Clinical Trial/Experimental Study

Medicine

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Mechanistic insights into heart failure induction in ovariectomized rats

The role of mitochondrial dysfunction

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Abstract

Background: Premature menopause is recognized as a factor that increase the risk of heart failure (HF). However, the fundamental pathophysiology concerning cardiac dysfunction remains inadequately understood.

Methods: This study investigated whether cardiac function was altered in ovariectomy (OV) rats compared to controls. Female rats (n = 12) were randomly assigned into 2 groups: control (sham operation) and bilateral OV group.

Results: The echocardiographic analysis revealed that the E and E/A were significantly decreased, while the deceleration time was significantly increased in the OV group compared to the control group, indicating the presence of HF in the OV rats. ATP levels in the myocardium were significantly decreased, and oxidative DNA damage was elevated in the OV group compared to the control group. Furthermore, the mRNA levels of peroxisome-proliferator-activated receptor-gamma (PPARγ) co-activator-1 alpha (PGC-1α) and CR6 interacting factor 1 (Crif1) were reduced in the OV group.

Conclusion: These findings suggest that OV may induce HF through mechanisms linked to mitochondrial dysfunction.

Abbreviations: 8-OHdG = 8-hydroxydeoxyguanosine, ATP = adenosine triphosphate, Crif1 = CR6 interacting factor 1, HF = heart failure, OV = ovariectomy, PGC-1α = PPARγ co-activator-1 alpha, PPARγ = peroxisome-proliferator-activated receptor-gamma.

Keywords: cardiac dysfunction, mitochondria, ovariectomy

1. Introduction

Estrogen, a well-known for cardioprotective benefits, exhibits a significant inverse relationship with cardiovascular risk in women.[1] Clinical data suggests that premature menopause, stemming from conditions such as ovariectomy (OV), elevates the risk of heart failure (HF) and mortality, underscoring the clinical relevance of understanding the consequences of bilateral OV.[2,3] Given the pivotal role of mitochondria in energy metabolism, they are considered a primary target of estrogen within the myocardium.[4] However, the precise mechanisms by which OV influences cardiac function, particularly concerning mitochondrial dysfunction, remain elusive, thus begging further exploration.

Therefore, this study aimed to clarify the effects of OV on cardiac function in rats. To assess mitochondrial function, myocardial ATP levels, mRNA expression of peroxisome-proliferator-activated receptor-gamma (PPARγ) co-activator-1 alpha (PGC1α) and CR6 interacting factor 1 (Crif1) which are critical regulators of mitochondrial function, as well as the levels of 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage.

2. Materials and methods

2.1. Animals and housing

All animal procedures were approved by the Eulji University Institutional Animal Care and Use Committee (EUIACUC13-08) in Daejeon, Republic of Korea. Female Wistar rats (8-weeks old) were obtained from DBL Co., Ltd (Eumseong-gun, Chungbuk-do, Republic of Korea) and housed at the Animal Care Center of Eulji University under controlled environmental conditions. The rats were housed in pairs at 23°C ± 1°C under a 12-hour light/12-hour dark cycle and were provided free access to water and a standard rat chow diet from Harlan Laboratories (Madison).

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2.2. Subject and procedure

All rats (N = 12) were randomly assigned to 2 groups: sham-operated rats (control group) and OV group, with 6 rats in each group. Bilateral OV was performed on the rats at 8-weeks of age. Standard echocardiography measurements and calculations were conducted 10 weeks after OV. All rats were fasted overnight and then decapitated under CO_2 anesthesia. Blood samples and myocardial tissues were collected from left ventricles of all rats. Serum samples were stored at – 80°C for later analysis. Myocardial tissues were snap-frozen immediately after collection and stored at – 80°C until further analysis.

2.3. Real-time polymerase chain reaction

A total of 10 to 30 mg of myocardial tissue was homogenized by using a polytron homogenizer (Fisher Scientific Inc., Pittsburgh). The homogenate was stored for 5 minutes to allow the complete dissociation of nucleoprotein complexes. Homogenates were mixed with 0.2 mL chloroform per 1 mL of TRI Reagent (Molecular Research Center Inc., Cincinnati) and vigorously shaken for 15 seconds. Total ribonucleic acid (RNA) isolated from the homogenates following the manufacture's instructions. Equal amounts of total RNA were reverse transcribed into complementary deoxyribonucleic acid (cDNA) using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules). Real-time polymerase chain reaction was performed in a 20 μL reaction mixture containing 1 μg of cDNA, 10 pmol forward primer, 10 pmol reverse primer, and 10 μL SYBR Green Supermix (Bio-Rad) using a CFX96 Real-Time Polymerase Chain Reaction Detection System (Bio-Rad Laboratories [Singapore] Pte Ltd., Singapore). The threshold cycle (Ct) values for each target mRNA were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, and the relative expression levels of each target gene (PGC1α and Crif1) were calculated using CFX Manager software version 1.5 (Bio-Rad).

2.4. Measurement of myocardial ATP level

The mitochondrial function was evaluated by measuring ATP levels in addition to the mRNA levels of PGC1α and Crif1. Myocardial tissue samples (approximately 10 mg each), obtained by snap-freezing, were homogenized in a mammalian cell ATP lysis solution (Perkin–Elmer, Waltham). The homogenates were centrifuged at 12,000 rpm at 4°C for 5 minutes, and the supernatants were transferred to fresh 1.5 mL tubes. The ATPlite assay was performed following the manufacturer's instructions (Perkin–Elmer). The luminescence emitted from the ATP-dependent luciferase reaction was measured using a 2030 multi-label reader (Perkin–Elmer).

2.5. Measurement of oxidative stress marker

The levels of 8-OHdG, a ubiquitous marker of oxidative stress (oxidative DNA damage), were measured. Genomic DNA was

Table 1

Echocardiographic parameters of rats after 10 wk of ovariectomy.

Variables	Control group	Ovariectomy group	*P*–value
Heat rate, bpm	237 ± 7	228 ± 11	.222
E, cm/s	86 ± 5	55 ± 21	.001*
A, cm/s	37 ± 2	42 ± 7	.544
E/A	2.3 ± 0.2	1.3 ± 0.2	.004*
DT, ms	141 ± 8	409 ± 1	.001*
LVPWd, cm	0.22 ± 0.02	0.21 ± 0.10	.694
LVIDd, cm	0.62 ± 0.01	0.61 ± 0.10	.318
LVPWs	0.20 ± 0.01	0.22 ± 0.10	.583
LVIDs, cm	0.31 ± 0.02	0.32 ± 0.01	.615
EF, %	65 ± 8	61 ± 2	.453

Values are expressed as mean ± SD (n = 6).

A = atrial wave in diastole, DT = deceleration time, E = early wave in diastole, EF = ejection fraction, LVIDd = left ventricular intraventricular dimension at diastole, LVIDs = left ventricular intraventricular dimension at systole, LVPWd = left ventricular posterior wall at diastole, LVPWs = left ventricular posterior wall at systole.

*Statistically significant.

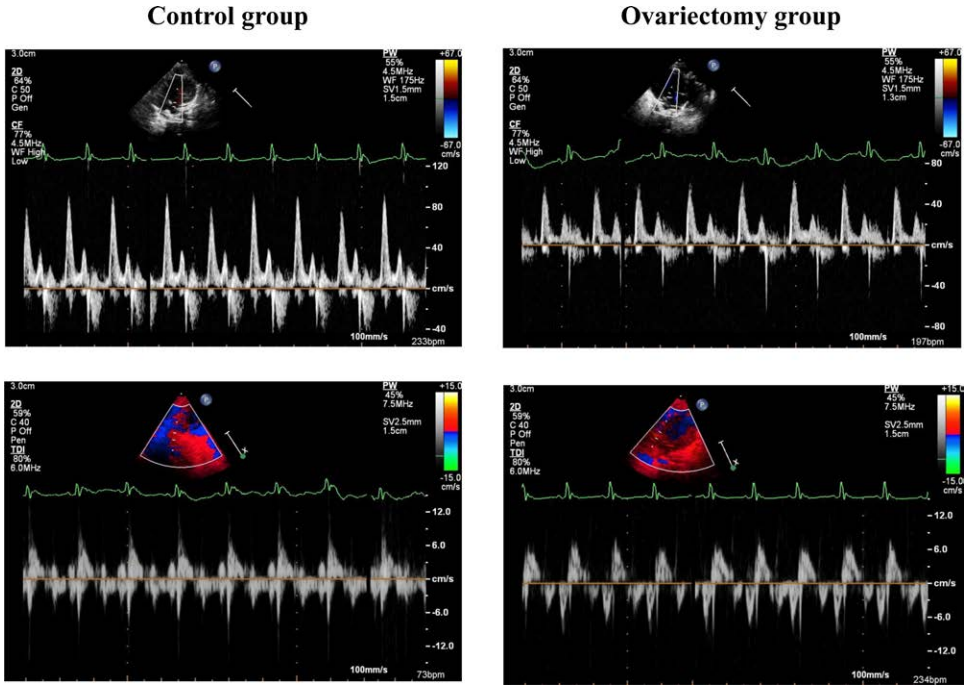

Figure 1. The echocardiographic image comparing parameters of cardiac structure and function between control and ovariectomy group. Left upper panel: mitral valve inflow velocity pattern in the control group. Left lower panel: tissue doppler of mitral annular velocity pattern in the control group. Right upper panel: mitral valve inflow velocity pattern in the ovariectomy group. Right lower panel: Tissue doppler of mitral annular velocity pattern in the ovariectomy group.

extracted from the myocardium using a DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia). The levels of 8-OHdG were assayed spectrophotometrically according to the manufacturer's instructions (Oxiselect Oxidative DNA Damage ELISA Kit, Cell Biolabs Inc., San Diego). The optical density (OD) of 8-OHdG was measured at 450 nm (OD 450 nm), where a decrease in OD correspond to increased reactive oxygen species damage.

2.6. Echocardiographic data

Echocardiographic parameters including chamber size, systolic ejection fraction (%), peak early diastolic filling (E), late diastolic filling (A), E/A, and the deceleration time (DT) were assessed using Vivi-7 system (GE Healthcare) and performed on anesthetized rats.

2.7. Statistical analysis

Statistical analyses were conducted using SPSS software. Data were expressed as mean ± standard deviation, and differences between groups were analyzed using Student *t*-test. A *P* value of <.05 was considered statistically significant.

3. Results

3.1. The data of cardiac function

In Table 1, echocardiographic assessments revealed significantly decreased E and E/A ratios, as well as increased DT in the OV group compared to those in the control group. Especially, the E was decreased to 55 ± 21 cm/s in the OV group versus 86 ± 5 cm/s in the control group, while DT increased to 409 ± 1 ms in the OV group versus 141 ± 8 ms in the control group indicating HF with preserved systolic function. Importantly, structural parameters showed no significant differences between OV and control groups, indicating maintained cardiac structure in the Figure 1.

3.2. The data of mitochondrial function

The expression levels of PGC1α and Crif1 genes were significantly reduced in the OV group (0.3 ± 0.1 vs 1.0 ± 0.1, $P < .05$ and 0.6 ± 0.1 vs 1.0 ± 0.1, $P < .05$, respectively) compare to those in the control group, supporting the presence of impaired mitochondrial biogenesis and mitochondrial dysfunction. The OD of 8-OHdG was significantly lower in the OV

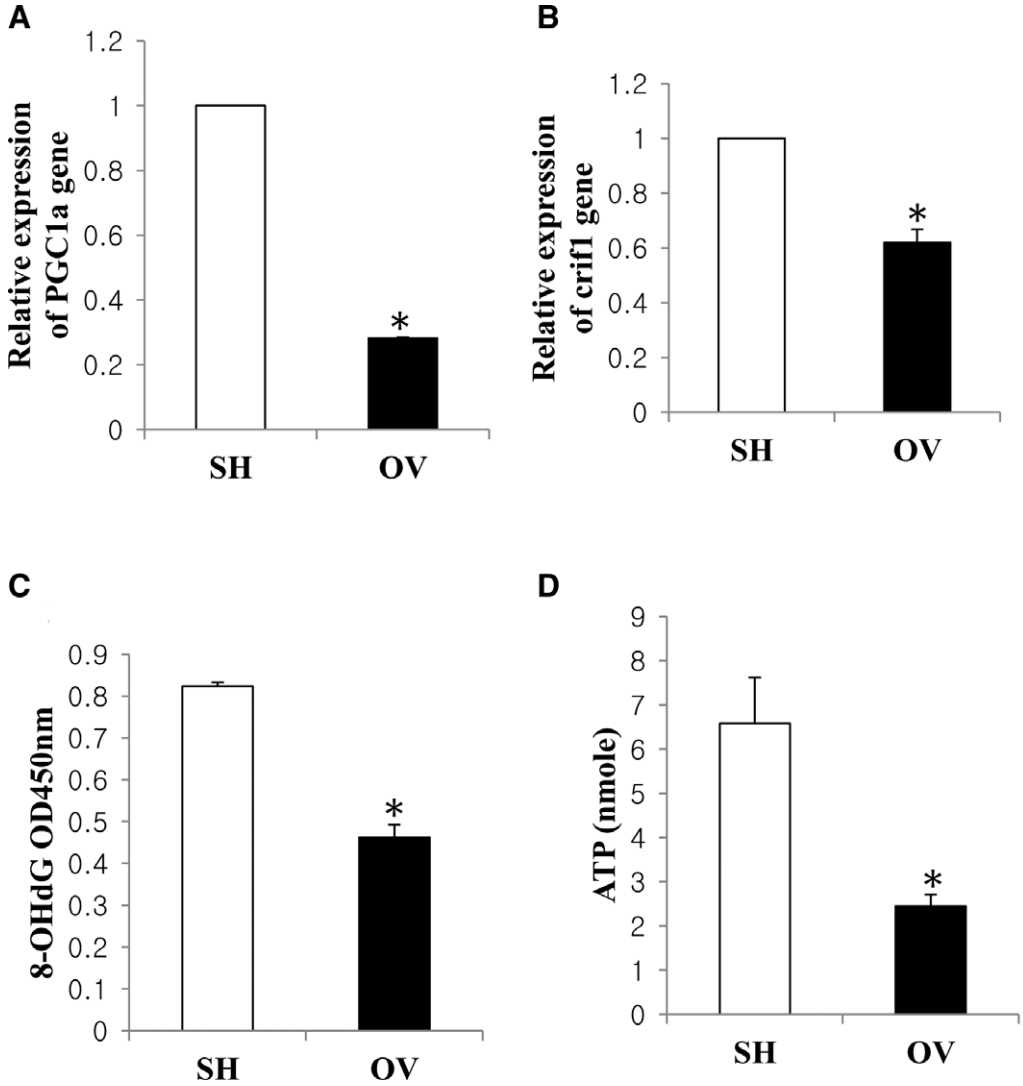

Figure 2. The mRNA expression levels of PGC1α gene (A) and crif1 gene (B), optical density of reactive oxygen species damage marker 8-OHdG (C), and ATP levels (D) in the myocardia in the control (SH) and ovariectomy (OV) group. 8-OHdG = 8-hydroxydeoxyguanosine, ATP = adenosine triphosphate, crif1 = CR6 interacting factor 1, OD 450 nm = optical density of 8-OHdG as measured at 450 nm, OV = ovariectomy, PGC1α = peroxisome-proliferator-activated receptor-γ (PPARγ), co-activator-1α, SH = sham. *$P < .05$.

group compared with those in the control group (0.4 ± 0.1 vs 0.8 ± 0.1, $P < .05$) indicating increased myocardial oxidative stress in the OV group. ATP levels were significantly lower in the OV group compared to those in the control group (2.5 ± 0.7 nmol vs 6.5 ± 0.8 nmol, $P < .05$) in Figure 2.

4. Discussion

The results of present study suggest that OV reduces mitochondrial function, leading to HF with preserved systolic function without structural changes. Previous studies have demonstrated that estrogen influences mitochondrial function and enhances energy metabolism in cardiomyocytes.[4–7] Thus, the reduction in estrogen levels due to OV likely results in mitochondrial dysfunction, which manifests as impaired cardiac function.[4–7] These findings further support the notion that mitochondrial dysfunction is a pivotal mechanism driving HF. Mitochondria, the powerhouses of cardiomyocytes, are critical for ATP generation.[8–10] The observed reduction in ATP levels aligns with a decreased expression of PGC1α and Crif1 genes, which are essential for maintaining mitochondrial function.[11,12] PGC-1α serves as a co-transcription regulator by directly related with estrogen receptor to modulate mitochondrial energy metabolism, encompassing fatty acid oxidation, oxidative phosphorylation, tricarboxylic acid cycle, and mitochondrial biogenesis.[13] Our data showed consistent with previous data that estrogen related receptor to be a regulator of cellular metabolism via PGC-1α.[14] This study also underscores the clinically cardiac structure and function using echocardiogram for significance of estrogen's cardioprotective effects and highlights the potential need for interventions targeting mitochondrial function to mitigate cardiovascular risks associated with premature menopause.

5. Conclusion

Further investigation into the mechanisms behind the deteriorated mitochondrial function observed in the OV state, along with exploring therapeutic strategies to enhance mitochondrial efficiency in women experiencing premature menopause, could provide valuable insights. Such research may be instrumental in preventing or treating HF with preserved systolic function and other related cardiovascular complications in these patients.

Author contributions

Conceptualization: Ki-Woon Kang.
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Funding acquisition: Ki-Woon Kang.
Investigation: Ki-Woon Kang, Seong-Kyu Lee.
Methodology: Ki-Woon Kang, Seong-Kyu Lee.
Software: Ki-Woon Kang.
Supervision: Seong-Kyu Lee.
Writing – original draft: Ki-Woon Kang.
Writing – review & editing: Ki-Woon Kang, Seong-Kyu Lee.

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