Post-doctoral fellowship – FINAL REPORT

Finding a novel role for the mineralocorticoid receptor in monocytes and its practical applications

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Background

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 which are not involved in electrolyte transport. Following the observation by Brilla et al in 1992 that aldosterone causes interstitial cardiac fibrosis, there has been a growing body of literature to show that inappropriate MR activation in the heart 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. The number of deaths from heart failure is also growing, with a 20% increase from 2006 to 2011 such that its prognosis is poorer than common forms of cancer. 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 benefit cardiac failure in landmark clinical trials. In addition to improving heart failure, MRAs have been shown to ameliorate a range of other conditions, including hypertension-related left ventricular hypertrophy, coronary circulatory dysfunction in diabetes mellitus, obesity-associated cardiac diastolic dysfunction and western diet-induced arterial stiffening. Aside from cardiovascular disease, spironolactone has also been shown to improve microvascular endothelial function and reduce proteinuria in patients with chronic kidney disease or diabetic nephropathy. The pleiotropic benefits of MRAs reflect their ability to block oxidative stress, inflammation and fibrosis induced by inappropriate MR activation in the heart, vasculature and kidney. However, use of MRA is limited by renal side effects, including hyperkalemia, in up to 10% of patients. Furthermore, the exact mechanism of the observed protection remains unclear.

Our laboratory, led by Dr Morag Young, has had a twenty-year track record in studying the role of MR in cardiovascular disease. The development of transgenic mice models has allowed us to examine the role of the MR in a cell-specific manner in heart failure. Her work has demonstrated that activation of the MR specifically in macrophages is a key mediator of cardiac inflammation and fibrosis. This 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 have chosen to study peripheral monocytes, which are precursors of macrophages, in patients with heart failure as it is not feasible to obtain human cardiac macrophages.

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.

Hypothesis

We hypothesise that mineralocorticoid receptor (MR) antagonist 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.

Research Questions

1. Do MR antagonists modulate the gene expression profile of monocytes in heart failure?
2. Can monocyte profiles be used as biomarkers of heart failure severity and response to MR antagonist therapy?
3. Do the altered monocyte genes represent novel MR target genes that may be utilised in the design of tissue-selective MR modulators?

Methods

This is a prospective study of peripheral blood monocytes in patients with recently diagnosed heart failure who have an indication for MR antagonist treatment with either spironolactone or eplerenone. They are recruited from the MonashHeart Early Intervention Systolic Heart Failure Clinic at Monash Health, after initial stabilisation on baseline heart failure medications. Patients with underlying inflammatory diseases or ongoing changes in medications other than the MR antagonist are excluded.

Enrolled patients are examined clinically (history, examination, NYHA functional class), biochemically (serum biomarkers including FBE, UEC, LFT and CRP as well as renin and aldosterone to exclude underlying primary aldosteronism) and with echocardiography for the assessment of baseline cardiac structure and function. An additional blood sample for monocyte extraction is processed in my host laboratory using Histopaque Ficoll gradient centrifugation and purification by negative magnetic labelling (Miltenyi). RNA is extracted for gene profiling using a high throughput PCR array card that allows the simultaneous measurement of 60,000 gene targets (SurePrint G3 Human Gene Expression Microarrays; MHTP Molecular Genetics platform).

A MR antagonist (either spironolactone or eplerenone) is commenced at the end of the first visit. Enrolled patients are followed prospectively and have their examination repeated at 3 months: both clinically, biochemically and with surveillance echocardiography, for the assessment of improved cardiac structure, function and functional capacity. Their monocyte gene expression profile is re-analysed to identify genes whose expression differed before and after MR antagonist treatment.

Results

Microarray analysis of paired monocyte/macrophage samples from 6 patients collected before commencement of spironolactone and after 3 months of treatment identified 137

differentially expressed genes, 87 upregulated and 50 downregulated (fold-change >1.5, $p<0.05$).

RT-PCR was performed to validate changes in mRNA expression of the candidate genes identified by microarray. Of all genes investigated, only CAV1 was significantly different after spironolactone treatment (Mean diff.= -0.619, $P<0.01$). CAV1 expression decreased by a similar proportion in concordance with the microarray results.

THP1 human leukocyte cells were treated with aldosterone (aldo), aldosterone plus spironolactone (aldo+spiro) or aldosterone plus eplerenone (aldo+eple). mRNA levels for each target gene were compared to untreated controls to determine if candidate genes were directly MR responsive. In contrast to values reported for human heart failure monocyte gene expression, no statistically significant changes in CAV1 were detected in THP1 cells in response to any treatment, however visual inspection suggests that the aldo+eple group has a lower expression of CAV1.

Discussion

This study identified changes in the expression of 137 genes that may be associated with MR antagonism in the monocytes of heart failure patients. 87 of these genes were upregulated in response to MR antagonism, whilst 50 were downregulated. CAV1 was identified as a gene downregulated by spironolactone treatment, confirmed by RT-PCR, Changes in CAV1 expression could not be replicated in cell culture experiments, although a larger sample size is needed.

The main limitation of the study is a high level of heterogeneity in the genetic background and lifestyle factors of the patient cohort. A review of the literature reveals that the inter-individual differences in patient characteristics observed in this study can influence monocyte gene expression.

More patients need to be recruited and grouped into more homogenous cohorts 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.

Future directions

CAV1 and other genes whose expression changed in response to MR antagonism will be investigated for their response to MR activation. MR-containing cell lines, including primary macrophages, will be treated with MR agonist and antagonist, and the expression of our genes of interest will be evaluated. The identification of key MR target genes which positively respond to MR antagonist treatment will aid the development of more selective heart failure therapeutics.

To extend our research findings, these gene markers can also be analysed in other conditions where MR antagonists offer benefit, such as diabetic nephropathy and hypertensive heart disease. A simple test of monocytes in affected patients before and after treatment with MR antagonists may help to dissect the molecular pathways involved in the pathogenesis and treatment of these diseases.