ESA Ken Wynne Postdoctoral Research Award
2016 final report

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I am grateful to the Endocrine Society of Australia for its generous support of my research into the cardiovascular impact of mineralocorticoid receptor antagonists in **"Finding a novel role for the mineralocorticoid receptor in monocytes and its practical applications in heart failure"**.

Introduction

The mineralocorticoid receptor (**MR**) is a steroid hormone receptor that is classically known to regulate sodium and potassium flux in the renal tubules. However it is also expressed in non-epithelial tissues including the heart where inappropriate MR activation leads to tissue inflammation, fibrosis and ultimately heart failure. Heart failure is a major public health problem with 30,000 patients diagnosed in Australia each year and a 20% increase in death from 2006 to 2011. There is clearly an urgent need for new insights and novel strategies to aid its management.

MR antagonists (**MRA**), spironolactone and eplerenone, have been shown to reduce the morbidity and mortality of cardiac failure in landmark clinical trials. However, the use of MRA is limited by renal side effects, especially hyperkalemia. An understanding of the cellular mechanisms of MRA activity may help design a tissue-selective MRA with less side effects. Recent transgenic mice models has shown that activation of the MR specifically in macrophages is a key mediator of cardiac inflammation and fibrosis. The preclinical data shows that macrophage-specific deletion of the MR and prevention of macrophage recruitment in transgenic mice models ameliorates cardiac inflammation and fibrosis and is thus cardio-protective.

To translate this body of work into a clinical setting, we need to study macrophages in patients with heart failure. However, as macrophages reside in tissues and heart tissue is difficult to obtain, we chose to study their precursors, the monocytes, as a surrogate marker. In cardiac failure, circulating monocytes produce proinflammatory cytokines which lead to an imbalance between cardiac wound healing and pathological tissue inflammation and fibrosis. The monocytes involved display functional plasticity with pro- or anti-inflammatory phenotypes. The features of monocytes which predominate in chronic heart failure have been characterised as molecular signatures using gene expression analysis in both animal models and patients with heart disease. Monocytes also express the MR; it is therefore plausible that their gene expression profile can be evaluated as a biological readout of pathological MR activation in heart failure.

Hypothesis:

We hypothesise that MRA therapy in patients with heart failure will lead to an alteration in the gene expression profile of peripheral monocytes, which will reveal a novel role of the MR in human monocyte function.

Aims:

1. Determine the effect of MRAs on monocyte gene expression in patients with heart failure;
2. Characterise these altered genes as novel MR target genes;
3. Demonstrate a correlation between changes in the gene expression profile of monocytes and markers of cardiac structure and function.

Methods:

We prospectively recruited patients with recently diagnosed heart failure and reduced left ventricular ejection fraction who have an indication for MR antagonist treatment with either spironolactone or eplerenone from the Heart Failure Clinic at Monash Health. Enrolled patients had their blood samples were collected before and after 3 months of spironolactone treatment. Cardiac function and patient functional capacity were also assessed. Monocyte phenotype was analysed using flow cytometry and alteration in monocyte gene expression following spironolactone treatment determined by microarray. Validation of eight selected genes was performed using reverse transcriptase polymerase chain reaction (RTPCR).

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Results:
Due to significant difficulties in recruitment and patient eligibility, only 4 patients with chronic heart failure could be analysed for this pilot study. Paired blood samples showed that there was no significant change in levels of sodium, potassium, creatinine, eGFR, white cell count, monocyte count, lymphocyte count or CRP before and after spironolactone treatment. Whilst flow cytometry did not reveal a change in monocyte phenotype, a > 2-fold change ($P < 0.0001$) in 217 genes was identified using microarray and multiple testings correction by the Westfall-Young permutation procedure. Many of the genes identified are implicated in cell signalling, metabolism, the immune system, extracellular matrix organisation, and the cell cycle. Of these, 32 genes had features relevant to cardiovascular disease, monocytes, inflammation, or fibrosis in peer-reviewed articles. Notably, 5 of the down-regulated genes were validated using RTPCR and represented meaningful changes based on published literature, consistent with the cardiovascular protection afforded by MR blockade. The identified genes include:

- ARG1(11.2-fold change, $p < 0.0001$) = arginase 1; expression in mouse macrophages increased post-myocardial infarction (MI) and associated with cardiac fibrosis;
- CXCL8 (23-fold, $p < 0.0001$) = CXC motif chemokine ligand 8; level raised post-MI in humans;
- DEFA4 (83-fold, $p < 0.0001$) = defensin alpha 4; up-regulated in acute inflammation in humans;
- HSD11B1 (5.2-fold, $p < 0.0001$) = hydroxysteroid 11-beta dehydrogenase 1; knockout improves ventricular function following MI in mice; and
- NAMPT (2-fold, $p < 0.002$) = nicotinamide phosphoribosyltransferase; level increased in patients with coronary artery disease.

Whilst the heart failure study is ongoing in an expanded population of patients, it provides proof of concept that monocyte gene expression analysis can be correlated with MR activity *in vivo*.

Future directions
More patients need to be recruited to consolidate the preliminary data. Furthermore, clinical data from a longer period of follow up is required so that gene expression changes can be correlated with heart failure parameters. This will permit the custom design of a platform for monocyte gene profiling as a novel, non-invasive biomarker of heart failure severity and treatment efficacy. Genes whose expression changed in response to MR antagonism will be validated as novel MR target genes *in vitro*. MR-containing cell lines, including a macrophage cell line, will be treated with MR agonist and antagonist, and the expression of our genes of interest will be evaluated. The identification of key MR- regulated genes which positively respond to MR antagonist treatment may represent new targets in the development of tissue-selective heart failure therapeutics.

Publications relating to this award
Nickson C, Yang J and Young M. Mineralocorticoid receptor signalling in heart failure: from experimental animals to the clinic. (in preparation)
Second manuscript will be drafted by September 2016 with updated results from an expanded cohort of patients.

Students relating to this award
Dr Clare Nickson – BMedSci (hon – H1)
Mr Jaynen Yong – in progress

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