OSTEOGENIC DIFFERENTIATION IN HUMAN ADIPOSE-DERIVED STEM CELLS EFFECTS MODULATED BY HYPERBARIC OXYGENATION

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Background. This is the first work evaluating the influence of HBO on the proliferation and osteogenic differentiation of human Adipose-Derived Stem Cells (hadscs).
Methods. Hadscs were exposed daily for 60 minutes, and up to 21 days, at 2,4 ATA and 100% O_2. The effects of elevated pressure (hyperbarism, HB) or elevated oxygen (hyperoxia, HO) alone on stem cells proliferation and differentiation were contextually analyzed. Hadscs were either used as undifferentiated cells (1) or as osteogenic-committed cells (2). 1. Cells were exposed to osteogenic stimuli for 7 days before starting treatments. 2. The undifferentiated cells were cultured with osteogenic differentiation factors, alone or in the presence of the pro-inflammatory cytokines. Cell proliferation was evaluated by means of the MTT assay at 7, 14, and 21 days of culture, whereas osteogenic differentiation was assessed by gene expression analysis of osteogenic markers and quantification of extracellular calcium deposition after Alizarin Red S staining.
Results. HBO did not affect cell proliferation and osteogenic differentiation when hadscs were exposed to osteogenic stimuli for 7 days before treatment. Similarly, proliferation and osteogenic properties of hadscs did not differ between HBO, HB, and HO conditions when treatments started contextually to the osteogenic differentiation of the cells.
HBO significantly decreased proliferation when hadscs were cultured in osteogenic differentiation medium supplemented with the pro-inflammatory cocktail. Remarkably, the reduction in cell proliferation was accompanied by an increase in osteogenic differentiation, as demonstrated by elevated calcium deposition and up-regulation of osteogenic markers.
Conclusions. Our data seem to indicate that the exposure of hadscs to HBO under in vitro simulated inflammatory conditions enhances differentiation towards the osteogenic phenotype, providing evidence of the potential application of HBO in all those processes requiring bone regeneration.