Brown Recluse Spider Envenomation: A Prospective Trial of Hyperbaric Oxygen Therapy

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ABSTRACT

Objectives: Loxosceles reclusa (brown recluse) spider bites can produce severe skin lesions that may necessitate extensive surgical repair. This study delineated the effects of hyperbaric oxygen (HBO) therapy on these lesions by performing a prospective controlled animal study.

Methods: After approval by the Institutional Animal Care and Use Committee, 41 New Zealand white rabbits received 64 intradermal injections of 73 μL of raw venom extract mixed with physiologic buffered saline (Dulbecco's solution). Control injections were made with buffer. The animals were divided into 5 groups: 1) venom and no HBO; 2) venom and 1 immediate HBO treatment (100% O₂); 3) venom and immediate HBO with 10 treatments (100% O₂); 4) venom and then delayed (48 hr) HBO therapy with 10 treatments (100% O₂); and 5) venom and immediate hyperbaric treatment with normal inspired O₂ for 10 treatments (84% O₂). Three animals in group 2 also received a control sodium citrate buffer injection. HBO treatments were at 2.5 atm absolute (ATA) for 90 minutes twice daily. Daily measurements were made of the lesion diameter, and skin blood flow using a laser Doppler probe.

Results: There was no significant effect of HBO on blood flow at the wound center or 1–2 cm from the wound center. Standard HBO significantly decreased wound diameter at 10 days (p < 0.0001; ANOVA), whereas hyperbaric treatment with normoxic gas had no effect. Histologic preparations from 2 animals in each group revealed that there were more polymorphonuclear leukocytes in the dermis of all the HBO-treated animals when compared with the venom-alone and sodium-citrate controls.

Conclusion: HBO treatment within 48 hours of a simulated bite from L. reclusa reduces skin necrosis and results in a significantly smaller wound in this model. The mechanism appears unrelated to augmented local blood flow between treatments.

Key words: hyperbaric oxygen therapy; HBO; brown recluse; spider bite; envenomation; wound healing.

dering viable skin. The eschar falls off, exposing fatty necrosis 1–3 cm deep and eventually leaving a stellate ulceration ranging 1–30 cm in diameter.\(^4\) Healing can require 1–6 months by means of epithelialization, granulation, and contracture. Secondary skin grafting or plastic surgery may be required.\(^4\)

The therapy for these bites is based on few controlled human or animal studies. Recent case series evidence shows that hyperbaric oxygen (HBO) therapy may be efficacious in managing the dermatonecrosis associated with \(L\). \(reclusa\). Svendsen reported accelerated healing in 6 patients with at least class III or IV envenomations\(^5\) up to 2–6 days post bite with a twice daily regimen of HBO at 2.0 atm absolute (ATA) for 60–90 minutes for 3 days in a Sechrist monoplace chamber. Kendall and Caniglia reported similar results in 47 cases with an average of 5.6 treatments. Both reported no third-degree skin slough, scarring, or need for surgery, grafting, or hospitalization.\(^6\) Maynor et al. electively treated 14 adult patients out of 40 who had suspicious lesions at 2–2.5 ATA for 90 minutes twice a day for an average of 7 treatments. All the patients healed without scarring, disability, or the need for skin grafting.\(^7\) Strain et al. performed the first controlled animal study and found significantly increased histologic healing with twice-a-day HBO therapy (2 ATA for 2 hr) compared with control.\(^8\) However, in another rabbit study, Phillips et al. compared HBO, dapsone, cyproheptadine, and control; they found no lesion size or histopathologic benefit with any of these therapies.\(^9\)

We sought to further delineate the effect of HBO on wound healing after \(L\). \(reclusa\) envenomation in a controlled rabbit model primarily by assessment of wound size, in vivo blood flow, and wound histopathology.

### METHODS

#### Study Design:

We used an evaluator-unblinded, controlled rabbit model of \(L\). \(reclusa\) envenomation to evaluate the effect of immediate and delayed HBO therapy on experimental wound size, blood flow, histopathology, venom sphingomyelinase activity, tissue polymorphonuclear leukocyte (PML) count and tissue lipid peroxidation up to 10 days after injury.

#### Animal Subjects:

Adult New Zealand white rabbits from the Duke vivarium were caged individually in an environmentally controlled room under a 12:12 lighting schedule with food and water as needed. The animals weighed on average 2.5 kg and were at least 12 months old. Prior approval for the study was obtained from the Duke University Animal Care and Use Committee.

#### Experimental Protocol:

Captured spiders were frozen at the University of Arkansas and the venom apparatus was dissected and homogenized in 20 mmol TRIS*-buffered saline (pH 7.4). Centrifugation removed all insoluble material. The venom protein extract was mixed with 25 mmol sodium citrate, at pH 5, to be placed in 10 different miniature vials to achieve individual concentrations of 0.21 μg/mL and a total shipment amount of 1.4 mg. This was shipped to Duke University Medical Center in a Styrofoam container on dry ice. On arrival the venom was stored at \(-70^\circ\)C until it was used.

To determine the proper dosage of venom necessary to produce a 2-cm lesion, 1 test animal was injected intradermally 3 times over a shaved area of the back using 5, 10, and 20 μg of venom. A 20-μg injection resulted in a 2-cm diameter lesion within 3 days, whereas the lower amounts caused insignificant lesions.

Thereafter prior to each study, the animal was shaved over both flanks to 6 cm from the midline 24 hours in advance to allow for any resulting changes in blood flow to subside. No animal instrumentation or sedation was used. On the day of injection, the venom protein extract was thawed and kept on ice until used. To prepare a single injection, 73 μL of raw extract was mixed with 73 μL of double-strength (2X) Dulbecco’s physiologically buffered saline (PBS) and then with an additional 54 μL of normal-strength (1X) Dulbecco’s PBS to make a total amount of 200 μL. The PBS (1X) had the following molar concentrations: CaCl\(_2\) 0.9 mmol; KCl 2.68 mmol; KH\(_2\)PO\(_4\) 1.47 mmol; MgSO\(_4\) 0.491 mmol; NaCl 136.7 mmol; Na\(_2\)HPO\(_4\) 15.22 mmol. For 2X PBS, the concentrations are doubled. This amount was injected intradermally within the shaved area with a 1-mL tuberculin syringe using a 26-gauge needle.

One untreated control animal did not receive an injection (i.e., no venom or sodium citrate injection) or HBO treatment. This animal was sacrificed for the myeloperoxidase portion of the study. The remaining animals received intradermal injections; 25 received only 1 venom injection and 16 received 2 venom injections. Duplicate injections were assigned randomly. All injections were made at least 7 cm apart. The rabbits were randomized to the following 5 treatment groups:

1. \(L\). \(reclusa\) venom and no HBO treatment \(n = 15; 6\) animals with 2 venom injections;\(^\star\)

2. Venom and 1 immediate HBO treatment at 2.5 ATA (100% \(O_2\)) \(n = 6; 3\) animals with 2 venom injections;\(^\star\)

3. Venom and immediate HBO for 10 treatments at 2.5 ATA (100% \(O_2\)) \(n = 9; 2\) animals with 2 venom injections;\(^\star\)

4. Venom and then delayed HBO (48 hr) for 10 treatments;\(^\star\)

\(\star\ \text{TRIS} = \text{tris(hydroxymethyl)aminomethane.}\)
FIGURE 1. Wound diameter vs treatment. Abbreviations are as follows: VenContr: venom control, no hyperbaric oxygen (HBO) treatment; DelHBO: 10 HBO treatments beginning 48 hours after venom injection; ImHBO10: 10 HBO treatments beginning immediately after injection; ImHBO1: a single HBO treatment immediately after injection; NaCitCon: sodium citrate control injection; 8%O2Con: 10 hyperbaric treatments while breathing 8.4% O2 to provide a normoxic inspired O2 tension. Wound diameter varied significantly with time (p < 0.0001). Wounds treated with ImHBO1, ImHBO10, or DelHBO were significantly smaller (p < 0.0001). Wound diameters in the 8%O2Con group were not significantly different from those in the untreated animals.

Measurements: The induced lesions were subsequently evaluated with the following methods without blinding of the investigator.

Gross Wound Size: Daily photographs were made documenting the gross appearance for 10 days. The diameter of the wounds for all groups were measured daily for 10 days using a centimeter ruler according to previously standardized Auer–Hershey wound classifications. We measured the black necrotic lesions or eschar in each animal. As most of the wounds were of roughly circular shape, diameter measurement was adequate to assess wound size, following the precedent of a previous report. If wounds were irregular in shape, the maximum diameter was used.

Wound Blood Flow: Laser Doppler Vasomedic BPM-2 (St. Paul, MN) with P403 right-angle probe flow measurements were made to evaluate superficial skin blood flow. These measurements were taken at the center of each lesion as well as 1 and 2 cm from the center in 4 quadrants. The 1- and 2-cm readings were 4 each/lesion and the mean was taken for each group.

Histological Examination: After euthanizing 1 animal/group for groups 1–4 at 3 days post venom injection, and 2 animals/group for groups 1–4 at 10 days post venom injection, skin was obtained from the center, and 1 and 2 cm from the center, yielding 36 gross tissue samples. These 36 samples were divided to equally represent treatment groups 1–4, at 3- and 10-day gross tissue samples placed in glutaraldehyde. In addition, the animals euthanized at 3 days post injection were not used in the daily wound size and blood flow measurement arms of the study because they could not be followed for the whole 10 days.

Myeloperoxidase Assay: To assess the tissue PML...
content, myeloperoxidase assays were performed on the frozen skin (wound margin) of the 3-day (1 animal/group; 2 specimens/animal) and 10-day (2 animals/group; 2 specimens/animal) samples from groups 1–4 to yield 12 specimen pairs. The specimens were homogenized using a Kinematica GmbH CH-6010 (Luzern, Switzerland) in phosphate buffer and then put in a Branson #250 opsonifier (Shelton, CT) for 5 times at 5 minutes each. The samples received 1% Hetab, and were frozen and thawed with liquid N$_2$ followed by sonication. The centrifuged supernatants were assayed using phosphate buffer, o-dianisidine, and hydrogen peroxide. The liquid samples were placed in a Beckman Du-64 spectrophotometer (DU Beckman Instruments, Wilmington, DE). The resulting data were expressed as a change in absorbance/min/mg protein, as 1 unit of myeloperoxidase activity equals that degrading 1 µmol of peroxide/min.

Malondialdehyde (MDA) Assay: To determine whether HBO had an effect on the amount of lipid peroxidation induced by L. reclusa venom, normal rabbit skin was homogenized, then mixed with venom and exposed to HBO. One New Zealand white rabbit was anesthetized with ketamine/diazepam and then an area of skin (1.5 g) was excised. This was homogenized in a Kinematica-GmbH CH-6010 homogenizer and put in a Branson opsonifier and then mixed with 8 mL PBS to facilitate dissolution. This produced 8 mL homogenized skin + PBS, of which 6 mL subsequently was mixed with 900 µL of raw venom. Two mL of this skin–PBS was kept on ice. The other 6 mL was divided to produce 300 µL venom/
FIGURE 4. Low-power section of the wound of an untreated animal on day 3. The wound crater is evident with attached eschar.

2 mL for skin-PBS + venom and 600 µL venom/4 mL skin-PBS + venom + HBO. The 4 mL of skin-PBS + venom + HBO was split evenly into 2-mL groups and placed under ice in a Bethlehem experimental treatment chamber (SA-1) (0.1696 m³) and the first group was exposed to 100% O₂ at 4 ATA and the second group at 2.5 ATA, both for 90 minutes. Two rabbits were shaved, and each was given 3 injections in small increments out of the 2-mL groups: 1) homogenized skin, 2) homogenized skin + venom, 3) homogenized skin + venom + HBO at 4.0 ATA.

FIGURE 5. Same section as Fig. 4 at higher power. The junction between the eschar and the wound crater can be seen. There is moderate leukocyte infiltration in the subeschar tissue.

The remaining homogenized specimens from each 2-mL group were used in an assay for sphingomyelinase-D. The assay was performed on the previously mentioned groups in addition to regular control venom and venom treated with 2.5 ATA. Each sample was then preincubated with HEPES† and CaCl₂. Then 14C-sphingomyelin in choline (Sigma, St. Louis, MO) was mixed with each preincubation sample. The reaction was terminated with the addition of chloroform, KCl, and methyl chloride. The samples were then centrifuged and 500-µL aliquots of each sample’s supernatants were used to obtain counts in the gamma spectrometer. Sphingomyelinase-D is cleaved by endogenous phospholipase-D to produce free choline measured in counts per minute (cpm).

Data Analysis: Statistical evaluation was performed on wound diameters and blood flow measurements using repeated-measures ANOVA. Post-hoc comparisons were made using the Bonferroni–Dunn adjustment for multiple comparisons. In the evaluation of the myeloperoxidase, MDA, and sphingomyelinase data, the sample sizes were too small for statistical comparisons; descriptive statistics are reported.

RESULTS

Survival: Two animals (from groups 1 and 4) died 2 days post venom injection and were subsequently excluded from the study (i.e., not used for data collection). It was presumed that they had succumbed to systemic loxoscelism because they both received 2 injections each.

Gross Wound Appearance: The HBO-treated animals had significantly smaller wounds than did the control animals (p < 0.0001). The animals treated with 8.4% O₂ at 2.5 ATA had wounds that were not different from those in the untreated animals. However, both delayed and immediate HBO treatment regimens, which produced supranormal O₂ levels, dramatically decreased the size of L. reclusa lesions (Fig. 1). All lesions had some evidence of necrosis, ranging from control animals with complete black eschars to HBO-treated animals with small lesions with red granulation bases.‡

Wound Blood Flow: However, there was no significant effect of HBO on wound blood flow as measured by laser Doppler at the center and 2 cm from the center (Figs. 2 and 3, respectively).

†HEPES = N-2-hydroxyethylpiperazine-N-2-ethanesulfonic [acid].
‡The authors will provide representative photographs of the wounds to interested readers on request.
Histology: We also looked at necrosis in the 3-day post-injection histologic sections showing increased numbers of PMLs in those animals treated with HBO compared with the untreated control animals (Figs. 4–7). For example, the delayed HBO-treated wound centers showed more consistent dense accumulations of PMLs (panniculitis) in the dermis and fascial layers than those in any of the control lesions. The control lesions had a larger, more complete central eschar or necrosis, even to the exclusion of all fatty layers in addition to necrotic myocytes. The HBO-treated groups had more underlying deep granulation tissue evidenced by new fibroblasts than did the control group. Sections of lung tissue showed no difference in the overall number of PMLs between the controls and all HBO-treated animals.

Myeloperoxidase: Wound margin samples were obtained from the animals in all groups except those receiving 8.4% O₂ during HBO therapy (group 5). Duplicate assays from each animal were compared for assay consistency. The duplicate assays were within 20% of each other. The mean results at 3 days post "bite" (units = change in absorbance/min/mg protein = 1 U myeloperoxidase activity) for these groups were as follows:

Normal skin: 1.24
Venom—no HBO: 1.31
Venom—HBO (1 immediate): 1.98
Venom—HBO (10 immediate): 2.45
Venom—HBO (10 delayed): 0.76

These results support the observation in this study and by other authors of increased PMLs on histologic specimens in animals that were immediately treated with HBO.¹⁰⁻¹⁴ However, the small sample size prohibits definitive statements.

The mean results at 10 days post "bite" were as follows:

Venom—no HBO: 0.189
Venom—HBO (1 immediate): 0.097
Venom—HBO (10 delayed): 0.176

MDA: Single wound margin samples were obtained from the animals in all groups except those receiving 8.4% O₂ during HBO therapy (group 5). The mean results at 10 days post "bite" (units = nmoles/mg protein) for these groups were as follows:

Buffer—HBO (1 immediate): 0.60
Venom—no HBO: 0.43
Venom—HBO (1 immediate): 0.33
Venom—HBO (10 immediate): 0.70
Venom—HBO (10 delayed): 0.47

These preliminary results do not suggest major differences in MDA content at 10 days.

Sphingomyelinase: The results of the preliminary homogenized skin studies initially show that the HBO
treated specimens had smaller lesions than did the venom + skin control specimens. At days 2, 4, and 6, the wound diameters in the animals injected with skin and venom were 6.5, 10, and 12 cm, respectively. In the animals injected with skin and venom pretreated with 4 ATA (HBO) the diameters were smaller: 1, 1.5, and 2 cm, respectively. Homogenized skin alone produced no lesion. There was no demonstration of decreased sphingomyelinase-D levels in our study samples.

**DISCUSSION**

Microdissection of venom sacs of *L. reclusa* has demonstrated alkaline phosphatase, hyaluronidase, protease, esterase, and collagenase. Most of these enzymes promote the swift diffusion of venom through tissues; however, the dermonecrotic component of *L. reclusa* venom is sphingomyelinase-D, a complex phospholipase of 32,000 da that causes a severe cytotoxic reaction in vitro.

Sphingomyelinase-D attaches to, structurally alters, and degrades sphingomyelin on human red blood and other cellular membranes in the presence of calcium, leading to cell lysis, hemolysis, and death. This leads to the release of inflammatory mediators such as arachidonic acid and prostaglandins, followed by platelet aggregation and serotonin release, resulting in microvascular thrombosis and ischemia. Most important, there is a significant chemotactic infiltration of PMLs, leading to intravascular clotting, degeneration of vessel walls, hemorrhage into the dermis, and liquefaction and abscess formation in 3–5 days. Finally, there is amplification of the initial inflammatory reaction by the intrinsic vascular cascade that is dependent on the mediators C-reactive protein and amyloid P component, as well as complement inhibition in producing the local and systemic reactions of necrotic arachnids. In contrast, snake and bee venoms produce a lesser necrotic amplification reaction in spite of much higher levels of hyaluronidase and phospholipase A2.

There continues to be controversy regarding the definitive therapy for cutaneous loxoscelism. Few controlled human studies have demonstrated that any one therapeutic modality decreases the chance of dermatonecrosis. Conservative measures include local cleansing, sterile dressings, cryotherapy, elevation, and administration of antipruritic, analgesic, and sedative medication.

Intraleisional, oral, and systemic steroids do not augment wound healing because of vasoconstriction and increased intraleisional pressure. Early wide excision is expensive, disabling, and disfiguring; furthermore, the extent of venom distribution margins is difficult to define. Dapson, traditionally used for leprosy therapy, has been used with some success in treating these dermatonecrotic lesions. However, the administration of dapson usually misses the surge of PMLs 1 hour post bite, and it can cause seizures and hemolytic anemia. The most promising therapy, intraleisional antivenin, inhibits the activity of sphingomyelinase-D. The obvious disadvantage is that it is effective only if given in the first 24–48 hours post bite; however, because these bites can appear initially innocuous, most patients are seen much later. Presently, antivenin is not commercially available. For those patients bitten by the brown recluse spider who go on to form necrotic ulcers, HBO administration may offer a safe and effective mode of therapy. The most common side effects of HBO administration include ear, sinus, and pulmonary barotrauma, as well as the benign O2 toxicity seizure.

Possible mechanisms for a beneficial effect of HBO in the healing of the bites of *L. reclusa* include the sequestration of PMLs away from the major wound sites, the inactivation of sphingomyelinase or venom, the direct inhibition of PML adherence at the cellular–endothelial interface and subsequent decreased effects of any venom-induced ischemia–reperfusion injury, and the increasing O2 delivery to ischemic tissue.

Our preliminary observations using homogenized skin + venom pretreated with HBO might suggest that the HBO mechanism in this injury could be some inactivation of a venom component. However, we could show no difference in the levels of sphingomyelinase-D between the HBO-treated and control groups (N = 2). This could be secondary to the type of assay chosen and to the presence in the venom of sphingomyelinase-C, which can compete with sphingomyelinase-D. We also cannot exclude the possibility that the mechanical action of the bubbles or the environment of the chamber also might have had an inactivating effect on the venom.

The pathophysiologies of ischemic perfusion injury and *L. reclusa* bites share the increased adherence of PMLs to microvascular endothelium and subsequent basement membrane penetration and interstitial tissue penetration. These PMLs produce reactive O2 metabolites, i.e., hydroxyl, superoxide, and peroxide, as well as myeloperoxidase, an enzyme that increases the production of hypochlorous acid and N-chloroamines, known cytotoxic oxidants. Zamboni et al. recently found that HBO provided a protective rather than an exacerbation effect for reperfusion injury, by decreasing venular PML adherence, myeloperoxidase, and arteriolar vasoconstriction. Thom and Ebubken recently observed in animal models of carbon monoxide (CO) poisoning that if HBO was given post CO exposure, there was a dramatic decrease in B2 integrins (CD11-18) and therefore PML endothelial binding, reducing the effects of ischemia–reperfusion injury. The accumulation of PMLs within the intravascular and interstitial tissue of *L. reclusa* bites may be vitally dependent on vascular endothelial adherence and could explain the ischemic injury pattern responsible for the ultimate necrosis.
We observed more preliminary PML activity in the HBO-treated animals when comparing histologic and myeloperoxidase assays of our wound tissue sections. This was noted by the more consistent dense accumulations of PMLs (panniculitis) in the dermal and fascial layers. This seems paradoxical, in light of inhibition of PML binding in other models by HBO\(^{17-30}\) and that sphingomyelase-D causes a massive influx of PMLs into the wound area in venom control animals. This could be explained by our limited number of study samples for each treatment group \((N = 2)\), and warrants future prospective large-sample animal studies.

Hyperbaric oxygen could minimize wound size in the bites of \(L.\) reclusa by 3 possible mechanisms. First, the increased \(P_2\) in wound extracellular fluid may prevent ischemic necrosis that might otherwise occur in the penumbra of the envenomed area by extending the usual \(O_2\) gradient from 30–80 \(\mu\)m away from the vessel walls.\(^{40}\) This increased \(O_2\) tension also could enhance capillary endothelial cell (angiogenesis) into new wounds. Wound margin fibroblasts require a tissue \(P_2\) of 40–55 torr to replicate and produce a collagen matrix strong enough to support angiogenesis and extension into the wound.\(^{40-42}\) Fibroblasts are also attracted to new wound centers by mitogen-releasing macrophages. This supports the use of intermittent HBO with normobaric intervals. It also has been shown that PMLs have bursts of \(O_2\) consumption after phagocytizing bacteria. The provision of extra \(O_2\) via HBO in a normally hypoxic wound could facilitate phagocytic killing of microorganisms.\(^{43}\)

**LIMITATIONS AND FUTURE QUESTIONS**

The rabbit model that we used was an excellent one in which to study the effects of HBO on the lesions of \(L.\) reclusa. To our knowledge, no controlled human study has addressed HBO therapy for this injury; however, several case reports have supported the use of HBO.\(^{5-8}\) Our animal lesions were similar to those of humans as supported by our pathophysiology review. Furthermore, previous authors have found rabbit skin, though not identical, to be a close approximation to human skin regarding dermal thickness, underlying layers, and texture.\(^{20,22,24}\)

The effect on ultimate healing (>10 days) was not determined in this study because all rabbits were killed after 10 days according to our animal committee protocol. However, our observations of the successful outcome of the lesions in the first 3–10 days support the use of HBO in the therapy of the bites of \(L.\) reclusa. The sample sizes of the myeloperoxidase, MDA, and sphingomyelinase assays were inadequate to draw definitive conclusions. However, these preliminary findings should help guide future studies with larger sample sizes.

The measurement methods used to assess lesion size (diameter) were based on previously recognized \(L.\) reclusa wound protocols set by Auer and Hershey.\(^{10}\) As our lesions were uniformly circular, no difference in results would have occurred comparing total wound surface areas. The measurement method for blood flow using laser Doppler readings proved to be limited as it produced day-to-day biologic variability secondary to motion artifact. The study was also limited because the observer was not blinded to solution used during lesion induction at the time of wound measurement.

This study provides important prospective data related to the effect of HBO alone in \(L.\) reclusa bites. This study opens many intriguing possibilities for future studies. Does HBO affect the inactivation on the toxic venom component sphingomyelinase? What role does HBO play in the manipulation of PML function, and how does that affect the healing of \(L.\) reclusa bites? Perhaps a future study using much larger sample sizes would compare HBO and a PML monoclonal antibody or other PML inhibitor to assess whether any difference in lesion sizes occurs.

**CONCLUSIONS**

In this study, there was a positive effect of HBO on reducing the size of lesions caused by \(L.\) reclusa venom. HBO was effective not only when given immediately in varying doses, but also when it was delayed by 2 days. When given immediately after induced \(L.\) reclusa bites, a single HBO treatment was as effective as multiple HBO treatments. These beneficial effects were not simply due to the effect of hyperbaric treatment alone. Since using an inspired \(P_2\) of 160 torr in a hyperbaric environment (2.5 ATA) did not result in any effect on wound size, the mechanism appears to be due to the effects of hyperoxia at increased pressure, rather than any nonspecific effect of hyperbaric normoxic exposure.

There was no indication that blood flow in the vicinity of the wound was altered as a result of HBO therapy. Also, PML accumulation in these induced lesions seems to be enhanced by immediate HBO administration. Our animal model results support the use of HBO in the therapy of the bites of \(L.\) reclusa. For those individuals who either see the \(L.\) reclusa spider during the bite or develop a typical ≥2-cm diameter lesion 2 days after being in a place consistent with the spider’s habitat, we recommend the initiation of HBO therapy with at least 1 treatment. Whether a single HBO treatment is as effective as multiple treatments for \(L.\) reclusa bites in humans remains to be proven.

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