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Molecular mechanisms of inflammation-induced cardiac dysfunction in a rat model of rheumatoid arthritis

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ABSTRACT

Heart failure with a preserved ejection fraction (HFpEF) accounts more than 50% of all heart failure cases. In comorbid conditions associated with HFpEF such as obesity, hypertension and diabetes mellitus, chronic systemic inflammation has been proposed as the underlying causal mechanism of cardiac dysfunction. However, confounding factors associated with these comorbid conditions preclude the understanding of the independent contribution of inflammation to the molecular mechanisms underlying the different phenotypes of HFpEF. This thesis comprises a series of animal and cell culture studies designed to advance our understanding of the impact of systemic inflammation on the cellular pathways involved in the development of HFpEF.

Left ventricular (LV) diastolic dysfunction is the primary abnormality associated with HFpEF. Both passive LV stiffness and active processes contribute to LV diastolic dysfunction. Cardiac fibrosis contributes to passive LV stiffness. Independent of confounding factors, the role of inflammation in cardiac fibrosis and its contribution to LV diastolic dysfunction remain uncertain. In the first study, using a model of collagen induced arthritis (CIA), I determined the role of systemic inflammation, and the effects of inhibiting circulating tumour necrosis factor alpha (TNF-α) with etanercept, on the mRNA expression of genes associated with myocardial fibrosis and markers of LV geometry and function measured by echocardiography in 70 Sprague-Dawley rats. I showed that after inoculating rats with collagen, inflammation caused an increase in the LV relative gene expression of pro-fibrotic genes including collagen I (*Col1*, $p < 0.0001$), lysyl oxidase (*LOX*, $p = 0.002$), and transforming growth factor β (*TGFβ*, $p = 0.03$) compared to control rats, consistent with the increased relative wall thickness, a marker of LV concentric remodelling. I also showed that the relative gene expression of matrix metalloproteinase 2 (*MMP2*), which degrades extracellular matrix components and contributes to collagen turnover, was increased after exposing rats to inflammation compared to controls ($p = 0.04$). Inhibiting circulating TNF-α prevented the inflammation-induced increased expression of pro-fibrotic genes, collagen turnover genes and development of LV concentric remodelling. Despite preventing the increased profibrotic gene expression and concentric remodelling, inhibiting circulating TNF-α did not prevent the inflammation-induced LV diastolic dysfunction. These data suggest that inflammation-induced fibrosis and dysregulated LV extracellular matrix remodelling contributes to LV concentric remodelling, and this process is mediated by TNF-α. However, inflammation-induced LV diastolic dysfunction is likely independent of myocardial fibrosis.

Besides changes in the extracellular matrix composition, changes in the cardiomyocyte spring protein, titin, also contribute to LV passive stiffness and LV diastolic dysfunction. Therefore, in the second study I assessed the role of inflammation and inhibiting circulating TNF-α, on the genes regulating the molecular

pathways involved in titin phosphorylation in 73 Sprague-Dawley rats exposed to CIA and to TNF-α inhibition. LV relative mRNA expression of vascular cell adhesion molecule 1 (*VCAM1*, $p = 0.007$), pentraxin 3 (*PTX3*, $p < 0.0001$), and inducible nitric oxide synthase (*iNOS*, $p = 0.0003$) were increased in CIA rats compared to controls, indicating local microvascular inflammation and immune cell activation in response to collagen inoculation. In turn, the relative LV gene expression of *sGCα2* and *sGCβ2* were decreased, suggesting downregulation of the nitric oxide-regulated soluble guanylate cyclase-cyclic guanosine monophosphate (NO-sGC-cGMP) signalling pathway, implicated in titin phosphorylation. Inhibiting circulating TNF-α prevented collagen-induced changes in *VCAM1*, *iNOS, sGCα2* and *sGCβ2* expression. Collagen inoculation increased the LV relative gene expression of *PP5*, which codes for the protein that dephosphorylates the N2B stretch element of titin, making it less compliant. Like the echocardiographic measures of LV diastolic dysfunction, inflammation-induced increased *PP5* expression was not prevented by inhibition of TNF-α. These results suggest that inflammation-induced LV diastolic dysfunction may be mediated by increased PP5 expression and increased dephosphorylation of the N2B stretch element of titin that is independent of TNF-α signalling, rather than by titin phosphorylation via the TNF-α-induced downregulation of NO-sGC-cGMP signalling.

Active processes within the cardiomyocyte contribute to the development of LV diastolic dysfunction. Recent evidence suggests subclinical systolic dysfunction in HFpEF, even when ejection fraction is normal. The impact of inflammation on active processes that may contribute to LV diastolic and early-stage systolic dysfunction is uncertain. In the third study, in 68 Sprague-Dawley rats exposed to CIA and subsequent TNF-α inhibition, and in a human adult cardiomyocyte cell line (AC16) exposed to TNF-α, I assessed the role of TNF-α on the expression of genes associated with the active cellular processes, including calcium homeostasis, oxidative stress, apoptosis and myosin heavy chain isoforms, that may be implicated in the active processes of impaired LV diastolic and systolic function. Inflammation did not alter the LV mRNA expression of *SERCA2a* or *S100A1*, genes involved in calcium handling in rats. In AC16 cells exposed to TNF-α, mRNA expression of SERCA2a was not altered. These results suggest that systemic inflammation and TNF-α does not impair calcium reuptake in LV diastolic dysfunction. In the CIA model, consistent with the preserved ejection fraction, systemic inflammation and inhibiting TNF-α did not alter the expression of markers of mitochondrial oxidative stress (*SOD2*) or apoptosis (*BAX/Bcl2*). In AC16 cells exposed to TNF-α, there was a small increase in expression of markers of apoptosis (*BAX/Bcl2*), however there was a substantial increase in the expression of *SOD2*, the superoxide dismutase that inactivates reactive oxygen species, which likely explains the minimal increase in apoptosis. Taken together, these results indicate that the energy-dependent active process is not compromised in the early stages of inflammation-induced cardiac dysfunction. Systemic inflammation impaired systolic function, as

myocardial deformation (global longitudinal strain) and motion (global longitudinal velocity) were impaired in the CIA rats. Consistent with this finding, systemic inflammation resulted in a shift in the expression patterns of myosin heavy chain (MyHC) genes from fast-twitch to slow-twitch, as the ratio of *Myh6/Myh7* was decreased in the CIA rats, which may account for the impaired myocardial systolic function. Inhibiting circulating TNF-α prevented the inflammation-induced impairments in LV systolic function and the MyHC isoform shift. These results suggest that inflammation-induced systolic functional changes result from MyHC isoform shifts, which may be, at least in part, mediated by TNF-α.

In addition to TNF-α, the inflammatory cytokine, interleukin 6 (IL-6), has also been implicated in inflammation-induced cardiac dysfunction. In the fourth study, in 68 Sprague-Dawley rats exposed to CIA, I investigated the role of IL-6, by using the IL-6 receptor blocker tocilizumab, on expression of genes implicated in the passive and active processes involved in LV diastolic and systolic dysfunction. Blocking circulating IL-6 did not prevent any of the inflammation-induced increases in LV relative mRNA expressions of pro-fibrotic genes, the genes involved in titin regulation, or the genes involved in the active mechanisms of myocardial relaxation or contraction. These findings are consistent with the echocardiographic results that showed blocking IL-6 did not prevent the inflammation induced increases in concentric remodelling (relative wall thickness), impaired relaxation (e') or increased filling pressures (E/e') or impaired myocardial deformation and motion. However, the inflammation induced increase in the LV mRNA expression of *LOX*, which codes for the enzyme that cross-links collagen, was partially offset in the IL-6 blocker rats, which may have contributed to the partial reduction in LV stiffness (e'/a'). Taken together, the effect of IL-6 receptor blockers on inflammation-induced changes in cellular mechanisms underlying LV diastolic and systolic dysfunction remain inconclusive, likely, in part, because of the complex signalling of IL-6 and the immunogenic effects of using a human monoclonal antibody.

In conclusion, the findings reported in this thesis advance our understanding of several molecular mechanisms whereby inflammation induces LV diastolic and systolic dysfunction. Evidence is provided that circulating TNF-α is implicated in the development of LV fibrosis and the resultant concentric remodelling, but that LV concentric remodelling is not the main catalyst for the development of LV diastolic dysfunction. This thesis further showed that inflammation may induce hypophosphorylation of titin that contributes to LV diastolic dysfunction, likely via increased expression of PP5 and not primarily via TNF-α mediated nitric oxide-mediated cGMP signalling. This thesis also provides evidence that diastolic calcium reuptake and the energy-dependent active process of diastolic relaxation is not compromised in the early stages of inflammation-induced LV diastolic dysfunction and that these processes do not contribute to early-stage systolic dysfunction. Lastly this thesis provides evidence that TNF-α contributes to early-stage systolic dysfunction in HFpEF, by disrupting myocardial contractile properties.