Characterisation of Placental Mesenchymal Stromal Cells and their Role in Cord Blood Transplantation.

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Research conducted in the Division of Haematology

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A thesis submitted for the degree of Doctor of Philosophy

Department of Medicine

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The University of Adelaide

March 2010

THE UNIVERSITY OF ADELAIDE
AUSTRALIA

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ACKNOWLEDGMENTS

I express sincere appreciation to Dr. Ian Lewis for giving me the chance to learn from him and to contribute to the interesting working undertaken to determine optimum method to enhance engraftment in cord blood transplantation. I am grateful to Professor L. B. To for the constructive criticism and helpful advice. I sincerely thank the staff of The Women's & Children's Hospital for providing cord blood unit and placenta samples. I appreciate all the help received from the members of the animal house of IMVS for providing and housing experimental mice.

I am thankful to the members of the Haemopoietic Expansion Laboratory Pamela Dyson, Rick Tocchetti and Sonia Young for providing timely help. I am thankful to the friendly members of the RDA laboratory Diana Salerno, Petra Neufing, Michelle Perugini, Sonya Diakiw, Chung Kok and Tessa Sadras who were always supportive. I appreciate the guidance received from Electra Iona and Karen Ambler (Molecular Pathology Laboratory), Tony Cambarei, Alan Bishop, Sandy Macintyre and Ghafar Sarvstani (Detmold Family Trust Cell Imaging Centre) and Dr. Peter Self (Adelaide Microscopy) who familiarised me with the instruments and techniques. I express my sincere thanks to the members of the Board for the Dawes Scholarship.

I thank the almighty God, my friends and family for being there to listen and provide the moral support.

Smita Hiwase

ABSTRACT

Cord blood transplantation (CBT) is an alternative to unrelated bone marrow transplantation in pediatric patients, while in adult patients the limited cell dose in cord blood (CB) unit results in delayed engraftment. To circumvent cell dose limitation, various methods have been investigated. Ex-vivo expansion of hematopoietic stem cells (HSC) is feasible but does not enhance engraftment due to HSC exhaustion. Use of double cord blood transplantation (DCBT) shows improved engraftment when compared to single unit transplantation, with median neutrophil engraftment at day 23 for recipients of DCBT, compared to 26-27 days for recipients of single cord blood transplantation (SCBT). However, engraftment is ultimately derived from single CB unit with reducing rates of chimerism seen up to day 100. The HSC share an intimate relationship with the BM microenvironment. Myeloablative conditioning using chemotherapy and radiotherapy may damage the microenvironment, which may contribute to delayed engraftment especially when the cell dose is limited. On the other hand mesenchymal stromal cells (MSC) could be used to restore this microenvironment. MSC are non-cycling cells having fibroblastic morphology, which express mesenchymal markers (CD73, CD105, CD90, CD29, and CD44), lack hematopoietic markers (CD45 and CD34) and differentiate into mesodermal lineages. MSC have been isolated from different tissues and show comparable characteristics to BM MSC. Recently, the placenta has been identified as a potential source of MSC and may have advantages to BM MSC due to a higher expansion potential and stronger immunosuppressive properties. This study has characterized cells obtained from the foetal aspect of the placenta and evaluated whether co-transplantation of placental MSC would enhance engraftment. Plastic adherent cells isolated from the placenta demonstrated typical characteristics of MSC. In 6 individual experiments, 4 cohorts of 24 NOD/SCID mice were evaluated. Cohort 1 received 5×10^4 $CD34^+$ cells from unit (U) 1 (SCBT); cohort 2 received 5×10^4 $CD34^+$ cells from U1+ 4×10^4

MSC (SCBT+MSC); cohort 3 received 2.5 $\times$ 10^4 $CD34^+$ cells from U1+ 2.5 $\times$ 10^4 $CD34^+$ cells from U2 (DCBT); cohort 4 received 2.5 $\times$ 10^4 $CD34^+$ cells from U1+ 2.5$\times$ 10^4 $CD34^+$ cells from U2+ 4 $\times$ 10^4 MSC (DCBT+MSC). Haemopoietic engraftment evaluated after 6-8 weeks was similar in recipients of SCBT and DCBT. MSC co-transplantation demonstrated enhanced engraftment in DCBT (51.8 ± 6.8% vs. 14.9 ± 6.5%; p=0.04) with an increased trend in SCBT (48.7 ± 7.7% vs. 17.5 ± 6.1%; p=0.07). In DCBT, co-transplantation of placental MSC reduced single CB unit dominance. Self-renewal capacity of engrafted HSC was assessed by serial transplantation in secondary recipients. Secondary recipients were infused with engrafted human cells from primary mice transplanted with or without MSC. In secondary transplantation of 17 evaluable mice, 13 engrafted at levels of 1-6.5%. Despite enhanced engraftment in primary mice, long-term engraftment capacity was unaltered with MSC co-transplantation. Furthermore, to study the potential mechanisms behind enhanced engraftment, eGFP transduced placental MSC and PKH-26 red labelled CB $CD34^+$ cells were traced in live mice. Imaging studies showed MSC migrated to the pelvic region and improved CB $CD34^+$ homing. Co-transplantation of placental MSC enhanced CB engraftment and may act by improving homing of $CD34^+$ cells.

PREFACE

Cord blood transplantation (CBT) in adults is restricted due to the limiting cell dose. Co-transplantation of bone marrow (BM) mesenchymal stromal cells (MSC) has been shown to enhance engraftment of CBT. Although BM MSC are well characterised are a rare population and their numbers decrease with age. Recently the placenta has been explored as a potential source of MSC. However there is limited literature available regarding its use in CBT. This study characterises the adherent cell population obtained from the placenta and investigates the role of these cells in CBT in non-obese diabetic/severely immuno-deficient (NOD/SCID) mice.

Chapter I

The literature review and the basis for setting the hypothesis of the study are discussed in detail. It also states the objectives to be achieved.

Chapter II

This chapter deals with the procedures describing the tissue selection, dissociation procedures and isolation of cells from the placenta. This is followed by investigating the phenotype of the tissue isolated primary cells and culture expanded adherent cells. The differentiation potential of these adherent cells has been demonstrated. The cell morphology, proliferation, and karyotype are also described. This chapter concludes that the adherent cells obtained from the foetal aspect of the placenta are non–haemopoietic progenitors, capable of self-renewal, differentiating into at least three mesenchymal lineages (bone, cartilage, fat) and expressing common MSC markers while lacking HSC markers. These cells also possess fibroblastic morphology demonstrating similar characteristics to BM MSC.

Chapter III

This chapter describes primary and serial transplantation studies in the NOD/SCID mice model. Here the method of isolating $CD34^+$ haemopoietic progenitor cells from CB unit and

cryopreservation is described, along with detailed explanations of the experiments conducted on NOD/SCID mice. This chapter deals with results from 6 individual primary transplantation experiments, in which 4 cohorts were evaluated. Cohort 1 received 5×10^4 $CD34^+$ cells from unit (U) 1 (SCBT); cohort 2 received 5×10^4 $CD34^+$ cells from U1+ 4×10^4 MSC (SCBT+MSC); cohort 3 received 2.5×10^4 $CD34^+$ cells from U1+ 2.5×10^4 $CD34^+$ cells from U2 (DCBT); cohort 4 received 2.5×10^4 $CD34^+$ cells from U1+ 2.5×10^4 $CD34^+$ cells from U2+ 4×10^4 MSC (DCBT+MSC). Co-transplantation of MSC from the placenta demonstrated enhanced engraftment in DCBT with an increased trend in SCBT. Moreover, in DCBT, co-transplantation of placental MSC reduced single CB unit dominance. This chapter also describes the serial transplantation experiments in secondary recipients. It demonstrated that despite enhanced engraftment in primary mice, long-term engraftment capacity was unaltered with co-transplantation of the placental MSC.

Chapter IV

There is limited published literature addressing the homing of the MSC.This chapter describes a live imaging assay to study the migration of placental MSC to delineate the mechanism of HSC supportive role. This chapter explains in detail the gene manipulation of the placental MSC to transduce green fluorescent protein which was imaged into the mice after IV injections at various intrvals. Co-transplantation of placental MSC enhances haemopoietic engraftment by increasing homing and retention of the CB $CD34^+$ cells to the haemopoietic site. This chapter also shows that the preincubation of MSC with anti-CXCR4 antibody, neither inhibited its migration to the pelvic region nor altered the engraftment of CB $CD34^+$.

Chapter V

This chapter summarizes the observations and findings conducted during the research project. Placental MSC demonstrate similar morphological, immunophenotypical and differentiation characteristics to BM MSC. This study has demonstrated that at equivalent cell

dose single and DCBT leads to similar engraftment. There was improved engraftment in mice that received placental MSC in both settings. The co-transplantation of MSC leads to reduced dominance of single CB unit in DCBT. How these results fit in with the work of other researchers is also discussed in detail. This chapter also summarises the attempts to understand the mechanism of MSC homing to BM by the blocking of CXCR4 by T140 peptide. Furthermore, limitations of the study are stated, and the direction for future work described.

ABBREVIATIONS

ANCs	absolute neutrophil count
AHSCT	allogeneic haemopoietic stem cell transplantation
Bp	base pairs
BFU-E	burst forming unit erythroid
BM	bone marrow
BMP	bone morphogenetic protein
BMT	bone marrow transplantation
BSA	bovine serum albumin
BSC	bio-safety cabinet
CB	cord blood
CBT	cord blood transplantation
CD	cluster of differentiation
CFU	colony forming unit
CFU-F	colony forming unit –fibroblast
CFU-GM	colony forming unit granulocytes and macrophage
CFU-GEMM	colony forming unit granulocyte, erythroid, macrophages and megakaryocyte
CPD	cumulative population doubling
DCBT	double cord blood transplantation
DMEM	Dulbecco's minimum essential media
DMSO	dimethyl sulfoxide
DNA	deoxy ribonucleic acid.
ECM	extra cellular matrix
EDTA	ethylenediamine tetra acetic acid
EPO	erythropoietin

ESC	embryonic stem cells
FACS	fluorescence activated cells sorting
FCS	foetal calf serum
FITC	fluorescein isothiocyanate
FSC	forward scatter
G-CSF	granulocyte-colony stimulating factor
GFP	green fluorescent protein
GM-CSF	granulocyte –macrophage-colony stimulating factor
GVHD	graft versus host disease
Gy	Gray
HBSS	Hank's balanced salt solution
HEK	human embryonic kidney
HGF	haemopoietic growth factors
HLA	human leucocyte antigen
HSC	haemopoietic stem cells
HSCT	haemopoietic stem cell transplantation
Ig	immunoglobulin
IL-1	interleukin-1
IMDM	Iscove's minimum defined media
IMVS	Institute of Medical and Veterinary Sciences
ITS	insulin transferring selenous
ISCT	International Society for Cellular Therapy
IVIS	in-vivo imaging system
LB	Luria broth
MACS	magnetic activated cell sorting

MNCs	mononuclear cells
MoAB	monoclonal antibody
MPP	multi potent progenitors
MRI	magnetic resonance imaging
MSC	mesenchymal stromal cells
MSCV	murine stem cell virus
MUD	matched unrelated donor
NOD-SCID	Non obese diabetic-severe combined immune deficient
O_2	oxygen
PB	peripheral blood
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PC5	phyco erythrin-cyanine 5
PD	population doubling
PDT	population doubling time
PE	phyco erythrin
PDGF	placental-derived growth factor
REC	research ethics committee
RO	reverse osmosis
ROI	region of interest
SC	sideward scatter
SCBT	single cord blood transplantation
SCT	stem cell transplantation
SCF	stem cell factor
SDF-1	stromal derived factor-1

SCS	sodium chloride /sodium citrate
STR	short tandem repeats
TEM	transendothelial migration
TNC	total nucleated cells
TRM	transplantation related morbidity
TPO	thromboprotein
UBMT	unrelated bone marrow transplantation
UCB	umbilical cord blood
URD	unrelated donor
URDT	unrelated donor transplantation
VCAM	vascular adhesion molecule
VLA-4	very late antigen -4
VLA-5	very late antigen-5

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PUBLICATION

Hiwase SD, Dyson PG, To LB, Lewis I. Co-transplantation of placental mesnchymal stromal cells enhances single and double cord blood engraftment in non obese diabetic/severe combined immune deficient mice. Stem Cells 27 (9):2293-300.

CONFERENCE PRESENTATIONS

International conference

An oral presentation at the European Bone Marrow Transplantation (EBMT) 2008 Congress held in Florence, Italy, 30 March - 2 April, 2008. Co-transplantation of placental derived mesenchymal stromal cells produces superior engraftment of umbilical cord blood compared to double unit umbilical cord blood transplantation. Smita. Hiwase, Pamela. Dyson, S. Young, L.B. To, Ian. Lewis* IMVS (Adelaide, AU)

National conferences

An oral presentation "Placental Derived Mesenchymal Stromal Cells Enhances Umbilical Cord Blood Engraftment" at the conference HAA-2007 (HSANZ/ANZSBT/ASTH-2007 ASM) held 14-17 October 2007- at Gold Coast Convention & Exhibition Centre, Surfers Paradise, Queensland.

2008 Postgraduate Research Expo

Poster presentation "Determination of the optimal technique to increase stem cell dose for cord blood transplantation" held at the National Wine Centre during 2008 Postgraduate Research Expo conducted by the Faculty of health Sciences.

Division of Haematology Seminar Presentation

An oral presentation “Determination of the optimal technique to increase stem cell dose for cord blood transplantation” held at IMVS August 2007.