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Effect of 17-β-estradiol administration during adriamycin-induced cardiomyopathy in ovariectomized rat

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Abstract

The incidence of cardiovascular diseases in humans differs in relation to the age of the patient. Although women suffer less than men from cardiovascular disorders during 15–55 years, after this period the incidence is equivalent in both sexes. This data suggests a cytoprotective effect of estrogens against cardiovascular disease. The estrogens, especially 17-β-estradiol, are important antioxidant molecules with potential cytoprotective properties during oxidant/antioxidant disbalance induced by oxidative stress. Oxidative stress is often the underlying mechanism during vascular alterations and cardiac damage.

The present study evaluated the role of ovariectomy and/or 17-β-estradiol administration on antioxidant status and lipid peroxidation during cardiac injury induced by adriamycin. Different parameters were measured, including hemodynamic response (arterial pressure and cardiac frequency), lipid peroxidation products (malondialdehyde), protein carbonylation, antioxidant status (reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase), and cardiac injury (creatinine kinase, lactate dehydrogenase, aspartate and alanine aminotransferase).

Our study showed that 17-β-estradiol reduced all of the parameters related to oxidative stress and cardiac injury in ovariectomized rats treated with adriamycin.

Keywords: 17-β-estradiol; Cardiomyopathy; Oxidative stress; Ovariectomy; Adriamycin

1. Introduction

Cardiovascular diseases cause a high rate of mortality in the human population (Wenger, 1997; Pijna and Buchter, 2003). Nevertheless, women between 25 and 55 years old suffer less than the corresponding male population from cardiovascular disorder. After this period, the incidence of cardiovascular disease is equivalent in both sexes (Ryan, 1976; Dimitrova et al., 2002). Numerous studies have shown that the presence of estrogens plays an important role in the protection against cardiac injury. Several hypotheses have been suggested to explain the beneficial effects of 17-β-estradiol. One of the more accepted hypotheses is related to its antioxidant properties during oxidative stress in cardiac and endothelial injury (Korantzopoulos et al., 2003; Bureau et al., 2003; Obata, 2002; Sugishita et al., 2003). The beneficial effect of estrogens may be also explained through the modulation of the renin–angiotensin system (Wassmann et al., 2001; Allen and Tresini, 2000; Tamir et al., 2002).

The administration of estrogens has been successfully used during cardiovascular dysfunction in postmenopausal women (Collins, 2002; Ganz, 2002; Sitruk-Ware and Ibarra de Palacios, 1989). Different investigators have recently observed that the prolonged treatment with estrogens may be associated with a high risk of cancer in endometrium, uterus, or breast (Bolego et al., 1997). Nevertheless, it has been also shown a beneficial effect of estrogens in different experimental studies involving ovariectomized animals (Hernández et al., 2000; Geary and

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Asarian, 1999). In these studies, estradiol regulates the weight, arterial mean pressure, nitric oxide production, and different parameters related to oxidative stress induced by ovariectomy.

Doxorubicin (adriamycin, AD), an anthracycline antibiotic, is a potent broad-spectrum chemotherapeutic agent that is effective against a wide range of human neoplasm as breast cancer, multiple myeloma, lymphoblastic leukaemia, ovarian cancer, and other (Kuku et al., 2005; Lamont et al., 2005; Signorelli et al., 2005; Oktem et al., 2004). However, the clinical uses of AD have been restricted owing to its serious cardiotoxic effects (Gewirtz, 1999; Singal and Iliskovic, 1998). Given by different routes of administration, provides an ideal model in rats and other rodents, of oxidative stress (Bertazoli et al., 1972). Recently, it has demonstrated that oxidative stress plays a key role in adriamycin-induced failure (Ahmed et al., 2005; Kumar et al., 2002; Singal et al., 1997).

In the present study, we evaluated the cardioprotective properties of 17-β-estradiol in experimental cardiomyopathy induced by ovariectomy in the presence or not of adriamycin in rats. We analyzed biochemical parameters indicative of oxidative stress and heart damage.

2. Material and methods

2.1. Animals

All animal care and procedures were in accordance with the European Community Council Directive of 24 November 1986 (86/609/ECC) and the R.D. 223/1988. All these guidelines were approved by the Bioethic Committee of the University of Cordoba. Female Wistar rats ($n=49$) of ten weeks old weighing between 200 and 220 g at the beginning of the study were purchased from Charles River (Barcelona, Spain). They were subjected to controlled conditions of temperature (22±2 °C), humidity, light cycle (12 h light : 12 h dark), and were provided with food (Purina®, Barcelona, Spain) and water ad libitum. The rats were divided into seven groups: 1) control, 2) sham operated, 3) OVX, 4) AD, 5) OVX+AD, 6) OVX+17βE_2 and 7) OVX+AD+17βE_2.

2.2. Treatments

The ovariectomy was done using a bilateral procedure as described by Pomeau-Delille (Poumeau-Delille, 1953). The treatments were administered to animals after verification of the absence of the estrous cycle in the rat; vaginal smears were daily collected for 7 days. This period was usually lasting twenty days. The animals from control and sham operated maintained a normal body weight during all the study.

Adriamycin (Pharmacia and Upjohn, Michigan, USA) was administered intraperitoneally twice (at 0 and 9 days) at a dose of 10 mg/kg body weight. 17-β-estradiol was administered subcutaneously at a dose of 2.5 mg/kg body weight in dimethylsulfoxide every two days until sacrifice. The solvent had no effect on the studied parameters. The animals were sacrificed under anesthesia 18 days after the administration of treatments according to previous studies (Montilla et al., 2000). Blood samples were obtained from the vascular trunk of the neck. The plasma fraction was used for the determination of different parameters related to cardiac damage. The whole heart was removed and frozen in liquid N_2 until use. After thaw, the tissue was immediately homogenated in 20 mM Tris–HCl pH 7.4 and centrifuged at 10,000 ×g for 10 min at 4 °C. The samples were immediately processed following the procedures described below.

2.3. Hemodynamic parameters

The arterial mean pressure and cardiac frequency were measured using a cylindrical device for the measure of pressure (LE 5100 Pressure Cylinder) coupled to a digital device (LE 5000 Digitalis Pressure Meter). The procedure required that the animal was maintained calm in order to get homogenous data along all the measurements. There were carried out ten–fifteen measures from each animal. The values of control have high reproducibility in our experiments.

2.4. Lipid peroxidation

The levels of lipid peroxidation products were determined using a commercial assay (LPO-586, Oxis Byoxitech, Oregon, USA). The heart was homogenated in 20 mM Tris–HCl pH 7.4 and centrifuged at 10,000 ×g for 15 min at 4 °C. The assay is based on the reaction of a chromogenic reagent, *N*-methyl-2 phenylindole, with malondialdehyde at 45 °C. The results were expressed in relation to the protein content.

2.5. Protein carbonylation

The measurement of protein oxidation was done following a modification of the procedure described by Oliver et al. (1987). Briefly, heart homogenate was deproteinized (v/v) with 500 μl 20% trichloroacetic acid for 10 min at 4 °C. After centrifugation of the samples at 10,000 ×g for 5 min, the supernatant was incubated with 500 μl of 10 mM dinitrophenylhidrazine in 2 N HCl for 1 h at room temperature. Samples were precipitated again with 500 μl of 20% trichloroacetic acid for 10 min at 4 °C. After desproteinization, the samples were centrifuged at 10,000 ×g for 5 min. The supernatant was extracted twice with 1 ml of ethanol/ethylacetate (1 : 1) (v/v). It was added a volume (1 ml) of 6 M guanidine, and the mixture was incubated for 15 min at 37 °C. The samples were measured at 360 nm using a coefficient of extinction $\varepsilon=21\ M^{-1}\ cm^{-1}$.

2.6. Reduced glutathione

Reduced glutathione (GSH) content was determined using a commercial assay GSH-400 (Byoxitech Oregon, USA). Briefly, all mercaptans present in the sample of heart homogenate reacted with 4-chloro-1-methyl-7-trifluoromethyl-quinolinium methylsulfate in a first chemical reaction. The second step consisted on the β-elimination reaction under alkaline conditions of the product from the first reaction into a chromogenic thione-derived product.

2.7. Glutathione peroxidase, catalase, and superoxide dismutase

Liver was homogenated following the procedure described above. Glutathione peroxidase was measured according to the method by Flohé and Gunzler (1984). The procedure is based on the capacity of glutathione peroxidase to block free radical generation by ter-butylhydroperoxide (1 mM) in the presence of GSH (5mM), NADPH (0.5 mM) and glutathione reductase (0.125 U/ml) in 50 mM potassium phosphate buffer at pH 7.7. Glutathione peroxidase activity was inversely proportional to the consumption of NADPH. Catalase was determined following the method described by Aebi (1984). Catalase was measured in the homogenate by the rate of decomposition of H_2O_2 (10 mM) to water and molecular oxygen measured at 240 nm. Superoxide dismutase (SOD) activity was assessed according to the method published by Sun et al. (1988). The assay is based on the inhibition of nitroblue tetrazolium reduction, with xanthine–xanthine oxidase system used as a superoxide generator. The activity of SOD was evaluated by measurement of absorbance at 420 nm of the sample. All the measurements were made in relation to the protein content of the sample.

2.8. Nitric oxide production in heart

Nitric oxide production was evaluated using a commercial, non-enzymatic assay from Byoxitech Oregon, USA. This assay is based on the chemical reduction of nitrate to nitrite by granulated cadmium that reacts with the chromogenic Greiss reagent.

2.9. Parameters related to cardiac injury

Different parameters related to cardiac injury, such as the activity of creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were automatically analyzed in plasma (Axon, Bayer Diagnostic, Tarrytown, NY, USA).

Table 1
Analysis of creatinine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in plasma of ovariectomized (OVX) rats treated or not with 17-β-estradiol ($17\beta E_2$) or adriamycin (AD)

	AST	ALT	CK	LDH
	UA/L ($\times 10^{-2}$)	UA/L	UA/L ($\times 10^{-3}$)	UA/L ($\times 10^{-2}$)
Control	1.24±0.152	24.9±2.9	3.29±0.274	8.27±1.99
Sham operated	1.24±0.154	25.2±2.8	3.34±0.263	8.34±2.06
OVX	1.77±0.134[a]	30.6±2.9[b]	5.65±0.848[a]	9.82±0.11[b]
AD	3.35±0.33[a]	40.2±1.5[a]	6.87±0.740[a]	12.3±1.45[a]
OVX+AD	3.70±0.341[a,c,f]	45.8±2.7[a,c,e]	7.31±3.202[a,c]	15.1±4.4[a,c]
OVX+$17\beta E_2$	1.32±0.193[c]	24.0±2.9[c]	3.38±0.408[c]	7.86±0.77[c]
OVX+AD+$17\beta E_2$	2.04±0.184[d]	34.0±5.5[d]	3.70±0.933[d]	10.3±1.33[d]

Values are expressed as the mean±S.E.M. ([a]$P \le 0.001$; [b]$P \le 0.01$ vs. sham operated), ([c]$P \le 0.001$ vs. OVX), ([d]$P \le 0.001$ vs. OVX+AD) and ([e]$P \le 0.001$; [f]$P \le 0.01$ vs. AD).

Table 2
Analysis of lipid peroxides, reduced glutathione (GSH) and carbonyl protein content in cardiac homogenates in heart tissue of ovariectomized (OVX) rats treated or not with 17-β-estradiol ($17\beta E_2$) or adriamycin (AD)

	Lipid peroxides	GSH	Carbonyl protein
	(μmol/mg protein)	(μmol/mg protein)	(nmol/mg protein)
Control	5.513±0.65	6.983±0.20	1.04±0.223
Sham operated	5.509±0.57	6.991±0.46	1.04±0.111
OVX	9.875±0.96[a]	3.556±0.25[a]	3.44±0.157[a]
AD	11.80±0.45[a]	2.159±0.25[a]	4.04±0.303[a]
OVX+AD	13.27±0.78[a,b,e]	1.597±0.17[a,b,e]	6.39±1.098[a,c,f]
OVX+$17\beta E_2$	5.433±0.17[b]	6.907±0.40[b]	1.64±0.069[b]
OVX+AD+$17\beta E_2$	4.792±0.34[d]	6.013±0.21[d]	2.26±0.177[d]

Values are expressed as the mean±S.E.M. ([a]$P \le 0.001$ vs. S.O), ([b]$P \le 0.001$; [c]$P \le 0.01$ vs. OVX), ([d]$P \le 0.001$ vs. OVX+AD) and ([e]$P \le 0.001$; [f]$P \le 0.01$ vs. AD).

2.10. Statistical analysis

All results are expressed as the mean±S.E.M. Differences between groups were assessed by the one-way analysis of variance (ANOVA). If the variances between groups were homogenous (Levene's test), groups were subjected to the multiple comparison Bonferroni's test. If the equal variances were not assumed, the significance was evaluated by the Dunnett's T3 test. The statistical significance was set at $P \le 0.05$ and indicated using superscript symbols in the tables.

3. Results

3.1. Evaluation of cardiac injury

The parameters related to cardiac injury are shown in Table 1. Ovariectomy or adriamycin enhanced significantly cardiac injury compared with sham operated animals ($0.001 \le P \le 0.01$). In addition, ovariectomy and adriamycin exerted an additive effect in the rise on AST and ALT in plasma from ovariectomy+adriamycin-treated rats ($P \le 0.001$). 17-β-estradiol administration had a significant cytoprotective

Table 3
Analysis of glutathione peroxidase, catalase and superoxide dismutase (SOD) in heart tissue of ovariectomized (OVX) rats treated or not with 17-β-estradiol ($17\beta E_2$) or adriamycin (AD)

	Glutathione peroxidase	Catalase	SOD
	(AU/mg protein)	(AU/mg protein)	(AU/mg protein)
Control	2.17±0.234	0.0425±0.0061	5.20±0.606
Sham operated	2.16±0.157	0.0419±0.0044	5.09±0.655
OVX	1.30±0.102[a]	0.0210 ±0.0021[a]	2.84±0.183[a]
AD	1.10±0.066[a]	0.0132 ±0.0044[a]	2.23±0.265[a]
OVX+AD	0.85±0.095[a,b,e]	0.0113±0.0010[a,b]	1.37±0.126[a,b,e]
OVX+$17\beta E_2$	2.10±0.154[b]	0.0425±0.0009[b]	4.54±0.353[b]
OVX+AD+$17\beta E_2$	2.00±0.192[c]	0.0411±0.0028[c]	5.64±0.610[c]

Values are expressed as the mean±S.E.M. ([a]$P \le 0.001$ vs. S.O), ([b]$P \le 0.001$ vs. OVX), ([c]$P \le 0.001$ vs. OVX+AD) and ([e]$P \le 0.001$ vs. AD). AU: Activity Unit.

effect on ovariectomy and ovariectomy + adriamycin-treated rats ($P \leq 0.001$).

3.2. Evaluation of oxidative stress

Table 2 shows different parameters related to oxidative stress. Ovariectomy and adriamycin enhanced lipid peroxides and carbonyl protein content in the heart ($P \leq 0.001$). In addition, ovariectomy and adriamycin exerted an additive effect on the rise of lipid peroxides and carbonyl protein content in heart from ovariectomy + adriamycin-treated rats ($0.01 \leq P \leq 0.001$). Administration of 17-β-estradiol had a significant cytoprotective effect in ovariectomy and ovariectomy + adriamycin-treated rats ($P \leq 0.001$).

3.3. Evaluation of antioxidants status

GSH, glutathione peroxidase, catalase, and superoxide dismutase content in heart tissue are showed in Tables 2 and 3. All of them followed a similar profile with ovariectomy and/or adriamycin experimental intervention. Ovariectomy and adriamycin decreased GSH content (Table 2) and catalase, superoxide dismutase, and glutathione peroxidase activities in heart tissue ($P \leq 0.001$) (Table 3). In addition, ovariectomy and adriamycin exerted an additive effect in the depletion of glutathione peroxidase, GSH and superoxide dismutase activities in heart from ovariectomy + adriamycin-treated rats ($P \leq 0.001$). The administration of 17-β-estradiol recovered the antioxidant status in ovariectomy and ovariectomy + adriamycin-treated animals ($P \leq 0.001$).

3.4. Evaluation of hemodynamic parameters

Ovariectomy, adriamycin, and ovariectomy + adriamycin-treated rats had increased significantly arterial mean pressure in rats ($0.05 \leq P \leq 0.001$) (Table 4). 17-β-estradiol administration reduced significantly arterial mean pressure and cardiac frequency in ovariectomy and ovariectomy + adriamycin-treated rats ($0.01 \leq P \leq 0.001$). Ovariectomy reduced the production of nitric oxide in heart ($P \leq 0.001$). By contrast, adriamycin enhanced significantly cardiac frequency and nitric oxide production in heart from experimental animals ($P \leq 0.001$ and $P < 0.01$, respectively). The administration of 17-β-estradiol increased nitric oxide in ovariectomy rats that was reduced in ovariectomy + adriamycin-treated rats ($0.05 \leq P \leq 0.01$).

Table 4
Analysis of arterial mean pressure (PAM), cardiac frequency and nitric oxide production in ovariectomized (OVX) rats treated or not with 17-β-estradiol ($17\beta E_2$) or adriamycin (AD)

	Arterial mean pressure	Cardiac frequency	Nitric oxide
	(mm Hg)	(s.p.m)	(μM/mg protein)
Control	130±16.2	387±33.9	0.0433±0.00122
Sham operated	129±12.5	386±32.28	0.0434±0.00087
OVX	136±5.1[c]	366±35.6	0.0401±0.00046[a]
AD	144±12.4[b]	446±11.0[a]	0.0499±0.00102[a]
OVX+AD	149±2.4[a,d]	463±27.9[a,d]	0.0457±0.00167[b,d,h]
OVX+17βE$_2$	130±3.2[d]	338±18.5[e]	0.0434±0.00151[e]
OVX+AD+17βE$_2$	133±6.1[f]	337±24.8[f]	0.0426±0.00193[g]

Values are expressed as the mean±S.E.M. ([a]$P \leq 0.001$; [b]$P \leq 0.01$; [c]$P \leq 0.05$ vs. sham operated), ([d]$P \leq 0.001$; [e]$P \leq 0.01$ vs. OVX), ([f]$P \leq 0.001$; [g]$P \leq 0.05$ vs. OVX+AD) and ([h]$P \leq 0.001$ vs. AD).

4. Discussion

Women between 25 and 55 years old suffer less than the corresponding male population from cardiovascular disorders. Different experimental studies have shown that the presence of estrogens plays an important role in the protection against cardiac injury. In our study, the administration of adriamycin or ovariectomy induced cardiomyopathy associated with the depletion of antioxidant status and exacerbation of oxidative stress in rats. The treatment with 17-β-estradiol recovered the antioxidant status, and prevented oxidative stress and cardiac injury induced by ovariectomy and/or adriamycin in rats.

The administration of anthracyclines, such as adriamycin, induces cardiomyopathy associated with an exacerbation of oxidative stress (Gewirtz, 1999; Singal and Iliskovic, 1998; Keizer et al., 1990). The present study has been focused to elucidate the role of 17-β-estradiol on oxidative stress and cardiac injury induced in rats. In our conditions, ovariectomy and/or administration of adriamycin reduced antioxidant status, enhancing oxidative stress, cardiac injury and hemodynamic disturbances. The cardiac injury induced by ovariectomy suggests that estrogens may be related to the maintenance of correct cardiac function. This relationship has already been suggested by other investigators in similar experimental models (Bolego et al., 1997; Hernández et al., 2000; Persky et al., 2000). The depletion of estrogens, progest and their metabolites by ovariectomy is also able to induce nephropathy (Montilla et al., 2000). Ovariectomy induced a significant increase in the concentration of lipid peroxides and carbonyl protein, as well as a clear reduction in GSH, catalase, superoxide dismutase and glutathione peroxidase content in cardiac tissue. The induction of oxidative stress in ovariectomy rats has been related to the deficiency of 17-β-estradiol (Ruiz-Larrea et al., 1993) or overexpression of angiotensin AT_1 receptors (Persky et al., 2000; Milsted et al., 1998). Ovariectomy induces hypertension and free radical generation in endothelial cells (Wassmann et al., 2001). In this study, the administration of an angiotensin AT_1 receptor antagonist (irbesartan) recovered the vascular function induced by ovariectomy. Other studies have also observed enhanced cardiac tissue injury and arterial pressure dysfunction in ovariectomized rats (Persky et al., 2000; Milsted et al., 1998). Increased oxidative stress and reduced nitric oxide production during estrogen deficiency may also be the underlying mechanism for the reduction of cardiac frequency in ovariectomy rats (Hernández et al., 2000). The negative impact of ovariectomy on nitric oxide production has been previously documented (Khorram et al., 2002; Goetz et al., 1994). In concordance, the administration of 17-β-estradiol abolished the reduction of nitric oxide production in heart induced by ovariectomy. The administration of estradiol to ovariectomy rats

enhanced the activity of nitric oxide synthase and nitric oxide production (Goetz et al., 1994).

In our study, we observed that ovariectomy exacerbated oxidative stress and reduced cardiac function in adriamycin-treated rats. Ovariectomy enhanced arterial mean pressure, oxidative stress and renal damage (urea, creatinine, and proteinuria) in adriamycin-treated rats (Montilla et al., 2000; Sakemi et al., 1997). Although ovariectomy or adriamycin induced cardiac injury, both noxious strategies exerted an opposite effect on cardiac frequency and nitric oxide production in heart. This observation may be related to a different underlying mechanism of cell damage or systemic effect of ovariectomy or adriamycin in rats.

The exogenous administration of 17-β-estradiol has an important beneficial effect against oxidative stress and cardiac injury in ovariectomy+adriamycin-treated rats. The administration of estradiol led to a remarkable reduction of the level of lipid peroxides and carbonyl protein with a significant recovery of GSH, glutathione peroxidase, catalase, and superoxide dismutase in cardiac homogenate from ovariectomy and ovariectomy+adriamycin-treated animals. A previous study has suggested that 17-β-estradiol may exert antioxidant activity or enhance the expression of antioxidant enzymes (Kim et al., 1998). Estradiol also protects against oxidative stress in cardiomyocytes induced by ischemia–reperfusion and hypoxia (Kim et al., 1994; McHugh et al., 1998; Becker et al., 1999). The antioxidant properties of estradiol have been related to the phenolic A ring of its structure (Mooradian, 1993). The hydroxyphenolic structure of estradiol can donate hydrogen atoms from its hydroxyl phenolic group to lipid radicals, blocking the lipid peroxidation chain reaction (Green et al., 1997). In this context, estradiol is able to abolish the cytomegalovirus-induced free radical generation by an independent receptor-mediated signal in human coronary artery smooth muscle cells (Speir et al., 2000). In this study, estradiol showed antioxidant properties even in the presence of the estrogen receptor inhibitors such as tamoxifen or ICI182,780. In addition, they demonstrated that estradiol-derived metabolites without the phenolic hydroxyl group, like 3-methoxyestrone, did not reduce free radical generation induced by H_2O_2 exposition or human cytomegalovirus infection in human coronary artery smooth muscle cells (Speir et al., 2000). Other studies have shown that progestagens without phenolic ring, such as progesterone or medroxyprogesterone acetate, and estradiol in the presence of antiestrogens such as ICI182780 were not able to prevent Cu-induced LDL oxidation (Hermenegildo et al., 2002).

The administration of estradiol plays an important role in the prevention of the atherosclerosis through the regulation of antioxidant enzyme expression and free radical production (Strehlow et al., 2003). Our study showed that 17-β-estradiol prevented the reduction of GSH content, superoxide dismutase, catalase, and glutathione peroxidase activities, and prevented oxidative stress and cardiac injury in ovariectomized rats. Tanaka et al. (2000) have shown that the rise of estrogen levels was related to enhanced SOD expression in monocytes. The reduction of hypertension by 17-β-estradiol may be related to the enhancement of nitric oxide production in ovariectomy rats. Estrogens bind to specific receptors (ERα and ERβ) expressed in endothelial cells and vascular smooth muscle cell membranes. Prolonged treatment with estrogens increases the expression of prostacyclin synthase and endothelial nitric oxide synthase (eNOS) (Lau, 2002). On the other hand, estrogens interact with vasoactives substances, such as the renin–angiotensin system, exerting a beneficial effect against coagulation and lipid profile (Geary and Asarian, 1999). As expected, the administration of 17-β-estradiol enhanced nitric oxide production in ovariectomy animals. Nevertheless, the estrogen reduced nitric oxide production in adriamycin-treated rats. This different effect of 17-β-estradiol may be a consequence of a different underlying mechanism of cell damage induced by ovariectomy or adriamycin in rats.

The data presented show that the beneficial effect of estrogens on oxidative stress in our experimental groups can possibly be carried out by different pathways: i) Direct action, blocking the production of reactive species and inhibiting the oxidative cascade. Situation characterized by reduction in damage oxidative biomarkers (lipid peroxidation products and protein carbonylation). ii) Indirect action, stimulating the genic expression (Kim et al., 1998) and the activity of antioxidant enzymes (SOD, glutathione peroxidase and catalase).

Moreover and although numerous studies show the pleiotropic and beneficial effects of estradiol, other studies have also suggested a toxic effect after prolonged administration or the generation of metabolites that could have noxious effects (Liehr, 1997). Estrogens have been administered in order to counteract the dysfunctions that take place in the women during and after the menopause. Nevertheless, different studies have recently demonstrated that the replacement therapy with estrogens can increase the risk of cancer (Blumel et al., 2004; Espeland et al., 2004). In this sense, it has been suggested that prolonged treatments with natural and synthetic estrogens as well as their metabolites can induce genetic mutations, DNA breaks, poliploidy and aneuploidy (Joosten et al., 2004). It has been also observed that the daily intake of equine estrogens plus medroxyprogesterone increased the risk of venous thromboembolic events and gallbladder disease in postmenopausal women (Grady et al., 2002). Moreover, some randomized studies did not find beneficial effect of substitution hormonal therapy on cardiovascular risk, whereas other works showed an increase of the risk of fatal stroke in women hormone-treated (Wenger, 2005; Gutiu, 2000-2001). These studies strongly suggest that the problem of the effects of hormone therapy on cardiovascular system is still in dispute, and estrogen replacement therapy should be administered with cautions in order to avoid undesirable side effect of the treatment. In this sense, further studies are required to elucidate the beneficial therapeutic dosage of estrogen administration in order to avoid the potential noxious effect.

In summary, our results showed that estrogen deficiency by ovariectomy leads to a depletion of antioxidant status, exacerbation of oxidative stress and a deterioration of cardiac function. The exacerbation of cardiac injury by ovariectomy in adriamycin-treated rats suggests that the depletion of estrogens

may unmask an underlying cardiac susceptibility to cell damage. The administration of 17-β-estradiol reduced oxidative stress and cardiac injury in animals subjected to ovariectomy and ovariectomy + adriamycin. In conclusion, our findings show the beneficial effect of 17-β-estradiol in maintaining cardiac function in control and adriamycin-treated animals. The reduction of oxidative stress by 17-β-estradiol supports that its protective effect is mostly related to the antioxidant capacity of the molecule.

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