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Hyperbaric oxygen results in an increase in rabbit calvarial critical sized defects

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Objective. This study was undertaken to evaluate whether the effects of hyperbaric oxygen (HBO) therapy could alter the critical size for spontaneous healing of a bone defect in the rabbit calvarial model.

Study design. An animal trial of 12 weeks duration was conducted using 20 New Zealand white rabbits, which were randomly divided into 2 groups of 10 animals each. Calvarial defects were created in the parietal bones of each animal bilaterally. Defects were critical-sized, 15 mm on one side and supra-critical-sized, 18 mm on the contralateral side. Group 1 received a 90-min HBO treatment sessions at 2.4 absolute atmospheric pressure (ATA) per day for 20 consecutive days. Group 2 served as a control without any HBO treatment sessions. Five animals in each group were sacrificed at 6 and 12 weeks. Data analysis included qualitative assessment of the calvarial specimens, post-sacrifice radiographs, as well as histomorphometric analysis to compute the amount of regenerated bone within the defects. ANOVA and paired sample *t* test were used for statistical analysis.

Results. Both radiographic analysis and histomorphometric analysis demonstrated that HBO-treated animals had significantly more new bone within their defects compared with the control group ($P < .001$). There was no statistically significant difference between the percentage of new bone forming in the 15-mm and 18-mm HBO-treated defects. There was no difference between the 6-week and the 12-week HBO-treated groups. HBO is effective in enhancing the bony healing of full thickness critical sized as well as supra-critical-sized defects in the rabbit calvarial model.

Conclusion. Bone regeneration was significantly greater in the HBO-treated animals regardless of the defect size. HBO may have increased the diameter of the rabbit critical-sized calvarial defect to more than 18 mm.

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A critical-sized defect is by definition the smallest full thickness osseous wound that will not heal spontaneously during the lifetime of an animal.¹ Such a defect requires an adjunctive technique to permit its complete bony healing. The rabbit calvarial critical-sized defect model has been used to study the efficacy of a variety of bone substitute materials in promoting defect healing.^{2,3}

Hyperbaric oxygen (HBO) has been used to aid in the healing of hypoxic or compromised wounds⁴⁻⁶ such as hypoperfused grafts, radiation-induced side effects,⁷ and necrotizing anaerobic bacterial infections.⁸ Further, Muhonen et al.⁹⁻¹¹ have demonstrated that HBO treated rabbits have more osteoblastic activity and osteogenic potential in their irradiated distracted mandibles when

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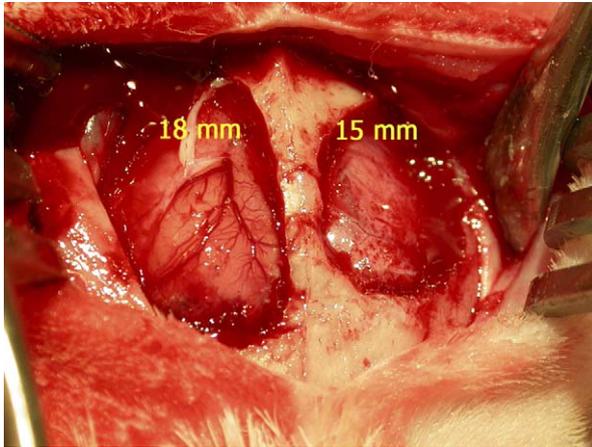


Fig. 1. Critical- (15 mm) and supra-critical- (18 mm) sized defects created in the rabbit parietal bones.

compared to a non-HBO-treated group. Consequently we hypothesized that HBO treatment would promote the healing of a rabbit critical-sized calvarial defect, possibly even allowing a supra-critical sized defect to heal.

MATERIALS AND METHODS

Animals

Twenty adult, skeletally mature, male New Zealand White rabbits weighing 3 to 4 kg were randomly divided into 2 groups of 10 animals. Group 1 was treated with HBO, while group 2 served as a control and did not receive any supplemental oxygen. Five animals from each group were sacrificed at 6 and 12 weeks.

Surgical procedures

The surgical protocol for this study was approved by the University of Toronto Animal Care and Ethics Committee (Protocol number 20005145). The surgical technique and analgesic and antibiotic protocols have been previously described.^{2,3} Each animal had bilateral full thickness calvarial defects created in its parietal bones. Defects were allocated randomly as critical-sized, 15 mm on one side and a supra-critical-sized, 18 mm on the contralateral side (Fig. 1). Standardization of defects was accomplished by using a trimmed template of the desired size. Wound closure was performed in layers.

HBO sessions

The 20 animals in group one (n = 10) underwent a 90-min HBO session at 2.4 absolute atmospheric pressure (ATA) per day 5 days a week for 4 weeks (20 days total). Pressurization and depressurization were done at a very slow rate (0.2 ATA per minute) in order to avoid barotrauma and potential discomfort. The control group, group 2 (n = 10) had no HBO sessions, breathing normobaric oxygen (NBO), otherwise referred to as *ambient*

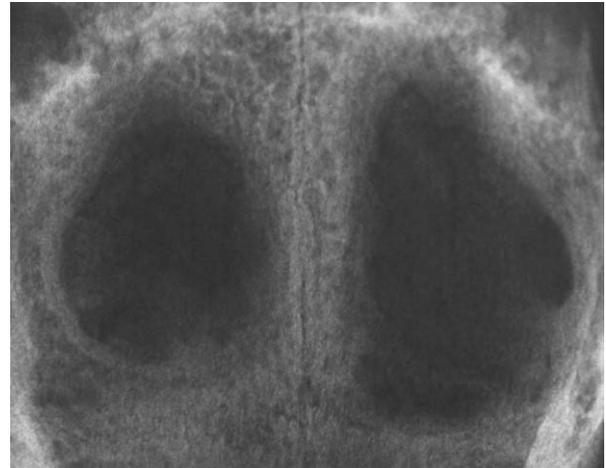


Fig. 2. Postsacrifice radiograph showing defects bilaterally in the parietal bones.

pressure room air, during the entire experimental period. HBO treatment sessions were begun 24 hours postoperatively in a monoplace chamber specially designed for small animal use. The test group was acclimatized to the HBO chamber one week preoperatively. This involved the animals being placed in the unpressurized hyperbaric chamber for 90 min/day for 5 days. The chamber had a glass window in its rear allowing the investigator to monitor the rabbits' behavior and comfort throughout the 90-min sessions. Pressurization and depressurization were done at a constant slow rate of 0.2 ATA/min in order to avoid barotrauma and potential discomfort.

Sacrifice and qualitative evaluation

Five animals from each group were sacrificed 6 weeks postoperatively and 5 from each group were sacrificed 12 weeks postoperatively. The parietal bones were divided from the rest of the cranium with an oscillating saw, carefully maintaining the intactness of the pericranium and the dura matter. Specimens were examined grossly for signs of inflammation, transilluminated, photographed, radiographed with a cephalostat (Fig. 2), and then fixed in 10% buffered formalin solution for 3 days before proceeding with the histological preparations.

Radiomorphometrics

Radiographs were digitized. An investigator blinded to the HBO status of the animals traced the areas of radiopacities within the defects. The percentages of radiopacities were calculated via Image Pro Plus 4.1 software for Windows (Media Cybernetics, Carlsbad, CA).

Histological evaluation

The specimens were decalcified using 45% formic acid and 20% sodium citrate for 4 weeks. The right and left parietal bones were separated through the

Table II. Percent new bone formation in the HBO (test) and NBO (control) groups

Sacrifice time Defect size Oxygen	6 Weeks				12 Weeks			
	15 mm		18 mm		15 mm		18 mm	
	HBO	NBO	HBO	NBO	HBO	NBO	HBO	NBO
Percent new bone formation	64.22	20.63	74.89	30.23	64.02	18.68	58.56	6.84
	49.60	22.77	43.36	45.33	58.06	10.07	52.58	20.03
	67.22	33.26	75.77	46.55	46.67	14.53	57.35	19.90
	53.94	22.64	49.34	24.48	55.39	10.02	50.46	24.16
	47.73	20.01	61.04	22.75	56.67	17.61	39.25	42.26
Mean	56.54	23.86	60.88	33.87	56.16	21.46	51.64	26.83
SD	8.74	5.31	14.65	11.37	6.25	18.20	7.67	15.36
SE	3.91	2.41	6.55	5.08	2.80	7.43	3.44	6.27

Percent new bone formation measured by calculating areas of new bone formed within the individual defect divided by the total defect area and multiplied by 100, with standard deviation (SD) and standard error (SE).

HBO, hyperbaric oxygen; NBO, normobaric oxygen.

defects in terms of signs of inflammation and integrity of the healing wound.

Radiomorphometrics (Table I)

Radiographs demonstrated more islands of radiopacities in the HBO group compared to the control group (Figs. 3 and 4) in both the 6- and 12-week specimens ($P < .001$). There were fewer radiopaque foci within the margins of the defects in the control group. In the control group the radiopacities tended to blend with the margins of the defects, whereas in the HBO group more radiopaque areas were evident both along the margins as well as in the center of the defects (Fig. 3). No differences were noted between the 15-mm and 18-mm defects ($P = .688$). The percentage of radiopacities were greater in HBO samples at 12 weeks when compared to those at 6 weeks ($P = .019$).

Histological evaluation

The healing of the defects in the control group was mainly by scar formation. There were a few bony islands scattered along the defect margins, which might have resulted from bone debris produced by drilling through bone. The healing of the HBO group produced a tissue regenerate with many blood vessels and cellular marrow spaces. The 12-week HBO samples tended to have more mature, trabecular bone whereas the 6 week samples had more woven bone and less trabeculated chunks of bone.

Histomorphometrics (Table II)

Histomorphometric analysis demonstrated more bone formation in the HBO group when compared to the control group ($P < .001$). Both critical- (15 mm) and supra-critical- (18-mm) sized defects healed with significantly more bone in the HBO group when compared with the control group. There was no significant difference between the percentage of new bone formed in the 15-mm and the 18-mm defects ($P = .520$), nor between the 6-week and 12-week groups ($P = .309$). (Figs. 5 and 6).

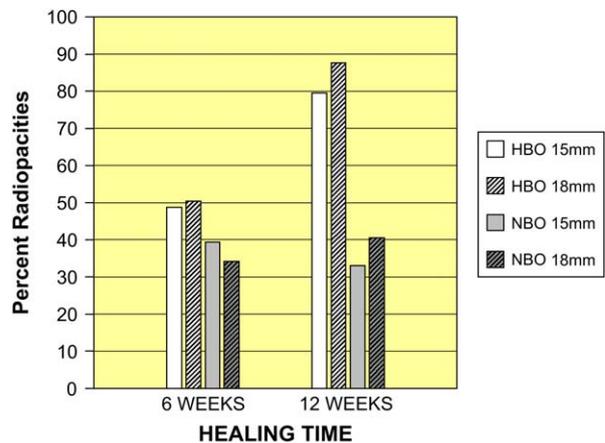


Fig. 4. Bar chart showing the differences in the mean radiopacity measurements within defects in the study groups (HBO = hyperbaric oxygen, NBO = normobaric oxygen).

DISCUSSION

One of the major problems encountered by surgeons who deal with large craniomaxillofacial defects is the difficulty of maintaining viability within bone-grafted tissue in order to ensure graft survival and eventual restoration of the defect. Microvascular reconstructive techniques provide one approach by supplying the graft with its own blood supply.¹² Harvesting such grafts results in significant morbidity of the donor site. Free autogenous bone-grafting techniques are associated with fewer donor-site complications but still have the limitation of the requirement of an adequately vascularized soft tissue bed in order to maintain graft viability. This is a major problem in patients who have received radiotherapy and extensive resection in order to control a malignant or an aggressive infectious condition.¹³

HBO has been used with success in treating hypoxic wounds and in anoxic conditions. Nilsson et al. have shown that HBO treatment would significantly increase

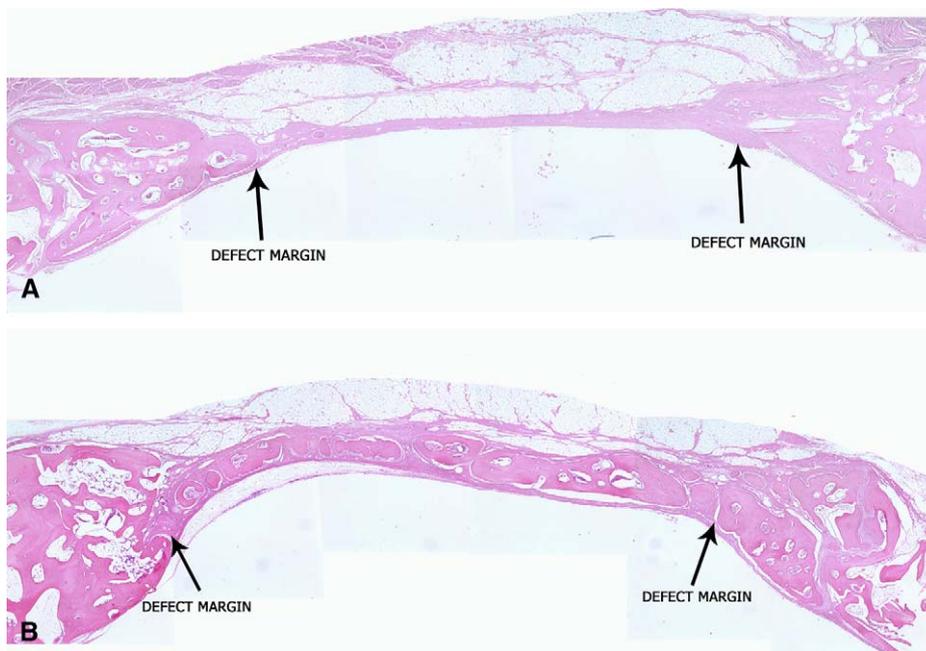


Fig. 5. 4× magnification hematoxylin and eosin stained sections. **A**, A control group specimen healed primarily with fibrous band of tissue. **B**, An HBO-treated animal sample showing bony healing of the defect.

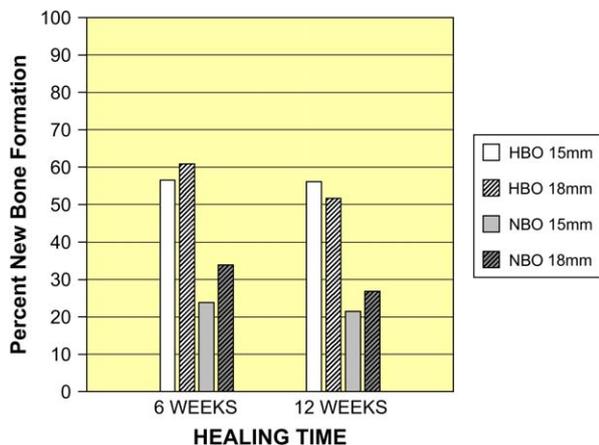


Fig. 6. Bar chart showing the differences in the means of new bone formation based on histomorphometric measurements in the study groups (HBO = hyperbaric oxygen, NBO = normobaric oxygen).

bone formation in the rabbit bone harvest chamber.^{11,14,15} The intent of this study was to evaluate the effects of HBO on osseous wound healing and to see if HBO can permit bony repair of critical sized defects.

The rabbit calvarial defect model is analogous in many ways to clinical maxillofacial reconstruction. There is an osseous defect with a periosteal blood supply and there is a membranous pattern of bone repair and healing. One difference, however, is the presence of a pulsatile dural

layer in the base of the rabbit calvarial model, which is not present in extracranial maxillofacial wounds.

A 15-mm defect was used in this animal model and defined as a critical-sized defect by Schmitz and Hollinger in 1986.¹ This was revised by Hollinger and Kleinschmidt in 1990 to be a defect having at most 10% of bony healing 10 years postoperatively.¹⁶ Further increases in the size of the defect beyond 18-mm would have required crossing the midline of the cranial vault, which posed a significant risk of lethal hemorrhage by potentially violating the sagittal sinus. Extending the defect to involve the temporal and the frontal bones might have altered the healing due to the involvement of the sutures.

Based on radiomorphometrics, there was a significant difference in the percentage of radiopacities in the HBO group at 6 versus 12 weeks. However, this was not reflected in the histomorphometric measurements, where the amount of bone in the defects was unchanged between 6 and 12 weeks. This finding can be explained by the fact that actual bone that is demonstrated histologically will not be evident radiographically unless it is considerably mineralized. Histological evaluation indicated a change in bone from woven to lamellar bone between 6 and 12 weeks, which would be expected to result in an increase in the radiodensity of the bone.

It could be argued that the differences observed between the two groups were due to the increased handling of the rabbits in the HBO chamber rather than the actual HBO treatment. This is unlikely as the

increased handling and confinement in the HBO chamber would result in increased stress to the HBO treated animals which would in turn be expected to adversely affect healing and not improve it. Further, the process of acclimatizing the HBO group of rabbits to the chamber for one week before the surgical procedure reduced the discomfort of the rabbits to being confined in the chamber and thus minimized stress.

HBO therapy was applied intermittently to minimize the theoretical blockade of hypoxia and lactate induced collagen synthesis and neovascularization^{17,18} as well as the differentiation of osteoprogenitor cells in the calvarial bone marrow and in the periosteal layer of the pericranium and the dura matter.¹⁹ A total of 20 HBO sessions were chosen because neovascularization reaches a plateau by 20 sessions.²⁰

CONCLUSIONS

The HBO treatment protocol described in this study seems to be an effective measure to enhance membranous bone healing in the rabbit calvarial critical sized defect model. The results reported here suggest that HBO treatment sessions as in the protocol described in this study significantly enhance bone formation within the critical-sized defect, even in the absence of an autograft or a bone substitute, possibly minimizing the amount or eliminating totally the bone graft required or bone substitute required to permit healing of the bony calvarial defect.

HBO may also increase the osteogenic, osteoinductive, and osteoconductive properties of bone regeneration materials indirectly by promoting early angiogenesis or by the genesis of a more viable soft tissue and hard tissue graft recipient wound for such materials. The rabbit calvarial critical-sized defect model is suitable for assessing the effectiveness of HBO therapy on cranio-maxillofacial bone repair and may be suitable for examining the effect of HBO in combination with various grafting materials or bone substitutes in the future.

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