Influence of hyperbaric oxygen on the initial stages of bone healing

Flaviana Soares Rocha, PhD,[a] Camilla Christian Gomes Moura, PhD,[b] Denise Betulucci Rocha Rodrigues, PhD,[b] Darceny Zanetta-Barbosa, PhD,[a] Karen Renata Nakamura Hiraki, PhD,[c] and Paula Dechichi, PhD[c]

Objective. The objective of this study was to evaluate, in a rat model, the effect of hyperbaric oxygen (HBO) on the healing of normal bone on day 7.

Study Design. Forty male rats were used, equally divided into two groups based on treatment and time of sacrifice: the control group had bone defects created; and the HBO group had bone defects and received HBO. HBO sessions were conducted daily, at 2.5 atmosphere absolute for 90 minutes, and the animals were euthanized after 1, 3, 5, or 7 days. Bone density, bone neoformation, and expression of Runt-related transcription factor 2 (Runx2) and tartrate-resistant acid phosphatase were evaluated.

Results. Computed tomography analysis revealed significant differences only at 3 days ($P = .01$) between the control and HBO groups. HBO treatment accelerated the initial events of bone repair, resulting in improved bone neoformation. Increased expression of Runx2 was observed, especially on days 5 and 7 in the HBO group, although not significantly. There was no significant difference ($P = .74$) in the number of tartrate-resistant acid phosphatase–positive osteoclasts between the control and HBO groups on day 7.

Conclusions. These results suggest that exposure to HBO enhances bone anabolism, reduces inflammation, and accelerates bone healing, with positive results in bone neoformation. Therefore, the aim of this study was to evaluate the effect of HBO on the healing of experimental defects created in normal bone, on the first 7 days, in a rat model. (Oral Surg Oral Med Oral Pathol Oral Radiol 2015;120:581-587)

Hyperbaric oxygen (HBO) is a treatment in which 100% oxygen is delivered to a patient at greater-than-normal atmospheric pressure at sea level. The mechanism of HBO action is that it increases the amount of oxygen dissolved in blood (oxygen tension), which, in turn, can increase the amount of oxygen delivered to hypoxic sites.[1] This may stimulate cellular proliferation and collagen synthesis with positive effects on healing.[2,3]

HBO therapy has already been successfully used to improve bone healing[4-6] because it also stimulates angiogenesis and osteogenesis.[4,7] The molecular mechanisms of HBO on bone formation may be mediated via increased osteogenic differentiation, which is related to Runt-related transcription factor 2 (Runx2) production.[8] Runx2 triggers the expression of major bone matrix protein genes, including the osteopontin and osteocalcin genes, at an early stage of osteoblast differentiation, and its actions sustain a supply of preosteoblasts.[8] Although HBO is considered a valuable adjunct for the treatment of bone defects,[4-6] the current knowledge about its influence on the initial stages of bone healing is limited. Therefore, the aim of this study was to evaluate the effect of HBO on the healing of experimental defects created in normal bone, on day 7, in a rat model.

MATERIALS AND METHODS

Animals and experimental groups

The sample consisted of 40 healthy male Wistar rats (*Rattus norvegicus*), weighing 250 to 300 g. The animals were kept in cages under light–dark period of 12 hours and controlled temperature conditions (22 ± 2°C), with standard food and water ad libitum. This study was approved by the Science and Ethics Committee from the Federal University of Uberlândia, Brazil, Protocol 028/12, and was performed in accordance with the Brazilian College for Animal Experimentation (COBEA). The animals were divided into two groups based on treatment and time of sacrifice: control group and HBO group, and the animals were euthanized 1, 3, 5, or 7 days after surgery.

Surgery and HBO

The animals received cephalosporin antibiotic prophylaxis (30 mg/kg, intraperitoneally) and received general

[a]Department of Oral and Maxillofacial Surgery, School of Dentistry, Federal University of Uberlândia, Uberlândia, MG, Brazil.
[b]Biomedical Sciences Institute, Federal University of Triângulo Mineiro, Uberaba, MG, Brazil.
[c]Biomedical Sciences Institute, Federal University of Uberlândia, Uberlândia, MG, Brazil.
Received for publication Feb 26, 2015; returned for revision Apr 30, 2015; accepted for publication May 11, 2015.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2015.06.039

Statement of Clinical Relevance

Bone healing is influenced by several factors, such as severity of injury, infection, age, health, and nutrition. Hyperbaric oxygen (HBO) is used to improve the repair, since it stimulates oxygenation, cell proliferation, and neovascularization, with positive results in osteogenesis.

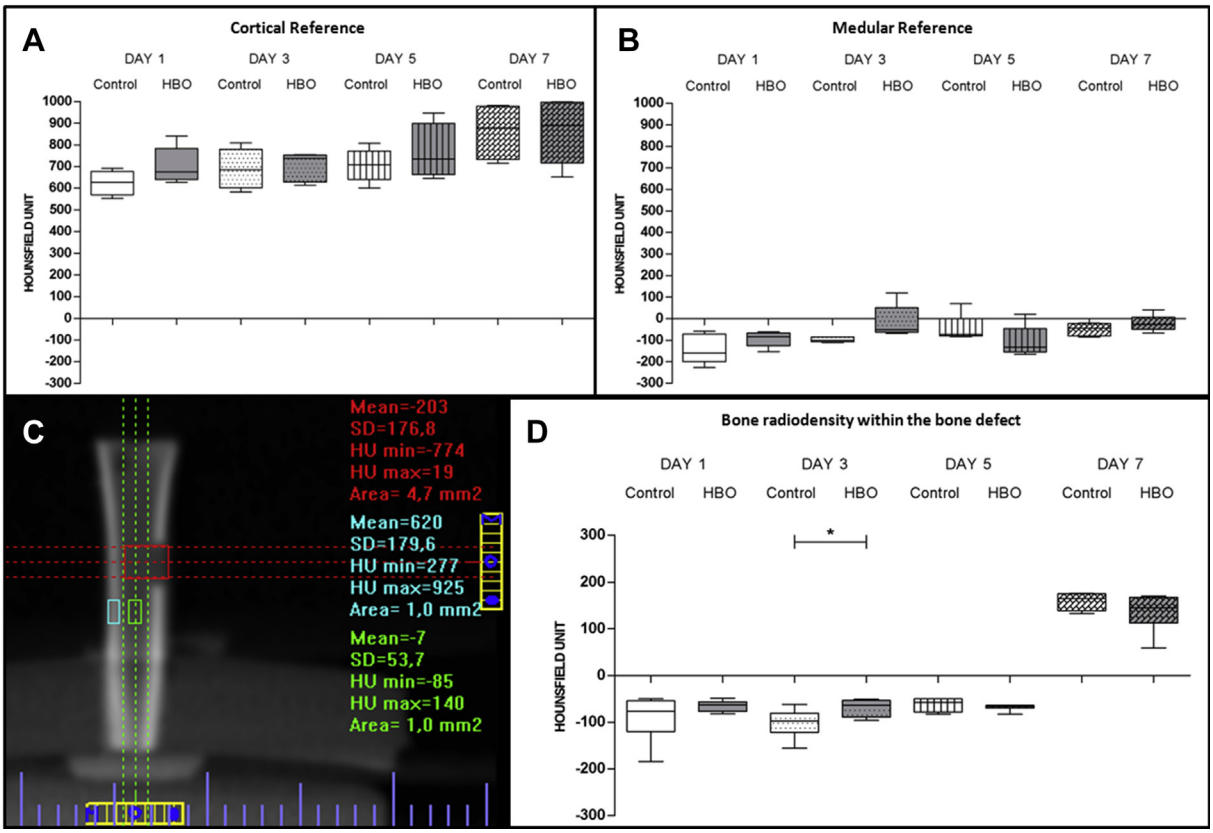

Fig. 1. Bone radiodensity references in cortical (**A**) and medullar (**B**) areas. Computed tomography image demonstrating the region of interest (ROI) delineation (**C**). Bone radiodensity in ROI in all evaluated groups (**D**).

anesthesia. Following shaving and antisepsis, the animal was positioned in the right lateral decubitus position, with the right femur exposed through a 2-cm longitudinal incision. Then, a full-thickness cortical bone osteotomy was made with a round bur, creating a 2.3-mm bone defect. During the procedure, constant irrigation with saline solution was maintained, and the suture was made using nylon 4.0. Treatment with HBO was carried out in a cylindrical pressure chamber (Ecobar 400, Ecotec Equipamentos e Sistemas Ltda, Mogi das Cruzes, SP, Brazil) at 2.5 ATA for 90 minutes. The HBO sessions started immediately after the surgical procedure and were conducted daily, depending on the subgroup to which each animal belonged. After 1, 3, 5, or 7 days, the animals were euthanized. The femurs were removed, fixed in 4% paraformaldehyde solution in 48 hours, and maintained in phosphate buffered solution.

CT analysis

The specimens were positioned in a standard device and scanned in a cone beam three-dimensional scanner (Gendex, GX-CB500-ICAT) at 7 mÁ, 120 kvp, and 0.125 voxel of resolution. From each specimen, three computed tomography (CT) images from the central region of the bone defect were selected. For image-specific calibration, one rectangle mark of 1 mm^2 (at a distance of 3 mm from the defect) of the medullar region and one from the cortical region of the bone were selected. After calibration, the region of interest (ROI) within the bone defect was delineated with a rectangle mark from the defect edges on cortical bone up to the inner surface of opposite cortex (see Figure 1C). The Hounsfield scale within these regions was obtained using specific software (i-CAT Vision, Imaging Sciences International, Penn Road, Hatfield, PA).

Histomorphometric analysis

After CT analysis, the specimens were decalcified in 10% ethylenediaminetetraacetic acid, dehydrated with graded ethanol, and embedded in paraffin. The longitudinal 5-μm histologic sections obtained were stained with hematoxylin and eosin (H&E) for morphologic analysis and Mallory trichrome for histomorphometric analysis. Mallory histologic images of bone defect were captured at ×4 magnification, using a binocular microscope, Nikon