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Influence of delayed hyperbaric oxygenation on recovery from mechanically induced damage

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Nelson AG, Wolf EG, Li B. Influence of delayed hyperbaric oxygenation on recovery from mechanically induced damage. Undersea & Hyperbaric Med 1994; 21(2):185–191.—This study examined whether subjecting a crushed muscle to a delayed intermittent hyperbaric oxygenation protocol would facilitate healing, the marker for healing being a return toward 100% uncrushed muscle in selected mechanical, morphologic, and biochemical parameters. Thirty-six rabbits (4 groups of 9) had the right lateral head of their gastrocnemius muscle surgically crushed. After surgery, the rabbits were exposed daily for 90 min 5 days/wk to either 100% O_2 at 243 kPa, 8.5% O_2 and 91.5% N_2 at 243 kPa, 100% O_2 at 101 kPa, or 21% O_2 at 101 kPa. Initial treatments were administered 16–18 h post-muscle crush. After 10 days of treatment, maximal twitch and tetanic tension of the crushed muscle and its contralateral counterpart were measured. The muscles were then removed and analyzed morphologically, and the activity of citrate synthase, phosphofructokinase, and glucose-6-phosphate dehydrogenase were measured. The treatment group means for any of the parameters measured were not significantly different from each other. The extent of muscle damage, however, was determined to be minor as the control group recovery threshold was approximately 80%. Thus, it seems that the treatment protocol used does not facilitate healing for this type of muscle crush injury.

rabbits, citrate synthase, phosphofructokinase, glucose-6-phosphate dehydrogenase, twitch tension, tetanic tension, crush injury, hyperbaric oxygenation

A common trauma seen in accidents is a severe mechanical compression of body tissues, commonly referred to as crush injury. Skeletal muscles are highly susceptible to damage after a crush, as this type of trauma can affect at least three systems involved in maintaining muscle vitality. First, the damage arises acutely from localized areas of ischemia or anoxia caused by either stopping blood flow completely or by rupturing vessels and creating edema regions. This ischemic damage is manifested in the muscle by necrosis of individual muscle cells. Second, the crush can directly induce muscle necrosis by destroying the individual cells through the mechanical exchange of energy. Finally, these acute damages may in turn lead to further damage by causing muscle atrophy and by remodeling the muscle's biochemical/

metabolic profile through destroying either the muscle nerve, the axon branches found within the muscle, or both.

Current treatments of crush injury at the acute stage include management as in any wound, prophylactic antibiotics, surgical debridement where necessary, and reduction of edema via fasciotomy. Long-term management of the trauma usually consists of anti-inflammatory therapy until the healing process is completed. A possible novel treatment for crush injuries is hyperbaric oxygenation (HBO) therapy. Jain (1) points out that HBO should be an ideal adjuvant to the basic acute treatments for crush injuries because HBO promotes wound healing and counteracts tissue hypoxia through elevating tissue P_{O_2} and by reducing edema via vasoconstriction and reduction of blood flow. In fact, this latter effect of HBO has been shown by Nylander (2) and Strauss et al. (3) to reduce muscle damage. Specifically, Nylander (2), using a tourniquet to produce ischemia, found that HBO reduced reperfusion edema and ischemic-based myonecrosis, and that these effects persisted for as long as 40 h post-HBO. Strauss et al. (3) found similar results using an artificially induced compartment syndrome. HBO in this case significantly reduced both myonecrosis and edema.

In addition to the apparent benefits derived from HBO in the acute management of a crush injury, other research suggests that continued HBO could be a beneficial addition to current standard care by reducing the recovery time needed for the muscle to return to normal. First, less repair should be necessary because the initial treatments would have reduced the severity of the ischemic base myonecrosis (2, 3), and continued treatments should help prevent any future ischemic damage arising while the vascular system is repaired. Furthermore, the effect on wound healing should promote a more rapid recapillarization of the damaged area by providing for increased P_{O_2} to reach the advancing cells (1).

Notwithstanding these indicators of the benefit of HBO in treating crush injuries, a controlled study has never been performed to ascertain if HBO would indeed be a beneficial treatment for the long-term management of muscle crush injuries. Therefore, it was the purpose of this study to determine if subjecting a crushed muscle to HBO would facilitate its rate of healing. It was hypothesized that HBO treatments would return the crushed muscle to its normal (100% of uncrushed) morphologic, biochemical/metabolic, and mechanical states in less time than exposure to ambient conditions.

Experimental design and methods

Animals

All work on animals in this study was performed after the proper authorization and approval was obtained from Brooks AFB Animal Care Committee, and the housing and care of the animals were according to USAF Regulation 169-2, HSD supplement 1. Thirty-six New Zealand white rabbits were used in this study. The median weight of the rabbits at the start and end of the study was 2.5 kg. The rabbits were randomly assigned to one of four groups of nine rabbits each.

Surgical procedures

All animals received the muscle crush. To create the crush injury, the rabbits were anesthetized with ketamine HCl (35 mg/kg i.m.) and xylazine (5 mg/kg i.m.) and the right hindlimb given a surgical preparation. During the surgery and crush the animals received further anesthesia by inhaling a mixture of oxygen and Fluothane. The lateral head of the right gastrocnemius was exposed and crushed twice at mid-belly. The crush was performed using two pair of welded-together, 6.25 in. Rochester-Carmalt intestinal clamps. The modified clamps provided a crush area approximately 1.5 cm in width. The clamps were closed as fully as possible, locked on the muscle, held for 60 s, released for 30 s, and clamped on again for 60 s. After the crush, the surgical incision was sutured closed, and postoperative analgesia (buprenorphine, 0.03 mg/kg i.m.) and antibiotics (Chloromycetin, 20 mg/kg i.m.) were provided for 3 days.

Experimental treatments

As mentioned above, each group of nine rabbits was randomly assigned to one of four treatments. Each animal began its individual treatment 16–18 h postsurgery. Treatment 1 (hyperbaric oxygen, HBO) protocol consisted of 90 min of breathing 100% O_2 at a pressure of 243 kPa (2.4 atm abs). This HBO protocol was chosen because it simulated the general treatment protocol currently used at the Davis Hyperbaric Medicine Facility at Brooks AFB to treat the majority of human patients, and previous work in our laboratory had shown this protocol to have a positive influence on skeletal muscle (4). The descent to 243 kPa took 5 min, and the ascent to normobaria after the treatment lasted 15 min. Both the ascent and descent were made in 100% O_2. Thus, the animals were in the hyperbaric chamber and exposed to 100% O_2 a total of 110 min each treatment period. Temperature within the hyperbaric chamber was maintained near 22°C by venting a continuous flow of 100% O_2 through the chamber. This venting procedure also prevented the build-up of expired CO_2. The venting flow rate was set at a flow that ensured that the O_2 content, determined by a polarographic O_2 analyzer, of the vented gases was greater than 95%. Treatment 2 (high pressure, HPS) followed the same protocol as HBO with one modification: Pressure was set at 243 kPa, but instead of breathing 100% O_2, HPS breathed a mixture of 8.5% O_2 and 91.5% N_2 during the entire time within the chamber. Treatment 3 (high oxygen, HIO) also received the same exposure protocol as HBO and HPS, except in this case the exposures were to 100% O_2 at 101 kPa (1 atm abs) during the entire time within the chamber. Finally, treatment 4 (control, CON) protocol was similar except in this case normobaric atmospheric air (101 kPa, 21.0% O_2, 0.03% CO_2, 79.0% N_2) was used.

Each 110-min treatment was performed daily. The treatments for each group proceeded as outlined above for 10 days, at which time the animals were euthanized and the lateral heads of the left and right gastrocnemius were harvested for analysis.

Tissue harvest

The muscle harvest occurred 12–16 h after the last treatment session (i.e., harvesting began in the morning of the day immediately following Day 10 of treatments).

The animals were anesthetized with ketamine (35 mg/kg i.m.) and xylazine (5 mg/kg i.m.) and the muscle exposed. Before harvest of the crushed muscle for morphologic and biochemical analysis, the muscle's twitch and fused tetanic tensions were recorded (*see* Mechanical measurements below). After the mechanical measurements, the muscle was removed, trimmed of connective tissue, weighed, and a portion of the mid-belly cut out and placed in 10% formalin buffer. The rest of the muscle was quick frozen in freon cooled by liquid nitrogen to below −80°C and stored at −75°C. These procedures were then repeated with the contralateral uncrushed left lateral gastrocnemius muscle head. After removal of the contralateral muscle, the animal was euthanized with sodium pentobarbital (40–60 mg/kg).

Mechanical measurements

For determination of the muscle's mechanical properties, the muscle's twitch and fused tetanic tensions were recorded. First, the muscle was surgically exposed, and the lateral head freed from the medial head and the surrounding connective tissue. The muscle's portion of the Achilles tendon was severed and clamped to the lever arm of a force transducer. The muscle was held at its normal resting length by securing the lower hindlimb via steel pins placed through the knee and ankle joints. The muscle was then stimulated directly via four stainless steel needle electrodes inserted at the proximal (two electrodes; one ventral and one dorsal) and distal (two electrodes; one ventral and one dorsal) ends of the muscle. To obtain a muscle twitch, the muscle was stimulated by a single 10-V square wave pulse 2.4 ms in duration. Four twitches were obtained with a minimum rest period of 30 s between twitches. After the twitch measurements, the muscle was allowed to recover for 1 min, after which maximal tetanic tension was recorded. Maximal tetanic tension was elicited using a 1 train/s for 500 ms with 100 impulses/train stimulation regimen for 5-s duration. Again, four tetanic measurements were made with a 1-min recovery period between stimulations. Finally, the muscle was allowed a 1-min recovery and then removed.

Tissue analysis

For morphologic analysis, the formalin fixed muscle pieces (both right and left) were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (5) to determine muscle pathology and with Masson trichrome (5) to highlight intramuscular collagen. These slides were subjectively evaluated and graded by an impartial observer (blind observation). The observer, a veterinarian pathologist, was told only the title of the purpose of the study, and requested to try to classify the slides into four groups based on the severity of injury. As an additional measure of the severity of muscle injury, the activity of the enzyme glucose-6-phosphate dehydrogenase (G6DH) was assayed following the technique of Wagner et al. (6).

To assay alterations in the muscle's biochemical/metabolic capacity, the activity of a marker enzyme from both the glycolytic pathway and the citric acid cycle was determined. The enzyme phosphofructokinase (PFK) served as an indicator of glycolytic capacity and was assayed following the procedures of Shonk and Boxer (7). For the citric acid cycle, the activity of citrate synthase (CS) was measured following the technique of Srere (8).

Statistical analysis

An analysis of variance was used to compare the differences between groups. For analysis, the values of the crushed muscle variables for each animal were expressed as a percentage of the values for the uncrushed muscle. The variables analyzed were muscle weight, tension magnitudes, and the activity levels of the three enzymes.

RESULTS

Rabbits had been chosen as the experimental model for this study because previous experience had shown them to be capable of surviving HBO without suffering death due to oxygen toxicity. Unfortunately, in this experiment the rabbits did not exhibit good survivability. Six animals died, death in each case being preceded by a 1- to 2-day bout of severe diarrhea. Death did not seem to be related to the pressure treatments because four of the six animals were in the unpressurized groups (HIO and CON). The animals had been purchased as certified pathogen free, and necropsy analysis failed to prove otherwise. The final number of animals in each group is as follows: HBO, $n = 7$; HPS, $n = 9$; HIO, $n = 6$; and CON, $n = 8$.

Mechanical measurements

The muscle crush caused a decrease in both maximal twitch and tetanic tension in all animals. After 10 days, maximal twitch tension of the crushed muscle ranged between 65 and 90% of that of the corresponding uncrushed muscle. Maximal tetanic tension exhibited a similar response, with the crushed muscle maximal tetanic tension ranging between 58 and 93% of the corresponding uncrushed maximal tetanic tension. Treatment with HBO, however, did not seem to influence either type of tension generation. As shown in Table 1, the mean relative tension generation was similar across the four groups.

Morphologic measurements

The muscle crush caused a decrease in muscle weight in all animals. After 10 days, the weight of the crushed muscle ranged between 72 and 97% of that of the

Table 1: Percent of Normal Uncrushed Muscle[a]

Measured Parameter	HBO, $n = 7$	HPS, $n = 9$	HIO, $n = 6$	CON, $n = 8$
Muscle weight	82 ± 10	89 ± 7	87 ± 6	86 ± 6
Twitch tension	83 ± 7	70 ± 7	83 ± 7	85 ± 9
Tetanic tension	80 ± 9	64 ± 16	78 ± 20	82 ± 12
PFK	48 ± 14	50 ± 18	35 ± 15	50 ± 17
CS	86 ± 14	76 ± 15	81 ± 6	82 ± 14
G6DH	337 ± 129	290 ± 114	463 ± 183	264 ± 99

[a]Values are expressed as mean % ± SD.

corresponding uncrushed muscle. Treatment with HBO, however, did not seem to influence either muscle weight. As shown in Table 1, the mean relative weight was similar across the four groups.

Histologic examination of the tissue provided results similar to that of the muscle weight data. Crushed muscles were easily distinguished from uncrushed muscles. Differences between the crushed muscles, however, were not sufficient to allow discrimination.

Enzyme measurements

The activity of G6DH was greater in each crushed muscle as compared to its contralateral counterpart. The mean relative amount of enzyme activity was not significantly different across the four groups (Table 1).

The muscle crush caused a decrease in the activity of both PFK and CS. Again, the mean relative activity of the crushed muscle was similar across groups for both enzymes (Table 1).

DISCUSSION

The purpose of this study was to determine if a protocol of HBO could increase the healing rate of a crushed muscle. The criterion for healing was based on a comparison of the crushed muscle with its uncrushed contralateral muscle. Facilitated healing was defined as when a crushed muscle displayed a higher percentage of normal (100% uncrushed) in selected mechanical, morphologic, and biochemical parameters. The data indicate that the HBO protocol used in this study was of no benefit in facilitating the restoration of the mechanical, morphologic, and biochemical profiles of a crushed muscle.

Although this study did not show HBO to be beneficial, extension of the results of this study should be done cautiously. As explained above, our rationale was based on previous studies in which, when initial hyperbaric treatments were performed, acute postinjury followed with repeated exposure separated by at most a 4-h interval (2, 3). In this study the initial hyperbaric exposures were 16–18 h postinjury and performed once daily. The discrepancy in time and number of sessions was due to problems experienced in using rabbits as the experimental model. To have a painless muscle crush (no verbal or motor response during surgery) it was necessary to put the animals into very deep levels of anesthesia. As a result, the animals did not regain consciousness or recover from the surgery for 4–6 h. Initial attempts were made to expose the animals during acute postsurgery followed by twice daily exposures separated by 4 h. Unfortunately, with this protocol almost 50% of the animals died and it was not until we switched to once daily exposures that the deaths subsided. Therefore the study was repeated as specified here with the animals allowed overnight recovery and once daily exposures. While this practice increased the survival rate of the animals, it is possible that this length of delay could have limited the effectiveness of HBO treatment. As mentioned above, Nylander (2) and Strauss et al. (3) have shown that acute and frequent exposure to HBO significantly reduces the extent of muscle necrosis acute postinjury. Therefore, the 16- to 18-h delay in this study probably reduced the therapeutic benefits which could have been obtained from

the treatments. Moreover, the negative results seen with the delayed treatments strengthen the recommendations of Nylander (2) and Strauss et al. (3) for early, frequent HBO treatments as a therapy for crush injuries.

An additional point for consideration is the degree of injury induced in this study. The tension generation levels in all groups 10 days postinjury are at approximately 80%. A return to this level of capacity in this short time suggests that the severity of the actual injury might be classified as low to moderate. Thus it is possible that any therapeutic benefits of HBO are not detectable in situations where a crush injury is not severe.

This study suggests that further investigations into the use of HBO as a treatment for muscle crush injuries should be concentrated on severe injuries where initial treatments can be administered during the acute postinjury stages. It is also recommended that an animal model other than rabbits be used.

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