Journal of Molecular and Cellular Cardiology 49 (2010) 801–811

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

An association between gene expression and better survival in female mice following myocardial infarction

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ARTICLE INFO

Article history:
Received 14 January 2010
Received in revised form 28 July 2010
Accepted 2 August 2010
Available online 6 August 2010

Keywords:
Myocardial infarction
Microarray
Heart failure
Sex/gender

ABSTRACT

Following myocardial infarction, the prognosis for females is better than males. Estrogen is thought to be protective, but clinical trials with hormone replacement failed to show protection. Here, we sought to identify novel mechanisms that might explain this sex-based difference. By diverging from the traditional focus on sex hormones, we employed a conceptually novel approach to this question by using a non-biased approach to measure global changes in gene expression following infarction. We hypothesized that specific gene programs are initiated in the heart following infarction that might account for this sex-based difference. We induced small, medium, and large infarcts in male and female mice and measured changes in gene expression by microarray following infarction. Regardless of infarct size, survival was better in females, while mortality occurred 3–10 days following infarction in males. Two days following infarction, males developed significant ventricular dilation, the best predictor of mortality in humans. Three days following infarction, we measured gene expression by microarray, comparing male versus female and sham versus surgery/infarction. In general, our results indicate a higher relative level of gene induction in females versus males and identified programs for angiogenesis, extracellular matrix remodeling, and immune response. This pattern of gene expression was linked to less pathologic remodeling in female hearts, including increased capillary density and decreased fibrosis. In summary, our results suggest an association between improved survival and less pathologic remodeling and the relative induction of gene expression in females following myocardial infarction.

1. Introduction

In humans, cardiovascular disease, including myocardial infarction (MI), is a predominantly male disease until 65–70 years of age. Statistics from the American Heart Association indicate that in 2004 the overall death rate from cardiovascular diseases was 335.1/100,000 for white males, but only 238.0/100,000 for white females with similar trends in black men and women [1]. In general, women have a lower age-adjusted incident rate and death rate for coronary heart disease (CHD). For people 45–64 years of age the CHD incident rate was three-fold higher in men versus women and the death rate for CHD was 1.7-fold higher in men [1]. Although women have a higher 1-year mortality rate following MI (age ≥ 40 years, 18% in men, 23% in women [1]), comparing outcomes following MI in men and women is confounded by the fact that: women have MIs at older ages, women are 55% less likely to participate in cardiac rehabilitation, and women are less likely to recognize the signs and symptoms of MI and therefore more likely to delay treatment [1]. However, once diagnosed with heart failure, a common outcome following MI, survival rates are significantly higher in women than men [1]. The lower death rates in women initially suggested that estrogen might protect against heart disease, but the results of the Heart and Estrogen/Progestin Replacement Study follow-up (HERS II) and Women's Health Initiative (WHI) clinical trials found either no protection or an increased risk of cardiovascular disease with hormone replacement therapy [2,3].

To date, most animal studies have focused on sex hormones, estrogen and testosterone, to address the issue of sex-based differences following MI. In male and female mice subjected to gonadectomy with hormone replacement (either testosterone or estrogen in both males and females), surgically induced MI produced higher mortality in mice receiving testosterone (male and female) due to cardiac rupture 3–5 days following MI [4–7], as well as significantly more contractile dysfunction and ventricular dilation [4–7].

Non-standard abbreviations: MI, myocardial infarction; CHD, coronary heart disease; SWMI, segmental wall motion index; HERS II, Heart and Estrogen/Progestin Replacement Study; WHI, Women's Health Initiative; LAD, left anterior descending coronary artery.

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doi:10.1016/j.yjmcc.2010.08.002

Cumulatively, these studies generally indicated that estrogen protects the heart from long-term remodeling following MI, whereas testosterone increases the risk of cardiac rupture immediately following MI and adversely affects long-term remodeling [4–7].

A number of studies have addressed estrogen function in the heart and cardiac myocytes, attempting to identify the mechanisms leading to the better outcomes observed in females with cardiovascular disease. Female estrogen receptor-β-deficient mice had worse outcomes following MI, suggesting that estrogen is required for adaptation to ischemic injury [8]. Others demonstrated that estrogen inhibits cardiac hypertrophy [9] and prevents myocyte apoptosis [10], suggesting possible protective mechanisms. Another study showed that estrogen lengthened cardiac myocyte repolarization and reducing automaticity in females following MI [11]. Conversely, some studies found that while estrogen inhibits apoptosis, it also increases ventricular remodeling following MI [12]. In total, experiments focused on the sex hormones have identified some potential mechanisms explaining estrogen mediated protection post-MI, but more work is needed.

Here, we examined the sex-based difference following MI to understand why females have better outcomes. Rather than focus on sex hormones, we employed a non-biased approach to measure global changes in gene expression following infarction. We hypothesized that specific gene programs are initiated in the heart following MI that might account for this sex-based difference. In summary, we found an association between improved survival and a higher level of gene induction, with specific programs for angiogenesis, extracellular matrix remodeling, and immune response, in female mice following MI.

2. Materials and methods

Male and female C57BL/6 mice (12–15 weeks of age) from Jackson Laboratories were used for all experiments. The use of animals conformed to the PHS Guide for Care and Use of Laboratory Animals and was approved by Sanford Research/USD Institutional Animal Care and Use Committee.

Detailed methods describing coronary artery ligation surgery, measurement of cardiac function, microarray analysis, and measurements of capillary density, heart and myocyte size, and fibrosis are contained in the supplemental methods.

A complete, annotated version of the array data contained in this manuscript is available at GEO (http://www.ncbi.nlm.nih.gov/geo/query), accession number GSE23294.

3. Results

3.1. Survival was better in female versus male mice following myocardial infarction (MI)

To examine sex-based differences in ventricular remodeling following ischemic injury, we induced MI in male and female C57BL/6 mice by ligation of the left anterior descending (LAD) coronary artery. Ligations of the LAD were made at distal, mid-level, or proximal sites to produce small, medium, and large infarcts respectively (Supplemental Fig. 1). Two days following surgery infarct size was measured non-invasively by echocardiography using segmental wall motion index (SWMI) [13]. Regardless of infarct size, small (~15% of left ventricular area), medium (~22%), or large (~30%), survival was higher in females (Fig. 1). Only one death was observed in all of the sham surgery groups ($n = 39$). For small, medium, and large infarcts, SWMI was not different between the sexes (Figs. 1A–C) indicating that the initial infarct size was similar at each ligation site. Interestingly, mortality occurred in both groups only between 3 and 10 days post-infarction, suggesting the rapid development of a non-compensatory acute heart failure leading to death.

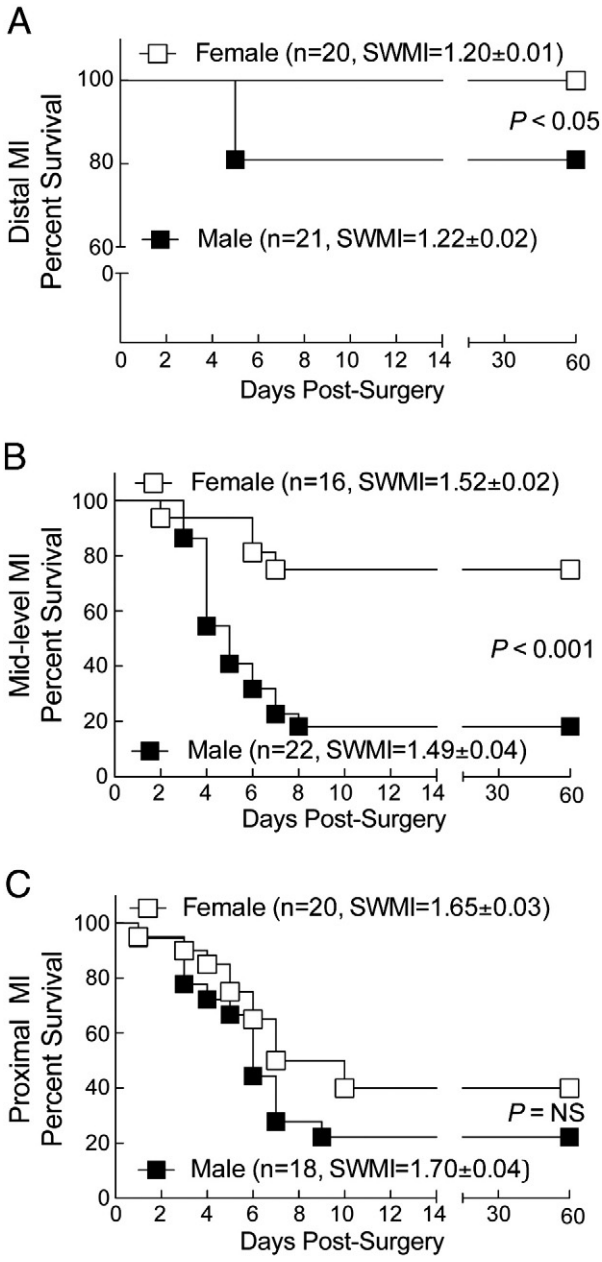

Fig. 1. Survival was better in female versus male mice following myocardial infarction (MI). Survival curves following induction of (A) small, (B) medium, and (C) large infarcts in male and female C57BL/6 mice, 12–15 weeks of age. Two days after surgery, cardiac function and segmental wall motion index (SWMI) were measured by echocardiography. Survival data were analyzed using log-rank (Mantel–Cox) test with a median survival.

3.2. Segmental wall motion index (SWMI) predicted infarct size and functional impairment

To confirm our non-invasive measurements of infarct size (SWMI) and further validate the survival benefit observed in females following MI, we correlated SWMI scores with infarct size. SWMI determined 2 days following MI correlated significantly with infarct size measured 60 days following MI (Fig. 2A). Our results also indicated that ejection fraction, measured by echocardiography 2 days following MI, was inversely correlated with SWMI (Figs. 2B–C).