Sex-Specific Impact of Aldosterone Receptor Antagonism on Ventricular Remodeling and Gene Expression after Myocardial Infarction

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Abstract

Aldosterone receptor antagonism reduces mortality and improves post-myocardial infarction (MI) remodeling. Because aldosterone and estrogen signaling pathways interact, we hypothesized that aldosterone blockade is sex-specific. Therefore, we investigated the impact of eplerenone on left ventricular (LV) remodeling and gene expression of male infarcted rats versus female infarcted rats. MI and Sham animals were randomized to receive eplerenone (100 mg/kg/day) or placebo 3 days post-surgery for 4 weeks and assessed by echocardiography. In the MI placebo group, left ventricular end-diastolic dimension (LVEDD) increased from 7.3 ± 0.4 mm to 10.2 ± 1.0 mm ($p < 0.05$) and ejection fraction (EF) decreased from 82.3 ± 4% to 45.5 ± 11% ($p < 0.05$) in both sexes (p = NS between groups). Eplerenone attenuated LVEDD enlargement more effectively in females (8.8 ± 0.2 mm, $p < 0.05$ vs. placebo) than in males (9.7 ± 0.2 mm, p = NS vs. placebo) and improved EF in females (56.7 ± 3%, $p < 0.05$ vs. placebo) but not in males (50.6 ± 3%, p = NS vs. placebo). Transcriptomic analysis using Rat_230–2.0 microarrays (Affymetrix) revealed that in females 19% of downregulated genes and 44% of upregulated genes post-MI were restored to normal by eplerenone. In contrast, eplerenone only restored 4% of overexpressed genes in males. Together, these data suggest that aldosterone blockade reduces MI-induced cardiac remodeling and phenotypic alterations of gene expression preferentially in females than in males. The use of transcriptomic signatures to detect greater benefit of eplerenone in females has potential implications for personalized medicine.

Keywords: myocardial infarction, heart failure, sex, aldosterone antagonism, gene expression

Introduction

Myocardial infarction (MI) is a major cause of left ventricular (LV) remodeling and, thus, of heart failure and sudden cardiac death.[1–3] Several lines of evidence supporting a key role for aldosterone in LV remodeling provided the rationale for clinical trials that demonstrated clinical efficacy of aldosterone receptor antagonists to treat patients with established heart failure and MI.[4,5]

There is accumulating clinical and experimental evidence that female sex may play a significant role in cardiovascular responsiveness to the aldosterone signaling pathway. For example, serum levels of aldosterone correlate with LV hypertrophy and LV mass index in women but not in men,[6] and in rodent models, activation of estrogen receptors protects the cardiovascular system against the detrimental effects of aldosterone-salt treatment, including effects on blood pressure, cardiac hypertrophy, and vascular fibrosis.[7]

Of critical importance, clinical and experimental studies have demonstrated that estrogen and mineralocorticoid receptors are both expressed in cardiac myocytes, fibroblasts, and vascular cells,[8,9] and sexual dimorphism in neurohumoral activation may influence cardiac remodeling, response to therapy,[10–13] and survival.[14] Accordingly, we predicted that females would be more responsive to aldosterone receptor blockade post-MI compared with males. We addressed this hypothesis by administering eplerenone to rats of both sexes following MI.

Methods

Animal model

MI induced by permanent coronary artery ligation or sham surgery was performed in female and male 6-month-old Wistar rats. Briefly, the rats were intubated and ventilated with 2% isoflurane in oxygen using a rodent respirator (Model 683; Harvard Apparatus, Inc., MA, USA). The heart was exposed via a left lateral thoracotomy, and the left anterior descending coronary artery was permanent ligated 2–3 mm below its origin with a 6–0 silk suture. The chest was closed, and the rat was allowed to recover under care.

The animals were randomly assigned to receive either eplerenone (100 mg/kg/day in food; Research Diets, Inc., NJ, USA) or placebo starting from 3 days post surgery and up to sacrifice 4 weeks later. The 3-day post-MI time point was chosen for the initiation of treatment with eplerenone due to higher mortality within 48 hours. The institutional animal care and use committee of The Johns Hopkins University School of Medicine approved all protocols and experimental procedures.

Echocardiographic measurements

Echocardiographic measurements were obtained at baseline, 5 days, 2 weeks, and 4 weeks. Echocardiographic assessments were performed in anesthetized (2% isoflurane inhalation) rats using a Sonos-5500 Echocardiogram (Philips, MA, USA) equipped with a 15-MHz transducer. Cardiac dimensions such as left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) and fractional shortening (FS) were recorded from M-mode images using averaged measurements from three to five consecutive cardiac cycles according to the American Society of Echocardiography. Ejection fraction (EF) was calculated from parasternal long-axis view using Simpson's modified single plan method.

Tissue collection

At the end of the study, rat hearts were harvested for further analysis. The hearts were weighted and the basal portion, free of fibrotic tissue, was flash-frozen in liquid nitrogen for RNA isolation and microarray analysis. The remaining tissue was fixed with Streck Fixative Tissue (Streck Laboratories Inc., NE, USA) for histology. Slides were prepared with Masson's

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trichrome stain to assess the presence and extent of fibrosis and myocardial scar. Infarct size was calculated as the percentage of LV perimeter in four transverse slices at least 2 mm apart, as previously described.[15] The size of MI was determined using the public domain NIH Image program (NIH-Image version 1.30v for Windows) developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image. To quantify the percentage area of fibrosis, an image-processing software (Imaging Processing Toolkit-5.0; Reindeer Graphics, NC, USA) and Adobe Photoshop-CS2 (Adobe Systems Inc., San Jose, CA, USA) were used to assess the slides as previously described with minor modifications.[16] The percentage of fibrosis was calculated using the following formula:

$$\%\text{fibrosis} = \text{fibrotic area}/(\text{fibrotic area} + \text{healthy area}) \times 100$$

RNA isolation and microarray analysis

Total RNA from the heart tissue was purified using the RNeasy Mini-Kit (Qiagen Inc., Valencia, CA, USA). The quality of extracted RNA was tested with the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). OD 260/280 ratio was in the range of 1.8–2.1 for all samples. The RNA was transcribed into cDNA, biotin-labeled, and hybridized to the Rat_230–2.0 Array from Affymetrix (Affymetrix, Inc., Santa Clara, CA, USA).

Microarray raw data were normalized with robust multiarray analysis (RMA) in R (available at www.r-project.org) and further analyzed with Decision Site Functional Genomics from Spotfire (Palo Alto, CA, USA), GeneSifter from VizXLabs (Seattle, WA, USA), GoMiner (Genomics and Bioinformatics Group [GBG] of LMP, NCI, NIH and the Medical Informatics and Bioimaging group of BME, Georgia Tech/Emory University),[17] Cluster, and Tree view (Eisen Lab, Berkeley, CA, USA).[18] A threshold of >1.4 fold-change (FC) and $p < 0.05$ (*t*-test) were used to identify differentially expressed genes. Gene clusters were manually selected using Tree view software based on (1) the unsupervised hierarchical gene classification tree, (2) a minimal number of 40 genes, and (3) a minimal correlation coefficient of 0.6.

Validation

Probe sets were designed for a subset of differentially expressed genes identified in this study; each gene and each sample were run in triplicates. First, QuantiGene assay (Panomics, Inc., CA, USA)[19] was performed to validate changes in three selected genes in the female group, and expression of beta-actin was used for normalization of the data. Second, real-time RT-PCR was performed to validate changes in four selected genes in both groups. First-strand cDNA was synthesized from 1 μg total RNA using the High-Capacity cDNA Reverse-Transcription Kit (Applied Biosystems, Inc., Foster City, CA, USA), and ribosomal 18S RNA served as the housekeeping gene. We used TaqMan probes labeled with 6-carboxyfluorescein (FAM) for real-time RT-PCR reactions, according to manufacturer's protocol (Applied Biosystems, Inc., Foster City, CA, USA). Data were analyzed by the threshold cycle (Ct) relative quantification method.

Statistical analyses

All values are shown as mean ± SEM. Echocardiographic parameters during the 4-week follow-up were compared within and between groups using one-way ANOVA for repeated measurements and two-way ANOVA followed by the Holm–Sidak test, respectively. For a given parameter, $p < 0.05$ was considered significant. All tests were carried out using Sigma Stat 3.5 (Jandel, San Rafael, CA, USA).

Results

Effects of MI on cardiac remodeling

Sex comparisons of body weight and infarct size are presented in *Table 1*. Although female rats were significantly smaller than male rats, as assessed by body weight, the impact of MI was similar in placebo conditions, resulting in similar infarct sizes (42 ± 4% vs. 48 ± 4%, p = NS) and cardiac fibrosis (29 ± 3% vs. 34 ± 1%, p = NS) in males and females, respectively (*Figures 1A* and *1B*). MI led to LV remodeling, as evidenced by LV chamber enlargement, and reduced EF and FS. As with infarct size, the degree of remodeling was similar between the two sexes (*Figure 2*).

Neither early mortality (within 48 hours; 28% vs. 28.5%, $p = 1$, Fisher exact test) nor total mortality at the end of the study (33% vs. 28.5%, $p = 1$, Fisher exact test) was different between both sexes.

Effects of eplerenone on cardiac remodeling

Eplerenone treatment significantly prevented LV remodeling, preferentially in female rats than in male rats. The increases in both LVEDD (43 ± 5.5% vs. 29 ± 6.2%, $p < 0.05$) and LVESD (142 ± 15.3% vs. 78 ± 7.6%, $p < 0.05$) were less in eplerenone-treated females versus placebo (*Figures 2A* and *2B*, respectively). In addition, the reduction in EF (*Figure 2C*) due to MI was ameliorated by eplerenone in females (47 ± 4.7% vs. 29 ± 4.1%, $p < 0.05$). Conversely, neither the changes in LVEDD (39 ± 4.8% vs. 26 ± 2.3%, p = NS) and LVESD (122 ± 8.9% vs. 91 ± 12.4%, p = NS) nor the reduction of EF (51 ± 4.1% vs. 38 ± 7.5%, p = NS) were statistically influenced by eplerenone relative to placebo in the male group. By week 4, changes in FS in both sexes were not modified by treatment (*Figure 2D*).

Finally, eplerenone also reduced infarct size (*Table 1*) in females (from 48 ± 4% to 33 ± 3%, $p < 0.05$) but not in males (from 42 ± 2% to 35 ± 4%, p = NS). Similarly, the impact of eplerenone in cardiac fibrosis (*Figure 1A*) showed reduction in the female group (from 34 ± 1% to 27 ± 2%, $p < 0.05$) but not in the male group (from 29 ± 3% to 28 ± 3%, p = NS).

Impact of eplerenone on MI-induced gene expression

Transcriptomic analysis was performed in order to investigate molecular correlates of the phenotypic changes observed between male and female rats. MI caused upregulation of 397 genes in females and 201 genes in males and downregulation of 74 and 252 genes in respective groups (*Tables S1–S9*, supporting information).

The expression profile of these genes was analyzed for both sexes using hierarchical clustering. Five major clusters of genes, each with a specific expression profile, were identified (*Figure 3*). Gene expression modifications after MI were congruent for most of these genes in both groups (clusters A, B, and E). More specifically, cluster A illustrates genes downregulated after MI in both sexes and reset to normal in the female group only, such as potassium channel subfamily-K, member2 (Kcnk2), sodium channel voltage-gated, type-IV, beta (Scn4b), or inositol (myo)-1 or 4-monophosphatase 2 (Impa2). Cluster B contains genes upregulated in both sexes and reset to normal in the female group only, such as thrombospondin-4 (Thbs4), transforming growth factor beta-1-induced transcript 1 (Tgfb1i1), collagen

			Male		Female	
			Sham (7)	MI (6)	Sham (7)	MI (5)
Baseline	BW (g)	Placebo	537 ± 20.3*	542 ± 11.8*	351 ± 11.2	357 ± 12.8
		Treated	532 ± 17.1*	551 ± 28.4*	364 ± 11.3	349 ± 7.4
4 weeks	BW (g)	Placebo	537 ± 20.2*	546 ± 11.5*	365 ± 13	360 ± 9.4
		Treated	541 ± 18.2*	547 ± 28.3*	356 ± 9.9	350 ± 10.4
	MI size (%)	Placebo	–	42 ± 2	–	48 ± 4
		Treated	–	35 ± 4	–	33 ± 3†

Results are presented as mean ± SEM.
*$p < 0.01$ versus female group.
†$p < 0.05$ versus female control.
BW = body weight; MI size = infarct size.

Table 1. Sex comparison of placebo and treated rats at baseline and at 4 weeks post-MI.

type-I (Col1a1), lamin gamma-1 (Lamc1), tissue inhibitor of metalloproteinase 1 and 2 (Timp1 and Timp2), fibrillin 1 (Fbn1), and extracellular matrix protein 1 (Ecm1). Cluster E depicts genes upregulated in both groups and not affected by eplerenone treatment such as actin-alpha cardiac muscle 1 (Actc1), integrin beta-1 (Itgb1), or gap junction protein alpha 1 (Gja1) also known as connexin 43. Interestingly, we detected divergent expression changes of some genes in both sexes after MI (Cluster C and D). Cluster C showed genes upregulated after MI only in the female group and reset to normal after eplerenone treatment such as insulin growth factor 1 (Igf1) or cyclin D1 (Ccnd1). Cluster D contains genes downregulated only in males after MI and not restored to normal level by eplerenone treatment. Representative genes for this cluster were tropomyosin-1 alpha (Tpm1), activated leukocyte cell adhesion molecule (Alcam), or insulin-like growth factor binding protein 5 (Igfbp5). For each cluster, gene expression profiles and corresponding gene ontology (GO) annotations are detailed in *Figure S1* (supporting information). Finally, other highly induced genes after MI were natriuretic peptide precursor type-B (Nppb) and Fc gamma receptor 2 beta (FcG).

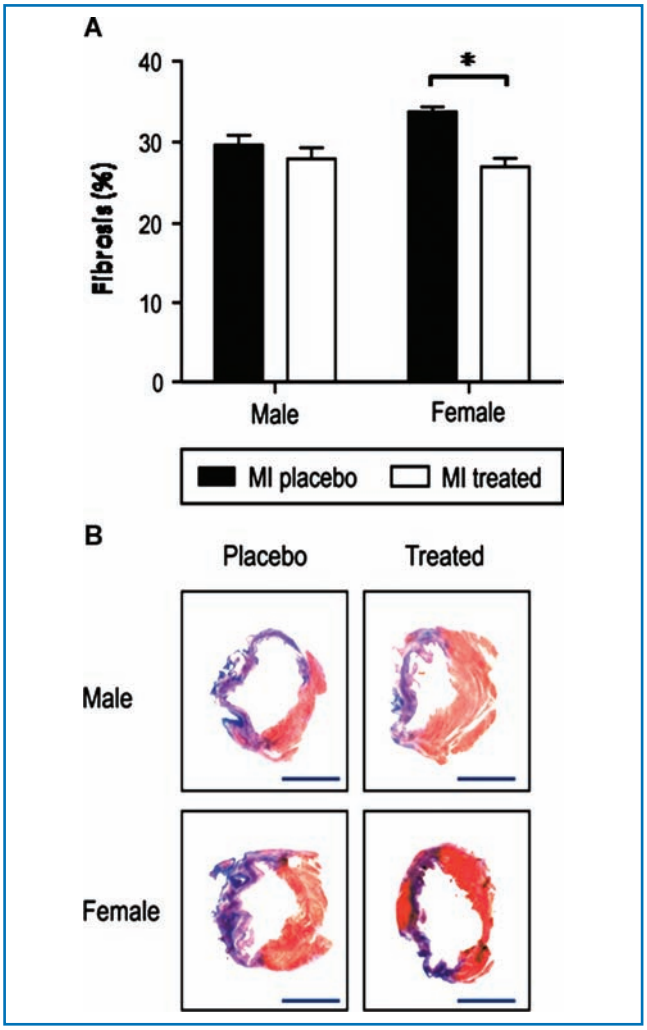

Figure 1. (A) Bar graphs showing percentage of cardiac fibrosis in male and female infarcted rats; *$p < 0.05$ versus placebo, same sex. Values are means ± SEM. (**B**) Trichrome-stained histological cross-sections of myocardial tissue from male placebo (top left) versus male treated (top right) and female placebo (bottom left) versus female treated (bottom right). Scale bar corresponds to 5 mm.

Following this, the impact of eplerenone treatment was examined in a sex-specific manner. Consistent with the reduced efficacy of eplerenone in male rats (*Figures 4A* and *4B*), only 4% of 252 underexpressed genes and none of the upregulated genes were normalized. Eplerenone had a substantial impact on the transcriptomic response to MI in female rats (*Figures 4C* and *4D*). Of the 397 genes upregulated by MI, 48% were downregulated and restored to normal. Similarly, of the 74 genes that were downregulated, 24% were restored to normal levels.

GO annotation was used for categorization of genes according to their involvement in different biological processes. The contribution of genes that were affected by eplerenone treatment after MI in male and female rats to various biological processes in the cell is illustrated in *Figures 5A* and *5B*, respectively. The expression level of genes involved in potassium ion transport and fatty acid metabolism that were downregulated post-MI for both sexes were restored to normal level only in female treated animals. We also detected a remarkable number of genes that were upregulated post-MI and restored to normal level by eplerenone in the female group only. Among those, genes involved in inflammation such as complement component 5 receptor 1 (C5ar1) and serine peptidase inhibitor, clade G, member-1 (Serping1), and pancreatitis-associated protein (PAP) were highly represented, as well as genes involved in extracellular matrix remodeling such as collagen type-I (Col1a1) and III (Col3a1).

Other highly overexpressed genes after MI, which were normalized by eplerenone, were periostin (Postn), fast myosin alkali light chain (RGD620885), FcG, and monoamine oxidase A (Maoa). Tgfb1i1, which is involved in androgen receptor binding

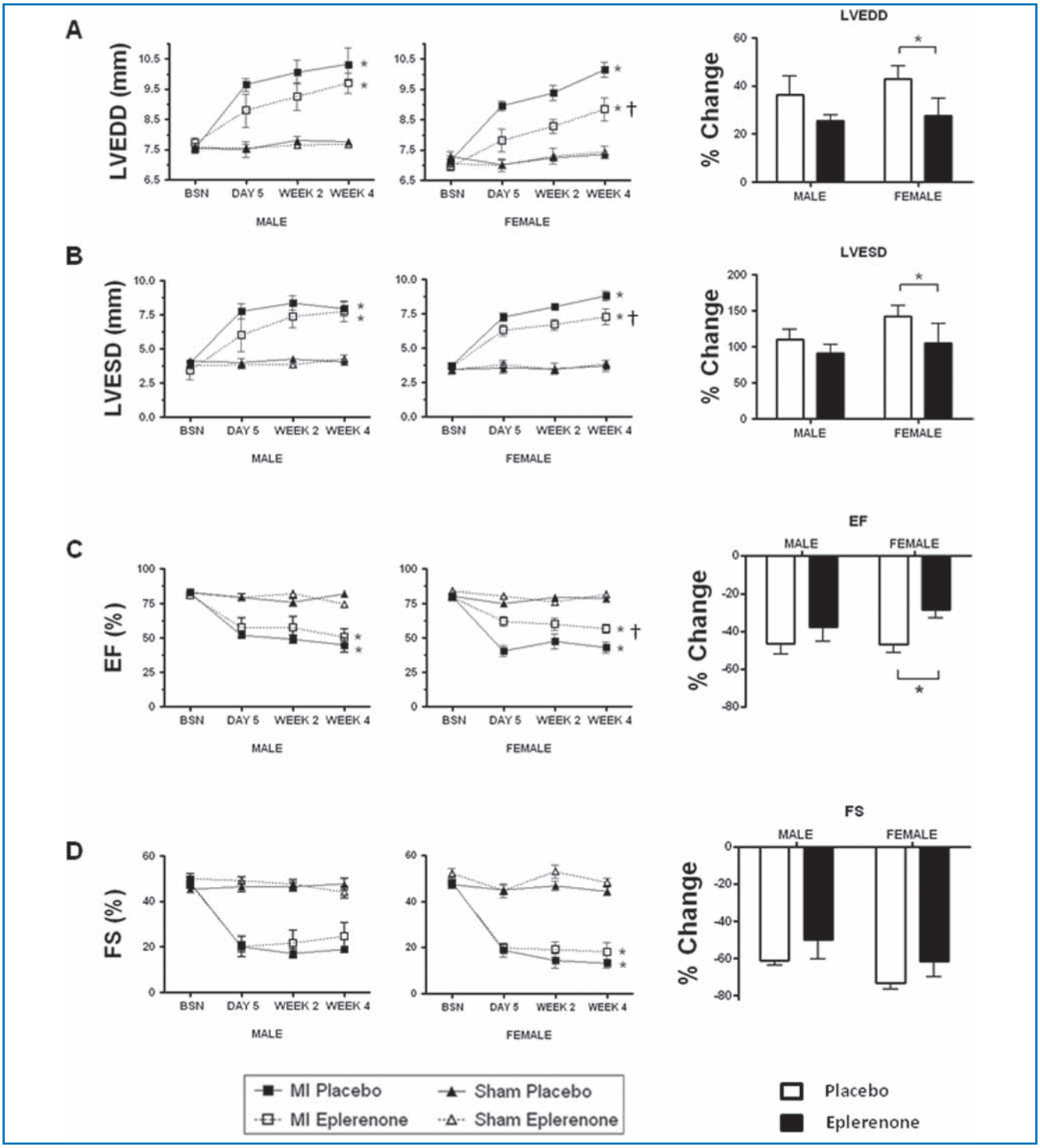

Figure 2. Line graphs showing changes in LVEDD (A), LVESD (B), EF (C), and FS (D) over time in male and female rats after MI or Sham surgery. *$p < 0.05$ versus BSN, same sex; †$p < 0.05$ versus placebo, same sex, same time point. Bar graphs showing percentage of changes in male and female infarcted rats at week 4. *$p < 0.05$ versus placebo, same sex. Values are means ± SEM.

and which was upregulated in both groups after infarction, was unaffected by treatment.

We next examined the transcriptomic changes in three biologically relevant candidate pathways. *Figures 6A* and *6B* illustrate the impact of eplerenone on genes related to renin–angiotensin–aldosterone (RAA) system and gonadal hormones (estrogen, progesterone, and androgens), respectively. Interestingly, angiotensin-II receptor expression displayed a trend towards divergent transcriptional pattern between both sexes. Eplerenone induced the downregulation of angiotensin-II receptor type-1 (Agtr1b) (FC = −1.1, $p = 0.07$, *t*-test) and upregulation of angiotensin-II receptor type-2 (Agtr2) (FC = +1.1, $p = 0.09$, *t*-test) in the female group but not in the male group. Moreover, genes related to sex hormones were only affected in females;

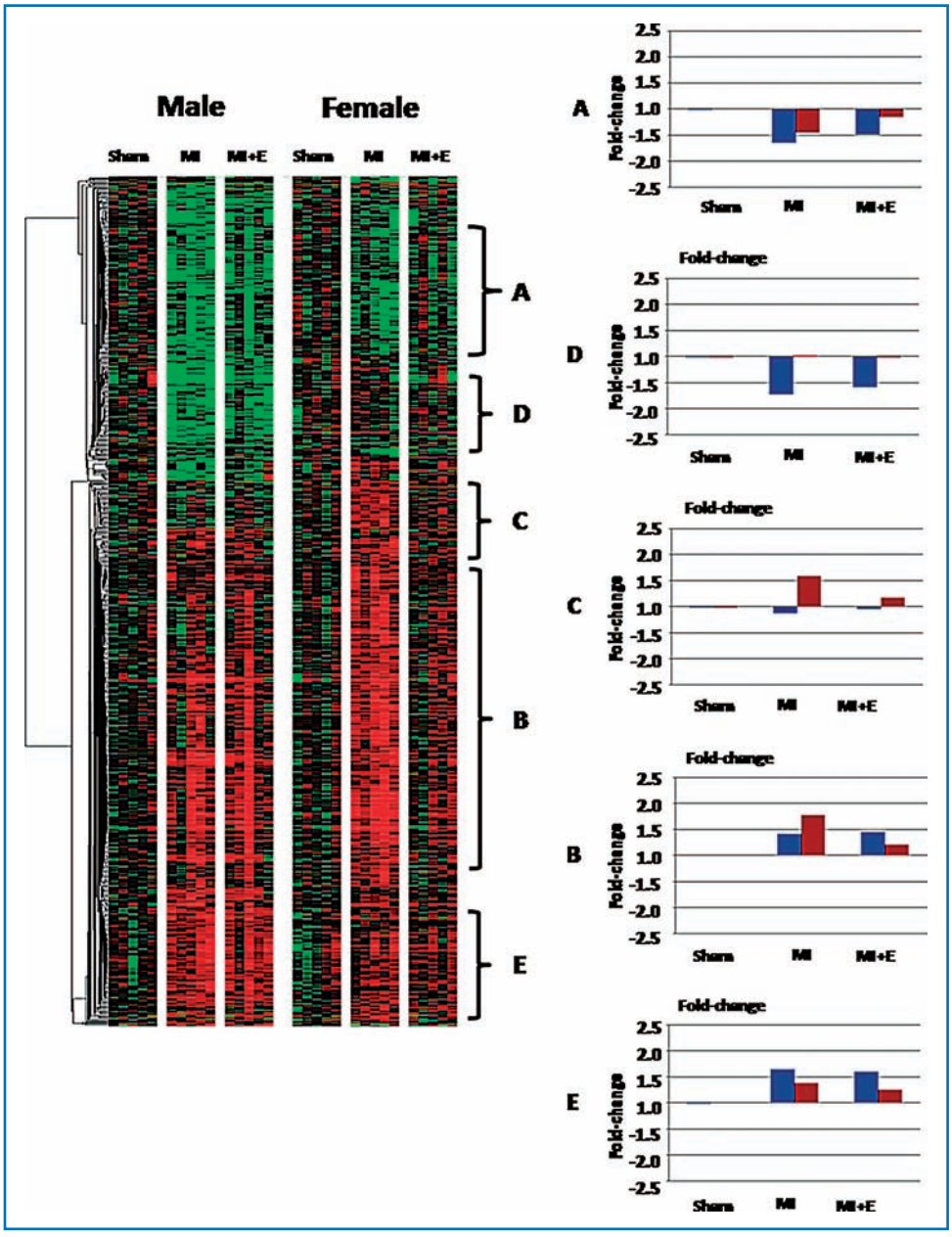

Figure 3. Left: Hierarchical clustering of the genes differentially expressed after MI. Expression data are centered on genes so that the mean value for each Sham group is zero. For each gender, red and green colors represent up- and downregulation relative to Sham, respectively. Right: Cluster-specific analysis of the mean expression profile in male and female rats. Blue and red bars correspond to male and female animals, respectively. Gene expression level is expressed as the fold-change versus same-gender Sham group.

progesterone membrane component 1 was downregulated (FC = −1.2, $p = 0.03$, t-test), whereas progesterone receptor was upregulated (FC = −1.1, $p = 0.04$, t-test) by eplerenone. *Figure 6C* illustrates the impact of eplerenone on genes related to fibrosis. In females, most of the genes were clearly restored to normal levels after treatment, whereas the same genes stayed unaffected in treated males.

We selected seven genes for validation using independent assays. These included three differentially expressed genes (Thbs4, PAP, and FcG) in the female group and four other genes related to fibrosis (Timp1, matrix metalloproteinase 2 [MMP2], Col1a1, and Col3a1). In all cases, independent quantitative validation confirmed microarray results (*Figures S2* and *S3*, supporting information).

Discussion

The major new finding of this study is that aldosterone receptor antagonism with eplerenone preferentially restores altered gene expression to normal in female rats compared with male rats post-MI. In addition, eplerenone tended to be more potent at attenuating LV chamber enlargement and EF reduction in female rats rather than male rats. Alterations in gene expression occurred not only at a global level but also in specific biologically plausible pathways, notably the RAA and fibrosis-inducing pathways. These findings have important pathophysiologic and therapeutic implications for sex-specific approaches to post-MI therapeutics.

Sex-related differences in response to eplerenone therapy

Sex-specific differences regarding the relationship of aldosterone with cardiac structure and function are described in humans.[6,20] In a large cohort of subjects without history of MI or heart failure, Vasan et al.[6] reported that serum aldosterone levels positively correlated with LV wall thickness and relative wall thickness in women but not in men. Similarly, aldosterone levels negatively correlated with LVEDD in women but not in men. These findings demonstrate a positive association between aldosterone serum level and concentric LV remodeling patterns in women but not in men. Together with these observations, our results suggest that aldosterone pathway activation may have a more direct involvement in the LV remodeling process in females than in males.

Interactions between estrogen and aldosterone metabolism are a potential mechanism to explain our results. Both estrogen and RAA systems are present and functional in the myocardium and exert their effect through nongenomic and genomic pathways. In epithelial systems, rapid nongenomic response to estrogen and aldosterone stimulation are mediated through a common pathway involving protein kinase C.[21] Ovariectomy worsens cardiac remodeling after MI in association with an increase in angiotensin-II and aldosterone plasma levels.[22] Estrogen supplementation reduces the intensity of post-MI LV remodeling in both castrated male rats and ovariectomized female rats, whereas testosterone had an exact inverse effect.[23] Furthermore, activation

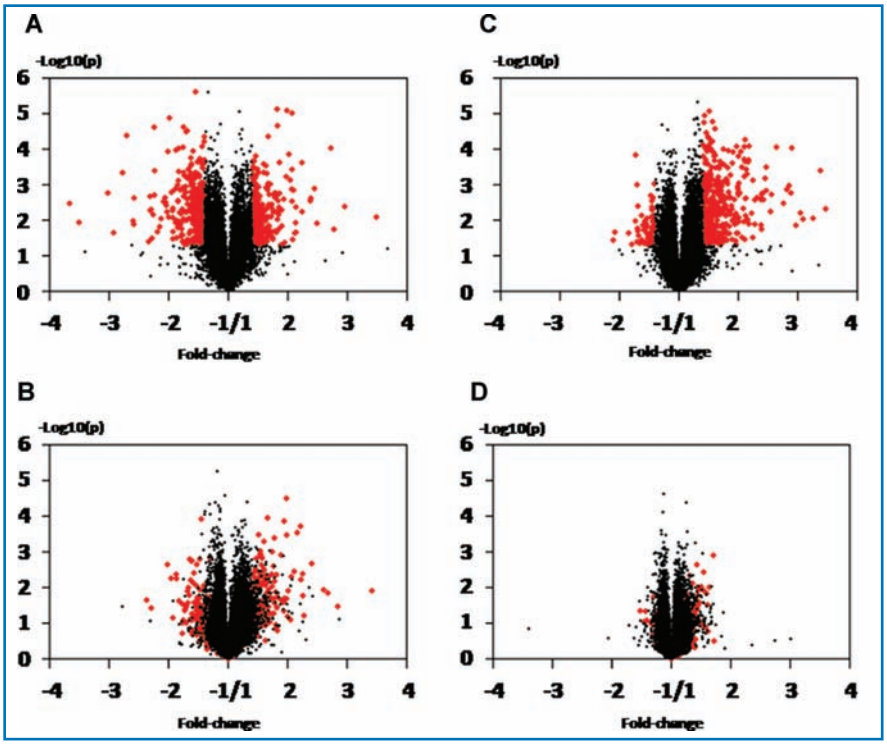

Figure 4. Volcano plot graphs showing the significance and magnitude of change in expression in the male (A, B) and female (C, D) groups. Expression profile of the Sham nontreated group is compared with expression profile of the MI nontreated group (**A**, **C**) and the MI treated group (**B**, **D**), respectively. Differentially expressed genes are selected based on their statistical significance ($p < 0.05$, *t*-test) and variation in amplitude (fold-change <−1.4 or >1.4) are shown in red. X-axis: Fold-change of gene expression levels between the two compared groups. Y-axis: *p* is the *p*-value obtained from the comparison (*t*-test) of gene expression levels in the two groups.

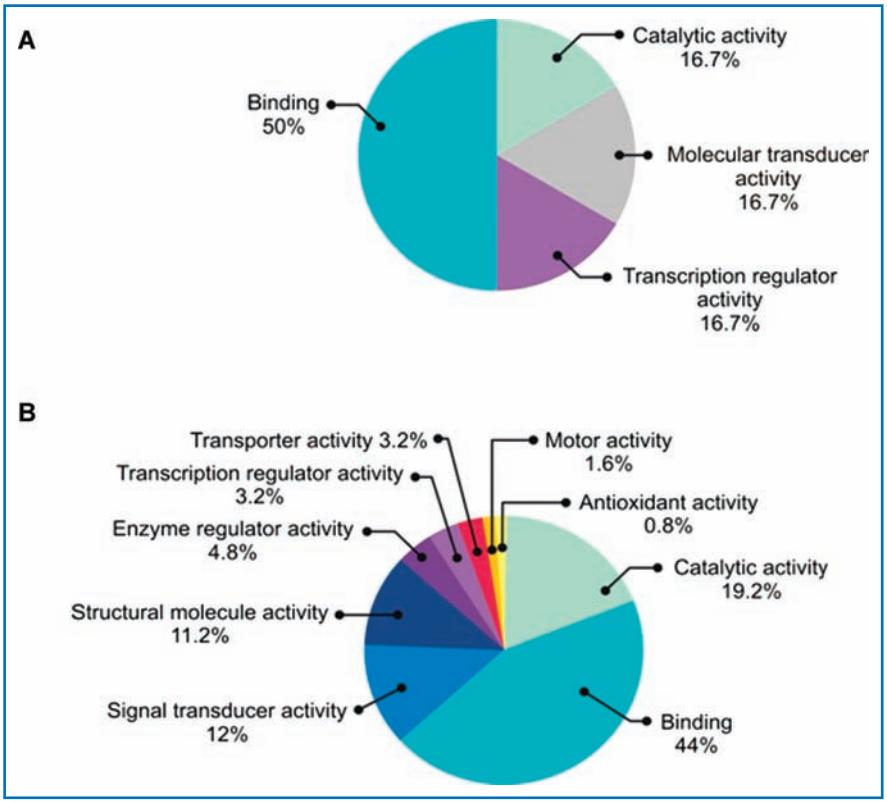

Figure 5. Pie charts showing distribution of genes differentially expressed after eplerenone therapy in male (A) and female (B) infarcted rats on the basis of their involvement in different biological processes (>1.4 fold, $p < 0.05$). Data in pie charts are expressed as a percentage of total genes.

of either estrogen receptor alpha or beta has been shown to protect aldosterone-salt-treated rats against hypertension, cardiac hypertrophy, and vascular fibrosis through abolition of aldosterone-induced cardiac gene expression changes.[7] These data suggest that estrogen interacts with the aldosterone pathway and modulates its biological effects.

Whether sex-specific responsiveness to eplerenone therapy post-MI exists in humans is unclear. The Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) trial is the major clinical investigation of eplerenone in patients with acute MI and LV dysfunction.[5] Consistent with our findings, sex-specific analysis on all-cause mortality showed a trend towards a greater benefit for women treated with eplerenone as compared with men at 30 days (interaction $p = 0.089$). However, these results were not verified at 16 months, and the other primary endpoint, defined as death for cardiovascular cause or hospitalization for cardiovascular events, trended toward a greater benefit for men at 16 months (interaction $p = 0.08$). In the Randomized Aldactone Evaluation Study (RALES) trial,[4] which investigated the effect of spironolactone on symptomatic heart failure patients, treatment benefit was not different between men and women. These findings should be interpreted cautiously as women represented only 30% of the patients enrolled in these studies and sex-based comparison of the treatment efficacy was not prespecified.

Previous studies have reported the effects of eplerenone on post-MI LV remodeling in the male rat. Interestingly and consistent with the present findings, two previous studies in rats treated with high salt diet[24,25] did not observe a significant reduction in infarct size between treated and placebo male rats. In contrast, one of these studies[25] did report a significant reduction in LV diameter and improved EF in treated male rats versus placebo male rats. As no previous study examined male and female animals head to head, ours is the first to explore whether a sex preference exists in response to aldosterone antagonism.

Transcriptomic changes

At the transcriptomic level, most (but not all) of the MI-related gene expression changes were similar in male and female groups. These changes involved numerous molecular pathways such as fatty acid metabolism, potassium ion transport, inflammation, extracellular matrix remodeling, and apoptosis or cell

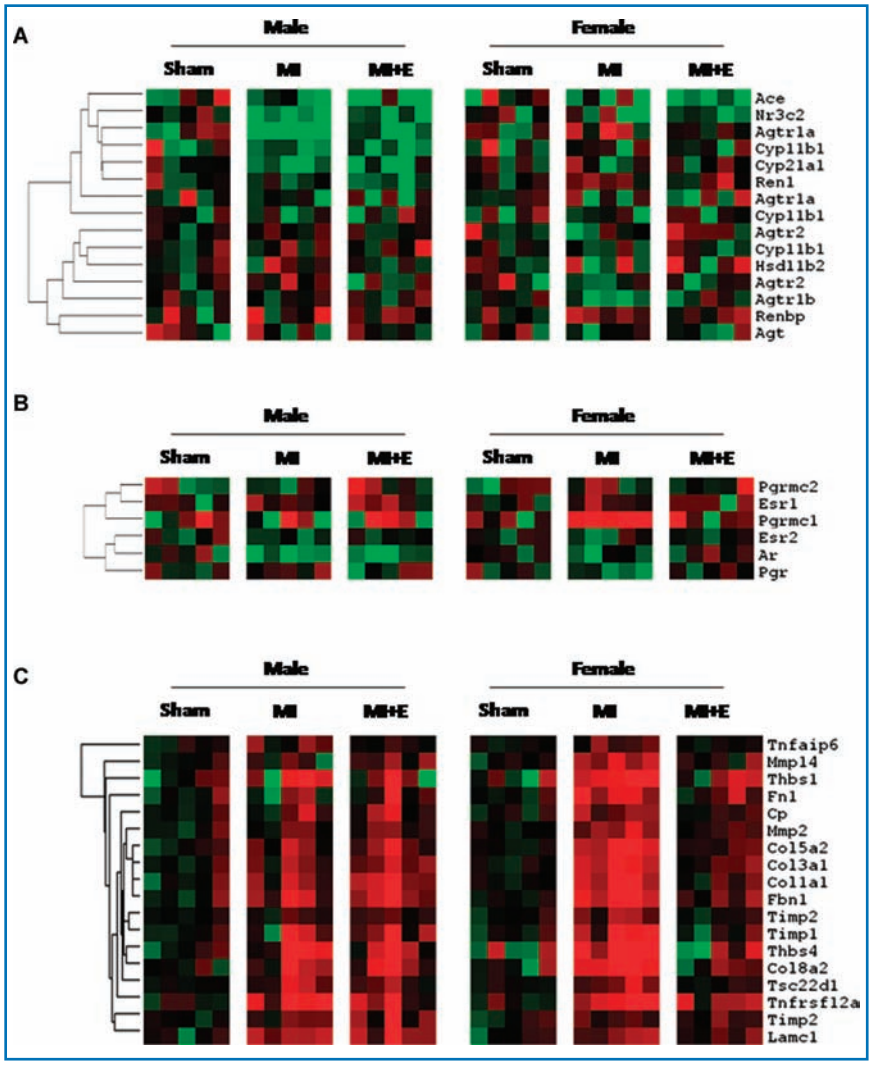

Figure 6. Representative heat map of the impact of eplerenone after MI or Sham surgery on genes related to RAA system (A), gonadal hormones (B), and fibrosis (C) in male and female rats. Expression data are centered on genes so that the mean value for each Sham group is zero. For each gender, red and green colors represent up- and downregulation relative to Sham, respectively. Genes are indicated using their official symbol (gene name is provided in *Table S10*, supporting information).

growth. Consistent with previous findings, genes involved in fatty acid metabolism were downregulated post-MI, whereas genes involved in collagen synthesis and inflammatory response were overexpressed.[26–28]

Accumulating evidence indicates that the myocardial Ang-II-generating pathway is also activated in MI.[29] Indeed, increased cardiac expression of angiotensinogen, Angiotensin converting enzyme (ACE) and Angiotensin-II subtype 1 (AT1) receptor proteins, ACE activity, and Ang-II content have been previously described in infarcted hearts,[29] and our study is consistent with these findings. At the transcriptomic level, reversal of a significant proportion of expression changes induced after MI paralleled improvement of LV function and reduction of infarct size in female rats, whereas eplerenone had very limited effect on the cardiac transcriptome in male rats. Importantly, when specific biologically relevant pathways were interrogated, such as genes involved in inflammatory responses and extracellular matrix formation, the preferential effect in females relative to males was born out.

Fibrosis is an important component of post-MI remodeling. Recent studies have shown that inhibition of MMPs can prevent early LV dilation in mice post-MI,[30] and infarcted mice with deficiency of MMP9 have decreased incidence of early myocardial rupture[31] and attenuated LV dilation.[32] Induction of fibrosis is one of the best documented effects of aldosterone on the heart. Aldosterone infusion increases expression of Col1a1 and Col3a1 and MMP2 and leads to cardiac fibrosis.[33] This phenomenon is reversed by eplerenone,[34] supporting a mineralocorticoid receptor-mediated effect. In humans, administration of spironolactone equally reduces post-MI LV remodeling with concomitant reduction of plasma procollagen type-III aminoterminal peptide level, a blood marker of fibrosis.[35] In our study, gene expressions of Col1a1 and Col3a1, Timp1, Timp2, and MMP2 were increased in both sexes following MI, yet reset towards normal in female only. In addition, numerous other genes coding collagen network proteins or MMPs displayed the same expression profile and thus represent new putative targets of mineralocorticoid receptor activation.

Furthermore, aldosterone stimulates inflammatory response and its infusion in rats induces cardiac infiltration of inflammatory cells and upregulation of both glycosylated 91kDa glycoprotein (GP91phox), a subunit of NADPH oxidase, and nuclear factor-kB (NF-kB), alterations that are reduced by spironolactone.[36] Consistent with these findings, GP91phox, several genes coding for complement proteins, and numerous genes preferentially expressed in inflammatory cells such as CD14 or lipopolysaccharide-binding protein (LBP) were upregulated after MI and reset to normal only in female rats treated with eplerenone. These results suggest that eplerenone also reduces the infiltration of inflammatory cells following MI in female but not male rats.

With regard to estrogen-specific pathways, we noted that peroxisome proliferator activated receptor gamma-coactivator-1 (PPARGC1B), a tissue-specific coactivator of nuclear receptors expressed at high level in the heart and skeletal muscle, was upregulated after MI and reset to normal level by eplerenone treatment in female rats only. Kressler et al.[37] showed that PPARGC1B coactivates estrogen receptor-alpha (ESR1). Yeast 2-hybrid analysis showed ligand-dependent binding of the 2 LXXLL motifs of PPARGC1B to the ligand-binding domain of ESR1. The PPARGC1B LXXLL motifs and the ESR1 ligand-binding domain are necessary for ESR1 coactivation. PPARGC1B has been reported to be downregulated after hypoxia or pressure overload in the heart, and the activation of PPARGC1B has shown to reduce myocardial infarct size, prevent or attenuate cardiac fibrosis, and reduce apoptosis.[38] Conversely, Lygate et al.[39] reported that PPARG activator, rosiglitazone, did not modulate LV remodeling, but was associated with increased mortality post-MI in rats. Therefore, the role of PPARG in heart failure remains controversial, and the reason for the observed differences in the results are likely to be related to the differences in study design.[40] All these findings suggest that this pathway might be important for further investigation.

Our results confirm that aldosterone is an important mediator of LV remodeling occurring after MI and also suggest different levels of activation of the aldosterone pathway in both sexes. Females have shown being more sensitive to aldosterone stimulation and, consequently, to mineralocorticoid receptor inhibition.

Sex-related differences in LV remodeling

At the transcriptomic level, sex-specific gene expression changes following MI were detected. Several of these changes affected genes modulating cardiomyocyte growth. Interestingly, Igf1 was upregulated in the female but not in the male group after MI. Upregulation of Igf1 following MI has been previously reported in animal models.[41,42] Igf1 induces LV hypertrophy *in vivo* in normal rats, and overexpression of Igf1 attenuates LV remodeling following MI in mice through reduction of cell death.[43] In cardiomyocytes, Igf1 has been shown to increase gene expression of Ccnd1, a cell cycle regulator that can induce *in vitro* cardiomyocyte proliferation,[44,45] which was also found upregulated in our study after MI in the female group only. Interestingly, we also observed downregulation of Igfbp5 in the male group but not in the female group. Igfbp5 binds to Igf1 with high affinity and inhibits the Igf1–Igfr1 interaction.[46] A previous study reported downregulation of cardiac expression of Igfbp5 after MI in pigs.[47] Taken together, these results identify Igf1 signaling pathway as a putative candidate to explain sex-specific differences in post-MI LV remodeling.

Limitations

There are several limitations to be acknowledged in this study. First, sex-related differences in the pharmacokinetics of eplerenone have been described in rats due to more extensive metabolism in males as compared to females.[48] Thus, the observed differences in response to eplerenone therapy could be related to higher steady-state plasma concentration of eplerenone in female rats. Nevertheless, the dose used in our study (100 mg/kg/day in food) is comparable to other studies in male rats and has proven to achieve effective mineralocortiocoid receptor antagonism.

Second, eplerenone treatment was only initiated 3 days post-MI. This time point was selected based on the high mortality during the first 48 hours post-MI; however, this should not represent selection bias as the early mortality post-MI was not different between both sexes.

Finally, the first echocardiographic measurements were taken at 5 days instead of the day of initial therapy. It is possible that at this time point, eplerenone treatment may have impacted the cardiac function in a different way in female and male rats; however, the purpose of this study was to focus on LV remodeling at 4 weeks and therefore this experiment was not designed to analyze eplerenone efficacy on early remodeling post-MI.

In summary, our data suggest a trend toward selective responsiveness of aldosterone receptor antagonist in females compared with males post-MI. These findings support the hypothesis that the mineralocorticoid pathway may be more active in females with LV dysfunction and therefore have important therapeutic implications. Further, our data highlight the idea that transcriptomic analyses have the potential to serve as a valuable biomarker gauging therapeutic responses.

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Conflict of Interest

None.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Heat maps (A–E) displaying the genes differentially expressed between SHAM and MI placebo (columns) and their corresponding GO annotations (rows). Associations between genes and GO annotations are depicted in dark blue. The analysis is restricted to GO annotations defined as statistically significant ($p < 0.05$) using GoMiner software.

Figure S2. Fold changes of three genes: thrombospondin-4 (Thbs4; black bar), pancreatitis-associated protein (PAP; white bar) and Fc gamma receptor (FcG; hatched bar) reassayed by QuantiGene Assay. The fold induction represents the average of triplicate samples. (**A**) MI placebo versus Sham placebo and (**B**) MI treated versus MI placebo.

Figure S3. Validation of the sex-specific gene expression changes for the fibrosis pathway using quantitative PCR. Relative gene expression levels for collagen type I (col1a1), collagen type III (col3a1), matrix metalloproteinase 2 (MMP2), and tissue inhibitor metalloproteinase 1 (timp1) were measured for Sham animals treated with placebo (Sham), infarcted animals treated with placebo, and infarcted animals (MI) treated with eplerenone (MI + E) in female (**A**) and male groups (**B**). For each sex group, data are centered so that the mean expression value of the Sham group is 1. Results are displayed as the average and standard error of mean of triplicate samples. $^*p = 0.05$ versus Sham.

Table S1. List of 201 significantly upregulated genes in male infarcted rats versus noninfarcted rats (paired *t*-test, $p < 0.05$, FC > 1.4).

Table S2. List of 397 significantly upregulated genes in female infarcted rats versus noninfarcted rats (paired *t*-test, $p < 0.05$, FC > 1.4).

Table S3. List of 252 significantly downregulated genes in male infarcted rats versus noninfarcted rats (paired *t*-test, $p < 0.05$, FC > 1.4).

Table S4. List of 74 significantly downregulated genes in female infarcted rats versus noninfarcted rats (paired *t*-test, $p < 0.05$, FC > 1.4).

Table S5. Overlap: Genes that were significantly upregulated in male and female rats after myocardial infarction (paired *t*-test, $p < 0.05$, FC > 1.4).

Table S6. Overlap: Genes that were significantly downregulated in male and female rats after myocardial infarction (paired *t*-test, $p < 0.05$, FC > 1.4).

Table S7. Genes that were underexpressed in male rats after myocardial infarction and upregulated with treatment ($p < 0.05$, FC > 1.4).

Table S8. Genes that were activated in the female group after myocardial infarction and showed lower activity after eplerenone treatment ($p < 0.05$, FC > 1.4).

Table S9. Genes that were downregulated in female rats after MI and upregulated by treatment with eplerenone ($p < 0.05$, FC > 1.4).

Table S10. Description of the abbreviations in *Figure 6* (Clusters A, B, and C).

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