ulceration.
- There is also evidence that NPWT may salvage compromised or ischemic tissue adjacent to the necrotic area.

![Figure 1.](image1.png) **Surface Area**

**Figure 1.** Surface area (square millimetres) of wounds resulting from injection of 1 µl of venom.

![Figure 2.](image2.png) **Surface Area**

**Figure 2.** Surface area (square millimetres) of wounds resulting from injection of 0.1 µl of venom.
Our experimental study showed an accelerated healing time in the three animals treated with NPWT as compared with the controls. There was a significant decrease in wound surface area by 50% of the maximum within 48 hours in both 0.1 μl and 1 μl dosages of venom in the treated wounds as compared with the control wounds. Ulcers did initially develop, despite the almost immediate application of vacuum to the sites. This, most likely, reflects cell death of those initial cells, which took up the venom upon introduction into the tissue. Upon death of the cells, the venom and intra-cellular contents were most likely drawn out of the tissue by the applied pressure gradient. An assay for the venom was not available at the time, but when fluid was taken from the VAC canister during the treatment period and reinjected into the skin of the pigs, a small ulcer developed. This result supports the concept that the enhanced diffusion gradient created by the VAC caused the venom to migrate out of the tissue and into the VAC collecting system (3). While this is a preliminary study on a few animals in which the progress of wound healing was measured only by a reduction in surface area over time, it does offer a promising adjunct for difficult spider bites that the patient cases were able to corroborate.

There is very little in the literature regarding animal models for brown recluse envenomation. A previous study using New Zealand white rabbits, an acceptable model for the study of loxoscelism therapeutic regimens, failed to show a decrease in eschar size or coagulopathy comparing colchicine, dapsone, trimethadione, and diphenhydramine therapies (10). Although, the use of brown recluse spider venom on a white rabbit model has been described in the literature (11–13), we chose a larger model so that we could use the purified venom, which could not be used on a smaller animal. However, five Chester pigs died either immediately after injection of the venom in an almost anaphylactoid manner or which had died in the following morning. This result illustrates the potency of the purified brown recluse venom on a larger animal and the capability of potentially inducing such severe clinical complications as necrotising fasciitis (14), intravascular hemolysis (15), hemolytic anemia (16,17), and, rarely, disseminated intravascular coagulation (18) that are sometimes seen in humans and in pediatric patients, in particular.

These studies suggest an important role for NPWT in influencing the crucial cellular processes associated with the inflammatory response to injury. In loxoscelism, wounds resemble the so-called ‘zones of injury’ as seen in burn wounds where the inner necrotic tissue is surrounded by a neighbouring zone of ischemic injury, characterised by poor local blood flow and a hypoxic environment. Histological evidence suggests that a pronounced inflammatory response occurs following envenomation from brown recluse spiders. The principal findings include the presence of a mixed inflammatory cell infiltrate together with coagulative tissue necrosis and vasculitis. We suggest that treatment with NPWT improves the underlying pathophysiology of these wounds by improving the local blood flow and dermal perfusion, modulating the inflammatory response, extracting toxic substances and ultimately salvaging tissue and providing a healthy environment for wound healing.

CONCLUSION

Loxoscelism is associated with significant morbidity. Envenomation may culminate in chronic non healing, dermonecrotic ulcers. This study shows that the use of negative pressure wound therapy may significantly improve healing of these lesions in an experimental model. We recommend that this therapy can be considered when treating patients suspected of sustaining brown recluse spider bites.

REFERENCES


