EFFECT OF HYPERBARIIC OXYGEN THERAPY ON MEDIAL COLLATERAL LIGAMENT HEALING IN A RABBIT MODEL

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Introduction
Hyperbaric oxygen (HBO) therapy has been shown to enhance bone, muscle, skin, and wound healing, particularly in conditions of ischemia and low oxygen tension. Preliminary data show that HBO may be beneficial in the treatment of muscle-tendon and ligament injury. However, the effect of hyperbaric oxygen therapy on ligament healing has not been thoroughly investigated. We had reported the positive effects of HBO on ligament in vitro [1]. The purpose of this study was to use biomechanical and histological analyses to assess the effect of hyperbaric oxygen on the healing of the medial collateral ligament in a rabbit model.

Materials and Methods
Sixty-four male rabbits were divided into eight groups of eight animals each. The experimental group (32 rabbits) was subjected to hyperbaric oxygenation at 2.5 atmospheres absolute for 2 hours daily, while the control group (32 rabbits) did not undergo hyperbaric oxygenation. Rabbits underwent a standardized surgical severance of the right medial collateral ligament, whereas the left medial collateral ligament was not surgically lacerated. Rabbids from each group were euthanized at 2, 4, 8, and 12 weeks postoperatively for biomechanical testing (6 rabbits) and histologic analysis (2 rabbits). After the specimens were clamped, an axial tensile force was applied at a crosshead rate of 7.2 mm/min using a material testing machine (Bionix 858, MTS Company, Minneapolis, MN, USA). The relationship between force and displacement was recorded simultaneously at an increment of 0.05mm using MTS Teststar II software. To assess the effect of hyperbaric oxygen therapy on the healing rate of ligament at various periods, the magnitude of force at failure for each individual specimen was selected for comparison. Using the other intact limb in the same rabbit as a control, the percentage of failure of the normal medial collateral ligament of the each rabbit was calculated. Statistical analysis was done using a two-tailed t test for discrete variables. A significant difference was reported at p < 0.05.

Histological analysis: Two sacrificed animals from each group were sent for histological analysis. After fixing in 10% buffered formalin and embedding in paraffin, the specimens were stained by standard Masson’s trichrome stain, hematoxylin and eosin procedure for subsequent examination. By the method of trichrome stain, collagen fibers were identified by blue color stain and muscle fibers were identified by red color stain.

Results
Fig. 1a illustrates the typical diagram of force and displacement versus time in the biomechanical tensile test. The displacement time curve characterizes the displacement as it increases at a constant rate of 7.2 mm/min, which is consistent with the preset testing condition mentioned previously. Fig. 1b illustrates the corresponding diagram of force versus displacement, showing the load magnitude increased linearly with increasing displacement before failure. However, force magnitude declined significantly once maximal force was reached. Following the failure of MCL ligament, the fluctuation phenomenon of force value was observed. It was considered due to the “damping” effect of the tibia mounted on the MTS load cell. Using the intact left medial collateral ligament as a control, the mean percentage of failure load at 2, 4, 8, and 12 weeks were 20.6%, 49.1%, 63.9%, and 76.3%, respectively, in HBO group, whereas the mean percentage of failure load were 13.5%, 25.3%, 36.5%, and 57.3%, respectively, in the non-HBO group. The mean percent failure load of the severed medial collateral ligament from the HBO group was significantly higher than that of the non HBO group at 2, 4, 8, and 12 weeks, respectively. The mechanical strength of the two groups begins to differ significantly as early as 2 weeks and through 4, 8 and 12 weeks of ligament healing. In this animal model, hyperbaric oxygen therapy clearly enhances the mechanical properties of early ligament healing in clinical practice. Evidence of the beneficial effect of hyperbaric oxygen on ligament healing using the rabbit model has not been previously reported. The results are measured biomechanically and using conventional light microscopy demonstrate an increased rate of tensile strength development and collagen formation in the hyperbaric oxygen treated animals as compared to the controls. There are no biochemical or cell biology studies so that there is no information on mechanisms. The mechanism of their effect remains unclear. It would be interesting to have carried the experiment out over a longer period of time to determine if the trends of the treated and non-treated group eventually reach the same level or if the hyperbaric treated animals had a permanent increase in ligament strength. The results of this study suggest that hyperbaric oxygen therapy enhances regenerative collagen formation in injured ligaments. The repaired ligaments had better biomechanical performance and earlier histological evidence of healing in the HBO-treated group. Limitations of this study include: (1) absence of a dose related analysis of hyperbaric oxygen therapy, and (2) the mechanism responsible for the effect of HBO on ligament healing was not investigated. Despite these limitations our findings still suggest that hyperbaric oxygen therapy has great potential for use in shortening the healing time in injured ligaments.

Discussion & Conclusion
The remodeling power and duration of human ligamentous healing is more similar to that of rabbits than rats. Ligament scars in humans and rabbits are thought to require as much as 12 months or more to complete remodeling [2, 3]. Previous studies found that the tensile strength of the injured ligament reached only 60% of control at one year [3, 4]. In this study, we used an established rabbit model of medial collateral ligament healing [2] and biomechanical and histologic analyses of the healed segments to investigate the effect of HBO on ligament healing. Using the other intact medial collateral ligament in the same rabbit as a control, we found that the mean percent failure load of the severed medial collateral ligament of the HBO group was significantly higher than that of the non HBO group at 2, 4, 8, and 12 weeks, respectively. The mechanical strength of the two groups begins to differ significantly as early as 2 weeks and through 4, 8 and 12 weeks of ligament healing. In this animal model, hyperbaric oxygen therapy clearly enhances the mechanical properties of early ligament healing in clinical practice. Evidence of the beneficial effect of hyperbaric oxygen on ligament healing using the rabbit model has not been previously reported. The results are measured biomechanically and using conventional light microscopy demonstrate an increased rate of tensile strength development and collagen formation in the hyperbaric oxygen treated animals as compared to the controls. There are no biochemical or cell biology studies so that there is no information on mechanisms. The mechanism of their effect remains unclear. It would be interesting to have carried the experiment out over a longer period of time to determine if the trends of the treated and non-treated group eventually reach the same level or if the hyperbaric treated animals had a permanent increase in ligament strength. The results of this study suggest that hyperbaric oxygen therapy enhances regenerative collagen formation in injured ligaments. The repaired ligaments had better biomechanical performance and earlier histological evidence of healing in the HBO-treated group. Limitations of this study include: (1) absence of a dose related analysis of hyperbaric oxygen therapy, and (2) the mechanism responsible for the effect of HBO on ligament healing was not investigated. Despite these limitations our findings still suggest that hyperbaric oxygen therapy has great potential for use in shortening the healing time in injured ligaments.

References