Preventive Effect of Erythropoietin on Cardiac Dysfunction in Doxorubicin-Induced Cardiomyopathy

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Background—Doxorubicin is a highly effective antineoplastic drug, but its clinical use is limited by its adverse side effects on the heart. We investigated possible protective effects of erythropoietin against doxorubicin-induced cardiomyopathy.

Methods and Results—Cardiomyopathy was induced in mice by a single intraperitoneal injection of doxorubicin (15 mg/kg). In some cases, human recombinant erythropoietin (5000 U/kg) was started simultaneously. Two weeks later, left ventricular dilatation and dysfunction were apparent in mice given doxorubicin but were significantly attenuated by erythropoietin treatment. Erythropoietin also protected hearts against doxorubicin-induced cardiomyocyte atrophy and degeneration, myocardial fibrosis, inflammatory cell infiltration, and downregulation of expression of GATA-4 and 3 sarcomeric proteins, myosin heavy chain, troponin I, and desmin. Cyclooxygenase-2 expression was upregulated in doxorubicin-treated hearts, and that, too, was attenuated by erythropoietin. No doxorubicin-induced apoptotic effects were seen, nor were any changes seen in the expression of tumor necrosis factor-α or transforming growth factor-β1. Antiatrophic and GATA-4 restoring effects of erythropoietin were demonstrated in the in vitro experiments with cultured cardiomyocytes exposed to doxorubicin, which indicated the direct cardioprotective effects of erythropoietin beyond erythropoiesis. Cardiac erythropoietin receptor expression was downregulated in doxorubicin-induced cardiomyopathy but was restored by erythropoietin. Among the downstream mediators of erythropoietin receptor signaling, activation of extracellular signal-regulated kinase was reduced by doxorubicin but restored by erythropoietin. By contrast, erythropoietin was ineffective when administered after cardiac dysfunction was established in the chronic stage.

Conclusions—The present study indicates a protective effect of erythropoietin against doxorubicin-induced cardiomyopathy. (Circulation. 2006;113:535-543.)

Key Words: cardiomyopathy ■ cells ■ heart failure

The antineoplastic drug doxorubicin is effective in the treatment of a broad spectrum of hematogenous and solid human malignancies, but its clinical use is limited by its adverse side effects: irreversible degenerative cardiomyopathy and congestive heart failure.1,2 The efficacy of doxorubicin as a cytotoxic agent for the treatment of various human tumors has prompted a search to find treatments that reduce or prevent the risk of doxorubicin-induced cardiomyopathy and congestive heart failure.3–5 So far, however, the ability of these treatments to protect the heart from doxorubicin-induced damage has been varied and limited.

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The cytokine erythropoietin is produced by the adult kidney and is indispensable for the proliferation, survival, and differentiation of erythroid progenitor cells. Erythropoietin receptors (EPORs) also have been identified in nonhematopoietic tissues, including the heart.7,8 Previous studies have shown that erythropoietin plays a protective role in the brain,9,10 spinal cord,11,12 and skeletal muscle.13 In addition, recent studies suggest that erythropoietin also exerts a cardioprotective effect against infarction and ischemia-reperfusion injury.14–16 In those studies, erythropoietin administration before or during ischemia significantly enhanced left ventricular (LV) contractility and cardiac recovery after myocardial ischemia/reperfusion. It was suggested that a cellular mechanism that contributed to this protection was the inhibition of cardiomyocyte apoptosis, which resulted in a reduction in infarct size within the area at risk. These findings, which suggest that erythropoietin acts as a tissue-protective cytokine in the heart, prompted us to investigate the possible protective effect of erythropoietin against heart disease with a nonischemic origin. We hypothesized that the cytoprotective action of erythropoietin would enable it to block progression of the cardiomyopathy induced by doxorubicin. To
test that idea, in the present study, we examined the effect of erythropoietin on doxorubicin-induced cardiomyopathy and investigated the specific mechanisms of its effects.

Methods

Experimental Protocols

This study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society. Cardiomyopathy was induced in male 10-week-old C57BL/6J mice (Chubu Kagaku, Nagoya, Japan) by a single intraperitoneal injection of doxorubicin (doxorubicin hydrochloride, Kyowa Hakko) at a dose of 15 mg/kg. The presence of doxorubicin-induced cardiomyopathy was confirmed both functionally and histologically in all mice that were given no therapeutic intervention by the observation of decreased LV function (by echocardiography and cardiac catheterization) and degenerative change in cardiomyocytes.2 In sham-treated mice, the same volume of saline was injected in a similar manner. Erythropoietin treatment entailed 3 intraperitoneal injections of recombinant human erythropoietin (Chugai Pharmaceutical Co) at a dose of 5000 U/kg with a 5-day interval between injections. The dose of erythropoietin was within a close range of known dosages for organ protection.14–17 Untreated control groups were given the same volume of saline. Erythropoietin was administered either prophylactically or after establishment of doxorubicin-induced cardiomyopathy (Figure 1).

Protocol 1 (Prophylactic Treatment)

In this protocol, treatment with erythropoietin was started just after mice were injected with doxorubicin. Mice were randomly assigned to receive (1) doxorubicin alone (n=11), (2) doxorubicin plus erythropoietin (n=10), (3) saline alone (n=10), or (4) saline plus erythropoietin (n=6). Two weeks later, all of the mice were killed with an overdose of pentobarbital after physiological examination. Cardiac specimens were then subjected to histological, immunohistochemical, and molecular biological analyses.

Protocol 2 (Late Treatment)

In this protocol, erythropoietin treatment was started 2 weeks after doxorubicin injection. At that time, mice were assigned to erythropoietin-treated groups or an untreated group (n=10). For this assignment, echocardiography was done to reduce the bias between the groups. Mice were administered erythropoietin at the same dose and frequency of injection as in protocol 1 (n=10), at a dose that was 10-fold higher (50 000 U/kg) with the same frequency (n=6; Figure 1). Four weeks after doxorubicin injection, mice were examined as described for protocol 1.

Protocol 3 (In Vitro Study)

Cardiomyocytes were isolated from 1-day-old neonatal C57BL/6J mice as reported previously.18 The cardiomyocytes were plated on laminin-coated dishes or in slide glass chambers and incubated in D-MEM (Sigma) containing 10% FBS (Sigma) at 37°C for 48 hours. The cells were then treated with doxorubicin at the concentration of 0.1 μmol/L or with saline of the same volume, and erythropoietin was simultaneously added to the medium at the concentration of 0, 0.5, 1, 5, 50, or 500 U/mL. Twenty-four hours later, the cells were used for morphometric and biochemical analyses.

The cardiomyocytes in slide glass chambers were fixed in 4% paraformaldehyde, permeabilized with 0.05% Triton-X, and stained with rhodamine phalloidin and Hoechst 33342 (both from Molecular Probes). Digital images captured with a laser-confocal microscope system (LSM510, Zeiss) were used for morphometric analysis with Adobe Photoshop 7.0 (Adobe Systems Inc). Proteins extracted from cardiomyocytes on dishes were used for Western blotting.

Figure 1. Experimental protocols. Protocol 1: To examine effect of prophylactic use of erythropoietin (EPO) on doxorubicin (DOX)-induced cardiomyopathy, erythropoietin administration was started just after doxorubicin injection. Protocol 2: To examine the therapeutic effect of erythropoietin on established doxorubicin-induced cardiomyopathy, erythropoietin was started at 2 weeks after doxorubicin injection, when cardiac dysfunction is already apparent.

Blood Cell Count and Measurement of Plasma Concentration of Erythropoietin

Blood was drawn from the inferior vena cava when the animals were euthanized and used for blood cell count and measurement of plasma concentration of erythropoietin by radioimmunoassay with the previously described method.19 This radioimmunoassay not only detects human erythropoietin but also cross-reacts with murine erythropoietin.

Physiological Studies

Animals were anesthetized via nasal mask with halothane (induction, 2%; maintenance, 0.5%) in a mixture of N₂O and O₂ (0.5 L/min each). Echocardiograms were then recorded with an echocardiographic system (Aloka) equipped with a 7.5-MHz imaging transducer as reported previously.20 After cardiac echocardiography, the right carotid artery was cannulated with a micrometerometer-tipped catheter (SPR 407; Millar Instruments) and advanced into the aorta and then into the LV to record pressure and ±dP/dt.

Histological Analysis

After the echocardiography, each heart was removed and cut into 3 transverse slices. Of these, the middle slice was fixed in 10% buffered formalin and embedded in paraffin, after which 4-μm-thick sections were stained with hematoxylin-eosin or Sirius red F3BA (0.1% solution in saturated aqueous picric acid; Aldrich). Quantitative assessments, including cell size and fibrotic area, were performed with a multipurpose color image processor (LUZEX F: Nireco) with 20 randomly chosen high-power fields in each heart.
Immunohistochemical Analysis
Deparaffinized sections from the middle third of the ventricle were incubated with a primary antibody against EPOR (H-194; Santa Cruz), Flk-1 (Santa Cruz), or CD45 (Pharmingen). An ABC kit (DAKO) was then used for the immunostaining with diaminobenzidine serving as the chromogen. Nuclei were counterstained with hematoxylin.

Western Blotting
Lysates from heart tissues were used for Western blot analysis. Proteins were separated and transferred to membranes by standard protocols, after which they were probed with antibodies against EPOR (M-20; Santa Cruz), GATA-4, myosin heavy chain (MHC), troponin I (all from Santa Cruz), desmin (Sigma), cyclooxygenase (COX)-2 (Santa Cruz), and transforming growth factor-β1 (TGF-β1; Promega). The activation of Akt, extracellular signal-regulated protein kinase (ERK), and signal transducer and activator of transcription 5 (STAT5) was assessed with antibodies against Akt, phospho-Akt, phospho-ERK, and phospho-STAT5 (all from Cell Signaling). Procaspase-3 and the activated form of caspase-3 were evaluated with anti-caspase-3 antibody (Santa Cruz). Three to 5 hearts from each group were used for this assay.

The blots were visualized by means of chemiluminescence (ECL, Amersham), and the signals were quantified by densitometry. α-Tubulin (analyzed with an antibody from Santa Cruz) served as the loading control.

ELISAs
Levels of tumor necrosis factor-α (TNF-α) in the myocardium were assayed with an ELISA (R&D Systems). Three hearts from each group were used for this assay.

In Situ Nick End-Labeling
Terminal dUTP nick end-labeling (TUNEL) assays were performed in sections with an ApopTag kit (Intergene) mainly according to the instructions of the supplier. Mouse mammary tissue served as a positive control.

Electron Microscopy
Cardiac specimens were immersion-fixed overnight in phosphate-buffered 2.5% glutaraldehyde (pH 7.4), postfixed for 1 hour with 1% osmium tetroxide, dehydrated through a graded ethanol series, and embedded in Epon medium. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in an electron microscope (H700, Hitachi).

Statistical Analysis
Value are shown as mean±SEM. The significance of differences between groups was evaluated with t tests or 1-way ANOVA followed by the Newman-Keuls multiple comparison test. Values of P<0.05 were considered significant.

Results

Effect of Prophylactic Use of Erythropoietin on Doxorubicin-Induced Cardiomyopathy
Using Protocol 1 (Figure 1), we initially evaluated the effect of prophylactic erythropoietin administration on doxorubicin-induced cardiomyopathy.

Survival Rate, Blood Cell Count, and Plasma Concentrations of Erythropoietin
Two weeks after doxorubicin administration, all of the mice of each group were alive. Blood cell counts conducted when mice were euthanized revealed no significant difference in hematocrit between groups in the present model (53.1±2.6% in sham without erythropoietin, 58.6±1.4% in sham with erythropoietin, 53.5±3.1% in doxorubicin without erythropoietin versus 57.4±2.2% in doxorubicin with erythropoietin, P=NS between each group). Plasma erythropoietin concentration of the erythropoietin-treated mice was significantly higher (30.9±2.0 mIU/mL) than that of the saline-treated mice (17.1±0.86 mIU/mL, P<0.05) in doxorubicin-administered mice, however.

Physiological Studies
The results of our physiological studies are summarized in Figure 2. Echocardiography and cardiac catheterization performed 2 weeks after doxorubicin (DOX) injection (protocol 1) using echocardiography and cardiac catheterization: *P<0.05 vs untreated sham group (doxorubicin− and erythropoietin−); #P<0.05 vs untreated doxorubicin group (doxorubicin− and erythropoietin−). LVDD indicates LV diastolic diameter; LVDs, LV systolic diameter; %FS, percent fractional shortening; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; and HR, heart rate.

The results of our physiological studies are summarized in Figure 2. Echocardiography and cardiac catheterization performed 2 weeks after doxorubicin administration showed that mice receiving doxorubicin alone had significant cardiac functional deterioration characterized by enlargement of the LV cavity and signs of decreased cardiac function, ie, increased LV diameter and end-diastolic pressure and decreased LV fractional shortening and ±dP/dt, compared with sham animals. Treatment with erythropoietin significantly mitigated the doxorubicin-induced impairment of cardiac function. Administration of erythropoietin to sham animals had no effect on cardiac function.

Pathological Studies
We observed no significant difference in the heart-to-body weight ratios among the groups (sham 3.71±0.05 mg/g; erythropoietin alone 3.73±0.08 mg/g; doxorubicin alone 3.86±0.10 mg/g; doxorubicin plus erythropoietin 3.67±0.10 mg/g). On the other hand, examination of transverse sections
of hearts stained with hematoxylin-eosin (Figure 3A) showed that the transverse diameter of cardiomyocytes from the group receiving doxorubicin alone was significantly smaller than in the sham group (13.7 ± 0.1 versus 14.6 ± 0.1 μm) and that erythropoietin exerted a significant protective effect against doxorubicin-induced atrophy (transverse diameter 14.4 ± 0.1 μm; Figure 3B). Similarly, when we then assessed cardiac fibrosis using Sirius red–stained sections (Figure 3A), we found that the amount of fibrosis was significantly higher in the group receiving doxorubicin alone than in the sham group (1.01 ± 0.10% versus 0.42 ± 0.05%) and that the doxorubicin-induced fibrosis was significantly reduced by erythropoietin (0.72 ± 0.07%; Figure 3B). In addition, immuno- 

nohistochemical analysis revealed that doxorubicin initiated significant infiltration of the myocardium by CD45-positive leukocytes (9.8 ± 1.1 versus 5.2 ± 0.6 cells/high-power field in the sham group). Again, erythropoietin attenuated the effect of doxorubicin, reducing doxorubicin-induced CD45-positive leukocyte infiltration to 6.1 ± 0.7 cells/high-power field. There was no significant difference in the number of Flk-1-positive cells between the groups (Figure 3).

Electron microscopic examination confirmed that doxorubicin caused degenerative changes in cardiomyocytes. These changes were characterized by myofibrillar derangement and disruption and increased numbers of subcellular organelles (Figure 3C). Notably, these degenerative changes were significantly attenuated by treatment with erythropoietin.

**TUNEL-Positive Cells and Caspase-3 Detection**
TUNEL-positive cells were observed among both cardiomyocytes and noncardiomyocytes from all 4 groups, but we found no significant difference in the incidence of TUNEL-positive cells between mice that received doxorubicin and those that did not, and erythropoietin had no significant effect on the incidence of TUNEL-positive cells (supplemental Figure IA). In addition, we detected no activated caspase-3 in any of the groups (supplemental Figure IB), and although electron microscopy revealed degenerative changes in cardiomyocytes from doxorubicin-treated mice, no typically apoptotic cells were seen (Figure 3C). Taken together, these results indicate that apoptosis is not involved in the present model of doxorubicin-induced cardiomyopathy.

**Expression of GATA-4 and Sarcomeric Proteins**
GATA-4 is a key transcriptional factor that regulates expression of sarcomeric proteins in the heart.21,22 We found myocardial levels of GATA-4 to be reduced significantly by doxorubicin (Figure 4), which is consistent with previous reports.23,24 Likewise, levels of 3 sarcomeric proteins, MHC, troponin I, and desmin, were significantly downregulated by doxorubicin. The inhibitory effect of doxorubicin on the expression of all 4 of these proteins was completely reversed by erythropoietin.

**Cytokine Production and COX-2 Signal in Myocardium**
Of the cytokines tested, doxorubicin only affected the expression of COX-2. We found doxorubicin to have no significant effect on myocardial expression of TNF-α or TGF-β1, but expression of COX-2 was markedly upregulated by doxorubicin, and that effect was largely reversed by erythropoietin (Figure 5).

**Expression of EPOR and Its Downstream Mediators**
Our Western blot analysis also showed that EPOR expression is diminished in doxorubicin-treated hearts but is greatly enhanced by erythropoietin treatment (Figure 6A, left panels). Consistent with that finding, immunohistochemical analysis showed EPORs to be expressed on cardiomyocytes and to be more strongly expressed in erythropoietin-treated hearts (Figure 6A, right panels).
Phosphatidylinositol 3-kinase (PI3K)/Akt, receptor-associated Janus family tyrosine kinase (Jak)/STAT, and ERK/mitogen-activated protein kinase (MAPK) are all known to be downstream mediators of EPOR signaling in cardiac cells both in vitro and in vivo. We found that neither doxorubicin-induced cardiomyopathy nor the beneficial effects of erythropoietin were related to activation (phosphorylation) of Akt or STAT5 (Figure 6B). On the other hand, ERK phosphorylation, and thus its activation, was markedly inhibited by doxorubicin, and that effect was significantly attenuated by erythropoietin. This implies that the ERK pathway is substantially involved in the protective effect exerted by erythropoietin against doxorubicin-induced cardiomyopathy.

**Effect of Erythropoietin on Established Doxorubicin-Induced Cardiomyopathy**

Using protocol 2 (Figure 1), we next examined the therapeutic effect of erythropoietin on established cardiac dysfunction resulting from doxorubicin-induced cardiomyopathy. In this experiment, erythropoietin treatment was started 2 weeks after doxorubicin injection, when cardiac dysfunction was already apparent; animals were then examined 2 weeks later. Under these conditions, we found erythropoietin to have no

### LV Geometry, Function, and Histology 4 Weeks After Administration of Doxorubicin to Mice With and Without Erythropoietin (Protocol 2)

<table>
<thead>
<tr>
<th></th>
<th>DOX (n=10)</th>
<th>DOX+EP0 (n=10)</th>
<th>DOX+10-Fold EPO (n=6)</th>
<th>DOX+2× Frequency of</th>
</tr>
</thead>
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<tr>
<td>LVdD, mm</td>
<td>3.4±0.11</td>
<td>3.3±0.10</td>
<td>3.3±0.10</td>
<td>3.2±0.08</td>
</tr>
<tr>
<td>LVdS, mm</td>
<td>2.7±0.13</td>
<td>2.6±0.10</td>
<td>2.6±0.14</td>
<td>2.4±0.08</td>
</tr>
<tr>
<td>%FS</td>
<td>20±1.4</td>
<td>22±1.2</td>
<td>22±1.9</td>
<td>23±2.9</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.0±0.64</td>
<td>3.6±0.58</td>
<td>3.3±0.49</td>
<td>3.3±0.76</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>437±20</td>
<td>444±22</td>
<td>501±31</td>
<td>478±58</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>5944±560</td>
<td>5596±527</td>
<td>6044±393</td>
<td>6363±288</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>5265±600</td>
<td>5569±619</td>
<td>5774±418</td>
<td>6067±259</td>
</tr>
<tr>
<td>Myocyte size, μm</td>
<td>13.5±0.21</td>
<td>13.4±0.28</td>
<td>13.5±0.49</td>
<td>13.8±0.65</td>
</tr>
<tr>
<td>% Fibrosis</td>
<td>1.20±0.08</td>
<td>1.12±0.11</td>
<td>0.97±0.15</td>
<td>1.00±0.07</td>
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</tbody>
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DOX indicates doxorubicin; EP0, erythropoietin; LVdD, LV diastolic diameter; LVdS, LV systolic diameter; %FS, percent fractional shortening; and LVEDP, LV end-diastolic pressure.

There were no significant differences in any parameter between the groups.
beneficial effects (Table). We also found that neither a 10-fold dose nor 2 times the frequency of injection of erythropoietin affected the outcome (Table).

**Effect of Erythropoietin on Cultured Cardiomyocytes**

Cardiomyocytes became significantly atrophic after doxorubicin administration. Erythropoietin treatment significantly prevented such doxorubicin-induced atrophic reaction in cardiomyocytes (Figure 7A). Erythropoietin affected the cardiomyocytes in a dose-dependent manner, but its maximal benefit was attained at the concentration of 5 U/mL. Western blot analysis revealed that doxorubicin significantly reduced expression of GATA-4 and MHC in cultured cardiomyocytes, which were restored by the erythropoietin treatment (Figure 7B).

**Discussion**

**Protective Effect of Erythropoietin Against Doxorubicin-Induced Cardiomyopathy**

The present study provides the first evidence of the beneficial effects of erythropoietin on cardiac dysfunction resulting from doxorubicin-induced cardiomyopathy, a nonischemic cardiomyopathy. The prominent pathological findings were that erythropoietin prevented doxorubicin-induced atrophic degeneration of cardiomyocytes and cardiac fibrosis. Thus, the mechanisms of the action of erythropoietin on the heart apparently differ from the mechanism seen in cases of acute myocardial infarction and ischemia/reperfusion, in which erythropoietin reportedly enhances the survival of ischemic cardiomyocytes.14-16 In addition, both in vivo and in vitro findings of the present study proved the direct cardioprotection by erythropoietin that is exerted independently of its erythropoietic effect. On the other hand, the results obtained with protocol 2 indicate erythropoietin to be ineffective for treating doxorubicin-induced cardiac dysfunction once it has become established.

**Mechanisms of the Beneficial Effects of Erythropoietin**

Our findings suggest that several factors contribute to the cardioprotective effects of erythropoietin against
doxorubicin-induced cardiomyopathy. The first is that erythropoietin exerts an antiatrophic/degenerative effect on cardiomyocytes. Sarcomeric proteins, including MHC, troponin I, and desmin, are important for the structural integrity and function of cardiomyocytes, and their myocardial expression is reportedly downregulated by doxorubicin, an effect we confirmed in the present study. Our new finding is that erythropoietin significantly restores the expression of sarcomeric proteins in the presence of doxorubicin. Moreover, GATA-4 is a key regulator of heart development that also is involved in the antiatrophic effect of erythropoietin, although such a mechanism has not been proved. Furthermore, it was recently shown that GATA-4 is depleted in doxorubicin-induced cardiotoxicity. The results of the present in vivo study not only confirm that earlier finding but also demonstrate that erythropoietin restores GATA-4 expression in the presence of doxorubicin. Our in vitro study using cultured cardiomyocytes, in which the erythropoietic effect of erythropoietin was not involved, confirmed the antiatrophic and GATA-4- and MHC-restoring effects of erythropoietin on cardiomyocytes.

Some recent findings indicate that apoptosis among cardiomyocytes is a leading cause of cardiac dysfunction in doxorubicin-induced cardiomyopathy. Seeking evidence of doxorubicin-induced apoptosis, we conducted a series of TUNEL assays, electron microscopic examinations, and analyses of myocardial caspase-3 activation but detected no effect of doxorubicin or erythropoietin on the incidence of apoptosis. Thus, our findings suggest cardiomyocyte apoptosis is not important for disease progression in the present model. Erythropoietin has been reported to have an angiogenic effect; however, we detected no doxorubicin-induced reduction in capillary density, nor did erythropoietin promote capillary outgrowth, which indicates no mechanistic role for angiogenesis in doxorubicin-induced cardiomyopathy or the beneficial effects of erythropoietin. We also observed that doxorubicin stimulates myocardial infiltration by inflammatory cells and fibrosis and that erythropoietin prevents these pathological processes. The anti-inflammatory properties of erythropoietin were previously observed in the central nervous system, where erythropoietin exerted a protective effect after cerebral ischemia. In the heart, induction of a powerful inflammatory mediator is reportedly associated with heart failure. In that regard, doxorubicin is known to induce COX-2 expression in cardiomyocytes; moreover, inhibition of COX-2 reportedly improves cardiac function in doxorubicin-induced cardiomyopathy. COX-2 occupies a central position in the biosynthesis of proinflammatory prostaglandin E2, prostacyclin, and thromboxane A2. In the present study, we noted that doxorubicin strongly induced myocardial COX-2 expression and that this effect was largely inhibited by erythropoietin. This suggests doxorubicin likely initiates cardiac inflammation and fibrosis by inducing COX-2 expression, whereas erythropoietin may produce its antifibrotic effect by suppressing that expression; however, such mechanistic linkage has not been proven. In addition, previous studies indicate that TNF-α and TGF-β1 are potent stimulators of inflammation and fibrosis in the failing heart, although their involvement in doxorubicin-induced cardiomyopathy was challenged in one recent report. Consistent with the latter report, we found no significant doxorubicin- or erythropoietin-induced changes in the expression of TNF-α and TGF-β1. Our immunohistochemical and Western blot analyses showed strong myocardial expression of EPORs, which confirms earlier reports. We also found that doxorubicin significantly downregulated myocardial EPOR expression and that this inhibitory effect was completely blocked by erythropoietin. The mechanism by which doxorubicin downregulates EPOR expression is entirely unknown, although doxorubicin reportedly inhibits calcium-independent phospholipase A2. It is thus tempting to speculate that inhibition of phospholipase A2 by doxorubicin critically impairs plasma
membrane function, which in turn inhibits insertion of 
EPO R into the cardiomyocyte plasma membrane.

In hematopoietic and cardiac cells, EPO signaling can 
couple the PI3K/Akt, Jak/STAT, and ERK/MAPK signaling 
pathways.14,36 The present findings suggest that altered 
signaling via ERK, but not Akt or STAT5, is involved in 
doxorubicin-induced cardiomyopathy. This is consistent with 
a very recent study that showed a significant reduction of 
activated ERK (p-ERK) during the chronic stage of 
doxorubicin-induced cardiomyopathy (3 weeks after doxoru-
bicin injection).37 Another study using isolated rat heart 
subjected to excessive LV wall stress (induced by balloon 
inflation) showed an involvement of MAPK (p38 and ERKs) 
in the activation of GATA-4 binding to DNA.38 We therefore 
suggest that erythropoietin exerts its beneficial effects via the 
ERK/MAPK signaling pathway, which was otherwise inhibited 
by doxorubicin.

Conclusions

The present study suggests a protective effect of erythropoi-
etin against doxorubicin-induced cardiomyopathy. Erythro-
poietin attenuated doxorubicin-induced atrophic degeneration 
of cardiomyocytes and myocardial fibrosis. On the other 
hand, late administration of erythropoietin had no beneficial 
effect in our protocol. This may suggest that erythropoietin 
treatment for doxorubicin-induced cardiomyopathy should be 
administered prophylactically, although the results do 
not deny the possible therapeutic efficacy of the other regimens 
of late treatment.

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Disclosures

None.

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**CLINICAL PERSPECTIVE**

Doxorubicin is a highly effective, widely used antineoplastic drug in cancer chemotherapy; however, the dose-dependent cardiotoxicity of this agent, irreversible degenerative cardiomyopathy (doxorubicin-induced cardiomyopathy) progressing to congestive heart failure, limits its clinical application. Erythropoietin is an essential hormone for normal erythropoiesis, and recombinant human erythropoietin (rhEPO) has revolutionized the treatment of anemia associated with chronic renal failure, chemotherapy, and surgery. In the present study, we found a novel cardioprotective role of rhEPO in a murine model of doxorubicin-induced cardiomyopathy. RhEPO significantly improved cardiac dysfunction of doxorubicin-induced cardiomyopathy when used prophylactically. The mechanisms involved appear to be an antiatrophic effect on cardiac myocytes and an antifibrotic effect on myocardium of rhEPO, both of which were independent of its erythropoietic effect. Although the use of erythropoietic agents is already established as a standard care for patients with chemotherapy-related anemia, the results of the present study imply the benefit of prophylactic use of rhEPO, irrespective of the presence or absence of anemia, on some population of patients receiving doxorubicin, eg, patients given a high dose of doxorubicin or patients at risk for developing heart failure. However, additional studies are necessary to establish the efficacy and safety of rhEPO regimens in clinical practice for patients treated with doxorubicin.
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