Targeted overexpression of mitochondrial catalase protects against cancer chemotherapy-induced skeletal muscle dysfunction

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The American Cancer Society predicts that approximately 230,000 new cases of invasive breast cancer will be diagnosed this year in the US (1). These new cases are in addition to the 2.8 million women with confirmed breast cancer diagnoses. Doxorubicin is a chemotherapeutic anthracycline commonly prescribed to treat numerous human malignancies, including breast cancer in postmenopausal women (6, 33). The combined effect of both cancer and chemotherapy leads to disabling muscle weakness and fatigue for patients (18, 35). The loss of strength in combination with constant fatigue is a burden on cancer patients undergoing chemotherapy. Doxorubicin, a standard chemotherapy drug used in the clinic, causes skeletal muscle dysfunction and increases mitochondrial H2O2. We hypothesized that the combined effect of cancer and chemotherapy in an immunocompetent breast cancer mouse model (E0771) would compromise skeletal muscle mitochondrial respiratory function, leading to an increase in H2O2-emitting potential and impaired muscle function. Here, we demonstrate that cancer chemotherapy decreases mitochondrial respiratory capacity supported with complex I (pyruvate/glutamate/malate) and complex II (succinate) substrates. Mitochondrial H2O2-emitting potential was altered in skeletal muscle, and global protein oxidation was elevated with cancer chemotherapy. Muscle contractile function was impaired following exposure to cancer chemotherapy. Genetically engineering the overexpression of catalase in mitochondria of muscle attenuated mitochondrial H2O2 emission and protein oxidation, preserving mitochondrial and whole muscle function despite cancer chemotherapy. These findings suggest mitochondrial oxidants as a mediator of cancer chemotherapy-induced skeletal muscle dysfunction.

Reduced cardiorespiratory fitness, or peripheral muscle fatigue can all contribute to functional impairment. Prior research suggests that deteriorating physical function in cancer patients may be due to an effect specifically on skeletal muscle, resulting in peripheral muscle weakness and fatigue. Using a healthy rodent model, the chemotherapy drug doxorubicin has been shown to decrease both hindlimb and respiratory muscle strength, along with accelerating fatigue (11, 13, 14). Chemotherapy alone has a detrimental effect on muscle function. However, little is known how functional capacity is affected by chemotherapy in the context of cancer, more closely resembling what happens to patients in the clinic. Potential mediators of chemotherapy-induced muscle weakness and fatigue are reactive oxygen species (ROS). Exposure to the chemotherapeutic agent doxorubicin leads to an increase in ROS in skeletal muscle, as evidenced by elevated cytosolic oxidant activity (13) and markers of protein oxidation (13, 36). Mitochondrial H2O2-emitting potential is elevated in both skeletal and cardiac muscle following doxorubicin administration (12, 26), and treatment of cultured myotubes or rats with a cell-permeable mitochondrial-targeting peptide (MTP-131) that reduces ROS production also attenuates doxorubicin-induced oxidant production and accumulation of oxidation markers (15, 26). Collectively, these findings suggest that mitochondria are the major source of ROS production in skeletal muscle in response to doxorubicin treatment.

The objective of this study was to determine the exact nature and extent to which mitochondrial function is impacted by cancer chemotherapy, specifically in skeletal muscle. It was hypothesized that chemotherapy in the context of cancer would inhibit skeletal muscle mitochondrial respiration, leading to an increase in H2O2-emitting potential. To determine whether increased mitochondrial H2O2 production is the primary factor leading to compromised muscle function in response to cancer chemotherapy, a genetic mouse model (MCAT) in which catalase is targeted to the mitochondria was employed. Mitochondrial function was evaluated in permeabilized fiber bundles (PfFBs) from soleus muscle of wild-type mice in an immunocompetent cancer rodent model exposed to breast tumor cells (E0771) and the chemotherapeutic agent doxorubicin. The results indicate that cancer chemotherapy impairs mitochondrial respiration in hindlimb skeletal muscle. Interestingly, exposure to tumor cells and chemotherapy individually increases mitochondrial H2O2-emitting potential, whereas the combination of cancer chemotherapy diminishes the effects of either treatment alone. Scavenging of mitochondrial oxidants...
abolishes the mitochondrial dysfunction caused by cancer chemotherapy and protects against contractile dysfunction.

METHODS

**Overview of experimental design.** Figure 1A illustrates the experimental design. Female wild-type and heterozygous mice expressing the human catalase gene targeted specifically to the mitochondrial matrix in striated muscle (MCAT) (34) on a C57BL/6N background were ovariectomized at 12 wk of age to simulate menopause. Following recovery from the surgery, mice assigned to the tumor-bearing (TB) groups were injected subcutaneously on the right pad of the fourth mammary gland with mouse breast cancer cells (E0771) suspended in phosphate-buffered saline (PBS). When the tumor reached a mean size of 100–150 mm² (Fig. 1B), mice assigned to the doxorubicin (DOX) + TB groups received a single intraperitoneal injection of doxorubicin (DOX; 20 mg/kg in PBS), as described previously (12, 13). Control animals received the same volume of vehicle (PBS). Mice were monitored and weighed daily. Functional measurements and PmFBs were prepared from soleus muscles 72 h postinjection. Tibialis anterior (TA) muscles were frozen in liquid nitrogen for Western blot analysis. Experimental groups included WT control (CTRL; n = 10), WT tumor bearing (TB; n = 10), WT doxorubicin (DOX; n = 10), WT TB + DOX (n = 10), MCAT CTRL (n = 12), MCAT TB (n = 10), MCAT DOX (n = 11), and MCAT TB + DOX (n = 10).

**Reagents and cell lines.** Doxorubicin was purchased from Bedford Laboratories (Bedford, OH). Protease and phosphatase inhibitors were purchased from Roche Diagnostics (Indianapolis, IN). RIPA Lysis and Extraction Buffer was purchased from ThermoFisher Scientific (Waltham, MA). The Oxidized Protein Western blot detection kit was purchased from Abcam (Cambridge, UK). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The mouse breast cancer cells (E0771) were originally developed at Wake Forest University Health Sciences and provided by Dr. Lee Jones at Duke University. E0771 cells were maintained as monolayer cultures in RPMI Medium 1640 (Gibco) supplemented with 10% fetal bovine serum and 1% antibiotic antimycotic solution and incubated at 37°C in a humidified 5% CO₂/air-injected atmosphere.

**Animal care.** All mice were housed in the Department of Comparative Medicine at East Carolina University in a temperature- and light-controlled room and given free access to food and water. All procedures were approved by the university’s Institutional Animal Care and Use Committee. For the ovariectomy procedure, mice were anesthetized and incision sites shaved and cleaned with iodine solution. Standard aseptic procedures were observed. Dorsal incisions were made in the lumbar region to reveal the dorsal fat pads covering the ovaries. Ovaries were removed through cauterization. After ovariectomy, muscle incisions were sutured and the skin incisions closed with sterile suture wound clips. Mice were given meloxicam (5 mg/kg orally) prior to surgery and 24 h postsurgery. Wound clips were removed 7 days following surgery.

Following recovery from the ovariectomy procedure, mice were inoculated subcutaneously on the right pad of the fourth mammary gland with 100 µl of 5 × 10⁵ E0771 cells suspended in PBS using a 22-gauge needle. Tumor growth was monitored every other day in two perpendicular dimensions parallel with the surface of the mice using a slide caliper. Skeletal muscle was obtained from anesthetized mice by intraperitoneal injection with ketamine-xylazine (90 and 10 mg/kg). Following surgery, mice were euthanized by cervical dislocation under anesthesia.

**Determination of body composition.** Measurements of fat and lean body mass were determined using the EchoMRI-500 (Houston, TX) in accordance with the manufacturer’s instructions.

**Permethylized fiber bundle preparation.** Procedures were performed as described previously (2, 12, 30). In brief, fiber bundles from the soleus muscle were separated with fine forceps in ice-cold buffer X (in mM: 50 K-MES, 35 KCl, 7.23 K₂EGTA, 2.77 Ca₃EGTA, 20 imidazole, 20 taurine, 5.7 ATP, 14.3 PCr, and 6.56 MgCl₂-6H₂O, pH 7.1). Once separated, fiber bundles were permethylated in buffer X with 30 µg/ml saponin for 30 min and then washed in ice-cold buffer Z (in mM: 105 K-MES, 30 KCl, 1 EGTA, 10 KH₂PO₄, 5 MgCl₂-6H₂O, and 0.5 mg/ml BSA, pH 7.1) until analysis.

**Mitochondrial respiration.** High-resolution oxygen consumption measurements were conducted using the OROBOROS Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) at 37°C with an initial chamber concentration of 300–350 µM oxygen. All experiments were run in buffer Z containing 10 mM creatine monohydrate and 25 µM blebbistatin to inhibit contraction, as described previously (30). Concentrations of individual substrates for respiration protocols were palmitoyl-CoA (50 µM), carnitine (5 mM), malate (2 mM), ADP (4 mM), pyruvate (1 mM), glutamate (10 mM), and succinate (10 mM). At the end of each protocol, cytochrome c (10 µM) was added to test for mitochondrial membrane integrity, and any PmFBs that generated a >10% increase in respiration following the addition were not included in data analysis. At the conclusion of each experiment, PmFBs were washed in dH₂O and dried via freeze-drying (Labconco, Kansas City, MO). Polarographic oxygen measurements are expressed as picomoles per second per milligram dry weight.

**Mitochondrial H₂O₂ emission.** H₂O₂ emission was measured fluorometrically at 37°C via Amplex Ultra Red [10 µM/horseradish peroxidase (1 U/ml)] detection system (Eₜ₁₀/Eₑₐ₅₆ = 565/600)]. Fluorescence was monitored by a fluorolog-3 spectrofluorometer (Horiba)
Jobin Yvon, Edison, NJ) with temperature control and magnetic stirring. For each protocol, a background fluorescence rate was established in the presence of a PmFB following the addition of subsequent substrates. After correcting for the rate of change in background fluorescence, the concentration of H$_2$O$_2$ (pmol) was calculated from previously established resorufin fluorescence intensity standard curves with known concentrations of H$_2$O$_2$. Following each experiment, PmFBS were dried and weighed as described above. Concentrations of individual substrates/inhibitors for H$_2$O$_2$ emission protocols were succinate (10 mM), pyruvate (1 mM), carnitine (5 mM), auranofin (AF; 1 μM), and bis-chloroethyl nitrosourea (BCNU; 100 μM).

**RESULTS**

Mitochondrial respiration was measured sequentially in the presence of maximal palmitoyl-CoA consumption during respiration supported by complex I (pyruvate/glutamate) and complex II (succinate) substrates (Fig. 2A). Doxorubicin administration alone also reduced both complex I- and complex II-supported respiration. No further reduction in respiration in skeletal muscle of healthy rodents (12), affecting both complex I- and complex II-supported respiration. To determine the combined effect of tumor cells and doxorubicin on skeletal muscle mitochondrial function, PmFBS were isolated from the soleus muscle following exposure. Mitochondrial respiration of various complexes in the electron transport system (ETS) was measured sequentially.

Mitochondrial citrate synthase activity was similar among groups (data not shown), suggesting that cancer/chemotherapy did not influence muscle mitochondrial density. Tumor-bearing WT mice showed a significant decrease in maximal ADP-stimulated respiration supported by complex I (pyruvate/glutamate) and complex II (succinate) substrates (Fig. 2A). Doxorubicin administration alone also reduced both complex I- and complex II-supported respiration. No further reduction in respiration was observed in tumor-bearing mice treated with doxorubicin. Interestingly, during respiration supported by fatty acid oxidation, maximal ADP-stimulated respiration was reduced only in the cancer plus chemotherapy treatment group.

MCAT mice exposed to tumor cells alone were protected from the decrease in maximal ADP-stimulated oxygen consumption during respiration supported by complex I (pyruvate/glu

**Fig. 2. Cancer chemotherapy impairs mitochondrial respiratory capacity in skeletal muscle.** Permeabilized myofibers were prepared following exposure to tumor cell (TB) and doxorubicin (DOX) administration in both wild-type mice [control (CTRL), n = 9; TB, n = 9; DOX, n = 10; TB + DOX, n = 9; A] and mice expressing catalase in the mitochondrial matrix (MCAT; CTRL, n = 11; MCAT TB, n = 10; MCAT DOX, n = 11; MCAT TB + DOX, n = 10; B). Mitochondrial respiration was measured sequentially in the presence of maximal palmitoyl-CoA + carnitine (P/Car), malate (Mal), ADP (D), pyruvate (Pyr), glutamate (Glut), and succinate (Succ). Data are means ± SE. *P < 0.05 vs. CTRL.
glutamate) and complex II (succinate) substrates (Fig. 2B). However, doxorubicin treatment induced a decrease in maximal respiration in MCAT mice (Fig. 2B) similar to that in WT mice (Fig. 2A). A similar trend was evident for the group exposed to both tumor cells and doxorubicin, although none reached statistical significance (P = 0.1 vs. CTRL; Fig. 2B), suggesting that the presence of catalase did provide at least some protection.

Cancer chemotherapy alters mitochondrial H$_2$O$_2$-emitting potential. The chemotherapeutic agent doxorubicin is known to increase oxidant activity in skeletal muscle (13), specifically altering mitochondrial H$_2$O$_2$-emitting potential (12). Few studies have determined whether skeletal muscle from tumor-bearing animals has altered mitochondrial H$_2$O$_2$-emitting potential, which could lead to elevated oxidant activity and ultimately dysfunction. We evaluated the H$_2$O$_2$-emitting potential of mitochondria in PmFBs following exposure to both tumor cells and doxorubicin.

WT tumor-bearing mice display a significant increase (~22%) in mitochondrial H$_2$O$_2$-emitting potential in PmFBs during respiration supported by the complex II substrate succinate (Fig. 3A), which elicits superoxide production at complex I (due to reverse electron flow) and potentially at complex II (28, 31). Doxorubicin alone increased the rate of mitochondrial H$_2$O$_2$-emitting potential by ~94%. Surprisingly, cancer combined with chemotherapy completely blunted the effects of either treatment alone, generating an H$_2$O$_2$-emitting potential no different from controls. H$_2$O$_2$ production by the pyruvate dehydrogenase complex (10) was not different between groups (data not shown).

To determine the potential effect of cancer chemotherapy on the mitochondrial redox-buffering system in skeletal muscle, succinate-mediated H$_2$O$_2$ production was studied in the presence of inhibitors of both glutathione reductase (BCNU) and thioredoxin reductase (AF), as described previously (10). In the presence of both reductase inhibitors, matrix H$_2$O$_2$ scavenging is inhibited, and thus H$_2$O$_2$ emission reflects total H$_2$O$_2$ production (i.e., emission = production − scavenging). The scavenging index was calculated by subtracting the emission rate (+Succ rate) from the total H$_2$O$_2$ production (+AF/BCNU rate). Compared with controls, the scavenging index was decreased for all treated groups (Fig. 3A), suggesting that the ability to scavenge oxidants is decreased with cancer chemotherapy.

Heterozygous mice expressing skeletal muscle mitochondrial catalase in the matrix were completely protected from the increase in mitochondria H$_2$O$_2$ production caused by cancer chemotherapy (Fig. 3B). As expected, removal of the mitochondrial redox-buffering system by inhibitors (AF/BCNU) in MCAT mice had minimal effect on H$_2$O$_2$ emission (Fig. 3B).

Cancer chemotherapy promotes oxidative modifications of muscle proteins. Findings from our previous work have shown the chemotherapeutic agent doxorubicin alone increases oxidative modifications of myofibrillar proteins in skeletal muscle (13). Elevated cellular oxidants can result in posttranslational modifications of proteins that can affect whole muscle function. We evaluated the levels of reactive carbonyl derivatives in tibialis anterior muscle following exposure to both tumor cells and doxorubicin.

Tumor-bearing WT mice showed a significant increase in hindlimb muscle global protein carbonylation (Fig. 4A). Administration of the chemotherapeutic agent doxorubicin alone or in combination with tumor cells induced a similar increase in levels of reactive protein carbonyls. Heterozygous mice expressing skeletal muscle mitochondrial catalase in the matrix were completely protected from the increase in global protein carbonylation caused by cancer chemotherapy (Fig. 4B). These findings suggest that the presence of mitochondrial catalase in skeletal muscle is protective of cancer chemotherapy-induced oxidative damage.

Function of soleus following cancer chemotherapy. To determine the possible effect of cancer chemotherapy on whole muscle function, measurements of contractile properties were performed on the contralateral soleus muscle. Soleus weight was decreased significantly in WT mice exposed to doxorubicin, along with a decrease in calculated cross-sectional area (Table 1). A similar trend was evident for the tumor-bearing WT mice, although the group did not reach statistical significance (P = 0.1 vs. CTRL; Table 1).

The absolute force-frequency relationship of the soleus muscle is displayed in Fig. 5A. In WT mice, maximal isometric tetanic force was decreased in all experimental groups by

Fig. 3. Mitochondrial H$_2$O$_2$ emission in wild-type and mitochondrial catalase-expressing mice following exposure to cancer chemotherapy. Mitochondrial H$_2$O$_2$-emitting potential of permeabilized myofibers from wild-type (A) and mitochondrial catalase-expressing mice (MCAT; B) exposed to tumor cells (TB) and chemotherapy (DOX). Substrate conditions were in the presence of maximal succinate (Succ) and inhibitors of both Thioredoxin reductase and glutathione reductase [auranofin/bis-chloroethyl nitrosourea (+AF/BCNU)]. Scavenging index (SI) indicates calculated index of maximal scavenging. Data are means ± SE. Wild-type CTRL, n = 9; TB, n = 9; DOX, n = 10; TB + DOX, n = 8; MCAT CTRL, n = 8; MCAT TB, n = 8; MCAT DOX, n = 10; MCAT TB + DOX, n = 10. *P < 0.05 vs. CTRL.
12–14% compared with controls (Fig. 5B). When normalized for cross-sectional area, specific force was not different between groups (Fig. 5, C and D).

MCAT mice were not protected against the loss of whole muscle weight following cancer chemotherapy (Table 2). Exposure to tumor cells or doxorubicin alone caused a ~13–16% decrease in soleus weight. The combined effect of cancer chemotherapy produced a similar decrease in soleus weight and cross-sectional area in MCAT mice (Table 2).

Interestingly, MCAT mice at baseline were characterized by lower maximal isometric tetanic force compared with wild-type mice at baseline (compare Fig. 6B, CTRL, with Fig. 5B, CTRL). In contrast with WT mice, cancer chemotherapy did not cause a decrease in maximal isometric tetanic force in MCAT mice, nor did exposure to doxorubicin alone or in combination with tumor cells (Fig. 6, A and B), suggesting that the presence of catalase in the mitochondria did provide some protection against the loss of absolute muscle force.

Table 1. Soleus muscle characteristics of female C57BL6/N wild-type following exposure to tumor cells and DOX

<table>
<thead>
<tr>
<th></th>
<th>CTRL (n = 10)</th>
<th>TB (n = 10)</th>
<th>DOX (n = 10)</th>
<th>TB + DOX (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, (L₀) cm</td>
<td>1.23 ± 0.008</td>
<td>1.24 ± 0.03</td>
<td>1.22 ± 0.015</td>
<td>1.24 ± 0.014</td>
</tr>
<tr>
<td>Weight, g</td>
<td>0.0079 ± 0.0002</td>
<td>0.0072 ± 0.0002†</td>
<td>0.0064 ± 0.0002*</td>
<td>0.0077 ± 0.0003</td>
</tr>
<tr>
<td>Cross-sectional area, cm²</td>
<td>0.0060 ± 0.0001†</td>
<td>0.0055 ± 0.0001†</td>
<td>0.0049 ± 0.0002*</td>
<td>0.0059 ± 0.0002</td>
</tr>
</tbody>
</table>

Data are means ± SE. TB, tumor bearing; DOX, doxorubicin; CTRL, control. *P < 0.05 vs. CTRL; †P = 0.1 vs. CTRL.
posed mice in conjunction with a decline in scavenging capac-
skeletal muscle of both tumor-bearing and doxorubicin-ex-
remains elevated. Scavenging H2O2 in the mitochondrial ma-
potential is diminished when tumor-bearing mice receive doxo-
bering female rodents exhibit significant decreases in activities of complex I, complex II, and complex IV of the ETS from hindlimb muscle of lung (9) and intestinal cancer models (7).

Exposing healthy wild-type mice to chemotherapy alone decreases mitochondrial respiration. Following doxorubicin administration, soleus mitochondrial NADH- and FADH2-supported respiratory capacity is diminished. These findings are consistent with our previous work and other groups demonstrating that doxorubicin causes skeletal muscle mitochondrial dysfunction in a time-dependent manner (12, 17, 26).

Although doxorubicin alone can decrease the tumor burden on the rodent breast cancer model by reducing tumor volume (25), the combined effect of both the tumor and chemotherapeutic drug is not without negative effects. Cancer chemotherapy significantly decreased skeletal muscle mitochondrial respiratory capacity in wild-type mice. Interestingly, the effects were not additive, as respiratory capacity was depressed in the cancer chemotherapy experimental group to approximately the same degree as cancer and chemotherapy alone (~20%). Very few studies have assessed mitochondrial function in non-

**DISCUSSION**

The present experiments provide novel insight into how the combined insults of cancer and chemotherapy affect mitochondria of skeletal muscle. Tumor bearing, the chemotherapeutic agent doxorubicin, and the combination of tumor cells and doxorubicin diminish both complex I- and complex II-supported respiration in skeletal muscle of wild-type mice. Furthermore, mitochondrial H2O2-emitting potential is elevated in skeletal muscle of both tumor-bearing and doxorubicin-exposed mice in conjunction with a decline in scavenging capacity. Surprisingly, the increase in mitochondrial H2O2-emitting potential is diminished when tumor-bearing mice receive doxorubicin, although global protein oxidation in skeletal muscle remains elevated. Scavenging H2O2 in the mitochondrial matrix partially protects against impaired skeletal muscle mitochondrial function and contractile dysfunction caused by cancer and chemotherapy. These findings provide evidence that elevated mitochondrial H2O2 emission may be a contributing

underlying mechanism by which cancer chemotherapy causes debilitating muscle weakness and fatigue.

Cancer alone can negatively affect skeletal muscle mitochondrial respiration. Both complex I- and complex II-supported respiration was impaired in permeabilized myofibers from soleus hindlimb muscle of tumor-bearing mice. Few studies have assessed skeletal muscle mitochondrial respiratory capacity in the immunocompetent mouse model of breast cancer utilizing E0771 cells. However, similar findings have been established in other rodent cancer models using isolated mitochondria from hindlimb muscles. Tumor-bearing female rodents exhibit significant decreases in activities of complex I, complex II, and complex IV of the ETS from hindlimb muscle of lung (9) and intestinal cancer models (7).

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Although doxorubicin alone can decrease the tumor burden on the rodent breast cancer model by reducing tumor volume (25), the combined effect of both the tumor and chemotherapeutic drug is not without negative effects. Cancer chemotherapy significantly decreased skeletal muscle mitochondrial respiratory capacity in wild-type mice. Interestingly, the effects were not additive, as respiratory capacity was depressed in the cancer chemotherapy experimental group to approximately the same degree as cancer and chemotherapy alone (~20%). Very few studies have assessed mitochondrial function in non-tumor-bearing tissue in a preclinical rodent model of combined cancer chemotherapy. The majority of studies indirectly assess mitochondria by focusing on the apoptotic effects of anticancer drugs, particularly through the mitochondrial permeability transition pathway (20). In cardiac muscle, alterations in mitochondrial apoptotic gene expression (e.g., Bcl3, Bad, and Casp7) and elevated caspase-3 activity are indicators of mito-

chondrial dysfunction in an immunocompetent female rat breast cancer model (8, 16). These studies imply that mitochondria in noncancerous cells are negatively affected by cancer chemotherapy. Our present work provides new data on how combined cancer chemotherapy affects mitochondria of skeletal muscle, suggesting a critical role in the impaired physical function experienced by patients.

Mitochondrial H2O2 emission and protein carbonylation, a global marker of protein oxidation, were increased in skeletal muscle from wild-type mice following exposure to both tumor cells and doxorubicin alone. Evidence of elevated oxidants in other rodent cancer models are indicated by increased markers of protein oxidation in hindlimb muscle of tumor-bearing mice (4), suggesting a disruption in the redox balance of the cell.

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**Table 2. Soleus muscle characteristics of female heterozygous MCAT following exposure to tumor cells and DOX**

<table>
<thead>
<tr>
<th></th>
<th>MCAT CTRL (n = 11)</th>
<th>MCAT TB (n = 10)</th>
<th>MCAT DOX (n = 11)</th>
<th>MCAT TB + DOX (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (L0), cm</td>
<td>1.29 ± 0.016</td>
<td>1.28 ± 0.018</td>
<td>1.26 ± 0.009</td>
<td>1.31 ± 0.011</td>
</tr>
<tr>
<td>Weight, g</td>
<td>0.0084 ± 0.0001</td>
<td>0.0073 ± 0.0002*</td>
<td>0.0070 ± 0.0002*</td>
<td>0.0074 ± 0.0002*</td>
</tr>
<tr>
<td>Cross-sectional area, cm²</td>
<td>0.0062 ± 0.0001</td>
<td>0.0054 ± 0.0001*</td>
<td>0.0053 ± 0.0001*</td>
<td>0.0053 ± 0.0003*</td>
</tr>
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</table>

Data are means ± SE. MCAT, mice expressing catalase in the mitochondrial matrix; TB, tumor bearing; DOX, doxorubicin. *P < 0.05 vs. MCAT CTRL.
Although cancer alone can jeopardize the redox buffering of a cell, the majority of studies point to chemotherapy as the main culprit. Min et al. (26) reported elevated H$_2$O$_2$ emission in both PmFBs from cardiac and skeletal muscle following doxorubicin administration, along with elevated markers of protein oxidation. Elevated H$_2$O$_2$-emitting potential in skeletal muscle induced by doxorubicin is likely due to redox modifications within the matrix (e.g., the ETS, redox-buffering system) (12). If persistent, elevated H$_2$O$_2$ emission can shift the redox environment of the cell to a more oxidized state. The findings of the present study also suggest that oxidant scavenging is depressed within the mitochondria. Consistent with these findings, prolonged exposure to the burden of tumor-bearing or chemotherapy has previously been shown to decrease superoxide dismutase (23) and glutathione (24, 27) content. Collectively, these findings suggest that mitochondrial redox buffering circuits are negatively affected by cancer and/or chemotherapy, compromising the ability to maintain the proper redox state of key structural and functional proteins in skeletal muscle.

Surprisingly, the combined effect of cancer with chemotherapy blunted the increase in mitochondrial H$_2$O$_2$ emission in skeletal muscle caused by the two insults individually despite a significant decrease in mitochondrial respiratory capacity. The reduced H$_2$O$_2$-emitting potential was not accounted for by an increase in mitochondrial scavenging capacity, suggesting that the combined effect of cancer chemotherapy decreased H$_2$O$_2$ production. Tumor size was not different with doxorubicin injection alone but elevated at earlier time points (12). The lower H$_2$O$_2$ production coincided with lower membrane potential and greater susceptibility to Ca$^{2+}$-induced opening of the permeability transition pore, both of which are indicative of compromised mitochondrial function. In the present study, global protein oxidation remained elevated with combined cancer chemotherapy, consistent with the notion that the lower H$_2$O$_2$ production did not reflect an improvement in mitochondrial function. The collective implications of these findings are of course constrained by the limited time course of such studies. Determining exactly how the combination of cancer and chemotherapy specifically modulates superoxide/H$_2$O$_2$ production and overall mitochondrial function in skeletal muscle mitochondria over time will require further experiments.

The chemotherapeutic agent doxorubicin alone caused the mice to lose weight following exposure, as shown previously (11–13, 32). Whole body fat and lean mass were significantly decreased, a phenomenon that occurs in the clinic. Catabolism and muscle wasting are significant contributors to the decline in physical function in cancer patients (38). Decrements in femoral quadriceps muscle thickness are detected by ultrasound within the first 4 wk of chemotherapy (21). The loss of...
Both oxidative and nitrosative stress are associated with muscle mass contributes to the muscle weakness caused by cancer chemotherapy and plays a role in the observed drop in absolute force of wild-type mice exposed to tumor cells and doxorubicin. In the present study, there was no change in specific force, which is the absolute force normalized for muscle cross-section. Our findings suggest that cancer chemotherapy affects skeletal muscle function via two pathways: 1) the loss of muscle mass and 2) a decline in contractile function, both of which can directly compromise the physical performance of patients.

To determine whether the elevated H$_2$O$_2$-emitting potential in skeletal muscle may mediate the mitochondrial dysfunction caused by cancer chemotherapy, mice that express mitochondrial-targeted catalase in skeletal muscle (MCAT) were exposed to tumor cells and doxorubicin. The increase in mitochondrial H$_2$O$_2$-emitting potential following tumor cell inoculation or doxorubicin administration alone was completely blunted in MCAT mice. Elevated protein oxidation in skeletal muscle caused by cancer chemotherapy was ablated in MCAT mice. In addition, the cancer chemotherapy-induced decline in mitochondrial respiration was restored in MCAT mice, suggesting that mitochondrial H$_2$O$_2$ may be a mechanism contributing to the mitochondrial dysfunction induced by cancer chemotherapy. Not only were MCAT mice protected on a cellular level from the negative effects of cancer chemotherapy, but the cancer chemotherapy-induced decrease in skeletal muscle isometric tetanic force was blunted in MCAT mice. The protection against the decline in contractile function occurred despite the MCAT mice exhibiting cancer chemotherapy-induced loss of muscle mass. It is important to note that although overexpression of catalase did appear to protect against the loss of maximal isometric tetanic force induced by cancer chemotherapy, maximal force was initially lower in MCAT controls relative to wild-type controls. Previous reports have demonstrated that a more oxidized cellular redox state increases maximal force in single fibers, an effect that is prevented by inclusion of an oxidant-buffering agent (3, 22).

Therefore, the presence of catalase would be predicted to reset the maximal force to a lower value, consistent with the observed findings. The implication is that optimal force depends on the degree of thiol oxidation within proteins of the contractile machinery and that the added oxidant-buffering capacity provided by catalase held this new redox set point despite elevated H$_2$O$_2$ production induced by the cancer chemotherapy.

Mitochondrial-targeted antioxidants are a viable intervention for protecting against the negative effects of cancer chemotherapy. Administration of the mitochondrial-targeted peptide MTP-131 also protects against the loss of skeletal muscle function caused by doxorubicin (26). MTP-131 is thought to exert its cytoprotective effects at least in part by reducing H$_2$O$_2$ emission (37). Nonmitochondrial targeted approaches appear to be less effective, as several studies have utilized the antioxidant NAC (N-acetylcysteine) to replenish the loss of glutathione caused by cancer chemotherapy and found little to no protection against mitochondrial dysfunction in tumor-bearing mice (9) or following doxorubicin administration (29, 39).

In summary, the findings of the present study suggest that increased mitochondrial ROS production is a significant contributor to the development of cancer chemotherapy-induced mitochondrial and skeletal muscle dysfunction. Our current findings and the work of others (26) indicate that mitochondrial-targeted antioxidants may be an effective adjunct therapeutic approach for cancer patients undergoing chemotherapy to help prevent muscle weakness and loss. The reduction in muscle mass and function is both an acute and long-term problem for cancer patients (17, 19), and thus limiting these distressing side effects could lead to improved therapy retention rates and enhanced quality of life for patients long term.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

11. Gilliam LA, Ferreira LF, Bruton JD, Myolan JS, Westerblad H, St Clair DK, Reid MB. Doxorubicin acts through tumor necrosis factor...


18. Min K, Kwon OS, Smude AJ, Wiggs MP, Sollane KJ, Christou DD, Yoo JK, Hwang MH, Szeto HH, Kavazis AN, Powers SK. Increased mitochondrial emission of reactive oxygen species and calpain activation are required for doxorubicin-induced cardiac and skeletal muscle myop


29. Szeto HH. Development of mitochondria-targeted aromatic-cationic pep

