



Use of hyperbaric oxygen in hematopoietic cell transplantation to aid post-transplant recovery

Alain Mina¹ & Omar S Aljitawi^{*,2}

¹Department of Hematology & Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

²Division of Hematology/Oncology & Bone Marrow Transplantation Program, University of Rochester Medical Center, Rochester, NY 14642, USA

*Author for correspondence: Tel.: +1 585 276 6259; Fax: +1 585 276 2576; omar.aljitawi@urmc.rochester.edu

“In this future pilot clinical trial concept patients will receive HBO therapy not only before stem cell infusion, but also afterward. In a separate approach, we are working on developing EPOR small-molecule inhibitors.”

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The concept of using hyperbaric oxygen (HBO) in hematopoietic cell transplantation (HCT) was initially developed as a new approach to improving umbilical cord blood (UCB) transplantation outcomes by improving the process of hematopoietic stem/progenitor cell (HSPC) homing and engraftment. UCB has been a reliable source of HSPC especially in minorities that are under-represented in international registries of HLA-matched donors [1]. Though an attractive option, UCB has its limitations including a limited number of cells available in each UCB unit necessary for successful engraftment [2], impaired hematopoietic stem cells (HSC) homing and increased rates of engraftment failure [3]. Numerous efforts have aimed at improving the clinical outcomes of this modality either through *in vitro* expansion of UCB HSC [4], studying its defective homing and trying to optimize it [5], or bypassing the whole process through direct injection of UCB stem cells into the superior posterior iliac crest [6]. These different approaches have been associated with variable degrees of success. Also, they are associated with graft manipulation and significant logistical support is needed to implement these approaches, which makes such approaches costly. The cost issue is important as patients who lack matched sibling donors might have haploidentical donors as an option in addition to UCB stem cells [7].

To understand how HBO works in improving HSPC homing and engraftment, it is important to understand how EPO impacts HSPCs. EPO is a hormone that has been shown to play a role in the development and selective differentiation of hematopoietic progenitor cells *in vivo*; it induces differentiation into the erythroid subtype and suppresses nonerythroid routes of differentiation [8]. In addition, an opposite relationship has been suggested between EPO levels and HSPC bone marrow (BM) homing, which stems from the observation that soon after birth, and coinciding with the increase in blood oxygenation, circulating stem cells decline precipitously, in conjunction with a decrease in EPO blood concentration [9,10]. These observations led us to investigate a new role for EPO in HSC differentiation and BM homing that might have clinical implications on UCB transplants and engraftment outcomes [11,12]. Approximately, 20% of UCB CD34+ cells express EPO receptor (EPOR) and exposing these cells to the EPO was shown to decrease the rates of UCB stem cell *in vitro* transmigration toward the chemokine SDF-1 which mediates UCB HSC homing to the BM [11]. This negative effect on *in vitro* transmigration was negated by treating these cells with an EPOR neutralizing antibody or by depleting EPOR via RNA interference [11]. Accordingly, to improve HSPC homing during HCT, we proposed to lower EPO levels at time of HSPC cell infusion. Given its known effects on lowering EPO in healthy volunteers [13], we proposed the approach of treating the host with HBO prior to HSPC infusion.

We initially investigated the effect of HBO treatment on UCB CD34+ cell homing and engraftment. Immune deficient mice that were sublethally irradiated were treated with 2 h of HBO therapy (100% oxygen at 2.5 atmospheric pressure) prior to UCB CD34+ cell infusion, which was planned to occur 6 h from the start of HBO treatment. Treatment with HBO was shown to favor engraftment kinetics and was associated with improvement in all lineage engraftment [14]. These preclinical findings provided the basis for examining HBO therapy in clinical HCT transplantation. Initial studies were focused on examining HBO safety and tolerability, with secondary focus on efficacy by comparing engraftment kinetics in HBO treated patients with historic controls. In the following sections, we review published data from our experience in utilizing HBO therapy in HCT.

Safety & tolerability

The tolerability to HBO therapy in HCT has been examined in two settings; in the setting of autologous HCT [15] and UCB transplantation [11,12]. In the autologous HCT setting, 19 out of 20 patients completed HBO therapy [15], only one patient did not complete therapy secondary to ear discomfort. In UCB transplantation, HBO was well tolerated with 14/15 patients completing therapy, while one patient did not complete the last 10 min of the planned 90 min HBO therapy secondary to nausea attributed to concomitant medications [11]. These studies demonstrate that HBO incorporation into HCT is feasible and that HBO therapy is well tolerated. Also, no safety concerns were noted upon long-term follow up in UCB transplantation setting [12].

Efficacy

In the study evaluating HBO treatment in autologous HCT, our primary aim was not only to assess the safety of HBO but also to determine its effects on hematopoietic reconstitution [15]. The median time to neutrophil recovery was found to be 11 days in both the historic cohort and the HBO group, but the Kaplan–Meier estimates did indicate that time to neutrophil recovery was 1 day shorter in the HBO treated group ($p = 0.005$). Also, the median time to platelet recovery was 18 days in the control group compared with 16 days in the HBO group ($p < 0.0001$). Though the number of transfused platelet units and packed RBCs were similar in both groups, the median duration of G-CSF use was less in the HBO group (6 days) compared with the control (8 days) and the difference was statistically significant. As expected, EPO was significantly reduced 6 h following HBO therapy compared with pre-HBO values. However, EPO levels were not examined in the historic cohort for optimal examination of HBO effects on EPO. One interesting finding was that the rates of mucositis were significantly lower in the HBO treated group compared with the historic group (26.3 vs 64.2%, respectively; $p = 0.002$) [15].

In the UCB transplant study, findings were also encouraging in the HBO subgroup [11,12]. The median time to neutrophil recovery was shorter in the HBO group (15 vs 19.5 days), but the difference was not statistically significant ($p = 0.31$). Also, a shorter median duration to platelet recovery was experienced in the HBO group compared with controls (36.5 vs 38 day), but that was not statistically significant either ($p = 0.96$). Though rates of neutrophil recovery were not different between the two groups, rates of platelet recovery were significantly higher in the HBO group compared with the historic control group (100 vs 70.7%; $p = 0.02$).

Survival was also compared at two clinically important time points [12]. At 6 months, 100% of HBO treated patients were still alive compared with 67% of the control group ($p < 0.0001$) with mortality in the latter group being attributed largely to relapse (53.8%) and infection (30.8%). At the 1-year time point, the HBO group lost its survival benefit (overall survival: 62.3 vs 56.5%; $p = 0.43$). Given the improvement in survival at the 6-month time point, we evaluated immune-reconstitution as a potential driver for improved early survival in the HBO treated cohort. Indeed, we found significant improvement in early (day +100 post-transplant) CD19 immune-reconstitution in the HBO group compared with controls ($p < 0.001$) [12]. In addition, a trend toward early reconstitution of natural killer cells (CD16/65) was observed in the HBO group compared with controls ($p = 0.091$) [12].

Interestingly, the extent of EPO suppression correlated with clinical outcomes [12]. For example, using a ratio of 8-h post-HBO EPO level to baseline (pre-HBO)-EPO level, the HBO cohort was separated into >80% ratio ($n = 4$) group versus <80% ratio group ($n = 10$). Those with ratio <80% had a trend toward improved progression free survival ($p = 0.09$) [12]. Also, patients who neither relapsed nor died at the 1-year mark had a median ratio of 0.45 compared with 0.91 in those who died or relapsed ($p = 0.09$), further supporting that the degree of EPO suppression might correlate with improved clinical outcomes [12].

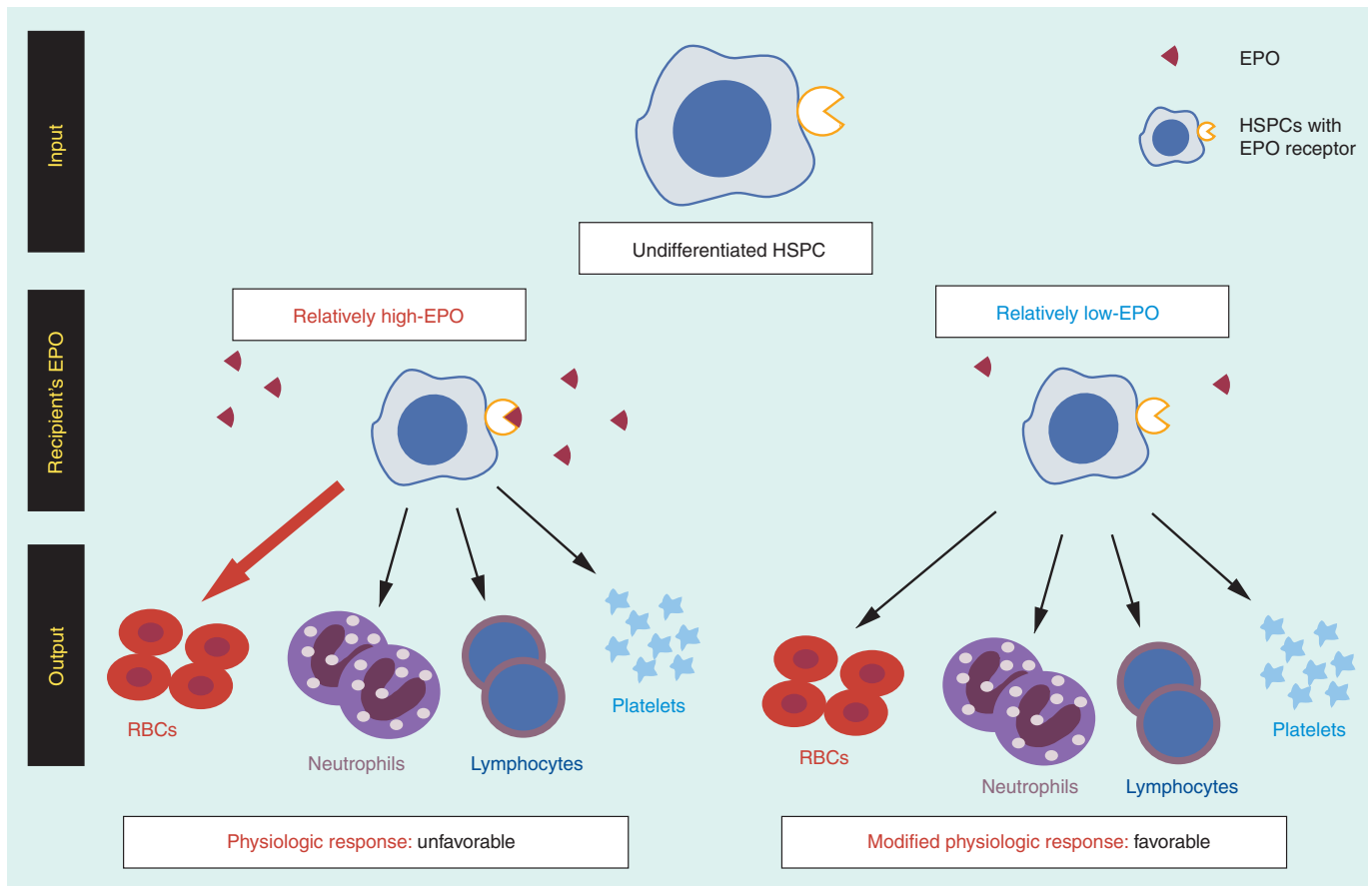


Figure 1. Erythropoietin at time of hematopoietic stem/progenitor cell transplantation.
 EPO: Erythropoietin; HSPC: Hematopoietic stem/progenitor cell; RBCs: Red blood cells.

Current state

HCT is not a physiologic process by design, whereas the physiologic response to varying degrees of BM myeloablation is upregulation of EPO [16] to maintain the oxygen carrying capacity through erythropoiesis. This erythroid bias, though physiologically appropriate, is not intended in HCT where multi-lineage differentiation potential is preferred. Since EPO treatment results in erythroid differentiation in hematopoietic CD34+ cells as has been reported by other groups [8] and our group [11] and since EPO treatment of UCB CD34+ cells results in inefficient homing [11], we characterize HCT conditions at the time of HCT as 'unfavorable' because of elevated EPO levels at time of stem cell infusion. These 'unfavorable' conditions related to high EPO, could potentially explain why aplastic anemia patients undergoing UCB transplantation have increased risk of graft failure [17]. Also, it could potentially explain the delayed neutrophil engraftment seen in patients with sickle cell disease [18] in a recent study for gene therapy in which autologous CD34+ cells were mobilized, *ex vivo* transduced and transplanted following busulfan conditioning (Esrick *et al.*, validation of BCL11A as therapeutic target in sickle cell disease: results from the adult cohort of a pilot/feasibility gene therapy trial inducing sustained expression of fetal hemoglobin using post-transcriptional gene silencing, ASH 2019). In this study, neutrophil engraftment occurred on day +22, which is much delayed compared with other contemporary autologous transplant outcomes [15]. By treating recipients with HBO prior to stem cell infusion, we lower EPO, and thus restore recipient conditions to more 'favorable' conditions (Figure 1). Since HSPC BM homing is a transient process [19], any intervention that is aimed at controlling EPO production or EPOR signaling should be timed to coincide with the homing process.

Our data show that a simple intervention that targets an early and transient event like BM homing could have long-term effects on immune reconstitution and long-term post-transplant outcomes [12]. In theory, better homing and engraftment will lead to improved multilineage differentiation and immune-reconstitution. In our experience in UCB transplantation, early B-cell recovery and to lesser extent natural killer cell recovery seem to be improved

in HBO patients compared with historic patients [12]. What is unknown is if HBO has direct effects on the BM/thymus gland microenvironments that could result in the noticed effects of HBO on immune-reconstitution. Based on our encouraging preliminary data in the area of immune reconstitution in response to HBO treatment in HCT setting, we believe this area is worth further exploration.

One of the interesting observations in our UCB experience was the correlation between poor EPO response to HBO treatment and increased risk of death from treatment-related mortality and/or relapse [12]. This observation suggests that EPO response to HBO could be used for prognostic purposes and further supports targeting EPO at time of HCT transplantation to improve post-transplant outcomes by reducing relapse and treatment-related mortality.

Future directions

Though data from our pilot and retrospective review studies are encouraging, prospective data from randomized clinical trials are needed to answer many questions regarding the safety and efficacy of HBO therapy in different HCT settings. We are currently conducting two randomized Phase II clinical trials evaluating HBO effects on neutrophil recovery (primary) and on immune reconstitution (secondary). In one study, we are evaluating HBO therapy in multiple myeloma patients undergoing high-dose therapy melphalan and autologous HCT (NCT03398200). In the other study we are evaluating HBO therapy in patients undergoing UCB transplantation (NCT03739502).

Since EPO reduction by HBO is transient and does not last throughout the duration of the homing process, we are planning on evaluating repeated HBO treatments as an approach that could result in sustained low-EPO state during the homing process (up to 72 h post-stem cell infusion). In this future pilot clinical trial concept patients will receive HBO therapy not only before stem cell infusion, but also afterward. In a separate approach, we are working on developing EPOR small-molecule inhibitors. These inhibitors could be combined with HBO or used alone to improve HCT homing and engraftment.

Though the impact of HBO on HSPC homing and engraftment is expected to be more noticeable in the setting of UCB transplantation, it is expected to have positive effects on engraftment in other types of HCT transplantation as BM progenitors express EPOR and in theory are amenable to EPO signaling [20]. For example, in our pilot study in autologous HCT we did realize an improvement in neutrophil and platelet engraftment in comparison to our historic cohorts [15]. Accordingly, we are planning a pilot clinical trial in allogeneic HCT setting.

Some might have concerns regarding the feasibility of applying this intervention at a large scale. However, the availability of HBO chambers in large academic medical centers and having HBO chambers in close proximity to ones that do not have in house chambers certainly helps in implementing this intervention. Even in the absence of easy access to one, to invest in having one in house or in close proximity is not an expensive endeavor. Certainly, if data supports its efficacy in improving time to hematopoietic recovery, that will have cost saving effects that will help offset the initial cost of investing in having a chamber in house.

Conclusion

Targeting EPO using HBO at the time of HCT to improve HSPC homing and engraftment is a new direction in HCT. Accumulated data thus far confirm the safety of this approach in HCT setting and support ongoing and future studies aimed at assessing the efficacy of this approach in improving hematopoietic recovery and immune reconstitution post-HCT.

Financial & competing interests disclosure

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