CXCR4 Antagonism Reduces Cardiac Fibrosis and Improves Cardiac Performance in Dilated Cardiomyopathy

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Background: Myocardial fibrosis is a key pathologic finding in the failing heart and is implicated as a cause of increased ventricular stiffness and susceptibility to ventricular arrhythmia. Neurohormonal mediators such as aldosterone and angiotensin II are known to cause fibrosis in experimental models, however, clinical evidence for the reversal of fibrosis with relevant antagonists is limited. Recent studies suggest that inflammatory mediators may contribute to fibrosis. In dilated cardiomyopathy the mechanism for myocardial fibrosis is unclear and its implications on systolic function are not known.

Methods and Results: We studied the effect of a highly selective antagonist of SDF-1/CXCR4 signaling, AMD3100, on the development of cardiac fibrosis and cardiac function in mice with dilated cardiomyopathy due to cardiac-specific transgenic overexpression of the stress-kinase, Mst1. AMD3100 significantly attenuated the progression of myocardial fibrosis and this was accompanied by significant improvements in diastolic and systolic performance as evaluated in isolated Langendorff perfused hearts. AMD3100 reduced BNP mRNA expression but did not alter the expression of Ca²⁺ handling genes. CXCR4 antagonism also reduced the abundance of splenic CD4⁺ T cells.

Conclusion: This study demonstrates that CXCR4 pathway contributes to pathogenesis of cardiac fibrosis in dilated cardiomyopathy, and it represents a new potential therapeutic target in heart failure. The data also demonstrate that anti-fibrotic strategies can improve systolic performance.

Keywords: cardiomyopathy, chemokine receptor, CXCR4 antagonism, heart failure, cardiac fibrosis, cardiac performance, receptor antagonist

INTRODUCTION

Cardiac remodeling, incorporating structural, functional and molecular alterations are fundamental findings in the failing heart (HF). More specifically individual patterns of remodeling, together with extra-cardiac abnormalities, contribute to the particular clinical phenotype. Expansion of the extracellular matrix with deposition of proteins including collagen type I and III, is a common finding in the large majority of HF. The extent of myocardial fibrosis has been demonstrated to be an important contributor to ventricular stiffness and arrhythmogenesis.
in a cardiac-specific manner using the α background, and the stress kinase, Mst1, was overexpressed. In brief, Mst1 transgenic mice were generated on a C57BL/6 mice) were used in our study. The Mst1 transgenic mice have been described in detail previously (Yamamoto et al., 2003).

Animals
All experimental protocols were approved by the Alfred Medical Research and Education Precinct Animal Experimentation Ethics Committee under the guidelines of the National Medical and Health Research Council of Australia. To investigate the impact of CXCR4 antagonism as a preventive strategy in dilated cardiomyopathy, thirty-two male mice age 6 weeks (16 Mst1 mice and 16 wild-type C57BL/6 mice) were used in our study. The Mst1 transgenic mice have been described in detail previously (Yamamoto et al., 2003). In brief, Mst1 transgenic mice were generated on a C57BL/6 background, and the stress kinase, Mst1, was overexpressed in a cardiac-specific manner using the α-myosin heavy chain promoter. Animals develop a dilated cardiomyopathy phenotype which is established by 10 weeks of age. Animals were randomly allocated to receive the selective CXCR4 antagonist AMD3100 (6 mg/kg per day, Tocris Bioscience, Bristol, United Kingdom) or vehicle (H2O), delivered by a mini-osmotic pump (Alzet, Model 2004). The dose selected was based upon our previous work using another CXCR4 inhibitor, AMD3465 (Chu et al., 2011). The study included the following 4 intervention groups: control (C57BL/6 mice, vehicle mini-osmotic pump, n = 8); control+AMD3100 (C57BL/6 mice, AMD3100 mini-osmotic pump, n = 8); Mst1 (Mst1 mice, vehicle mini-osmotic pump, n = 8); and Mst1+AMD3100 (Mst1 mice, AMD3100 mini-osmotic pump, n = 8). All animals received a standard diet and were followed by weekly measurements of weight and monitored for a period of 12 weeks. This duration was selected based upon the hypothesis that antagonism of SDF-1/CXCR4 signaling would abrogate the development of the cardiac phenotype in Mst-1 transgenic mice, which is typically advanced by 18 weeks.

Functional Measurements
On the day before study completion, echocardiographic imaging of the left ventricle was performed under isoflurane anesthesia, using a PHILIPS IE33 ultrasound machine (Royal Philips Electronics, Amsterdam, The Netherlands) with a 15-MHz linear transducer. Off-line image analysis was performed in a blinded fashion. Before termination, arterial blood pressure was measured, using a 1.4 F microtipped transducer catheter (Millar) inserted through the carotid artery.

Gross Morphometry and Histological Analysis
Animals were killed at the end of the experiments by deep anesthesia and the heart, kidney and lung were rapidly excised, washed and weighed. Hearts were immersed in saline on ice before fixing half of the left ventricle in 10% formalin for paraffin sectioning and the remaining half was snap-frozen in liquid nitrogen for molecular biology. Ventricular tissue was prepared for paraffin sectioning. Four-micron paraffin sections were stained with Masson's Trichrome to evaluate the distribution and localization of collagen. The extent of fibrosis was measured in each of ten randomly chosen fields per animal in perivascular and interstitial areas with ImagePro Plus software (Adapt Electronic Solutions Pty Ltd., Moorabbin, VIC, Australia) using an Olympus BH2 microscope with results expressed as a percentage of blue area in each screen at a magnification of 400×. Perivascular and interstitial collagen volume fraction of the Masson's Trichrome stained tissue were measured separately. All collagen surrounding an intramyocardial coronary artery was considered as perivascular collagen. Vessels that were located in scars were excluded from the analysis. Image analysis was performed by an investigator who was unaware of the assigned treatment group.

Isolated Left Ventricular Performance
Left ventricular performance was investigated in isolated Langendorf perfused hearts. The investigator performing the studies was unaware as to group allocation. Mice were...
anesthetized with an intraperitoneal injection of sodium pentobarbitone (50 mg/kg), a thoracotomy was performed and hearts rapidly excised into ice-cold perfusion fluid. The aorta was cannulated on a shortened blunt 21 gauge needle and perfusion initiated at a constant pressure of 80 mmHg on the apparatus. Thebesian fluid accumulation into the left ventricle was vented via a polyethylene drain through the apex of the heart, and a fluid-filled balloon constructed from polyvinyl chloride film was introduced into the left ventricle through the septum. The perfusate was immersed in warmed perfusate in a jacketed bath maintained at 37 °C and bubbled with a mix of 95% O2/5% CO2 at 37°C to provide a pH of 7.4. Organ bath and perfusate temperatures were continuously monitored using a three channel Physitemp TH-8 digital thermometer (Physitemp Instruments Inc., Clifton, NJ, United States). Ventricular pressure signals were recorded on a four-channel MacLab data acquisition system (ADInstruments). Left ventricular pressure was digitally processed to yield systolic, end-diastolic and developed (LVDP) pressures, heart rate, and the peak positive and negative differentials of pressure change with time (+dP/dt and −dP/dt, respectively). The capacity of the left ventricular to respond to increasing preload was subsequently determined during stepwise increases in the left ventricular diastolic (balloon) pressure.

**Molecular Biology**

Real-time PCR was performed to determine collagen types I and III, brain natriuretic peptide (BNP), sarcoplasmic reticulum ATPase type 2a (SERCA2a), phospholamban (PLN), ryanodine receptor, sodium calcium exchanger (NCX) and SDF-1 mRNA expression in the heart. Total RNA was isolated using the TRIzol (Invitrogen) purification system and reverse transcribed using TaqMan reagents (Applied Biosystems). Real-time PCR (ABI Prism 7300 sequence detection system, Applied Biosystems) was conducted using a 50-ng template. The primer sequences for real-time PCR included the primers sequences for real-time PCR included collagen I: (fwd) 5′-TCTGGAGTGTTCAGGGAGACAT-3′ and GAPDH (fwd: 5′-ACAGCAACTCCCACTCTTCC-3′, rev: 5′-CCTCTCTTGGTCAGTGGTCC-3′). Expression values were determined by calculating the ΔΔCt value for each reaction, and data were expressed as fold difference compared with control.

**Flow Cytometry**

To investigate the potential role of T cell activation in the pathophysiology of the Mst1 cardiac phenotype and the effects of CXCR4 antagonism, we performed flow cytometric analysis of splenic homogenates. Splenic tissue was homogenized and cells were pelleted and then suspended in FACS buffer. Cells were subsequently stained for CD4, CD25, and FoxP3 (eBioscience) according to the manufacturer’s instructions, followed by flow cytometric analysis (BD FACS Canto LSR II flow cytometer).

**Statistical Analyses**

The procedures were carried out using the Statistical Package for the Social Sciences (SPSS) software (SPSS 17.0 for Windows, SPSS, Chicago, IL, United States). Values are presented as the mean ± SEM. As appropriate, between group comparisons were performed using Student t-test for normally distributed data. Comparisons where relevant between 3 or more groups were performed with ANOVA with Tukey’s post hoc analysis. A p-value of <0.05 was considered to be significant.

**RESULTS**

**Effects of CXCR4 on Cardiac Morphometry and Fibrosis**

The primary objective of the study was to investigate the effect of the CXCR4 antagonist, AMD3100, on ventricular structure and function in mice with dilated cardiomyopathy. Gross morphometry demonstrated a lower body weight in Mst1 compared to controls (p < 0.05) and, interestingly, this appeared to be abrogated in Mst1 treated with AMD3100 (p < 0.05, Table 1). In keeping with the original description, Mst1 mice did not demonstrate evidence of compensatory cardiac hypertrophy as evidenced by a lack of increase in the heart to body weight ratio (Table 1). Consistent with the cardiomyopathy phenotype,

**TABLE 1 | Gross morphometry.**

|               | Control       | AMD3100      | Mst-1         | Mst-1 +AMD3100 |
|---------------|---------------|--------------|---------------|----------------|
| Body weight (gm) | 35.6 ± 0.6    | 35.8 ± 1.1   | 31.5 ± 1.0*   | 35.7 ± 0.6†    |
| Heart weight (mg) | 170 ± 10      | 161 ± 4      | 165 ± 4       | 172 ± 8        |
| Heart:BW       | 4.8 ± 0.4     | 4.7 ± 0.2    | 5.4 ± 0.1     | 4.8 ± 0.2      |
| Lung weight (mg) | 197 ± 17      | 208 ± 1      | 218 ± 4*      | 215 ± 7        |
| Lung:BW        | 5.6 ± 0.2     | 5.9 ± 0.4    | 6.9 ± 0.3*    | 6.0 ± 0.3†     |
| Kidney weight (mg) | 250 ± 12      | 245 ± 9      | 221 ± 5      | 215 ± 6        |
| Kidney:BW      | 7.1 ± 0.3     | 7.1 ± 0.3    | 6.9 ± 0.3     | 6.2 ± 0.1      |

Data are mean ± SEM (n = 5–8); BW, body weight; *p < 0.05 vs. control; †p < 0.05 vs. Mst-1.
Mst1 mice displayed evidence of lung congestion (Table 1) as reflected by an increase in lung weight and lung weight indexed to body weight. In Mst1 mice treated with AMD3100 there was no change in total lung weight, however, the body weight indexed lung weight was lower as shown in Table 1 ($p < 0.05$).

Quantitative analysis of myocardial sections demonstrated significant accumulation of perivascular fibrosis (control vs. Mst1: 2.7 ± 0.2 vs. 12.3 ± 0.3%, $p < 0.001$) and interstitial fibrosis (control vs. Mst1: 0.2 ± 0.1 vs. 4.1 ± 0.1%, $p < 0.001$) in cardiomyopathy mice as represented in Figure 1. The accumulation of both perivascular fibrosis and interstitial fibrosis were markedly attenuated in Mst1 mice treated with AMD3100 (both $p < 0.001$ vs. Mst1), whilst AMD3100 alone was without effect in control mice.

**Effect of CXCR4 Antagonism on Cardiac Function**

As shown in Table 2, cardiomyopathic mice exhibited significant left ventricular dilatation in conjunction with significantly reduced systolic performance, compared to wild type mice as assessed by echocardiography. Furthermore, cardiac specific Mst1 overexpression was accompanied by left ventricular dilatation and reduced wall thickness. CXCR4 antagonist administration was associated a significant attenuation in ventricular wall thinning, however, fractional shortening was not changed (Table 2). AMD3100 alone was without effect on any echocardiographic index of left ventricular size or function. Consistent with significantly reduced contractile function, hemodynamic evaluation showed that Mst1 mice had a lower systolic blood pressure (control vs. Mst 1: 92 ± 3 vs. 75 ± 2 mmHg, $p < 0.01$) and an increased left ventricular end-diastolic pressure compared to wild type mice (Control vs. Mst 1: 5.4 ± 0.3 vs. 11.3 ± 0.9 mmHg, $p < 0.05$). AMD3100 did not alter systolic blood pressure in control mice (95 ± 2 mmHg) or in Mst-1 mice (70 ± 5 mmHg). LVEDP was also unaltered by ADM3100 in control (6.6 ± 1.3 mmHg) or Mst-1 mice (12.0 ± 2.3 mmHg).

To investigate cardiac function in greater detail and in the absence of the potentially confounding influence of anesthesia, we next evaluated left ventricular performance using isolated Langendorff perfused hearts ($n = 4$ per group). As shown in Table 3, both peak positive and negative $dP/dt$ were significantly reduced in Mst1 mice compared to controls. Administration
in mice with cardiomyopathy (III gene expression in the failing heart was significantly increased by RT-PCR. Consistent with the histological data, collagen I and III mRNA abundance. Treatment with the CXCR4 antagonist alone in wild type mice was without effect.

To complement the histological studies on myocardial fibrosis, we first characterized the secretion of matricellular proteins and their degradation (Packer, 2018). Diffuse cardiac fibrosis is a structural hallmark of ventricular remodeling, contributing to an increase in ventricular stiffness (Westermann et al., 2008; Ellis et al., 2014). Pathophysiologically, increased myocardial stiffness causes an increase in ventricular filling pressures which in turn contributes to the symptoms of HF via pulmonary congestion (Maeder et al., 2010; Houists and Lewis, 2014). We found that lung weight and indexed lung weight was elevated in Mst-1 mice consistent with pulmonary congestion, and AMD3100 reduced the indexed lung weight, consistent a reduction in pulmonary congestion. However, there was no change in lung weight per se and without an assessment of lung water the interpretation of the change in indexed lung weight remains speculative. Remodeling of the myocardial interstitium by cardiac fibroblasts, and other cells, represents the net balance between the secretion of matrix collagen proteins and their degradation by proteolytic enzymes including the matrix metalloproteinases (Frangogiannis, 2012). The origin of secretory cardiac fibroblasts, is complex and potentially includes contributions from resident cardiac fibroblasts, recruited circulating fibrocytes, vascular pericytes, endothelial to mesenchymal transition, and pericardial cells (Krenning et al., 2010; Furtado et al., 2014). Activation of the renin-angiotensin-aldosterone system has been proposed to be a principal driver of cardiac fibrosis. Whilst, the use of angiotensin converting enzyme inhibitors, angiotensin receptor antagonists and aldosterone antagonists provides clear benefit in HF, evidence for an anti-fibrotic action in patients is limited (Brilla et al., 2003; Kitzman et al., 2010). In the current study, Mst1 mice had evidence of ventricular dilatation and reduced systolic function which was not altered by AMD3100. We observed some evidence for attenuation of ventricular wall thinning although this was not accompanied by a change in fractional shortening.

**Myocardial Gene Expression**

To address the possibility that the anti-fibrotic action of CXCR4 antagonism is mediated via an immunomodulatory action we performed FACS analysis of splenic homogenates. CD4 positive cells represented 16.9 ± 1.3% of splenic cells in and this was modestly reduced in Mst-1 mice (12.4 ± 1.2%, p = 0.05). In Mst-1 mice, treatment with the CXCR4 antagonist reduced the frequency of CD4+ cells to 7.2 ± 1.2% (p < 0.05). Splenic regulatory T cell abundance in Mst-1 mice was unaffected by AMD3100, as reflected by the relative proportions of Foxp3+CD4+ cells (Mst1 vs. Mst-1 +AMD3100: 10.3 ± 1.2 vs. 9.4 ± 1.9%, p = ns) or CD25+CD4+ cells (Mst1 vs. Mst1+AMD: 8.8 ± 0.9 vs. 8.9 ± 1.0%, p = ns).

**DISCUSSION**

Our findings demonstrate that activation of the SDF-1/CXCR4 axis plays an important role in the structural and functional remodeling of the failing heart. In mice with experimental dilated cardiomyopathy we showed that administration of AMD3100, a highly selective CXCR4 antagonist, was associated with a marked reduction in perivascular and interstitial fibrosis, supported by a reduction in the expression of collagen I mRNA. These findings are consistent with reports of increased heart failure in patients treated with dipeptidyl peptidase-4 inhibitors, given that these drugs augment SDF-1 levels by inhibiting degradation (Packer, 2018). Diffuse cardiac fibrosis is a structural hallmark of ventricular remodeling, contributing to an increase in ventricular stiffness (Westermann et al., 2008; Ellis et al., 2014). Pathophysiologically, increased myocardial stiffness causes an increase in ventricular filling pressures which in turn contributes to the symptoms of HF via pulmonary congestion (Maeder et al., 2010; Houists and Lewis, 2014). We found that lung weight and indexed lung weight was elevated in Mst-1 mice consistent with pulmonary congestion, and AMD3100 reduced the indexed lung weight, consistent a reduction in pulmonary congestion. However, there was no change in lung weight per se and without an assessment of lung water the interpretation of the change in indexed lung weight remains speculative. Remodeling of the myocardial interstitium by cardiac fibroblasts, and other cells, represents the net balance between the secretion of matrix collagen proteins and their degradation by proteolytic enzymes including the matrix metalloproteinases (Frangogiannis, 2012). The origin of secretory cardiac fibroblasts, is complex and potentially includes contributions from resident cardiac fibroblasts, recruited circulating fibrocytes, vascular pericytes, endothelial to mesenchymal transition, and pericardial cells (Krenning et al., 2010; Furtado et al., 2014). Activation of the renin-angiotensin-aldosterone system has been proposed to be a principal driver of cardiac fibrosis. Whilst, the use of angiotensin converting enzyme inhibitors, angiotensin receptor antagonists and aldosterone antagonists provides clear benefit in HF, evidence for an anti-fibrotic action in patients is limited (Brilla et al., 2003; Kitzman et al., 2010). In the current study, Mst1 mice had evidence of ventricular dilatation and reduced systolic function which was not altered by AMD3100. We observed some evidence for attenuation of ventricular wall thinning although this was not accompanied by a change in fractional shortening.

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**TABLE 2** | Echocardiographic parameters.

|                  | Sham Control | Control +AMD3100 | Mst-1 | Mst-1+AMD3100 |
|------------------|--------------|------------------|-------|---------------|
| IVSd, mm         | 0.73 ± 0.02  | 0.70 ± 0.01      | 0.63 ± 0.01* | 0.63 ± 0.01* |
| LVDDc, mm        | 4.13 ± 0.01  | 3.97 ± 0.03      | 3.47 ± 0.06* | 4.40 ± 0.06* |
| LVPWd, mm        | 0.72 ± 0.01  | 0.75 ± 0.03      | 0.67 ± 0.01* | 0.82 ± 0.02** |
| IVSs, mm         | 1.27 ± 0.04  | 1.20 ± 0.03      | 0.76 ± 0.02* | 0.99 ± 0.02** |
| LVIDs, mm        | 2.69 ± 0.08  | 2.60 ± 0.07      | 3.37 ± 0.08* | 3.39 ± 0.03* |
| LVPWs, mm        | 1.09 ± 0.03  | 1.15 ± 0.02      | 0.78 ± 0.03* | 1.03 ± 0.01** |
| FS (%)           | 35 ± 2       | 35 ± 2           | 23 ± 2*      | 23 ± 1*       |

IVSc, interventricular septal diastolic thickness; LVDDc, left ventricular internal diameter at end diastole; LVPWd, left ventricular posterior wall thickness at end diastole; IVSs, interventricular septal systolic thickness; LVIDs, left ventricular internal diameter at end systole; LVIDs, left ventricular posterior wall thickness at end systole; FS, fractional shortening. Values are means ± SE (n = 6); *P < 0.05 vs. Sham Control; †P < 0.05 AMD3100 vs. Mst-1.
TABLE 3 | Contractile function in isolated perfused hearts.

| Groups          | Basal LV Balloon pressure (5 mmHg) | LV Balloon pressure (10 mmHg) | LV Balloon pressure (20 mmHg) |
|-----------------|------------------------------------|-----------------------------|-----------------------------|
|                 | +dP/dt (mmHg/s)                    | −dP/dt (mmHg/s)             | +dP/dt (mmHg/s)             | −dP/dt (mmHg/s)             |
| Control         | 4717 ± 216.3                       | −3305.0 ± 143.7            | 4693.7 ± 238.9              | −3336.7 ± 113              |
| Mst1            | 3367.6 ± 113.9*                    | −2670.0 ± 137.1*           | 3492.9 ± 104*              | −2511.1 ± 155.5           |
| Mst1+AMD3100    | 4145.2 ± 306.8†                    | −3217.3 ± 140.1†           | 4188 ± 181.6†              | −3141.6 ± 99.2†           |
|                 |                                    |                            | 4601 ± 320.9†              | −3357.2 ± 172.6           |
|                 |                                    |                            | 3258 ± 111.7*              | −2344.8 ± 129.3*          |
|                 |                                    |                            | 3677.6 ± 233.3             | −2966.6 ± 111.1†          |

Data are mean ± SEM (n = 7–8 per group); *p < 0.05 vs. C57BL6; †p < 0.05 vs. Mst1.

Immune activation, potentially triggered by elevated oxidative stress and cardiomyocyte damage is also an important contributor to cardiac fibrosis. Amongst the mediators of fibrosis, recruited macrophages play an important role via secretion of TGFβ, together with other cytokines, which stimulate fibroblast proliferation and differentiation, together leading to the release of chemotactic, and other pro-inflammatory signals (Glezeva and Baugh, 2014). We previously demonstrated an increase in the expression of stromal derived factor-1 (SDF-1) in the setting of experimental and clinical heart failure (Chu et al., 2010), however, it remained unclear as to whether this was a mediator of fibrosis. Conceptually, SDF-1 acting via its cognate receptor, CXCR4 may increase fibrosis by stimulating fibroblast proliferation and recruitment or alternately may stimulate inflammation via T cell recruitment (Bryant et al., 2012).

We also previously showed that selective CXCR4 antagonism attenuated the development of cardiac fibrosis in experimental hypertension (Chu et al., 2011).

Recent studies performed in pressure overload and mineralocorticoid excess models of cardiac hypertrophy and fibrosis increasingly suggest a key role for activation of inflammatory cells and cytokines. In particular, CD4+ T cells have been strongly implicated as a driver of cardiac fibrosis (Laroumanie et al., 2014; Nevers et al., 2015), likely through pro-inflammatory Th1 and Th17 subsets and their respective cytokine mediators including IL-6, IL1β, TNF, and IL17 (Madhur et al., 2010; Amador et al., 2014) which may cause fibrosis by activation of resident cardiac fibroblasts. Consistent with this immunological paradigm, we found that CXCR4 antagonism led to a significant reduction in the abundance of splenic CD4+ T cells. This finding is consistent with previous studies showing that SDF-1 activates T cells (Nanki and Lipsky, 2001). Furthermore,
The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. The SDF-1/CXCR4 axis has previously been studied in the setting of myocardial infarction (MI) and ischemia reperfusion injury. Elevated SDF-1 expression occurs early after MI, contributing to the recruitment and retention of endothelial progenitor cells arising from the bone marrow (Aiuti et al., 1997; Shintani et al., 2001). Within bone marrow, the SDF-1/CXCR4 axis plays a role in the retention of progenitor cells (Peled et al., 1999), and its inhibition has been shown to promote the mobilization of stem cells. Within this framework, single dose administration of CXCR4 antagonists have been shown to augment recovery from ischemic injury (Jujo et al., 2013; Hsu et al., 2015). CXCR4 antagonism has been shown to promote the mobilization of endothelial precursor cells and mesenchymal stem cells with improvements in cardiac performance potentially mediated by enhanced angiogenesis, reduced apoptosis or reduced inflammation (Jujo et al., 2013; Hsu et al., 2015).

We found that left ventricular systolic function, assessed by dP/dt, in isolated perfused hearts was improved in AMD3100 treated Mst-1 mice compared to Mst-1 mice alone. However, this was not mirrored by any changes in systolic function as assessed echocardiographically or via cardiac catheterisation. It is possible that the effects of general anesthesia may have masked the presence of functional differences in intact animals. We did not assess the end-systolic pressure volume relationship, a load insensitive measure of contractile performance. Our study is consistent with several imaging studies which report an inverse correlation between the extent of myocardial fibrosis and indices systolic performance, although this remains controversial (Donekal et al., 2014; Jacquier et al., 2014; Tandon et al., 2015).

Mechanistically it has been proposed this observation could be explained by several pathophysiologic mechanisms. These include the combined effects of cardiomyocyte loss together with replacement fibrosis; the effects of impaired coronary flow reserve in the context of perivascular fibrosis; or increased contractile afterload in the setting of interstitial fibrosis. Interestingly it has also been shown that myofibroblasts may signal via paracrine mechanisms to cardiomyocytes, to reduce Ca2+ handling and contractility (LaFramboise et al., 2007; Vasquez et al., 2010; Cartledge et al., 2015). In the current study we did not observe any change in the expression of SERCA2a, phospholamban, ryanodine receptor or the sodium calcium exchanger, which are key regulators of calcium handling. Similarly, there was no evidence of altered ventricular mass which would argue against a potential anti-apoptotic action. We did not directly assess the extent of cardiomyocyte apoptosis in this study. Apoptosis, driven by transgenic overexpression of Mst-1 has been shown to be elevated in Mst-1 mice (Yamamoto et al., 2003), and accordingly would be unlikely to be modified by pharmacologic intervention. Given that we did not observe any effect of AMD3100 on cardiac structure or function in control mice, we did evaluate the effects of AMD3100 alone on myocardial gene expression or ventricular function in isolated perfused hearts.

Taken together, the present study shows, for the first time, that chronic CXCR4 antagonism can exert an anti-fibrotic action which is accompanied by evidence of improved cardiac performance in non-ischemic, dilated cardiomyopathy. Further studies are warranted to examine the potential utility of manipulation of the SDF-1/CXCR4 axis as a clinical therapeutic strategy in heart failure.

AUTHOR CONTRIBUTIONS
DK and P-YC designed the study. P-YC, HK, MJ and DH conducted the study. All authors contributed to analysis and manuscript preparation. Study funding was acquired by DK.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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