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Hyperbaric oxygen treatment effects on *in vitro* cultured umbilical cord blood CD34⁺ cells

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Abstract

Background aims. Umbilical cord blood (UCB) provides an alternative source for hematopoietic stem/progenitor cells (HSPCs) in the treatment of hematological malignancies. However, clinical usage is limited due to the low quantity of HSPCs in each unit of cord blood and defects in bone marrow homing. Hyperbaric oxygen (HBO) is among the more recently explored methods used to improve UCB homing and engraftment. HBO works by lowering the host erythropoietin before UCB infusion to facilitate UCB HSPC homing, because such UCB cells are not directly exposed to HBO. In this study, we examined how direct treatment of UCB-CD34⁺ cells with HBO influences their differentiation, proliferation and *in vitro* transmigration. *Methods.* Using a locally designed HBO chamber, freshly enriched UCB-CD34⁺ cells were exposed to 100% oxygen at 2.5 atmospheres absolute pressure for 2 h before evaluation of proliferative capacity, migration toward a stromal cell–derived factor 1 gradient and lineage differentiation. *Results.* Our results showed that HBO treatment diminishes proliferation and *in vitro* transmigration of UCB-CD34⁺ cells. Treatment was also shown to limit the ultimate differentiation of these cells toward an erythrocyte lineage. As a potential mechanism for these findings, we also investigated HBO effects on the relative concentration of cytoplasmic and nucleic reactive oxygen species (ROS) and on erythropoietin receptor (Epo-R) and CXCR4 expression. HBO-treated cells showed a relative increase in nucleic ROS but no detectable differences in the level of Epo-R nor CXCR4 expression were established compared with non-treated cells. *Discussion.* In summary, HBO amplifies the formation of ROS in DNA of UCB-CD34⁺ cells, potentially explaining their reduced proliferation, migration and erythrocytic differentiation.

Key Words: *hematopoietic stem/progenitor cells, hyperbaric oxygen, stem cell transplantation, umbilical cord blood*

Introduction

Since the first successful bone marrow transplant was performed in the 1950s, great strides have been taken in the field of transplantation [1]. Currently, bone marrow transplantations have become routine in the treatment of hematological malignancies. Growing demand and advancements have led to the use of umbilical cord blood (UCB) as an alternative source of hematopoietic stem/progenitor cells (HSPCs). UCB has many benefits related to its relatively simple means of procurement and storage, decreased incidence of graft-versus-host disease, and less stringent criteria for human leukocyte antigen matching [2]. Despite these benefits, the use of UCB is limited by its small quantity of cells in collected units and impaired bone marrow homing [3], which increase post-transplant complications related to delayed immune reconstitution after UCB [4–6], resulting in higher post-transplant infection [7]. To this end, the majority of research on UCB transplantation works to increase the efficacy of collection, develop a means of *ex vivo* expansion and enhance bone marrow homing [8]. Hyperbaric oxygen (HBO) treatment in particular is among the newer methods of augmenting UCB homing that has yet to be fully explored.

It has been shown that HBO treatment of mice before UCB-CD34⁺ infusion significantly improves engraftment [9], an effect attributed to HBO effects on lowering systemic erythropoietin (EPO) [10]. From these studies, it is evident that HBO treatment on the prospective environment can properly enhance

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engraftment. Because HBO effects on EPO are transient, there is interest in retreating the host with HBO after UCB-CD34$^+$ cell infusion to further enhance homing and engraftment. HBO's direct effect on CD34$^+$ cells is a matter that has yet to be fully investigated, however. Thus, in this study, we explored the direct effects that HBO treatment might have on UCB-CD34$^+$ cells to further its potential in improving UCB bone marrow transplantation.

Methods

Isolation of CD34$^+$ cells

Fresh UCB units were obtained from St. Louis Cord Blood Bank and immediately enriched for CD34$^+$ cells using EasySep Human Cord Blood CD34 Positive Selection Kit (Stemcell Technologies) as described previously [10]. Isolated cells were incubated overnight in StemSpan SFEM (Stemcell Technologies) supplemented with 1:100 cytokine cocktail CC110 (Stemcell Technologies) before proceeding with treatment or analysis.

Fluorescence-activated cell sorting

Cells were washed with phosphate-buffered saline (PBS) and blocked for Fc receptors by using FcR Blocking Reagent (Miltenyi Biotec), and stained with antibodies including anti-CD34-FITC (clone AC136, Miltenyi Biotec), anti-EPOR-PE (clone 38421, R&D Systems), anti-CXCR4-APC (Miltenyi Biotec), anti-CD41-FITC (clone HIP8, STEMCELL Technologies), anti-CD71-VioBlue (clone AC102, Miltenyi) and CD33-PE (clone WM53, BioLegend) for 10 min at 4°C. Ten thousand events were acquired for each sample using the LSRII flow cytometer (BD Biosciences) and data was analyzed using FACSDiva software.

HBO treatment

UCB-CD34$^+$ cells received HBO treatment in a locally designed HBO chamber with 100% oxygen for 2 h (as per standard HBO therapy) at 37 psi (2.5 atmospheres absolute) at room temperature. HBO-treated UCB-CD34$^+$ cells were examined 24 h after the end of HBO treatment.

CellTrace proliferation assay

The CellTrace Violet Cell Proliferation Kit (Invitrogen) was used to measure proliferation of HBO-treated and untreated UCB-CD34$^+$ cells up to 48 h after the end of HBO treatment. Cells were prepared according to the CellTrace kit protocol and proliferation was assessed at 0, 24 and 48 h via flow cytometry (LSR II; BD Biosciences). Data were analyzed using FlowJo software.

Assessment of CD34$^+$ cell differentiation

The phenotypes of cultured HBO-treated and untreated UCB-CD34$^+$ cells was examined at 0, 24 and 48 h at the end of HBO treatment and analyzed by flow cytometry (LSR II, BD Biosciences) by examining CD71 (erythroid lineage), CD41 (megakaryocytic lineage) and CD33 (myeloid lineage) expression.

Colony forming unit assay

HBO-treated and untreated UCB-CD34$^+$ cells were washed in Dulbecco's PBS and plated in triplicate in MethoCult Optimum medium (Stemcell Technologies) with 300 cells/mL medium in one 35-mm culture dish (Stemcell Technologies). Colony numbers were counted using an inverted microscope after 10–12 days cultured at 37°C with 5% CO_2. The number of each of the following colony types was counted: colony forming unit–granulocyte, –macrophage, –granulocyte-macrophage and erythroid burst-forming units.

Transmigration assay

HBO-treated and untreated UCB-CD34$^+$ cells were cultured in complete medium at 37°C for 24 h followed by transmigration assay in a two-compartment chamber separated by a membrane with 8-μm pore size in a 24-well transwell system (Falcon). In the upper chamber 8×10^4–1×10^5 cells in 100 μL RPMI1640 + 10% fetal bovine serum were seeded after one wash in Dulbecco's PBS, and the lower chamber was filled with 125 ng/mL stromal cell–derived factor 1 (SDF-1; R&D systems) in 600 μL of RPMI 1640 +10% fetal bovine serum. Cells were incubated for 4 hours at 37°C and 5% CO_2. Migrated cells were collected from the lower chamber and counted via FACS (Attune NxT flow cytometer; Thermo Fisher) and the percentage of migrated cells was calculated.

Oxidative stress detection

The CellROX Oxidative Stress assay (Thermo Fisher) was used to assess HBO-treated and untreated UCB-CD34$^+$ cells before HBO and 24 h after the end of treatment. CellROX Green and Deep Red reagents measuring reactive oxygen species (ROS) level in the DNA and cytoplasm, respectively, were added at a concentration of 1 μmol/L per 10^5 cells in separate flow tubes. Cells were incubated at 37°C for 30 min in the dark, washed and pelleted by centrifugation for 5 min at 300*g*, then resuspended in Dulbecco's PBS and immediately assessed via flow cytometry (LSR II; BD Biosciences). Results were reported as fold change compared with control normalized to 1.