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Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation

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ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
10 October 2014
Accepted 11 October 2014
Available online xxx

Keywords:
Bone formation
Hyperbaric oxygen therapy
Osteonecrosis of the jaw

ABSTRACT

Objectives: Hyperbaric oxygen therapy (HBO) has been used as an adjunctive therapy in the treatment of radiotherapy or bisphosphonate-induced osteonecrosis of the jaw however the effect of HBO on osteoblast formation and mineralisation has not been extensively studied. The current study therefore examined the effects of HBO, elevated pressure or elevated oxygen alone on osteoblast differentiation and bone nodule formation.

Methods: Saos-2 human osteoblast cells were exposed to HBO (2.4 ATA, 97.9% O_2, 90 min per day), elevated pressure alone (2.4 ATA, 8.8% O_2, 90 min per day) or elevated oxygen alone (1 ATA, 95% O_2, 90 min per day) after culturing under normoxic or hypoxic conditions and osteoblast differentiation and bone formation assessed by alkaline phosphatase activity and calcein incorporation. Expression of key regulators of osteoblast differentiation and bone matrix proteins were assessed by quantitative PCR.

Results: Daily exposure to HBO accelerated the rate of osteoblast differentiation as determined by increased alkaline phosphatase activity and expression of type I collagen and Runx-2 mRNA during the early stages of culture. HBO also augmented bone nodule formation in hypoxic conditions. HBO had a more pronounced effect on these key markers of osteoblast differentiation than elevated oxygen or pressure alone.

Conclusions: The data from this study shows that daily HBO treatment accelerated the rate of osteoblast differentiation leading to an increase in bone formation.

Clinical significance: These studies add to our understanding of HBO's reparative action in osteonecrotic bone loss. In addition to stimulating angiogenesis HBO may also improve surgical outcomes through a direct beneficial effect on osteoblast differentiation generating a larger bone mass available for reconstruction.

1. Introduction

Blood vessel integrity and bone homeostasis are often disrupted in patients receiving high dose bisphosphonates or head and neck radiotherapy. This is associated with the formation of necrotic areas of alveolar bone causing pain and loss of function. Several studies have shown a beneficial effect of hyperbaric oxygen therapy (HBO) on the skeleton[1–5] and HBO has been used to promote healing in osteonecrosis, bone grafts and dental implants.[6–8]

Changes in oxygen partial pressure directly impact on osteoblast function with hypoxia being associated with decreased osteoblast formation and mineralisation *in vitro*.[9]

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http://dx.doi.org/10.1016/j.jdent.2014.10.006
0300-5712/

Please cite this article in press as: Al Hadi H, et al. Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation. Journal of Dentistry (2014), http://dx.doi.org/10.1016/j.jdent.2014.10.006

HBO rapidly delivers oxygen to areas of ischaemic tissue damage by elevating plasma oxygen concentration.[10] The subsequent increase in oxygen tension is thought to promote tissue regeneration through multiple mechanisms including changes in vascular reactivity, angiogenesis, free radical production, cytokine synthesis and modulation of the immune response.[11] Therefore by promoting capillary proliferation HBO may indirectly help restore osteoblast formation at formerly hypoxic sites in the jaw. In addition to indirectly promoting osteoblast activity it is possible that HBO may also have direct actions on osteoblasts that further enhance HBO's regenerative capacity. However the direct effect of HBO on osteoblast formation and function has not been examined. The aim of this paper is therefore to examine if HBO has a direct effect on markers of osteoblast differentiation and bone nodule formation in normoxic and hypoxic conditions.

2. Media and reagents

2.1. Cell culture

Saos-2 human osteoblast-like cells were obtained from ECACC (Porton Down, UK) (ECACC cat. no.89050205) and cultured in Dulbecco's minimum essential medium supplemented with 10% charcoal stripped foetal calf serum (Autogen Bioclear, UK) 2 mmol/l glutamine, 100 IU/ml benzylpenicillin and 100 mg/ml streptomycin all from Sigma (Poole, Dorset, UK). All incubations were performed at 37 °C in 5% CO_2 or equivalent. Cultures were fed every 2–3 days by replacing half the medium with fresh reagents.

To generate hypoxic (2% O_2) or normoxic (21% O_2) conditions cells were incubated in airtight chambers prepared at the Diving Diseases Research Centre (DDRC, Plymouth, UK). Chambers were flushed with appropriate gas mixtures for 90 min and then sealed. Chambers were re-gassed daily with appropriate O_2 concentrations. Cells were exposed to HBO (97.9% O_2, 2.1% CO_2, 2.4 ATA), elevated pressure alone (2.4 ATA, 8.8% O_2, 2.1% CO_2, and 89.1% N_2) or elevated oxygen alone (95% O_2, 5% CO_2) daily for 90 min to replicate the duration of treatment received by hyperbaric therapy patients. The oxygen and CO_2 concentrations used in the elevated pressure group were designed such that the partial pressures experienced by the cells in this group at 2.4 atmospheres absolute (ATA) were equivalent to 21% O_2 and 5% CO_2 at normal atmospheric pressure.

An overview of the experimental design can be seen in Fig. 1. Exposures were performed in airtight stainless steel culture chambers that were flushed for 4 min with relevant gas mixes and then pressurised to 2.4 ATA over 2 min as needed (Fig. 1). Following treatment cultures were returned to normoxic or hypoxic conditions as necessary.

2.2. Cell proliferation assay

Proliferation was measured using a Cell Titre 96 AQeous non-radioactive cell proliferation assay according to manufacturer's instructions (Promega, UK). Absorbance was measured at 490 nm (Molecular Devices, USA). Standard curves were

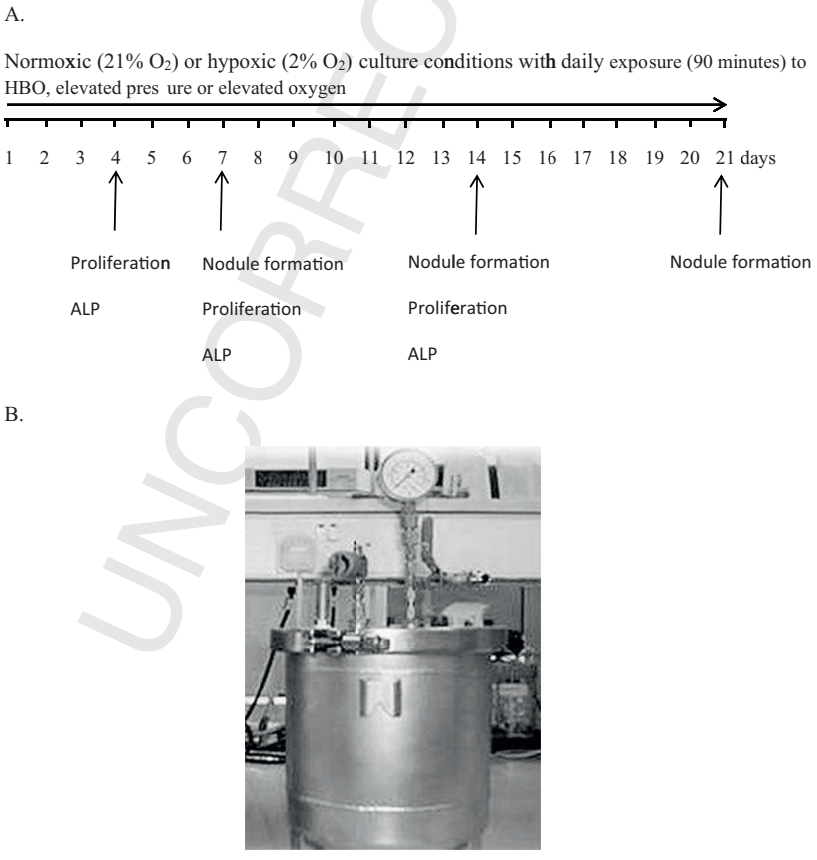

Fig. 1 – Schematic of treatment and assay regimes (A). Image of hyperbaric culture chamber used in HBO exposures (B).

Please cite this article in press as: Al Hadi H, et al. Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation. Journal of Dentistry (2014), http://dx.doi.org/10.1016/j.jdent.2014.10.006