Published in final edited form as:

Free Radic Biol Med. 2010 December 15; 49(12): 2068–2077. doi:10.1016/j.freeradbiomed.2010.10.686.

Cardiac-Specific Overexpression of Catalase Attenuates Paraquat-Induced Myocardial Geometric and Contractile Alteration: Role of ER Stress

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Abstract

Paraquat, a quarternary nitrogen herbicide, is a highly toxic prooxidant resulting in multi-organ failure including the heart via generation of reactive oxygen species although the underlying mechanism has not been well elucidated. This study examined the influence of cardiac-specific overexpression of catalase, an antioxidant detoxifying H₂O₂, on paraquat-induced myocardial geometric and functional alterations, with a focus on ER stress. FVB and catalase transgenic mice were administrated paraquat for 48 hrs. Myocardial geometry, contractile function, apoptosis, and ER stress were evaluated using echocardiography, edge-detection, caspase-3 activity and immunoblotting. Our results revealed that paraquat treatment significantly enlarged LV end-diastolic and systolic diameters, increased LV mass and resting myocyte length, reduced fractional shortening, cardiomyocyte peak shortening, maximal velocity of shortening/relengthening and prolonged relengthening duration in FVB group. While catalase transgene itself did not alter myocardial geometry and function, it mitigated or significantly attenuated paraquat-elicited myocardial geometric and functional changes. Paraquat promoted overt apoptosis and ER stress as evidenced by increased caspase-3 activity, apoptosis and ER stress markers including Bax, Bcl-2, GADD153, calregulin and phosphorylation of JNK, IRE1 α and eIF2 α , all were ablated by catalase transgene. Paraquat-induced cardiomyocyte dysfunction was mitigated by the ER stress inhibitor tauroursodeoxycholic acid. Moreover, the JNK inhibitor SP600125 reversed paraquat-induced ER stress as evidenced by enhanced GADD153 and IRE1 α phosphorylation. Taken together, these data revealed that catalase may rescue paraquat-induced myocardial geometric and functional alteration possibly via alleviating JNK-mediated ER stress.

Keywords

Myocardium; geometry; contractile function; prooxidant; ER stress; apoptosis

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INTRODUCTION

Oxidant balance is essential to the maintenance of physiological cardiac structure and contractile function. A plethora of physiological processes including mitochondrial respiration, inflammatory response and enzymatic reaction orchestrate the balance between reactive oxygen species (ROS) production and endogenous antioxidant defense, a rather complex defense system composed of antioxidant molecules and enzymes to counteract the damaging effects of ROS by converting more reactive species to less reactive and less damaging forms [1–3]. The antioxidant reserve often becomes inadequate under pathological conditions leading to ROS accumulation-triggered oxidative stress, myocardial geometric and functional defects [2]. Although a number of mechanisms have been postulated for oxidative stress-induced myopathic changes including interrupted mitochondrial damage, intracellular Ca^{2+} homeostasis, oxidative modification of essential cardiac contractile proteins and direct cardiac toxicity of ROS [2,4], the mechanism of action behind “oxidative cardiomyopathy” has not been clearly elucidated. Recent evidence from our lab as well as others has depicted a pivotal role of endoplasmic reticulum (ER) stress in oxidative stress-associated cellular damage and cardiac dysfunction [5,6]. Counterintuitive findings were also seen where ER stress may trigger ROS production [7] and redox deviation [8], suggesting a complex interplay between oxidative stress and ER stress. Up-to-now, three classes of ER stress transducers have been identified including inositol-requiring protein-1 (IRE1), the protein kinase RNA (PKR)-like ER kinase (PERK)-translation initiation factor eIF-2 α pathway and transcription factor-6 (ATF6) [9,10]. Similar to oxidative stress, ER stress has been shown to participate in the pathogenesis of a wide variety of diseases such as neurodegenerative disorders, diabetes, alcoholism, and ischemia reperfusion heart disease [9–11].

Paraquat is a highly toxic nitrogen herbicide prooxidant leading to multiple organ failure [12]. The bipyridyl herbicide acts as a redox cycling compound to produce reactive oxygen species (ROS) using xanthine oxidase and glutathione reductase to reduce molecular oxygen (O_2) to superoxide anion (O_2^-). O_2^- formed by paraquat radicals in turn produces hydrogen peroxide (H_2O_2) and H_2O_2 -dependent hydroxyl radical by at least two mechanisms: 1) dismutation via superoxide dismutase (SOD) and 2) reaction with Fe^{2+} from the $[\text{4Fe-4S}]^{2+}$ cluster [13–15]. Intentional or accidental ingestion of paraquat is frequently fatal due to the failure of multiple organs with an extremely high fatality rate (30 – 70%) [16]. Paraquat has been shown to overtly compromise myocardial survival and contractile function, *en route* to cardiopulmonary failure [17–19]. To better understand the mechanism of action behind paraquat-induced myocardial dysfunction and the impact of antioxidant protection, this study was designed to evaluate the effect of transgenic overexpression of antioxidant catalase, which detoxifies H_2O_2 into H_2O and O_2 , on paraquat-induced myocardial geometric and contractile alterations. In an effort to elucidate the possible cellular mechanisms involved in catalase and paraquat-induced myocardial alterations, apoptosis and ER stress were evaluated in FVB and catalase myocardium following paraquat exposure.

MATERIALS AND METHODS

Experimental animals and paraquat treatment

The experimental protocol described in this study was approved by the Animal Use and Care Committees at the University of Wyoming (Laramie, WY, USA). Cardiac-specific overexpression catalase mice were used as described [20,21]. FVB littermates were used as wild-type. A primer pair derived from the MHC promoter and rat catalase cDNA was used for identification of catalase transgene with the reverse sequence of 5'-aat atc gtg ggt gac ctc

aa-3' and the forward sequence of 5'-cag atg aag cag tgg aag ga-3'. All mice were housed in a temperature-controlled room under a 12hr/12hr-light/dark and allowed access to food and tap water *ad libitum*. Six to eight-month-old male FVB and catalase mice were administered 75 mg/kg paraquat (i.p., methyl viologen, Sigma, St. Louis, MO, USA) and were examined 48 hrs later [19].

Catalase Activity

Tissues were homogenized in 1% Triton X-100 in an assay buffer described below using a variable-speed tissue tearer (Biospec Products, Racine, WI, USA) at 20,000 rpm for 30 sec on ice. The homogenates were centrifuged at $6000 \times g$ at 4°C for 20 min. The supernatant was diluted with 1.5 volumes of the assay buffer (50 mM KH_2PO_4 /50 mM Na_2HPO_4 , pH 7.0). The enzyme activity was determined by the method described previously [21,22]. Briefly, in a cuvette 2 ml of sample was added and the reaction was initiated by adding 1 ml 30 mM H_2O_2 and the change in absorbance at 240 nm was monitored at 25°C for 1 min. A portion of the remaining sample was used for protein determination. Specific activity is expressed as $\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$ protein.

Echocardiographic assessment

Cardiac geometry and function were evaluated in anesthetized (Avertin 2.5%, 10 $\mu\text{l}/\text{g}$ body weight, i.p.) mice using a 2-D guided M-mode echocardiography (sonos 5500) equipped with a 15-6 MHz linear transducer. Diastolic and systolic left ventricular (LV) dimensions were recorded from M-mode images using method adopted by the American Society of Echocardiography [2]. Fractional shortening was calculated from end-diastolic diameter (EDD) and eye-systolic diameter (ESD) using the equation of $(\text{EDD}-\text{ESD})/\text{EDD}$. Estimated echocardiographic LV mass was calculated as $(\text{LVEDD} - \text{septal wall thickness} + \text{posterior wall thickness})^3 - \text{LVEDD}^3 \times 1.055$, where 1.055(mg/mm^3) is the density of myocardium[2].

Cell isolation

After ketamine/xylazine sedation, hearts were rapidly removed from anesthetized mice and mounted onto a temperature-controlled (37°C) Langendorff system. After perfusing with a modified Tyrode solution (Ca^{2+} free) for 2 min, the heart was digested for 16 – 20 min with 0.9 mg/ml Liberase Blendzyme 4 (Hoffmann-La Roche Inc., Indianapolis, IN, USA) in the modified Tyrode solution. The modified Tyrode solution (pH 7.4) contained the following (in mM): NaCl 135, KCl 4.0, MgCl_2 1.0, HEPES 10, NaH_2PO_4 0.33, glucose 10, butanedione monoxime 10, and the solution was gassed with 5% CO_2 -95% O_2 . The digested heart was then removed from the cannula and left ventricle was cut into small pieces in the modified Tyrode's solution. Tissue pieces were gently agitated and pellet of cells was resuspended. Extracellular Ca^{2+} was added incrementally back to 1.20 mM over a period of 30 min. Isolated cardiomyocytes were used for study within 8 hrs of isolation. Only rod-shaped cardiomyocytes with clear edges were selected for mechanical studies [19].

Cell mechanics

Mechanical properties of myocytes were assessed using an IonOptix™ soft-edge system (IonOptix, Milton, MA, USA) as previously described [19]. Myocytes were placed in a chamber mounted on the stage of an Olympus IX-70 microscope and superfused (~2 ml/min at 25°C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl_2 , 1 MgCl_2 , 10 glucose and 10 HEPES. Myocytes were field stimulated at 0.5 Hz. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR_{90}) and maximal velocities of shortening/relengthening ($\pm \text{dL}/\text{dt}$). To directly assess the role of ER stress on cardiomyocyte contractile function in response to

paraquat, cardiomyocytes were treated with paraquat (100 μ M) for 3 hrs in the presence or absence of the ER stress inhibitor tauroursodeoxycholic acid (TUDCA, 500 μ M) [5] prior to mechanical function assessment.

Caspase-3 assay

Caspase-3 activity was measured as described [23]. Briefly, 1 ml PBS was added to a flask containing myocardial homogenates prior to centrifugation at 10,000 g at 4°C for 10 min. The supernatant was discarded and homogenates were lysed in 100 μ l of ice-cold cell lysis buffer [50 mM HEPES, pH 7.4, 0.1% CHAPS, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.1% NP40]. The assay was carried out in a 96-well plate with each well containing 30 μ l of cell lysate, 70 μ l of assay buffer (50 mM HEPES, 0.1% CHAPS, 100 mM NaCl, 10 mM DTT and 1 mM EDTA) and 20 μ l of caspase-3 colorimetric substrate Ac-DEVD-pNA (Sigma). The 96-well plate was incubated at 37°C for 1 hr, during which time the caspase in the sample was allowed to cleave the chromophore p-NA from the substrate molecule. Absorbency was detected at 405 nm with caspase-3 activity being proportional to color reaction. Protein content was determined using the Bradford method. The caspase-3 activity was expressed as picomoles of pNA released per μ g of protein per minute.

Western blot analysis

Myocardial protein was prepared as previously described [23]. Samples containing equal amount of proteins were separated on 10% SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad, Hercules, CA, USA) and transferred to nitrocellulose membranes. The membranes were blocked with 5% milk in TBS-T, and were incubated overnight at 4°C with anti-caspase-12 (1:500), anti-Bax (1:500), anti-Bcl-2 (1:1,000), anti-JNK (1:1,000), anti-phosphorylated JNK (pJNK, Thr183/Tyr185, 1:1,000), anti-CHOP (GADD153, 1:1,000), anti-Bip (1:1,000), anti-Calregulin (alreticulin, 1:1,000), anti-IRE1 α (1:500), anti-phosphorylated IRE1 α (pIRE1 α , Ser724, 1:1,000), anti-eIF2 α (1:1,000), anti-phosphorylated eIF2 α (peIF2 α , Ser51, 1:500), and anti-GAPDH (1:1,000, loading control) antibodies. After immunoblotting, the film was scanned and the intensity of immunoblot bands was detected with a Bio-Rad Calibrated Densitometer. To assess the role of JNK on paraquat-induced ER stress, cardiomyocytes were pre-treated with the JNK inhibitor SP600125 (20 μ M) prior to paraquat (100 μ M) challenge for 3 hrs.

Data Analysis

Data were presented as Mean $\pm$ SEM. Statistical significance ($p < 0.05$) for each variable was estimated by a one-way analysis of variance (ANOVA) or t-test, where appropriate. A Tukey's test was used for the *post hoc* analysis when required.

RESULTS

Echocardiographic properties of FVB and catalase mice with or without paraquat treatment

Measurement of catalase activity revealed significantly elevated enzymatic activity only in hearts but not in brain, liver, kidney and skeletal muscles (gastrocnemius) (Fig. 1), validating the cardiac-specificity of the transgene overexpression. Heart rate and LV wall thickness were not significantly affected by the catalase transgene or paraquat treatment. Paraquat significantly increased EDD, ESD and calculated LV mass as well as suppressed fractional shortening in FVB mice. Catalase overexpression itself did not affect the geometric or functional parameters tested although it mitigated paraquat-induced change in EDD, ESD and fractional shortening as well as significantly attenuated the increased LV mass following paraquat exposure (Fig. 2).

Effect of catalase on paraquat-elicited cardiomyocyte contractile response

Neither paraquat nor catalase overexpression exhibited any overt effect on cell phenotype (data now shown). The resting cell length was comparable between the FVB and catalase mice. Paraquat significantly increased the resting cardiomyocyte cell length in FVB although not in catalase murine hearts. Cardiomyocytes from FVB mice receiving paraquat exposure displayed significantly depressed PS, reduced $\pm$ dL/dt and prolonged TR₉₀ associated with normal TPS. Interestingly, cardiac-specific overexpression of catalase abrogated paraquat-induced decrease in PS and $\pm$ dL/dt as well as prolongation of TR₉₀ without eliciting any mechanical effect by itself (Fig. 3).

Effects of catalase on paraquat-induced apoptosis

To examine the mechanism(s) of action behind catalase-elicited protection against the prooxidant paraquat-induced myopathic changes, myocardial apoptosis was examined in FVB and catalase hearts with or without *in vivo* paraquat challenge. Results shown in Fig. 4 indicate that caspase-3 activity was significantly elevated in paraquat-treated FVB mice. Consistent with its mechanical response, catalase transgene nullified the paraquat treatment-induced caspase activation while displaying minimal apoptotic effect itself in the absence of paraquat challenge. Consistent with the caspase-3 activity, Western blot analysis showed that paraquat significantly upregulated the expression of Bax, Bcl-2 and phosphorylated JNK without affecting expression of caspase-12 and pan JNK. Catalase itself did not affect the expression of caspase-12, Bax, Bcl-2, JNK and phosphorylated JNK as well as the pJNK-to-JNK ratio although it reconciled paraquat-induced changes in the expression of Bax, Bcl-2 and JNK phosphorylation (absolute value or normalized to pan JNK expression) (Fig. 5). These data indicated a likely beneficial effect of the catalase transgene against apoptosis and that the transgene is not innately harmful.

Western blot analysis for ER stress markers

Western blot analysis revealed that paraquat significantly upregulated the expression of the ER stress markers GADD153/CHOP, calregulin and phosphorylation of IRE1 α and eIF2 α (both absolute and normalized value to pan protein) without affecting the expression of pan IRE1 α and pan eIF2 α . Catalase transgene itself failed to affect the expression of these proteins (or their phosphorylation) although it prevented paraquat-induced increase in GADD153/CHOP, calregulin, pIRE1 α and peIF2 α (both absolute and normalized value to pan protein). Expression of Bip, pan IRE1 α and pan eIF2 α was not affected by either catalase transgene or paraquat (Fig. 6).

Effect of ER stress inhibition on paraquat-induced changes in cell mechanics

To further examine the role of ER stress in paraquat-induced cardiomyocyte contractile defects, freshly isolated cardiomyocytes from FVB mice were treated with paraquat (100 μ M) for 3 hrs in the absence or presence of the ER stress inhibitor TUDCA (500 μ M) [5]. Somewhat similar to *in vivo* findings, paraquat significantly decreased PS and $\pm$ dL/dt as well as prolonged TR₉₀ without affecting resting cell length and TPS in murine cardiomyocytes. Interestingly, TUDCA ablated paraquat-induced cardiomyocyte mechanical defects without any effects themselves (Fig. 7). These data provided direct evidence for a likely role of ER stress in paraquat-induced cardiac contractile dysfunction.

Role of JNK signaling in paraquat-induced ER stress

Our data revealed concurrent JNK phosphorylation and overt ER stress in cardiomyocytes following paraquat challenge. To examine if JNK signaling plays a role in paraquat-elicited ER stress, freshly isolated cardiomyocytes from FVB mice were pre-treated with the JNK inhibitor SP600125 (20 μ M) for 1 hr before exposure to paraquat (100 μ M, 3 hrs) followed

by assessment of ER stress. Our data depicted that SP600125 ablated paraquat-induced elevation in GADD153 expression and IRE1 α phosphorylation (both absolute and normalized value to pan protein) but not calregulin expression in cardiomyocytes. Neither SP600125 nor paraquat altered expression of IRE1 α (Fig. 8). These data favored a role of JNK signaling in paraquat-induced ER stress.

DISCUSSION

Data from our present study revealed that the antioxidant catalase mitigated the herbicide prooxidant paraquat-induced cardiac geometric alteration, myocardial contractile dysfunction, apoptosis and ER stress. In addition, paraquat-induced cardiomyocyte dysfunction was mitigated by the ER stress inhibitor tauroursodeoxycholic acid while the JNK inhibitor SP600125 reversed paraquat-induced ER stress. These findings support the previous observation that paraquat exerts devastating cardiac anomalies and compromises cardiac contractile function [17–19]. More importantly, these results favor a beneficial role of the H₂O₂ detoxifying endogenous antioxidant enzyme catalase and possibly other antioxidants in paraquat or other prooxidant(s)-elicited undesirable effects on cardiac geometry, contractile function, and apoptosis. The correlation between ER stress and myocardial geometry/function in response to catalase transgenic overexpression and paraquat challenge suggest a possible interplay between ER stress and oxidative stress, at least in our current experimental setting.

The major hallmarks of “oxidative cardiomyopathy” include cardiac hypertrophy and myocardial contractile dysfunction [2,24–26]. This is supported by our current findings of enlarged LV size (EDD and ESD although not wall thickness), LV ventricular mass, resting cardiomyocyte cell length, decreased fractional shortening and compromised cardiomyocyte shortening capacity (decreased PS/ $\pm$ dL/dt and prolonged TR₉₀) in FVB mice following paraquat challenge. Given the well-known capacity of paraquat to promote generation of superoxide anion and other ROS [12], ROS accumulation and subsequent development of oxidative stress are perceived to be the main culprit factor underneath cardiac geometric and contractile alterations following paraquat challenge. ROS is known to stimulate myocardial growth, matrix remodeling and cellular dysfunction by turning on a broad array of hypertrophic signaling and transcription factors [26]. Cardiac hypertrophy in response to free radical accumulation and oxidative stress may become maladaptive and contribute to cardiac contractile dysfunction. More importantly, our observation that catalase transgene mitigated paraquat-induced cardiac geometric and functional changes, indicating a likely role of H₂O₂ in the onset and development of these myopathic changes. H₂O₂ was previously shown to stimulate tyrosine kinase Src, GTP-binding protein Ras, PKC, mitogen-activated protein kinases (extracellular response kinase and extracellular signal-regulated kinase, and JNK [26]. High levels of H₂O₂ are associated with activation of JNK and p38 to induce apoptosis [26]. In our hand, we found stimulated JNK phosphorylation following paraquat treatment, which was obliterated by the cardiac-specific overexpression of catalase. None of the other organs tested exhibited an enhanced catalase activity although liver displayed a much higher catalase activity overall. This observation validates our transgenic model and is in line with the previous finding [21]. The elevated JNK phosphorylation following paraquat consolidated the possible role of JNK activation in paraquat-induced cardiac hypertrophy and contractile dysfunction. This notion was substantiated by the fact that JNK inhibition attenuated paraquat-induced ER stress (evidenced by GADD153 and IRE1 α phosphorylation). Collectively, a couple of scenarios may be considered for the beneficial effects of catalase under the prooxidant environment. First, catalase may elicit its cardioprotective effect against paraquat-induced myopathic changes through its antioxidant and anti-apoptotic properties. In our hands, catalase effectively lessened paraquat-induced apoptosis supported by the caspase-3 activity, Bax, Bcl-2 and JNK phosphorylation. Our

data did not favor a major role of Caspase-12 in paraquat-elicited oxidative stress. The somewhat surprisingly upregulated expression of Bcl-2 may reflect a compensatory response of this anti-apoptotic signal in response to paraquat-induced oxidative stress. Secondly and perhaps more importantly, catalase may offer myocardial protection against paraquat exposure-induced cardiac geometric and functional changes through mitigating myocardial ER stress. This is supported by our observation that catalase transgene effectively dampened paraquat-elicited ER stress markers GADD153/CHOP, calregulin and phosphorylation of IRE1 α and eIF2 α , all of which play an essential role in the maintenance of physiological cardiac survival, structure and function [9,10]. It is somewhat surprising that paraquat-induced elevation of calregulin was unaffected by JNK inhibition. Such discrepancy may be attributed to difference between *in vivo* and *in vitro* settings and possibly involvement of unique ER stress signaling pathways. Nonetheless, our observation of ER stress change appears to coincide well with changes in apoptosis, cardiac geometry and contractile function in response to paraquat challenge in FVB and catalase mice.

Our result revealed for the first time presence of myocardial ER stress following paraquat treatment and protection of ER stress inhibition against paraquat-induced cardiomyocyte dysfunction. These data favor a possible role of ER stress downstream of oxidative stress. ER is an extensive intracellular membranous network involved in Ca²⁺ storage, Ca²⁺ signaling, glycosylation and trafficking of membrane and secretory proteins. Recent findings have demonstrated the role of ER stress in the pathogenesis of a number of cardiovascular diseases such as diabetes, ischemia reperfusion-induced heart damage and alcoholic cardiomyopathy [10,11,27]. Data from our current study revealed upregulation of GADD153/CHOP and activation of IRE1 α and eIF-2 α , two of the major ER-resident transmembrane proteins sensing ER stress, in the hearts following paraquat challenge. Although ER stress has been reported to trigger ROS production [7] and redox deviation [8], earlier findings from our lab as well as others have depicted a pivotal role of ER stress in oxidative stress-induced cellular damage and cardiac dysfunction [5,6]. This is also supported by the finding that oxidative stress or oxidative stress inducers such as alcohol may enhance expression of the unfolded protein response (UPR) target protein GADD153/CHOP, the induction of which in the UPR is highly dependent upon the PERK/eIF-2 α pathway [10,11]. It was indicated that GADD153/CHOP may serve as a marker of PERK activity in the UPR [28]. Our finding that catalase reconciled paraquat-induced changes in GADD153/CHOP as well as phosphorylation of IRE1 α and eIF-2 α suggests the possible contribution of ER stress to paraquat (prooxidant)-induced cardiac anomalies. Last but not the least, our data do not favor a role of the ER chaperone Bip (or GRP78), which interacts with all three ER stress sensors, PERK/eIF-2 α , ATF6 and IRE1, in the catalase and/or paraquat-elicited myocardial response. It should be mentioned that our study may not provide evidence with regards to the precise interplay between ER stress and oxidative stress following paraquat challenge. Further study is warranted to elucidate the causal-effect relationship between the oxidative stress and ER stress in cardiac pathology.

In our study, we demonstrated that catalase significantly alleviated paraquat-induced myocardial dysfunction associated with retardation of cardiac geometric changes. This is likely due to the antagonism of catalase against the pleiotropic effect of oxidative stress following paraquat treatment. In light of the catalase-elicited protection against paraquat-induced cardiac remodeling, contractile dysfunction, apoptosis and ER stress, our data favor the therapeutic value of antioxidants against aberrant myocardial remodeling and function under oxidative stress. It is imperative that we understand the cellular changes under oxidative stress in order to maximize the use of antioxidant treatment, for example, in targeting a specific intracellular organelle or stress signaling molecule [10].

Acknowledgments

This work was presented in part in the abstract form during Experimental Biology 2010 in Anaheim, CA. The founder mice of catalase transgenic line were kindly provided by Professor Paul N. Epstein from University of Louisville (Louisville, KY, USA). This work was supported in part by NIH 1R01 AA013412 and 5P20 RR016474 (JR).

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Fig. 1. Catalase activity in heart, brain, liver, kidney and skeletal muscle (gastrocnemius) from catalase (CAT) transgenic mice compared with their non-transgenic FVB controls. Mean $\pm$ SEM, $n = 7-8$ mice per group, * $p < 0.05$ vs. FVB group.

Fig. 2.

Echocardiographic properties of FVB and catalase transgenic (CAT) mice treated with or without paraquat (75 mg/kg, i.p.) or vehicle for 48 hrs. A: Heart rate (beat per minute); B: LV wall thickness; C: LV end diastolic diameter (EDD); D: LV end systolic diameter (ESD); E: Fractional shortening (%); and F: calculated LV mass. Mean $\pm$ SEM, $n = 6-8$ mice per group, * $p < 0.05$ vs. FVB group, # $p < 0.05$ vs. FVB-paraquat group.

Fig. 3. Cardiomyocyte contractile properties in FVB and catalase (CAT) transgenic mice treated with or without paraquat (75 mg/kg, i.p.) or vehicle for 48 hrs. A: Resting cell length; B: Peak shortening (normalized to cell length); C: Maximal velocity of shortening ($+dL/dt$); D: Maximal velocity of relengthening ($-dL/dt$); E: Time-to-peak shortening (TPS); and F: Time-to-90% relengthening (TR_{90}). Mean $\pm$ SEM, $n = 72 - 87$ cells per group; * $p < 0.05$ vs. FVB group, # $p < 0.05$ vs. FVB- paraquat group.

Fig. 4. Caspase-3 activity in myocardium from FVB and catalase (CAT) transgenic mice treated with or without paraquat (75 mg/kg, i.p.) or vehicle for 48 hrs. Mean $\pm$ SEM, n = 4 per group; * p < 0.05 vs. FVB group, # p < 0.05 vs. FVB- paraquat group.

Fig. 5. Western blot analysis of apoptotic markers in myocardium from FVB and catalase (CAT) transgenic mice treated with or without paraquat (75 mg/kg, i.p.) or vehicle for 48 hrs. A: Representative gel blots depicting expression of caspase-12, Bax, Bcl-2, JNK, phosphorylated JNK (pJNK) and GAPDH (used as loading control); B: Caspase-12; C: Bax; D: Bcl-2; E: JNK; F: Phosphorylated JNK (pJNK); and G: pJNK-to-JNK ratio. Mean $\pm$ SEM, n = 4–5, * p < 0.05 vs. FVB group, # p < 0.05 vs. FVB- paraquat group.

Fig. 6. Western blot analysis of ER stress markers in myocardium from FVB and catalase (CAT) transgenic mice treated with or without paraquat (75 mg/kg, i.p.) or vehicle for 48 hrs. A: Representative gel blots depicting expression of GADD153(CHOP), Bip, calregulin, pan and phosphorylated IRE1α and eIF2α as well as GAPDH (loading control); B: GADD153(CHOP); C: Bip; D: Calregulin; E: pan IRE1α; F: phosphorylated IRE1α (pIRE1α); G: pIRE1α-to- IRE1α ratio; H: pan eIF2α; I: phosphorylated eIF2α (pEIF2α); and J: pEIF2α-to-eIF2α ratio. Mean ± SEM, n = 4–5, * p < 0.05 vs. FVB group, # p < 0.05 vs. FVB- paraquat group.

Fig. 7.

Effect of the ER stress inhibitor TUDCA on paraquat-induced cardiomyocyte contractile defects. Freshly isolated cardiomyocytes from normal FVB mice were incubated with paraquat (100 μM) in the presence or absence of TUDCA (500 μM) for 3 hrs. A: Resting cell length; B: Peak shortening (normalized to resting cell length); C: Maximal velocity of shortening (+ dL/dt); D: Maximal velocity of relengthening (- dL/dt); E: Time-to-peak shortening (TPS) and F: Time-to-90% relengthening (TR₉₀). Mean ± SEM, n = 33 cells from 4 mice per group, * p < 0.05 vs. control group, # p < 0.05 vs. paraquat group.

Fig. 8. Effect of the JNK inhibitor SP600125 on paraquat-induced ER stress markers. Freshly isolated cardiomyocytes from normal FVB mice were pretreated with SP600125 (20 μ M) for 1 hr before incubation with paraquat (100 μ M) for 3 hrs. A: Representative gel blots depicting expression of GADD153 (CHOP), calregulin, pan and phosphorylated IRE1 α (GAPDH was used as the loading control); B: GADD153 (CHOP); C: Calregulin; D: pan IRE1 α ; E: phosphorylated IRE1 α (pIRE1 α); and F: pIRE1 α -to-IRE1 α ratio. Mean $\pm$ SEM, n = 3–5, * p < 0.05 vs. control group, # p < 0.05 vs. paraquat group.