Baroreflex Sensitivity and Oxidative Stress in Adriamycin-Induced Heart Failure

Eneida Rabelo, Kátia De Angelis, Patrícia Bock, Tânia Gatelli Fernandes, Fábio Cervo, Adriane Belló Klein, Nadine Clausell, Maria Cláudia Irigoyen

Abstract—Adriamycin cardiotoxicity is associated with oxidative stress in the presence of globally depressed cardiac function. It is unknown if there is a similar profile with early diastolic changes and how it relates to baroreflex control of circulation. In this study, we evaluated baroreflex control of circulation in adriamycin-treated Wistar rats compared with controls, using invasive blood pressure recording processed by a data acquisition system (CODAS, 1 KHz). Baroreflex sensitivity was evaluated by modulating blood pressure with phenylephrine and sodium nitroprusside. Oxidative stress was quantified by chemiluminescence and by glutathione peroxidase enzyme activity. Diastolic dysfunction was characterized by increased left ventricle end-diastolic pressure in adriamycin-treated rats compared with controls with preserved ascending aortic flow. Baroreflex sensitivity in response to blood pressure elevation and reduction were similar in adriamycin (−2±0.27 and −3.19±0.56 bpm/mm Hg) and control rats (−1.35±0.15 and −2.52±0.39 bpm/mm Hg). Chemiluminescence was higher (20450±1286 versus 16517±1020 counts per second/mg protein) and glutathione peroxidase activity was lower (456.2±4.3 versus 76.4±6.9 µmol·min⁻¹·mg⁻¹ protein) in adriamycin rats compared with controls. Inverse correlations were observed between glutathione peroxidase activity and left ventricle end-diastolic pressure (r=−0.72, P=0.02), between baroreflex sensitivity to phenylephrine and left ventricle end-diastolic pressure (r=−0.77, P=0.004), and between chemiluminescence and baroreflex sensitivity to sodium nitroprusside (r=−0.75, P=0.02), whereas a positive correlation was observed between baroreflex sensitivity to sodium nitroprusside and glutathione peroxidase activity (r=0.7, P=0.04). Thus, adriamycin led to increased left ventricle end-diastolic pressure without changes in baroreflex sensitivity, and associated increased oxidative stress appeared to be related to reduction of reflex control of circulation. (Hypertension. 2001;38[part 2]:576-580.)

Key Words: heart failure ■ blood pressure ■ baroreflex ■ oxidative stress ■ adriamycin ■ rat

Adriamycin-induced cardiac toxicity remains a major limitation to its use as an antineoplastic agent. In humans, this cardiac toxicity appears to be dose dependent. Total dose of 550 mg/m² of adriamycin is considered the upper limit to be used, because of the high risk for cardiotoxicity. The clinical course of adriamycin-induced heart failure may vary, but mortality rates can reach 20%.1

Several mechanisms are thought to be involved in the development of adriamycin-induced heart failure. Experimental studies devoted to this issue indicate that oxidative stress injury, inflammatory injury, calcium overload, and sympathetic overdrive are likely to be involved once a systolic dysfunction is present.2,3 In fact, impairment of baroreflex control of circulation has been demonstrated in adriamycin-treated rabbits in the presence of systolic left ventricular dysfunction.4,5 However, as diastolic dysfunction usually occurs before systolic dysfunction in the course of heart failure, early diagnosis focusing on diastolic performance may lead to the interruption of further adriamycin administration to avoid clinically relevant cardiac impairment.

In most experimental studies,3,6,7 adriamycin clearly causes both diastolic and systolic congestive failure. In this study, we used a total dose of 13 mg/kg of adriamycin in Wistar rats to test if early changes in cardiac performance (before overt systolic impairment) are related to changes in oxidative stress profile and in reflex control of circulation.

Methods

Animals
Male Wistar rats weighing 150 to 210 g were acquired from the Animal Facility from Federal University of Rio Grande do Sul, Brazil. Animals were kept in appropriate containers, with normal rat chow and water ad libitum. Room temperature was kept between 20°C to 25°C with light/dark cycles of 12 hours. All surgical procedures and protocols used were in accordance with the Guide-
lines for Ethical Care of Experimental Animals and were approved by the International Animal Care and Use Committee.

**Adriamycin-Induced Cardiotoxicity Protocol**
Cardiotoxicity was induced by intraperitoneal injections of adriamycin for a cumulative dose of 13 mg/kg in 6 injections over a 2-week period. Before each administration, the weight of the animal was taken to recalculate dosage. Monitoring of weight continued once a week thereafter until the end of experimentation. At the end of 3 weeks, after the last adriamycin injection, animals were hemodynamically assessed and killed by decapitation. Their livers were weighted, and their hearts were excised to study the myocardial oxidative profile.

**Cardiovascular Assessments**
For studies of the baroreflex control of circulation, 2 catheters filled with 0.06 mL saline were implanted under ether anesthesia into the femoral artery and vein (PE-10, Clay Adams) for direct measurements of arterial pressure and drug administration, respectively. One day after catheter placement, measurements were performed with conscious animals allowed to move freely during experiments. The arterial cannula was connected to a strain-gauge transducer (Narco Bio-Systems Miniature Pressure Transducer RP 1500), and blood pressure signals were recorded during a 20-minute period by a microcomputer equipped with an analog-to-digital converter board (CODAS, 1-KHz sampling frequency, Dataq Instruments Inc). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean arterial pressure (MAP) and heart rate (HR). Increasing doses of phenylephrine (0.05 to 0.8 g/mL) and sodium nitroprusside (5 to 20 g/mL) were given as sequential bolus injections (0.1 mL) to produce pressure responses ranging from 5 to 40 mm Hg. A 3- to 5-minute interval between doses was necessary for blood pressure to return to baseline. Baroreflex sensitivity was evaluated by a mean index relating changes in HR to the changes in MAP and was expressed as bpm/mm Hg as described elsewhere. Blood pressure responses to phenylephrine were evaluated in both group of conscious rats by increase of MAP caused by progressively higher doses (0.05 to 0.8 g/mL) of intravenous phenylephrine injections.

For left ventricle pressure and cardiac output evaluations, the rats were anesthetized with a mixture of ketamine (100 mg/kg IM) and diazepam (1.5 mg/kg IM). A small cervical anterior incision was made to allow dissection of the right carotid artery. Then, a polyethylene saline-filled cannula was inserted into the artery and advanced under continuous wave monitoring up to the left ventricle. Left ventricle end-diastolic pressure (LVEDP) signals were processed as described above for arterial pressure recording. The mean of 20 points in each record was taken for calculations. Immediately after, the trachea was cannulated with a Gelco tube and the ventilation controlled with a Harvard ventilator for small animals (model 683). Transonic 2SB flow probes (Transonic Systems) were implanted in the ascending thoracic aorta through a thoracotomy performed at the third right intercostal space. Animals were allowed to recover for 1 day before study. The ultrasonic volume flow sensor was connected to a T206 Transonic Flowmeter, and the flow signals were continuously recorded by a CODAS system in conscious animals.

**Oxidative Stress Profile**
After animals were killed by decapitation, the hearts were immediately removed, rinsed in saline, trimmed to remove fat tissue, visible connective tissue, and the atria. Ventricles were cut in small pieces and placed in ice-cold buffer. Ventricles were homogenized in an ultra-Turrax blender using 1 g of tissue for 5 mL of 150 mmol/L potassium chloride and 20 mmol/L phosphate buffer, pH 7.4. The homogenates were centrifuged at 600 g for 10 minutes at −2°C. Chemiluminescence assay was performed with an LKB Rack Beta Liquid Scintillation Spectrometer 1215 (LKB Producer AB, Bromma) in the in-out-of-coincidence mode at room temperature (25°C to 27°C). The supernatants were diluted in 140 mmol/L KCl and 20 mmol/L phosphate buffer, pH 7.4, and added to glass tubes that were placed in scintillation vials; 3 mmol/L t-butyl hydroperoxide was added, and chemiluminescence was determined up to the maximal level of emission. Glutathione peroxidase (GPx) activity was measured by the method of Del Maestro.10 Sample aliquots were added to the assay mixture of 1 U/mL glutathione reductase and 2 mmol/L glutathione in 1 mL phosphate buffer. Mixtures were preincubated at 37°C for 30 minutes. Subsequently, NADPH and t-butyl hydroperoxide were added to final concentrations of 155 and 580 μmol/L, respectively, and the change in absorbance of 340 nm was recorded at regular intervals over 4 minutes. One enzyme unit was defined as the amount of GPx required to oxidize 1 μmol NADPH in 1 minute at 25°C and was calculated on the basis of molar absorbance for NADPH at 340 nm. Proteins were assayed by the method of Lowry and associates.11

**Statistical Analysis**
Data are presented as mean±SEM. Comparisons between the 2 groups (saline and adriamycin treated) were performed by Student’s t tests. For analysis of repeated measures (vascular reactivity), ANOVA was used followed by Student-Newman-Kewls test. Pearson correlation was used to study association between variables. The significance level was established at P<0.05.

**Results**

**General Characteristics**
In the group of animals receiving adriamycin, a mortality rate of 30% was observed. A final number of 8 and 6 animals formed the adriamycin and control study groups, respectively. In the adriamycin group, phenotypic characteristics were noted such as changes in animal’s fur and lethargy. In this group, there was a significant decrease in weight in the last week of experimentation compared with controls (236±5.8 versus 254±5.1 g, respectively; P=0.04). Finally, the liver/body weight ratio measured at the end of the experimental period was significantly higher in controls compared with adriamycin-treated rats (3.5±0.2 versus 4.7±0.5 in controls, P=0.04).

**Hemodynamic Assessments**
Arterial pressure and HR signals were obtained from undated animals. 20 minutes before baroreflex sensitivity measurement experiments (see below). In adriamycin-treated rats, there was a significant decrease in the systolic, diastolic, and MAP compared with the values in the saline group (MAP, 116±2 versus 124±2 mm Hg in controls). Basal tachycardia was observed in adriamycin-treated rats in relation to control rats (Table).

Baroreflex sensitivity evaluated during phenylephrine administration was similar in adriamycin and control rats. No differences were observed in response to decreasing MAP in both groups (Table). Reduced baseline values of MAP observed in adriamycin-treated rats were accompanied by a lower increase in blood pressure in response to progressively higher doses of phenylephrine. Hyporeactivity in a wide range of MAP response was observed, as shown in the Figure, A.

A significant increase in the LVEDP in adriamycin-treated rats compared with the control group (22±2 versus 8±2 mm Hg in controls) was observed. However, cardiac output, measured by the ascending aortic flow, was similar in both groups (Table).
**Oxidative Stress Assessments**

Membrane lipid peroxidation as assessed by chemiluminescence showed a significant increase in heart homogenates from animals receiving adriamycin. These changes were accompanied by a significant reduction in GPx activity in this group (Table). Also, myocardial GPx activity was inversely correlated with chemiluminescence values ($r = -0.72, P = 0.01$).

Correlation analysis involving all animals studied showed a significant inverse relationship between GPx activity and LVEDP ($r = -0.72, P = 0.02$). Moreover, GPx activity was directly correlated to the baroreflex sensitivity to MAP decrease induced by sodium nitroprusside ($r = 0.7, P = 0.04$). Interestingly, chemiluminescence was negatively correlated with baroreflex-induced HR responses to nitroprusside ($r = -0.75, P = 0.02$) (Figure, B). Finally, baroreflex sensitivity evaluated by HR responses to increasing MAP was negatively correlated to LVEDP ($r = -0.77, P = 0.004$) (Figure, C).

**Discussion**

In this study, we showed that before the development of overt systolic dysfunction, there is evidence of diastolic impairment and increased oxidative stress in adriamycin-treated rats (13 mg/Kg), which, in turn, appeared to be associated with changes in baroreflex control of the circulation.

Previous studies in Sprague-Dawley rats treated with a cumulative dose of adriamycin of 15 mg/kg showed myocardial cell damage, depressed systolic pressure, ascites, and increase in LVEDP, leading to cardiomyopathy and congestive heart failure.$^{3,6}$ Experimental models have been developed in rats$^{12,13}$ and rabbits,$^{14}$ but most of these studies have been devoted to investigation of myocardial structure and metabolism. Although some hemodynamic features common to congestive heart failure induced by various factors have been described, no relationship was established between those and biochemical changes in this particular adriamycin model. In this study, we focused on early impairment of cardiac performance illustrated by increased LVEDP with preserved cardiac output as assessed by aortic flow, and its relationship to oxidative stress and cardiocirculatory reflex control.

Siveski-Iliskovic and coworkers$^{6,7}$ have previously demonstrated that adriamycin reduces blood pressure in anesthetized rats. Blood pressure reduction associated with heart failure has been described in chronic myocardial infarction$^{15}$ and, in other experimental models of cardiac failure,$^{16}$ associated with depressed cardiac output. In the present study, a small reduction of blood pressure was observed, and no impairment of cardiac output was detected, as evaluated by direct measurement of aortic flow. However, this hypotension was accompanied by an increasing basal HR in adriamycin-treated rats, suggesting that HR changes could be due to baroreflex buffering interference.$^{17}$ Basal tachycardia observed in the early phase of myocardial infarction or different states of heart failure may also be attributed to the overactivity of the sympathetic nervous system to the reduced cardiac efficiency. It is likely that this increase in the HR may be secondary to the increase of sympathetic activity at the heart, which may have contributed to the normal cardiac output (aortic flow) observed in the adriamycin-treated rats in the present experiment. The increase in pressure responsiveness to phenylephrine in adriamycin-treated rats reinforces this possibility. In fact, the increased HR observed after myocardial infarction shown by others suggest modulation by propanolol,$^{15}$ and the tachycardia in heart failure has been attributed to an enhancement of sympathetic tonus.$^{18}$

Changes in HR could indicate reflex alterations modulated by the baroreceptors. Indeed, impairment of baroreflexes has been shown in heart failure.$^{19}$ However, the time-course changes of baroreflex control of HR and sympathetic activity after myocardial infarction shown by others suggest modulation by cardiopulmonary afferents, because cardiopulmonary reflex responses changed from an improvement in the first day (24 hours) to an impairment in 10 days after coronary occlusion.$^{20}$ Evidence of impairment of reflex control of circulation by sinoaortic and cardiopulmonary afferents has been showed in different studies.$^{21}$ More specifically, impairment of baroreflex control of circulation was previously demonstrated in adriamycin-treated rabbits.$^{4,5}$ In rats, reduced baroreflex responses were observed in conditions of un-
changed cardiac output or LVEDP, but in presence of increased total peripheral resistance. In the present study, we did not observe changes in baroreflex control of HR, although baseline values of MAP were reduced and basal HR was increased. Moreover, cardiac output was normal compared with controls, yet in the presence of increased LVEDP.

In the present experiments, increased lipid peroxidation and decreased GPx indicate increase in oxidative stress. In fact, Adriamycin has been shown to cause myocardial antioxidant deficit in different animal species. Moreover, a decrease in GPx activity has been indicated as a key defect in the pathogenesis and progression of adriamycin-induced heart failure. The inverse relationships observed between GPx and chemiluminescence and between GPx activity and LVEDP reinforce the possibility of increased oxidative stress in hearts of adriamycin-treated rats. Indeed, the reflex response to nitroprusside administration was positively correlated with GPx activity and negatively correlated with chemiluminescence, suggesting that an increase in oxidative stress of the heart is associated with a reduced reflex response to blood pressure decreases. It is possible that changes in the heart produced by changes in oxidative profile may be related with the changes in baroreflex sensitivity, probably by modulating cardiopulmonary afferents. The observed increased LVEDP clearly indicates a role for the cardiac reflexes. The inversely correlated values of LVEDP and reflex responses to phenylephrine may give support to this idea. Decreased parasympathetic activity and decreased myocardial adrenergic sensitivity at the time of sympathetic hyperactivity, as well as the reduced pressure responsiveness to phenylephrine observed in adriamycin-treated rats, may suggest earlier changes in autonomic control of heart, before changes in cardiac output.

Thus, we showed in this study that 13 mg/kg of adriamycin administered during 2 weeks induces diastolic dysfunction 6 weeks later, without changes in cardiac output or baroreflex control of HR. However, the observed increase in oxidative stress seems to be associated with reduced reflex control of the circulation, reinforcing the role of oxidative profile not only in the metabolic and biochemical changes but also in hemodynamic features of adriamycin-induced cardiomyopathy.

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