Effects of Supplemental Oxygen and Hyperbaric Oxygen on Tendon Healing in a Rat Model

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Systemic supplemental oxygen therapy (SOT) and hyperbaric oxygen therapy (HBOT) have been shown to positively impact wound healing. The purpose of this study was to evaluate the effects of SOT and HBOT on tendon healing in a rat tendon model. The right patellar tendon of 90 male Sprague-Dawley rats was completely sectioned. Animals were randomized to receive HBOT, SOT, or room air therapy. Animals were sacrificed at 3- and 6-weeks postoperatively. The ultimate tensile strength in axial extension was compared between groups. Statistical significance was calculated using the Student's t-test. The SOT group exhibited the highest tensile strength at both time-points, although HBOT was the only treatment that exhibited a statistically significant increase in tensile strength between time-periods ($p = 0.006$). There was no statistical difference in ultimate tensile strength when the three groups were compared at the 3- or 6-week time-points. Results presented here cannot support the premise that intermittent HBOT or SOT significantly increases the healing of tendon repairs. (Journal of Surgical Orthopaedic Advances 20(4):225–229, 2011)

Key words: Hyperbaric Oxygen Therapy, Supplemental Oxygen Therapy, Oxygen, Tendon Healing, Wound Healing

Introduction

Oxygen plays a pivotal role in tissue healing, as well as modulating a wide variety of cellular responses through reactive oxygen species (1–5). Tissue healing relies heavily on collagen deposition, which provides the matrix for angiogenesis and tissue remodeling. Oxygen is essential for the hydroxylation of proline and lysine during the synthesis and cross-link formation of collagen. It is this formation that is ultimately responsible for the tensile properties of healing wounds (2, 4, 5).

Systemic oxygen therapy can be provided under pressure, via hyperbaric oxygen, or at normobaric pressures via supplemental oxygen. The therapeutic efficacy of systemic hyperbaric oxygen therapy (HBOT) has been investigated with regard to many disease processes (6, 7). The use of HBOT in some animal models has shown accelerated collagen synthesis and concomitant ligament and tendon healing (8, 9). However, there is limited evidence to suggest that HBOT increases the ultimate tensile strength of tendons or ligaments (10, 11).

HBOT has been popularized due to its use by professional athletes to help promote injury healing at accelerated rates (7). Unfortunately, HBOT is not widely available, and its use adds considerably to health-care expenditures (approximately $300 to $400 for 90 minutes) (6).

Systemic supplemental oxygen therapy (SOT) has been clinically shown to decrease surgical-wound infection rates and increase wound ultimate tensile strength(12–16). However, the use of SOT for tendon healing has never been reported previously in the literature. This investigation sought to compare the effects of systemic HBOT and SOT on tendon healing in a rat model. The hypothesis was that rats receiving HBOT and SOT would show improved tendon healing as evidenced by increased tendon ultimate tensile strength when compared to those receiving no additional oxygen therapy.

Methods

This study was approved by our Institutional Animal Care and Use Committee and complied with the Guide for

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the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1978). Ninety male Sprague-Dawley rats (rattus norvegicus), weighing between 350 to 500 g, were randomly assigned to three groups of thirty to either receive HBOT, SOT, or normobaric room air therapy (RA). Randomization occurred prior to the performance of any surgical procedures.

Description of Procedure

We utilized an established rat patella tendon laceration model described by Ishii et al. (8, 9). Each animal was anesthetized with ketamine and xylazine and given a subcutaneous injection of gentamicin preoperatively. The right hind limb was shaved and prepped in a sterile fashion. A 1- to 2-cm longitudinal skin incision was made over the anteromedial aspect of the knee exposing the patellar tendon. A complete transection was made at the mid-substance of the tendon equidistant from the origin and insertion (Figure 1). After patellar tendon laceration, the gap was less than or equal to 1 mm in all rats. The skin was closed with two simple 4-0 nylon sutures. After recovery from anesthesia all of the animals were returned to their cages. All animals were housed in individual cages in the same room, fed a standard pellet diet, and given water ad libitum.

Treatment Regimens

HBOT (Class C Monoplace Hyperbaric System, Pacific Coast Welding and Machine Inc, Chula Vista, CA) was initiated on the third day following surgery. The HBOT regimen involved intermittent 60-minute sessions of oxygen (100% FiO2) delivered at two atmospheres. SOT was initiated starting on the third day following surgery and consisted of intermittent 60-minute sessions of oxygen (100% FiO2) delivered at approximately 1 atmosphere. The same chamber was used to for both the HBOT and SOT groups. The elevation of the veterinary lab where the treatments were given is approximately 4,000 feet, coinciding with 0.86 atmospheres of pressure according to Universal Industrial Gases, Inc. Beginning on the third day following surgery the rats receiving RA were removed from their individual cages and placed together in enclosed cages for 60 minutes in an attempt to match the activity level of the treated groups. The treatments for all groups (HBOT, SOT, RA) were given on the same days for consistency.

Harvesting Procedure

On postoperative day 20 (the 3-week time-point), 15 rats from each of the three groups were sacrificed. At this time-point each group had completed 10 treatment sessions. The patellar tendon was harvested as a patella-patellar tendon-tibia complex (Figure 2). The patella-patellar tendon-tibia complex was wrapped in saline soaked towels, placed in individual containers, and kept in a 34° Fahrenheit refrigerator overnight for mechanical testing on post-harvest day 1. Each tendon was refrigerated for approximately 24 hours prior to mechanical testing. On the day of testing each tendon was removed from the refrigerator and kept in its individual container at room temperature for approximately 1 hour prior to testing.

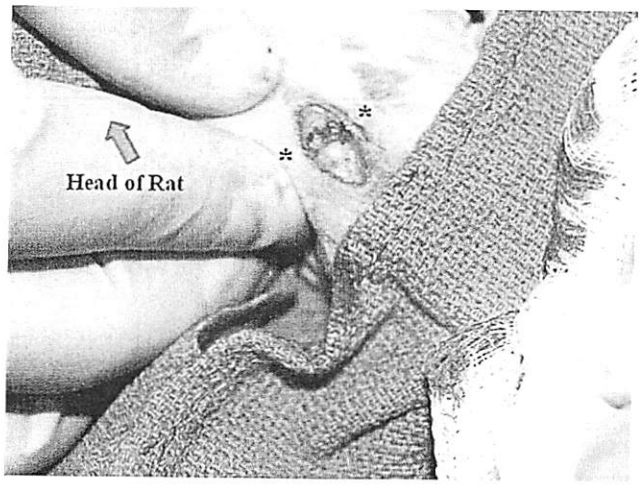

FIGURE 1 Surgical Laceration of Right Patellar Tendon (asterisks denote where laceration was made at the midpoint of the patellar tendon).

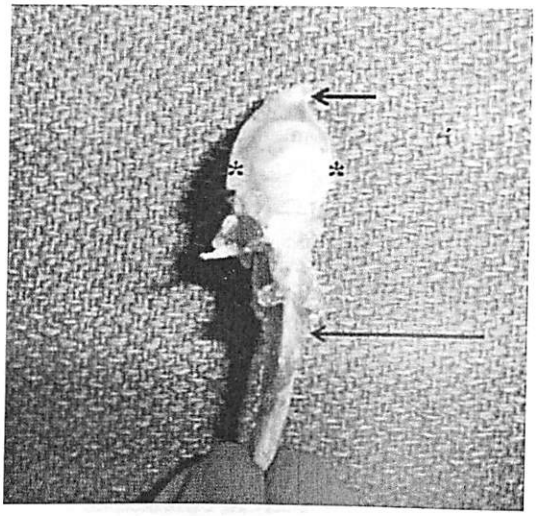

FIGURE 2 Patella-Patellar Tendon-Tibia Complex specimen (Short arrow points to the patella, tendon laceration filled with scar tissue is between asterisks, long arrow points to tibia).

The remaining 15 rats per group continued to receive therapy as described above for a total of 20 treatments prior to being sacrificed on postoperative day 41 (the 6-week time-point). Tendons were harvested in a fashion identical to that described above.

Mechanical Testing

Each specimen was tested to failure in axial extension using an Instron 5866 material-testing system (Norwood, MA). The patella and tibia were clamped and fixed longitudinally to the material-testing apparatus (Figure 3) and a preload of 2 N was applied. Load to failure was then performed at a rate of 1 mm/min until ultimate tensile failure occurred. The load at which failure occurred was recorded as the ultimate tensile strength for the tendon.

Statistical Analysis

A priori power analysis ($\alpha = 0.05$ and $\beta = 0.20$) determined a minimum of 10 rats per group, per time period tested were necessary to identify a difference of 20% in the average ultimate tensile strength (in newtons) of the patellar tendons between treatment and control groups. We considered that a 20% increase in tendon strength would be clinically significant although, to our knowledge, there have been no prior studies evaluating this phenomenon. The ultimate tensile strength and the mean change in strength between time-points with 95% confidence intervals (CI) were compared for each treatment group. Initially, treatment groups were compared to each other at the 3- and 6-week time points. Subsequently, the tensile strength of tendons within treatment groups was also analyzed. All statistical analyses were performed utilizing the Student's t-test. Statistical significance was pre-determined for p values <0.05.

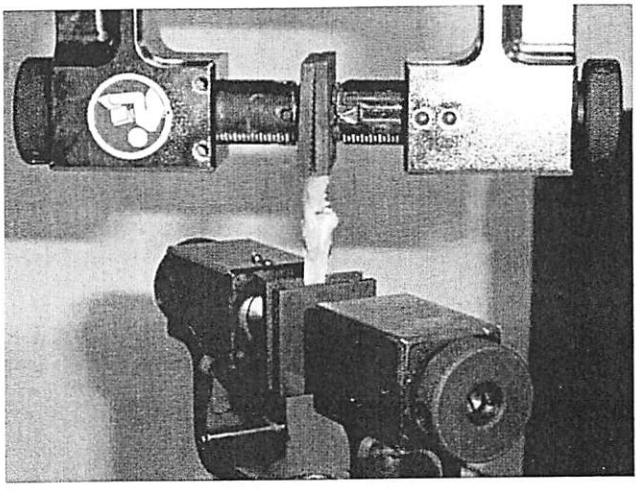

FIGURE 3 Specimen fixed to Instron Machine in axial tension prior to mechanical testing (Patella is fixed to the top clamp and the tibia is fixed to the bottom clamp).

Results

Upon gross inspection at the time of harvesting, the patellar tendons showed complete scar tissue healing at the laceration site in all specimens at 3 and 6 weeks. At 3 weeks postoperatively, the average ultimate tensile strength for the RA group was 64.0 ± 12.5 N, while the mean for the SOT and HBOT groups were 65.1 ± 12.7 N and 56.0 ± 13.8 N, respectively. There was no statistical difference when comparing the individual groups for this time-point. At this time-point, 33 of the 45 specimens failed at the patella laceration site.

At 6 weeks postoperatively, the mean ultimate tensile strength for the RA group was 67.7 ± 10.7 N, while the average for the SOT and HBOT groups were 75.4 ± 15.5 N and 68.8 ± 9.6 N, respectively. There was no statistical difference when comparing the individual groups from this time-point. At this time-point, 31 of the 45 specimens failed at the patella laceration site.

There was an increase in the mean ultimate tensile strength from 3 weeks to 6 weeks within each group (Table 1). For the RA group the average increase was 3.7 N (95% CI −4.96 to 12.40) and for the SOT and HBOT groups the average increase was 10.3 N (95% CI −0.24 to 20.98) and 12.8 N (95% CI 3.90 to 21.64), respectively. The mean increase in tensile strength was only statistically significant for the HBOT group ($p = 0.006$), although there was a trend toward significance for the SOT group ($p = 0.055$).

Discussion

The goal of this investigation was to evaluate the efficacy of supplemental oxygen therapy on tendon healing in a small animal model. We hypothesized that rats receiving

TABLE 1 Mean change in ultimate tensile strength within each treatment group from the 3- to the 6- week time points

	3-week mean ± standard deviation (newtons)	6-week mean ± standard deviation (newtons)	Mean Change (newtons)	P-value
RA	64.0 ± 12.5	67.7 ± 10.7	3.7	0.388
SOT	65.1 ± 12.7	75.4 ± 15.5	10.3	0.055
HBOT	56.0 ± 13.8	68.8 ± 9.6	12.8	0.006

RA — Room Air group.
SOT — Supplemental Oxygen Therapy group.
HBOT — Hyperbaric Oxygen Therapy group.

HBOT and SOT would have increased ultimate tensile strength of the patellar tendon after surgical transection compared with control tendons. Findings presented here cannot substantiate a significant effect for supplemental systemic oxygen therapies on tendon healing. Although HBOT did result in a statistically significant increase in tensile strength between the 3- and 6-week time-points, and a similar trend was appreciated for SOT, no statistical differences in tensile strength were appreciated between the experimental groups and the RA controls.

Prior investigations have studied the effect of HBOT on tendon healing. In two separate studies, Ishii and colleagues examined the effect of HBOT on tendon healing in a similar model (8, 9). Subjectively, the rats that received HBOT demonstrated improved gross appearance of the tendon laceration and more rapid filling and ordering of collagen fibers on histological analysis when compared to rats receiving room air conditioning 1–2 weeks postoperatively. Objectively, the rats that received HBOT had significantly increased levels of pro-α(I) mRNA (the main component of type I collagen) 1 to 2 weeks postoperatively. Both studies concluded that intermittent HBOT therapy could enhance collagen synthesis.

There are a limited number of mechanical studies in animals measuring the effect of HBOT on tendon and ligament strength. Horn et al. (11) examined the effect of HBOT in a rat model of surgically lacerated medial collateral ligaments by comparing ligament strength and stiffness at 2, 4, 6, and 8 weeks postoperatively. At 4 weeks, a statistically greater force was required to cause failure of the ligaments that had been exposed to HBOT compared to those that had not. There were no additional increases seen at the other time-points. Hsu and colleagues investigated the effect of HBOT on collagenase-induced tendinopathy in the rabbit patellar tendon. After 10 weeks, the ultimate tensile load was 34.8% greater in the tendons that had received HBOT compared to the control.

Several authors have also investigated the effect of SOT on soft tissue healing in humans and animals (12, 16). These studies have shown that SOT may diminish the incidence of surgical wound infections (12), and increase the tensile strength of reparative tissue in healing wounds (16). Despite the encouraging findings reported in prior investigations on HBOT and SOT, similar results could not be substantiated in the present study. No statistically significant difference could be identified between treatment groups at any time-point under study, and the majority of tensile failures occurred at the injury site irrespective of treatment.

Limitations of this study include the 3-day time delay between surgically created patellar laceration and the start of oxygen therapy. Some evidence suggests that tissue hypoxia may be most severe immediately following an injury (17). Although the use of intermittent oxygen therapy is typical for a regimen of multiple treatments in humans, it may have been too infrequent to yield significant results in this investigation. As no prior evidence exists supporting the optimal timing of SOT, treatment sessions were identical to those described for the HBOT in previous studies. Furthermore, mechanical testing at 3- and 6-week time-points may have allowed too much time for healing in order to appreciate a significant difference between groups. Horn et al. had been able to demonstrate a significant difference at 4-weeks postoperatively in a similar rat model (11). Based on these findings it was felt that 3- and 6-week time-points would be appropriate periods for mechanical testing. Finally, many tendons did not fail at the surgically created laceration site, impairing our ability to definitively determine the tensile strength at the site of injury.

Conclusion

This research draws important conclusions regarding the use of systemic hyperbaric oxygen therapy or supplemental oxygen therapy to effect accelerated tendon healing. Results presented here may indicate that previously published findings may not be reasonably applied to the clinical realm in terms of their impact on patellar tendon healing or tendon strength (8–12, 16). In light of these findings, the increased cost of systemic oxygen administration, particularly hyperbaric oxygen therapy, might not be justified following patellar tendon repairs.

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