Original Article

Chronic use of 17β-Ethinyl estradiol on cardiovascular hemodynamic profile: "Friend or foe"?

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Abstract

Introduction: The effects of ovariectomy (Ovx), menopause, and estrogen replacement on the hemodynamic remain controversial. This study employed the technique of impedance cardiography analysis to measure the effect of chronic use of estrogen replacement on cardiovascular hemodynamic in the Ovx rats. **Materials and Methods:** Colony-bred adult Ovx female Sprague-Dawley rats were randomized into three groups: 17 β-Ethinyl Estradiol treated ovariectomized group (OvxE), vehicle treated ovariectomized group (OvxV), and Sham Operated (SO). Animals received 17 β-Ethinyl Estradiol (17 β-EE) once daily for 90 days. Cardiovascular hemodynamic parameters such as left ventricular ejection time (LVET), pre-ejection period (PEP), Systolic time interval (STI), cardiac output (CO), cardiac index (CI), stroke volume (SV), and stroke volume index (SVI) were assessed 24 h after last treatment on 7, 15, 30, 60, or 90 days. **Results:** Compared to SO group, Ovx with or without estrogen replacement did not significantly affect the mean blood pressure, CO, CI, SV, and SVI. No significant changes were observed in LVET and PEP from SO. Treatment with estradiol increased the STI by 66.62% and 53.60% ($P < 0.05$), from control after 60 and 90 days, respectively. Blood velocity, base impedance (Zo) and maximum change in impedance during systole (Zt) corresponding to time-varying fluid volume (blood) remained within normal limits of variation. **Conclusion:** These results demonstrate that on long-term administration of estrogen significantly increased STI in rats.

Key words: 17 β-ethinyl estradiol, ovariectomized, systolic time interval

INTRODUCTION

Post-menopausal estrogen replacement therapy has been associated with a decrease in cardiovascular morbidity and mortality.[1,2] However, the heart and estrogen/progestin replacement study has not shown any beneficially cardiovascular effect with an early increase in the incidence of risk of coronary heart disease, i.e., estrogen therapy has been shown to exert prothrombotic effect.[3,4] The cardio-protective effect of estrogen can be understood on the basis of different possibility that have been proposed which includes autonomic nervous system modulation, improvement in carbohydrate and lipoprotein metabolism, inhibition of vascular smooth muscle proliferation, and improving the vasodilatory potential by enhancement of nitric oxide (NO)-dependent and independent effects.[5-8] Chronic estrogen deprivation leads to cardiac hypertrophy, Left Ventricular (LV) remodeling associated with arterial stiffening, increase in LV relative wall thickness, and increase in blood viscosity.[9-11]

Estrogen decreases resistance to blood flow in various

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vascular beds.[12] Endothelium-dependent coronary artery vasodilatation is enhanced by estrogen treatment in Ovx monkey.[13,14] Estrogen therapy exerts beneficial effects in ischemia-reperfusion injury and cardiac remodeling by normalizing wall tension and inhibiting post-infarction left ventricular dilatation.[15-18] The technique of impedance cardiography analysis provides unique index of myocardial contractile a measure of left ventricular performance. It helps to distinguishes two phases of systole: The PEP and the LVET. As cardiac function deteriorates, the PEP lengthens while the LVET shortens, resulting in an increase in the ratio.

Short- and long-term hemodynamic effects of estrogen significantly increase of mean, systolic and diastolic pressures were observed after menopause.[19, 20] Estrogen decreases late systolic blood pressure in post-menopausal woman. There is improvement in aortic functions on acute administration of 17 β-EE. But studies of long-term use have shown no significant effect on BP.[21-24] Controversial study done by Hayward reported significant change in HR after menopause, the result of which was opposed by Saab *et al*.[25]

Therefore, our objective was to explore the long-term estrogen replacement on cardiovascular hemodynamic using impedance cardiography in rats. In this study, we calculated systolic time interval (STI), which is the ratio of the PEP (time from onset of electrical systole to onset of mechanical systole) to LVET (duration of mechanical systole).

MATERIALS AND METHODS

Materials

17 β-EE was procured from Sigma Chemical (USA) and all other chemicals were of analytical grade.

Animals and treatment

The experimental protocol used in this study was approved by the Institutional Animal Ethical Committee. Colony-bred adult virgin female Sprague-Dawley rats at 12 weeks of age (body weight: 180-200 g) maintained under standard conditions with alternate 12 h light/dark periods at 22°C and free access to pellet diet and tap water as per committee for the purpose of control and supervision on experiments on animals guidelines were used. The animals were kept in polypropylene cages containing rice husk. Minimum six numbers of adult female rats were randomly assigned into three groups. Animals in Groups 1 and 2 were bilaterally ovariectomized, whereas, rats in group 3 were subjected to SO. After a 7-day rest to allow for natural elimination of endogenous hormones, rats in groups 1 were administered 17 β-EE (150 μg/kg/day) by oral gavage[26] once daily for 90 days (dissolved in sesame oil to a final concentration of 0.15 mg/ml), whereas all the remaining rats received vehicle only for the same duration. Approximately, 24 h after the last treatment on days 7, 15, 30, 60, and 90 rats were anesthetized bilateral overiectomy using dorsal approach was performed,[27] for both 17 β- EE and vehicle treated rats at 12 weeks of age sparing SO. Long-term treatment with estrogen to Ovx females may be arguably an animal model for menopause. Twenty-four hour after last treatment for 7, 15, 30, 60, or 90 days trachea of each fasted rat, maintained on a heating table (37°C; Hugo Sachs Electronic, Germany), was cannulated under pentobarbital anesthesia.[28] Non-restored Ovx females or sham-operated intact females were sacrificed at the days the estrogen replacement therapy females were examined and sacrificed. Intermittent positive pressure respiration with oxygen-atmospheric-air-mixture (2.5 ml, 120/min) was maintained using Animal Ventilator (Harvard Apparatus, USA).

Hemodynamics

Common carotid arteries were dissected free and mean blood velocity was measured in right carotid artery with 20 MHz pulsed-Doppler flow probe (CBI-8000; Crystal Biotech, Hopkinton).[29] Left carotid artery was cannulated with heparinized (50 U heparin/ml) polyethylene cannula connected to pressure transducer for measurement of systolic and diastolic pressure using data acquisition and analysis system MP100 with Biopac Acknowledge software, version 3.7.3 and mean arterial blood pressure was calculated.[30] EBI 100C module is utilized to measure Z (t) directly and electrocardiogram was recorded using ECG-100C (NICO-100C, Biopac System). After 5-minute of equilibration/stabilization time, hemodynamic parameters were recorded for 20 min. Impedance cardiography-based cardiac hemodynamic is measured by incorporating a precision high-frequency current source and electrodes are placed (I1, I2, V1, V2) subcutaneously in the region of neck and thorax (near xiphisternum and mid-axillary line); thus, it measures the voltage across the tissue volume connected to transducers and amplifiers. STI between electrical and mechanical systole = PEP/LVET (PEP; time interval between ventricular depolarization and aortic valve opening) and (LVET; time interval between aortic valve opening and closing) were calculated.

Statistical analysis

All the values have been reported as the Mean ± standard error mean (SEM) in all the groups. Comparisons between different groups were performed by two-way ANOVA with Newman-Keuls multiple comparison test and Microcal software version 6.0 was used for data analysis[31] and differences were considered significant at $P < 0.05$.

RESULTS

STI

To assess left ventricular performance suggesting chamber dynamics and the presence of possible chronotropic and inotropic actions, the STI was found to increase consistently in 17 β-EE-treated Ovx rats in comparisons to vehicle control. The STIs obtained in Ovx rats are 0.80 ± 0.01, 0.82 ± 0.09, 0.71 ± 0.04, 0.93 ± 0.13, and 0.95 ± 0.1 after 7th, 15th, 30th, 60th, and 90th days of treatment, respectively, by 17 β-EE, whereas STI obtained in case of vehicle control is 0.61 ± 0.03 and that of sham-operated one is 0.78 ± 0.08 [Table 1].

PEP

PEP is the interval from the onset of ventricular depolarization to the beginning of the left ventricular ejection. PEP was 0.05 ± 0.002, 0.06 ± 0.01, 0.06 ± 0.002, 0.06 ± 0.002, and 0.06 ± 0.003 s for OvxE after 7th, 15th, 30th, 60th, and 90th days of treatment, respectively, and 0.06 ± 0.005 for vehicle-treated and 0.06 ± 0.002 for sham-operated rats [Table 1].

LVET

LVET reflects the duration that the aortic valve remains opened; thus, it is directly related to stroke volume (SV)[32-34] obtained with treatment of OvxE was 0.07 ± 0.003, 0.074 ± 0.01, 0.077 ± 0.01, 0.07 ± 0.01, and 0.074 ± 0.01 on 7th, 15th, 30th, 60th, and 90th day of treatment, respectively, whereas baseline with OvxV was 0.095 ± 0.06 s.

The cardiac output (CO) observed with treatment of OvxE was 47 ± 4.3, 44 ± 6.6, 46 ± 7.2, 48 ± 9.7, and 38 ± 8.3 on 7th, 15th, 30th, 60th, and 90th day of treatment, respectively, whereas baseline reading observed was 39 ± 5.5. OvxE treatment increased the heart rate (364 ± 20) on 7th day from that of control (320 ± 18); however, on 15th day of treatment, it reduced to (360 ± 23). Further reduction in heart rate was observed on 30th, 60th, and 90th day of treatment from that of 7th day. This suggests that OvxE modulates the heart rate, albeit not significantly from that of baseline/control. Moreover, OvxE treatment modulated the Mean blood pressure (MBP). Increase in MBP was observed on 7th, 15th, 30th, and 60th day of treatment. However, MBP came down to normal on 90th day of treatment. Also, concordant increase and decrease in Systolic and diastolic blood pressure were observed. These results suggest that 17 β-EE treatment can increase CO if administered for longer duration with slight modulation of MBP, blood flow velocity, cardiac index (CI), SV, and stroke volume index (SVI) related cardiac parameter and the effect of 17 β-EE treatment on CO, CI, SV, SVI, Velocity, Zo sec, and dZ/dtmax in Sham, OvxV, and OvxE rats on 7th, 15th, 30th, 60th, and 90th day are summarized in Table 2.

DISCUSSION

It has been shown that with 17 β-EE replacements, there is an increase in systemic arterial compliance[35-37] in post-menopausal women which reflected to be increase in STI because of peripheral vasodilatation. In addition to its reduction in the after load, estrogen can acutely

Table 1: Effect of 17β-Ethinyl estradiol treatment on heart rate, mean blood pressure, systolic blood pressure, diastolic blood pressure, left ventricular ejection time, pre-ejection period, and systolic time interval in Sham, ovariectomy vehicle and 17 β-Ethinyl estradiol rats on 7th, 15th, 30th, 60th and 90th days

Parameter	Sham	Vehicle control (OvxV)	17β-Ethinyl estradiol (OvxE) 150 µg/kg				
			7	15	30	60	90
HR beat/min	378±5	320±18	364±20	360±23	305±18	335±22	344±19
MBP mmHg	103±2	117±10	132±7	137±10	134±9	135±7	112±15
SBP mmHg	126±3	131±12	150±9	153±16	146±9	156±5	119±16
DBP mmHg	95±2	110±6	110±6	124±7	120±10	120±7	112±12
LVET s	0.076±0.01	0.095±0.006	0.07±0.003	0.074±0.01	0.077±0.01	0.070±0.01	0.074±0.01
PEP s	0.06±0.002	0.06±0.005	0.05±0.002	0.06±0.01	0.06±0.002	0.06±0.002	0.06±0.003
STI	0.78±0.08	0.61±0.03	0.80±0.01	0.82±0.09	0.71±0.04	0.93±0.13*	0.95±0.1*

Values are the mean±SEM *P<0.05, as compared to control, n=Minimum 6 numbers, HR: Heart rate; MBP: Mean blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LVET: Left ventricular ejection time; PEP: Pre-ejection period; STI: Systolic time interval; OvxV: Vehicle treated ovariectomized group; OvxE: 17 β-Ethinyl estradiol treated ovariectomized group

Table 2: Effect of 17β-Ethinyl estradiol treatment on cardiac output, cardiac index, stroke volume, stroke volume index, thoracic impedence and dZ/dtmax in Sham, ovariectomy vehicle and 17 β-Ethinyl estradiol rats on 7th, 15th, 30th, 60th and 90th days

Parameter	Sham	Vehicle control (OvxV)	17β-Ethinyl estradiol (OvxE) 150 μg/kg				
			7	15	30	60	90
CO ml/min	52.8±5.3	39±5.5	47±4.3	44±6.6	46±7.2	48±9.7	38±8.3
CI ml/min/100 g	24.6±2.1	17±2.3	20±1.7	21±3.6	21±3.8	25±4.6	20±4.3
SV ml/beat	0.19±0.01	0.21±0.07	0.14±0.01	0.12±0.2	0.15±0.02	0.14±0.03	0.12±0.04
SVI ml/beat/100 g	0.071±0.03	0.09±0.03	0.06±0.01	0.06±0.01	0.07±0.01	0.08±0.01	0.11±0.05
Velocity mm/s	18±4.2	16.17±1.4	13.1±2.3	13.8±3.0	8±1.5	15.2±2.4	16.7±2.2
Zo s	−28±3.9	−22±2.1	−21±1.8	−24±4.4	−19±2.1	−24±4.4	−17±10.7
dZ/dtmax ohms/s	2.1±0.3	1.8±0.48	2.0±0.3	2.4±0.6	1.7±0.4	2.3±0.4	1.5±0.3

Values are the mean±SEM **P*<0.05, as compared to control, *n*=minimum 6 numbers, CO: Cardiac output; CI: Cardiac index; SV: Stroke volume; SVI: Stroke volume index; LVET: Left ventricular ejection time; Zo: Thoracic impedence; dZ/dtmax: Maximum change in thoracic impedence during systole; OvxV: Vehicle treated ovariectomized group; OvxE: 17 β-Ethinyl estradiol treated ovariectomized group

increase CO and ejection fraction. In our studies, CO increased following 7, 15, 30, or 60 days of treatment; however, it restored to its range in vehicle control group following 90 days of treatment. STI can be influenced by factors like heart rate. Our study precludes the possible subtle impact of the marginal increase in heart rate with increase in STI following 90 days of treatment with estrogen. Combined effect of both increase in heart rate and MBP contributed at least in part to the increase in STI. STI has been found to increase with reduction in pre-load, with negative inotropy and in conditions of left ventricle diseases. STI should be viewed as a measure of chamber performance which under the most rigorous condition could be applied as a measure of contractility.[38] The mechanisms by which 17 β-EE affects cardiac and aortic geometry on ovariectomized rats are unclear. This study demonstrates that long-term use of 17 β-EE in female rats significantly increases systolic time index/STI.

It has been shown in the various perfusion studies on heart that at the moderate after load, there is decrease in negative dP/dt, whereas, the duration of cardiac relaxation is increased.[39] Estrogens have negative inotropic (direct effect) on mammalian heart due to reduced L-type voltage-sensitive calcium channel current.[40] Animal studies on gonadectomized rats show that with the long-term use of estrogen, there is decrease in contractile performance of heart[39] which has been corroborated by further studies on rabbit papillary muscles of heart, which shows decrease in isometric force of contraction.[41] Long-term estrogen study on human heart observed that estrogen can increase cardiac mass[42-44] as well as increase NO formation causing sustained coronary artery vasodilatation.[45] This is due to enhancement of endothelial function via NO-dependent pathway through non-genomic and other post-transcriptional mechanisms.[46,47]

LVET is inversely proportional to heart rate[48,49] which correlate with our data and observation in this study and is directly proportional to MBP[50] and condition which decreases pre-load[51] such as vasodilatation, SV,[32-34] end diastolic volume, and positive and negative inotropic drugs.[52] The cellular basis of estrogen may be mediated by the initiation or modification of protein synthesis and is presumed to be mediated by the nuclear translocation of cytosolic estrogen receptors present in vascular smooth muscle cells, myocardium,[53,54] and vascular endothelium.[55] Moreover, nuclear translocation of the estrogen receptor (and physiological actions of estrogens) in the cardiovascular system is reported to be absent following oophorectomy in female baboons.[54]

It is not clear till date whether the long-term protective effects of estrogen replacement therapy in women involve any direct effects of estrogens on the heart as described in the earlier studies. The long-term estrogen administration has several benefits such as causing reduction in the development of atherosclerosis[56,57] and improvement of the vasodilatory potential of normal and atherosclerotic vasculature.[58-60] However, the decreased potential for calcium entry through the L-type channel could provide some beneficial effect during either global or regional myocardial ischemia.[61,62]

CONCLUSION

Very little is known about the net effect of long-term estrogen administration on the electrical or mechanical consequences of myocardial functions. The results obtained from the present studies suggest that sex hormonal levels can have important influences on cardiac function and biochemistry. Precise mechanisms and physiological significance await further exploration. Moreover, STI should be considered an important hemodynamic parameter in cardiovascular system. Several drugs modulate or influence the hemodynamic functions of heart such as heart rate, pre-load, ejection fraction, after load, ejection fraction, etc. If all these parameters are taken together for assessing the myocardial contractibility and ventricular performance along with STI, then it could yield a more comprehensive and valid analysis of ventricular performance. Moreover, STI can be used as an important parameter in LV systolic function as it can be easily and accurately measured in clinical settings.

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