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RESEARCH ARTICLE

Hyperbaric oxygen affects endothelial progenitor cells proliferation in vitro

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Abstract

Hyperbaric oxygen is a clinical treatment that contributes to wound healing by increasing fibroblasts proliferation, collagen synthesis, and production of growth factors, inducing angiogenesis and inhibiting antimicrobial activity. It also has been shown that hyperbaric oxygen treatment (HBO), through the activation of nitric oxide synthase promotes an increase in the nitric oxide levels that may improve endothelial progenitor cells (EPC) mobilization from bone marrow to the peripheral blood and stimulates the vessel healing process. However, cellular mechanisms involved in cell proliferation and activation of EPC after HBO treatment remain unknown. Therefore, the present work aimed to analyze the effect of HBO on the proliferation of pre-treated bone marrow-derived EPC with TNF-alpha. Also, we investigated the expression of ICAM and eNOS by immunochemistry, the production of reactive species of oxygen and performed an in vitro wound healing. Although 1h of HBO treatment did not alter the rate of in vitro wound closure or cell proliferation, it increased eNOS expression and decreased ICAM expression and reactive oxygen species production in cells pre-treated with TNF-alpha. These results indicate that HBO can decrease the inflammatory response in endothelial cells mediated by TNF-alpha, and thus, promote vascular recovery after injury.

Keywords: cell therapy; endothelial progenitor cell; hyperbaric oxygen; inflammation; tumor necrosis factor-alpha; wound healing

Introduction

EPC recruitment from bone marrow to the peripheral blood occurs under pathological conditions like trauma or ischemia. The homing of these cells to the injury site is related to growth factors and chemokines like VEGF (vascular growth factor) and CXCL12 (C-X-C chemokine Ligand 12), but also in response to nitric oxide (NO) (De Agostini et al., 1990; Libby et al., 2002; Gotlieb, 2005). NO is produced in endothelial cells by endothelial nitric oxide synthase (eNOS) and its role on endothelial function, and homeostasis maintenance is related to vasodilatation potential, inhibiting platelet aggregation and leukocyte adhesion (Tousoulis et al., 2012). NO also regulates the mobilization of EPC increasing CD34/VEGFR2 positive cells mobilization from bone marrow to peripheral blood and their differentiation in vivo to endothelial cells (Özüyaman et al., 2005).

HBO therapy involves the supply of 100% of O_2 in a chamber under pressure higher than 1ATA. It has a high participation in producing NO, which stimulates eNOS in cerebral cortex, pulmonary tissue and increasing the wound healing process due to the mobilization of EPC to the peripheral blood (Asl et al., 2015; Geng et al., 2015; Sunkari et al., 2015). HBO treatment contributes to wound healing by increasing fibroblasts proliferation, collagen synthesis,

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Abbreviations: CXCL12, C-X-C chemokine ligand 12; CXCR4, C-X-C chemokine receptor 4; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; FBS, fetal bovine serum; FGF-B, fibroblast growth factor-B; HBO, hyperbaric oxygen; ICAM, intercellular cell adhesion molecule; IGF, insulin growth factor; NO, nitric oxide; ROS, reactive oxygen species; TNF-alpha, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

growth factors concentration, promotion of angiogenesis and its antibacterial capacity. HBO also modulates inflammatory response since it reduces leukocytes activation and the release of some cytokines, such as tumor necrosis factor (TNF-alpha) (Rits et al., 2013).

However, it is not entirely understood of how HBO treatment affects EPC proliferation and stimulation to vascular repair. Therefore, an in vitro study using EPC treated with hyperbaric oxygenation may help to elucidate how the oxidative stress impacts on these cells, evaluating whether this treatment may work as a potential additional therapy to vascular recovery after endothelial damage.

Materials and methods

Animals

C57BL/6J male mice 5–6 weeks old were obtained from CEMIB/UNICAMP. All experiments were carried under protocol (4171-1) approved by the Research Ethics Committee from State University of Campinas.

Mononuclear cells isolation and EPC differentiation

Bone marrow was obtained from 7 to 8 weeks old male mice. Animals were euthanized under anesthesia (100 mg/kg de ketamine e 16 mg/kg de xylazine), followed by cervical dislocation. Bone marrow from femur, tibia and humerus bones were washed with DMEM (Dulbecco's Modified Eagle's medium) containing 10% of fetal bovine serum (FBS—Nutricell) and 1% de penicillin (100 U/L)/streptomycin (0.1 g/L) (PS—Nutricell). Mononuclear cells were isolated using Ficoll (Ficoll Paque Plus—GE-Healthcare). Cells were placed in flasks (25 cm^2) pre-coated with porcine gelatin 1% (Sigma–Aldrich, USA) and cultivated with EGM-2 (Endothelial Growth Medium-2, Lonza, Switzerland), supplemented with the kit for endothelial cultures containing VEGF, FGF-B, IGF, EGF, ascorbic acid, heparin, hydrocortisone and 2% FBS according to the manufactory, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 3 days, the initial cell culture was washed, and non-adherent cells were removed, then the cells were subsequently cultivated for more 30 days (five passages) in EGM medium exchanged every 2 days until differentiated into EPC.

Flow cytometry for EPC characterization

Approximately 1×10^6 EPC cultivated for 30 days were used for each group (n = 3 in duplicate), control or incubated for 24 h with 10 ng/mL of TNF-alpha. Cells were trypsinized and suspended in PBS/BSA (2%), incubated with CD34-FITC (eBioscence, Thermo Fischer, USA), CD31-PE (BD Biosciences, USA) and CD45-APC (eBioscence, Thermo Fischer) for 1 h. The cells were centrifuged, and the pellet was suspended in PBS/BSA (2%) with paraformaldehyde (4%) and analyzed by flow cytometer using a BD FACSCalibur^{TM} System. Data obtained was analyzed using software FlowJo (FlowJo, EUA).

Hyperbaric Oxygen (HBO)

The hyperbaric chamber was gently provided by Professor Dr. Selma Giorgio, in *Leishmania* Lab from Department Animal Biology, UNICAMP (Campinas, São Paulo, Brazil). Cell culture plates with 300 μL of medium/well were positioned without the lid inside the HBO chamber. The experiments were performed at 23°C, 100% O_2 and 2,5ATA for 60, 120 or 180 min.

Determination of the effect of HBO and TNF alpha on cell proliferation

Approximately 2×10^4 EPC/well (n = 4/group) were seeded on a 24 well plate and treated in the HBO chamber for different times (60, 120, 180 min) to verify their proliferation and any morphology alteration.

Twenty-four, 48, and 72 h after HBO exposition, cells were washed with PBS, trypsinized and counted in the Neubauer chamber. Cells were counted in duplicate, using a Microscope Olympus BX60 (camera QColor 3).

For the morphological analysis, cells were cultivated in round glass coverslips pre-coated with 1% gelatin, fixed with acetone after the treatments and stained with hematoxylin-eosin (HE). The coverslips were mounted using Cytoseal 60 (*Richard Allan Scientific*), and images were taken using the AxioVision software, in 100x magnification of microscope Zeiss Observer Z.1 (Zeiss, Germany).

Treatment TNF-alpha

EPC were treated with TNF-alpha in a concentration of 10 ng/mL for 24 h to mimic endothelial activation induced an inflammatory response according to Sainson et al. (2008). After the treatment, the medium was exchanged and the cells treated with HBO as described above.

Determination of the expression of ICAM and eNOS using immunocytochemistry

2×10^4 cells were seeded in round glass coverslips (n = 4/group) in a 24 wells plate. After 48 h, cells were fixed with cold acetone for 20 min. Cells were incubated for 2 h with primary polyclonal antibody NOS-3 *rabbit* (SC-654) (Santa Cruz Biotechnology, USA), for endothelial oxide nitric synthase (eNOS) or anti-*rat* CD54 (ICAM) (R&D Systems,

USA). Coverslips were washed with PBS and cells were incubated for 45 min with secondary antibodies, diluted in 1:250 PBS, *goat anti-rabbit* FITC (Sigma–Aldrich), for eNOS, and *goat anti-rat* FITC (Sigma–Aldrich), for ICAM-1. Nuclei were counterstained with DAPI (Sigma, USA) for 15 min. The coverslips were mounted using FluoroShield^{TM} (Sigma–Aldrich) and analyzed in microscope Olympus BX600 with 100× magnification. Four images of each well (whole field) were obtained by camera Olympus Optical U-ULH and analyzed using software QCapture 4.0. The fluorescence intensity was measured using the software Fiji Image J (U. S. National Institutes of Health, Bethesda, Maryland, USA).

ROS detection assay

The reagent DCFDA (2′, 7′-dichlorofluorescein diacetate) from the Cellular Reactive Oxygen Species Detection Assay Kit (5 μM) (Invitrogen, USA) was used to determine whether ROS concentration could be altered in EPC cultures after HBO treatment and/or incubation with TNF-alpha. DCFDA is oxidized by ROS, producing the fluorescent compost DCF (2′-7′ dichlorofluorescein) that can be measured by the spectrophotometer (485 nm).

Approximately 10^4cells/well were seeded overnight with Dulbecco Modified Eagle medium (DMEM) without phenol red and FBS in a clear-bottom 96-well microplate (n = 4/group). Negative control (no cells, only media plus DCFDA); positive control (cells plus 10 μM of hydrogen peroxide plus DCFDA); blank control (adherent cells with media only, no DCFDA); adherent cells plus DCFDA; adherent cells plus DCFDA and TNF-alpha (10 ng/mL), the cell were incubated with DCFDA (5 μM) 30 min, microplates were submitted or not to HBO and the fluorescence measured by the spectrophotometer.

The ROS values were calculated by subtracting blank readings (negative control) from all measurements and, determining fold change related to control.

In vitro wound healing assay

To verify whether HBO could affect cell migration after being incubated with TNF-alpha (10 ng/mL), an in vitro wound-healing assay was performed using a scratch assay on an EPC monolayer (Liang et al., 2007). 5×10^4 cells/mL were seeded per well (n = 3/group) in a 24-well plate, and a pipet tip was used to scrape in a straight line to create a "scratch." Cells were treated for 24 h with 10 ng/mL of TNF-ALPHA and treated or not with HBO for 60 min. Images were taken at the beginning (T_0) and regular intervals of 6h (T_6, T_{12}, T_{18}, and T_{24}) to determine the rate of cell migration during 24 h. The migration path was followed with a Zeiss Observer Z.1 microscope and image analysis done with Zeiss Zen software. In each image, three linear distances (on top, middle, and bottom) were measured using the Fiji Image J software, and then the average distance was calculated for each time. To calculate the closure rate, accordingly to Bartolini et al. (2013), average distances obtained from times T_6, T_{12}, T_{18} and T_{24} were compared to time T_0, for each group, by the following equation: [(wound area T_0 − wound area T_n)/wound area T_0] × 100, where n can be represented by 6, 12, 18, or 24 h.

Statistical analysis

All the results were presented as mean ± SD of at least three independent experiments. The statistical significance was determined by analysis of variance (One-way ANOVA test) followed by Tukey test. The difference between values was considered statistically significant when the $P > 0.05$.

Results

Characterization of EPC using flow cytometry

Previous work from our laboratory demonstrated that mononuclear cells isolated from mice bone marrow and cultivated using media endothelial growth media differentiated into EPC with late EPC characteristics and were positive for CD34, CD133, VEGFR-2, CD31, and VE-cadherin (CD144) (Carneiro et al., 2015). Our cells were differentiated following the same protocol. Briefly, mononuclear cells were isolated from murine bone marrow and cultivated in plates pre-covered with porcine gelatin 1% (Sigma–Aldrich) and EGM-2 (Lonza, Swiss), after 30 days, cells were characterized by flow cytometry using the antibodies for cell markers CD34, CD31 and CD45 (Figure 1A). More than 20% of the control cells were positive for CD34, and 25% were positive for CD31 (Figure 1B), and 95% of the cell were CD45 negative. It shows that these cells presented the expected endothelial characteristics as previously described by our group.

Determination of HBO and TNF-alpha effect on cell proliferation and morphology

We tested three different exposure times to HBO (60, 120, and 180 min) and analyzed the cells 24, 48, and 72 h after the exposure. EPC were stained with hematoxylin-eosin (HE) to evaluate their morphology (Figure 2A). It has been described that EPC has a fusiform morphology (Asahara et al., 1999) similar to those we verified in the control group and groups of EPC treated for 1 or 2 h with HBO. However, after the treatment for 180 min, the cells presented a round shape and 72 h after the treatment the initial morphology was recovered.

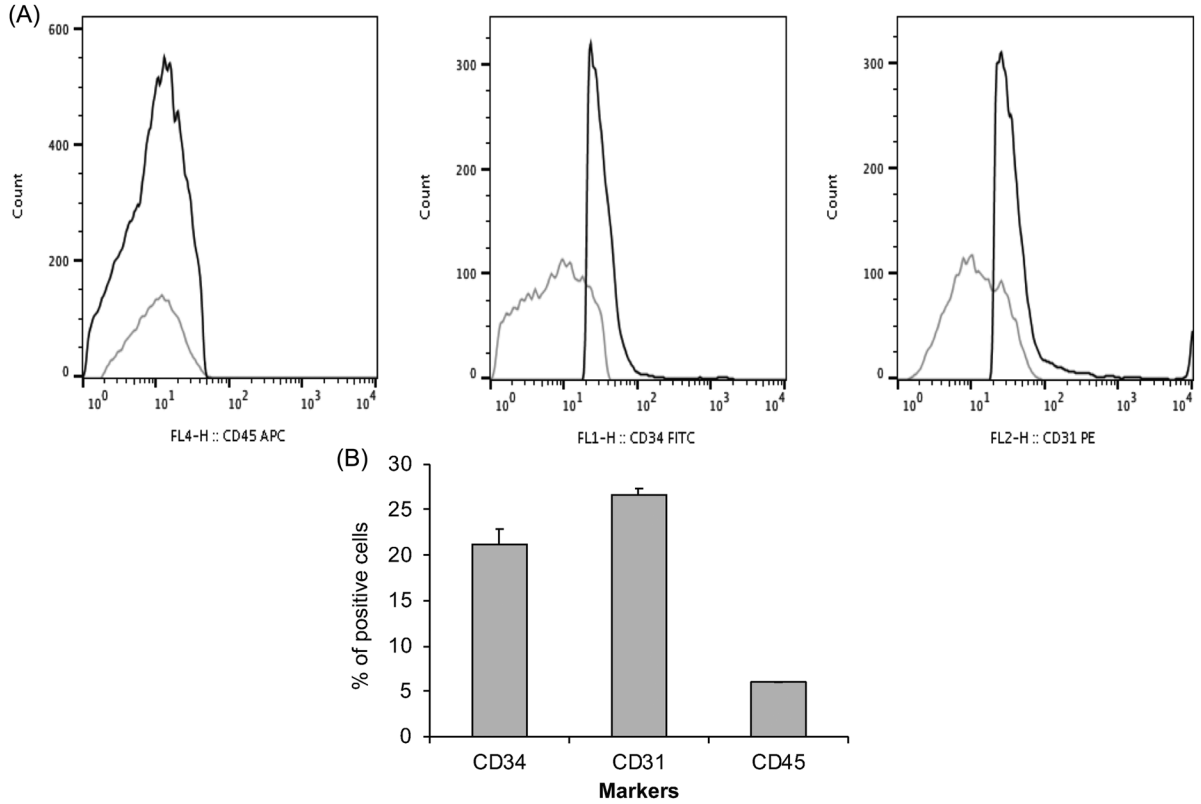

Figure 1 Characterization of EPC by flow cytometry. (A) Gating profile. CD45, CD34 and CD31 expression were determined on EPC cultivated for 30 days. Cells (n = 2 in duplicate) were labeled with CD34, CD31, and CD45 antibodies and analyzed by flow cytometry. Gray line means control and black line, sample. (B) Quantification of CD34, CD31, and CD 45 cells (n = 2 in duplicate). The values are mean ± SD expressed in percentage related to a total of 50,000 events acquired (*$P < 0.05$).

To determine cell proliferation, we counted the cells using a Neubauer chamber 24, 48, and 72 h after HBO treatment (Figure 2B) and compared the results with EPC from the control group (not treated). We observed that EPC proliferation of the group treated for 60 min had no significant difference when compared to control, but it decreased significantly after 3 h of HBO treatment (35%, 24%, and 22% with $P < 0.001$), 24, 48, and 72 h, respectively.

HBO reduces the TNF-alpha effect on EPC proliferation

We incubated the cells 24 h with TNF-alpha previously to the treatment with or without HBO, and after 24, 48, and 72 h, we analyzed cell morphology and proliferation, by using HE staining and counting the cell number, respectively. The morphology of TNF-alpha treated cells was altered after 24 h but recovered after 48 h. Treatment with HBO prevented these alterations induced by TNF-alpha (Figure 3A).

Proliferation decreased by around 30% in TNF-alpha treated cells after 24, 48, and 72 h. The treatment for 1 h with HBO recovered the proliferation levels to control levels at all times with $P < 0.05$ (Figure 3B).

Effect of HBO in ICAM expression

Endothelial lesion leading to thrombosis is accompanied by an increased ICAM expression, a marker of inflammatory stress (Torres and Sanjuliani, 2013). ICAM participates in the leukocytes adhesion and their migration to the subendothelial layer may occlude partial or entirely the arterial vessel and therefore lead to a thrombotic event (Zhong et al., 2018).

We analyzed ICAM expression using immunocytochemistry (Figure 4A), and the fluorescence was quantified using Image J. We verified that EPC treated only with TNF-alpha had an increase of 35% with $P < 0.05$ of ICAM expression. When we treated these activated cells with HBO for 60 min and analyzed ICAM expression 48 h later, we observed that the levels returned to control, indicating that HBO treatment can help recover the inflammatory response induced by TNF-alpha (Figure 4B). We also performed a control experiment using only 100% of oxygen for one hour, and we observed that oxygen alone did not affected ICAM in controls cells but decreased the effect of inflammatory effect of TNF alfa treatment significantly to intermediary values

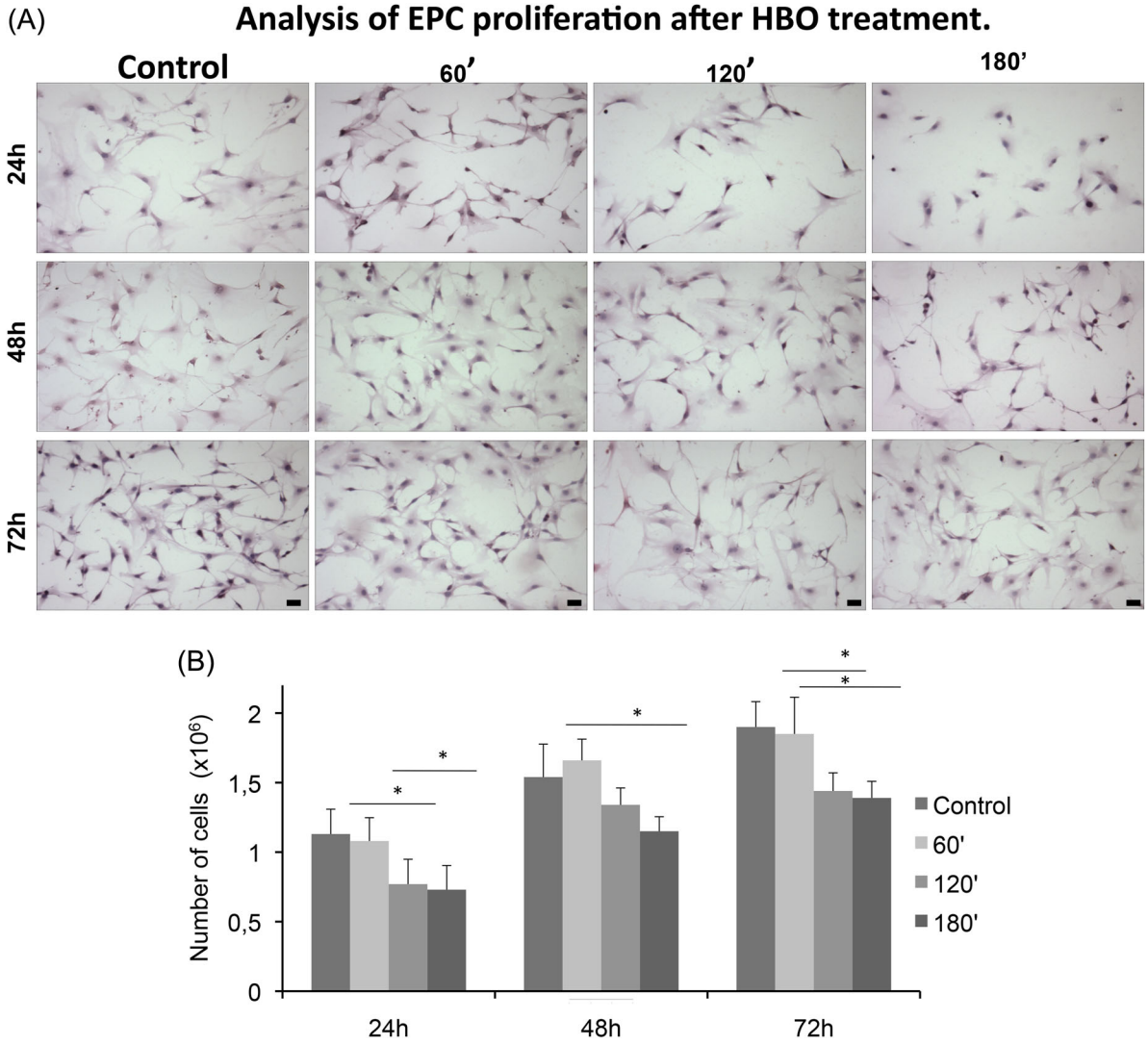

Figure 2 Analysis of EPC morphology and proliferation, after HBO treatment. (A) Morphological analysis of EPC 24, 48, and 72 h after HBO treatment. Cells (n = 4/group) were fixed with acetone and stained with HE. Bar scale: 50 μm. 100× magnification. (B) EPC proliferation 24, 48, and 72 h after HBO treatment. Cells (n = 4/group) were counted in duplicate using a Neubauer chamber. The values are mean ± SD, $^{*}P > 0.01$, and $^{**}P > 0.001$.

when compared to TNF-ALPHA treated with HBO (supplemental Figure S1).

Effect of HBO in eNOS expression

The enzyme eNOS increases NO production, which can inhibit platelet aggregation and ameliorate endothelial repair through the EPC recruitment to the peripheral blood. Thus, the presence of NO is considered to be a marker for good endothelial health (Aicher et al., 2003; Liu and Velazquez, 2008).

We assessed the effect of HBO treatment for 60 min, 48 h after the exposure in eNOS expression using immunocytochemistry (Figure 5A) and determined the expression by immunofluorescence, measured by Image J (Figure 5B).

The eNOS levels were not altered by TNF-alpha treatment for 24 h, but after treatment with HBO, eNOS increased 25% with $P < 0.05$, also indicating the beneficial effect of HBO in the endothelial function.

Effect of TNF-alpha and HBO in ROS production

In physiological conditions, reactive oxygen species (ROS) are subproducts of oxidative phosphorylation inside the mitochondria (Taniyama and Griendling, 2003;

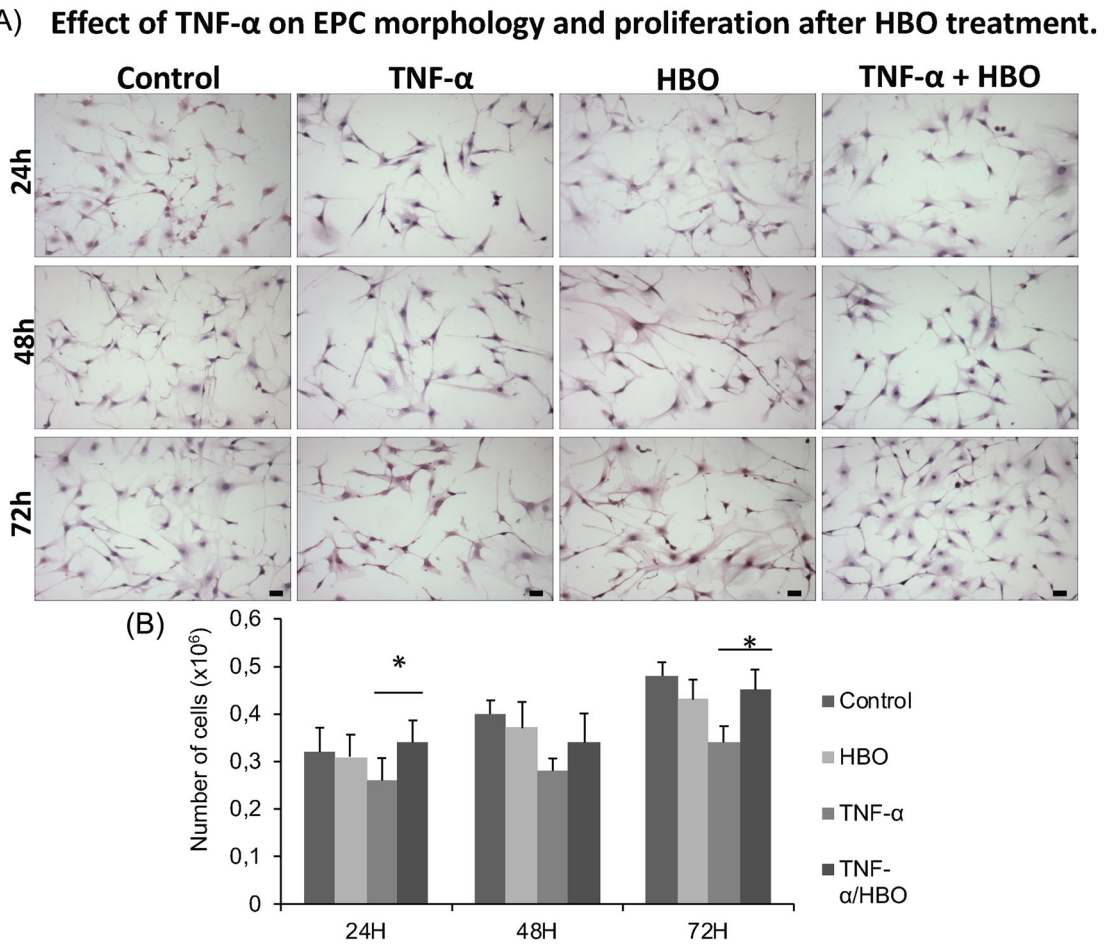

Figure 3 Effect of TNF-alpha on EPC morphology and proliferation, after HBO treatment. Morphological analysis of EPC 24, 48, and 72 h after HBO exposure. Cells (n = 4/group) were incubated for 24 h with TNF-alfa, exposed 60 min to HBO, and then fixed with acetone and stained with HE. Bar scale: 50 μm. 100× magnification. (B) EPC proliferation 24, 48, and 72 h after incubation with TNF-alpha and HBO exposure. Cells (n = 4/group) were counted in duplicate using a Neubauer chamber. The values are mean ± SD, $^{*}P < 0.05$.

Liu et al., 2014; Zhou et al., 2014; Rönn et al., 2017). However, in inflammatory conditions, activated macrophages secrete metalloproteinases that increases ROS concentration and the intracellular oxidative stress promoted by this situation can blockade eNOS activity (Vaddi et al., 1994).

We performed the ROS detection assay to verify whether HBO could decrease the concentration of these molecules in EPC in the presence of the pro-inflammatory cytokine. We observed that HBO treatment reduced the production of ROS by 40% when compared to control. Also, TNF-alpha alone did not induce an increase in ROS, but in the treatment with TNF-alpha followed by 60 min with HBO the levels of ROS were similar to HBO alone. That indicates that the TNF-alpha did not increase ROS production or inhibit the positive effect of HBO on the production of ROS (Figure 6).

Effect of HBO on wound healing in vitro

Wound healing was analyzed by following the proliferation and migration of EPC in a scratch assay for 24 h (Figure 7A). The cells were incubated or not with TNF-alpha for 24 h and HBO for 60 min. We did not observe statistical differences between all the groups analyzed in this assay (Figure 7B). This indicates that the concentration of TNF-alpha and the HBO exposure time analyzed were not sufficient to alter cell migration and proliferation, but it may affect the physiological activity of EPC promoting endothelial recovery.

Discussion

Hyperbaric oxygen treatment is an adjuvant therapy that contributes to wound healing process, inhibiting ROS

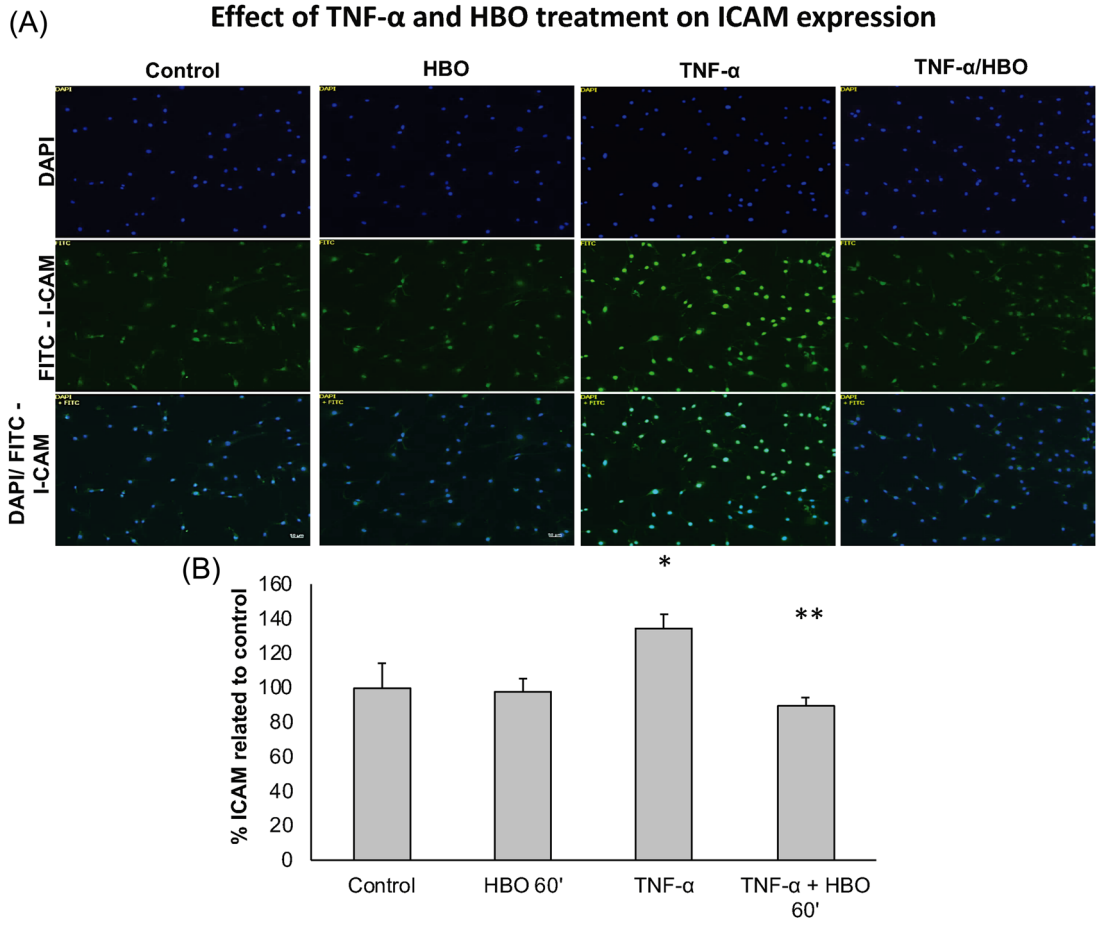

Figure 4 Effect of TNF-alpha on ICAM expression after HBO treatment. (A) EPC immunostaining of ICAM after HBO treatment and TNF-alpha incubation. ICAM was detected using ICAM antibody and using FITC as secondary antibody, and nuclei were counterstained using DAPI. N = 4/group. Bar scale: 50 μm. 100× magnification. (B) Quantification of ICAM-FITC fluorescence intensity in EPC measured by using the software Fiji. N = 4/group. The results were expressed as a percentage related to control. The values are mean ± SD, $^{*}P < 0.05$.

production and stimulating angiogenesis. In vivo, HBO has been shown to mobilize EPC from bone marrow through activation of eNOS on NO production (Goldstein et al., 2006). Also, HBO reduces the expression of adhesion molecules, like ICAM-1 decreasing inflammation after endothelial lesion (Sunkari et al., 2015). TNF-alpha stimulates leukocyte adhesion mainly by the upregulation of adhesion molecules on the endothelial cell surface like intercellular adhesion molecule type 1 (ICAM-1), E-selectin, and vascular cell adhesion molecule type 1 (VCAM-1). It also increases TF expression in these cells inducing a prothrombotic effect (Mackay et al., 1993). Also, TNF-alpha increases adhesion of EPC on endothelial cells inducing recruitment of these cells to promote vessel recovery after injury (Prisco et al., 2014).

Our results showed that HBO treatment for 60 min with 2,5ATA did not affect cell morphology and proliferation, but in groups treated for 120 and 180 min with HBO, proliferation was significantly decreased after 24 and 72 h. Cell morphology was only altered after 180 min of HBO, which suggests that this exposure time to 2,5 ATA may be toxic to these cells. Treatments for 120 and 180 min affected morphology and cell proliferation; thus we decided to use the treatment for 60 min since the intention was to use a non-toxic treatment for the cells.

Cell proliferation was also analyzed in the presence of TNF-alpha. We used TNF-alpha in the cultures in a non-toxic concentration (10 ng/mL) (Sainson et al., 2008) for 24 h before the experiment to induce an inflammatory state in these cells. We observed a decrease in cell proliferation even after 72 h of the treatment. However, HBO was able to revert this anti-proliferative effect in all times. Godman et al. (2010) showed that HBO treatment enhances the expression of cytoprotective agents in endothelial cells, related to inflammatory signaling pathways. This might explain the

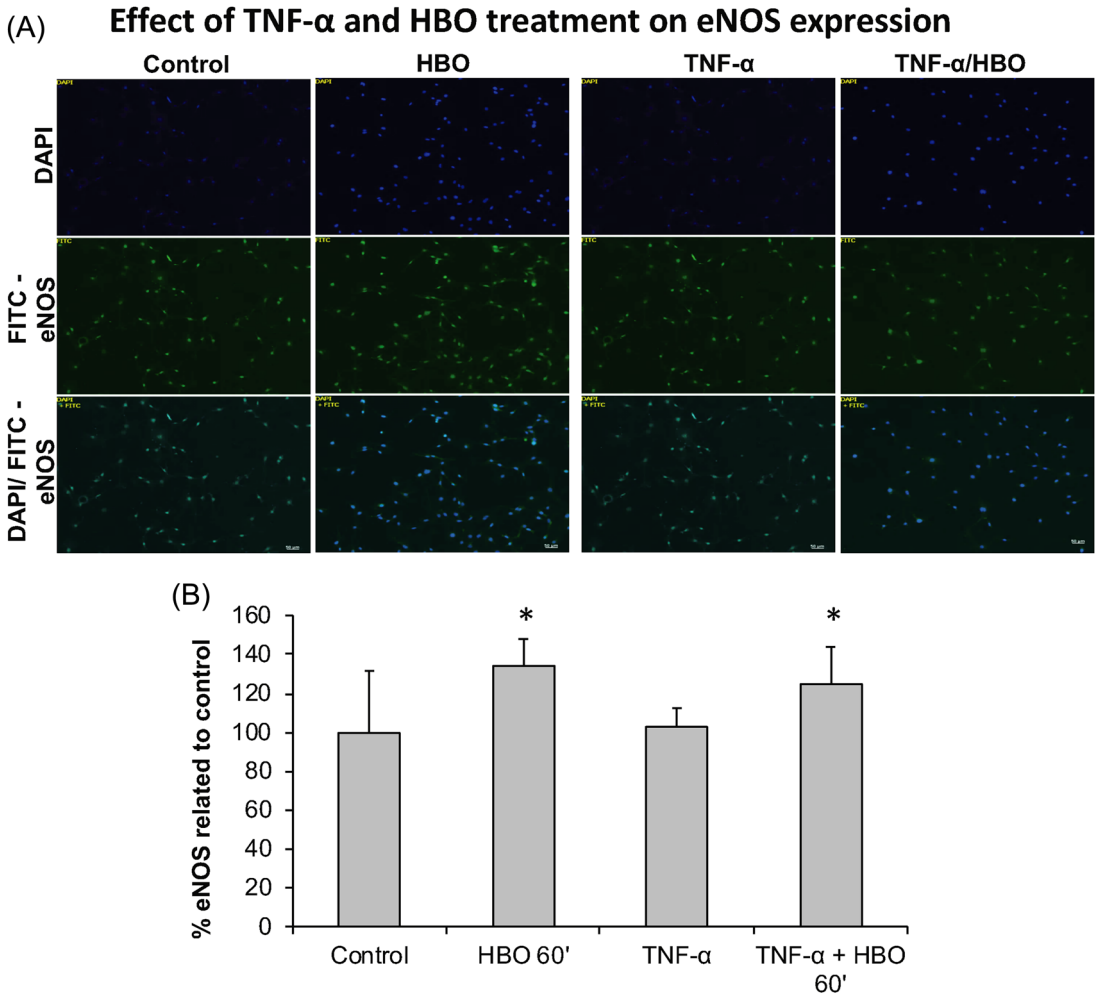

Figure 5 Effect of TNF-alpha on eNOS expression after HBO treatment. (A) EPC immunostaining of eNOS after HBO treatment and TNF-alpha incubation. eNOS was detected using eNOS antibody and FITC as secondary antibody, and nuclei were counterstained using DAPI. N = 4/group. Bar scale: 50 μm. 100× magnification. (B) Quantification of eNOS-FITC fluorescence intensity in EPC measured by using the software Fiji. N = 4/group. The values are mean ± SD and were expressed as a percentage related to control. $^{*}P < 0.05$.

positive effect on cell proliferation observed in the presence of TNF-alpha and HBO.

To analyze the HBO effects on activated endothelial cells, we investigated the expression of ICAM-1 and eNOS on EPC treated with TNF-alpha. Our data showed that HBO for 60 min increased eNOS and decreased ICAM-1 expression on EPC. The reduction of ICAM-1 expression improves the inflammatory background since this molecule contributes to the thrombotic event (Tousoulis et al., 2012). The activity of eNOS represents an adequate functionality of endothelial cells since NO is one of the molecules responsible for vessel homeostasis and repair (Aicher et al., 2003). In vivo, ICAM is increased after arterial injury and injection of EPC can decrease ICAM and promote the increase of eNOS expression, inducing vascular regeneration (Godoy et al., 2015). Although EPC treated for 60 min does not alter their proliferation, it triggers NO production, which maintains the non-adherence characteristic of a healthy endothelium and diminishes the ICAM expression, which could contribute to the thrombotic event. Hyperoxia alone (100% oxygen) without pressure decreased ICAM expression, but the use of HBO decreased this expression more efficiently. Almzaiel et al, (2015), observed that treatment with 2.5 ATA HBO increased the anti-inflammatory cytokine IL-10 in neutrophils and promoted its apoptosis, also increasing these cells engulfment what may regulate inflammation, and also that hyperoxia alone altered the inflammatory response in neutrophils.

TNF-alpha caused a decrease in cell proliferation after 48 h. This cytokine had a prejudicial effect on EPC, where

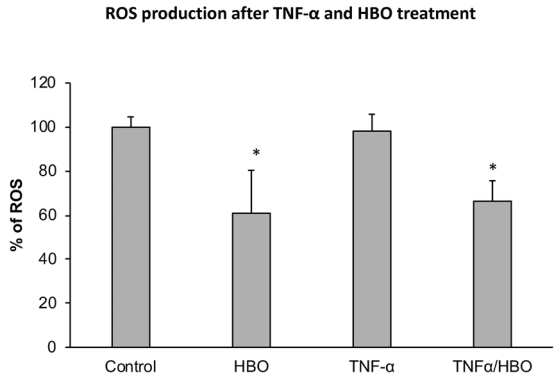

Figure 6 ROS production after HBO treatment. EPC (n = 4/group) were incubated with TNF-alpha and treated with HBO for 60 min. DCFDA was used as a substrate, which degrades in the presence of ROS and produces green fluorescent products that can be detected by spectrometry. Data were normalized to control, and the values are mean ± SD. $^{*}P < 0.05$.

the number of cells counted was smaller than control. However, HBO treatment was able to revert this anti-proliferative effect 24 h later. Godman et al. (2010) showed that HBO treatment enhances the expression of cytoprotective agents in endothelial cells, related to inflammatory signaling pathways. In the wound-healing assay, neither HBO nor TNF-alpha affected the cell monolayer healing process, mostly due to the exposition time to HBO used in the assay and the time after the exposition that we used.

The study of Wang et al. (2011) suggested that HBO treatment could enhance ROS concentration in cell cultures and therefore increase oxidative stress. Godman et al. (2010), however, showed that the treatment provided increased expression of antioxidants agents, which can suppress the cell damage caused by ROS. Our results demonstrated that HBO decreased ROS production in control cells and this decrease was maintained even when the cells were pre-

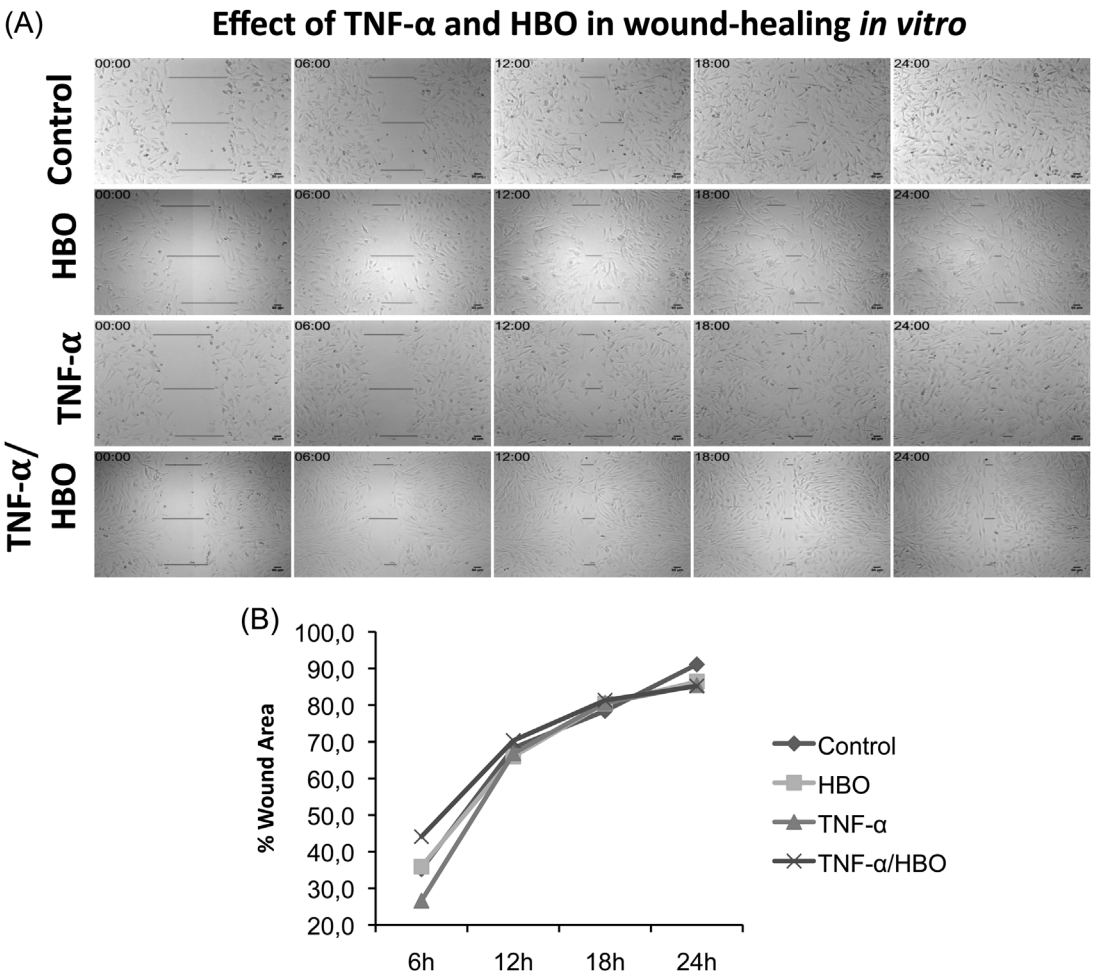

Figure 7 Effect of TNF-alpha and HBO in wound-healing in vitro. (A) Time-lapse of the scratch assay in EPC monolayer treated or not with TNF-alpha and HBO. The recovery was followed for 24 h. N = 3/group. Bar scale: 50 μm. 100× magnification. (B) Quantification of the percentage of lesioned area in the EPC monolayer related to control analyzed in every 6 along 24 h. The values are mean ± SD. N = 3/group.

treated with TNF-alpha, revealing its relevance to become an additional treatment to therapies involving vascular regeneration and remodeling.

Conclusions

This study aimed to provide a better understanding of HBO effects on EPC proliferation, morphology and the molecules involved in the inflammatory process. Our results showed that HBO for 1 h could provide an improvement in the inflammatory status induced by TNF-alpha by decreasing the expression of the adhesion molecule ICAM, ROS production and inducing eNOS activity what may promote vascular regeneration, ameliorating the inflammatory response and reestablishing the endothelium after an injury.

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J.C.B. elaborated the project, isolated the cells and performed all the assays with the cells. L.H.F.J and G.D.C helped with cell isolation and culture, M.S.S helped with histological assays, S.G helped with the assays using HBO. C.C.W helped with the cell culture and reviewed the article; C.P.V was responsible for the overall project helped to write the article and with the wound-healing assay. This work was supported by grants from Coordination for the Improvement of Higher Education Personnel (CAPES) to J.C.B, L.H.F.J and G.D.C. C.P.V and S.G were supported by São Paulo Research Foundation (FAPESP) (2012/23640) and (2015/23767-0) respectively. C.C.W and M.S.S. to National Council for Scientific and Technological Development (CNPq). C.C.W (308368/2016-9).

Disclosure of interest

The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Effect of 100% oxygen on ICAM expression.