

Hyperbaric oxygen treatment decreases pain in two nerve injury models

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ABSTRACT

Hyperbaric oxygen (HBO) treatment has been used clinically to treat a variety of ailments, including severe burns and carbon monoxide poisoning, and in research settings has produced promising results when used to treat animal models of inflammatory pain. However, studies examining neuropathic pain or nerve injury models have been limited to physiological assessments and not whether the pain condition improves. The purpose of this study was to evaluate the effect of HBO on two common models of neuropathic pain, L5 ligation and chronic constriction injury (CCI) of the sciatic nerve. Following surgical manipulations, animals demonstrating mechanical hyperalgesia were randomly assigned to either HBO treatment or control for 90 min treatment sessions, after which mechanical sensitivity was assessed at 15 min and 6 h post. Daily HBO sessions, with assessments 15 min post-treatment, continued for two weeks, followed by 5 days of assessment only. The results indicated that both models demonstrated significant improvement in response to treatment over the course of the two-week period, with CCI animals recovering more quickly and maintaining this recovery throughout the post-treatment period. Hyperbaric oxygen treatment appears to be successful in relieving neuropathic pain for an extended period of time, and future research should be aimed at investigating the precise mechanisms underlying this positive effect.

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Hyperbaric oxygen (HBO) treatment involves the process of applying 100% oxygen at atmospheric pressures greater than sea level. It has been used to treat a variety of ailments, including severe burns and carbon monoxide poisoning. However, within recent years its use for the treatment of disease has increased (Jain, 2004). For instance, HBO has been assessed for its effects on multiple sclerosis, migraines, and cerebral ischemia (Bennett and Heard, 2001; Carson et al., 2005; Bennett et al., 2008). Of more recent interest is the effect it may have on pain. Previous research has indicated a decrease of inflammation and inflammatory pain in animal models which would suggest its application to other pain conditions such as pain resulting from nerve injury (Sumen et al., 2001; Wilson et al., 2007). Unfortunately, little research has focused on the effect of HBO treatment on neuropathic pain.

The majority of nerve injury and hyperbaric treatment research has had conflicting results. For example, research utilizing a sciatic

nerve crush injury indicated that animals which received treatment with hyperbaric oxygen at pressures of at least 2.5 atmospheres absolute (ATA) experienced enhanced regeneration of the crushed nerve during the first few days following injury and treatment (Haapaniemi et al., 1998). Bajrović et al. (2002) found that, when using a similar model, HBO therapy did not dose dependently effect regeneration and when examining a crush of the sural nerve, HBO did not effect regeneration in distal axons in comparison to the control group. Research focusing on recovery of motor functioning after a nerve crush injury has been inconclusive as well. For instance, functional recovery following a nerve crush injury and HBO therapy was not improved by treatment (Haapaniemi et al., 2002), yet Zamboni et al. (1995) found that when the sciatic nerve was transected and then repaired HBO significantly improved motor functioning. Finally, in a commonly used model of neuropathic pain, the chronic constriction injury of the sciatic nerve, it was found that HBO improved blood flow, decreased edema, and prevented cellular damage of the mitochondria and other organelles as compared to animals which did not receive treatment (Mychaskiw et al., 2005). Surprisingly, this study did not quantify the effects of HBO treatment on pain, and no other study has attempted to utilize this model or examine neuropathic pain and HBO.

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The purpose of the present study was to investigate and quantify the effects of hyperbaric oxygen treatment on neuropathic pain. In an attempt to obtain a more complete understanding two different nerve injury models were utilized: the chronic constriction injury model of the sciatic nerve and the L5 spinal nerve ligation model. It was hypothesized that HBO treatment would significantly decrease pain as compared to control treated animals. No research has been conducted examining the L5 model and hyperbaric oxygen treatment, so it was difficult to hypothesize that one model would respond better to HBO treatment than another. However, literature indicates that the CCI model may have a greater inflammatory component (Clatworthy et al., 1995), thus it was hypothesized that the CCI model would respond better to treatment.

1. Method

1.1. Subjects

The experiment used 104 male Sprague-Dawley rats, ranging between the ages of 60 and 80 days at the start of the experiment. Animals were group housed in a temperature-controlled room with a 12 h light/dark cycle. Food and water were provided *ad libitum*. All protocols were approved by the local Institutional Animal Care and Use Committee of the University of Texas at Arlington and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983).

1.2. Induction of neuropathic pain conditions

Two different neuropathic pain conditions were utilized for this study, the L5 spinal nerve ligation model ($n = 31$) (Kim and Chung, 1992) and the chronic constriction injury (CCI) model ($n = 73$) (Bennet and Xie, 1988). In brief, all animals were anaesthetized with isoflurane in 100% O₂ (3% induction, 2% maintenance). Animals receiving the L5 ligation were placed in the prone position to allow access to the left L4–L6 spinal nerves. Under magnification, approximately one-third of the L6 transverse process was removed. The L5 nerve was identified and carefully dissected free from the adjacent L4 spinal nerve and then tightly ligated using a 6-0 silk suture. For animals in the CCI group, a blunt dissection of the biceps femoris was made at the mid-thigh level on the left leg, and the sciatic nerve exposed. Three sutures of 4.0 chromic cat gut were loosely tied around the sciatic nerve, just proximal to the trifurcation, and spaced approximately 1 mm apart. Finally, the wound for all animals (both L5 and CCI models) was treated with an antiseptic solution, the muscle layer was sutured, and the wound was closed with wound clips. Animals displaying L4 damage (i.e. impaired motor functioning of the left hind paw) following the L5 ligation were removed from further experimentation ($n = 1$).

1.3. Hyperbaric oxygen treatment

All animals were randomly assigned to either a treatment group or control group. Hyperbaric oxygen treatment consisted of placing animals inside a hyperbaric treatment chamber where they received 100% oxygen at a pressure of 2.4 ATA. During each treatment animals were slowly brought from room pressure to depth (5–7 min), they received a 90 min treatment, and were then resurfaced to normal room pressure again (5–7 min). This protocol was chosen as it reflects a typical clinical treatment regimen for chronic wounds treated in a monoplace chamber. Therefore, each treatment lasted in total approximately 100–104 min. The control group was simply placed inside the hyperbaric treatment chamber for approximately 100 min, and did not receive any treatment.

1.4. Mechanical paw withdrawal threshold testing

Animals were placed within a Plexiglas chamber (20 cm × 10.5 cm × 40.5 cm) on top of a mesh screen which allowed easy administration of the mechanical stimuli. Animals were given a 10 min habituation period prior to each test session. Mechanical thresholds were determined for each hind paw utilizing eight von Frey monofilaments (4.01, 5.78, 10.19, 19.40, 40.77, 80.98, 137.60, and 261.04 mN) and the up/down procedure as explained by Dixon (1980). Each trial started by applying the von Frey force of 10.19 mN for 1 s to the plantar surface of the right and then left hind paw. If a withdrawal response was not observed then the next highest force of monofilament was applied, however if a withdrawal was observed then the next lowest force was applied. This protocol was continued until no withdrawal of the paw was observed at the highest force (261.04 mN), or until four stimuli had been administered following the initial withdrawal response. Withdrawal thresholds were calculated using the following formula: $[X_{th}] \log = [vFr] \log + ky$, where $[vFr]$ is the force of the last von Frey used, $k = .2591$ which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. If an animal did not respond to the highest von Frey monofilament then $y = 1.00$, and the withdrawal threshold was calculated to be 474.03 (maximum possible value). Threshold testing was performed three times during each testing period, and the scores were averaged to determine the mean mechanical paw withdrawal threshold for both the left and right hind paws.

1.5. Experimental protocol

Prior to induction of the neuropathic pain condition all animals received a baseline mechanical paw withdrawal threshold (MPWT) test. Pre-surgical criteria dictate that animals displaying mechanical hyperalgesia at the baseline test be removed from the study; however this is extremely rare and no animals met such criteria in this study. Therefore, all animals included had an average mechanical threshold of 474.03 (the maximum possible value) at baseline. Animals then received either the L5 ligation or CCI surgery as outlined above. All animals were given a brief recovery period (3 days for L5 animals and 5 days for CCI animals), and then received a pre-treatment MPWT test. Animals were included for further experimental analysis if they displayed at least a 50% difference from baseline MPWT testing. At this point, 10 rats from the L5 group and 51 rats from the CCI group were removed due to not meeting inclusion criteria. Immediately following the initial pre-treatment MPWT testing animals received hyperbaric treatment according to their experimental condition. Upon removal from the hyperbaric chamber animals were immediately placed into the Plexiglas chambers for MPWT testing, and an experimenter blind to treatment condition carried out testing.

MPWT testing occurred twice on the first day of treatment, once immediately after treatment, and again 6 h following treatment. Then, all animals continued to receive treatment according to their experimental condition for the next 13 days, receiving MPWT testing immediately following treatment. Finally, animals continued to receive MPWT testing for 5 days following the cessation of treatment. Therefore, animals were treated daily for 14 days where they received daily MPWT testing, and were further monitored for another 5 days, resulting in a 19-day testing protocol.

2. Results

The final number of animals included in experimental analysis after pre-treatment MPWT testing occurred was 20 for the L5 ligation group ($n = 10$ for both control and treatment conditions),

and 22 for the CCI group ($n = 10$ for control, $n = 12$ for treatment conditions). Due to baseline pre-treatment differences, the mean MPWT scores from each rat were converted daily into percent change from pre-treatment values with the following formula: $[(\text{daily MPWT average} - \text{pre-treatment MPWT average}) / (474.03 - \text{pre-treatment MPWT average})] \times 100$. Scores were converted in order to ease interpretation of data, and by normalizing resulted in a more conservative estimate of difference.

Prior to analyses, datum was screened for outliers and assumptions of normality. No outliers were detected and the data met assumptions. Two (Treatment Condition: control vs. treated) \times 20 (Time: day 1 of treatment through day 5 of post-treatment) mixed factorial analysis of variances (ANOVA) were used to analyze the effect of hyperbaric oxygen treatment on percent change of threshold values across time in animals with either the L5 surgery or CCI surgery. Bonferroni post hoc analyses were used when necessary. The ANOVA for the L5 group revealed a significant main effect of Time, $F(19,342) = 5.01$, and Treatment Condition, $F(1,18) = 18.32$, and a significant Time \times Treatment Condition interaction, $F(19,342) = 3.14$ (all three findings at $p < .001$) (Fig. 1). The ANOVA for the CCI group indicated a significant main effect for Time $F(19,380) = 1.89$, $p = .014$, and Treatment Condition, $F(1,20) = 14.76$, $p = .001$, and a significant Time \times Treatment Condition interaction, $F(19,380) = 2.13$, $p = .004$ (Fig. 2). The results indicate that animals receiving hyperbaric oxygen treatment (of both nerve injury conditions) had significantly less mechanical hypersensitivity than those that did not receive treatment. Furthermore, as indicated by the post hoc analyses, HBO treated animals displayed decreased mechanical hypersensitivity at nearly every time point after the start of treatment, and this was also significant during the post-treatment time period.

A 2 (Treatment Condition: control vs. treated) \times 2 (Surgery Condition, L5 vs. CCI) \times 20 (Time: day 1 of treatment through day 5 of post-treatment) mixed factorial ANOVA was used to examine potential differences between the surgical conditions response to hyperbaric oxygen treatment across time. A main effect of Surgery Condition was not found, $F(1,38) = 3.10$, $p = .08$, indicating no overall differences between the L5 and CCI surgery; however a significant Time \times Treatment Condition \times Surgery Condition interaction was found, $F(19,722) = 1.66$, $p = .038$. Bonferroni post hoc

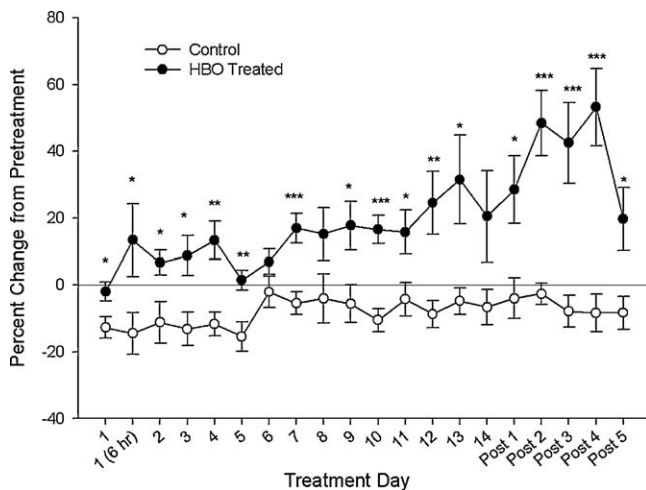


Fig. 1. Effect of hyperbaric oxygen (HBO) treatment on the L5 model of neuropathic pain. Scores represent the average percent change (\pm SEM) from pre-treatment mechanical threshold measures. Data indicate that hyperbaric oxygen treatment significantly decreases neuropathic pain across time and that animals receiving hyperbaric oxygen treatment had significantly more improvement at nearly every time point. * $p < .05$, ** $p < .01$ and *** $p < .001$.

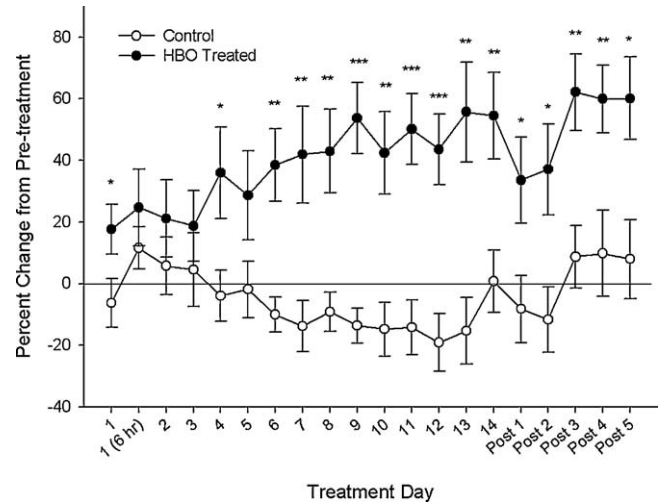


Fig. 2. Effect of hyperbaric oxygen (HBO) treatment on the chronic constriction injury (CCI) model of neuropathic pain. Scores represent the average percent change (\pm SEM) from pre-treatment mechanical threshold measures. Data indicate that hyperbaric oxygen treatment significantly decreases neuropathic pain across time and that animals receiving hyperbaric oxygen treatment had significantly more improvement at nearly every time point. * $p < .05$, ** $p < .01$ and *** $p < .001$.

analyses indicated that the control groups of both surgery conditions did not differ significantly from each other across all of the time points. The treated CCI group displayed significantly more improvement than the treated L5 group immediately following treatment on day 1, and again on day 6, day 8 through day 11, day 14, and post-treatment day 5. The results indicate that the CCI group responded to treatment sooner than the L5 group and the treatment effect was maintained longer as well. These findings suggest that the L5 group and CCI group responded differently to hyperbaric oxygen treatment.

3. Discussion

The purpose of this study was to quantify the effects of hyperbaric oxygen treatment on neuropathic pain. The results suggest that hyperbaric oxygen (HBO) treatment decreases neuropathic pain, and this is consistent in two of the most common nerve injury models. Overall there was not a significant main effect of surgery type, however differences between the two models exist with regards to treatment response over time. Animals with the chronic constriction injury (CCI) experienced a decrease in mechanical hypersensitivity earlier than the animals with the L5 ligation. The effect of this treatment also appeared to maintain itself longer for the CCI group than for the L5 group.

The exact mechanisms of hyperbaric therapy on neuropathic pain are unknown. No studies to date have specifically investigated the effects of HBO on neuropathic pain; rather they have only measured other markers such as nerve regeneration and functionality. It is possible that HBO treatment decreases pain by relieving inflammation that is commonly found in nerve injury models. Several studies have indicated that inflammation is a key component to neuropathic pain. Using the CCI model, Clatworthy et al. (1995) tested for thermal hyperalgesia in animals that received either daily injections of dexamethasone or saline. Those that received dexamethasone displayed significantly less thermal hyperalgesia than saline treated animals. In the same study, they also conducted an experiment where they applied cotton sutures soaked in either Freund's adjuvant or saline instead of the chronic cat gut that is typically used in the CCI model. Animals that received adjuvant soaked sutures displayed significantly higher

levels of thermal hyperalgesia. Other studies have suggested that injections of corticosteroids or anti-inflammatory cytokines decreases hyperalgesia in the L5 model and CCI model respectively (Wagner et al., 1998; Li et al., 2007). These results suggest that inflammation is a key factor for hyperalgesia to exist in neuropathy models. Previous research indicates that HBO decreases acute inflammation produced by carrageenan (Sumen et al., 2001; Wilson et al., 2006), which therefore suggests that HBO treatment may decrease the inflammation associated neuropathy and thus decrease pain.

Additionally, the activity of immunological cells such as mast cells, macrophages, and T cells is increased at the site of nerve injury. Each of these cells is capable of producing inflammatory cytokines, thus furthering inflammation at the site (Moalem and Tracey, 2006). HBO has been shown to decrease the circulating T cell ratio and decrease the functioning of macrophages, therefore decreasing the numbers of inflammatory cytokines released by these cells, and decreasing inflammation in the body (Brenner et al., 1999). Overall, the data suggest that inflammation is an important aspect of the development of neuropathic pain and hyperbaric oxygen therapy decreases inflammation and activity of inflammatory cells. It is therefore possible that hyperbaric oxygen treatment decreases neuropathic pain through anti-inflammatory mechanisms.

A large number of animals in the CCI group were not included in the study due to not meeting inclusion criteria for mechanical sensitivity. Research has indicated that the CCI model is less sensitive to mechanical sensitivity testing as compared to the L5 model. However, the CCI model is much more sensitive to the hot and cold plate (Kim et al., 1997). It is possible that our findings are limited due to the select group of CCI animals that were responsive to mechanical threshold testing. We are confident however that the time course and choice to exclude animals was appropriate, as Kim et al. found a significant mechanical departure from baseline by day 5, and the effect was maintained without a decrease through four weeks. Their findings would indicate that the effect of CCI on mechanical pain is long lasting, and therefore our findings are due to a treatment effect and not simply the animals recovering on their own. It would be of interest therefore to compare the effects of the CCI and L5 models' response to HBOT on the cold and/or hot plate in the future.

Also of interest was that on the final day of testing (day 5 post), the L5 group showed a drastic reduction in their improvement, while the CCI group continued to have significantly less mechanical pain. Testing was not continued beyond this point to examine how long the CCI model maintained its improvement of neuropathic pain. Future research should be directed towards examining the longevity of this effect, and additionally attempt to determine why the L5 group began to exhibit increased pain responses again.

The HBO treatment protocol used reflects a typical clinical treatment regimen for chronic wounds treated in a monoplace chamber. The therapeutic benefit of using a HBO protocol that resembles an intermittent procedure used clinically in multiplace chambers is not known. Animals were treated daily for 14 days, yet the majority of hyperbaric treatments for animals, in an attempt to avoid the possibility of oxygen poisoning, do not last longer than about a week. Previous analysis of human participants have found that the risk of seizure or central nervous system oxygen toxicity is extremely low (less than .05%), however these studies only examined 1 day protocols (Plafki et al., 2000; Hampson and Atik, 2003). A study on Sprague-Dawley rats examining the effect of HBO on streptozotocin induced diabetic neuropathy gave daily treatments for four weeks, at 2 ATA, and found no indication of hyperbaric oxygen toxicity (Low et al., 1988). Furthermore, animals in the present study were monitored

daily, and none showed any indications of seizures or toxicity, suggesting that the extended time course did not affect the animals negatively.

In conclusion, neuropathic pain typically does not respond well to conventional treatments, and few patients receive total relief of their pain. Treatments include either opioids or non-steroidal anti-inflammatories, which have side effects or run the risk of dependence, or anti-depressants, which are usually given in less than effective doses (Harden and Cohen, 2003). Hyperbaric oxygen treatment appears to be successful in relieving neuropathic pain for an extended period of time, and thus should be considered as an alternative measure to the therapies that are currently utilized. Future research should be aimed at investigating the precise mechanisms underlying the effect of HBO treatment on neuropathic pain, in addition to determining the time course of pain alleviation.

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