

Coronary Vascular and Aortic Endothelial Permeability During Estrogen Therapy: A Study in DOCA-Salt Hypertensive Ovariectomized Rats

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Summary

Cardiovascular disease (CVD) is a major source of morbidity and mortality in the Western World. Premenopausal and estrogen-treated postmenopausal women have a lower incidence of CVD. It has been suggested that circulating endogenous estrogens are probably responsible for this protection. This study investigated the hypothesis that the reduction of endothelial permeability is responsible for cardioprotective effects of estrogen in hypertensive animals. Forty-four rats were ovariectomized and divided into five groups: groups 1, 2 and 4 received DOCA-salt and groups 3 and 5 received normal saline (N/S) injection for four weeks. Then, in groups 4 and 5 the blood pressure was measured. Group 1 received estradiol valerate and in groups 2 and 3 continued with DOCA-salt and N/S injection for six weeks, respectively. Endothelial permeability was measured by Evans Blue extraction method. There was no significant difference in endothelial permeability in coronary circulation in estrogen-treated group and controls (12.97 ± 2.32 vs. 9.96 ± 1.01 , respectively). Also, aortic endothelial permeability in DOCA-salt hypertensive rats did not change significantly after estrogen treatment (28.34 ± 3.65 vs. 41.60 ± 5.98). This study showed that the cardioprotective effects of estrogen in DOCA-salt hypertensive animals are not mediated by a reduction of endothelial permeability.

Key words

Aorta • Coronary artery • Endothelial permeability • Estrogen • Hypertension

Introduction

Cardiovascular disease (CVD) is a major source of morbidity and mortality in the Western world (Keany 2000). CVDs are the leading cause of death among women, accounting for nearly 30 % of deaths (Lerner and Kannel 1986). The incidence in women have been found to lag 10 years behind those in men (Ho and Mosca 2002), because they lose their protection against CVD after the menopause (Sullivan and Fowlkes 1996). It has

been suggested that circulating endogenous estrogens are probably responsible for this protection (Ho and Mosca 2002, Sullivan and Fowlkes 1996). However, the mechanisms mediating this protection remain obscure. Although estrogens can favorably affect lipid profiles (Tolbert and Oparil 2001, Miller *et al.* 1991, Walsh *et al.* 1991, Walsh and Sacks 1991), only 25-50 % of the antiatherogenic effects of estrogens are attributable to its effects on lipid metabolism (Gruchow *et al.* 1988, Stampfer *et al.* 1991, Grady *et al.* 1992).

It has been observed that endothelial cell injury is responsible for the development of atherosclerosis. Ross and Glomset (1973) proposed that atherosclerosis resulted from arterial response to chronic injury. They reported that changes in injured endothelium lead to a disruption of its permeability characteristics and permit the interaction between the blood and arterial wall. Therefore, the endothelium plays a central role in the process of atherosclerotic disease (Anderson 1999, Britten *et al.* 1999). The accumulation of atherogenic lipoproteins in the arterial wall intima constitutes a fundamental event in the atherogenesis (Nielsen 1996, Williams and Tabas 1995). It is possible that estrogens may inhibit atherosclerosis process by direct effects on the arterial wall (Wagner *et al.* 1991).

Since the incidence of CVDs related to hypertension is significantly lower in premenopausal women than in men of similar age (Isles *et al.* 1992) and hypertension is associated with functional and morphological alterations of the endothelium (Kennedy and Tedgiu 1995), this study was designed to test the hypothesis that the reduction of endothelial permeability is responsible for cardioprotective effects of estrogens in hypertensive animals.

Methods

Animals

The experiment was carried out in female Wistar rats of body weight 175 ± 15 g fed a commercial pellet diet for rodents. The animals were housed four per cage with a 12-h light/dark cycle. All animals were obtained from the Pasteur Institute of Iran.

Ovariectomy surgery

Rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg). A longitudinal incision (0.5-1.0 cm) was made in the midline area of lower abdomen. A small peritoneal incision was made and the ovaries were removed.

Experimental design

After recovery, the animals were randomly divided into five groups. Hypertension was induced by DOCA-salt treatment as previously described (Huang *et al.* 1992). DOCA (Iran Hormone Co, Iran) was injected 30 mg/kg of body weight subcutaneously, twice a week, tap water for drinking was replaced by 1 % NaCl throughout the treatment period. Estradiol valerate

(Aboureihan Co, Iran) was injected 2 mg/week, i.m. In control groups, normal saline (N/S) was injected with the same volume. The groups were as follow:

Group 1 (n = 11): DOCA-salt for four weeks and DOCA-Salt + estradiol valerate for six weeks.

Group 2 (n = 8): DOCA-salt for four weeks and DOCA-Salt + N/S injection for six weeks.

Group 3 (n = 11): N/S injection for ten weeks.

Group 4 (n = 7): DOCA-salt for four weeks only.

Group 5 (n = 7): N/S injection for four weeks only.

Blood pressure measurement

After four weeks, the animals of groups 4 and 5 were anesthetized with an intraperitoneal injection of ketamine. A polyethylene catheter was inserted into the right common carotid artery and direct blood pressure was measured by a physiograph (Bioscience, England). Comparison of blood pressure measurement in these groups showed that DOCA-salt hypertension was induced successfully. After ten weeks, the remaining animals were subjected to direct blood pressure measurement. Comparison of blood pressure between groups 2 and 3 showed that induced hypertension continued throughout the experiment.

Measurement of endothelial permeability

Coronary and aortic endothelial permeability were measured in groups 1 and 2. Endothelial permeability was determined by extravasation of injected Evans Blue dye (EB) as previously described (Hulthen *et al.* 1996). Briefly, EB diluted in normal saline (20 mg/ml) was administered through the catheter. After 20 min, rats were sacrificed. Heart and aorta (to the beginning of renal arteries) were isolated and cleaned from surrounding connective tissues. They were then weighed immediately and put into formamide solution (aorta: 2 ml, heart: 4 ml) for 24 h at room temperature for EB dye extraction. The extracted amount of EB and plasma EB diluted in formamide, was determined by a spectrophotometer (Secomam, France) at 620 nm wavelength. The results were plotted on standard of EB in 0.2 to 10 $\mu\text{g/ml}$ formamide. Concentration of EB in these tissues was expressed in $\mu\text{g/gram}$ wet weight ($\mu\text{g/g}$ ww) tissue and tissue/plasma EB ratio.

Statistical analysis

Data are reported as means \pm S.E.M. Student's t-test was used for comparison between two groups. The

difference between three groups was analyzed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

Blood pressure (Table 1)

Blood pressure measurements of group 4 (DOCA-salt for four weeks) and group 5 (N/S injection) showed that mean arterial pressure (MAP) significantly

increased in DOCA-salt treated rats compared to the controls. These data indicated that hypertension was induced successfully in rats by the DOCA-salt treatment. At the end of the experiment, there were significant differences in MAP between groups 1 (DOCA-salt + estradiol) and 2 (DOCA-salt) from group 3 (N/S injection). These data indicated that hypertension continued in DOCA-salt treated rats. The results also showed that MAP in DOCA-salt hypertensive animals did not change significantly after estrogen treatment.

Table 1. Systolic, diastolic and mean arterial pressure (mm Hg) in particular experimental groups.

Group	n	Systolic BP	Diastolic BP	Mean arterial pressure
Group 1	11	160.4±4.1	112.7±3.7	128.6±3.72
Group 2	8	161.6±4.1	120.4±5.2	134.1±5.84
Group 3	1	133.6±4.7*	103.9±4.2*	113.8±4.26*
Group 4	7	156.4±5.6 [#]	117.1± 6.7 [#]	130.2± 6.21 [#]
Group 5	7	118.6± 4.6	90.7± 4.8	100.0±2.20

Data are expressed as Mean ± S.E.M.* significant difference ($P < 0.05$) between groups 1 and 2.
[#] significant difference ($P < 0.05$) between groups 4 and 5.

Table 2. Quantitative extravasation of EB and heart or aorta/plasma EB ratio.

Group	n	Aorta EB (µg/g ww)	Heart EB (µg/g ww)	Aorta/plasma EB ratio	Heart/plasma EB ratio
Group 1	11	41.60±5.98	12.97±2.32	0.256±0.068	0.817±0.087
Group 2	8	28.34±3.65	9.96±1.01	0.094±0.017	0.539±0.097

Data are expressed as Mean ± S.E.M.

Endothelial permeability (Table 2)

There was no significant difference in endothelial permeability in coronary circulation (expressed as quantitative extravasation of EB and tissue/plasma EB ratio) between estrogen-treated hypertensive rats and hypertensive rats. Moreover, aortic endothelial permeability in DOCA-salt hypertensive rats did not also significantly change after estrogen treatment.

Discussion

The effect of estrogen on coronary vascular and aortic endothelial permeability was the objective of this study. Results showed that coronary vascular and aortic endothelial permeability did not change significantly after

estrogen treatment.

The cardioprotective effects of estrogen are supported by several epidemiological studies, which have been prompted by the recommendation for the widespread use of postmenopausal replacement therapy (Lobo and Speroff 1994). It has traditionally been assumed that the antiatherogenic effect of estrogen is due to amelioration of the serum lipid pattern (Miller *et al.* 1991, Walsh *et al.* 1991, Walsh and Sacks 1991, Lobo and Speroff 1994, Tolbert and Oparil 2001). However, recent studies suggested that direct effects of estrogen on blood vessels may contribute significantly to the cardioprotective effects (Mendelsohn 2002). Both endothelial cells and vascular smooth muscle cells possess estrogen receptors and they are physiological

targets for estrogen action (Karas *et al.* 1994, Farhat *et al.* 1996).

Endothelial dysfunction is a primary defect in essential hypertension, which is present even before the elevation of blood pressure (Panguina *et al.* 2000). Hypertension is associated with functional and morphological alterations of the endothelium (Lüscher 1994), and endothelial dysfunction creates a vicious cycle that enhances propensity to the development of atherosclerosis, regardless of the initial cause of the hypertensive process (Panguina *et al.* 2000). A major hypothetical change in endothelial function is the increase of endothelial permeability (Ross *et al.* 1990), particularly to atherogenic lipoproteins (Thubrikar *et al.* 1992, Nielsen *et al.* 1992, Nordestgaard and Nielsen 1994). In hypertension, the permeability of both the endothelium and media are altered (Kennedy and Tedgui 1995).

There is a link between endothelial permeability and progression of atherosclerosis (Nielsen 1996). It has been suggested that the alterations in endothelial permeability and suppression of the accumulation of atherogenic lipoproteins may be important for the estrogenic effects (Hough and Zilvermit 1986, Wagner *et al.* 1991). In this study we tested the hypothesis that reduced endothelial permeability may account for the cardioprotection of premenopausal and postmenopausal women who received estrogen. According to our results, estrogen could not change endothelial permeability in DOCA-salt hypertensive rats. Our results agree with the study of Haarbo *et al.* (1994) who observed that plasma lipid-independent antiatherogenic effect of estradiol is not mediated through an effect on aortic permeability to LDL, but this effect is related to the metabolism of lipoproteins after they had entered the arterial wall. Also,

Robert *et al.* (1997) showed that supraphysiological concentrations of 17-beta estradiol can increase LDL accumulation in the artery wall. However, another study reported a 50 % reduction of basal LDL accumulation rate and a 25 % decrease in endothelial layer permeability in arteries from estradiol-treated animals (Walsh *et al.* 2000). Another *in vitro* study suggested that an important effect of estradiol treatment is protection of arterial wall from modified LDL-mediated injury (Gardner *et al.* 1999). There have been conflicting reports as to whether estrogens are capable of modulating arterial permeability.

It should be considered that endothelial cells can release several relaxing and constricting factors which are important for the dynamic properties of the arterial wall. Endothelial cells release nitric oxide (NO), vascular endothelial growth factor (VEGF) or endothelin-1 that can separately affect the endothelial permeability (Toborek and Kaiser 1999). Estrogen can change these factors and thus affect the vascular wall. Maintenance and upregulation of endothelial NO production seems to be an ideal mediator of the beneficial cardiovascular effect of estrogen, due to its multiple vasculoprotective actions (Rubanyi *et al.* 2002). Estrogen can also increase serum levels of VEGF (Agrawal *et al.* 2000) which is involved in the atherosclerosis process in humans (Sumino *et al.* 2000).

We conclude that the cardioprotective effects of estrogen in hypertensive animals are not mediated by the reduction of endothelial permeability.

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