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Females Exhibit Relative Resistance to Depressive Effects of TNF on the Myocardium

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Abstract

Background—Tumor necrosis factor- α (TNF) plays a critical role in myocardial dysfunction following acute injury. It is unknown, however, if a gender-specific response to TNF infusion exists in isolated rat hearts. Elucidating such mechanisms is important to understanding the myocardial gender differences during acute injury. We hypothesize that females will exhibit a relative resistance to TNF-induced myocardial dysfunction compared to males and that menstrual cycle would influence the degree of female myocardial resistance to TNF-induced myocardial functional depression.

Methods—Adult male, proestrus female, and metestrus/diestrus female hearts were subjected to 60 minutes of TNF infusion at 10,000 pg/ml-min via Langendorff. Myocardial contractile function (left ventricular developed pressure, and the positive/negative first derivative of pressure) was continuously recorded.

Results—10,000pg/ml min of TNF markedly depressed myocardial function in males compared with other doses of TNF. Myocardial function was significantly decreased in males compared to females following TNF infusion. Additionally, both the proestrus and metestrus/diestrus females exhibited equal resistance to TNF-induced myocardial dysfunction.

Conclusion—Our study shows that females exhibit a significantly greater degree of resistance to TNF induced myocardial depression. Moreover, data from this study suggests that fluctuations in estrogen during the reproductive cycle may have little to no influence on TNF-induced myocardial depression.

Keywords

Gender differences; TNF; myocardial function; testosterone; estrogen

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INTRODUCTION

Gender differences have been noted in the myocardial response to acute injuries such as burns (1), trauma (2), sepsis (3), and myocardial infarction (4). Studies have consistently shown that females exhibit greater degree of cardioprotection, and much of this protection is attributed to estrogen and its receptors (5–7). Acute (8) as well as chronic (9) administration of estrogen has been shown to provide protection from myocardial ischemia/reperfusion (I/R) injury. Conversely, there is evidence to suggest that endogenous testosterone exacerbates myocardial dysfunction following major trauma (10) and acute ischemic conditions (11), but the mechanisms by which testosterone contributes to cardiac dysfunction under such conditions are much less understood (5).

The inflammatory response, which is mediated by pro-inflammatory cytokines such as tumor necrosis factor- α (TNF) (12), contributes significantly to the myocardial dysfunction and loss of cardiomyocytes observed following acute injury. TNF, whose levels rise significantly during acute injury (13), may play an important role in the development of heart failure. A direct correlation between functional capacity, survival, and circulating TNF levels has been found (14). TNF appears to depress myocardial function via cytotoxicity, oxidant stress, myocyte apoptosis, disruption of excitation-contraction coupling, and induction of other cytokines. In fact, the heart itself produces significant amounts of TNF during acute injury (15,16). Although levels of circulating TNF are most pronounced during infection and endotoxemia, studies have shown that elevated levels of myocardial TNF and other cytokines are present following ischemia-reperfusion (17), myocardial infarction (15), and burn trauma (18).

It has also been shown that levels of TNF following acute injury vary according to gender. Female rats were shown to have lower cardiomyocyte secretion of TNF, as well as decreased serum concentrations of TNF after burn injury (1) compared to males. In a related clinical study, Schroder et al found women to have lower TNF levels during sepsis (19). Sex hormones may be responsible for the gender differences in cytokine levels and in the myocardial response to acute injury, given that estrogen has been reported to suppress the release of TNF from macrophages after trauma-hemorrhage and to decrease the susceptibility to subsequent sepsis (20), while endogenous testosterone might increase pro-inflammatory cytokines following I/R injury (11). It is not known, however, if there is a gender-specific response in the myocardium to isolated TNF. Our previous study has shown improved post-ischemic myocardial function associated with TNFR1 signaling resistance in females (21). Therefore, we hypothesized that 1) females, compared to males, will maintain a greater degree of resistance to TNF induced myocardial functional depression, and 2) proestrous females will exhibit a greater degree of cardioprotection compared to metestrus/diestrus females.

MATERIAL AND METHODS

Animals

Age matched normal male (280–350 g, 9–10 wk) and female (200–250 g, 9–10 wk) Sprague-Dawley rats (Harlan, Indianapolis, IN) were fed a standard diet and acclimated in a quiet quarantine room for 1 week before experiments. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Indiana University. All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85-23, revised 1985).

Isolated heart preparation (Langendorff)

Rats were anesthetized using pentobarbital sodium (60 mg/kg ip) and heparinized (500 U ip), and the hearts were rapidly excised via a median sternotomy and placed in a 4°C Krebs-

Henseleit solution. The aorta was cannulated, and the heart was perfused (70 mmHg) with oxygenated (95% O₂ and 5% CO₂) Krebs-Henseleit solution (37°C). A 20 ml syringe was filled with 400 ng/ml TNF dissolved in perfusate (Krebs-Henseleit solution) and secured in the infusion pump. The syringe and tubing were connected to a two-way stop-cock above the aortic root. During equilibration, the coronary flow was measured by collecting pulmonary artery effluent, and the infusion rate and volume were preset to ensure that 10,000 pg/ml-min was delivered during infusion. Data were continuously recorded with a PowerLab 8 preamplifier/digitizer (AD instruments, Milford, MA) and an Apple G4 PowerPC computer (Apple Computer, Cupertino, CA). The left ventricular developed pressure (LVDP) and end diastolic pressure were measured, and the maximal positive and negative values of the first derivatives of pressure (+dP/dt and -dP/dt) were calculated with the PowerLab software.

Dose Response

A dose response curve was created to determine the concentration of TNF that would cause similar myocardial dysfunction as that seen during ischemia/reperfusion injury when used on experimental groups. Isolated male rat hearts were subjected to the same infusion protocol: 15-minute equilibration period, 60 minutes of TNF infusion, and a 30-minute post-infusion period. Male hearts were infused with TNF concentrations of either 1000 pg/ml-min (n=3), 2000 pg/ml-min (n=4), 4000 pg/ml-min (n=3) or 10,000 pg/ml-min (n=5); male controls (n=5) were infused with a perfusate vehicle.

Experimental Groups

Rats were divided into three groups with TNF infusion: normal males (n=5), proestrus females (n=5), and metestrus/diestrus females (n=5). The female rats were utilized in the proestrus and metestrus stage of the menstrual cycle to elucidate the effect of estrogen on myocardial function following TNF infusion. The stage of their menstrual cycle was assayed via the wet vaginal smear method as previously described (22). Isolated hearts were subjected to the same infusion protocol: a 15-minute equilibration period and a 60-minute infusion period at 10000 pg/ml-min TNF, without 30-minute post-infusion period. Controls were infused with a perfusate vehicle.

Statistical Analysis

All reported values are mean $\pm$ SEM. Data were compared with a two-way ANOVA with Bonferroni post-test or unpaired Student's *t* test when appropriate (GraphPad Software, San Diego, California USE). A two-tailed probability value <0.05 was considered statistically significant.

RESULTS

TNF-depressed myocardial function

Myocardial function was impaired by directly TNF infusion (Figure 1). The post-infusion period was intended to allow TNF to further induce myocardial depression. However, during this post-infusion period it was observed that the control group also showed progressive myocardial depression. The greatest disparity in myocardial dysfunction between the 10,000 pg/ml/min group and vehicle control group was seen immediately after cessation of infusion at the 75-minute mark, at which time myocardial function in the control hearts was kept stable.

Our data indicated that TNF infusion with 10,000 pg/ml-min dosage caused significant LVDP, +dP/dt, and -dP/dt depression compared with the controls. This concentration of TNF resulted in about 30% myocardial functional depression as exhibited by LVDP, $\pm$ dP/dt (Figure 2).

Males exhibit increased myocardial dysfunction during TNF infusion compared to females

During exposure to 10,000 pg/ml-min of TNF, male myocardial function progressively decreased. Myocardial function (LVDP, +dP/dt) in males, which was kept stable during the 15 min equilibration period, was significantly depressed after 10 minutes of TNF infusion (Figure 3) and was decreased by 25.7% of LVDP, 25.4% of +dP/dt and 33.2% of -dP/dt at the end of experiments (Figure 3, 4). However, TNF induced-myocardial functional depression was not markedly noted in females.

Estrous cycle does not affect TNF induced myocardial dysfunction

During TNF exposure, there was no significant difference in myocardial function between the proestrus (9.5% of LVDP, 6.8% of +dP/dt, 9.3% of -dP/dt depression) and metestrus/diestrus females (9.3% of LVDP, 2.2% of +dP/dt, 10.5% of -dP/dt depression) (Figure 3, 4).

DISCUSSION

Previous studies have shown gender differences exist not only in the myocardial response to acute injury, but in the circulating levels of pro-inflammatory cytokines, particularly TNF (1). However, it is not clear whether this gender differences in the myocardial response to acute injury are due to different levels of TNF production in males and females. In this present study, we demonstrated that when exposed to equivalent doses of TNF: 1) males exhibited greater myocardial functional depression compared with females; 2) there were no significant differences in myocardial dysfunction between the proestrus and metestrus/diestrus females.

Gender differences have been noted in many disease pathologies, such as hepatic disorder (23), trauma-hemorrhage induced small intestinal dysfunction (24), ischemia/reperfusion- or endotoxin-induced cardiac injury (21,25), and inflammation stimulated by sepsis or shock (26). Gender or sex hormones affect the production of inflammatory cytokines in these and other disease states and complicate organ dysfunction and (7). Accumulated evidence from animal experiments and clinical studies has indicated that females have a higher degree of protection against injury as compared to males (27). In this study we assessed whether the magnitude of TNF-induced myocardial depression is also gender-specific. Clearly, males showed significantly greater myocardial dysfunction when compared with either proestrus or metestrus females. It suggests that gender-specific differences do exist in the TNF-induced myocardial dysfunction with the relative greater degree in male hearts.

TNF acts biologically through TNFR1 and TNFR2, both of which are present in the cardiomyocytes. The majority effects of TNF on myocardial dysfunction and cardiac myocyte apoptosis are initiated by TNFR1. Although our previous study has shown that TNFR1 signaling is resistance in female hearts (21), there were no gender differences observed in the expression of TNFR1 and TNFR2 in cardiac myocytes from our group and others. It suggests that gender differences in TNF-induced myocardial dysfunction are not due to the magnitude of TNFR1 and/or TNFR2. However, several studies do suggest that testosterone and estrogen are mediators of the intracellular signaling cascade initiated by TNF (Figure 5).

Overwhelming data has indicated that the relative cardioprotection conferred to females appears to be mediated through estrogen. Based on the results of this study, however, no significant difference was observed in the TNF-induced functional depression between the proestrus and metestrus/diestrus myocardium. Although Jarrar and colleagues showed that female hormonal fluctuations during the reproductive cycle were strong factors influencing the survival following trauma-hemorrhage (28), fluctuations in estrogen appear to have little influence on the myocardial response to TNF. However, our data suggests that another sex

hormone, testosterone, may play a role in myocardial response to injuries and may exacerbate TNF-induced myocardial dysfunction.

Testosterone, though less well studied, is believed to have detrimental effects on the myocardium by not only regulating an increase in intracellular calcium levels, but by increasing p38 MAPK activation, and inducing apoptosis(4). Indeed, our previous study has indicated that chronic endogenous testosterone had a deleterious effect in the normal isolated heart subjected to I/R. Testosterone depletion (castration) or subcutaneous testosterone blockade (flutamide treatment) improved post-ischemic myocardial function and decreased myocardial TNF, IL-1 β and IL-6 production, activation of p38 MAPK, and expression of apoptotic-related proteins caspase-1, -3 (11). Additionally, our group also demonstrated that acute exogenous testosterone treatment worsened myocardial functional recovery associated with the increase in activation of p38MAPK, JNK, and expression of caspase-3 (29).

In summary, gender differences have been implicated in TNF-induced myocardial functional depression following acute injury. Given that there was no significant difference in TNF-induced myocardial dysfunction between the proestrus and non-proestrus groups, it appears that estrogen itself may not play as crucial a role in myocardial protection as previously thought. In fact, testosterone may play a more important role in mediating the deleterious effects imposed by TNF on the myocardium. However, further investigation is needed to elucidate the mechanisms behind gender mediated TNF-induced myocardial dysfunction, especially the detrimental effects of testosterone on the myocardium. Understanding these mechanisms may allow for the design of novel therapies to attenuate TNF induced heart failure.

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Figure 1.

Myocardial function of males infused with TNF at 1000 (n=3), 2000 (n=4), 4000 (n=3), and 10000 (n=5) pg/ml-min. Male controls (n=5) were infused with Krebs-Henseleit solution. Infusion duration was one hour and was initiated after a 15-minute equilibration period. A and D, LVDP; B and E, +dP/dt; C and F, -dP/dt. All results are mean $\pm$ SEM of raw data. D, E and F are represented as the percent of equilibration (eq). **P<0.01 10000 pg/ml-min vs. control, ***P<0.001 vs. 10000 pg/ml-min vs. control.

Figure 2.

Myocardial functional depression of males infused with TNF at 1000 (n=3), 2000 (n=4), 4000 (n=3), and 10000 (n=5) pg/ml-min measured immediately upon cessation of infusion period (one hour in duration). Male controls (n=5) were infused with Krebs-Henseleit solution. Results are represented as the percent depression of equilibration. A, percent LVDP depression; B, percent +dP/dt depression; C, percent -dP/dT depression. **P<0.01 vs. control, ***P<0.001 vs. control; #P<0.05 vs. other groups with TNF infusion at 1000, 2000 and 4000 pg/ml-min; ##P<0.01 vs. other groups with TNF infusion at 1000, 2000 and 4000 pg/ml-min.

Figure 3.

Myocardial function of males (n=5), proestrus females (n=5), and metestrus females (n=5) infused with TNF at 10,000 pg/ml-min. Male controls (n=5) were infused with Krebs-Henseleit solution. A and D, LVDP; B and E, +dP/dt; C and F, -dP/dt. All results are mean $\pm$ SEM. D, E, and F are represented as the percent of equilibration (eq). *P<0.05 male vs. control, **P<0.01 male vs. control, ***P<0.001 male vs. control; #P<0.05 male vs. proestrus, ##P<0.01 male vs. proestrus, ###P<0.001 male vs. proestrus.

Figure 4.

Myocardial functional depression of males (n=5), proestrus females (n=5), and met/diestrus females (n=5) infused with TNF at 10,000 pg/ml·min measured immediately upon cessation of infusion period (one hour in duration). Male controls (n=5) were infused with TNF-free Krebs-Henseleit solution. Results are represented as the percent depression of equilibration. A, percent LVDP depression; B, percent +dP/dt depression; C, percent -dP/dT depression. **P<0.01 vs. control and females, ***P<0.001 vs. control and females ; #P<0.05 vs. met/diestrus.

Figure 5.

TNF signaling cascade mediated via TNFR1. Uppercase 'T' signifies activation by testosterone, whereas lower case 't' signifies deactivation or inhibition by testosterone. Uppercase 'E' signifies activation by estrogen, whereas lower case 'e' signifies deactivation or inhibition by estrogen.