ALPHA-L-IDURONIDASE TRANSDUCED MESENCHYMAL STEM CELLS AS A THERAPY FOR THE TREATMENT OF CNS DEGENERATION IN MUCOPOLYSACCHARIDOSIS TYPE I MICE

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Abstract

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disorder that is characterised by a deficiency in the α-*L*-iduronidase (IDUA) enzyme, resulting in the accumulation of undegraded heparan sulphate and dermatan sulphate glycosaminoglycans (gags) within the lysosome of nearly every cell. MPS I is a multi-tissue and organ disease, presenting with profound mental retardation and skeletal abnormalities. Haematopoietic stem cell (HSC) transplant and enzyme replacement therapy, two clinically available forms of treatment, are able to correct the soft tissue aspects of MPS disease, but have had a limited effect on the more complex skeletal and neurological symptoms. Stem cell therapy utilizing mesenchymal stem cells (MSC) has the potential to overcome these limitations due to their ability to differentiate into cells that are the major sites of MPS pathology.

MSCs naturally produce and secrete significantly higher levels of multiple MPS enzymes than HSCs *in vitro* and can be engineered to over-express multiple MPS enzymes using a lentiviral system. MSCs were found to secrete up to 5,559 fold greater IDUA enzyme after lentiviral transduction *in vitro*, suggesting a greater potential to cross-correct MPS pathology than HSCs. Lentiviral transduction was stable and persistent *in vitro*, and over-expression of MPS enzyme did not affect MSC *in vitro* differentiation down osteogenic, adipogenic, chondrogenic or neurogenic lineages.

Systemically administered human derived MSCs distribute widely, to multiple MPS I affected organs, including the brain, and persist *in vivo* for at least two months post administration. Significantly elevated brain and serum IDUA activity was observed two and six months post administration, respectively, and was associated with sustained functional improvements in

neuromuscular strength, motor control, coordination and spatial learning. MSCs were found to limit astroglial activation and modulate brain inflammatory gene expression of *Cd68*, *Gfap* and *Tnf in vivo*. Vertebral body width also returned towards normal. However, no improvement in gag storage or elevations of IDUA were observed in other tissues.

For the first time, this thesis has investigated the biochemical and behavioural changes due to *i.v.* administered hMSCs in MPS I mice. This thesis demonstrates that MSCs can exert added neurological improvements in MPS I pathology through exhibiting a combined effect between their superior enzyme secretion and anti-inflammatory effects. While minimal changes were noted in MPS I associated somatic pathology, MSCs could be administered in combination with already implemented ERT and/or BMT, which have both shown resolution in patients stored gag, therefore providing additional clinical benefits to MPS I children.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Abbreviations

IDUA/*IDUA*/*Idua*	α-L-iduronidase
α-MEM	Minimum essential medium (α modified)
AAV	Adeno-associated-virus
BMT	Bone marrow transplant
BBB	Blood brain barrier
Cd68	CD68 antigen
CNS	Central nervous system
Ccl3	Chemokine (C-C motif) ligand 3
CS	Chondroitin sulphate
CycpA	Cyclophilin A
DAPI	4’,6-diamidino-2-phenylindole
DS	Dermatan sulphate
DMEM	Dulbecco’s modified eagle medium
ERT	Enzyme replacement therapy
FCS	Foetal calf serum
FACS	Fluorescence-activated cell sorting
FITC	Fluorescein isothiocyanate
GT	Gene therapy
GFAP/*Gfap*	Glial fibrillary acidic protein
gag(s)	Glycosaminoglycan(s)

GvHD	Graft-versus-host disease
Hep	Heparin
HSC(s)	Haematopoietic stem cell(s)
HS	Heparan sulphate
hBM	Human derived bone marrow
hDP	Human derived dental pulp
HA	Hyaluronan
i.v.	Intravenous
Ifnγ	Interferon gamma
Il1β	Interleukin 1 beta
KS	Keratan sulphate
M6P	Mannose-6-phosphate
M6PR	Mannose-6-phosphate receptor
MSC(s)	Mesenchymal stem/stromal cell(s)
MPS	Mucopolysaccharidosis
MPS I	Mucopolysaccharidosis type I
PBS	Phosphate buffered saline
SDT	Substrate deprivation therapy
SHIRPA	SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment
SUMF1	Sulphatase modifying factor 1
Tgfβ1	Transforming growth factor beta 1

Tnfα	Tumor necrosis factor alpha