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Functional regulation of osteoblastic MC3T3E-1 cells by hyperbaric oxygen treatment

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Abstract

Objectives

The purpose of the present study was to examine the influence of hyperbaric oxygen (HBO) on the function of osteoblastic MC3T3-E1 cells.

Design

Murine MC3T3-E1 cells were exposed to HBO treatment (at 2.5 absolute atmospheric pressure with 100% oxygen, 90 min per day) for 28 days. Alkaline phosphatase (ALP) staining, activity, and calcium (Ca) content were measured. Gene expression of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hypoxia-inducible factor-1α (HIF-1α), type 1 collagen (COL1), and osteocalcin (OCN) was assessed using real-time quantitative polymerase chain reaction after a single HBO exposure for 1.5, 6,

and 12 h. Furthermore, adenosine triphosphate (ATP) levels were measured using a luminescent cell viability assay.

Results

ALP activity and Ca content were higher in the HBO group compared to those in the control group. Gene expression of bFGF, COL1, and OCN was upregulated in the HBO group; however, that of VEGF and HIF-1α significantly decreased in the HBO group in comparison with that in the control group. ATP levels were significantly higher in the HBO group compared to those in the control group.

Conclusions

These findings suggest that HBO accelerates bone formation by increasing the ATP levels of osteoblasts, and bFGF can act as a substitute for VEGF in vascularization by HBO application.

Introduction

Hyperbaric oxygen (HBO) treatment is an adjunctive method for therapeutic purposes in which 100% oxygen with an atmospheric pressure of > 2.0 absolute atmosphere inside a chamber is used (Grim, Gottlieb, Boddie, & Batson, 1990). HBO treatment was used to treat various diseases, such as decompression sickness (Moon & Sheffield, 1997), intoxication by carbon monoxide (Garrabou et al., 2011), arterial embolism (Moon, de Lisle Dear, & Stolp, 1999), and nonischemic chronic diabetic ulcers (Kessler et al., 2003). With regard to bone regeneration, Lindstrom, Gullichsen, Lertola, and Niinikoski (1998) reported that HBO treatment had a positive effect on the management of crush injuries in patients with intramedullary nailed simple tibial shaft fractures. Animal studies showed that HBO enhances bone regeneration in calvarial defects (Jan et al., 2006), and the ossification and angiogenesis of the regenerated bone was accelerated by HBO in a distracted alveolar bone area in dogs (Inokuchi et al., 2010). An in vitro study showed that alkaline phosphatase (ALP) activity and collagen synthesis increased under 90% oxygen hyperoxic conditions (Tuncay, Ho, & Barker, 1994). Hadi,

Al, Smerdon, and Fox (2015) also demonstrated that a HBO exposure (at 2.4 absolute atmospheric pressure, 90-min with 90% oxygen) can induce osteoblast differentiation. With regard to angiogenesis, HBO induced microvascular perfusion, which was observed using laser Doppler imaging in murine dermal wound healing (Sheikh, Rollins, Hopf, & Hunt, 2005). HBO also possesses useful effects on neovascularization in healing of burn injury, in the early stage (Bilic et al., 2005).

Vascular endothelial growth factor (VEGF) (Ferrara & Davis-Smyth, 1997) and basic fibroblast growth factor (bFGF) (van Wijk & van Kuppevelt, 2014) are the major angiogenic factors. VEGF expression is known to increase under hypoxic conditions due to the effects of hypoxia-inducible factor-1 (HIF-1α) (Krock et al., 2011, Ramakrishnan et al., 2014). Izumino et al. (2020) showed that HBO accelerates bone regeneration in a bone defect model of mouse calvaria, and the protein concentration of VEGF in exudates from the injured area was less in the HBO group compared to that in the control group; however, the bFGF concentration was higher in the HBO group compared to that in the control group. However, the effects of HBO on VEGF, bFGF, and HIF-1α expression in osteoblastic cells remains unclear.

The purpose of the present study was to examine the effect of a single HBO exposure on the gene expression of VEGF, bFGF, HIF-1α, type 1 collagen (COL1), osteocalcin (OCN), and the adenosine triphosphate (ATP) level in murine MC3T3-E1 cells. The alkaline phosphatase (ALP) staining, activity, and calcium (Ca) content were also investigated.

Section snippets

Cell culture

Osteoblastic cell line MC3T3-E1 cells were purchased from European Collection of Animal Cell Cultures (Salisbury, UK), and cultured in alpha minimum essential medium (Sigma-Aldrich, Saint Louis, MO, USA) with 10%

fetal bovine serum (Daiichi Chemical, Tokyo, Japan), 1 mg/mL amphotericin-B (ICN Biomedicals Corp, Costa Mesa, CA, USA), 240 ng/mL kanamycin (Meiji Seika, Tokyo, Japan), and 500 ng/mL penicillin (Sigma-Aldrich) at 37 °C in 5% CO_2. The medium was changed twice weekly and cells were

ALP staining

On day 7, positive staining for ALP was detected in the HBO group. However, ALP staining was not observed until day 14, and weak positive staining was detected in 21 days in the control group. The intensity of ALP staining was higher in the HBO group compared to that in the control group throughout the experimental period (Fig. 2).

ALP activity

ALP activity in the HBO group was significantly higher compared to that in the control group at 7, 14, 21, and 28 days after the beginning of culture. ALP activity

Discussion

Several atmospheres and treatment durations were investigated for animal bone regeneration using HBO. Hayashi et al. (2016) reported that 2.5 absolute atmosphere for 120 min HBO treatment can stimulate the murine calvarial bone repair. It was also reported that calvarial bone defects in mice exhibited a significant increase in regenerated bone volume in the HBO group in comparison with that in the control group by 90-min HBO treatment at 2.5 absolute atmosphere (Izumino et al., 2020). However,

Conclusions

It has been suggested that HBO accelerates bone formation by increasing the ATP levels of osteoblasts. During HBO treatment, bFGF plays a critical role in promoting angiogenesis for bone regeneration as a substitute for VEGF.

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CRediT authorship contribution statement

Masato Kaku: Conception and design of the study, Drafting the article, Final approval of the version to be submitted. Jin Izumino: Acquisition of data, Drafting the article, Final approval of the version to be submitted. Taeko Yamamoto: Acquisition of data, Revising the article critically for important intellectual content, Final approval of the version to be submitted. Yuka Yashima: Acquisition of data, Revising the article critically for important intellectual content, Final approval of the

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Conflict of interest

The authors declare that they have no conflict of interest.

References (24)

- D. Wu *et al.*
  Effects of hyperbaric oxygen on proliferation and differentiation of osteoblasts from human alveolar bone

  Connective Tissue Research
  (2007)
- O.C. Tuncay *et al.*
  Oxygen tension regulates osteoblast function

  American Journal of Orthodontics and Dentofacial Orthopedics
  (1994)
- A.M. Jan *et al.*
  Hyperbaric oxygen results in an increase in rabbit calvarial critical sized defects

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics
(2006)

- T. Asano *et al.*
  Hyperbaric oxygen induces basic fibroblast growth factor and hepatocyte growth factor expression, and enhances blood perfusion and muscle regeneration in mouse ischemic hind limbs
  Circulation Journal
  (2007)
- I. Bilic *et al.*
  Effects of hyperbaric oxygen therapy on experimental burn wound healing in rats: a randomized controlled study
  Undersea & Hyperbaric Medicine
  (2005)
- N. Ferrara *et al.*
  The biology of vascular endothelial growth factor
  Endocrine Reviews
  (1997)
- G. Garrabou *et al.*
  Hyperbaric oxygen therapy for carbon monoxide poisoning
  Intensive Care Medicine
  (2011)
- P.S. Grim *et al.*
  Hyperbaric oxygen therapy
  The Journal of the American Medical Association
  (1990)
- H. Hadi *et al.*
  Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation
  Journal of Dentistry
  (2015)
- K. Hayashi *et al.*
  The facilitatory effects of hyperbaric oxygen treatment on membrane bone wound healing in a rat calvarial defect model
  Undersea & Hyperbaric Medicine
  (2016)