Protective Effect of Carvedilol on Adriamycin-Induced Left Ventricular Dysfunction in Children With Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Adriamycin (ADR) is a potent chemotherapeutic agent widely used in the treatment of childhood acute lymphoblastic leukemia (ALL); its clinical use is limited owing to its marked cardiotoxicity. The present study investigated the possible protective role of carvedilol on ADR-induced left ventricular dysfunction in children with ALL.

Methods and Results: Fifty children with newly diagnosed ALL were included in this study. They were divided into 2 equal groups: 1) ADR; and 2) ADR + carvedilol. Patients were evaluated with conventional 2-dimensional echocardiographic examination (2D), pulsed tissue Doppler (PTD), and 2-dimensional longitudinal strain echocardiography (2DS) before and after therapy. Plasma lactic dehydrogenase (LDH), creatine phosphokinase (CPK), and troponin I levels were also determined before and after therapy. ADR treatment reduced left ventricular systolic dysfunction as assessed by a significant decrease in fractional shortening (FS) (2D) and global peak-systolic strain (GPSS; 2DS). In addition, ADR treatment significantly increased plasma troponin I and LDH. Pretreatment of ADR-treated patients with carvedilol resulted in a significant increase in FS (2D) and GPSS (2DS). Furthermore, carvedilol pretreatment inhibited ADR-induced increase in plasma troponin I and LDH.

Conclusions: These results suggested a protective role of carvedilol against ADR-induced cardiotoxicity. (J Cardiac Fail 2012;18:607–613)

Key Words: Adriamycin, carvedilol, leukemia, echocardiography.

Adriamycin (ADR) is an anthracycline chemotherapeutic agent widely used in the treatment of pediatric malignancies, including childhood acute lymphoblastic leukemia (ALL). ADR therapy has led to successful treatment of childhood cancer with improved survival rates.1 However; its use is frequently associated with both acute and chronic cardiotoxicity manifested as cardiac arrhythmias and congestive heart failure, respectively.3–4 The main mechanism of ADR-induced cardiotoxicity is generally accepted as reactive oxygen species (ROS) formation which ultimately results in cardiomyocytes apoptosis.5–7

Carvedilol is a nonselective adrenergic blocker acts on β1, β2, and α1 receptors. It also has potent antioxidant (∼10 times more potent than α-tocopherol) and antiapoptotic properties.8–11 Certain carvedilol metabolites found in human plasma also exhibit antioxidative activity ∼50–100-fold greater than carvedilol itself. These unique properties of carvedilol may be important in preventing progressive deterioration of left ventricular (LV) dysfunction and chronic heart failure in ADR-induced cardiomyopathy.12

Recent animal studies and experimental observations showed that carvedilol prevented the development of ADR-induced cardiotoxicity. However, there are very few clinical trials concerning the prophylactic role of carvedilol on ADR-induced cardiomyopathy.7,13 Therefore, the present work aimed to conduct a randomized clinical trial to...
further investigate the possible protective role of carvedilol on ADR-induced LV dysfunction in children with ALL undergoing intensive chemotherapy.

Methods

This study was a prevention, parallel-assignment, randomized, and clinical study.

Population of the Study

Fifty consecutive patients with a new diagnosis of ALL presenting to the Hematology and Oncology Unit, Pediatric Department, Faculty of Medicine, Tanta University, Tanta, Egypt, from March 2008 to March 2010 were included in this study. All of the children met the inclusion criteria.

Inclusion and Exclusion Criteria

Children were 6–12 years old, newly diagnosed with ALL confirmed by complete blood picture, bone marrow examination, immunophenotyping assessed by flow cytometry, and fluorescence in situ hybridization (FISH) technique. Normal LV systolic function in all patients was assessed by conventional 2-dimensional echocardiography (2D) at the beginning of the study. All of the patients accepted to be enrolled in the study after signing an informed consent form.

Exclusion criteria were previous chemotherapy or radiotherapy, the presence of any cardiac disease either congenital or acquired, any cardiac lesion detected in baseline echocardiography, any associated systemic disease, such as renal dysfunctions and hepato-cellular insufficiency, and/or medications that can affect cardiac function, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, and other beta-blockers.

Randomization

Patients were equally randomly assigned to receive either ADR only (ADR group) or carvedilol before ADR (ADR + carvedilol group).

Up- and Down-Titration

All patients were subjected to the following protocol of therapy in induction of remission: vincristine: 1.5 mg/m² intravenously (days 8, 15, 22 and 29); L-asparaginase: 10,000 IU/m², intramuscularly (3 times a week for 9 doses); prednisone: 60 mg m⁻² d⁻¹ orally in 3 divided doses (days 1–28), then tapered to 30 mg m⁻² d⁻¹ (days 29–32) and 15 mg m⁻² d⁻¹ (days 32–35), and discontinued on day 36; ADR: 30 mg m⁻² (days 8, 15, 22, and 29) given by slow intravenous infusion over 6 hours; triple intrathecal therapy was administered to the Echopacs workstation with Q analysis software version 4.0.3 (General Electric, Waukesha, Wisconsin, USA) for processing.

Assessment of Plasma Enzymes

Plasma total LDH and total CPK activities were determined with the use of commercial kits from Randox (UK) and Spineepe (Spain), respectively.

Total LDH activity was assessed according to the method of Henry (1974). That method depends on the reaction of lactate with nicotinamide-adenine-dinucleotide (NAD). The NADH formed is measured spectrophotometrically at 340 nm. The increase in absorbance is measured at 1-minute intervals for 3 minutes. Plasma total LDH activity was calculated as units per liter (U/L).

Total CPK activity was determined according to the method of Abbot et al. (1984). That method is based on the transphosphorylation of adenosine diphosphate to adenosine triphosphate through a series of coupled enzymatic reactions; NADH produced is directly proportional to the CPK activity. The increase in absorbance at 1-minute intervals was recorded for 3 minutes at 340 nm. Plasma total CPK activity was calculated as units per liter (U/L).

The assessment of troponin I blood levels (ng/mL) was performed with the Chiron Bayer ACS 180 chemiluminescent diagnostic test by El-Safwa Laboratory, Tanta, Egypt.

Data Evaluation and Statistical Analysis

Results are expressed as the mean ± SD. All statistical analyses of the results were performed by intention-to-treatment...
method. Comparison of baseline characteristics between before ADR and before ADR + carvedilol was performed with the \( \chi^2 \) test and the unpaired \( t \) test. Comparison of before and after therapy was performed with the paired \( t \) test. Differences between the after ADR group and the after ADR + carvedilol group is compared with the unpaired \( t \) test. The level of significance was set at \( P \leq .05 \). Statistical analysis was performed using SPSS software for Windows, version 14.0.

### Results

#### Baseline Clinical Characteristics of Study Patients

The baseline clinical characteristics of the patients are summarized in Table 1. There were no significant differences regarding age, sex, hemoglobin levels, heart rate, systolic blood pressure, fractional shortening (FS), and body mass index between the patient group before ADR and the patient group before ADR + carvedilol.

#### Effect of ADR and Carvedilol Pretreatment on 2D-Derived Parameters Examined in Children With ALL

Treatments of ALL children with 30 mg/m\(^2\) ADR (days 8, 15, 22, and 29 after remission induction) caused a significant \((P = .01)\) decrease (16.25\%) in the FS measured 1 week after the last ADR dose compared with the values of before ADR treatment (Table 2; Fig. 1). On the other hand, pretreatment of ALL children with carvedilol for 5 days before every dose of ADR caused a significant \((P = .0015)\) increase (14.9\%) in FS measured 1 week after the last ADR dose compared with the values after ADR treatment (Table 2; Fig. 1). Treatments of ALL children with either ADR only or ADR + carvedilol caused nonsignificant changes in the LV diastolic function E, A, and E/A measured 1 week after the last ADR dose compared with the values before ADR treatment and the values after ADR treatment (Table 2; Fig. 1).

#### Effect of ADR and Carvedilol Pretreatment on PTD-Derived Parameters Examined in Children With ALL

Treatments of ALL children with either 30 mg/m\(^2\) ADR (days 8, 15, 22, and 29 after remission induction) or ADR + carvedilol caused a nonsignificant changes in tissue Doppler peak mitral annulus systolic velocity (s), tissue Doppler mitral flow early-phase filling velocity (e), and tissue Doppler peak atrial-phase filling velocity (a) measured 1 week after the last ADR dose compared with the values before or after ADR treatment and the values after ADR treatment (Table 3).

### Table 1. Baseline Clinical Characteristics of the Study Patients

<table>
<thead>
<tr>
<th></th>
<th>Before ADR</th>
<th>Before ADR + Carvedilol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>9.5 ± 2.6</td>
<td>8.5 ± 2.9</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>12:13</td>
<td>11:14</td>
</tr>
<tr>
<td>Hb (%)</td>
<td>11.4 ± 2.4</td>
<td>12.3 ± 1.2</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>85 ± 7.0</td>
<td>83 ± 9.0</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>100 ± 15</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>FS (%)</td>
<td>40 ± 4.62</td>
<td>34 ± 4.53</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>30 ± 5</td>
<td>32 ± 8</td>
</tr>
</tbody>
</table>

Hb, hemoglobin; HR, heart rate; SBP, systolic blood pressure; FS, fractional shortening; BMI, body mass index.

Data are in ratio or mean ± SDM of 25 patients.

### Table 2. Effect of ADR and Carvedilol Pretreatment on Conventional Echocardiography–Derived Parameters Examined in Children With Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th></th>
<th>ADR</th>
<th>ADR + Carvedilol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>FS</td>
<td>40 ± 4.62</td>
<td>33.5 ± 6.24*</td>
</tr>
<tr>
<td>E</td>
<td>0.732 ± 0.009</td>
<td>0.733 ± 0.01</td>
</tr>
<tr>
<td>A</td>
<td>0.683 ± 0.036</td>
<td>0.663 ± 0.041</td>
</tr>
<tr>
<td>E/A</td>
<td>1.904 ± 0.403</td>
<td>1.966 ± 0.389</td>
</tr>
</tbody>
</table>

FS, fractional shortening; E, peak early filling velocity; A, peak atrial-phase filling velocity.

Data are expressed as mean ± SDM of 25 patients.

*Significantly different from respective before-treatment group \((P \leq .05)\).

### Fig. 1. Effect of adriamycin (ADR) and carvedilol pretreatment on fractional shortening (FS), derived from conventional echocardiography of pediatric patients with acute lymphoblastic leukemia. Data are expressed as mean ± SDM of 25 patients. *Significantly different from respective before-treatment group \((P \leq .05)\). @Significantly different from after-ADR treatment group \((P \leq .05)\).
Table 3. Effect of Adriamycin (ADR) and Carvedilol Pretreatment on Pulsed Tissue Doppler—Derived Parameters Examined in Children With Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ADR Before</th>
<th>ADR After</th>
<th>ADR + Carvedilol Before</th>
<th>ADR + Carvedilol After</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX</td>
<td>22.13 ± 3.36</td>
<td>13.28 ± 3.5*</td>
<td>18.48 ± 3.94</td>
<td>20.8 ± 2.63*–1</td>
</tr>
<tr>
<td>A4C</td>
<td>18.91 ± 4.08</td>
<td>17.27 ± 3.97</td>
<td>18.88 ± 3.5</td>
<td>17.61 ± 2.66</td>
</tr>
<tr>
<td>A2C</td>
<td>16.87 ± 6.88</td>
<td>14.75 ± 3.38</td>
<td>16.47 ± 3.78</td>
<td>21 ± 2.704*–1</td>
</tr>
<tr>
<td>GPSS</td>
<td>18.65 ± 2.9</td>
<td>15.1 ± 1.769*</td>
<td>17.44 ± 2.92</td>
<td>19.3 ± 1.96*–1</td>
</tr>
</tbody>
</table>

Table 4. Effect of Adriamycin (ADR) and Carvedilol Pretreatment on Plasma Troponin I, LDH, and CPK Levels Measured in Children With ALL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ADR Before</th>
<th>ADR After</th>
<th>ADR + Carvedilol Before</th>
<th>ADR + Carvedilol After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin I</td>
<td>156%</td>
<td>70.2%</td>
<td>166%</td>
<td>77.3%</td>
</tr>
<tr>
<td>LDH</td>
<td>230%</td>
<td>115.2%</td>
<td>238%</td>
<td>118.4%</td>
</tr>
<tr>
<td>CPK</td>
<td>280%</td>
<td>125.8%</td>
<td>289%</td>
<td>130.9%</td>
</tr>
</tbody>
</table>

Effect of ADR and Carvedilol Pretreatment on Plasma Troponin I, LDH, and CPK Levels Measured in Children With ALL

Treatment of ALL children with 30 mg/m² ADR (days 8, 15, and 29 after remission induction) caused a significant (P values .005 and .006) increase (~100% and 156%) in plasma levels of both troponin I and LDH, respectively, measured 1 week after the last ADR dose compared with the values before ADR treatment (Table 5). On the other hand, pretreatment of ALL pediatric patients with carvedilol for 5 days before every dose of ADR caused a significant (P values .0008 and .0001) decrease (62% and 57%) in plasma levels of both troponin I and LDH, respectively, measured 1 week after the last ADR dose compared with the values after ADR treatment (Table 5).

Discussion

The result of this study revealed that ADR treatment reduced LV systolic dysfunction as assessed by a significant decrease in FS and GPSS. In addition, ADR treatment significantly increased plasma troponin I and LDH. Pretreatment of ADR-treated patients with carvedilol resulted in a significant increase of FS and GPSS. Furthermore, carvedilol pretreatment inhibited ADR-induced increase of plasma troponin I and LDH.

ADR-caused decrease in FS is in agreement with most earlier studies of ADR-induced cardiotoxicity. However, some earlier studies have demonstrated that left ventricular ejection fraction (LVEF) and FS are insensitive to detect early ADR-induced cardiomyopathy.

There was no significant difference in diastolic function (E, A, and E/A) measured after treatment with ADR in our patients. Similar results were reported by Ewer et al. (1994). On the other hand, Galderisi et al. (2007) found that E, A, and E/A ratio were more sensitive than FS to the cardiotoxic effect of ADR. This difference in the results could be because they studied late cardiotoxicity 1 year after ADR treatment. The most likely explanation of our results regarding diastolic function is that the parameters of systolic function represent a better tool for detecting early cardiac changes associated with ADR in the pediatric population. There are, however, a number of other considerations that also must be taken into account. Young patients may demonstrate changes in diastolic function relatively later in the course of their disease than adults.

Much less likely is the fact that our patients received ADR by continuous infusion (6 hours), rather than the standard treatment schedule, although in such patients systolic and diastolic functions may behave differently.
In the present study, the PTD parameters s, e, and e/a showed no significant differences after ADR therapy. These results disagree with earlier studies which found that tissue Doppler parameters were sensitive to changes induced by ADR.24–27

The significant reduction in GPSS after ADR treatment was in agreement with Migrino et al. (2009), who came to the same results in experimental animals.18 Cheung et al. (2010) also showed impairment of GPSS and mechanical dyssynchrony in children after anthracycline therapy with normal LVEF.28

The results of the present study demonstrated that coadministration of carvedilol before ADR treatment preserves LV systolic functions. Pretreatment of ADR-treated patients with carvedilol resulted in a significant increase in FS. Furthermore, carvedilol pretreatment inhibited ADR-induced increase in plasma troponin I and LDH. The effect of carvedilol is best shown by the results of 2DS, where the

![Fig. 3. Longitudinal strain echocardiography in pediatric patients with acute lymphoblastic leukemia: (A) before treatment; (B) adriamycin-induced cardiotoxicity; (C) carvedilol-induced cardioprotection in adriamycin-treated patients.](image)

**Table 5. Effect of Adriamycin (ADR) and Carvedilol Pretreatment on Plasma Troponin I, Lactic Dehydrogenase (LDH), and Creatine Phosphokinese (CPK) Levels Measured in Children With Acute Lymphoblastic Leukemia**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>ADR</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin I (ng/mL)</td>
<td>ND</td>
<td>0.061 ± 0.05*</td>
<td>ND</td>
<td>0.023 ± 0.018*&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>373.7 ± 197.64</td>
<td>956.8 ± 358.52*</td>
<td>205 ± 63.98</td>
<td>409.8 ± 258.78&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>50.6 ± 25.67</td>
<td>47.6 ± 20.45</td>
<td>64.6 ± 41.47</td>
<td>70.26 ± 58.28</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SDM of 25 patients.
*Significantly different from respective before-treatment group (P ≤ .05).
<sup>1</sup>Significantly different from after-ADR treatment group (P ≤ .05).
peak-systolic strain in the 3 apical views and the GPSS became significantly higher, almost approaching ALX or exceeding both apical 2 chambers and GPSS measured before the treatment with ADR + carvedilol.

The reason for the cardioprotective effects of carvedilol in ADR-induced cardiomyopathy is not fully known. However, cardioprotection may be attributed to through the potent antioxidant activity of carvedilol. Both carvedilol and its metabolites were shown to have antioxidant effects. It was reported that ROS in failing heart were reduced by administration of carvedilol. Therefore, oxidative stress is considered to be the major pathogenic mechanism of ADR-induced cardiotoxicity. Antioxidant properties of carvedilol may be responsible for the beneficial effects of the drug that help in the prevention of cardiomyopathy. However, the protective effect of carvedilol itself cannot be ruled out. Sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2) may be another key factor in ADR-induced cardiotoxicity. ADR causes down-regulation of SERCA2 mRNA in animals with cardiac dysfunction. Carvedilol restores SERCA2 promoter activity in myocytes and can block the down-regulation of SERCA2 gene expression independently from its beta-blocking activity. Because apoptosis plays a highly significant role in ADR-induced cardiomyopathy, the antiapoptotic properties of carvedilol could be another important factor in protection from ADR-induced cardiomyopathy. In addition, mitochondrial dysfunction has a significant role in ADR-induced cardiotoxicity. Earlier studies have shown that carvedilol prevents mitochondrial dysfunction.

The results of the present study should be of interest for pediatric cancer patients, who are now surviving the disease in greater numbers and who are more commonly receiving treatment regimens that include risks of acute and subacute cardiac toxicity from ADR.

### Study Limitations

This study was performed during the induction phase and 1 week after the last ADR dose, so it did not check the late and chronic phases of ADR treatment on the cardiac functions. Consequently, the effect of carvedilol on the late phase of ADR-induced cardiotoxicity was not studied. In addition, the follow-up time of this clinical investigation was rather short which most likely affects the findings reported. The study also concentrated on the left side of the heart and did not evaluate the right side of the heart or pulmonary pressure.

### Conclusion

The results of this study suggested that carvedilol could be used to protect the heart against the cardiotoxic effects of ADR. In addition, this research would anticipate that concomitant treatment with carvedilol during initial chemotherapy could be implemented widely for most cancer patients submitted to chemotherapy with potential cardiotoxic side effects. In view of the present study results, large-scale studies are recommended to allow further assessment of the protective role of carvedilol in ADR-induced cardiotoxicity in cancer patients.

### Acknowledgment

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### Disclosures

None.

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