Overexpression of CYP2J2 provides protection against doxorubicin-induced cardiotoxicity

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of CYP2J2 provides protection against doxorubicin-induced cardiotoxicity. Am J Physiol Heart Circ Physiol 297: H37–H46, 2009. First published May 8, 2009; doi:10.1152/ajpheart.00983.2008.—Human cytochrome P-450 (CYP) epoxygenases are predominant enzymes responsible for the epoxidation of endogenous arachidonic acid (AA) to four regioisomeric epoxyeicosatrienoic acids (EETs). Recently, we demonstrated that these eicosanoids protect myocardium from ischemia-reperfusion injury. The present study utilized transgenic (Tr) mice with cardiomyocyte-specific overexpression of human CYP2J2 to investigate protection toward toxicity resulting from acute (0, 5, or 15 mg/kg daily for 3 days, followed by 24-h recovery) or chronic (0, 1.5, or 3.0 mg/kg biweekly for 5 wk, followed by 2-wk recovery) doxorubicin (Dox) administration. Acute treatment resulted in marked elevations of serum lactate dehydrogenase and creatine kinase levels that were significantly greater in wild-type (WT) than CYP2J2 Tr mice. Acute treatment also resulted in less activation of stress response enzymes in CYP2J2 Tr mice (catalase 750% vs. 300% of baseline, caspase-3 235% vs. 165% of baseline in WT vs. CYP2J2 Tr mice). Moreover, CYP2J2 Tr hearts exhibited less Dox-induced cardiomyocytes apoptosis (measured by TunEL) compared with WT hearts. After chronic treatment, comparable decreases in body weight were observed in WT and CYP2J2 Tr mice. However, cardiac function, assessed by measurement of fractional shortening with M-mode transthoracic echocardiography, was significantly higher in CYP2J2 Tr than WT hearts after chronic Dox treatment (WT 37 ± 2%, CYP2J2 Tr 47 ± 1%). WT mice also had larger increases in β-myosin heavy chain and cardiac ankyrin repeat protein compared with CYP2J2 Tr mice. CYP2J2 Tr hearts had a significantly higher rate of Dox metabolism than WT hearts (2.2 ± 0.25 vs. 1.6 ± 0.50 ng·min⁻¹·100 μg protein⁻¹). In vitro data from H9c2 cells demonstrated that EETs attenuated Dox-induced mitochondrial damage. Together, these data suggest that cardiac-specific overexpression of CYP2J2 limited Dox-induced toxicity.

cytochrome P-450 2J2; heart; function

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MATERIALS AND METHODS

Animals. Mice with cardiomyocyte-specific overexpression of human CYP2J2 and their wild-type (WT) littermate controls were utilized (48). All experiments used male and female mice aged 3–5 mo and weighing 25–35 g and were approved by the National Institute of Environmental Health Sciences/National Institutes of Health and University of Alberta Animal Care and Use Committees.

Treatment protocols. In the acute protocol, mice were randomly divided into three groups and received 0, 5, or 15 mg/kg Dox by intraperitoneal injections (Fig. 1A). Mice were treated with a single dose each day for 3 days (0, 24, and 48 h) and were killed by CO2 asphyxiation on the fourth day (72 h). Hearts were either isolated and perfused in the Langendorff mode to assess heart function (see below) or collected for histological and biochemical analyses.

In the chronic protocol, mice were randomly divided into three groups and received 0, 1.5, or 3.0 mg/kg Dox by intraperitoneal injections (Fig. 1B). Dox was administered twice a week for 5 wk for a total of 10 treatments. A 2-wk “washout” period was allowed after the last treatment, at which point cardiac function was assessed by echocardiography. Mice were then killed by CO2 asphyxiation, and cardiac specimens were analyzed. All studies were conducted by investigators who were blinded to treatment group assignments.

Biochemical analyses. At the end of each protocol, blood was drawn from the inferior vena cava to assess levels of lactate dehydrogenase (LDH) and creatine kinase (CK). Serum was collected within 2 h from clotted blood by centrifugation and analyzed with end point assay kits (Sigma Diagnostics, St. Louis, MO). LDH and CK activities were expressed as units per liter. Subcellular fractions were prepared by differential centrifugation from frozen hearts as described previously (15). Catalase activity was measured with a spectrophotometric assay kits (Sigma Diagnostics, St. Louis, MO). LDH and CK activities were expressed as %FS (LVDs/LVDd) × 100.

Histology and gene expression. Histological analyses were performed on hearts from both CYP2J2 Tr and WT mice as previously described (48). Briefly, hearts were removed, dissected, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned for examination. Sections were used for a terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL) procedure for detecting apoptotic cardiomyocytes as previously described (16). The percentage of TUNEL-positive nuclei was determined by counting 10 random fields. All tissues were stored at −80°C until preparation of RNA. Total RNA was isolated with a RNeasy Midi kit (Qiagen, Valencia, CA) and concentrated with a Microcon YM-30 column (Millipore, Billerica, MA). A formaldehyde agarose gel containing ethidium bromide was used to assess the quality of the RNA. Semi-quantitative PCR analysis was performed for alterations in gene expression. PCR primers for α-myosin heavy chain (α-MHC) were 5′-GAAGTTGAGCCGCTACATCAAGGCG-3′ (forward) and 5′-TCCAGTGTTTCTCTGAGGCCG-3′ (reverse); for β-myosin heavy chain (β-MHC) were 5′-GGCCAAACCTCTCTCTGG-3′ (forward) and 5′-CACCTTCTGATGGGCAAGCCTTCTC-3′ (reverse); and for γ-erythrocyte-3-phosphate dehydrogenase (GAPDH) were 5′-ATTACCAAGGAGCCAGCCTT-3′ (forward) and 5′-TACGCTTCTATGCTCACTC-3′ (reverse). The ability of WT and CYP2J2 Tr mice to metabolize Dox was evaluated in an HPLC assay. Briefly, microsomal proteins from WT and CYP2J2 Tr hearts (1 mg protein/ml) were incubated with Dox (500 nM) at 37°C for 60 min in a buffer containing 50 mM potassium phosphate, 1.15% KCl, pH 7, and 1 mM NADPH (17, 34). Control experiments were performed with the selective P-450 epoxidase inhibitor N-methylsulfonyl-(6-propargyl-oxophenyl)hexanamide (MS-PPOH) (50 μM; generously provided by Dr. J. Falck, University of Texas, Dallas, TX). The reactions were stopped by the addition of 300 μl of acetonitrile, and Dox was extracted with a chloroform and 2-propanol (1:1 vol/vol) procedure, dried, and redissolved in 120 μl of methanol. Samples were injected into the HPLC system for analysis.
into a Waters 712 WISP HPLC equipped with a Schima D RF-10AXL fluorescence detector (17, 34, 38). A C18 10-μm Bondapak column was utilized with a formic acid (0.05%):acetonitrile gradient mobile phase in reverse mode. All products were identified based on coelution with authentic standards. Standard curves prepared with Dox (0–1,000 ng/ml) were used to determine concentration differences and specific activity (excitation 470 nm, emission 560 nm).

Mitochondrial assessment. H9c2 cells (American Type Culture Collection, Manassas, VA) were cultured and grown in DMEM supplemented with 10% bovine serum albumin and antibiotics such as penicillin and streptomycin at 37°C in an atmosphere of 5% CO2-95% air. Cells were loaded with 150 nM tetramethylrhodamine ethyl ester (TMRE) (Invitrogen) for 30 min and then subjected to time-lapse air. Cells were loaded with 150 nM tetramethylrhodamine ethyl ester (TMRE) (Invitrogen) for 30 min and then subjected to time-lapse imaging for 60 min at 37°C and 5% CO2. A Zeiss Axios Observer Z1 inverted epifluorescence microscope was used to take z-stack images every minute with 200-ms exposure time. Cells were observed under a 40× objective, fluorescence was excited at the 555-nm line, and emission was recorded with a band-pass filter of 575–640 nm.

Cells were observed under a ×40 objective, fluorescence was excited at the 555-nm line, and emission was recorded with a band-pass filter of 575–640 nm. Changes in fluorescence were recorded in cells treated with vehicle (0.5% ethanol in PBS), Dox (10 μM), 11,12-EET (1 or 10 μM) or 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) (1 μM; generously provided by Dr. J. Falck, University of Texas). Mitochondrial morphology changes were visualized over the 60-min exposure period, and individual mitochondria were assessed for alterations to the elongated and filamentous appearance found in control cells. The term “punctate mitochondria” was used to describe both condensed and fragmented mitochondrial morphology as described elsewhere (35). Measurements were taken from four or five individual experiments, and intensities were quantified relative to background levels. Individual mitochondria were quantified in multiple images taken at similar magnifications with AxioVision Software (Carl Zeiss Imaging Solutions). Changes in fluorescence were expressed as percent change relative to baseline levels.

**RESULTS**

**Effects of acute Dox administration.** Serum CK and LDH activities increased in a dose-dependent manner after three consecutive daily administrations of Dox in both WT and CYP2J2 Tr mice (Fig. 2, A and B). However, overexpression of CYP2J2 resulted in lower serum CK and LDH activities after Dox treatment, indicative of reduced myocardial injury. Dox-mediated cardiotoxicity has been shown to involve production of reactive oxygen species (ROS) (1, 8). In turn, increased intracellular ROS activate endogenous antioxidant enzymes, such as catalase. Catalase is an important antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen. Acute Dox treatment resulted in significant increases in cardiac catalase activities in both WT and CYP2J2 Tr mice, although this increase was less pronounced in the latter group (Fig. 2C).

Postischemic cardiac performance was decreased in Dox-treated WT mice compared with vehicle-treated mice as measured by LVDP (21.8 ± 5% vs. 12.3 ± 3% for the 0 and 15 mg/kg groups, respectively; \( P < 0.05 \)) (Fig. 2D). CYP2J2 Tr mice exhibited improved postischemic cardiac function compared with WT mice, consistent with previously published results (48). Importantly, no decrease in LVDP was observed in CYP2J2 Tr hearts after Dox treatment (38 ± 3% vs. 36 ± 11% for the 0 and 15 mg/kg groups, respectively) (Fig. 2D).

**Statistical analysis.** Values are expressed as means ± SE. Statistical significance was determined by the unpaired Student’s t-test and one-way ANOVA followed by a Duncan’s test to assess multiple group comparisons. Values were considered significant if \( P < 0.05 \).
Evidence suggests that cellular apoptotic responses may be triggered subsequent to Dox exposure. Therefore, we further assessed cardiac injury by analyzing cytotoxic fractions for caspase-3 activity. Compared with mice treated with vehicle, caspase-3 activity was significantly higher in both WT and CYP2J2 Tr mice treated with Dox (15 mg/kg) (Fig. 3A). Importantly, caspase-3 activity was significantly higher in WT hearts than in CYP2J2 Tr hearts after Dox treatment (84 ± 8 vs. 55 ± 9 pmol·min⁻¹·mg protein⁻¹, respectively; \( P < 0.05 \)) (Fig. 3A). Consistent with these results, significant increases in TUNEL-positive nuclei were observed in WT hearts after acute treatment with Dox, whereas no significant changes were observed in CYP2J2 Tr hearts (Fig. 3B). However, similar to a recent report by Hiraumi et al. (21), we did not observe any significant histological changes with hematoxylin and eosin staining on repeated blinded analyses.

**Effects of chronic Dox administration.** Chronic treatment with Dox resulted in significant dose-dependent decreases in body weight in both WT and CYP2J2 Tr mice (Fig. 4, A and B). After Dox treatment was stopped and during the “washout” period, body weight stabilized and began to recover in all Dox-treated animals. Mice were killed at the end of the recovery period, after which heart weights were measured and compared with changes in body weight. As shown in Fig. 4C, heart weight-to-body weight ratios increased in a dose-dependent manner after Dox treatment. However, the magnitude of increase was significantly larger in WT mice than in CYP2J2 Tr mice at the highest Dox dose, suggestive of cardiac hypertrophy.

In contrast to the acute protocol, serum CK and LDH activities were not different in Dox-treated mice compared with vehicle control animals at the end of the chronic protocol (see Supplemental Data for this article). Additionally, no significant difference in caspase-3 activity was observed between Dox-treated and vehicle-treated mice in the chronic study (see Supplemental Data). To determine whether chronic Dox treatment resulted in significant changes to the cardiac structure, we examined expression levels of MHC and CARP in WT and CYP2J2 Tr mice. Analysis for MHC gene expression following chronic Dox treatment revealed an increased \( \beta \)-MHC-to-\( \alpha \)-MHC ratio in WT mice but not in CYP2J2 Tr mice (Fig. 4D). CARP is a transcriptional cofactor and structural component of the sarcomere involved in cardiogenesis and muscle injury (46). Studies have demonstrated that Dox treatment can induce CARP in vivo but repress CARP expression in cell culture systems (5, 60). As shown in Fig. 4E, chronic Dox treatment increased CARP expression only in WT mice. Together the data indicate that CYP2J2 Tr mice had less cardiac injury than WT mice after chronic Dox treatment.

To examine whether CYP2J2 can metabolize Dox, we incubated microsomes from CYP2J2 Tr and WT mouse hearts and measured Dox turnover. As shown in Fig. 4F, CYP2J2 Tr mice demonstrated a significantly higher rate of Dox metabolism (2.2 ± 0.25 ng·min⁻¹·100 μg protein⁻¹) compared with WT mice (1.6 ± 0.50 ng·min⁻¹·100 μg protein⁻¹). Importantly, the selective epoxygenase inhibitor MS-PPOH abolished the improved metabolism observed in CYP2J2 Tr mice (Fig. 4F). Thus the enzymatic activity was comparable in the two genotypes after treatment with MS-PPOH. Immunoblot analysis demonstrated that Dox treatment did reduce the expression level of CYP2J2 protein in hearts after chronic administration (Supplemental Data).

**Cardiac function after chronic administration of Dox.** To determine whether contractile function was affected by chronic Dox administration, LVDd and LVDs were assessed by transthoracic echocardiography at the end of the recovery period and LVFS was calculated. As shown in Fig. 5, there was no significant difference in these parameters between WT mice and CYP2J2 Tr mice in the vehicle control groups, indicating that CYP2J2 Tr mice had normal chamber dimensions and basal contractile function. Dox treatment caused significant dose-dependent increases in both LVDd and LVDs in WT mice (Fig. 5, A and B). In contrast, there were no significant changes in LVDd or LVDs in CYP2J2 Tr mice after Dox treatment. DOX caused a reduction in %FS in WT and CYP2J2 Tr mice, although the decrease in %FS was significantly less in CYP2J2 Tr mice (Fig. 5C). These data suggest that CYP2J2 Tr hearts had significantly better cardiac function than WT hearts after chronic Dox treatment. There were no significant changes in heart rate between the groups (Fig. 5D).

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1 The online version of this article contains supplemental material.
EETs limit mitochondrial damage caused by Dox in H9C2 cells. Recent reports indicate that mitochondria undergo dramatic fragmentation and dysfunction in response to Dox-induced toxicity (13, 21, 23, 43). To determine whether EETs can limit Dox injury, we investigated the mitochondrial morphology and membrane potential in H9c2 cells by real-time imaging. Mitochondria, which exhibit elongated and filamentous morphology in healthy control cells, became dramatically shorter and round after Dox exposure in H9c2 cells loaded with TMRE (Fig. 6 and Fig. 7A). Dox exposure resulted in the dissipation of fluorescence from the cells within 30 min (Fig. 7B), indicating changes in mitochondrial membrane potential. Pretreatment of H9c2 cells with 11,12-EET significantly attenuated the fragmentation and conversion of tubular mitochondria to punctate mitochondria (Fig. 6 and Fig. 7A). Blinded quantitative analysis revealed significantly higher relative fluorescence intensity in cells cotreated with 11,12-EET and Dox compared with Dox alone (Fig. 7B). Together these data suggest that EETs attenuated mitochondrial fission and slowed the collapse of the membrane potential. To confirm that the effect was mediated by EETs, we conducted experiments in the presence of 14,15-EEZE. This putative pan-EET receptor antagonist had no effect on mitochondria when administered alone and abrogated the effect of 11,12-EET in H9c2 cells (Figs. 6 and 7). In further analysis of downstream effects of mitochondrial dysfunction, Dox-induced caspase-3 activity was partially attenuated by co-treatment with EETs (Fig. 7C).

DISCUSSION

Several hypotheses have been put forth to explain the cardiotoxicity that limits the therapeutic use of Dox (37, 52, 53), including generation of free radicals in cardiomyocyte mitochondria. There is an increasing amount of literature reporting the functional significance of CYP monooxygenase enzymes in the heart. This is particularly true for CYP2J2, a primarily cardiac P-450 active in the epoxidation of AA to EETs (61). In the present study, we demonstrate that cardiac overexpression of the human CYP2J2 cDNA limits Dox-induced toxicity in
mice by maintaining LV function and increased Dox metabolism. Moreover, our in vitro data demonstrate direct protective effects of CYP2J2-derived metabolites, EETs, toward Dox-mediated mitochondrial damage.

Dox-induced injury can be indirectly monitored by the release of CK and LDH into the serum. No differences in baseline CK and LDH were observed between CYP2J2 Tr and WT mice, but acute Dox treatment resulted in significant

Fig. 5. Assessment of cardiac function after chronic Dox treatment. A and B: LV end-diastolic dimension (LVDd, mm; A) and LV endsystolic dimension (LVDs, mm; B) after chronic Dox treatment (0, 1.5, or 3.0 mg/kg); n = 12–17. *P < 0.05 Dox vs. control of the same genotype; ^P < 0.05 CYP2J2 Tr vs. respective WT. C: fractional shortening (FS) after chronic Dox treatment (0, 1.5, or 3.0 mg/kg). FS of the LV is expressed as %FS = (LVDD − LVDS)/LVDD × 100; n = 12–17. *P < 0.05 Dox vs. control of the same genotype; ^P < 0.05 CYP2J2 Tr vs. respective WT. D: heart rate (HR) after chronic DOX treatment (0, 1.5, or 3.0 mg/kg); n = 12–17. bpm, beats/min.

Fig. 6. Assessment of mitochondrial morphology in H9c2 cells. Representative frames from time-lapse series show H9c2 cells treated with vehicle (0.5% EtOH in PBS), 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE, 1 μM), Dox (10 μM), Dox (10 μM) + 11,12-epoxyeicosatrienoic acid (11,12-EET, 10 μM), or Dox (10 μM) + 11,12-EET (10 μM) + 14,15-EEZE (1 μM) at 0, 30, and 60 min. Mitochondrial morphology, filamentous and tubular shape, of the control cells remains unaltered during this time period. In contrast, Dox-treated cells exhibit significant punctate and fragmented mitochondrial morphology, marked by arrows, which is attenuated in EET-treated cells.
overexpressed in cardiomyocytes of CYP2J2 Tr mice and circulating EETs levels are similar between WT and CYP2J2 Tr mice (48), the data presented here infer a reduction in cardiac-specific CK and LDH as a result of CYP2J2 overexpression.

Evidence indicates that cardiomyocyte apoptosis plays a significant role in cardiac dysfunction in Dox-induced cardiomyopathy (39, 59). Here we observed that hearts from CYP2J2 Tr mice had reduced activation of caspase-3 and reduced TUNEL-positive cells after acute Dox administration, consistent with reports demonstrating the antiapoptotic effects of CYP2J2-derived EETs in other cell types (12, 26, 63). It is plausible that the increased level of EETs in the hearts of CYP2J2 Tr mice mediated this response or that CYP2J2 was involved in the metabolism of Dox. Although the exact antiapoptotic mechanism(s) of EETs is not known, it appears to involve p42/p44-MAPK and phosphatidylinositol 3-kinase/Akt pathways (12, 26, 63). Manifestation of acute injury results in functional decline in cardiac performance following Dox toxicity. In this regard, cardiac dysfunction was marginally evident during baseline perfusions as evidenced by decline in both inotropy and lusitropy before ischemic insult, consistent with other reports (58). However, the cardioprotective effect of CYP2J2 was prominent after ischemia-reperfusion, when the LVDP of Dox-treated CYP2J2 Tr mice did not differ significantly from that of vehicle-treated mice whereas Dox-treated WT mice had a significant decline in LVDP compared with vehicle-treated mice. These data are strongly suggestive of a protective effect of CYP2J2 in maintaining cardiac function after Dox administration.

To assess the influence of CYP2J2 on late events in Dox-mediated cardiotoxicity, we used a chronic Dox administration protocol. Our results revealed a general toxicity that occurred in both WT and CYP2J2 Tr mice and manifested as a significant decrease in body weight, most likely stemming from severe anorexia, poor oral intake, and dehydration. Interestingly, Dox-induced increases in heart weight-to-body weight ratios, β-MHC-to-α-MHC ratios, and CARP expression were greater in WT mice than in CYP2J2 Tr mice. While the functional role of CARP is not fully understood, evidence suggests its involvement in mechanical or stress responses, where it contributes to tissue repair (46). CARP appears to have a role in structural organization of sarcomeres as well as in the transcriptional machinery of cardiomyocytes, striated muscles, and vasculature (51). Increased expression of CARP has been observed in several cardiovascular injuries, such as LV dilated cardiomyopathy and pressure-overload hypertrophy (4, 46). Interestingly, opposing data are available regarding Dox-mediated regulation of CARP expression, with increased expression reported in vivo (60) and repression reported in vitro; these differences have been attributed to differences in timing and models utilized (46). Data presented here indicate that cardiac overexpression of CYP2J2 resulted in maintenance of control levels of CARP expression after chronic Dox administration, although the significance of this finding for the maintenance of overall cardiac function is unclear. Consistent with adverse effects, there was a decrease in cardiac function in WT mice that was either not apparent (LVdD and LVDs) or not as severe (LVFS) in CYP2J2 Tr mice. As such changes have been well documented in various models of DOX-induction heart failure (28, 29, 42, 44), the results found here are
indicative of a significant protection against Dox-induced cardiac dysfunction by CYP2J2 overexpression.

Evidence demonstrates that CYP enzymes are expressed in the heart, where they may participate in the metabolism of therapeutic agents, environmental toxicants, and endogenous compounds (10, 14, 48). Currently limited information is available regarding the regulation and role CYP enzymes play in the pathophysiology of heart diseases and cardiac drug metabolism. Our present results demonstrate that CYP2J2 Tr mice can limit the Dox-induced injury. Recent studies documenting that P-450-derived eicosanoids can affect cardiomyocyte function in vitro (25, 27, 32, 33, 40, 45, 62) and protect against ischemia-reoxygenation injury (19, 20, 48, 50) have led to the hypothesis that these metabolites may have important endogenous functions in the heart. However, the reduced injury observed in our CYP2J2 Tr mice may be partially contributed to the increased Dox metabolism compared with WT mice. Interesting data from H9c2 cell culture experiments show that Dox can induce CYP enzymes, notably CYP2J2 isoforms (65). The increased turnover of Dox in CYP2J2 Tr mice suggests a potential mechanism for the reduced toxicity observed in these animals. Considering that CYP enzymes can produce bioactive metabolites and metabolize foreign compounds, many important questions remain regarding the role of cardiac CYP.

Cellular excitation-contraction and mitochondrial energetics are tightly regulated in cardiac cells to meet the high energetic flux during cardiac work. Importantly, cellular stress conditions can result in distinct morphological changes that reduce mitochondrial dynamics influencing the energetic state of the cell (24). We recently demonstrated that a marked disorganization of cardiomyocyte ultrastructure following ischemia-reperfusion was significantly reduced in CYP2J2 Tr mice; moreover, EETs can minimize adverse effects of stress on mitochondrial function (30). Mitochondria are dynamic organelles that migrate through the cell and undergo continuous fusion or fission processes to maintain proper function and meet cellular demands (22). Significant decreases in fusion or increases in fission resulting from disease or toxicity can lead to punctate, fragmented mitochondria, which are thought to play a critical role in cellular dysfunction and death (22, 35, 64). Dox-induced toxicity can result in mitochondrial swelling and ultrastructural changes and alter function. Recently, Hiraumi et al. (21) demonstrated that Dox-induced mitochondrial damage can begin to occur at an early phase in cardiac injury before apoptotic changes. In the present study using an in vitro model, we demonstrate that Dox-increased mitochondrial fragmentation and membrane depolarization occur within 1 h before caspase-3 activation at 24 h in H9c2 cells. Consistent with our previous data, our present study demonstrates that EETs minimize the adverse effects of Dox on mitochondrial function. While these results are limited to our in vitro model, the implication that EETs can attenuate formation of punctate mitochondria is of particular significance. Indeed, the fact that EETs can inhibit apoptotic events and maintain mitochondrial function highlights an interesting dichotomy, where elevated EETs may provide benefit to reduction in cellular injury but can also be detrimental for cancer therapy. Further studies are needed to investigate how EETs might influence mitochondrial fission and fusion and, moreover, how this might affect in vivo cardiac function and cardioprotection and what implications there are for oncogenesis.

In summary, the results obtained here illustrate that cardiac CYP2J2 overexpression limits the progression of cardiac injury and preserves cardiac function in mice after treatment with Dox under two different administration protocols. Indeed, as assessed by various biochemical and functional end points, a more advanced progression toward development of cardiac injury was observed in WT mice compared with CYP2J2 Tr mice after treatment with Dox. These studies are the first to document a protective effect of CYP2J2 in Dox-induced cardiotoxicity and may have implications for treatment of cardiac injury.

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