—Original—

Effect of Gonadectomy and Angiotensin II Receptor Blockade in a Mouse Model of Isoproterenol-induced Cardiac Diastolic Dysfunction

Kuniya Asai[1], Koji Murai[2], Akihiro Shirakabe[1], Masataka Kamiya[2], Satsuki Noma[2], Naoki Sato[2], Kyoichi Mizuno[3] and Wataru Shimizu[2]

[1]Intensive Care Unit, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan
[2]Department of Cardiovascular Medicine, Nippon Medical School, Tokyo, Japan
[3]Mitsukoshi Health and Welfare Foundation, Tokyo, Japan

Background: Although heart failure (HF) with preserved ejection fraction (HFpEF) is more common in postmenopausal women than in men, the effect of sex hormones on cardiac diastolic function remains unclear. We examined the effect of gonadectomy with or without the angiotensin receptor blocker olmesartan (Olm) in an isoproterenol (ISO)-induced mouse model of left ventricular hypertrophy (LVH) and cardiac diastolic dysfunction.

Methods: ISO or ISO with Olm were administered for 28 days in sham-operated male and female, castrated (CAS), and ovariectomized (OVX) mice. LV ejection fraction (EF) and E/A ratio were analyzed by echocardiography, and the LV and lung weight corrected by tibial length were used as indices of LVH and lung congestion, respectively.

Results: On echocardiography, systolic function did not differ between the four groups. LV/tibial length (TL) and Lung/TL significantly increased in all groups. The LV/TL ratio was lower in castrated-ISO vs. Male-Sham-ISO but did not differ between Female-Sham-ISO and OVX-ISO. However, the Lung/TL ratio of OVX-ISO was greater than that of Female-Sham-ISO. Olm prevented LV hypertrophy in all groups. The decrease in E/A and increase in lung weight were improved by Olm in Male-Sham and OVX-ISO but not in the other groups.

Conclusion: These sex differences suggest that sex hormones play a pivotal role in modulating cardiac hypertrophy and diastolic dysfunction induced by chronic β-adrenoceptor stimulation, and thus affect the therapeutic potential of angiotensin receptor blockade. (J Nippon Med Sch 2021; 88: 113–120)

Key words: left ventricular hypertrophy, heart failure, postmenopause, angiotensin receptors, gonadal steroid hormones

Introduction

Sex differences in cardiovascular disease have been noted. Heart failure (HF) with a preserved ejection fraction (HFpEF) is more common in postmenopausal women than in men[1]. Left ventricular (LV) hypertrophy is more pronounced in women than in men in pressure overload hypertrophy states such as hypertension and aortic stenosis[2,3]. Sex differences in LV hypertrophy have also been observed in animal models of pressure and volume overload[4–6]. Females have a lower incidence of heart failure (HF), a higher rate of HF survival[5], and better function[6,7]. HFpEF accounts for 50% of all hospital admissions for heart failure[8] and is believed to be more common in women than in men[9]. Therefore, older age and female sex are considered risk factors for HFpEF.

Sympathetic nervous system (SNS) and renin-angiotensin system (RAS) activities increase in HF, and sex hormones may affect neurohumoral activities[10].

Sex differences have been observed in cardiac and peripheral responses to acute adrenoreceptor antagonists[11,12].

Correspondence to Kuniya Asai, MD, PhD, Intensive Care Unit, Nippon Medical School Chiba Hokusoh Hospital, 1715 Kamagari, Inzai, Chiba 270–1694, Japan
E-mail: kasai@nms.ac.jp
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Nevertheless, few studies have conducted detailed analyses of sex differences in LV hypertrophy response to increased chronic SNS activity. An understanding of sex differences is crucial for improving clinical management of HF and developing possible new sex-specific therapeutic options. Indeed, there are sex differences in the pharmacokinetics and pharmacodynamics of the most frequently used drugs in cardiovascular disease. A retrospective analysis of previously published drug trials revealed sex differences in the efficacy of a number of cardiovascular drugs, including RAS blockers[13].

The isoproterenol-induced HF mouse model is believed to mimic HFpEF[14]. Therefore, we examined the effect of chronic β-adrenergic receptor (AR) stimulation with isoproterenol on LV hypertrophy and cardiac diastolic dysfunction in male and female mice. To test for sex hormone interactions, castrated and ovariectomized mice were also given chronic isoproterenol infusion. In addition, we attempted to identify any differences in the effect of an angiotensin II receptor blocker (ARB), olmesartan (Olm), on prevention of LV hypertrophy and cardiac diastolic dysfunction in these mice.

Methods and Animals

Experimental Protocol

We purchased 132 male and female ddY mice (age, 7-8 weeks) weighing 24 to 32 grams from Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan). The mice were kept in a constant dark/light cycle (12 h each) and provided standard mouse chow and water *ad libitum.* All experiments were performed under anesthesia with sodium pentobarbital (50 mg/kg body weight, intraperitoneally). The care and treatment of experimental animals were performed in accordance with institutional guidelines. This study was approved by the Animal Care and Use Committee of Nippon Medical School (Approval number: 20-067).

Male mice were castrated (CAS) or sham-operated (Sham), and female mice were ovariectomized (OVX) or sham-operated at age 7-8 weeks. Mice were assigned to isoproterenol (ISO) or vehicle (Veh) groups at 2 weeks after surgery. ISO (30 μg/g/day), a β-AR stimulant, was administered with an osmotic minipump (Alzet Mini osmotic pump, model 2004; Durect Corporation, Cupertino, CA, USA) for 28 days, as described previously[15]. In the Olm group, another minipump was simultaneously implanted, and Olm was infused at 1 mg/kg/day for 28 days.

Echocardiography

Echocardiography was performed immediately before and approximately 28 days after administration with a high-frequency 15-MHz linear scanner (Sequoia, ACUSON®, Siemens Medical Solutions, Germany) under anesthesia with sodium pentobarbital (50 mg/kg body weight, intraperitoneally). In brief, mice were laid in supine position on a heating pad maintained at 37°C, and the anterior chest area was shaved. Warmed gel was applied to the anterior chest of the examined mice, and M-mode tracing was recorded from the short-axis view at the papillary muscle level. Pulsed-wave Doppler of mitral inflow was also measured[16,17]. M-mode measurements of LV internal diameter and pulsed-wave Doppler were performed from more than three beats and averaged. End-diastolic measurements were obtained at the time of the apparent maximal LV diastolic dimension. End-systolic measurements were obtained at the time of the most anterior systolic excursion of the posterior wall. LVEF was calculated by the cubed method as LVEF = [(LVDd)3 − (LVDs)3]/(LVDd)3, where d indicates diastole and s indicates systole. E/A ratio (ratio of E and A velocity at LV inflow) was calculated as an index of LV diastolic function. Echocardiography measurements and analyses were performed in a blinded manner.

Hemodynamic Measurements

Four weeks after administration of Veh or ISO with or without Olm, cardiac catheterization was performed under pentobarbital anesthesia. A 1.4-F catheter tip micromanometer (Millar Instruments®, Houston, TX, USA) was inserted into the right carotid artery. The data were recorded digitally, and aortic pressure (AP) was analyzed with a computer-based system (Notocord®; Croissy, France).

Heart and Lung Weight

After hemodynamic measurements, the heart was excised and weighed.

Lung weight was measured as an index of lung congestion, and the absolute weight was normalized to tibia length (TL).

Statistical Analysis

Data are presented as means ± SD. Multiple comparisons were performed by analysis of variance followed by Fisher's Least Significant Difference (LSD) test. Comparisons of two groups, between either Male-Sham and corresponding CAS mice, or Female-Sham and corresponding OVX mice, were made using Student's unpaired t-tests. Comparisons of two groups between male and female mice were also made using Student's unpaired t-

Table 1 Echocardiographic Findings in Male Mice at 4 Weeks After Treatment

	Male-Sham			CAS		
	Veh N = 6	ISO N = 6	ISO/Olm N = 4	Veh N = 6	ISO N = 5	ISO/Olm N = 4
WT (mm)	0.72 ± 0.09	0.83 ± 0.07*	0.69 ± 0.06§	0.76 ± 0.05	0.82 ± 0.06	0.71 ± 0.07§
LVIDd (mm)	4.04 ± 0.43	4.13 ± 0.52	4.18 ± 0.58	4.05 ± 0.42	3.99 ± 0.27	3.86 ± 0.23
EF (%)	69.6 ± 7.9	74.9 ± 3.9	67.8 ± 7.0	69.1 ± 8.0	73.8 ± 7.7	74.2 ± 3.4
E/A	1.80 ± 0.41	1.39 ± 0.17	1.82 ± 0.39§	1.73 ± 0.38	1.46 ± 0.26	1.39 ± 0.22
HR (bpm)	465 ± 87	662 ± 127*	589 ± 90	466 ± 112	643 ± 9*	588 ± 78

Values are mean ± SD.
Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham operated mice, CAS; Castrated mice, WT, Wall thickness; LVDd, LV dimension at end-diastole; EF, ejection fraction; E/A, ratio of E and A velocity at LV inflow; HR, heart rate.
*$p < 0.05$ Veh vs. ISO, §$p < 0.05$, ISO vs. ISO/Olm, †$p < 0.05$, Veh vs. ISO/Olm, #$p < 0.05$, Male vs. CAS.

Table 2 Echocardiographic Findings in Female Mice at 4 Weeks After Treatment

	Female- Sham			OVX		
	Veh N = 13	ISO N = 17	ISO/Olm N = 6	Veh N = 10	ISO N = 18	ISO/Olm N = 6
WT (mm)	0.69 ± 0.02	0.79 ± 0.06*	0.68 ± 0.05§	0.67 ± 0.02	0.79 ± 0.07*	0.67 ± 0.27§
LVDd (mm)	4.32 ± 0.09	4.18 ± 0.20	4.20 ± 0.07	4.29 ± 0.16	4.31 ± 0.25	4.24 ± 0.08
EF (%)	63.0 ± 2.3	72.8 ± 4.3*	77.4 ± 3.5	62.3 ± 3.2	70.1 ± 5.1	73.1 ± 7.6†
E/A	2.08 ± 0.24	1.56 ± 0.23*	1.45 ± 0.39†	2.07 ± 0.44	1.38 ± 0.22*,#	1.67 ± 0.25§
HR (bpm)	344 ± 42	604 ± 127*	640 ± 155†	331 ± 30	626 ± 86*	591 ± 131†

Values are means ± SD.
Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham operated mice, OVX; Ovariectomized mice, WT, Wall thickness; LVDd, LV dimension at end-diastole; EF, ejection fraction; E/A, ratio of E and A velocity at LV inflow; HR, heart rate.
*$p < 0.05$ Veh vs. ISO, §$p < 0.05$, ISO vs. ISO/Olm, †$p < 0.05$, Veh vs. ISO/Olm, #$p < 0.05$, Female vs. OVX.

tests. Statistical analyses were performed with the commercially available SPSS 22.0 software program (SPSS Japan Institute, Tokyo, Japan). Significance was defined as a P value of <0.05.

Results

Echocardiography

Wall thickness (WT) was significantly greater in ISO-treated than in vehicle-treated mice in both male and female mice, except for the CAS group. Olm treatment significantly prevented increase of WT in all groups. LVEF was not lower in ISO-treated mice, suggesting preserved LV systolic function in the four groups (**Table 1, 2**). E/A was significantly lower after chronic ISO, especially in female and OVX mice, and in OVX mice than in Female-Sham mice. Interestingly, Olm restored the decrease in E/A only in Male-Sham and OVX mice (**Table 1, 2**, **Fig. 1**).

Hemodynamic Measurements

In the Veh group, there were no significant differences in systolic AP between the four subgroups. After chronic ISO administration, systolic AP was similar between the four groups (Male-Sham: 91.6 ± 20.4, n = 6; CAS: 90.6 ± 29.6, n = 4; Female-Sham: 90.4 ± 15.5, n = 17; OVX: 96.0 ± 10.7, n = 18). Blood pressure dropped significantly in all four groups when Olm was administered simultaneously (Male-Sham: 74.4 ± 22.2, n = 4; CAS: 60.3 ± 17.2, n = 5; Female-Sham: 71.1 ± 7.6, n = 6; OVX: 70.0 ± 10.3, n = 6).

LV Weight

In the Veh groups (white bars in **Fig. 2**), LV weight (LVW)/TL in the Male-Sham group was significantly higher than in the Female-Sham group. After CAS, LVW/TL was significantly lower in male mice. However, there was no change in the LVW/TL of female mice after OVX, suggesting that any sex difference in LVW/TL did not persist after gonadectomy. LVW/TL significantly increased, by approximately 20% to 30%, in all four groups after chronic ISO administration (gray bars in **Fig. 2**). In

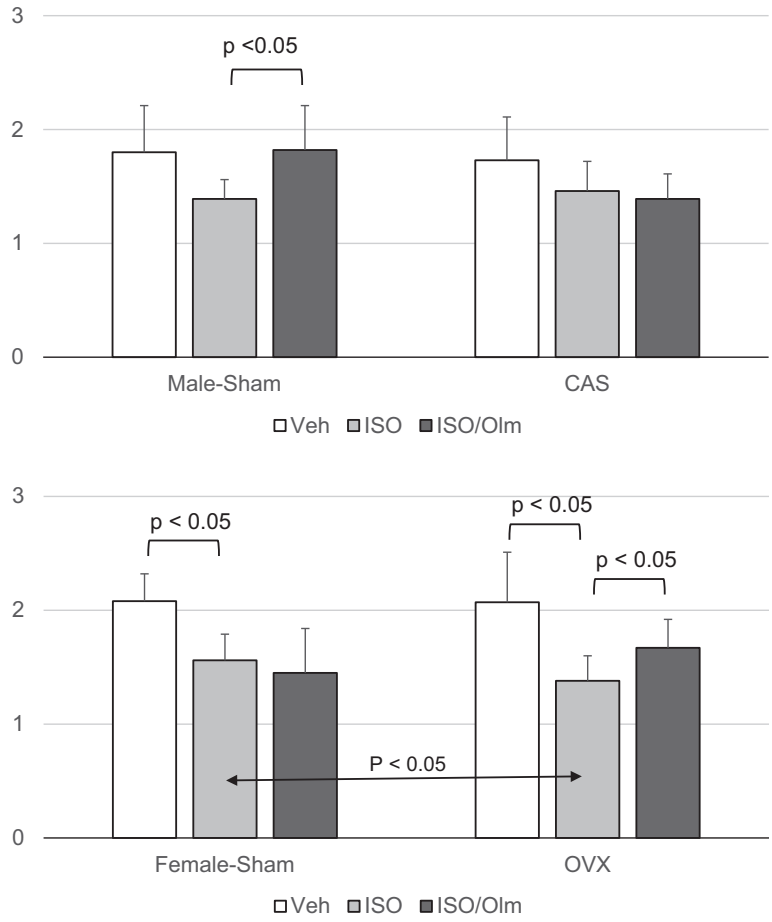

Fig. 1 E/A Ratio in Male (Sham and CAS) and Female (Sham and OVX) Mice.
Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham-operated mice, CAS; Castrated mice.

the Sham-ISO group, LVW/TL was significantly higher in males than in females. After gonadectomy, there was no difference in LVW/TL between the CAS-ISO and OVX-ISO groups.

CAS alone decreased LVW/TL (Male-Sham: 5.3 ± 0.5 vs CAS: 4.2 ± 0.9, P<0.05). In contrast, OVX alone did not affect LVW/TL. Therefore, there was no difference in LVW/TL between the Female-Sham and OVX groups (4.2 ± 0.4 vs 4.2 ± 0.3, respectively). After ISO administration, LVW/TL was also similar between the Female-Sham and OVX groups. The effect of ISO in LV hypertrophy was significantly diminished by simultaneous administration of Olm in all four groups. However, the effect of Olm on hypertrophy was particularly obvious after gonadectomy in both male and female mice (**Table 3, 4**).

Lung Weight

Lung weight (Lung)/TL was significantly higher in all groups after chronic ISO, indicating pulmonary congestion. Lung/TL after chronic ISO administration (gray bars in **Fig. 3**) was significantly lower in the Female-Sham than in the Male-Sham group. In the sham group, Lung/TL was lower in females than in males. However, the favorable effect observed in the Female-Sham group disappeared after OVX, and there were no differences in Lung/TL between the Male-Sham, CAS, and OVX groups (**Fig. 3**). Simultaneous administration of Olm (dark gray bars in **Fig. 3**) decreased Lung/TL in all four groups; the effect was highly significant in the OVX group (**Table 3, 4**).

Discussion

Randomized trials of a variety of promising drugs have not demonstrated a clear benefit in HFpEF patients[18]. Therefore, it is important to understand the pathogenesis of sex differences in HFpEF, to guide future treatment. A mouse model of isoproterenol-induced cardiac diastolic dysfunction is believed to mimic HFpEF[14]. Our data were consistent with those of previous reports. The present

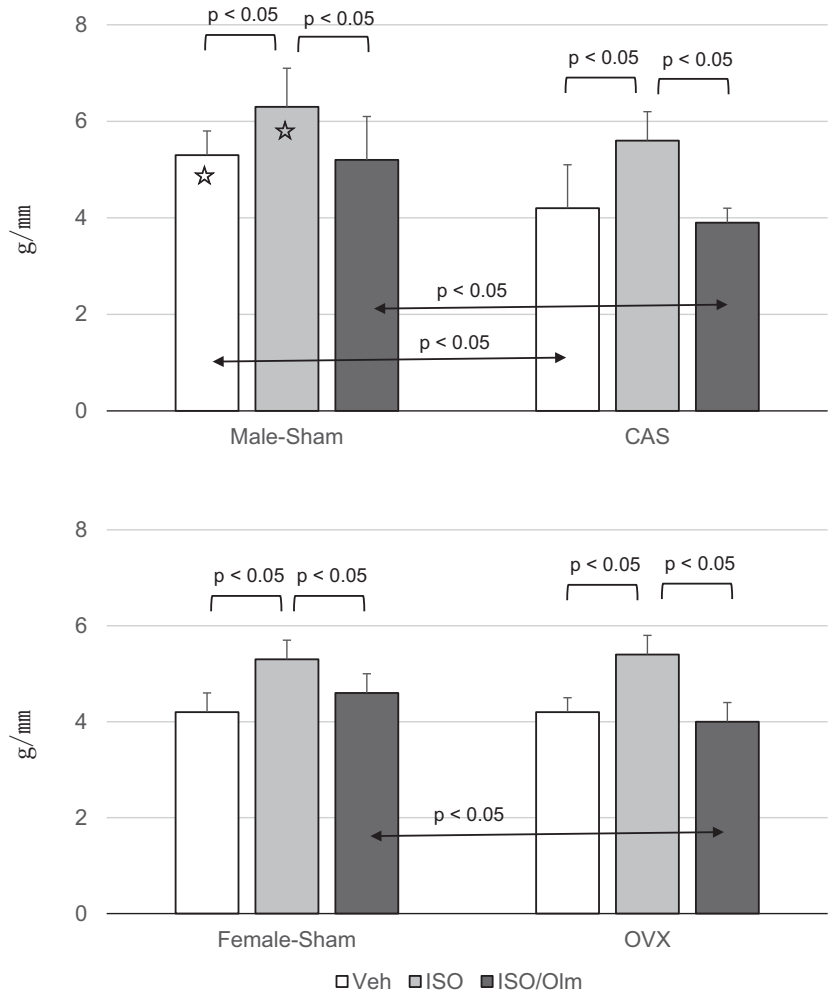

Fig. 2 Left Ventricular (LV) Weight/Tibial Length (TL) in Male and Female Mice. Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham-operated mice, CAS; Castrated mice
☆ $p < 0.05$, vs. corresponding Female mice.

model showed features of HEpEF, such as preserved EF, reduced diastolic function (decreased E/A ratio), and pulmonary congestion (increased lung weight). E/A ratio, which is used to assess diastolic function, is affected by heart rate, and its interpretation is limited. However, in this study there was no significant difference in heart rate among the four groups, or between the ISO and ISO-Olm groups.

In this study, chronic β-AR stimulation promoted LV hypertrophy in all groups; however, the effect was more striking in the Male-Sham group. The difference was abolished after castration in male mice, resulting in similar LVW/TL ratios for the CAS-ISO, Female-Sham-ISO, and OVX-ISO groups. This finding suggests that male hormones such as testosterone and SNS have a synergistic effect on LV hypertrophy.

Pulmonary congestion, indicating increased lung weight, was induced by chronic ISO administration in all four groups. However, after chronic ISO administration, pulmonary congestion was significantly less severe in Female-Sham than in Male-Sham mice. Furthermore, Lung/TL after chronic ISO administration was significantly higher in OVX mice than in Female-Sham mice, suggesting that female hormones had a favorable effect on pulmonary congestion. Consistent with this finding, E/A ratio was more impaired in OVX mice than in Female-Sham mice.

The effects of chronic ISO on LV hypertrophy and pulmonary congestion were diminished by Olm in all four groups. The favorable effect of Olm was stronger in the OVX and Male-sham groups.

RAS activity was stimulated by the adrenergic β-agonist isoproterenol[19]. Sex hormones and RAS are believed to be closely related. Premenopausal women may

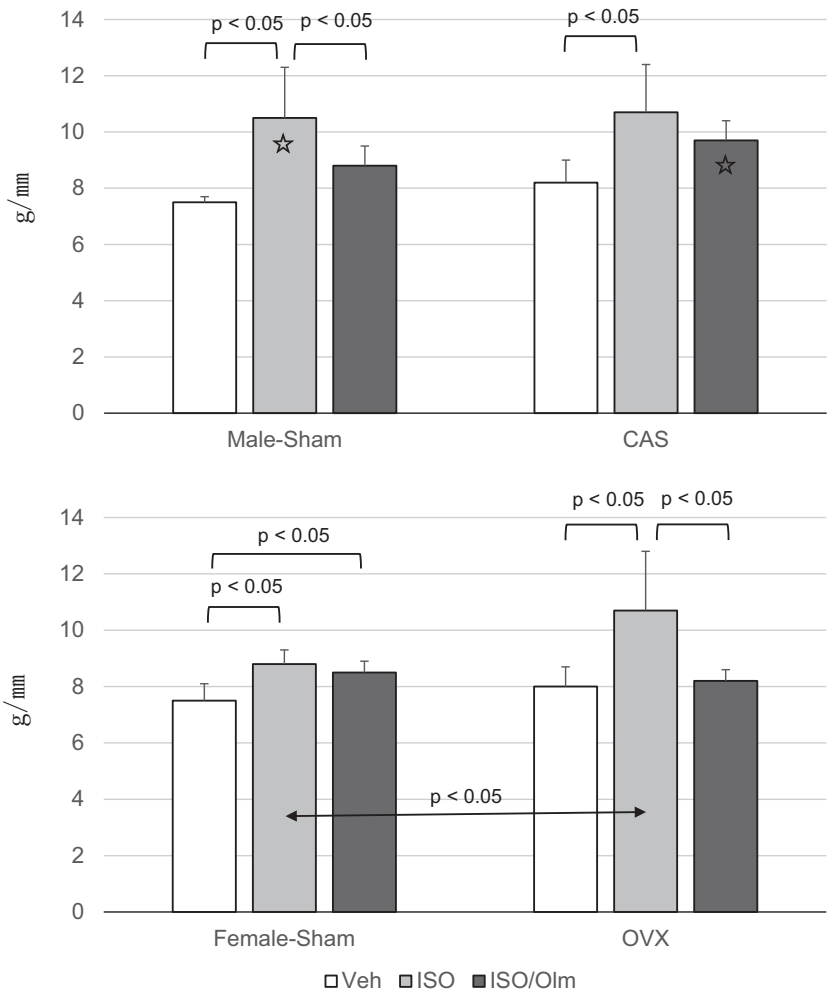

Fig. 3 Lung Weight/Tibial Length (TL) in Male and Female Mice.
Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham-operated mice, CAS; Castrated mice
☆ $p < 0.05$, vs. corresponding Female

Table 3 Left Ventricular (LV) Weight/Tibial Length (TL) and Lung Weight/TL Ratio in Male Mice

Male	LVW/TL (mg/mm)			Lung/TL (mg/mm)		
	Veh	ISO	ISO/Olm	Veh	ISO	ISO/Olm
Sham (n = 17)	5.3 ± 0.5	6.3 ± 0.8*	5.2 ± 0.9§	7.5 ± 0.2	10.5 ± 1.8*	8.8 ± 0.7§
CAS (n = 15)	4.2 ± 0.9	5.6 ± 0.6*	3.9 ± 0.3§	8.2 ± 0.8	10.7 ± 1.7*	9.7 ± 0.7
Sham vs CAS	P < 0.05	n.s.	P < 0.05	n.s.	n.s.	n.s.

Values are mean ± SD.
Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham operated mice, CAS; Castrated mice
*$p < 0.05$ Veh vs. ISO, §$p < 0.05$, ISO vs. ISO/Olm, †$p < 0.05$, Veh vs. ISO/Olm, #$p < 0.05$, Female vs. OVX.

benefit from estrogen-induced inhibition of the RAS[10]. Premenopausal and postmenopausal women on estrogen replacement therapy (ERT) have lower renin levels than do men and postmenopausal women without ERT[20]. Angiotensin-converting enzyme (ACE) activity also appears to be affected by estrogen in animal models[21] and humans[22]. Estrogens and ovariectomy decrease and increase AT1 R expression, respectively[23,24], and estrogens upregulate AT2R expression[25]. In contrast, some studies reported that testosterone increased angiotensinogen and plasma renin activity[26]; nevertheless, there are far fewer data from studies of estrogens.

Table 4 Left Ventricular (LV) Weight/Tibial Length (TL) and Lung Weight/TL Ratio in Female Mice

Female	LVW/TL (mg/mm)			Lung/TL (mg/mm)		
	Veh	ISO	ISO/Olm	Veh	ISO	ISO/Olm
Sham (n = 34)	4.2 ± 0.4	5.3 ± 0.4*	4.6 ± 0.4§	7.5 ± 0.6	8.8 ± 0.5*	8.5 ± 0.4†
OVX (n = 37)	4.2 ± 0.3	5.4 ± 0.4*	4.0 ± 0.4§	8.0 ± 0.7	10.7 ± 2.1*	8.2 ± 0.4§
Sham vs OVX	n.s	n.s	P < 0.05	n.s.	P < 0.01	n.s.

Values are mean ± SD.
Veh, Vehicle; ISO, Isoproterenol; Olm, Olmesartan; Sham; Sham-operated mice, OVX; Ovariectomized mice,
*p < 0.05 Veh vs. ISO, §p < 0.05, ISO vs. ISO/Olm, †p < 0.05, Veh vs. ISO/Olm, #p < 0.05, Sham vs. OVX.

It is unclear whether women and men respond differently to medications for heart failure. As described previously, the RAS is believed to be enhanced in postmenopausal women, and a beneficial effect of RAS blockade is expected. Indeed, several studies have shown that ARBs are more effective at lowering blood pressure in women than in men[27,28], and the superior event suppression rate of ARB, as compared with β-blocker, is more apparent in women[29]. In contrast, the beneficial effect of ARB on a combined mortality-morbidity endpoint was consistent among predefined patient subgroups, and ARB similarly improved outcomes in men and women[30,31]. However, studies of outcomes in women with HF are limited by the smaller proportion of women (typically around 20% to 25% of participants) enrolled in trials.

In our study, Olm improved Lung/TL, an index of pulmonary congestion, more effectively in male-sham and OVX mice, where the RAS system was enhanced. Although we examined the effects of gonadectomy, we did not investigate the effects of estrogen or testosterone. In the future, hormonal replacement therapy will be necessary for detailed evaluation of the effects of these hormones. Pathological and molecular biological approaches are needed to determine the mechanisms underlying these sex differences.

Conclusions

Sex differences were observed in chronic ISO-induced LV hypertrophy in an HF mice model. There were also sex differences in responses to Olm for prevention of LV hypertrophy and cardiac diastolic dysfunction. These findings support the hypothesis that ARB treatment is beneficial for postmenopausal women with HFpEF.

Conflict of Interest: The authors declare no conflicts of interest.

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