Mesenchymal Stem Cells for the Treatment of Myocardial Infarction-Induced Ventricular Dysfunction.

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James David Richardson

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Personal Bibliography

Full-text publications arising directly from work conducted toward this thesis:

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2. Cardiac magnetic resonance, transthoracic and transoesophageal echocardiography: a comparison of in vivo assessment of ventricular function in rats. **Richardson JD**, Bertaso AG, Frost L, Psaltis PJ, Carbone A, Koschade B, Wong DT, Nelson AJ, Paton S, Williams K, Azarisman S, Worthley MI, Teo KS, Gronthos S, Zannettino AC, Worthley SG. Lab Anim. 2013 Oct; 47(4):291-300. doi: 10.1177/0023677213494373
3. Impact of timing and dose of mesenchymal stromal cell therapy in a preclinical model of acute myocardial infarction. **Richardson JD**, Bertaso AG, Psaltis PJ, Frost L, Carbone A, Paton S, Nelson AJ, Wong DT, Worthley MI, Gronthos S, Zannettino AC, Worthley SG. J Card Fail. 2013 May;19(5):342-53. doi: 10.1016/ j.cardfail.2013.03.011. PMID: 23663817 [PubMed - in process]
4. Incremental Benefits of Repeated Mesenchymal Stromal Cell Administration Compared to Solitary Intervention in a Preclinical Model of Myocardial Infarction. **JD Richardson**, PJ Psaltis, L Frost, A Carbone, S Paton, AG Bertaso, AJ Nelson, DT Wong, MI Worthley, S Gronthos, A Zannettino, SG Worthley. Cytotherapy 2013

Manuscript in preparation:

5. **JD Richardson**, S Paton, PJ Psaltis, A Carbone, L Frost, S Gronthos, ACW Zannettino, SG Worthley. Prospectively-Isolated Hypoxic-Conditioned Mesenchymal Stem Cells Have Superior in vitro Capacity and Significantly Attenuate Myocardial Infarction-Induced Ventricular Dysfunction.

Abstracts arising from the Thesis:

1. **Richardson JD**, Frost F, Bertaso AG, Carbone A, Paton S, Nelson AJ, Psaltis PJ, Wong DT, Worthley MI, Gronthos S, Zannettino ACW, Worthley SG. Immediate Mesenchymal Stem Cell Therapy Provides Greater Attenuation of Myocardial Injury Than Deferred Treatment in Rats After Acute Myocardial Infarction. Heart, Lung & Circulation 2012; S21: S1-S142 (**CSANZ YIA finalist**)

2. **Richardson JD**, Paton S, Frost F, Carbone A, Bertaso AG, Nelson AJ, Psaltis PJ, Wong DT, Worthley MI, Gronthos S, Zannettino ACW, Worthley SG. Prospectively Isolated, Hypoxic-Preconditioned Mesenchymal Stem Cells Significantly Attenuate Myocardial Infarction-Induced Ventricular Dysfunction In Rats. Heart, Lung & Circulation 2012; S21: S1-S142.

3. **Richardson JD**, Frost F, Bertaso AG, Carbone A, Paton S, Nelson AJ, Psaltis PJ, Wong DT, Worthley MI, Gronthos S, Zannettino ACW, Worthley SG. Sequential Mesenchymal Stem Cell Interventions Produce Greater Myocardial Repair Than Solitary Treatment in Rats After Acute Myocardial Infarction. Heart, Lung & Circulation 2012; S21: S1-S142.

4. **Richardson JD**, Bertaso AG, Koschade B, Wong DT, Williams K, Frost F, Carbone A, Paton S, Nelson AJ, Psaltis PJ, Worthley MI, Teo KS, Gronthos S, Zannettino ACW, Worthley SG. Cardiac Magnetic Resonance, Transthoracic and Transoesphageal Echocardiography: A Comparison of In Vivo Ventricular Function Assessment in Rats. Heart, Lung & Circulation 2012; S21: S143-S316.

5. **Richardson JD**, Bertaso AG, Wong DT, Frost F, Carbone A, Nelson AJ, Psaltis PJ, Paton S, Koschade B, Williams K, Worthley MI, Teo KS, Gronthos S, Zannettino ACW, Worthley SG. Assessment of Regional Myocardial Function in Rats using 1.5T Cardiac Magnetic Resonance Imaging. Heart, Lung & Circulation 2012; S21: S143-S316.

Presentations & Prizes arising from the Thesis:

- Cardiac Society (CSANZ) 2012 Oral Presentations:
    - "Immediate Mesenchymal Stem Cell Therapy Provides Greater Attenuation of Myocardial Injury Than Deferred Treatment in Rats After Acute Myocardial Infarction."
    - "Sequential Mesenchymal Stem Cell Interventions Produce Greater Myocardial Repair Than Solitary Treatment in Rats After Acute Myocardial Infarction"
- Cardiac Society (CSANZ) 2012 Poster Presentations:
    - "Prospectively Isolated, Hypoxic-Preconditioned Mesenchymal Stem Cells Significantly Attenuate Myocardial Infarction-Induced Ventricular Dysfunction In Rats".
    - "Cardiac Magnetic Resonance, Transthoracic and Transoesphageal Echocardiography: A Comparison of In Vivo Ventricular Function Assessment in Rats".
    - "Assessment of Regional Myocardial Function in Rats using 1.5T Cardiac Magnetic Resonance Imaging"
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Abbreviations

αMEM	Alpha modification of Eagle's medium	ESV	End-systolic volume
µg	Microgram	FACS	Fluorescence-activated cell sorting
µL	Microlitre	FCS	Foetal calf serum
µm	Micrometre	FITC	Fluorescein isothiocyanate
µM	Micromolar	FS	Fractional Shortening
ANOVA	Analysis of Variance	g	Gram
AUC	Area under the curve	GFP	Green fluorescent protein
BM	Bone marrow	HBSS	Hanks' balanced salt solution
BMMNC	Bone marrow mononuclear cells	HGF	Hepatocyte growth factor
CD	Cluster of differentiation	HR	Heart rate
cDNA	Complementary deoxyribonucleic acid	IGF	Insulin-like growth factor
CFU-F	Colony forming units-fibroblast	IHD	Ischaemic heart disease
CI	Confidence interval	IL	Interleukin
CMR	Cardiac magnetic resonance	PBS	Phosphate buffered saline
CXCL12	Stromal cell-derived factor 1 (SDF-1)	LV	Left ventricle (or left ventricular)
DMEM	Dulbecco's modification of Eagle's medium	MACS	Magnetic-activated cell sorting
DMSO	Dimethyl sulphoxide	mg	Milligram
DNA	Deoxyribonucleic acid	MI	Myocardial Infarction
EC	Endothelial cells	SD	Standard deviation
EDD	End-diastolic dimension	MPC	Mesenchymal precursor cells
EDTA	Ethylenediaminetetraacetic acid	MSC	Mesenchymal stromal/stem cells
EDV	End-diastolic volume	n	Sample number
EF	Ejection Fraction	TGF	Transforming growth factor
ELISA	Enzyme-linked immunosorbent assay	VEGF	Vascular endothelial growth factor
EPC	Endothelial progenitor cells	v/v	Volume per volume
ESD	End-systolic dimension	w/v	Weight per volume

Thesis Abstract

Despite current treatment options, cardiac failure after myocardial infarction (MI) is associated with significant morbidity and mortality so highlighting a compelling clinical need for novel therapeutic approaches. Based on promising pre-clinical data, stem cell therapy has been suggested as a possible therapeutic strategy. Early studies largely utilised autologous bone marrow cells with only modest benefits observed in clinical trials. Of the alternative candidate cell types evaluated, mesenchymal stromal/stem cells (MSCs) have shown promise, however their clinical application for mainstream cardiovascular use is currently hindered by several important limitations. Consequently, this has prompted intense efforts to advance the therapeutic properties of MSCs through cell optimisation strategies.

Allogeneic sources of MSC appear to hold several important advantages over autologous bone marrow/BM mononuclear cells (BMMNC); (1) MSC can be derived from young, healthy donors thereby enhancing the absolute yield and functional biology of MSCs; (2) The cell product could be prepared well ahead of time, so making very early MSC treatment feasible, e.g. after primary percutaneous intervention, when myocardium remains viable; (3) MSC could be optimised to potentially advance their therapeutic efficacy.

The studies described in this thesis utilised all of the above features to address the primary aims of:

1. Reviewing the literature and writing a review regarding the optimisation of the cardiovascular therapeutic properties of MSC.
2. Develop an allogeneic MSC population optimised by the novel combination of prospective-isolation enrichment and hypoxic preconditioning. Furthermore, evaluate the in vivo function of optimised MSC compared to conventional plastic-adherent isolation of MSC (PA-MSC).

3. Develop a reliable non-invasive assessment of rat ventricular function using 1.5T cardiac magnetic resonance and evaluate this modality against conventional methods (transthoracic echocardiography) and novel modalities in rats (transoesophageal echocardiography).
4. Explore the impact of the timing of MSC intervention and cell dose after MI, now that immediate cell intervention is feasible clinically and these factors have not previously been investigated.
5. Explore the potential benefits of immediate and deferred MSC treatment after MI, two very different time points – a novel concept.

An allogeneic source of MPCs was derived from donor rat bone marrow. In contrast to conventional plastic-adherent isolation of MSC, an enriched and optimised MSC population prepared by prospective isolation of immature MPCs (via a CD45 immunodepletion step) and hypoxic preconditioning was established. In cell-based experiments, optimised MSC were compared to same-donor plastic-adherence isolated MSC and demonstrated superior in-vitro differentiation and colony forming capacity than PA-MSC.

To evaluate the effects of MSC treatment after MI in rats, highly accurate and reproducible imaging techniques are required. Cardiac magnetic resonance (CMR) is widely regarded as the gold standard modality, however the use of standard 1.5T “clinical” MR scanners in rodents has only been achieved by a handful of investigators worldwide and none have used contemporary MR techniques. CMR was then evaluated against conventional imaging modalities (transthoracic echocardiography) and novel methods in rats (transoesophageal echocardiography).

Allogeneic MSC permits immediate treatment, previously impossible with autologous stem cells, therefore this potentially important variable (timing) was assessed. Myocardial infarction was induced by ligation of the left anterior descending artery in rats. Optimised

MSC were then injected into the myocardium either immediately after MI or one week later, at one of two cell doses. This study provided an innovative comparison of these clinically relevant time points and demonstrated value at both times. Furthermore, greater efficacy was observed with immediate treatment, which displayed high sensitivity to MSC dose, with benefits largely localised to the infarct territory. Deferred treatment, though less effective, was not dose dependant and primarily influenced non-infarct myocardium.

Given the disparate, yet beneficial effects, of immediate and deferred MSC intervention the benefit of combining MSC treatment at both time points was investigated. Again, this was undertaken in the rat model of MI, with CMR determination of ventricular function. This novel study showed clinically relevant improvements in LV function and confirmed the differential distribution of MSC repair according to timing of cell intervention.

In summary, the studies described in this thesis provide new evidence outlining the merits of prospective isolation and hypoxic preconditioning of MSC. Furthermore they demonstrate the reparative effects of these cells and provide novel insights into the significance of timing of MSC intervention on efficacy and mode/distribution of effect, which can be further augmented through treatment both time points after MI.