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 (45.6±4.3 versus 76.4±6.9 µmol/min·mg 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 reduced 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 hemody-

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 measure-

Oxidative Stress Profile
After animals were killed by decapitation, the hearts were immedi-

Statistical Analysis
Data are presented as mean±SEM. Comparisons between the 2

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, respect-

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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-Kuels test. Pearson correlation was used to study association between variables. The significance level was established at P<0.05.

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Hemodynamic Assessments and Oxidative Stress Profile in Control and Adriamycin Groups

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Control</th>
<th>Adriamycin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic arterial pressure, mm Hg</td>
<td>144±3</td>
<td>134±2.5*</td>
<td>0.02</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mm Hg</td>
<td>107±2</td>
<td>100±2*</td>
<td>0.05</td>
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<tr>
<td>MAP, mm Hg</td>
<td>124±2</td>
<td>116±2*</td>
<td>0.02</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>342±9</td>
<td>391±13*</td>
<td>0.03</td>
</tr>
<tr>
<td>Tachycardic response, bpm/mm Hg</td>
<td>-2.52±0.39</td>
<td>-3.19±0.56</td>
<td>0.38</td>
</tr>
<tr>
<td>Bradycardic response, bpm/mm Hg</td>
<td>-1.35±0.15</td>
<td>-2.00±0.27</td>
<td>0.11</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>59±4</td>
<td>53±5</td>
<td>0.65</td>
</tr>
<tr>
<td>End diastolic pressure, mm Hg</td>
<td>8±2</td>
<td>22±2*</td>
<td>0.001</td>
</tr>
<tr>
<td>Chemiluminescence, cps/mg protein</td>
<td>16517±1020</td>
<td>20450±1286*</td>
<td>0.04</td>
</tr>
<tr>
<td>Glutathione peroxidase (µmol·min⁻¹·mg⁻¹ protein)</td>
<td>76.4±6.9</td>
<td>45.6±4.3*</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<0.05 vs control group.

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, ascerts, and increase in LVEDP, leading to cardiomyopathy and congestive heart failure. Experimental models have been developed in rats and rabbits, 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 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 and, in other experimental models of cardiac failure, 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.

Baseline 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 in rat, has been shown to be modulated by propanolol, and the tachycardia in heart failure has been attributed to an enhancement of sympathetic tonus.

Changes in HR could indicate reflex alterations modulated by the baroreceptors. Indeed, impairment of baroreflexes has been shown in heart failure. 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. Evidence of impairment of reflex control of circulation by sinoaortic and cardiopulmonary afferents has been showed in different studies. More specifically, impairment of baroreflex control of circulation was previously demonstrated in adriamycin-treated rabbits. In rats, reduced baroreflex responses were observed in conditions of un-
A, Blood pressure responsiveness to phenylephrine (0.05 to 0.8 μg/mL), expressed by the slope of the regression line relating increasing doses of phenylephrine and blood pressure increases in control and adriamycin groups. Inverse correlation obtained by linear regression in control and adriamycin groups; \( r = -0.75 \).

B, Chemiluminescence (cps/mg protein).

C, End-diastolic pressure (mmHg).

\( r = -0.77 \).

A, Blood pressure responsiveness to phenylephrine (0.05 to 0.8 μg/mL), expressed by the slope of the regression line relating increasing doses of phenylephrine and blood pressure increases in control and adriamycin groups. Inverse correlation obtained by linear regression in control and adriamycin groups; \( r = -0.75 \). In the present experiments, increased lipid peroxidation and decreased GPx indicate increase in oxidative stress. Moreover, cardiac output was normal compared with controls, yet in the presence of increased LVEDP.

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 LVEDP and chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and lung muscle.

B, Chemiluminescence (cps/mg protein).

C, End-diastolic pressure (mmHg).

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