Interstitial Fibrosis and Macrophages Persist in the Myocardium Following Removal of Left Ventricular Pressure Overload

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Introduction: Aortic stenosis can develop into Left ventricular pressure overload (LVPO). LVPO is associated with increased myocardial collagen content and stiffness. Patients undergoing alleviation of LVPO by surgical aortic valve replacement (SAVR) demonstrate reduced but persistent fibrosis. Little is known regarding the cellular mechanisms that contribute to ECM turnover following hemodynamic unloading of the myocardium. Also, patient biopsies and animal models of LVPO demonstrate an increased level of macrophages and a correlation between myocardial macrophage levels and LVPO-induced interstitial fibrosis. Whether and to what extent myocardial macrophages contribute to ECM turnover after unloading of the myocardium is unexplored. **Methods:** To induce LVPO, transverse aortic constriction (TAC) was performed on 12wk old C57Bl6 mice. After 4wks of LVPO, the TAC band was removed (unTAC) to alleviate pressure overload. Cardiomyocyte cross-sectional area (CSA), Collagen volume fraction (CVF), collagen hybridizing peptide (CHP), and total myocardial macrophage levels were assessed at five time points: Control no TAC, 4wk TAC, 4wk TAC+2wk unTAC (2wk unTAC), and 4wk TAC+4wk unTAC (4wk unTAC), and 4wk TAC+6wk unTAC (6wk unTAC). **Results:** First, at 4wk TAC, CSA increased by 47% compared to control. By 6wk unTAC, CSA returned to control levels. Second, a 204% increase in CVF was observed at 4wk TAC compared to control. CVF was similar between 4wk TAC and 2wk unTAC, but a significant decrease in CVF was observed at 4wk unTAC although it remained elevated compared to control. CVF remained elevated at 6wk unTAC compared to control. Third, proLOX was significantly increased in 4wk TAC compared to control. proLOX levels significantly decreased in 2wk unTAC and 4wk unTAC compared to 4wk TAC. However, proLOX significantly increased in 6wk unTAC. TIMP1 continued to increase during the unTAC time course. Finally, macrophage area increased by 259% at 4wk TAC compared to control, and early after unloading, a 240% increase was observed at 2wk unTAC compared to 4wk TAC. Macrophages remained elevated at 4wk unTAC and 6wk unTAC compared to control. **Conclusions:** After unloading the myocardium, hypertrophy fully regressed, but persistent fibrosis and myocardial stiffness were observed. Proteins implicated in collagen crosslinking and inhibiting collagen degradation were increased in 6wk unTAC demonstrating a shift in ECM homeostasis that favors persistent fibrosis. Furthermore, macrophage levels remained elevated at all TAC and unTAC time points over control. **Significance:** Our TAC/unTAC murine model mimics the AS/SAVR patient paradigm and emphasizes the importance of investigating new therapies to address persistent cardiac fibrosis. Future studies using this model will focus on elucidating macrophage phenotype and macrophage-dependent mechanisms of collagen turnover to determine if macrophages are a targetable therapy for inhibiting persistent fibrosis.