Doxorubicin-induced markers of myocardial autophagic signaling in sedentary and exercise trained animals

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Submitted 27 July 2012; accepted in final form 15 May 2013

Doxorubicin-induced markers of myocardial autophagic signaling in sedentary and exercise trained animals. J Appl Physiol 115: 176–185, 2013. First published May 23, 2013; doi:10.1152/japplphysiol.00924.2012.—Doxorubicin (DOX) is an effective antitumor agent used in cancer treatment. However, its clinical use is limited due to cardiotoxicity. Indeed, the side effects of DOX are irreversible and include the development of cardiomyopathy and ultimately congestive heart failure. Although many studies have investigated the events leading to DOX-induced cardiotoxicity, the mechanisms responsible for DOX-induced cardiotoxicity remain unknown. In general, evidence suggests that DOX-induced cardiotoxicity is associated with an increased generation of reactive oxygen species and oxidative damage, leading to the activation of cellular proteolytic systems. In this regard, the autophagy/lysosomal proteolytic system is a constitutively active catabolic process that is responsible for the degradation of both organelles and cytosolic proteins. We tested the hypothesis that systemic DOX administration results in altered cardiac gene and protein expression of mediators of the autophagy/lysosomal system. Our results support this hypothesis, as DOX treatment increased both the mRNA and protein levels of numerous key autophagy genes. Because exercise training has been shown to be cardioprotective against DOX-induced damage, we also determined whether exercise training before DOX administration alters the expression of important components of the autophagy/lysosomal system in cardiac muscle. Our findings show that exercise training inhibits DOX-induced cardiac increases in autophagy signaling. Collectively, our results reveal that DOX administration promotes activation of the autophagy/lysosomal system pathway in the heart, and that endurance exercise training can be a cardioprotective intervention against myocardial DOX-induced toxicity.

DOXORUBICIN (DOX) IS AN ANTHRACYCLINE that is a widely used and effective antitumor drug. However, DOX’s clinical use is limited by its negative side effects. In general, DOX can induce the development of cardiac cardiomyopathy and dysfunction, leading to congestive heart failure and death (29, 33, 58, 67). In this regard, numerous studies have investigated the signaling pathways responsible for DOX-induced cardiotoxicity. Our group and others have shown that acute DOX administration results in an increase in the generation of reactive oxygen species (ROS) from cardiac mitochondria, and that increased mitochondrial ROS may contribute to the detrimental downstream effects of DOX administration (3–5, 23, 28, 33). Specifically, DOX administration may lead to cardiac mitochondrial respiratory chain failure and increased ROS production. Furthermore, it is estimated that ~1–3% of the oxygen used by mitochondria is converted to ROS (7). Importantly, an increase in cellular ROS production above basal levels has a wide range of consequences within the cell, including activation of redox-sensitive transcription factors, oxidative modifications to protein structure, and ROS-induced increases in cellular calcium concentration, all of which can result in increased proteolytic processing (2, 6, 14–16, 57).

It is well established that endurance exercise training is an effective countermeasure capable of providing robust cardioprotection in a variety of potentially damaging conditions (i.e., ischemia–reperfusion injury and DOX administration) (8, 10, 12, 17, 21, 33, 53). Growing evidence indicates that among the myriad of cardiac adaptations exercise causes, alterations in cardiac mitochondria play an essential role in exercise-induced cardioprotection (32, 38). In this regard, our laboratory has demonstrated that endurance exercise training is a sufficient intervention to inhibit the DOX-induced increases in mitochondrial dysfunction and ROS production (33). However, the mechanism(s) by which endurance exercise training provides protection against DOX-induced cardiac dysfunction remains unclear.

In this regard, previous work demonstrates that four key proteolytic systems are activated in the heart as a result of DOX administration. Specifically, calpain, caspase-3, the ubiquitin-proteasome system, and the autophagy/lysosomal system are all activated in the heart as a result of DOX (28, 33, 42, 63, 67, 68). Our laboratory demonstrated that exercise-induced increases in endogenous antioxidants are sufficient to protect the heart from DOX-induced increases in calpain activation and apoptosis (33). While this work reveals that exercise can protect the heart against activation of these two proteolytic systems, the effect of exercise on regulating DOX-induced gene expression of key autophagy proteins is unknown.

Autophagy is responsible for the removal of defective organelles and cytosolic proteins for lysosomal degradation (22, 25, 67, 68). In general, autophagy occurs on a continuous basis at low levels in the myocardium and is an important renewal mechanism (67). However, under pathological conditions, autophagy can be greatly increased to promote a deleterious rate of protein removal. Therefore, the functional significance of autophagy in the heart is complex and appears to be dependent on the activation levels of the signaling pathways that regulate autophagy (44, 48, 49, 55). Specifically, studies show that basal levels of autophagy are required for normal cardiac function, and that deletion of specific autophagy genes can result in significant functional deficits to the cardiac tissue (47, 50). In contrast, pathologically high levels of autophagic signaling can result in the elimination of vital cellular organelles and proteins and can also result in cross talk with other pathways responsible for the removal of defective organelles and proteins by the ubiquitin-proteasome system, resulting in an increase in the generation of ROS. Furthermore, the increase in ROS production may contribute to the detrimental downstream effects of DOX administration (3–5, 23, 28, 33). Specifically, DOX administration may lead to cardiac mitochondrial respiratory chain failure and increased ROS production.
proteolytic systems to increase cell death (28, 55, 67, 68). Therefore, the aim of this study was to investigate the effects of DOX on signaling of the autophagy/lysosomal system in the heart of sedentary and exercise-trained animals.

**METHODS**

**Experimental Design**

Adult 6-mo-old male Sprague-Dawley rats were used in these experiments. The Animal Care and Use Committee of the University of Florida approved these experiments, and animals were housed at the University of Florida Animal Care facility according to guidelines set forth by the Institutional Animal Care and Use Committee. Animals were maintained on a 12:12-h reverse light-dark cycle and with access to rodent chow and water ad libitum throughout the experimental period. Rats were randomly assigned to one of four experimental groups (n = 6/group): 1) sedentary control (SED); 2) exercise trained, no drug treatment (EXTR); 3) sedentary, treated with DOX (SED-DOX); and 4) exercise trained, treated with DOX (EXDOX).

Animals assigned to exercise groups were habituated to running by increasing durations of treadmill exercise for 5 days (10, 20, 30, 40, 50 min/day on days 1–5). After 2 days of rest, animals then performed 5 consecutive days of treadmill exercise for 60 min/day at 30 m/min, 0% grade. This work rate represents an estimated 70% of maximum oxygen consumption (36). The EXDOX animals received DOX hydrochloride (20 mg/kg body wt ip) immediately after the final exercise bout, and animals were killed 24 h later. The SEDDOX animals received DOX hydrochloride (20 mg/kg body wt ip) 24 h before death. Saline was used as both the vehicle and the placebo treatment. These doses of DOX are human clinical doses of this drug that are pharmacologically scaled for use in rats (13, 31, 64). At the conclusion of the experimental period, animals in each group were acutely anesthetized with pentobarbital sodium (60 mg/kg ip). After a surgical plane of anesthesia was reached, the heart was removed, and a section from the left ventricle (~10 mg) was mounted in Tissue-Tek OCT compound (Sakura Finetek USA, Torrance, CA) by submerging it in liquid nitrogen-cooled 2-methylbutane and then stored at −80°C to be used for immunohistochemistry. The remaining left ventricular wall was frozen in liquid nitrogen and stored at −80°C for subsequent analyses.

**Biochemical Analyses**

- **Hematoxylin and eosin staining.** Sections from frozen heart samples were cut at 10 μm using a cryotome (Shandon, Pittsburgh, PA) and stained for hematoxylin and eosin for analysis of cardiac muscle damage.

- **Western blot analysis.** Heart muscle was homogenized 1:10 (wt/ vol) in 5 mM Tris (pH 7.5) and 5 mM EDTA (pH 8.0) with a protease inhibitor cocktail (Sigma, St. Louis, MO) and centrifuged at 1,500 g for 10 min at 4°C. The resulting supernatant (cytosolic) fraction was collected, and protein content was assessed by the method of Bradford (Sigma). Proteins (40 μg) were separated by polyacrylamide gel electrophoresis via 4–20% gradient polyacrylamide gels containing 0.1% sodium dodecyl sulfate for ~1 h at 200 V. After electrophoresis, the proteins were transferred to nitrocellulose membranes via the criterion’s system for 90 min at 65 V. To control for protein loading and transfer differences, membranes were stained with Ponceau S. Ponceau S membranes were scanned, and the lanes were quantified using the 440CF Kodak Imaging System (Kodak, New Haven, CT) to normalize Westerns to protein loading. Nonspecific sites were blocked for 2 h at room temperature in phosphate-buffered saline (PBS) solution containing 0.05% Tween and 5% nonfat milk. Membranes were then incubated overnight at 4°C with primary antibodies directed against the proteins of interest. Beclin-1 (Cell Signaling, Danvers, MA) was probed as a measurement of the induction of autophagy. Bcl-2 was probed as a marker of Beclin-1 activity (Santa Cruz, Santa Cruz, CA). Atg7 (Cell Signaling), Atg4 (Biosensis, Adelaide, Western Australia, Australia), Atg12, Atg12-Atg5 (Cell Signaling), and LC3 (Cell Signaling) were measured as markers of autophagosomal formation. Finally, the protein content of the lysosomal protease cathepsin L (Santa Cruz) was also measured. Following incubation with primary antibodies, membranes were washed extensively with PBS-Tween and then incubated with secondary antibodies (GE Healthcare, Pittsburgh, PA). After washing, a chemiluminescent system was used to detect labeled proteins (GE Healthcare). Membranes were developed using autoradiography film, and images of the film were captured and analyzed using the 440CF Kodak Imaging System (Kodak, New Haven, CT).

**RNA isolation and cDNA synthesis.** Total RNA was isolated from cardiac tissue with TRIzol Reagent (Life Technologies, Carlsbad, CA), according to the manufacturer’s instructions. RNA content (μg/mg muscle) was evaluated by spectrophotometry, and RNA (5 μg) was then reverse transcribed with the Superscript III First-Strand Synthesis System for RT-PCR (Life Technologies), using oligod(T)20 primers and the protocol outlined by the manufacturer.

**Real-time polymerase chain reaction.** One microliter of cDNA was added to a 25-μl PCR reaction for real-time PCR using Taqman chemistry and the Applied Biosystems (ABI) Prism 7000 Sequence Detection system (ABI, Foster City, CA). Relative quantification of gene expression was performed using the comparative computed tomography method (ABI, User Bulletin no. 2). This method uses a single calibrator sample for comparison of every unknown sample’s gene expression. ΔΔCT (ΔCT(calibrator) – ΔCT(sample)) was then calculated for each sample, and relative quantification was calculated as 2ΔΔCT. Hypoxanthine guanine phosphoribosyltransferase was chosen as a reference gene for heart muscle samples based on previous work showing unchanged expression with our experimental manipulations (18, 19, 60). Fivefold dilution curves were assayed on selected samples to confirm the validity of this quantification method for each gene. ATG4b, ATG7, ATG12, LC3, cathepsin B, cathepsin D, and cathepsin L mRNAs were assayed using predesigned rat primer and probe sequences commercially available from Assays-on-Demand (ABI).

**LC3 immunohistochemistry.** Sections from frozen heart samples were cut at 10 μm using a cryotome (Shandon, Pittsburgh, PA) and fixed in 4% paraformaldehyde for 20 min. Sections were then washed in PBS and blocked with 5% bovine serum albumin and 3% goat serum in PBS. LC3 and dystrophin primary (Cell Signaling) and secondary reagents were diluted in 1% bovine serum albumin. Sections were mounted with 4',6-diamidino-2-phenylindole fluorescent mounting medium, and images were acquired via a monochrome camera (Qimaging Retiga) attached to an inverted fluorescent microscope (Axiovert 200, Zeiss).

**Data Analyses**

Data are presented as means ± SE. Comparisons between groups for each dependent variable were made by a one-way ANOVA, and, when appropriate, Tukey honestly significantly different tests were performed post hoc. Significance was established at P < 0.05.

**RESULTS**

To determine the effects of DOX administration on cardiac markers of the autophagy/lysosomal signaling pathway, experimental animals were assigned to one of four groups: sedentary and exercise trained groups that received placebo or DOX. All animals in the exercise groups completed the exercise protocol without incident, with no noticeable differences in exercise performance and with no apparent complications.
DOX Administration Increases Cardiac Muscle Damage

Hematoxylin and eosin staining was performed histologically to visualize the cardiac muscle damage that occurs in response to DOX treatment. This histological analysis reveals that the SEDDOX animals exhibited damaged myofiber ultrastructure compared with all other groups (Fig. 1). Importantly, exercise training before DOX administration prevented this damage to the cardiac muscle.

DOX Administration Increases Markers of Autophagosome Initiation

Activation of the autophagic signaling pathway begins with the formation of an isolation membrane, which is the first step of autophagosome formation. This process is regulated by a system of autophagy proteins (Atg proteins). Beclin-1 is part of a phosphoinositide 3-kinase complex and seems to play an important role during the initial steps of autophagosome formation by mediating the localization of other Atg proteins to the isolation membrane (25, 34). Beclin-1 mRNA levels in cardiac muscle were elevated \( (P < 0.05) \) in the SEDDOX group compared with all other groups (Fig. 2A), and Beclin-1 protein levels were significantly increased in the SEDDOX group compared with the SED group \( (P < 0.05) \) (Fig. 2B). Additionally, we measured the protein levels of Bcl-2, an anti-apoptotic protein that binds to Beclin-1 to inhibit autophagy. Compared with SED and EXTR, the SEDDOX group had a significant decrease in cardiac Bcl-2 protein content \( (P < 0.05) \). In addition, there was a significant increase in the ratio of Beclin-1 to Bcl-2 in the SEDDOX group compared with all other groups \( (P < 0.05) \) (Fig. 2, C and D).

DOX Administration Increases Markers of Autophagosome Formation

Elongation of the double-membrane of the autophagosome requires the interaction of many Atgs. Specifically, Atg12, Atg5, Atg7, Atg4, and LC3 all play important roles in formation of the autophagosome. Our results show that Atg12 mRNA levels in the heart were significantly elevated \( (P < 0.05) \) in the SEDDOX group compared with EXDOX (Fig. 3A). In addition, Atg12 protein expression was increased in the SEDDOX group compared with both SED and EXDOX groups \( (P < 0.05) \) (Fig. 3B). Finally, the conjugation of Atg12 to Atg5 is a required step in the elongation of the autophagosome. Our results reveal that there was a significant increase in the Atg12-Atg5 product as a result of DOX administration \( (P < 0.05) \). However, exercise training combined with DOX treatment was sufficient to cause a significant reduction in Atg12-Atg5 \( (P < 0.05) \) (Fig. 3C).

Heart Atg7 mRNA expression was not significantly different between any group; however, Atg7 protein levels were significantly greater \( (P < 0.05) \) in the SEDDOX group compared with the SED and EXDOX groups (Fig. 4, A and B). Similarly, Atg4 mRNA demonstrated no significant difference between groups, while Atg4 protein expression was increased \( (P < 0.05) \) in the SEDDOX group compared with the SED and EXDOX groups and was significantly increased \( (P < 0.05) \) in the EXTR group compared with the SED group (Fig. 4, C and D). Finally, LC3 mRNA expression in the heart was increased \( (P < 0.05) \) in the SEDDOX group compared with all other groups (Fig. 5A). In addition, the ratio of LC3 II to LC3 I was also measured as a marker of LC3 cleavage and therefore activation. In the heart, the LC3 ratio was increased \( (P < 0.05) \) in the SEDDOX group compared with the SED and EXTR.
groups (Fig. 5B). In addition, compared with SED animals, staining of cardiac tissue for LC3 revealed a significant increase in LC3 accumulation in heart from sedentary animals exposed to DOX. Importantly, exercise training before DOX administration attenuated increased LC3 accumulation in the heart (Fig. 5C).

DOX Administration Increases Lysosomal Proteases

Cathepsin B, D, and L are all ubiquitously expressed lysosomal proteases that are charged with the removal of both organelles and nonmyofibril cytosolic protein aggregates. In the heart, we saw a significant increase in cathepsin B mRNA compared with the SED and EXDOX groups ($P < 0.05$). Also, hearts from the SEDDOX group had significantly ($P < 0.05$) higher levels of cathepsin L mRNA expression compared with all other groups. No significant differences were detected in cardiac cathepsin D mRNA. (Fig. 6, A–C). In addition, cathepsin L protein levels were significantly increased in the SEDDOX group compared with both SED and EXDOX ($P < 0.05$) (Fig. 6D).

**DISCUSSION**

**Overview of Principal Findings**

These experiments provide new and important information regarding the effects of DOX administration on markers of autophagic signaling in cardiac muscle and also reveal for the first time an inhibitory effect of exercise training on DOX-induced increases in autophagy signaling. We hypothesized that DOX administration would result in an increase in markers of autophagy, and that exercise training would attenuate increases in DOX-induced autophagic signaling. Our results support these predictions as DOX administration resulted in increased markers of autophagy in the heart, and exercise
training performed before DOX administration attenuated the increase in autophagy. A detailed discussion of these findings follows.

**DOX Administration Promotes Autophagosome and Lysosomal Protein Expression in the Heart**

Autophagy is a highly regulated process whose activation is required at basal levels to maintain homeostasis in cardiomyocytes and other cells. However, large increases in autophagic signaling can be detrimental to the heart. Specifically, during stressful conditions in cardiac myocytes, accelerated autophagy can damage the myocardium due to the inability to control the rate of autophagy, resulting in the unregulated breakdown of cytosolic proteins and vital organelles (i.e., mitochondria) (3–5, 10–12, 28, 33). In this regard, our previous work (33) has shown that DOX administration greatly increases ROS production in cardiac mitochondria, resulting in mitochondrial damage, which could serve as a stimulus for increased autophagy signaling.

Autophagy is characterized by the formation of double-membrane vesicles called autophagosomes, which sequester molecules and fuse with lysosomes to digest the contents of the vesicle. The initial stages of autophagosome formation consist of induction and nucleation of an isolation membrane known as a phagophore. In this regard, Beclin-1 is an important signaling protein for the induction of autophagy. Specifically, expression of Beclin-1 is sufficient to stimulate autophagy initiation (41). Our results show that DOX administration was sufficient to increase the expression of Beclin-1 in the heart. In addition, Bcl-2 is an anti-apoptotic protein that can form a complex with Beclin-1 to inhibit autophagy activation. Specifically, it has been demonstrated that dissociation of Bcl-2 from Beclin-1 facilitates the activation of autophagy (43, 62). Therefore, DOX-induced decreases in Bcl-2 protein content could facili-
tate the activation of autophagy. These results suggest that DOX administration can promote increases in autophagic signaling in the heart.

The initiation of autophagosome formation is followed by the expansion and maturation of the autophagosome. This process requires the interaction of several autophagy proteins. Specifically, Atg12 and Atg5 conjugate to form an essential complex that requires Atg7 and Atg10 to covalently conjugate Atg12 to Atg5 (25, 56). The Atg12-Atg5 complex interacts noncovalently with Atg16, and this complex then initiates the elongation of the membrane by recruiting LC3 after it has been cleaved by the cysteine protease Atg4 (1, 25, 35, 45, 46). Therefore, we measured the expression of several of these key autophagy proteins. It is important to note that the half-life of the autophagosome is ~10 min, and, therefore, relatively small changes in protein expression may be due to the rapid turnover of these structures (46). Nevertheless, our findings reveal that, compared with nontreated sedentary animals, DOX administration resulted in a significant increase in the expression of several key proteins involved in the elongation and maturation of the autophagosome.

After formation, the autophagosome is then directed to the lysosome. The two fuse to form an autolysosome, where the contents of the autophagosome are then degraded (27, 40, 55). Numerous lysosomal proteases exist (e.g., cathepsin B, D, L), and each plays a vital role in autophagy-related protein breakdown (9, 54). Specifically, cathepsin B, D, and L are key lysosomal proteases that are expressed in cardiac tissue (9, 61). During conditions of muscle damage, greater expression of cathepsins are often observed in tissues exhibiting high rates of protein turnover. Our data reveal that there is a significant increase in the expression of both cathepsin B and L in the heart following DOX administration. These results provide additional support for the hypothesis that DOX administration can initiate autophagic signaling, leading to the degradation of the autophagosome and its contents by lysosomal proteases, which results in DOX-induced detriments to the heart.
These changes confirm previous findings from other laboratories that have demonstrated an increase in autophagy signaling in response to DOX administration (20, 42, 59, 63, 67). Moreover, it has also been demonstrated that inhibition of autophagy via 3-methyladenine (3-MA) is sufficient to protect against DOX-induced dysfunction (42, 63). Specifically, Xu et al. (63) reported that administration of 3-MA in cardiomyocytes treated with DOX resulted in attenuation of both DOX-induced autophagy, as well as cell death. In addition, in an animal model of DOX-induced cardiac failure, Lu et al. (42) demonstrated an improvement in cardiac function and a reduction in mitochondrial dysfunction in DOX-treated animals that also received 3-MA. One proposed mechanism by which inhibiting autophagy protects against DOX-induced cardiac dysfunction is that DOX administration results in damage to the mitochondria, which induces Beclin-1 gene expression, resulting in autophagy and heart failure. Another proposed mechanism is that cross talk can exist between autophagy and other proteolytic systems, which could explain why inhibition of autophagy protects cardiac contractility. However, future studies are needed to determine the precise role of autophagy in DOX-induced cardiac failure due to the potential off-target effects of 3-MA.

Exercise Protects Against Dox-induced Increases in Autophagy/Lysosomal Signaling

Endurance exercise training has been shown to be an effective intervention providing cardioprotection against a variety of...
myocardial insults (8, 17, 26, 33, 39, 51, 52). In addition, the ability of exercise training to protect against DOX-induced cardiotoxicity is well described (3–5, 10–12, 30, 33). Specifically, Chicco et al. (11) demonstrated that exercise preconditioning can protect against DOX-induced decreased left ventricle systolic function, as well as a decreased rate of left ventricle relaxation. While it has been reported in cardiac and skeletal muscle that exercise training can increase autophagy, these changes appear to be acute and necessary to maintain muscle integrity and aid in the clearance of organelles and long-lived proteins (24). In this regard, it has been hypothesized that exercise may protect against DOX-induced toxicity by eliciting mitochondrial adaptations, which results in a phenotype that resists damage and apoptotic stimuli (24). Our group has previously shown that exercise training before DOX administration protects against DOX-induced increases in cardiac mitochondrial ROS, mitochondrial damage, proteolysis by calpain and caspase-3, and increased apoptosis (33). In addition, we have also shown that exercise training before DOX administration is sufficient to inhibit increased autophagic signaling in skeletal muscle (59).

A second potential mechanism to explain the inhibitory effects of exercise training on DOX-induced autophagy signaling may be due to the exercise-induced increases in the endogenous antioxidants SOD1, SOD2, glutathione peroxidase-1, and catalase. Indeed, previous work from our group demonstrates an exercise-induced increase in the protein expression of each of these antioxidants (33, 59). In this regard, it has been demonstrated in many cell types that the induction of autophagy is redox sensitive (37, 66). Indeed, increased mitochondrial ROS production due to DOX administration could be responsible for increased autophagic signaling. In addition, increased autophagy may also be responsible for increased ROS levels in the cell. For example, it has been demonstrated that the autophagosome can degrade cytosolic catalase (65). This decrease in intracellular catalase concentrations would result in an inability to remove hydrogen peroxide, which could result in increased ROS levels in the cell. Therefore, exercise could protect against DOX-induced autophagy by increasing catalase protein levels. Therefore, we hypothesized that exercise would also be a sufficient intervention to decrease markers of the autophagy/lysosomal system in the heart, which are upregulated as a result of DOX administration. Our results confirm this hypothesis as exercise training resulted in a significant reduction in DOX-induced increases in autophagy gene expression. Specifically, compared with seden-

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**Fig. 6.** Cathepsin B, D, and L were analyzed as markers of increased degradation by the lysosomal proteolytic system. Representative Western blots are shown above the graph. A: heart cathepsin B mRNA expression. ‡SEDDOX significantly (P < 0.05) different vs. SED and EXDOX. B: heart cathepsin D mRNA expression. C: heart cathepsin L mRNA expression. §SEDDOX significantly (P < 0.05) different vs. all groups. D: heart cathepsin L protein expression. ‡SEDDOX significantly (P < 0.05) different vs. SED and EXDOX.
tary DOX-treated animals, cardiac Beclin-1, Atg12, Atg4, Atg7, LC3, cathepsin B, and cathepsin L were all reduced in animals that were exercise trained before DOX administration. Together, these results suggest that cardiac autophagic signaling is increased following DOX administration, and that exercise is sufficient to block DOX-induced increased autophagy. Additional experiments are required to determine the exact mechanisms responsible for this exercise-induced protection against the DOX-induced acceleration of autophagy.

Conclusions

In summary, our study provides the first evidence that DOX administration is associated with increased expression of key autophagy proteins in the heart. In addition, we also demonstrated that exercise training before DOX administration can attenuate many of the DOX-induced changes to autophagy markers. Finally, the present work provides the experimental rationale for future studies to determine the functional consequences of increased autophagy in the heart following DOX administration.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


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