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EFFECT OF TRANSFORMING GROWTH FACTOR- β RECEPTOR ANTAGONISM IN MICE WITH CARDIAC RESTRICTED OVEREXPRESSION OF TUMOR NECROSIS FACTOR

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Abstract

The mechanisms that are responsible for the development of myocardial fibrosis in the inflammatory cardiomyopathy are unknown. Previously we have generated lines of transgenic mice with cardiac restricted overexpression of tumor necrosis factor (MHCsTNF mice), a pro-inflammatory cytokine. The MHCsTNF mice develop a heart failure phenotype that is characterized by progressive myocardial fibrosis, as well as increase levels transforming growth factor- β (TGF- β) mRNA and protein. In order to determine whether TGF- β mediated signaling was responsible for the myocardial fibrosis observed in the MHCsTNF mice, we treated MHCsTNF and littermate control mice from 4 to 12 weeks of age with a novel orally available TGF- β receptor antagonist (NP-40208). At the time of terminal study myocardial collagen content was determined using the picrosirius red technique, and LV systolic and diastolic function were determined using the Langendorff method. Treatment with NP-40208 resulted in a significant decrease in the nuclear translocation of Smad 2/3, a decrease in heart-weight to body-weight ratio, decreased fibrillar collagen content and decreased LV chamber stiffness in the MHCsTNF mice when compared to diluent treated controls. Treatment with NP-40208 had no discernable effect on LV systolic function, nor any effect on fetal gene expression in the MHCsTNF mice. Taken together, these observations suggest that sustained pro-inflammatory signaling in the adult heart is associated with a pro-fibrotic phenotype that arises, at least in part, from TGF- β mediated signaling, with resultant activation of Smad 2/3, leading to increased myocardial fibrosis and increased LV diastolic chamber stiffness.

Keywords

Tumor necrosis factor; transforming growth factor; myocardial fibrosis; transgenesi

INTRODUCTION

Previous studies from this and other laboratories have shown that cardiac restricted overexpression of tumor necrosis factor (TNF) consistently leads to the development of a heart failure phenotype characterized by left ventricular (LV) dilation and progressive myocardial fibrosis.¹⁻³ Although the progressive LV dilation in these mice has been attributed to TNF-induced activation of matrix metalloproteinases (MMPs) with subsequent degradation of fibrillar collagen, the mechanisms that are responsible for the progressive myocardial

fibrosis that accrues in these mice is not all understood. Given that TNF inhibits collagen gene expression and/or collagen synthesis in cardiac fibroblasts,^{4,5} the increased myocardial fibrosis observed in the transgenic models with cardiac restricted overexpression of TNF is unlikely to be a direct effect of TNF-mediated signaling. Of note, Feldman and colleagues have suggested the interesting possibility that MMP induced degradation products, or “matrikines,” are responsible for the progressive tissue fibrosis in their model of sustained TNF overexpression.^{6,7} A second possible explanation for the increased fibrosis observed in the TNF transgenic mice is that TNF mediated signaling increases the density of angiotensin type I receptors (AT₁) on cardiac fibroblasts,⁸ and that this increase in AT₁ receptor density sensitizes cardiac fibroblasts to the profibrotic actions of endogenous angiotensin II.⁵ Germane to the present discussion, we have shown that both transforming growth factor- β_1 (TGF- β_1) and TGF- β_2 mRNA and protein levels are significantly increased in the hearts of the MHCsTNF transgenic mice relative to littermate controls, raising the interesting possibility that TGF- β mediated signaling was responsible for the fibrosis observed in the MHCsTNF mice.

TGF- β binds to at least three specific cell-surface receptors, referred to as receptor types I, II, and III, which exist in virtually all mammalian cells, including fibroblasts. The Type I (T β RI) and type II TGF- β (T β RII) receptors are transmembrane receptors with serine/threonine kinase activity, whereas the type III receptor is a membrane-anchored proteoglycan (betaglycan) with a short cytoplasmic domain that is unlikely to mediate the biologic actions of TGF- β , and likely acts to present TGF- β to other receptors. TGF- β mediated cellular signaling is initiated by binding of the ligand to a transmembrane type II TGF- β receptor (T β RII). After ligand activation, the T β RII receptor recruits the type I receptor TGF- β receptor (T β RI) into a heterotetrameric receptor signaling complex. The constitutively active T β RII activates T β RI by phosphorylating serine and threonine residues in a conserved stretch of glycine and serine residues that precede the receptor kinase domain. Phosphorylation of the T β RI kinase results in subsequent downstream signaling predominantly by phosphorylation of cytoplasmic mediators belonging to the Smad family, with resultant translocation of phosphorylated Smad2/3 to the nucleus.⁹ Importantly, TGF- β can also signal through mitogen activated protein kinases (MAPK) in a Smad independent manner.

In order to determine whether TGF- β mediated signaling was responsible for the observed myocardial fibrosis observed in the in the MHCsTNF mice, we employed a novel orally available T β RI antagonist, NP-40208 (Scios Inc, Freemont, CA),¹⁰ that blocks TGF- β mediated signaling by inhibiting the intracellular kinase domain of T β RI. The results of this study suggest that TGF- β mediated signaling plays an important role in the structural remodeling and cardiac dysfunction that is observed in mice with cardiac-restricted overexpression of TNF.

METHODS

Mice

The studies described herein were performed on 12-week-old male with cardiac restricted overexpression of TNF (MHCsTNF) and littermate control mice lacking the TNF transgene. The characterization of the MHCsTNF mice used in these studies has been reported previously.^{1,11} Briefly, MHCsTNF mice develop progressive myocardial fibrosis from 8–12 weeks of age that is characterized by increased levels of TGF β_1 and TGF β_2 mRNA and protein.¹ All mice housed under standard environmental conditions and maintained on commercial chow and tap water ad libitum. All studies conformed with the principles of the National Institutes of Health “Guide for the Care and Use of Laboratory Animals,” and were approved by the Baylor College of Medicine Animal Care and Use Committee.

TGF- β Receptor Inhibition

To determine the significance of TGF- β signaling MHCsTNF and littermate control mice were treated from 4 to 12 weeks of age with diluent or 100 mg/kg body weight/day NP-40208 (Scios Inc, Freemont, CA), a novel 2,4-disubstituted pteridine that inhibits the intracellular kinase domain of the type I TGF- β receptor (T β RI).¹⁰ In an in vitro kinase assay NP-40208 inhibits the T β RI kinase with an IC₅₀ of 0.048 μ mol/L. When tested at 50 nmol/L the compound has no effect on T β RII kinase (data provided by Scios Inc.). The dose of NP-40208 chosen for these studies was based on previous studies which showed that 50–150mg/kg body weight/day was sufficient to achieve adequate blood levels of NP-40208 (data provided by Scios Inc.). NP-40208 was dissolved in deionized water and administered through drinking water.

TGF- β Mediated Signaling

To determine the specific effects of the NP-40208 on TGF- β -mediated signaling, we examined protein levels of Smad2/3 in nuclear and cytoplasmic fractions of the MHCsTNF and littermate control mouse hearts. Hearts were excised, snap-frozen in liquid nitrogen and stored at 80 °C until protein extraction. After measuring frozen heart weight, nuclear and cytoplasmic proteins were isolated using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce; Rockford, IL) according to the manufacturer's instructions. Protein levels for Smad2/3 were measured by Western blot analysis as described previously,¹² using a polyclonal Smad2/3 antibody (1:1000, Upstate, Lake Placid, NY). Western blots were incubated with an appropriate peroxidase-labeled secondary antibody (1:5000 dilution), and membranes were incubated with luminol using a commercially available kit (ECL plus, Amersham, IL), and exposed to chemiluminescent film. The films were scanned on a personal densitometer (Molecular Dynamics, Sunnyvale, CA) and band intensities were evaluated using ImageQuaNT 4.2a (Molecular Dynamics) software.

Cardiac Morphometrics and Myocardial Collagen Content

Cardiac hypertrophy was assessed by determining the heart-weight to body-weight ratio in littermate control and MHCsTNF mice at 12 weeks of age.¹ Myocardial collagen content was determined using the picrosirius red technique, as described.¹ Briefly, after completing the ex vivo studies described below, hearts were perfusion-fixed, embedded in paraffin and stained. The percent area of extracellular picrosirius red staining was computed from 3 random fields within the mid-myocardium in order to exclude large epicardial arteries and/or veins, as well as any cutting and/or compression artifacts.¹

Left Ventricular Function and Stiffness

Hearts from diluent and NP-40208 treated littermate control and MHCsTNF mice were isolated and perfused in the Langendorff mode, as described.¹³ Briefly, mice were injected intraperitoneally with heparin (10,000U/Kg; Sigma, St Louis, MO) and anesthetized with Avertin (16 μ l/g body weight of a 2.5% solution). The hearts were rapidly excised and perfused in a retrograde manner at a constant perfusion pressure of 80 mmHg with modified Krebs-Henseleit buffer containing (mmol): NaCl 118, NaHCO₃ 24, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.2, Glucose 11, Pyruvate 2. Buffer was oxygenated with 95% O₂ and 5% CO₂ at 37 °C, to yield a pH of 7.4. A thin cannula was pierced through the apex of the LV to vent thebesian drainage. A hand-made balloon (polyvinyl chloride film) connected to a polyethylene tube (PE50), was inserted into LV through mitral valve via an incision of left atrium and was connected to pressure transducer (ML844, ADInstruments, Colorado Spring CO). The hearts were paced from the right ventricle using Grass SD9 stimulator (Grass Instruments, Quincy, MA) at 420 beats per minute. Functional data were recorded at 1 KHz on a data acquisition system (PowerLab, ADInstruments, Colorado Spring CO). After 20

minutes of stabilization, the balloon was progressively inflated in 5- μ L increments until LV developed pressure (LVDP) became asymptotic and then began to decline. LVDP was calculated as the difference between peak-systolic pressure and LV end-diastolic pressure (LVEDP). A final pressure-volume exponential relationship obtained as described.¹⁴ The data for LVEDP were plotted as a function of increasing balloon volumes, and were plotted and a best-fit exponential curve ($P=b \cdot \exp(KcV)$), where V is volume, and P is LV pressure generated for each mouse. The volumes at a given pressure were averaged for animals in each group. The chamber stiffness constant (Kc) was determined by fitting the end-diastolic pressure volume curves from individual hearts to an exponential function.^{15,16} The LV chamber stiffness constant (Kc) was determined by fitting the end-diastolic pressure volume curves from individual hearts to an exponential function as described in detail elsewhere.^{15,16} In order to measure LV systolic function at a physiological filling pressure, the balloon was deflated to adjust LVEDP to 8–10 mmHg, and LV systolic pressure and LVEDP were measured.

Fetal Gene Expression

Northern blot analysis was performed on 15 μ g of total RNA isolated from the hearts of transgenic mice and littermate control mice.¹⁷ The characterization of the probes for α -myosin heavy chain (α -MHC), β -myosin heavy and SERCA2a have been reported previously.¹⁸

Statistical Analysis

Results are expressed as mean values $\pm$ SEM. Data were assessed with the use of a commercially available statistical software (STATVIEW version 5.0, SAS institute Inc., Cary, North Carolina). Differences at specific time points between groups were assessed using one-factor ANOVA, which was followed by Fisher's PLSD test for post-hoc analysis where appropriate. A p value < 0.05 was considered statistically significant.

RESULTS

TGF- β Mediated Signaling

We have shown previously that TGF- β mRNA and protein levels are increased in the hearts of MHCsTNF mice as they develop myocardial fibrosis.¹ To determine whether there were TGF- β mediated alterations in Smad 2/3 signaling in the hearts of MHCsTNF mice, we performed Western blot analysis of Smad 2/3 protein levels in the cytoplasmic and nuclear extracts. Figure 1 highlights two important findings. First, although the cytoplasmic levels of Smad 2/3 were similar ($p=0.33$) in the MHCsTNF and littermate control mice, there was a significant ($p=0.002$) increase in the level of Smad 2/3 in the nuclear fraction of the MHCsTNF mouse hearts. The ratio of nuclear to cytoplasmic Smad 2/3 was significantly higher ($p=0.018$) in the MHCsTNF mice than in the littermate controls, consistent with as well as a significant, consistent with activation of the TGF- β signaling in the MHCsTNF mouse hearts. Second, treatment with the T β RI antagonist NP-40208 significantly attenuated the increase in Smad 2/3 protein in the nuclear fraction compared to diluent treated MHCsTNF mice, as well as normalized the ratio of nuclear to cytoplasmic Smad 2/3 of the MHCsTNF mice when compared to diluent treated littermate controls. However, despite normalization of the nuclear to cytoplasmic ratio in the treated MHCsTNF mice, the absolute level of Smad 2/3 was still significantly greater ($p < 0.05$) than that observed in the littermate control mice. NP-40208 had no effect on the cytoplasmic nor nuclear distribution of Smad 2/3 in the littermate control mice.

Cardiac Morphometrics

Figure 2 shows that the heart-weight to body-weight ratio was significantly greater in the MHCsTNF mice than in the littermate control mice ($p < 0.001$), consistent with our previous findings.¹ Importantly, treatment with NP-40208 led to a small but significant decrease in the heart-weight to body-weight ratio in MHCsTNF mice ($p = 0.016$), whereas NP-40208 had no effect on the heart-weight to body-weight ratio in the littermate control mice. However, treatment with NP-40208 did not abrogate cardiac hypertrophy in the MHCsTNF mice, insofar as the heart-weight to body-weight ratio was still significantly greater than that observed in littermate control mice.

Myocardial Collagen Content

We have shown that myocardial fibrosis develops in the hearts of the MHCsTNF mice from 8–12 weeks of age.¹ Treatment with NP-40208 attenuated the increase in myocardial collagen content in the MHCsTNF mice at 12 weeks of age (Figure 3A). The group data summarized in Figure 3B, show that treatment with NP-40208 significantly ($p = 0.007$) attenuated the myocardial collagen content in the MHCsTNF mice when compared to diluent treated MHCsTNF mice. However, the myocardial collagen content in the NP-40208 treated mice after 12 weeks of age was still significantly greater ($p = 0.014$) than that observed in the littermate control mice at a comparable time point. Treatment with NP-40208 for 8 weeks resulted in a small, but non-significant decrease in myocardial collagen content in the littermate control mice.

Myocardial Function and Stiffness

As shown in the Table, there was no significant difference in LV systolic pressure, LVEDP, nor LV developed pressure (LVDP) in the littermate control and MHCsTNF mice at baseline. Moreover, treatment with NP-40208 had no effect on LV systolic pressure, LVEDP, nor LV developed pressure (LVDP) in the littermate control and MHCsTNF mice. In contrast, Figure 4 shows that there was a significant increase in diastolic chamber stiffness in MHCsTNF hearts when compared to littermate control hearts, consistent with the increased interstitial myocardial collagen deposition observed in these mice. As illustrated, there was a significant ($p < 0.05$) leftward shift in the pressure-volume relationship (Figure 4A), and a significant ($p < 0.05$) increase in the LV chamber stiffness constant (Figure 4B) in the MHCsTNF hearts, when compared to littermate control mice. Consistent with the effects of NP-40208 on myocardial collagen content, treatment with NP-40208 resulted in a rightward shift in the pressure-volume curve in the MHCsTNF mouse hearts when compared to untreated MHCsTNF hearts (Figure 5A), as well as a significant reduction ($p < 0.05$) in the LV chamber stiffness constant (Figure 5B). In comparison, treatment with NP-40208 had no effect on the pressure-volume relationship (Figure 5A), nor the chamber stiffness constant in the littermate control mice (Figure 5B). Importantly, the values for LV chamber stiffness in the NP-40208 treated MHCsTNF mice were not significantly different from those observed in treated and untreated littermate control mice (Figure 5B).

Fetal Gene Expression

As shown in Figure 6, Serca2a and α -MHC mRNA expression were both reduced, and the expression of β -MHC was increased in MHCsTNF mice when compared to littermate controls. Treatment with NP-40208 had no effect on the level of expression of Serca2a, α -MHC and β -MHC mRNA in the MHCsTNF mice.

DISCUSSION

The results of this study suggest that the TGF- β mediated signaling contributes to LV structural remodeling (fibrosis) and diastolic dysfunction in mice with cardiac-restricted overexpression of TNF (MHCsTNF). The following three lines of evidence support this statement. First, we observed increased nuclear localization of Smad 2/3 protein in the MHCsTNF mice when compared to littermate control mice, consistent with TGF- β induced Smad 2/3 activation (Figure 1). Importantly, treatment with NP-40208, a selective T β R1 antagonist, normalized the nuclear to cytoplasmic ratio of Smad2/3 when compared to littermate control mice (Figure 1); however, it bears emphasis that the absolute level of nuclear Smad2/3 protein was still significantly greater in the NP-40208 mice MHCsTNF when compared to diluent treated littermate controls, raising the possibility of TGF- β -independent Smad activation in the MHCsTNF, as has been reported recently for angiotensin II (reviewed in ¹⁹). In this regard, we have reported previously that there is increased activation of the renin angiotensin system, as well as increased angiotensin II peptide levels in the hearts of the MHCsTNF mice as they age.²⁰ That said, we cannot exclude the formal possibility that increased dosage and/or duration of treatment with NP-40208 might have further attenuated nuclear localization of Smad 2/3 in the MHCsTNF mice. Second, we observed that the increased myocardial fibrosis observed in the MHCsTNF mice was sensitive to inhibition with NP-40208 (Figure 3). Treatment with NP-40208 resulted in a significant attenuation of myocardial fibrosis in the MHCsTNF mouse hearts when compared to diluent treated MHCsTNF mice. However, the level of myocardial fibrosis in the NP-40208 treated mice was still significantly greater than that observed in diluent treated littermate control mice, consistent with the observation that Smad 2/3 nuclear translocation was not completely abrogated by NP-40208. Treatment with NP-40208 resulted in a small but significant decrease in the heart-weight to body-weight ratio of the NP-40208 treated MHCsTNF mice compared to diluent treated MHCsTNF mice (Figure 2). Nonetheless, the decrease the heart-weight to body-weight ratio was relatively modest (~ 10%) compared to the more robust (~ 40%) decrease in myocardial collagen in the NP-40208 treated MHCsTNF, suggesting that the cardiac hypertrophy observed in the MHCsTNF mice was not due to increased myocardial fibrosis alone. Finally, treatment of the MHCsTNF mice with NP-40208 for 8 weeks prevented the leftward shift of the pressure-volume relationship, as well as the increased LV chamber stiffness observed in diluent treated MHCsTNF mice (Figure 5). Indeed the values for LV chamber stiffness in the NP-40208 treated MHCsTNF mice were not different from those observed in diluent treated littermate control mice. Taken together, these observations suggest that sustained pro-inflammatory signaling in the adult heart is associated with a pro-fibrotic phenotype that arises, at least in part, from T β R1 mediated signaling, with resultant activation of Smad 2/3, leading to increased myocardial fibrosis and increased LV diastolic chamber stiffness. Interestingly, treatment with NP-40208 had no discernable effect on expression of the fetal gene pattern observed in the MHCsTNF mice (Figure 6), suggesting that the major pathophysiological role of TGF- β signaling in the MHCsTNF mice involves remodeling of the extracellular matrix.

TGF- β Signaling and Myocardial Fibrosis

TGF- β provokes a classical set of transcriptional responses that define the “profibrotic” phenotype, including increased myofibroblast transformation, increased collagen synthesis and decreased collagen degradation.²¹ Indeed several studies have demonstrated that TGF- β promotes collagen synthesis and/or deposition in cultured cardiac fibroblasts in vitro.^{22–24} Moreover, TGF- β antagonism with neutralizing antibodies, soluble TGF- β receptors, and over expression decorin or dominant negative TGF- β receptors has resulted in attenuation of tissue fibrosis can be attenuated in a variety of different tissues including the heart.^{25–27}

Further, expression of Smad7, an endogenous inhibitor of TGF- β induced signaling, attenuates experimentally induced lung and kidney fibrosis.^{28,29} Taken together, these observations suggest that sustained activation of TGF- β can lead to pathological tissue fibrosis and/or tissue dysfunction in a variety of organs, including the heart. The results of the present study both complement and extend these previous studies by showing for the first time that an orally available T β R1 antagonist, NP-40208, is sufficient to attenuate myocardial fibrosis, as well as decrease myocardial stiffness in the adult heart. Further, the present study provides provisional evidence that increased Smad 3 signaling is responsible for the increased myocardial fibrosis observed in the MHCsTNF mouse hearts, insofar treatment with NP-40208 resulted in a significant attenuation of Smad 2/3 nuclear translocation in the MHCsTNF mice. This statement notwithstanding, we cannot exclude a potential contributory role for Smad independent pro-fibrotic mechanisms, including activation of extracellular signal-regulated kinase,³⁰ the Rho/Rho-kinase signaling pathway,³¹ and connective tissue growth factor.^{32,33}

Although TGF- β mediated signaling has been shown to be important in myocardial fibrosis, it is worth noting a recent study in transgenic mice with cardiac restricted overexpression of a constitutively active TGF- β_1 mutant, which showed that the expression of this constitutively active mutant did lead to the development of ventricular fibrosis.³⁴ Moreover, fibrosis was not observed in hearts engrafted with skeletal myoblasts expressing high levels of TGF- β_1 .³⁵ While these latter two observations do not necessarily obviate a potentially important role for TGF- β as a mediator of cardiac fibrosis, they do suggest that TGF- β per se may be insufficient to promote fibrosis in the adult heart, and that the profibrotic actions of TGF- β may be the result of important time-dependent interactions between TGF- β and the complex milieu of growth factors and inflammatory cytokines (TNF, IL-1 β) that are expressed following tissue injury. Germane to the present discussion, we have shown previously that the myocardial fibrosis observed in the MHCsTNF mice is sensitive to treatment with losartan, an angiotensin type 1 receptor antagonist.²⁰ These latter observations are consistent with the point of view that TGF- β may be one of several important mediators that act in concert during the development the progressive myocardial fibrosis that occurs in the setting of sustained pro-inflammatory signaling.

In summary, this study suggests that the TGF- β pathway plays an important role in the development of the progressive myocardial fibrosis and LV chamber stiffness that occurs in the setting of sustained pro-inflammatory signaling. The results of the present study also raise the interesting possibility that the TGF- β pathway may represent a therapeutic target in inflammatory cardiomyopathies.

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Figure 1.

Nuclear and cytoplasmic Smad 2/3 levels in littermate control and MHCsTNF mice. Panel A: Representative Western blots of Smad 2/3 in nuclear and cytoplasmic fractions in diluent treated littermate control, NP-40208 treated littermate control, diluent treated MHCsTNF, and NP-40208 treated MHCsTNF mice. Panel B: Results of group data for Smad 2/3 levels in nuclear and cytoplasmic fractions and the ratio of nuclear to cytoplasmic Smad 2/3 in diluent treated littermate control (n=3 hearts), NP-40208 treated littermate controls (n=3 hearts), diluent MHCsTNF (n = 3 hearts) mice, and NP-40208 treated MHCsTNF (n = 4 hearts) mice. (Key: * p < 0.05 vs. diluent and NP-40208 treated littermate controls, † p < 0.05 vs. diluent treated MHCsTNF mice).

Figure 2.

Heart-weight to body-weight ratio in diluent treated littermate controls (n=7), NP-4-2-8 treated littermate controls (n=6), diluent treated MHCsTNF (n=6) mice, and NP-40208 treated MHCsTNF (n=6) mice. (Key * $p < 0.05$ vs. diluent and NP-40208 treated littermate controls, † $p < 0.05$ vs. diluent treated MHCsTNF mice).

Figure 3.

Effect of T β RI antagonism on myocardial fibrillar collagen content in littermate control and MHCsTNF mice. Panel A: Representative picrosirius red staining of myocardial samples from diluent treated littermate control, NP-40208 treated littermate control, diluent treated MHCsTNF, and NP-40208 treated MHCsTNF mice. Panel B: Results of group data for fibrillar collagen content (expressed as percentage of myocardial area) in diluent treated littermate control (n=5), NP-40208 treated littermate control (n=5), diluent treated MHCsTNF (n=8) mice, and NP-40208 treated MHCsTNF (n=7) mice. (Key * p < 0.05 vs. diluent and NP-40208 treated littermate controls, † p < 0.05 vs. diluent treated MHCsTNF mice).

Figure 4.

Diastolic pressure volume relationship and LV chamber stiffness in littermate control and MHCsTNF mice. Panel A: Diastolic pressure-volume relationship for littermate control and MHCsTNF mice. Panel B: Results of group data for LV chamber stiffness constant in littermate control (n=10) and MHCsTNF (n=10) mice. (Key: LM = Littermate; * $p < 0.05$ vs. diluent treated littermate controls; † $p < 0.05$ vs. diluent treated MHCsTNF mice)

Figure 5.

Effect of T β IR antagonism on diastolic pressure volume relationship and LV chamber stiffness in littermate control and MHCsTNF mice. Panel A: Diastolic pressure-volume relationship for diluent treated littermate control (n=7 hearts), NP-40208 treated littermate controls (n= 6 hearts), diluent treated MHCsTNF (n=7 hearts), and NP-40208 treated MHCsTNF (n=6 hearts) mice. Panel B: Results of group data for chamber stiffness constant in diluent treated littermate control (n=7 hearts), NP-40208 treated littermate control (n=6), diluent treated MHCsTNF (n=7), and NP-40208 treated MHCsTNF (n=6) hearts. (Key: LM = littermate; *p < 0.05 vs. diluent and NP-40208 treated littermate controls, † p < 0.05 vs. diluent treated MHCsTNF mice).

Figure 6.

Fetal gene expression in littermate control and MHCsTNF mice. Representative Northern blots for Serca2a, alpha-myosin heavy chain (α -MHC) and beta-myosin heavy chain (β -MHC) in diluent treated littermate control, NP-40208 treated littermate control, diluent treated MHCsTNF mice and NP-40208 treated MHCsTNF mice.

Table

Functional Parameters in Littermate Control and MHCsTNF Hearts

	Littermate		MHCsTNF		p-value
	Untreated (n=7)	Treated (n=6)	Untreated (n=7)	Treated (n=6)	
Systolic LVP (mmHg)	94.8 ± 2.4	98.8 ± 3.2	100.6 ± 2.4	100.2 ± 2.6	0.46
LVEDP (mmHg)	9.8 ± 0.4	9.5 ± 0.7	8.6 ± 0.5	9.8 ± 0.3	0.23
LVDP (mmHg)	85.0 ± 2.4	89.1 ± 3.8	93.0 ± 3.0	90.4 ± 2.4	0.24

Key: LVP = left ventricular pressure, LVEDP = left ventricular end-diastolic pressure, LVDP = left ventricular developed pressure.