Testosterone enhances early cardiac remodeling after myocardial infarction, causing rupture and degrading cardiac function

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Cavasin, Maria A., Zhen-Yin Tao, Ai-Li Yu, and Xiao-Ping Yang. Testosterone enhances early cardiac remodeling after myocardial infarction, causing rupture and degrading cardiac function. Am J Physiol Heart Circ Physiol 290: H2043–H2050, 2006.—Cardiac rupture can be fatal after myocardial infarction (MI). Experiments in animals revealed gender differences in rupture rate; however, patient data are controversial. We found a significantly higher rupture rate in testosterone-treated female mice within 1 wk after MI, whereas castration in males significantly reduced rupture. We hypothesized that testosterone may adversely affect remodeling after MI, exaggerating the inflammatory response and increasing cardiac rupture, whereas estrogen may be cardioprotective, attenuating early remodeling and reducing rupture rate. We studied the effect of gender and hormone manipulation on morphological and histological changes during early remodeling after MI in 4-wk-old male and female C57BL/6J mice and how these events could affect cardiac function. Females were randomly divided into J) sham ovariectomy + placebo (s-ovx + P), 2) s-ovx + testosterone (T), 3) ovx + P, and 4) ovx + T; males were divided into J) sham castration + P (s-cas + P), 2) s-cas + 17β-estradiol (E), 3) cas + P, and 4) cas + E. At 6 wk after gonadectomy and hormone manipulation, MI was induced. Mice were randomly killed 1, 2, 4, 7, and 14 days after MI. The left ventricle was weighed and sectioned for evaluation of MI size, infarct expansion index (IEI), and neutrophil infiltration. Transthoracic echocardiography was performed in conscious mice in the 14-day group before organ harvest. Cardiac rupture rate and IEI were significantly higher in testosterone-treated females and noncastrated males than in controls; these effects were accompanied by enhanced neutrophil infiltration and pronounced deterioration of cardiac function and left ventricular dilatation. Ovariectomy in females and estrogen supplementation in males did not confer significant protection from cardiac rupture, IEI, or neutrophil infiltration. We concluded that, in mice, high testosterone levels enhance acute myocardial inflammation, adversely affecting myocardial healing and early remodeling, as indicated by increased cardiac rupture, and possibly causing deterioration of cardiac function after MI, and, conversely, estrogen seems to have no significant protective effect in the acute phase after MI.

left ventricle; infarct expansion index; neutrophil infiltration; hormone manipulation

A FATAL COMPLICATION that occurs within 1 wk of acute myocardial infarction (MI) is rupture of the left ventricular (LV) free wall, which is responsible for 10–20% of patient deaths (2, 19). It is well known that healing and remodeling, seen as inflammatory cell infiltration, degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs), removal of dead cells, and, ultimately, scar formation, start to develop immediately after MI. Thus infarct healing and early remodeling may have a significant impact on the incidence of cardiac rupture and prognosis after MI (8).

Observational studies in humans have shown controversial gender differences in the incidence of cardiac rupture after MI, perhaps because of the difficulty in adjusting for age and risk factors. Shapira et al. (21) reported that the incidence of acute MI was far less in women <59 yr of age than in age-matched men and that cardiac rupture was rare. Other investigators reported that, in the clinical setting, risk of rupture was higher in women than in men, although cardiovascular risk factors were more prevalent in older women (2, 9, 20). Thus it is important to clarify the role of sexual hormones in the incidence of cardiac rupture, infarct expansion, and early remodeling.

Results in experimental animals have been more consistent. Gao et al. (7) showed a lower incidence of cardiac rupture in female mice than in males after MI, and Litwin et al. (14) reported that female rats show a different pattern of cardiac remodeling after MI, with less wall thinning and reduced abnormalities in LV diastolic filling. The present study is the third in our series describing gender and sexual hormone effects on cardiac function and remodeling after MI. We previously found a higher cardiac rupture rate, poorer cardiac performance, and enhanced remodeling after MI in male mice compared with females (4); however, this study only showed gender differences in early and late cardiac remodeling and chronic dysfunction without hormone manipulation or gonadectomy, and it did not clarify whether these observations were due to the presence of testosterone in males or to the possible beneficial effects of estrogen in females. We also found that the rate of rupture and dysfunction was significantly higher in testosterone- than in placebo-treated females and that castration in males significantly reduced rupture and improved function in the chronic phase after MI. However, our second study (3) showed that reduction of estrogen levels due to ovariectomy in females or estrogen supplementation in males had no significant effect on cardiac rupture, although it did affect the long-term prognosis. We also described the effects of estrogen and testosterone on chronic remodeling and cardiac dysfunction 4–12 wk after MI; however, this study did not involve the morphological changes or inflammatory process during the early phase after MI. These observations suggested that the previously reported gender differences in early remodeling may be due to the detrimental effects of testosterone, rather than the protective effects of estrogen. Thus we designed the
present study to focus on the effects of sexual hormones on the acute phase after MI and on the early remodeling process, i.e., 1–14 days after MI.

Cardiac rupture is the result of enhanced infarct expansion, which involves continuous wall stretch and stress combined with thinning and weakening of the myocardium. The long-term prognosis would be more favorable if high-risk groups could be identified and rupture prevented. Thus it is important to understand the mechanisms underlying cardiac rupture and how sexual hormones may influence infarct expansion and healing.

Using a mouse model of MI, we tested the hypothesis that testosterone may have adverse effects on myocardial healing, exaggerating the inflammatory response and, consequently, increasing the rupture rate, whereas estrogen may confer cardioprotection during early remodeling and reduce cardiac rupture. We studied the effect of gender and hormone manipulation on morphological and histological changes during early remodeling after MI and how these events could affect cardiac function.

METHODS

Animals. Four-week-old male and female C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were housed in an air-conditioned room with a 12:12-h dark-light cycle and given standard chow and free access to tap water. The study was approved by the Institutional Animal Care and Use Committee of Henry Ford Health System in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Gonadectomy. All surgical procedures were conducted with the animals under pentobarbital sodium anesthesia (50 mg/kg ip). Females were randomly subjected to bilateral ovariectomy (ovx) or sham ovariectomy (s-ovx); they were placed in the prone position, and males were randomly subjected to bilateral ovariectomy (ovx) or castration (cas) or sham castration (s-cas); they were placed supine, and the testes were removed or left intact via a pair of flank incisions. Females were divided into groups as follows: 1) s-ovx + placebo (P), 2) s-ovx + testosterone (T), 3) ovx + P, and 4) ovx + T. Males were randomly subjected to castration (cas) or sham castration (s-cas); they were placed supine, and the testes were removed or left intact via a low-middle abdominal incision. Males were divided into groups as follows: 1) s-cas + P, 2) s-cas + 17β-estradiol (E), 3) cas + P, and 4) cas + E. Testosterone or 17β-estradiol was administered by subcutaneous pellets (Innovative Research, Sarasota, FL) inserted on the day of surgery, which released a dose of 23 μg/day for 17β-estradiol (1.7 mg in 60 days) and 208 μg/day for testosterone (12.5 mg in 60 days). Our previous study (3) established that these doses provide significantly high levels of testosterone in females (15 times normal) and estrogen in males (10 times normal).

Induction of MI. At 6 wk after gonadectomy and hormone manipulation, animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and MI was induced by coronary artery ligation as described previously (5, 25). Briefly, mice were anesthetized, intubated, and ventilated with room air using a positive-pressure respirator (model 680, Harvard, South Natick, MA). A left thoracotomy was performed via the fourth intercostal space; the lungs were retracted to expose the heart, and the pericardium was opened. The left anterior descending coronary artery was ligated with an 8-0 silk suture near its origin between the pulmonary outflow tract and the edge of the left atrium. Acute myocardial ischemia was considered successful when the anterior wall of the LV turned pale and S-T segment elevation was obvious on the electrocardiogram. The lungs were inflated by an increase in positive end-expiratory pressure, and the thoracotomy site was closed. The animals were kept on a heating pad until they awakened. Sham MI surgery was performed on mice given sham gonadectomy.

Experimental protocol. Mice were anesthetized with pentobarbital sodium (50 mg/kg ip) and randomly killed 1, 2, 4, 7, and 14 days after MI. The heart was stopped at diastole by injection of 15% KCl and removed. The LV was weighed, sectioned transversely into three slices, rapidly frozen in isopentane, and stored at −70°C. Sections (6 μm thick) from each slice were stained with Gomori’s trichrome (15) to evaluate MI size and infarct expansion index (IEI) and with hematoxylin and eosin to evaluate neutrophil infiltration. Transthoracic echocardiography was performed before organ harvest in conscious mice in the 14-day group (26). LV systolic and diastolic dimensions (LVDs and LVDd) were measured from the M-mode view, and ejection fraction (EF) was calculated as follows

\[
EF(\%) = \left( \frac{LVA_d - LVA_s}{LVA_d} \right) \times 100
\]

where LVA and LVA are LV diastolic and systolic areas measured from the LV cross-sectional area (2-dimensional short-axis view).

MI size and IEI. To calculate MI size and IEI, LV slices were stained with Gomori’s trichrome. The circumference of the entire endocardium and epicardium, the thickness and length of the infarcted portion as well as the LV cavity area and the total LV area were determined using a computer-assisted planimeter (Sigma Scan, Jandel). The infarcted portion of the LV was expressed as a percentage of the total circumference (15). IEI, which represents acute dilatation and thinning of the infarcted area, was calculated as follows

\[
IEI = \left( \frac{LVA - LVA}{LVA} \right) \times 100
\]

where LVA and LVA are LV diastolic and systolic areas measured from the LV cross-sectional area (2-dimensional short-axis view).

\[
IEI = \left( \frac{LVA - LVA}{LVA} \right) \times 100
\]

× (noninfarcted septal wall thickness/infarcted LV free wall thickness)
Measurement of infiltrating neutrophils. A 6-μm section from each slice was stained with hematoxylin and eosin. Neutrophil infiltration was determined blindly. Twelve random points at the infarct border were observed under a light microscope (Nikon Labophot; ×400 magnification), and neutrophils in each section were counted. Average numbers were calculated using data from all 3 slices and 12 images per heart. The ability to distinguish neutrophils from macrophages or other cells is dependent on the morphological characteristics of their nuclei (11, 17). Neutrophils are 6–8 μm in diameter, with a nucleus consisting of 2–5 sausage-shaped lobes.

Statistical analysis. Values are means ± SE. The primary method of group comparison was ANOVA with repeated measures, with Student’s t-test used to compare only two groups. Most groups did not exhibit significant deviations from normality. When nonnormality was observed, a nonparametric test (Wilcoxon’s rank-sum test) was used. P < 0.05 was considered statistically significant. Mortality and cardiac rupture rates were compared using χ² tests or Fisher’s exact test for sparse data. Comparisons among females were as follows: 1) MI + s-ovx + P vs. MI + s-ovx + T and MI + ovx + P vs. MI + ovx + T (to test effects of testosterone in the presence or absence of significant levels of estrogen) and 2) MI + s-ovx + P vs. MI + cas + P vs. MI + cas + P (to test effects of reducing endogenous testosterone).

RESULTS

Mortality within 1 wk after MI. Figure 1 shows mortality and cardiac rupture rates within 1 wk after MI. A gender difference was observed in the rupture rate, with significantly more ruptures in “normal males” (s-cas + P) than in “normal females” (s-ovx + P). In addition, mortality rate due to cardiac rupture within 1 wk after MI was significantly higher in testosterone-treated females and noncastrated males. These results are in agreement with our previous study (3). Because rupture generally occurs 3–5 days after MI (22), only mice in the 7- and 14-day groups were considered in the evaluation of early mortality. Total number per group was as follows: 17 s-ovx + P, 30 s-ovx + T, 17 ovx + P, and 30 ovx + T for females and 26 s-cas + P, 18 s-cas + E, 19 cas + P, and 19 cas + E for males.

IEI and MI size. Although infarct size (30–40%) was similar among groups regardless of gender at each time point, IEI was

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Fig. 2. Representative left ventricular (LV) sections showing infarct expansion (enlarged LV cross-sectional area and thinning of the infarct wall on Gomori’s trichrome staining 4 days after MI. Magnification ×10. LV was visibly dilated and thinner in groups with higher testosterone levels [s-ovx + T (females) and s-cas + P (males)] than in controls.
significantly different among both males and females. Figure 2 shows representative Gomori’s trichrome-stained LV cross sections from mice killed 4 days after MI. This is when mortality peaks due to cardiac rupture (22). Figure 3 shows temporal changes in IEI in females and males 1–14 days after MI. IEI was significantly higher in testosterone-treated females regardless of ovariectomy than in placebo-treated females; whereas it decreased significantly in castrated males. Neither ovariectomy in females nor estrogen supplementation in males affected IEI.

Neutrophil infiltration at the infarct border. Figure 4 shows representative hematoxylin-and-eosin-stained sections of the LV infarct border 1 day after MI. Only one pair of female groups and one pair of male groups were chosen to show the significant differences in neutrophil accumulation caused by testosterone. Cumulative data for temporal changes in neutrophil accumulation at the infarct border are shown in Fig. 5. Cells from 12 random microscopic fields were blindly counted and averaged. A gender difference was observed in neutrophil infiltration 1 day after MI: normal males had significantly more neutrophils than normal females. Neutrophil infiltration at the infarct border 1 and 4 days after MI was significantly greater in testosterone- than in placebo-treated females regardless of ovariectomy. In males, castration alone significantly reduced infiltrating neutrophils 1, 2, and 4 days after MI, whereas estrogen supplementation had a significant effect 1 and 2 days after MI, reducing infiltrating neutrophils in sham-castrated males.

Cardiac morphology and function. LV mass corrected by body weight is shown in Fig. 6. LV mass was significantly higher 1 and 4 days after MI in nonovariectomized females given testosterone compared with placebo and significantly higher 2, 7, and 14 days after MI in ovariectomized females given testosterone compared with placebo. In males, estrogen supplementation significantly reduced LV mass 1 day after MI; however, castration had no significant effect at any time.

Figure 7 shows EF and LVDd in females and males 14 days after MI. As expected, MI alone significantly decreased EF and increased LVDd in males and females (sham MI vs. s-ovx + P or s-cas + P, respectively). There was a gender difference in cardiac function and LV dilatation, because EF was significantly lower and LVDd was significantly higher in normal males than in normal females. EF was significantly lower in testosterone-treated females compared with placebo regardless of ovariectomy, and pronounced LV dilatation was significant only in ovariectomized females. In males, castration alone significantly improved EF and decreased LVDd. Neither ovariectomy in females nor estrogen supplementation in males affected EF or LVDd.

DISCUSSION

This study was designed to examine the effects of sexual hormones on early cardiac remodeling during the acute phase of MI and describe some of the morphological changes and possible mechanisms involved in this process. We found that 1) supplemental testosterone increased cardiac rupture within 1 wk after MI in sham ovariectomized and ovariectomized females, and this was accompanied by a significant increase in IEI and neutrophil infiltration at the infarct border; 2) in general, LV mass was higher in the testosterone-treated groups, becoming significant at some time points; 3) testosterone impaired LV function and increased dilatation, as indicated by decreased EF and increased LVD as early as 14 days after MI; and 4) ovariectomy had no significant effect on any of these parameters. In males, 1) castration alone significantly reduced cardiac rupture within 1 wk after MI, and IEI and neutrophil infiltration at the infarct border were significantly decreased; 2) estrogen supplementation in noncastrated mice significantly reduced neutrophil infiltration 1 and 2 days after MI but had no effect on IEI and merely tended to decrease cardiac rupture rate compared with placebo; 3) LV mass was similar in all groups at all times, except 1 day after MI, when LV weight was significantly lower in sham-castrated estrogen-treated compared with placebo-treated animals; 4) castration alone significantly improved LV function and prevented dilatation, as indicated by increased EF and decreased LVD 14 days after MI; and 5) no additive effects were observed with castration + estrogen.

Effects of testosterone and estrogen on neutrophil infiltration and IEI during the acute phase after MI. Myocardial healing is a complex process that involves a number of overlapping events, including inflammatory cell recruitment, pre-existent ECM degradation by MMPs, new matrix deposition, and resolution of inflammation with formation of a mature scar. It has been well documented that MMP activity rapidly increases in the myocardium during infarction and remains
elevated during the healing process (23) and that the main source of these enzymes is neutrophils. We previously showed that maximal neutrophil infiltration at the infarct border occurred 2–4 days after MI and that activation of MMPs was temporally related to neutrophil accumulation (22). It has been reported that there is less inflammatory cell accumulation in hearts from infarcted female than male mice (7); however, it was not determined whether estrogen attenuated inflammation or whether testosterone promoted it. In the present study, we found that, in females, high levels of testosterone significantly increased neutrophil infiltration at the infarct border as early as 1 day after MI, comparable to the number of neutrophils seen in noncastrated males. In males, castration significantly decreased neutrophil infiltration 1, 2, and 4 days after MI. Our results may suggest that enhanced inflammation in the infarcted myocardium induced by high circulating levels of testosterone, which were measured in our previous study (3), could be the cause of the increased IEI and rupture rate. These findings are in agreement with those of Wang et al. (24), who recently showed that testosterone may promote the inflammatory response, because myocardial proinflammatory cytokine production (TNF-α, IL-1β, and IL-6) was decreased in castrated male rats and male rats treated with testosterone receptor blockers compared with noncastrated males after acute ischemia-reperfusion.

Although inflammatory response and cytokine release are essential to initiation of tissue repair, enhancement of this process and/or prolonged activation of cytokines and collagenases seems to be detrimental (6). It has recently been shown that targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture in infarcted mice (18). Taken together with our present findings, the colocalization of MMP-9 with infiltrating neutrophils (13) and the temporal relation of neutrophil accumulation to MMP activation after acute MI (22) may suggest that testosterone increases and/or prolongs myocardial inflammation after MI, producing excessive ECM degradation by MMPs (derived from neutrophils) and causing the increase in infarct expansion. Thus the delay in myocardial healing would be reflected by the increase in rupture events seen in testosterone-treated females as well as in noncastrated males.

On the other hand, estrogen has been reported to promote healing of endometrial and cutaneous wounds (1, 10), an effect that may be related to estrogen-stimulated release of growth factors such as transforming growth factor-β1 and fibroblast growth factor (12, 16). In our study, estrogen supplementation in noncastrated mice slightly decreased the number of rupture events, possibly because of the significant reduction in neutrophil infiltration, although we did not observe a detrimental effect due to ovariectomy in females. This leads us to think that
the lower rupture rate in normal females compared with normal males may not necessarily reflect a protective effect of estrogen but, rather, the absence of a negative effect due to the low circulating levels of testosterone.

Effects of testosterone and estrogen on cardiac function and LV mass and dilatation during the acute phase after MI. We found that, in addition to enhanced acute inflammation, testosterone also produced marked dilatation and decreased LV function in female mice, whereas castration produced opposite (beneficial) effects in males 14 days after MI. These findings are in agreement with a recent report showing improved postischemic functional recovery in castrated mice and in mice treated with testosterone receptor blockers compared with normal mice in a ischemia-reperfusion model (24) and indicate that somehow testosterone impairs LV function after the onset of MI. The integrity of the heart and its hemodynamic function depend on the biochemical stability of the ECM; because this integrity may be compromised by excessive inflammation and myocardial weakening, LV dilatation and functional deterioration may develop as early as 14 days after MI. However, it is important to point out that although estrogen did not offer any significant protection against cardiac rupture and infarct expansion immediately after MI, we previously reported that estrogen improves cardiac function and attenuates maladaptive remodeling in the chronic phase after MI (3), and these beneficial long-term effects may be due to reduced inflammatory cell infiltration in the viable myocardium.

Limitations of the present study. Expression or activity of MMPs or tissue inhibitors of metalloproteinases in the infarct border was not measured in this study. We based our conclusions on previous work in which increased neutrophil infiltration was associated with heightened MMP activity (22). We did not examine whether sexual hormones affect the quantity and quality of collagen at the infarct border. We believe that enhanced inflammation and increased MMPs may decrease the quantity and quality of the ECM, as reflected by the increase in IEI.

Conclusions. This study helps explain the dramatic differences in cardiac rupture previously observed between males and females, i.e., noncastrated and castrated males and females treated with or without testosterone. Our data suggest that testosterone promotes 1) excessive inflammation after infarction, as indicated by neutrophil infiltration, and 2) significant myocardial expansion, perhaps due to enhanced ECM degradation, thus delaying and/or impairing myocardial healing and causing a significant increase in LV dilatation
rupture events. These detrimental effects on early remodeling may contribute to the cardiac dysfunction and LV dilatation seen by 14 days after MI. On the other hand, ovariectomy in females does not seem to impair infarct expansion and rupture or LV dilatation and function, although estrogen prevented neutrophil infiltration and tended to decrease cardiac rupture in noncastrated males. We conclude that high levels of testosterone (endogenous or supplemental) enhance acute myocardial inflammation and early remodeling, which adversely affect myocardial healing and impair cardiac function in mice after MI, whereas estrogen does not seem to have a significant protective effect in the acute phase after MI.

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REFERENCES
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TESTOSTERONE EFFECTS IN EARLY CARDIAC REMODELING