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Mitigation of doxorubicin-induced cardiotoxicity by dichloroacetate: potential roles of restoration of PGC-1α/SIRT3 signaling and suppression of oxidative stress and apoptosis

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Abstract. – OBJECTIVE: Doxorubicin (DOX) is an effective chemotherapeutic agent used in the treatment of various neoplasms. Nevertheless, its therapeutic efficacy is hampered by life-threatening heart failure. Therefore, the current study was undertaken to investigate whether dichloroacetate (DCA), a metabolic and mitochondrial modulator, when administered at a therapeutically feasible dose could potentially reverse acute DOX cardiotoxicity. Furthermore, the possible underlying mechanisms of cardioprotection were also assessed.

MATERIALS AND METHODS: Different techniques were performed to assess cardiac injury like echocardiography, histopathology, transmission electron microscope, biomarkers of cardiac injury, and oxidative stress markers. Further, the expression levels of mRNA and protein were quantified by PCR and immunohistochemistry, respectively.

RESULTS: Echocardiography showed that mice that received DOX/DCA combination were protected against heart failure. Additionally, histopathology and transmission electron microscopy revealed structural damage alleviation by DOX/DCA combination, which was confirmed biochemically via significant suppression of elevated CK-MB and AST levels. Mechanistically, DOX dysregulated the expression of PGC-1α and SIRT-3 genes which are key to normal mitochondrial functioning. Of note, co-treatment with DCA effectively restored PGC-1α/SIRT-3 signaling and normalized the mitochondrial DNA index. Moreover, events downstream of DOX-triggered mitochondrial dysfunction such as oxidative stress and p53-dependent apoptosis were all abrogated by combination with DCA.

CONCLUSIONS: The present study is the first to provide in vivo evidence that DCA is effective in protecting against acute DOX cardiotoxicity. Additionally, the study highlights the potential of administering metabolic modulators to safeguard against DOX cardiotoxicity.

Key Words:
Dichloroacetate, Doxorubicin, Cardiotoxicity, Mitochondrial bioenergetics failure, PGC-1α, Apoptosis.

Introduction

Doxorubicin (DOX) is one of the most commonly used chemotherapeutic agents in the treatment of a broad spectrum of solid tumors and hematological malignancies, however, its clinical utility is frequently complicated by dose-dependent cardiotoxicity[1]. Cardiotoxicity usually manifests itself in the form of dilated cardiomyopathy and heart failure which is associated with disabling morbidity and higher mortality[2].

Doxorubicin-induced bioenergetics failure through myocardial metabolic remodeling or alteration in mitochondrial bioenergetics has been suggested as an important mechanism mediating cardiotoxicity[3]. The metabolic derangement is an important contributor to the development of DOX cardiotoxicity[4]. Both peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) and pyruvate dehydrogenase kinase 4 (PDK-4) genes played important functions in metabolism through regulating mitochondrial biogenesis and mitochondrial glucose oxidation, respectively. DOX could facilitate metabolic rewiring by suppressing gene expression of PGC-1α and upregulating PDK-4 expressions within cardiomyocytes. This shift in metabolism played a role in the progression of DOX-induced cardiotoxicity[5,6]. Furthermore, the crosstalk between several dysregulated pathways, such as mitochondrial dysfunction, oxidative stress, and the apoptotic pathway is probably the cause of DOX-induced

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cardiac damage rather than the dysregulation of a single pathway[3,7].

Dichloroacetate (DCA) is a chemical compound that has been used clinically for the treatment of lactic acidosis and genetic mitochondrial disease[8,9]. DCA suppresses glycolysis and shifts glucose metabolism toward mitochondrial oxidation by acting as a metabolic modulator that could explain the anti-neoplastic activity observed in various cancer types when administered either alone or in combination with other treatment modalities[10,11]. Interestingly, through modulation of myocardial metabolism, DCA has the potential to improve cardiac function and protect against heart failure progression[12]. Several studies[13,14] have shown that DCA is associated with improving cardiac function in a model of myocardial ischemia and right ventricular failure. Therefore, the current study was undertaken to evaluate the potential of DCA to improve cardiac function and protect against acute doxorubicin cardiotoxicity with the elucidation of the possible underlying mechanisms.

Materials and Methods

Drugs and Chemicals

Doxorubicin (Adricin®) was purchased from Hikma Pharmaceuticals (Cairo, Egypt). Sodium Dichloroacetate (DCA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). DCA solution was freshly prepared by dissolving in sterile normal saline immediately before mice injections.

Animals

Adult female albino mice (22-26 gram) were obtained from National Research Center, Giza, Egypt. Mice had free access to water and diet. Mice were acclimatized for 10 days before the start of the experiment. Animal procedures and animal care were conducted following the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Research Ethics Committee of the Faculty of Pharmacy, Tanta University, Egypt.

Experimental Design

Mice were randomly allocated into 4 groups (15 mice in each group) as follows:

1. Control group (saline).
2. Doxorubicin group (a single bolus dose of 20 mg/kg).
3. DCA group (200 mg/kg daily).
4. Combination of doxorubicin (a single bolus dose of 20 mg/kg) and DCA (200 mg/kg daily) group.

All treatments were administered by intraperitoneal injections. The dose of DOX-induced acute cardiotoxicity was previously reported[15]. The dose of DCA was selected based on a dose-finding preliminary study aiming at exploring the maximum tolerable dose that achieved optimal cardioprotection. Furthermore, previous studies[16,17] have also confirmed the tolerability of this dose. In addition, this dose in mice corresponds to 16.3 mg/kg/day in human[18], which is less than the therapeutic dose of DCA administered in various clinical trials, ranging from 25 to 50 mg/kg/day[8,9,18]. Daily DCA administration was initiated 4 days preceding doxorubicin administration. On the fifth day, DOX was administered 3 hours following DCA administration. The study was continued for additional 3 days following DOX administration to evaluate cardiotoxicity.

After drug administration, echocardiography was performed first on alive mice. After that, mice were weighed and euthanized to collect blood samples and heart tissue. Heart tissues were dissected and immediately weighted for calculation of heart weight to bodyweight ratio. Next, left ventricles were dissected for further experimental analysis such as RNA and DNA extraction, histopathological examination, immunohistochemistry, and oxidative stress markers to assess cardiotoxicity. RNA extraction was performed 24hr and 72hr to evaluate changes in gene expression as previously described in this model[6]. Serum samples were collected and stored at -80°C.

Echocardiography

Left ventricular M-mode echocardiography was performed on awake mice using a DC60 equipped with a 10-MHz transducer (Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, China) as previously described[19]. M-mode measurements, such as left ventricular fraction shortening (LVFS), left ventricular ejection fraction (LVEF), LV interventricular septal thicknesses at diastole (IVSd) and posterior wall thicknesses at diastole (LVPWd) were detected to evaluate cardiac function[19].

Histopathological Examinations

Specimen of the left ventricle of different groups were fixed in 10% neutral buffered for-

malin and processed as paraffin-embedded tissue sections of 5 μm thick sections. These sections were stained by hematoxylin and eosin (H&E), as previously reported[20]. Sections were examined under a light microscope, and images were obtained using a digital camera (Canon Inc., Tokyo, Japan).

Transmission Electron Microscope

Left ventricular tissues of different groups were processed to prepare ultrathin sections of tissue for TEM analysis as previously described[21]. Images were examined and photographed by JEOL JEM-2100 (JEOL Ltd., Tokyo, Japan).

Assessment of Cardiotoxicity Biomarkers

Commercially available kits for serum levels of creatine kinase-MB (Spectrum diagnostics, Cairo, Egypt) and aspartate aminotransferase (AST) (Biodiagnostic, Giza, Egypt) were used according to the manufacturer's instructions.

Measurement of Lipid Peroxidation

Lipid peroxidation was assessed by measuring the level of MDA in the left ventricular tissue homogenate using commercial kits (Biodiagnostic, Giza, Egypt).

Measurement of SOD Activity

According to the manufacturer's instructions, the superoxide dismutase enzyme activity in the left ventricular homogenate was measured using a commercially available kit (Biodiagnostic, Giza, Egypt).

Analysis of gene expression via Semiquantitative RT-PCR

RNA extraction and conventional RT-PCR were performed in the left ventricular tissue samples harvested 24hr and 72hr after DOX dose as previously described[22]. These time points were evaluated based on previous study[6]. The relative expression levels of Bax, p21, Bcl-2, PGC-1α, SIRT-3 were evaluated using primer sequences previously reported[23-27]. Band intensities were quantified relative to 18S rRNA as the loading control using the Image J program (http://imagej.nih.gov/ij/).

Immunohistochemical Examination

The immunohistochemical staining procedures for caspase-3 and p53 were performed using anti-caspase 3 active antibodies (1/1000, R&D Systems Inc., Minneapolis, MN, USA) and recombinant anti-P53 (1/1000, Abcam, Cambridge, MA, USA). The staining intensity was assessed and presented as a percentage of positive expression in a total of 1000 cells per 8 high-power fields[28].

Mitochondrial DNA Index

According to the manufacturer's instruction, the DNA of left ventricular tissue was separated using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The mitochondrial index was then calculated using previously described primer sequences for the 16S rRNA gene of the mitochondrial DNA (mtDNA) and the HK gene of nuclear DNA (nDNA)[29]. The relative amount of mtDNA and nDNA expression was compared and normalized to control.

Statistical Analysis

Data are presented as mean ± S.D. Multiple comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer as a post-hoc test using GraphPad Prism version 9.1.0 for Windows (GraphPad Software, La Jolla, CA, USA). Results were considered statistically significant at $p < 0.05$.

Results

Doxorubicin-Induced Cardiac Dysfunction Was Rescued by Dichloroacetate Treatment

We investigated the potential of DCA to protect against acute DOX cardiotoxicity. At first, left ventricular M-mode echocardiography of different groups was recorded to calculate different parameters indicative of left ventricular function (Table I). Echocardiography findings revealed that the DCA-treated group had similar LEFS and LVEF values to those of the control group. On the other hand, DOX treatment was associated with significant deterioration of LEFS and LVEF to 0.55 and 0.57-fold of their normal values, respectively. Significantly, DCA co-treatment prevented LV function decline by restoring LVFS and LVEF close to typical values. LVPWd and IVSd were significantly reduced by 25.3% and 39%, respectively, in the DOX group compared to the control group, while DCA co-treatment normalized these changes (Table I). Collectively, echocardiography findings highlighted the in vivo potential of DCA to prevent the deterioration of cardiac function upon DOX administration.

Table I. Echocardiographic parameters of mice treated with doxorubicin and/or DCA.

Groups	LVFS (%)	LVEF (%)	LVPWd (mm)	IVSd (mm)
Control	40.9% ± 2.9%	76.4% ± 5.4%	0.83 ± 0.058	0.87 ± 0.062
DOX	22.7% ± 4.1%*	43.9% ± 4.5%*	0.62 ± 0.076*	0.53 ± 0.058*
DCA	41% ± 1.2%#	76% ± 2.2%#	0.8 ± 0.05#	0.88 ± 0.06#
DOX/DCA	38% ± 1.7%#	69.8% ± 5.7%#	0.75 ± 0.054#	0.083 ± 0.064#

Data are represented here as mean ± S.D (n=3). *Denotes statistically significant from control, while #Denotes statistically significant from DOX.

Table II. Effect of different treatment groups on body weight, heart weight and heart to body weight ratio.

Groups	Initial weight (gm)	Final weight (gm)	Heart weight (mg)	Heart/body wt. (mg/gm.)
Control	24.2 ± 1.4	24.7 ± 1.3	117.4 ± 6.5	4.76 ± 0.24
DOX	23.8 ± 0.9	19.9 ± 1.4*	90.6 ± 12.8*	4.54 ± 0.5
DCA	24.9 ± 1.1	23.6 ± 2#	114.4 ± 7.9#	4.87 ± 0.33
DOX/DCA	23.3 ± 1.2	20.1 ± 2*	102.8 ± 10.8#	5.12 ± 0.39#

Data are represented here as mean ± S.D (n=15). *Denotes statistically significant from control, while #Denotes statistically significant from DOX.

Dichloroacetate Co-Treatment Reversed DOX-Induced Cardiac Atrophy and Improved Biomarkers of Myocardial Injury

Next, we tested whether preservation of myocardial function with DOX/DCA combination treatment was associated with protecting from cardiac atrophy and reducing cardiac injury biomarkers. Table II showed 20% and 23% declines in the total body weight and heart weight, respectively, following DOX treatment. Despite that DOX/DCA group showed a similar decline in body weight compared to DOX, partial recovery from cardiac atrophy was evident. Consequently, that was translated to a significant elevation of the combination arm's heart weight to body weight ratio compared to the DOX group.

Further, analysis of CK-MB and AST serum levels as biomarkers for cardiac injury post-DOX administration showed that DCA monotherapy did not show significant alterations in cardiac injury biomarkers. However, DOX treatment caused significant increments of serum levels of CK-MB and AST by 61% and 73%, respectively compared to control as in Table III. The levels of both biomarkers in DOX/DCA treated mice were effectively reduced to 21% for CK-MB and 37% for AST more than their control levels, indicating a significant amelioration of cardiac injury.

Effect of Dichloroacetate on the Histopathological Alteration of Cardiomyocytes of the Left Ventricular Tissue

For structural damage assessment, histopathological examination *via* H&E of the left ventricular tissues was carried out as in Figure 1A and showed normal myocardial fibers architecture with cigar-shaped nuclei in both control and DCA monotherapy groups. On the contrary, DOX-treated mice had myocardial degeneration in the form of myocardial fiber loss with sarcoplasmic eosinophilia and interstitial infiltration of mononuclear cells, primarily macrophages and lymphocytes. Co-treatment of mice with DCA showed marked restoration of typical myocardial structure with a noticeable decline of myocardial degeneration.

To delve deeply into subcellular damage induced by DOX treatment, we performed a TEM

Table III. Effect of different treatment groups on CK-MB and AST as cardiotoxicity biomarkers.

Groups	CK-MB (IU/L)	AST (IU/L)
Control	130.4 ± 10.7	95.4 ± 12.3
DOX	210.5 ± 14.3*	165.3 ± 9.9*
DCA	127.5 ± 15.9#	110.9 ± 9.8#
DOX/DCA	157.7 ± 10.6#	131.0 ± 12.9#*

Data are represented here as mean ± S.D (n=4). *Denotes statistically significant from control, while #Denotes statistically significant from DOX.

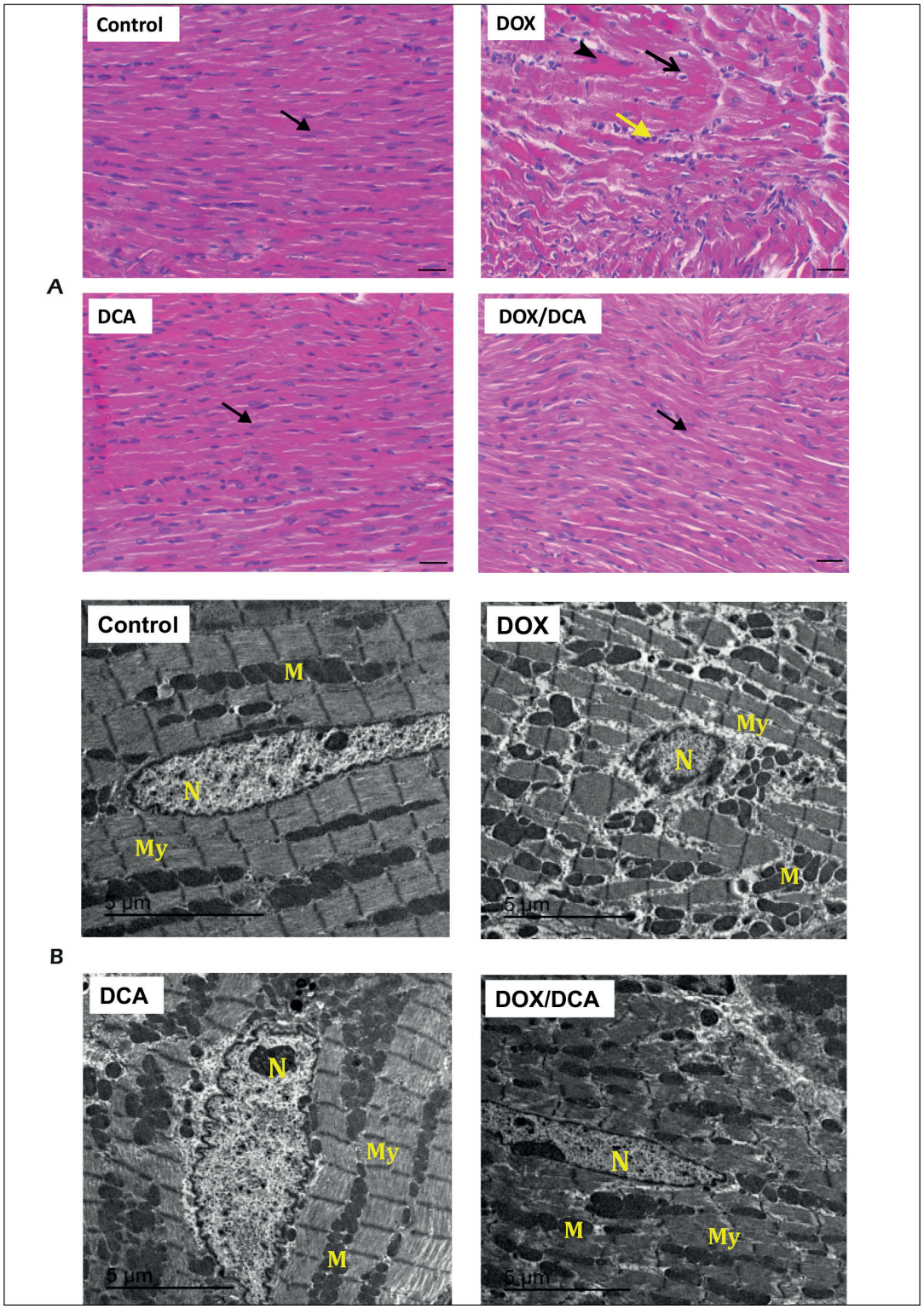

Figure 1. Effect of Dichloroacetate on histopathological alteration in mice subjected to acute doxorubicin intoxication. **A,** Photomicrograph of left ventricle sections stained by H&E of different treatment groups: control, doxorubicin, DCA, Doxorubicin/DCA combination. (*Black arrow:* myocardial fiber, *arrowhead:* sarcoplasmic eosinophilia, *yellow arrow:* inflammatory cell infiltration). Scale bar= 100 µm (200x). **B,** Representative transmission electron micrographs (TEM) of ultrathin sections of the left ventricular heart muscle of different treatment groups: control, doxorubicin, DCA, Doxorubicin/ dichloroacetate combination. (N: nucleus, M: mitochondria, My: Myocardial fibrils), Scale bar= 5 µm.

examination of the cardiomyocyte of left ventricular tissue (Figure 1B). Analysis of the control group showed a regular ultrastructure pattern characterized by a large rod-shaped nucleus with normal chromatin condensation and highly organized myofibrils with a regular striation pattern separated by multiple mitochondria. Further, DCA treatment had no adverse effect on the ultrastructure pattern of cardiomyocytes. On the other hand, DOX treatment severely impacted the normal ultrastructure. The nucleus showed dramatic alterations, such as significant nuclear shrinkage with the disappearance of a normal rod-shaped nucleus, irregular folding of the nuclear membrane, and abnormal chromatin condensation characteristic for apoptosis.

Moreover, loss of myofibril striation pattern and organization with irregular mitochondria that vary in size and shape were additional structural abnormalities evident in the DOX group. Of note, co-treatment with DCA partially restored the deleterious impact of DOX intoxication (Figure 1B). These results collectively indicated that DCA could antagonize DOX-induced structural alteration in the scope of overall myocardial damage and subcellular injury within myocardial cells.

Dichloroacetate Rescued Mitochondrial Dysfunction Through Normalization of the PGC-1α/SIRT3 Pathway

To gain mechanistic insights into how DCA could confer protection against DOX-induced cardiotoxicity, we investigated whether DCA may have rescued mitochondrial dysfunction by altering the expression of genes responsible for mitochondrial biogenesis and function, such as PGC-1α 72hr post- DOX administration as previously reported[6]. Results showed that DOX suppressed the mRNA level of PGC-1α by 42% compared to control. In contrast, the expression levels of PGC-1α mRNA in DCA monotherapy and combination treatment were increased by about 63% and 52%, respectively, relative to the control group (Figure 2A). Furthermore, we examined the expression of SIRT-3 as a PGC-1α downstream target, which could potentially modulate mitochondrial metabolism[30]. Results showed that DOX decreased SIRT-3 gene expression by 25% relative to control, whereas DCA/DOX combination upregulated SIRT-3 mRNA level by about 23% compared to the control level (Figure 2A).

Next, to investigate whether the effects of DCA co-treatment on gene expression could improve mitochondrial mass and function, we measured the mtDNA index (mtDNA/nDNA ratio) as an indicator. As shown in Figure 2B, only the DOX-treated group reduced mtDNA index compared to control, while DCA monotherapy or combination groups were similar to control.

Dichloroacetate Amelioration of DOX-Induced Oxidative Stress Involved the Restoration of SOD Activity

Given the role of both PGC-1α and SIRT-3 in increasing activity of mitochondrial SOD-2 enzyme[31], we investigated the impact of DOX and/or DCA treatments on the functional consequences of the downregulation of PGC-1α and SIRT-3 expression by DOX *via* measuring left ventricular SOD activity. Results showed that DOX reduced SOD activity by 25%. Interestingly, SOD activity was restored back to control levels by DOX/DCA combination (Figure 2C). Additionally, we evaluated the relative contribution of the restored SOD activity by DOX/DCA treatment. To this end, we measured the left ventricular MDA level as a marker of oxidative stress. Results showed that DOX monotherapy caused about a 53% increase in MDA levels. Of note, DOX/DCA combination could almost completely abrogate MDA elevation (Figure 2D). Collectively, these findings provided convincing pieces of evidence that DCA could antagonize DOX-induced mitochondrial dysfunction at least in part by the restoration of PGC-1α/SIRT3 signaling and the ensuing oxidative stress.

Dichloroacetate Suppressed p53 Protein Expression and Transcriptional Targets Induced by Doxorubicin Treatment

Based on previous findings, DCA could interfere with two upstream p53 regulators, mitochondrial dysfunction and ROS generation, and therefore have the potential to suppress p53 activity that is considered a major contributor to myocardial apoptosis and cardiac dysfunction post-DOX administration[32]. We tested whether DCA could confer protection against the activation of p53 signaling and the subsequent myocardial apoptosis.

Firstly, we analyzed p53 protein expression by immunohistochemistry across different treatment groups. Results revealed that both control and DCA monotherapy showed weak cytoplasmic expression of p53 within myocardial cells. Conversely, DOX treatment caused a marked 6.5 folds elevation in the cytoplasmic and nuclear p53 expression within myocardial cells compared to the control group (Figure 3A and 3B). Of note, DOX/DCA combination markedly suppressed

p53 expression to just 2.1-folds the control level (Figure 3A and 3B).

Secondly, we analyzed the expression of several p53 transcriptional targets by RT-PCR 24 hours post-DOX administration. We selected this time point as previous studies showed that elevation in the expression of p53 downstream genes is an early event that becomes most evident at this time point[6,33]. Results obtained by RT-PCR showed that the mRNA levels of p21 and Bax were elevated 3.07 and 1.49 folds, respectively, by DOX monotherapy, while that of Bcl-2 was downregulated by about 76% (Figure 3C). Notably, the effects of the DOX/DCA combination on the mRNA levels of Bax and Bcl-2 were similar to those of DCA monotherapy, while p21 expression was almost normalized (Figure 3C).

Dichloroacetate Inhibited Doxorubicin-Induced Caspase-3 Immunostaining

Finally, we measured the expression level of caspase-3 by immunohistochemistry to confirm the ability of DCA to abrogate DOX-induced apoptosis. Results showed that DCA monotherapy did not alter caspase-3 immunostaining compared to control (Figure 4A), whereas DOX caused an increase in the expression of caspase-3 by about 6.4-folds (Figure 4A, B). Of note, caspase-3 immunopositive cells dropped to approximately 1.8 folds by DOX/DCA combination relative to control (Figure 4A, B). Collectively, these findings further confirmed the ability of DCA to suppress p53 activity and apoptosis following DOX administration that may explain in part the mechanism of DCA to ameliorate acute DOX cardiotoxicity.

Discussion

The mechanisms of DOX-induced cardiotoxicity are multifactorial, with a complex interplay between different pathways that ultimately contribute to cardiac dysfunction[34]. In the current study, we have provided pieces of evidence showing that DCA

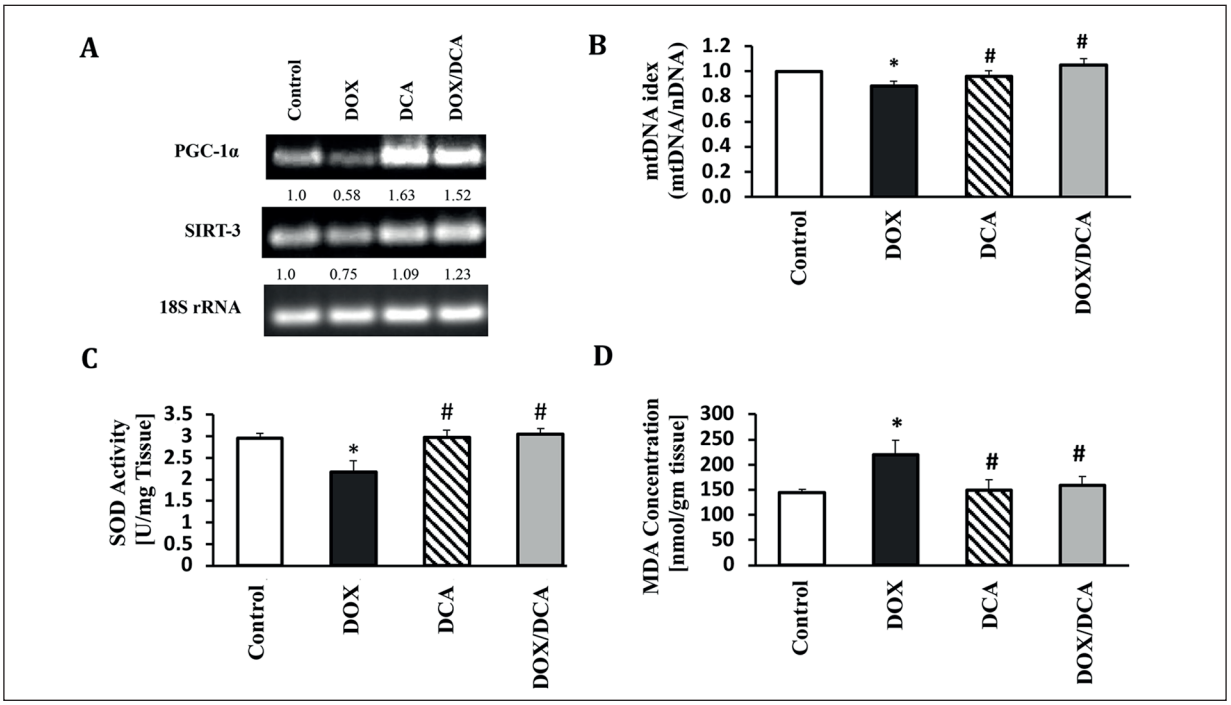

Figure 2. Restoration of doxorubicin-induced mitochondrial dysfunction and amelioration of oxidative stress by dichloroacetate co-treatment. **A,** Representative agarose gel electrophoresis of the effect of different treatment groups on left ventricular mRNA expression of PGC-1 and SIRT-3 that was performed at 72hr following doxorubicin dose with 18S rRNA as the loading control. Data are presented underneath each gene's panel reflect gene expression fold change relative to control. **B,** mtDNA index of different groups. Data are presented as mean ± SD (n=3). **C,** SOD activity measured as [U/mg wet tissue]. **D,** MDA concentration [nmol/gm. wet heart tissue]. Data are presented as mean ± SD (n=4). * or # denotes statistically significant difference from control or doxorubicin group respectively at $p < 0.05$.

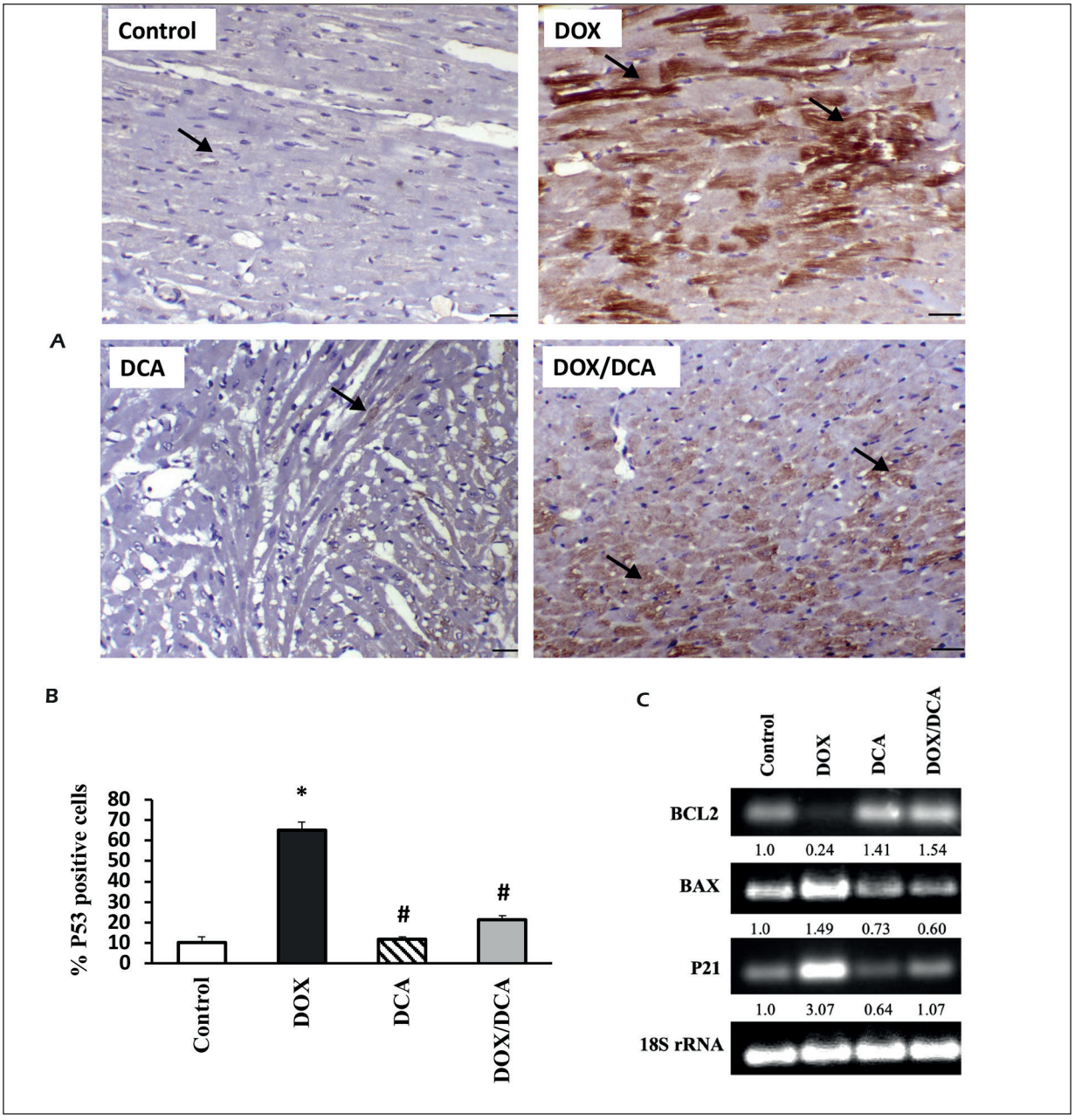

Figure 3. Dichloroacetate suppressed p53 activation in mice subjected to doxorubicin intoxication. **A,** Representative photomicrograph for the effect of different treatments on P53 protein expression by immunostaining within myocardial cells of the left ventricle of control, doxorubicin (DOX), DCA, and DOX/DCA combination groups (***arrows:*** positively stained myocardial cell), bar=100 µm (200x). **B,** Labeling index of P53 protein expressed as the percent of positive cells / 1000 cells in high power fields. Data are presented as mean ± SD (n=8). * or # denotes statistically significant difference from control or doxorubicin group respectively at $p < 0.05$. **C,** Representative agarose gel electrophoresis of the effect of different treatment groups on left ventricular mRNA expression of p53 downstream target genes: p21, Bax, and Bcl-2 that was performed 24hr following doxorubicin dose with 18S rRNA as the loading control.

could interfere with DOX acute cardiotoxicity *via* restoration of PGC-1α/SIRT3 signaling and abrogation of the ensuing oxidative stress and apoptosis.

PGC-1 α is considered the master regulator of mitochondrial biogenesis by co-activating many transcription factors responsible for regulating mitochondrial biogenesis and improving mitochondrial oxidative phosphorylation[35]. Furthermore, DOX treatment has previously been reported to be associated with a reduction of PGC-1α expression[6,36],

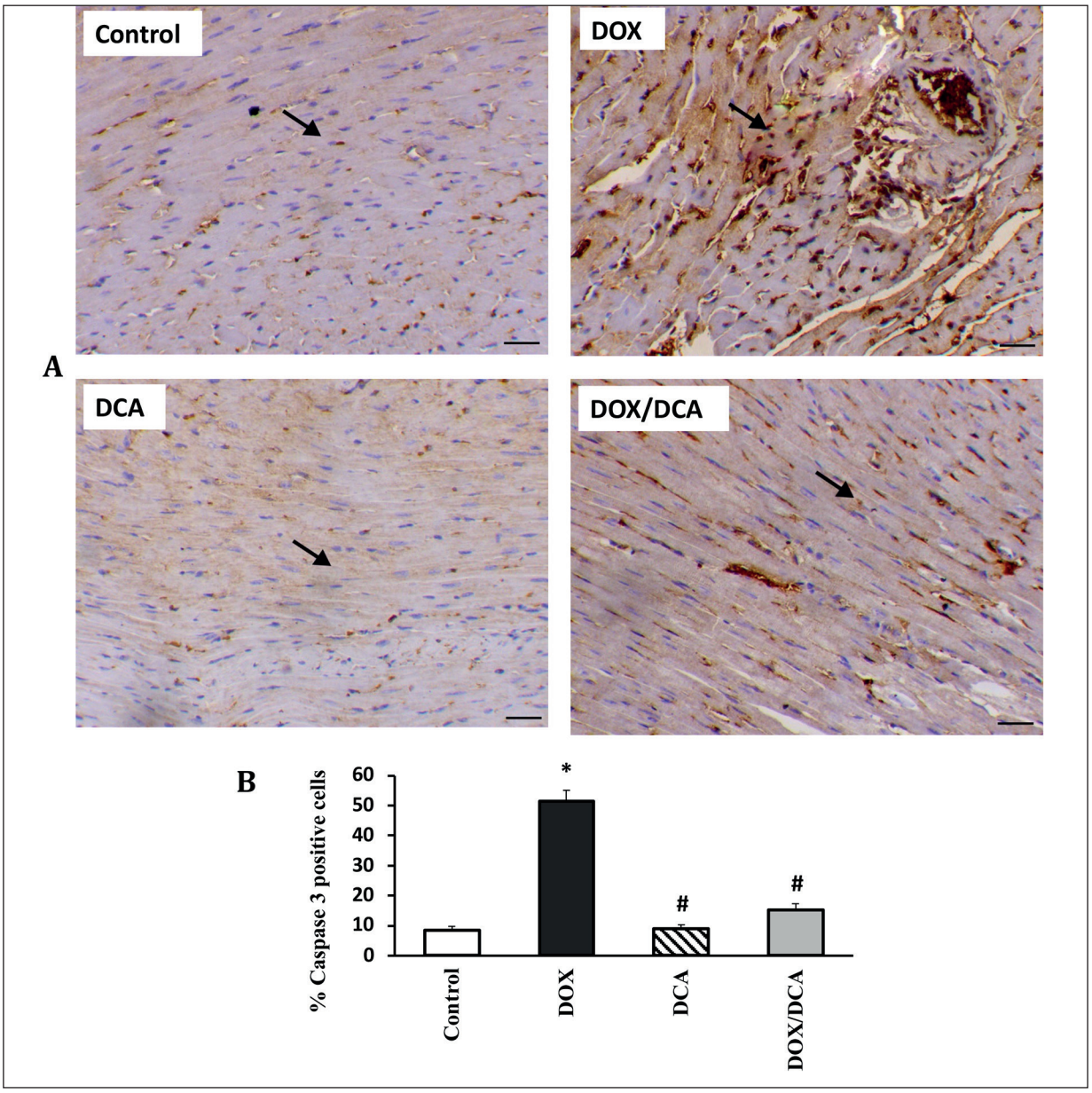

Figure 4. Dichloroacetate caused a decline in markers of doxorubicin-induced apoptosis. **A,** Representative photomicrograph for the effect of different treatments on caspase-3 expression by immunostaining within myocardial cells of the left ventricle of control, doxorubicin (DOX), DCA, and DOX/DCA combination groups (***arrows:*** positively stained myocardial cell), bar=100 µm (200x). **B,** Labeling index of caspase-3 expressed as the percent of positive cells/1000 cells in high power fields. Data are presented as mean ± SD (n=8). * or # denotes statistically significant difference from control or doxorubicin group respectively at $p < 0.05$.

and a growing body of evidence has shown that increased PGC-1α expression is associated with protection against DOX-induced cardiomyopathy[37].

Besides its metabolic role, PGC-1α has been linked to the modulation of other regulatory pathways either directly or indirectly. For example, PGC-1α upregulation is associated with reducing apoptosis in a model of cardiac hypertrophy[38] and DOX-induced cardiomyopathy[39], which confirms that both apoptotic machinery and mitochondrial activity are linked together. Furthermore, PGC-1α expression has been associated with a reduction in oxidative stress[30]. The relationship between ROS generation and mitochondria is bidirectional as ROS generation could also cause mitochondrial dysfunction, which leads to bioenergetics failure[3].

SIRT-3 is a member of class III histone deacetylases that exerts its activity mainly within the mi-

tochondria[30]. SIRT-3 is associated with improving mitochondrial metabolism through deacetylation and activation of mitochondrial proteins with metabolic activity such as the Krebs cycle and oxidative phosphorylation component of the mitochondria[40]. Besides its role in improving myocardial metabolism and enhancing myocardial function, its activity in protection against DOX-induced cardiotoxicity has been described both *in vitro*[41] and *in vivo*[42]. SIRT-3 overexpression was associated with increased SOD-2 activity and attenuated ROS generation by DOX *in vitro*[41].

The inhibitory effect of DCA on myocardial apoptosis in our study is consistent with previous studies reporting the ability of DCA to protect against cardiomyocyte cell death both *in vitro*[43] and in a rat model of right ventricular failure[14]. Both mitochondrial dysfunction and oxidative stress could contribute to DOX-induced activation of the p53 pathway and subsequent myocardial apoptosis, confirming that all these pathways are interlinked together[32]. Mechanistically, p53 activation following DOX modulated the expression of Bcl-2 family proteins by favoring the expression of the pro-apoptotic Bax while downregulating anti-apoptotic proteins like Bcl-2[44]. Of note, DOX has previously been shown to upregulate myocardial p53-p21 signaling *in vivo*[45]. Interestingly, reduction of p21 expression was associated with attenuation of DOX cardiotoxicity[46].

Numerous studies have shown the anti-tumor activity of DCA against many cancers either alone or in combination with other chemotherapeutics[11]. Additionally, DCA has been used successfully in conjunction with DOX to augment its cytotoxicity against cancer[47,48]. Given that cancers depend mainly on aerobic glycolysis rather than glucose oxidation as the mean of energy production and supplying cells with essential macromolecules for proliferation, DCA could reverse this glycolytic phenotype and exerts selective anti-tumor activity[11]. Further, anti-tumor efficacy and tolerability of DOX-DCA conjugate nanoformulation are observed in a murine melanoma model[49].

DCA is a cheap generic compound that has already been used in humans for more than 40 years[50]. In addition, the dose of DCA administered in this study has a translational potential as it corresponded to 16.3 mg/kg human dose[18] that is lower than the dose of DCA previously administered in humans for the treatment of lactic acidosis at a dose of 50 mg/kg[9]. These merits highlight the potential clinical benefits of DCA in combination with DOX.

Expanding evidence underscores the role of metabolic dysregulation as a major regulator of DOX cardiotoxicity that could potentially be targeted to antagonize DOX toxicity[4]. To illustrate, trimetazidine has shown promise to protect against cardiac dysfunction by reversing metabolic rewiring induced by DOX[51].

Conclusions

To the best of our knowledge, this study is the first to provide *in vivo* experimental evidence supporting the protective activity of DCA against acute DOX cardiotoxicity, which may provide a rationale for repurposing DCA as an adjunct therapy with DOX that could open the door for the investigation of metabolic modulators in protecting against DOX cardiotoxicity.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Goda AE: Project conceptualization, Methodology, Data curation & analysis, Writing- draft and editing, Writing final review and approval, Project Supervision. Saleh MF: Methodology, Data curation & analysis, Writing- draft and editing, Writing final review and approval. Elsayad ME: Writing- final review and approval, Project Supervision.

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