Chapter

Effect of Hyperbaric Oxygen on Hematopoietic Stem Cell Transplantation

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Abstract

In this chapter the accumulated evidence that supports the role of hyperbaric oxygen therapy (HBOT) in improving the process of hematopoietic stem/progenitor cell (HSPC) homing, engraftment, and immune-reconstitution will be reviewed. The underlying mechanism by which HBO modulates erythropoietin (EPO)/EPOR signaling to improve HSPC homing and engraftment will be described. Also the pre-clinical evidence and pilot clinical trial evidence that supports HBO role in improving HSPC homing and engraftment will be examined. Current and future clinical trial studies that stem from this concept will be detailed. Finally, areas that need future investigations to optimally utilize HBO in the field of HSPC transplantation will be described.

Keywords: hyperbaric oxygen therapy (HBOT), hematopoietic stem/progenitor cell (HSPC), homing and engraftment, hematopoietic stem/progenitor cell transplantation, pilot clinical trials, phase II clinical trials

1. Introduction

Allogeneic transplantation is the only curative approach for many hematologic malignant and nonmalignant disorders. Unfortunately, only 30% of patients will have a matched sibling donor [1]. However, well-matched donors (MUDs) are a suitable alternative for those who do not. In one study, well-matched MUDs were identified in 53% of those with Northern European ancestry, compared to only 21% of patients of other origin [2]. For patients without a histocompatible adult donor, transplant options include unrelated umbilical cord blood (UCB) transplantation or transplant from a haploidentical (haplo) donor [3]. Since the first successful UCB transplant in 1988 [4], UCB has been used as a graft source for over 40,000 patients with both malignant and nonmalignant diseases [5, 6].

As a graft source for transplantation, UCB has several practical advantages including ease of procurement, absence of donor risks, reduced risk of transmissible infections, and availability for immediate use [7]. UCB is also associated with a lower incidence of graft-versus-host disease (GVHD) despite HLA disparity [8]. Therefore, UCB extends the application of allogeneic transplant to ethnic minority populations who are underrepresented in donor registries [9]. Additionally, UCB transplantation is associated with reduced leukemia relapse in patients with evidence of minimal residual disease at time of transplant, suggesting a strong graft-versus-leukemia effect [10]. However, UCB units in themselves are limited in

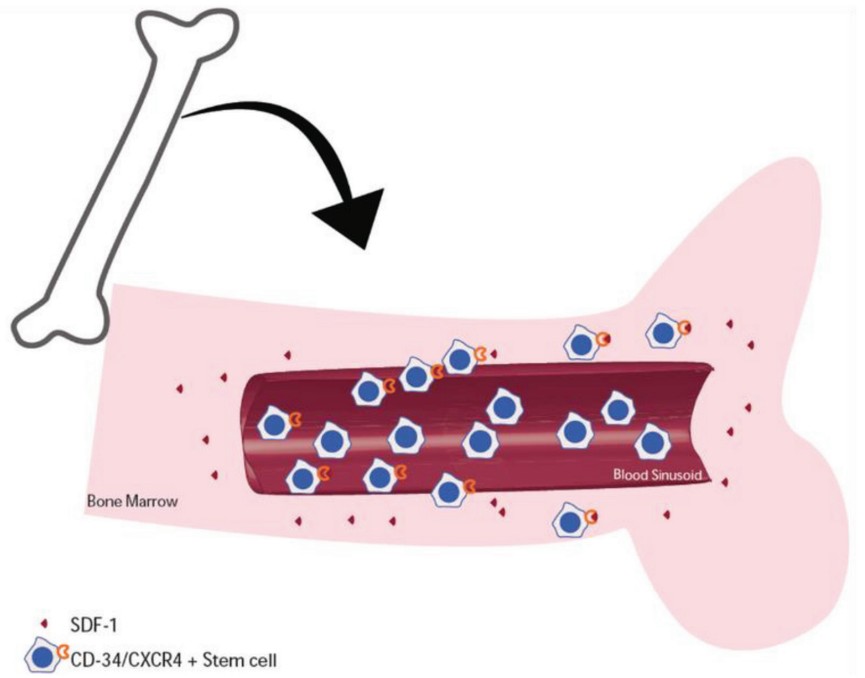

Figure 1.
Hematopoietic stem/progenitor cell (HSPC) homing to the bone marrow. This process is mediated by CXCR4 receptors on the surface of HSPCs and stromal cell-derived factor-1 (SDF-1) in the bone marrow.

cell doses available for optimal transplantation in adults. UCB stem cells also demonstrate defects in homing to the bone marrow (BM), implicating delayed recovery of neutrophil and platelet count and achieved engraftment, resulting in higher rates of graft failure [11]. This prolonged time to engraftment is also associated with delayed immune reconstitution after UCB transplantation [12–14], resulting in higher posttransplant infection rates [15]. Strategies to overcome these defects in homing and engraftment are clearly needed in order to make this potentially curative therapy more effective for patients. Additionally, such strategies might apply to other types of hematopoietic stem cell (HSC) transplantation, including autologous stem cell transplantation as well as allogeneic stem cell transplantation.

Homing is the first process by which circulating hematopoietic cells actively cross the blood/BM endothelium barrier to migrate into the BM compartment (**Figure 1**) [16]. This process is fairly rapid and occurs within hours and no longer than a day or two after stem cell infusion [16]. HSC homing is mediated in part by the binding of chemokine CXCR4 receptor on the surface of HSCs to their ligand, stromal cell-derived factor-1 (SDF-1) expressed by BM stromal cells [17]. Stem cell homing precedes engraftment, corresponding to proliferation and differentiation of hematopoietic stem cells (HSCs) to produce mature, functional hematopoietic cells within the BM [18]. One study claimed that only 18–20% of all intravenously transplanted stem cells, including different subsets, seeded in the BM, with UCB stem cell seeding even lower [19]. Another study demonstrated that human UCB stem cell seeding efficiency in NOD/SCID mice was found to be less than that for BM (4.4% versus 20%) [20].

2. Current methods to improve UCB HSPC homing

Due to the curative potential of UCB transplantation, several approaches have been investigated to improve UCB stem cell homing to the BM. In one study inhibition of CD26 peptidase activity by pretreating purified $CD34^+$ human CB cells with

Diprotin A significantly enhanced engraftment of HSCs from human UCB into NOD/SCID mice [21]. A CD26 peptidase inhibitor, sitagliptin, was investigated in a clinical trial with encouraging results in engraftment of adults with hematological malignancies after using a single unit UCB transplant [22]. Another strategy taken involved direct intrabone administration of cord blood cells into the superior-posterior iliac crest under rapid general anesthesia. Though this strategy produced impressive results in one study [23], another study showed contradictory results [24]. Therefore this procedure has not been widely accepted. In exploring further defects in cord blood stem cell homing, it was found that cord blood $CD34^+$ cells have reduced alpha(1,3)-fucosyltransferase (FucT) expression and activity causing a depletion of cord blood stem cell surface ligands necessary for interaction with adhesion molecules at time of stem cell homing [25]. Forcing fucosylation was found to be clinically feasible with encouraging engraftment efficiency data in the double UCB transplant setting [26]. Some of these interventions require significant logistical support, and some require graft manipulation; accordingly, there is an urgent need to identify safe and practical interventions to enhance UCB homing and engraftment for patients with hematologic malignancies who are undergoing allogeneic stem cell transplantation.

3. Pre-clinical data supporting HBO role in modulating EPO/EPOR signaling in HSCs

Previously published work implicating erythropoietin (EPO) in HSC homing led investigators to examine the role of EPO/EPOR signaling in HSC homing and engraftment in vitro and in vivo pre-clinical models. Gonzalez et al. demonstrated that circulating HSCs rapidly decline after birth [27]. Interestingly, the decline in HSCs correlated with low EPO blood concentration. Additionally, the decline in HSCs being attributed to HSC BM homing, these observations suggested a possible role for EPO in BM homing and clearance of HSCs from the infant's circulation following birth. Investigators have pursued HBO as a potentially safe approach to effectively lower EPO as previously published [28]. The hypothesis was that lowering EPO at the time of hematopoietic stem/progenitor cell (HSPC) infusion will result in improved bone marrow homing and subsequent engraftment. Studies examining HBOT effects on hematopoietic stem cells are limited. On the other hand, HBOT has been shown to have minimal, if any, effects on blood counts during steady-state conditions [29]. The previously published and accumulated pre-clinical data that supports EPO's role in UCB engraftment are summarized in the next section [30].

To understand EPO effects on UCB $CD34^+$, the expression of EPOR was assessed by flow cytometry. Analyses of 5 UCB units revealed that on average 6.5% of $CD34^+$ UCB cells express EPOR [30]. A significantly higher percentage of EPOR positive cells (45.7 ± 1.4%, **Figure 2**) was observed within the HSC (Lin^- $CD34^+$ $CD38^-$ $CD45RA^-$ $CD90^+$ $CD49f^+$ cells) population. EPOR positive cells were less among multipotent progenitor (MPP) (Lin^- $CD34^+$ $CD38^-$ $CD45RA^-$ $CD90^-$ $CD49f^-$ cells, 22.2 ± 0.3%) or the broader progenitor pool ($Lin^-CD34^+CD38^+$ cells, 25.1 ± 0.7%). To test whether a functional EPO-EPOR signaling cascade was activated in EPOR-expressing UCB $CD34^+$ cells, EPOR expression was depleted via RNA interference (RNAi), and the erythroid differentiation potential after culture in methylcellulose culture medium was compared to UCB $CD34^+$ cells without EPOR depletion. Depletion of EPOR expression by RNAi greatly reduced the size of erythroid colonies and UCB $CD34^+$ differentiation potential toward the erythroid lineage, indicating that EPO promotes functional EPO-EPOR signaling response in these cells [30].

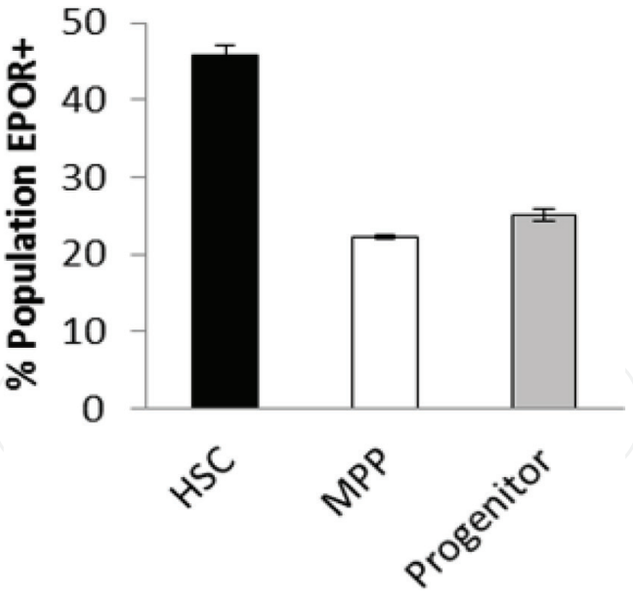

Figure 2.
Erythropoietin receptor expression on umbilical cord blood CD34⁺ cells and subsets (unpublished data).

As earlier studies potentially implicated EPO signaling in hematopoietic stem/progenitor cell (HSPC) homing [27], investigators tested if there were EPO-EPOR signaling effects on SDF-1-induced migration of UCB $CD34^+$ HSPC, by examining UCB $CD34^+$ $CD38^-$ cell transmigration toward an SDF-1 gradient after a preexposure of the cells to different concentrations of EPO. Exposure of UCB $CD34^+$ $CD38^-$ to EPO significantly reduced their SDF-1-induced directional migration. Blocking EPO signaling by anti-EPOR or anti-EPO antibodies rescued SDF-1-induced migration of UCB $CD34^+$ cells for both $CD34^+$ $CD38^-$ and $CD34^+$ $CD38^+$ populations [30].

HBO treatment has been shown to reduce systemic EPO levels in healthy volunteers [28]. As previous in vitro studies indicated that EPO-EPOR signaling inhibits SDF-1-induced migration of UCB $CD34^+$ cells, investigators examined whether HBO pre-treatment of mice prior to cell infusion enhances BM homing. First, investigators measured serum EPO levels in their murine transplant model 7 hours after HBO exposure (or 3 hours post UCB $CD34^+$ infusion). HBO exposure significantly reduced serum EPO levels compared to controls ($p < 0.0001$). In addition, a higher percentage of the UCB $CD34^+$ cells was seen in the BM of HBO-treated mice 3 hours posttransplant [30].

In the same murine model, investigators evaluated the impact of HBO treatment on peripheral blood, BM, and spleen retention at early time points (24–72 hours), which correlates with BM homing, and up to 4.5 months, which correlates with long-term engraftment. Efficient support of human cell engraftment has been reported in 6–8-week-old female NSG mice NOD/SCID/IL-2Rgc^{null} [31] model. Briefly, sublethally irradiated NSG mice, after 24 hours, were treated with HBO for 2 hours (HBO) or without HBO in the control group. Next, approximately 10^5 CD34-selected UCB cells were infused into each mouse 6 hours following the start of HBO. Mice were euthanized at different time points; peripheral blood, BM, and spleen tissue were harvested; and engraftment was analyzed by flow cytometry. The degree of engraftment was determined by measuring the percentage of human CD45-expressing cells. For HBO therapy, 100% oxygen was delivered at 2.5 atmospheres absolute (ATA) in a single-place chamber. In murine in vivo model, HBO-treated mice had significantly improved BM ($p = 0.0067$), peripheral blood ($p = 0.0131$), and spleen ($p = 0.0293$) engraftment [32], the impact of which was more pronounced toward later time points at 3 and 4 months.

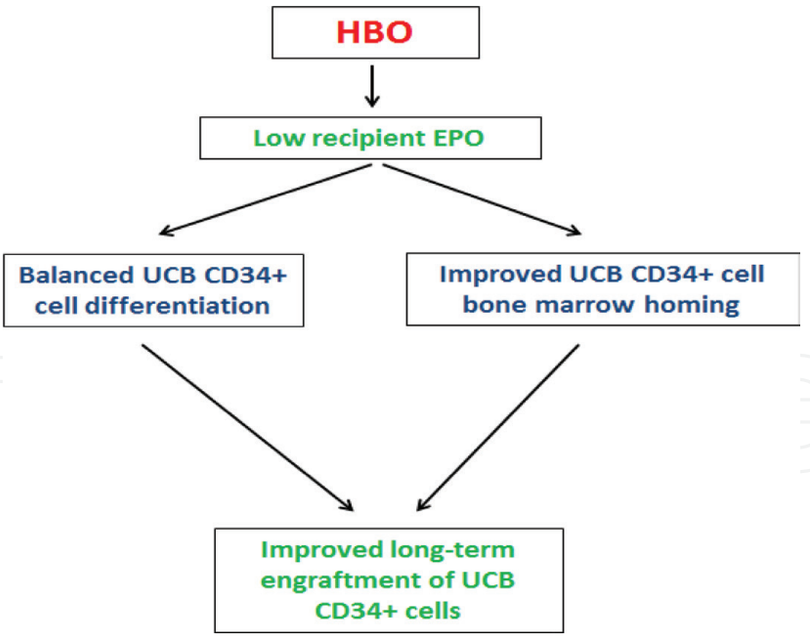

Figure 3.
The mechanisms by which hyperbaric oxygen therapy (HBO) affects hematopoietic stem/progenitor cell engraftment.

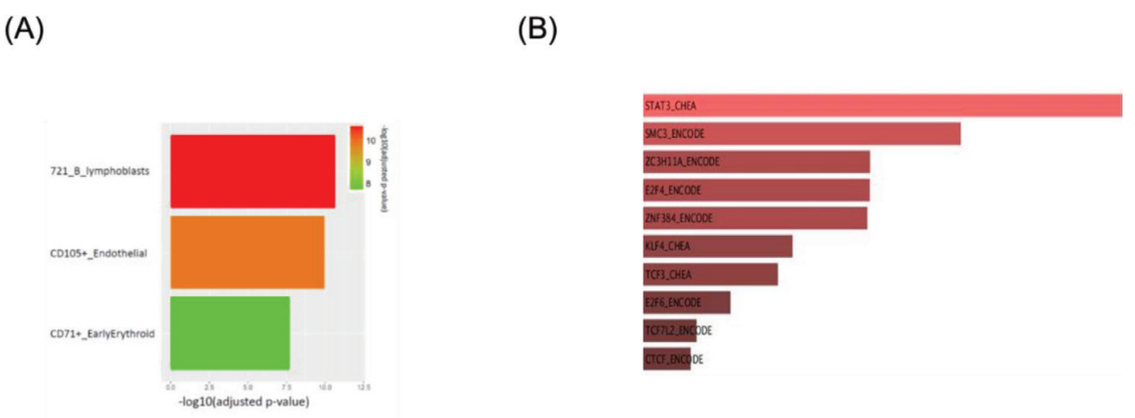

Figure 4.
Gene expression data analysis evaluating erythropoietin (EPO) treatment effects on UCB CD34⁺ cells. EPO treatment enriches CD71⁺ early erythroid cells (A) and correlates with active STAT3 signaling (B) (unpublished data).

EPO has been shown to impact hematopoietic progenitor cells differentiation [33]. Because HBOT lowers EPO levels in posttransplant, the impact of a low EPO environment induced by HBO on human UCB CD34⁺ cell differentiation was examined. HBO mice demonstrated significantly lower numbers of burst-forming unit-erythroid (BFU-E) (p = 0.043) and increasing numbers of colony-forming unit-granulocyte/macrophage (CFU-G/M) (p = 0.05) 1 week following transplant. Interestingly, despite reduced BFU-E in the in vivo experiments, investigators observed a favorable trend in red blood cell (RBC) time to transfusion independence (TTI) in their pilot study.

These findings suggest that lowering the recipient EPO levels favors UCB CD34⁺ engraftment by affecting two important HSC functions: BM homing and HSPC differentiation (**Figure 3**). Lower recipient EPO at the time of UCB CD34⁺ cell infusion results in less early erythroid differentiation of infused progenitor cells. This leads to early homing of undifferentiated UCB CD34⁺ cells to the BM, thus improving long-term multi-lineage engraftment. In confirmatory experiments utilizing

RNA-seq for transcriptional assessment, investigators found that EPO treatment of UCB $CD34^+$ cells enriches $CD71^+$ early erythroid cells, consistent with early erythroid commitment (**Figure 4**). In the same data set, EPO treatment was associated with signal transducer and activator of transcription 3 (STAT3) pathway activation (**Figure 4**). Importantly, signal transducer and activator of transcription 3 (STAT3) is a known downstream effector of EPOR signal transduction [34–37].

4. Pilot clinical data supporting HBO role in HSC transplantation

To date, two pilot clinical trials exploring HBO in UCB transplantation as well as autologous hematopoietic cell transplantation (HCT) have been completed. In both studies HBO was given in standard fashion at least 6 hours prior to HSCP infusion on day 0 of their transplant (**Figure 5**). The first aim of these studies is to examine the safety and tolerability of HBO in the setting of HCT. In addition, these studies explored the impact of HBO on blood count recovery as well as EPO levels posttransplant. Details of HBO therapy and the results of these studies are being summarized in the next three paragraphs.

4.1 Details of HBO therapy

After receiving routine clinical care on day 0 (the day of HSPC infusion), subjects were exposed to HBO for a total of 90 min after compression to 2.5 atmosphere absolutes (ATA) in a monoplace hyperbaric chamber (Model 3200/3200R, Sechrist Industries, Inc., USA), breathing 100% oxygen. The subjects spent 10–15 min during the compression and decompression phases and 10 min room air breaks for every 30 min of HBO treatment.

4.2 HBO in UCB transplantation

Based on the previously mentioned pre-clinical data, a pilot clinical trial investigating the safety of HBO in UCB transplant was initiated. Patients considered

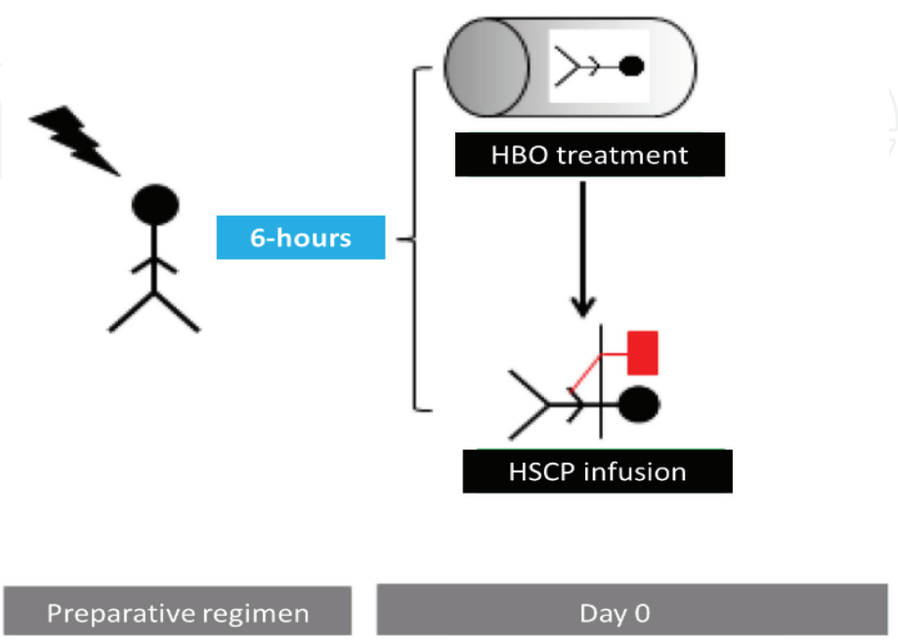

Figure 5.
Clinical trial schema incorporating hyperbaric oxygen (HBO) into hematopoietic cell transplantation.

		HBO (n = 15)	Historic (n = 48)	p value
Neutrophil recovery (n/%)	No	0%	6 (12%)	NS
	Yes	15 (100%)	42 (82%)	
Platelet recovery (n/%)	No	0%	15 (31%)	0.013
	Yes	15 (100%)	33 (69%)	
Median time to neutrophil recovery (range)		14 (6–45)	20.5 (571)	NS
Median time to platelet recovery (range)		37.5 (0–85)	38 (0–161)	NS

Table 1.
Blood count recovery in umbilical cord blood transplantation pilot study utilizing hyperbaric oxygen (HBO).

for either standard myeloablative conditioning (MAC) (higher intensity chemotherapy and radiation) or standard reduced intensity conditioning (RIC) (lesser intensity chemotherapy and radiation) UCB transplantation were enrolled. In this study, HBO treatment was administered on day 0 of the transplant. The treatment consisted of exposure to 100% oxygen at 2.5 ATA for a total of 2 hours, in a single see-through hyperbaric chamber. Six hours from the start of HBO, single or double UCB units are infused, and patients are followed daily for toxicity and blood count recovery. In addition to safety, neutrophil and platelet recovery and engraftment were investigated as efficacy end points. A total of 15 subjects have been treated; all have tolerated the procedure very well except for 1 patient who did not finish the last 10 min of therapy because of nausea thought to be secondary to a concomitant medication. In terms of efficacy, final data from the study indicate an encouraging median time to neutrophil recovery of 14 days compared to 20.5 in historic data (n = 48) and a median time to platelet count recovery of 37.5 compared to 38 in historic data (**Table 1**). HBO also resulted in improved day 100 survival (p = 0.051) and in improvement in the percentage of patients who demonstrated Neutrophil recovery was not significant platelet count recovery (p = 0.013). HBO also resulted in statistically significant reduction in median EPO level from baseline (−30.37 mU/ml+/−31.68, p = 0.004).

In a follow-up study, the long-term outcome of patients in this pilot HBO study in UCB transplantation was examined. Patients' outcome was compared to a historic control group. The 6-month survival in the HBO group was 100%, compared to 67.0% in the control group (95% CI 50.1–79.4%, p < 0.0001) [38]. HBO-treated patients had on average lower relapse and non-relapse mortality rates, and less chronic graft-versus-host disease (GVHD), but had increased acute GVHD. However, these differences were not statistically significant, probably because of the small sample size. In the HBO-treated cohort, immune-reconstitution analysis showed significant improvement in early B-cell recovery, with a trend toward improvement in early NK cell recovery. The ratio of 8 hours to baseline EPO levels was examined. A nonsignificant trend toward lower EPO values was found in those who did not relapse or die in year 1 than those who did die or relapse. Disease progression-free survival was also improved in those who had more than 80% reduction in EPO levels in response to HBO. This study highlights the long-term safety of HBO therapy when used prior to UCB transplantation. It also shows a relationship between HBO-induced EPO reduction, early NK cell recovery and posttransplant disease progression. Since lower rates of relapse have been reported in association with higher early NK cell recovery [39], it was hypothesized that by reducing EPO, HBO improves early NK cell recovery, and improved NK cell recovery slows down disease progression.

4.3 HBO in autologous HCT

Encouraged by the results of HBO in UCB transplantation, the same group conducted a pilot study in Auto-HSPC transplantation. A total of 20 patients were treated on the Auto-HSPC transplant study. HBO therapy was very well tolerated as 19 completed full therapy [40]. For efficacy comparison, HBO subjects were matched to historical controls from the same institution based on gender, age (within 5 years), disease type (multiple myeloma or lymphoma), and preparative regimen. The median time to neutrophil count recovery was 11 days in both cohorts, the HBO and control cohorts. However, time to neutrophil recovery was approximately 1 day sooner for HBO than historical controls taking into account the full distribution estimates of Kaplan-Meier estimator (log rank $p = 0.005$). The median time to platelet count recovery was 16 versus 18 days for the HBO and control cohorts, respectively (log rank $p < 0.0001$).

In a separate analysis, HBO effects on other outcomes of post-autologous transplantation were evaluated. In this analysis, the HBO cohort patients who completed HBO therapy ($n = 19$) were compared with historic patients ($n = 225$) [40]. The average days of GCSF use were 6 days in the HBO cohort compared to 8 days in controls ($p < 0.01$). Also, HBO patients had significantly less mucositis (26.3 versus 64.2%, $p < 0.01$).

5. HBO and stem cell mobilization

In the previous section, the effects of HBO on stem cell homing and engraftment posttransplant were reviewed. Interestingly, HBO can also help with stem cell/progenitor cell mobilization from the bone marrow [41]. However, the mobilized stem/progenitor cells exhibited characteristics of endothelial progenitor cells [42].

6. Current and future prospective

Incorporating HBO into HCT backbone represents a new direction in the field of HCT aiming at improving the outcome of HCT by improving HSPC homing and subsequent engraftment. Accumulated data suggest improvement in immune reconstitution too. Targeting EPO at the time of HSPC infusion represents a new understanding of EPO role in basic HSCP functions, including cell differentiation, transmigration, homing, and engraftment. Though these studies represent an early attempt at understanding EPO role in HSCP biologic functions and HBO's role in blocking EPO/EPOR signaling in HCT transplantation, the accumulated data seem to be promising. Currently, a phase II study investigating HBO in Auto-HCT is open for enrollment (ClinicalTrials.gov Identifier: NCT03398200). Another phase II study investigating HBO in UCB transplantation is expected to be open for enrollment in early 2019 (ClinicalTrials.gov Identifier: NCT03739502). Both of these studies are randomized prospective clinical trials that focus on investigating HBO effects on time to neutrophil recovery, platelet count recovery, blood and platelet transfusion requirements, and growth factor use. Additionally, both studies will be evaluating disease response posttransplant. Immune reconstitution will be examined in an attempt to correlate that to disease response posttransplant, hypothesizing that HBOT improves immune reconstitution which in turn will result in improved disease response to transplant. Finally, these studies will examine HBO effects on EPO and IL-15 levels posttransplant. The study in UCB transplantation will also focus on time to achieving full-donor chimerism as that might influence

disease control posttransplant. This wave of phase II studies will be essential in establishing the efficacy of such procedure in HCT and might lead to future phase III studies.

An additional area for future investigation is defining the optimal HBO schedule to effectively block EPO/EPOR signaling during HCT. In a previous study, one single HBO treatment 6 hours prior to HSPC infusion was used. It was noticed that EPO level rebounds as early as 24 hours after HBO treatment [30]; accordingly additional HBO therapy might keep EPO levels low for 48 hours, which is the duration during which homing occurs. To accomplish that, investigators will have to treat the recipients 24 hours after HSPC infusion, which means the infused HSPCs will be exposed to hyperbaric conditions. In their experience, direct $CD34^+$ cell exposure to HBO reduced their proliferation, impaired their in vitro transmigration, and reduced their erythroid differentiation [43]. These effects were statistically significant, but the biological effects were minimal which in theory should not influence UCB $CD34^+$ cell behavior significantly. Additionally, these direct HBO effects on UCB $CD34^+$ cells are desirable when it comes to the HSPCs that have already homed to the bone marrow as these effects might help with HSPC retention in the bone marrow.

Finally, in addition to reducing EPO and affecting EPO/EPOR signaling, HBO might have additional effects beyond EPO/EPOR signaling that might impact HSPC biologic functions.

7. Conclusions

Targeting EPO using HBO in hematopoietic cell transplantation is a new direction in the HCT field which will potentially have major impact on the outcome of HCT. By improving HSPC homing, engraftment, and immune reconstitution, HBO therapy will have the potential to improve the outcome of HCT by improving patient recovery and by reducing posttransplant complications related to infections. Overall, that might reduce the cost of HCT. Though data from pre-clinical and pilot clinical studies are encouraging, data from current and future phase II studies might show more definitive data in support of this application. Also future studies will be needed to examine HBO effects on bone marrow microenvironment elements.

Conflict of interest

No conflict of interest to declare.

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