Reduced in vivo high-energy phosphates precede Adriamycin-induced cardiac dysfunction


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ADRIAMYCIN (ADR) represents one of the most potent and extensively used anticancer drugs (46); however, its antineoplastic use can be compromised in practice by cardiotoxic side effects (17, 35). Although ADR-induced cardiotoxicity is usually subclinical, symptoms of heart failure (HF) can develop acutely during therapy (18, 33) or chronically (25, 34). In general, HF with systolic and diastolic abnormalities develops in 18–36% of patients receiving a cumulative ADR dose of 250–601 mg/m² (17, 35). A spectrum of cardiac metabolic (21, 39) and morphological (2, 6) abnormalities occurs following ADR treatment, but it is unclear which, if any of these, causes ADR cardiotoxicity. One metabolic abnormality that may contribute mechanistically to ADR cardiotoxicity is impaired creatine kinase (CK) energetics.

The CK reaction serves as the prime cardiac energy reservoir, quickly and reversibly converting adenosine diphosphate and phosphocreatine (PCr) to ATP and creatine (15, 44), where the PCr-to-ATP ratio (PCr/ATP) is commonly used to characterize a high-energy phosphate metabolism. An inhibition of CK impairs cardiac function or contractile reserve in normal hearts (13, 37), and an altered CK metabolism is observed in both experimental and human HF (14, 19, 23, 32, 47). In particular, an altered in vitro and in vivo CK energetics and a reduced cardiac PCr/ATP have been observed in ADR cardiotoxicity (8, 11, 24, 29). Whether the CK energetic decline contributes to ADR-induced cardiac dysfunction or is just another consequence of HF is still unresolved. This question has not been answered, in part, because there have been no serial studies performed in the same animals showing whether CK abnormalities precede or follow the development of contractile abnormalities in ADR cardiotoxicity.

³¹P magnetic resonance (MR) spectroscopy (MRS) and MR imaging (MRI) methods uniquely allow the serial noninvasive quantification of in vivo myocardial CK metabolites, ventricular anatomy, morphology, and function under physiological conditions (14, 19, 23, 32, 47). In vivo MRS/MRI techniques have been extended to the small dimensions and high heart rates of the mouse, enabling the quantification of murine cardiac CK energetics, anatomy, and function (7). The mouse heart exhibits similar in vivo PCr/ATP and measures of global function to those of the human heart (7), and the mouse has been frequently used to study ADR cardiotoxicity (5, 9, 10, 41). Recent experiments showed that the decline in cardiac PCr/ATP in pressure overload-induced murine HF precedes functional abnormalities (19). A causal role for impaired CK in ADR cardiotoxicity would be suggested if the energetic decline occurs before or at the same time as cardiac dysfunction, whereas a consequential role if decreased PCr/ATP occurs after cardiac dysfunction. Therefore, the aim of this study was to test the hypothesis that impaired CK energetics, indexed by a decreased cardiac PCr/ATP, precedes the development of ADR-induced systolic and/or diastolic dysfunction.

MATERIALS AND METHODS

Experimental animals. All studies were approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University School of Medicine. Adult male C57BL6 mice (weight, 30–40 g) were used and received intraperitoneal injection of ADR (5 mg/kg) weekly for a total of five injections. This ADR dose/scheme was
chosen after experimental screening of doses/schemes previously reported (5, 9, 10, 41) with some modifications. Control mice received normal saline instead of ADR.

MRI/MRS study. In vivo MRI/MRS studies were performed 6, 8, and 10 wk after the initiation of ADR administration. Mice were anesthetized with 1% isoflurane in a 50:50 oxygen and ambient air mixture and positioned prone on a Plexiglas platform with temperature control (37 ± 1°C). ECG leads, respiratory pad, and thermo couple (SA Instruments) were used to monitor basic vital parameters.

1H MRI and image-guided, spatially localized 31P MRS were used to study global left ventricular (LV) function and energetics, respectively, during the same examination, as previously reported (7). The probe set included 22-mm 1H MRI and 11-mm 31P MRS coils. Data were acquired on a Bruker Biospec MRS/MRI spectrometer equipped with a 4.7-T/40-cm Oxford magnet and a 12-cm (inner diameter) actively shielded BGA-12 gradient set capable of developing gradient strength of up to 400 mT/m. ECG and respiratory-gated multislice FLASH cine MRI (15 frames, echo time 1.79 ms, repetition time 30°) was used to acquire LV morphological and functional data. LV morphology and systolic function were quantified from LV short-axis slices and diastolic function from long-axis slices. Spatially localized 31P MRS was carried out using a one-dimensional chemical shift imaging sequence with a 16-mm field of view, 16 phase encoding steps in the direction perpendicular to the plane of the coil, 128 averages per phase encoding step, a modified BIR4 adiabatic excitation pulse of 60° flip angle, and an interpulse delay of 1 s. All mice awoke within ~1 min after the study.

MRI/MRS data processing. 1H MR images were analyzed with ImageJ software and 31P MR spectra with in-house custom software (1). The largest and smallest LV volumes during the cardiac cycle were visually identified as end-diastolic and end-systolic volumes, respectively. LV mass was the sum of the LV areas of all end-diastolic slices multiplied by the slice thickness and by 1.05 (cardiac specific gravity). The LV ejection fraction was calculated from the relative difference in end-diastolic and end-systolic volumes. Diastolic function was assessed by the magnitude of the peak filling rate. The values for diastolic filling rates were calculated as the difference in LV volumes of the sequential frames in cine acquisitions from a long-axis slice divided by the duration of the frame interval. Cardiac PCR/ATP was quantified from the integrated peak areas of PCr and [β-ATP] resonances from voxels intersecting the anterior LV wall and apart from chest skeletal muscle, as identified with 1H MR images as previously described (7). Voxel shifting was performed as necessary to avoid chest muscle contamination (3).

Statistical analysis. Data are expressed as means ± SD. One-way ANOVA tests with Bonferroni correction for multiple comparisons were used. The relationship between metabolic and functional parameters was evaluated with Pearson product-moment correlational analysis. A value of $P < 0.05$ was considered statistically significant (GraphPad Prism, version 4.06).

RESULTS

Fifteen animals received ADR, and the mortality was 53% at 6 wk and 67% at 10 wk (Fig. 1). Additional studies described in the supplemental material (posted with the online version of this article) suggest this early mortality is not due to structural cardiac damage or cell death and unrelated to typical ADR cardiotoxicity, consistent with prior reports. Thus five animals survived for all MRI/MRS studies between 6–10 wk. Representative MR images and cardiac 31P MR spectra are shown in Figs. 2 and 3. Six weeks of ADR treatment resulted in a cardiac energetic decline as evidenced by a reduction in mean cardiac PCr/ATP (1.79 ± 0.18 vs. 1.39 ± 0.30, control vs. ADR, respectively, $P < 0.05$, Fig. 4). At the same time, systolic and diastolic functional parameters in ADR mice remained similar to control values (Table 1).

Over the ensuing 2 wk, i.e., 8 wk after the first ADR exposure, a further energetic decline (Fig. 4) was accompanied by LV systolic and diastolic dysfunction. Specifically, the mean ejection fraction decreased to 55.9% ($P < 0.002$) and the mean peak diastolic filling rate decreased by ~40% from that of control mice ($P < 0.01$, Table 1).

After an additional 2 wk, i.e., 10 wk after the first ADR injection, mean cardiac PCr/ATP was 35% lower than that of control mice (1.79 ± 0.18 vs. 1.17 ± 0.18, control vs. ADR, respectively, $P < 0.003$, Fig. 4). LV ejection fraction was

![Fig. 1. Survival curve for Adriamycin (ADR)-treated mice. MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy.](http://ajpheart.physiology.org/)

![Fig. 2. Typical transverse short-axis 1H magnetic resonance images of a mouse thorax through the mid-left ventricle at end systole and end diastole.](http://ajpheart.physiology.org/)
significantly lower (53.2 ± 4.7%) than that of control mice (67.3 ± 3.9%, \( P < 0.05 \)), whereas end-systolic volumes trended higher and stroke volumes lower (Table 1). LV mass decreased by 22% and mean resting cardiac output by 23% \( (P < 0.05) \) (Table 1).

ADR administration was associated with an early significant decline in cardiac PCr/ATP and later significant decreases in mean LV filling rate, ejection fraction, and cardiac output. To determine whether the LV energetics correlated with the extent of contractile and morphological abnormalities, cardiac PCr/ATP was compared with the functional and morphological parameters. There was a significant correlation of in vivo cardiac PCr/ATP with peak filling rate, cardiac output, and ejection fraction (Fig. 5).

DISCUSSION

There are several novel observations from this in vivo murine study of serial changes in cardiac energetics and function following ADR administration. First, we observe that CK energetics decrease, as shown by lower cardiac PCr/ATP, early after ADR administration. Second, abnormalities of LV systolic and diastolic function appear weeks after the lower cardiac PCr/ATP, indicating that the impaired CK energetics is an early phenomenon that may contribute to subsequent LV dysfunction. Third, the systolic (LV ejection fraction and cardiac output) and diastolic (peak filling rate) dysfunction is significantly associated with the energetic abnormality (cardiac PCr/ATP).

Although ADR is an effective anticancer drug, cardiotoxicity occurs and limits the amount administered (17, 35). Energy metabolism, a requisite for normal cardiac function, is a likely target for ADR (38). CK functions as the major myocardial ATP reserve and temporal buffer (26, 43) and is impaired in experimental and human HF (14, 19, 23, 32, 47). Even though the existence of a relationship between impaired CK energetics and ADR cardiotoxicity was suggested years ago (8, 11, 38, 39), the chronological sequence of events necessary for establishing possible causality has not been defined in the same animals.

What are the possible mechanisms responsible for the energetic decline after ADR administration, and how can they interfere with cardiac function? The mechanisms of action of the anthracyclines against tumor cells remain controversial, although the generation of reactive oxygen species through the interaction with mitochondrial enzymes via their quinone ring is thought to be one likely contributing factor (36). ADR-generated reactive oxygen species may oxidize important enzymes containing sulfhydryl groups and may also interfere with DNA replication (36). ADR is known to oxidize sulfhydryl groups of CK and attenuate CK activity (22). Prior studies from cultured cardiomyocytes and in vivo hearts demonstrate reductions in the absolute and relative cardiac CK metabolite pool sizes following ADR exposure (11, 29). In addition, ADR alters the structure and function of purified mitochondrial CK (CKMt), leading to a dissociation of octamers into dimers and an inhibition of CKMt binding to the mitochondrial membrane (38). CKMt also mediates the formation of important multienzyme complexes, including the adenine nucleotide translocator and voltage-dependent anion channel-facilitating chemical energy transfer (4, 28). The disruption of the functional coupling of CKMt and adenine nucleotide translocator after ADR exposure, in part, may explain the reduction of the rate and efficiency of energy transfer (40). On the other hand, multiple abnormalities in myofibrillar CK (CKM) can contribute to the impaired overall inefficiency of the CK system as well. For
instance, ADR inhibits CKM gene expression (16) and causes an inhibition of the CKM isoform in vitro and in vivo (20, 44). Another reported response of the CK system to ADR is an increase in the B-isoform of CK (CKB), which occurs in some other forms of HF (30, 48). Although the antitumor effect of ADR may be related to interference with DNA synthesis (12, 31), the augmentation of CKB suggests that ADR does not interfere with all DNA processes. The effect of ADR inhibits CKM in vitro and in vivo (20, 44).

Table 1. LV morphology and function in ADR-treated mice

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HR, beats/min</th>
<th>EDV, μl</th>
<th>ESV, μl</th>
<th>SV, μl</th>
<th>EF, %</th>
<th>CO, ml/min</th>
<th>LV Mass, mg</th>
<th>PFR, μl/ms</th>
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<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>519 ± 26</td>
<td>49.7 ± 8.7</td>
<td>16.3 ± 3.5</td>
<td>33.4 ± 5.9</td>
<td>67.3 ± 3.9</td>
<td>17.4 ± 3.4</td>
<td>94.1 ± 16.4</td>
<td>0.56 ± 0.13</td>
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<tr>
<td>ADR (6 wk)</td>
<td>5</td>
<td>519 ± 29</td>
<td>45.5 ± 3.5</td>
<td>16.1 ± 3.5</td>
<td>29.4 ± 3.2</td>
<td>64.7 ± 6.1</td>
<td>15.3 ± 2.6</td>
<td>79.1 ± 12.3</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>ADR (8 wk)</td>
<td>5</td>
<td>508 ± 80</td>
<td>55.9 ± 11.2</td>
<td>24.4 ± 4.4</td>
<td>31.4 ± 7.6</td>
<td>55.9 ± 4.2</td>
<td>15.5 ± 2.4</td>
<td>78.0 ± 8.4</td>
<td>0.34 ± 0.12</td>
</tr>
<tr>
<td>ADR (10 wk)</td>
<td>5</td>
<td>525 ± 46</td>
<td>48.8 ± 9.8</td>
<td>23.0 ± 6.3</td>
<td>25.8 ± 4.7</td>
<td>53.2 ± 4.7</td>
<td>13.4 ± 1.9</td>
<td>73.8 ± 10.0</td>
<td>0.27 ± 0.08</td>
</tr>
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Values are means ± SD; n, number of mice. HR, heart rate; EDV, end-diastolic volume; ESV, end-systolic volume; SV, systolic volume; EF, ejection fraction; CO, cardiac output; LV, left ventricular; PFR, peak filling rate. *P ≤ 0.05 compared with control; †P ≤ 0.05 compared to 6 wk after 1st Adriamycin (ADR) injection.

In conclusion, ADR cardiotoxicity in the mouse is associated with an early, significant decline of in vivo cardiac PCr/ATP metabolism.

Even though these data were acquired in vivo under physiological conditions and provide new insights into ADR cardiotoxicity, there are distinct limitations of this study. First, the degree of systolic dysfunction, as measured by the LV ejection fraction, was modest and not to the degree at 10 wk that was likely to cause HF at that time. However, this degree of dysfunction is clinically important, since it would be sufficient to discontinue ADR therapy if observed in patients and it did allow testing of the proposed hypothesis. Second, although cardiac PCr/ATP is an important index of myocardial energetics, the rate of ATP flux through CK may be a more important factor underlying contractile dysfunction in HF (32, 47). However, at this time, in vivo measures of CK flux in the murine heart have not been reported. Thus, future studies to measure ATP flux through CK, as well as other purported potential energetic contributors to dysfunction, are needed. Finally, the early mortality observed here in the mouse is higher than that observed clinically today. It is important to point out that comparable mortality rates have been reported in prior murine studies of ADR cardiotoxicity (26, 42) and the fundamental observation that cardiac dysfunction is observed well after ADR administration parallels the clinical observation that ADR cardiotoxicity is generally a chronic condition often occurring long after ADR exposure.

In conclusion, ADR cardiotoxicity in the mouse is associated with an early, significant decline of in vivo cardiac energetics that occurs before systolic and diastolic abnormalities are detected by serial MRI examinations. The extent of systolic and diastolic dysfunction correlates with reduced cardiac PCr/ATP following ADR exposure, consistent with the hypothesis that abnormalities in cardiac CK energetics underlie mechanical abnormalities in ADR cardiotoxicity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
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