Cytotherapy, 2014; 16: 1280–1293

International Society for Cellular Therapy
ISCT®

Influence of a dual-injection regimen, plerixafor and CXCR4 on *in utero* hematopoietic stem cell transplantation and engraftment with use of the sheep model

A. DAISY GOODRICH[1], NICOLE M. VARAIN[1], CHRISTINE M. JEANBLANC[1], DONNA M. COLON[1], JAEHYUP KIM[2], ESMAIL D. ZANJANI[1] & PEIMAN HEMATTI[2,3]

[1]*Department of Agriculture, Nutrition, and Veterinary Science, University of Nevada-Reno, Reno, Nevada, USA,* [2]*Department of Medicine, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA, and* [3]*Carbone Cancer Center, University of Wisconsin Carbone Cancer Center, Madison, Wisconsin, USA*

Abstract

Background aims. Inadequate engraftment of hematopoietic stem cells (HSCs) after *in utero* HSC transplantation (IUHSCT) remains a major obstacle for the prenatal correction of numerous hereditary disorders. HSCs express CXCR4 receptors that allow homing and engraftment in response to stromal-derived factor 1 (SDF-1) ligand present in the bone marrow stromal niche. Plerixafor, a mobilization drug, works through the interruption of the CXCR4-SDF-1 axis. *Methods.* We used the fetal sheep large-animal model to test our hypotheses that (i) by administering plerixafor *in utero* before performing IUHSCT to release fetal HSCs and thus vacating recipient HSC niches, (ii) by using human mesenchymal stromal/stem cells (MSCs) to immunomodulate and humanize the fetal BM niches and (iii) by increasing the $CXCR4^+$ fraction of $CD34^+$ HSCs, we could improve engraftment. Human cord blood-derived $CD34^+$ cells and human bone marrow-derived MSCs were used for these studies. *Results.* When MSCs were transplanted 1 week before $CD34^+$ cells with plerixafor treatment, we observed 2.80% donor hematopoietic engraftment. Combination of this regimen with additional $CD34^+$ cells at the time of MSC infusion increased engraftment levels to 8.77%. Next, increasing the fraction of $CXCR4^+$ cells in the $CD34^+$ population albeit transplanting at a late gestation age was not beneficial. Our results show engraftment of both lymphoid and myeloid lineages. *Conclusions.* Prior MSC and HSC cotransplantation followed by manipulation of the CXCR4–SDF-1 axis in IUHSCT provides an innovative conceptual approach for conferring competitive advantage to donor HSCs. Our novel approach could provide a clinically relevant approach for enhancing engraftment early in the fetus.

Key Words: *hematopoietic stem cell transplantation, in utero transplantation, CXCR4, SDF-1, plerixafor, sheep model*

Introduction

In utero hematopoietic stem cell transplantation (IUHSCT) provides the opportunity for transplanting cells from an allogeneic donor into the early fetus to correct numerous genetic disorders of hematological, immunological and metabolic etiologies that could be diagnosed prenatally (1). IUHSCT offers the promise of the delivery of a healthy baby and preventing the consequences of the disease at its earliest stages. Furthermore, this procedure provides therapeutic advantages of a fetal environment such as acceptance of unmatched allogeneic donor cells in the pre-immune fetus and engraftment without the need for conditioning regimen in the rapidly expanding bone marrow (BM) niche. The fetal sheep is a relevant pre-clinical animal model for IUHSCT with a large body size and long gestation such that chronology of procedures and dosing of cells/cytokines/pharmaceuticals are easily translatable to the human clinical scenario (2). Rodent models of IUHSCT have also proved useful, especially with the availability of recipients lacking certain immune cells. As such, the murine anemic model and severe combined immunodeficient (SCID) model demonstrate better engraftment than normal mice after IUHSCT, similar to the observation of patients with SCID in whom donor cells have an advantage over recipient HSC for populating the niche (3,4). Unfortunately, the IUHSCT of human donor cells into immune competent models, mice (5) or sheep (6,7), results in only low levels of engraftment in those recipients that do engraft, which is also a key reflection of limitations facing patients in actual clinical settings.

Correspondence: **Peiman Hematti**, MD, WIMR 4033, 1111 Highland Avenue, Madison, WI 53705, USA. E-mail: pxh@medicine.wisc.edu

(Received 30 October 2013; accepted 27 May 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.05.025

Immunological hurdles to achieving clinically relevant levels of engraftment that have recently been identified include maternal alloantibodies, maternal T cells and recipient natural killer (NK) cells (8–10). We propose that access to the fetal BM HSC niche must also be of prominence, for engraftment in the absence of conditioning regimens is a competitive process between donor and recipient HSCs for populating limited niche space (11,12). We therefore hypothesized that vacating the fetal HSC niche before IUHSCT would increase available niche spaces for incoming donor cells. Standard conditioning regimens for vacating BM niches are prohibitively toxic at the fetal stage of development. Plerixafor (AMD3100; Sigma Aldrich, St Louis, MO, USA) is a drug that mobilizes HSCs out of the BM into the peripheral blood (PB) with no cytotoxicity, so that HSCs return to the BM niche when drug effects subside (13,14). BM stromal cells present stromal-derived factor 1 (SDF-1) (also known as C-X-C ligand 12 [CXCL12]), which functions as the ligand for the C-X-C receptor 4 (CXCR4) present on HSCs (15), whereas plerixafor, an antagonist for SDF-1, disrupts this ligand-receptor axis. Plerixafor has been administered to pediatric patients as young as 2 months of age (16). In this study, we explored a novel use for this drug and administered plerixafor just before injection of donor HSCs into the fetus. We estimated that at 4–6 h after dosing when the effects of plerixafor start to diminish (17), donor and recipient HSCs in circulation would home to the BM. In this manner, donor cells would have better access to the vacated recipient HSC niche and may have competitive advantage because of their high cell numbers in the bolus injection. In the use of the sheep model, we also proposed that transplanting human BM-derived mesenchymal stromal/stem cells (MSCs) would result in a "humanized" sheep HSC niche. MSCs are known to promote HSC engraftment and immune recovery after HSC transplantation, probably through the provision of hematopoietic supportive elements such as cytokines, matrix proteins and cell-to-cell contacts in the BM niche, while also modulating the immune response thereby promoting tolerance (18–24). Last, we tested the transplantation of HSCs with a larger fraction of $CXCR4^+$ cells in the $CD34^+$ population to evaluate the effect of the CXCR4 receptors in enhancing engraftment.

Methods

Cells for IUHSCT

Cord blood (CB) units deemed unfit for clinical use because of insufficient volume at Duke University Medical Center and BM from donors at the University of Nevada-Reno were collected at respective institutions after approval from their institutional review boards. All cells were cryopreserved until use. CB units were thawed and sorted before transplantation. $CD34^+$ cells were isolated through magnetic activated cell sorting (MACS) with the use of the CD34 MicroBead kit (Miltenyi Biotec, Auburn, CA, USA) according to manufacturer instructions. MACS-sorted populations for sheep transplantation typically were ~97% pure for $CD34^+$ by flow cytometry. MSCs used in these studies were generated from adult BM and met all criteria for MSC characteristics defined elsewhere (25). Cryopreserved MSCs were thawed ~2 weeks before use and expanded in culture. MSCs up to passage 7 were transplanted after digestion into single cells on the day of transplantation according to standard protocols (26,27).

Upregulation of CXCR4 receptors on HSCs

The chemokine receptor CXCR4 can be upregulated by hypoxia on PB cells (28). We simulated hypoxic conditions in a normoxic incubator (20% O_2, 37°C, 5% CO_2, humidified) through the inclusion of deferoxamine (DFX) (Sigma) in cell culture media as demonstrated by others (29). DFX inhibits the hydroxylation of a prolyl residue that is essential for the ubiquitination of Hypoxia-inducible factor 1-alpha, thereby mimicking hypoxia. A 60-mmol/L stock of DFX was made in Dulbecco's phosphate-buffered saline (Invitrogen, Carlsbad, CA, USA) and sterilized through a 0.22-μm filter. CB-derived cells were incubated in QBSF60 serum-free media (Atlanta Biologicals, Lawrenceville, GA, USA) containing a final concentration of 600 μmol/L DFX. Cell samples were analyzed by means of flow cytometry at 0, 24 and 48 h for the determination of cell surface expression of CD34 and CXCR4. Anti-human antibodies that were either fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated were purchased from BD Biosciences (San Jose, CA, USA).

Sheep transplantation procedures

Transplantation into fetal sheep was carried out at the University of Nevada-Reno Agriculture Experimental Station after receiving approval from our Institutional Animal Care and Use Committee. Whereas ultrasound-guided injections are considered minimally invasive, sheep must be anesthetized and immobilized to facilitate this procedure. Pregnant ewes on gestation days 53–75 after timed mating were fasted for 36 h, and water was also removed for the last 12 h. Anesthesia was induced initially with the use of Telazol (Zoetis, Kalamazoo, MI, USA) (2.2 mg/kg,