Pretreatment with angiotensin-converting enzyme inhibitor improves doxorubicin-induced cardiomyopathy via preservation of mitochondrial function

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Objective: Doxorubicin is a widely used chemotherapy drug, but its application is associated with cardiotoxicity. Free radical generation and mitochondrial dysfunction are thought to contribute to doxorubicin-induced cardiac failure. Angiotensin-converting enzyme inhibitors are commonly used as cardioprotective agents and have recently been shown in clinical studies to be efficacious in the prevention of anthracycline-induced heart failure. This study evaluated a mechanism for these protective effects by testing the ability of the angiotensin-converting enzyme inhibitor enalapril to preserve mitochondrial function in a model of chronic doxorubicin treatment in rats.

Methods: Sprague Dawley rats were divided into 3 groups and followed for a total of 10 weeks: (1) control-untreated, (2) doxorubicin treated, and (3) doxorubicin + enalapril treated. Doxorubicin was administered via intraperitoneal injection at weekly intervals from weeks 2 to 7. Enalapril was administered in the drinking water of the doxorubicin + enalapril group for the study duration.

Results: Doxorubicin treatment produced a significant loss in left ventricular contractility ($P < .05$), decrease in mitochondrial function via impairment of state-3 respiration, decrease in the cytosolic fraction of adenosine triphosphate, and up-regulation of free radical production. Enalapril significantly attenuated the decrease in percent fractional shortening ($P < .05$) and prevented the doxorubicin-associated reduction in respiratory efficiency and cytosolic adenosine triphosphate content ($P < .05$). Enalapril also abolished the robust doxorubicin-induced increase in free radical formation.

Conclusions: Administration of enalapril attenuates doxorubicin-induced cardiac dysfunction via preservation of mitochondrial respiratory efficiency and reduction in doxorubicin-associated free radical generation. (J Thorac Cardiovasc Surg 2011;142:396-403)

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Doxorubicin, an anthracycline, is a widely used cytotoxic agent for the treatment of human neoplasms, such as leukemias and Hodgkin's lymphoma. However, administration of doxorubicin is known to cause cardiomyopathy, eventually leading to congestive heart failure that almost invariably develops in patients receiving cumulative doses of doxorubicin more than 550 mg/m^2.[1] As a result of doxorubicin therapy, a growing number of patients in the United States survive childhood and adult cancers only to develop severe cardiac dysfunction after treatment.[2] These patients have few surgical options for treatment because they have a combination of both dilated and restrictive cardiomyopathies, and make poor heart transplant candidates because of their history of cancer. Development of medical therapies that aid in the management of cardiac dysfunction is thus paramount for the long-term survival of these individuals.

Several previous studies have implicated free radical formation and oxidative stress as central mechanisms underlying the cardiotoxic effects of doxorubicin.[3] As a result, many experimental drug treatments to ameliorate cardiotoxicity of doxorubicin have focused on the protective effects of antioxidants and metal chelators, which minimize free radical damage.[4] Although these substances have shown some benefit, they are difficult to administer because of the challenge of achieving constant plasma concentrations of antioxidant drugs and their poor uptake by the heart.[5]

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Abbreviations and Acronyms
ACE = angiotensin-converting enzyme
ADP = adenosine diphosphate
ATP = adenosine triphosphate
ETC = electron transport chain
FRL = free radical leak
NS = not significant
P/M = pyruvate/malate
RCR = respiratory control ratio
ROS = reactive oxygen species

Angiotensin-converting enzyme (ACE) inhibitors are a class of drugs that are routinely administered in the clinic and have clearly shown positive therapeutic profiles for the treatment of heart failure caused by a number of cardiovascular diseases.[6] ACE inhibitors possess free radical scavenger and antioxidant properties,[7] and have recently been shown in 2 prospective clinical studies to be efficacious in the prevention of anthracycline-induced heart failure when administered early after chemotherapy regimens.[8,9] Administration of ACE inhibitors in these trials and preclinical studies has not been linked with an increased rate of recurrent malignancy.[1,10] However, only a limited number of studies have investigated the mechanisms by which ACE inhibition can prevent anthracycline-induced cardiotoxicity.[7,11] The potential effects ACE inhibitors may have on prevention of anthracycline-induced mitochondrial dysfunction remain unknown. To answer these questions, this study aimed to (1) investigate in vivo the possible protective effects of the ACE inhibitors (enalapril) against doxorubicin-induced cardiac toxicity in a longitudinal model of treatment and (2) determine whether the improved cardiac function from ACE inhibitor therapy is due to improved mitochondrial function and reduced free radical generation. Our results show that the concurrent administration of ACE inhibitors with doxorubicin treatment not only ameliorates cytotoxic effects of doxorubicin but also prevents doxorubicin-induced free radical formation and preserves mitochondrial respiratory efficiency and cellular adenosine triphosphate (ATP) content.

MATERIALS AND METHODS

Animals

Twenty-four female Sprague Dawley rats at approximately 10 weeks of age were obtained from Charles River Laboratories (Wilmington, Mass). The animals were divided into 3 groups: a) control-untreated (n = 8), b) doxorubicin treated (Dox) (n = 8), and c) doxorubicin + enalapril treated (DE) (n = 8). Both Dox and DE groups received doxorubicin at a cumulative dose of 25 mg/kg administered weekly via intraperitoneal injection for 6 weeks. Enalapril pretreatment for the DE group was started 1 week before administration of the first doxorubicin injection and continued throughout the study and for an additional 3 weeks after the last doxorubicin injection (Figure 1, *A*). The dosage of enalapril was calculated on the basis of a previous study by Sanbe and colleagues[12] and set at a dose of 10 mg/kg/d for each animal as previously described.[12] A detailed description is included in the Supplemental Materials and Methods.

Assessment of Cardiac Function

Echocardiography studies were performed before treatment and immediately before animal sacrifice to determine left ventricular fractional shortening, using a 14.7-MHz transducer on a Sequoia C512 echocardiography system (Siemens, Malvern, Pa). A detailed description is included in the Supplemental Materials and Methods.

Tissue Histology

Heart and liver specimens were fixed in 4% paraformaldehyde and stained with hematoxylin–eosin for analysis by light microscopy. A detailed description is included in the Supplemental Materials and Methods.

Enzymatic Measurement of Caspase-3 and Caspase-9 Activities

Caspase-3 and caspase-9 activities were measured using fluorometric protease assay kits: Caspase-3/CPP32 and Caspase-9/Mch6 (Biovision, Mountain View, Calif). A complete description is included in the Supplemental Materials and Methods.

Mitochondrial and Cytosolic Isolation

Mitochondrial and cytosolic protein fractions were isolated using differential centrifugation as previously described. A detailed description is included in the Supplemental Materials and Methods.

Mitochondrial Respiration and H_2O_2 Generation

Mitochondrial oxygen consumption was measured at 37°C by polarography, with a Clark-type oxygen electrode (Oxytherm, Hansatech, Norfolk, UK) under identical conditions (same mitochondria, buffer composition, and substrate concentrations) to H_2O_2 production measurements. The rate of mitochondrial H_2O_2 production was assayed in freshly isolated mitochondria by a fluorometric method described by Barja.[13] A complete description is included in the Supplemental Materials and Methods.

Adenosine Triphosphate Content

Cytosolic homogenate isolated from heart was used immediately after isolation to determine ATP content using a bioluminescence ATP kit from Sigma-Aldrich Inc (St Louis, Mo). A complete description is included in the Supplemental Materials and Methods.

Statistical Analysis

Data were analyzed using a 1-way analysis of variance with Newman–Keuls post hoc comparison. All data are represented as average ± standard error of the mean.

RESULTS

Rats were studied longitudinally over 10 weeks (Figure 1, *A*). Control animals were compared with the Dox and DE groups. There was a 100% survival in both control and DE groups up to 10 weeks. In contrast, the Dox group experienced only a 75% survival (2 deaths) (Figure 1, *B*). These data show that a 10-week model of doxorubicin administration causes significant toxicity resulting in mortality and validates the time course for intervention.

Doxorubicin treatment was observed to cause a significant decrease in cardiac function, as quantified by depressed