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Doxorubicin causes diaphragm weakness in murine models of cancer chemotherapy

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Abstract

Introduction—Doxorubicin is a chemotherapeutic agent prescribed for a variety of tumors. While undergoing treatment, patients exhibit frequent incidents of dyspnea associated with respiratory muscle weakness. Cancer patients can receive doxorubicin chemotherapy through either intravenous (i.v.) or intraperitoneal (i.p.) injections. We hypothesized that respiratory muscle function would be depressed in a murine model of chemotherapy.

Methods—We tested this hypothesis by treating C57BL/6 mice with a clinical dose of doxorubicin (20 mg/kg) via i.v. or i.p. injection. Three days later we measured contractile properties of muscle fiber bundles isolated from the diaphragm.

Results—Doxorubicin consistently depressed diaphragm force with both methods of administration ($p < 0.01$). Doxorubicin i.p. exaggerated the depression in diaphragm force and stimulated tissue inflammation and muscle fiber injury.

Discussion—These results suggest that clinically relevant doses of doxorubicin cause respiratory muscle weakness and that the loss of function depends, in part, on the route of administration.

Keywords

chemotherapy; respiratory muscle; weakness; oxidative stress; inflammation

INTRODUCTION

Common side effects of cancer chemotherapy include dyspnea and exercise intolerance (1) that can persist for up to two years following therapy (2). Both of these side effects are clinical indicators of respiratory muscle weakness (3). Patients who have undergone chemotherapy exhibit decreased maximal inspiratory pressures as evidence of respiratory muscle weakness (4). These associations suggest chemotherapeutic agents might act on respiratory muscles to compromise contractile function.

A common chemotherapy drug, doxorubicin (adriamycin), is administered to cancer patients through two routes. In some clinical conditions, e.g. lymphomas and leukemias, doxorubicin is administered systemically by intravenous (i.v.) infusion (5,6). In contrast, doxorubicin is

directly administered into the abdominal compartment via intraperitoneal (i.p.) injection to treat ovarian cancer (7) and peritoneal carcinomatosis (8). Both routes of administration cause debilitating weakness and fatigue in patients (9,10).

Doxorubicin treatment increases circulating markers of inflammation in both patients (11) and rodent models of chemotherapy (12). At the tissue level, oxidant production is elevated in cardiac muscle following doxorubicin exposure (13-15). It follows that inflammatory mediators could also stimulate oxidant production in respiratory muscles following doxorubicin exposure and contribute to contractile dysfunction (16,17).

The effects of doxorubicin on respiratory muscle function have not been tested under any conditions and are the focus of this study. Our interest stems from prior observations that doxorubicin causes weakness of hindlimb muscles in mice (18). Similarly, we hypothesized that respiratory muscle function would be depressed in a murine model of chemotherapy. We tested this hypothesis by treating mice with a clinical dose of doxorubicin via i.v. or i.p. injection; three days later, we measured contractile properties of muscle fiber bundles isolated from diaphragm, the primary muscle of inspiration. Doxorubicin depressed diaphragm force, a response that was exaggerated by i.p. administration which stimulated muscle inflammation and injury. We conclude that doxorubicin chemotherapy can weaken respiratory muscles and that this response is influenced by the route of administration.

MATERIALS AND METHODS

Animal care

Studies of systemic doxorubicin administration were conducted at the University of Kentucky using six- to eight-week-old male C57BL/6 mice (Harlan, Indianapolis, IN, USA). Animals were maintained in the Division of Laboratory Animal Resources facility on a 12:12-h dark:light cycle and provided food and water *ad libitum*. All experiments were approved by the Institutional Animal Care and Use Committee.

Doxorubicin treatment

Mice were injected with doxorubicin i.v. or i.p. (20 mg/kg; Bedford Laboratories, Bedford, OH, USA). This dose is comparable to doxorubicin chemotherapy given to patients with small cell lung cancer (6) and has been shown to cause hindlimb muscle dysfunction in mice (18). The amount of doxorubicin was based on the conversion factor established by Freireich (19) which is derived from the relationship between body weight and surface area of the animal. Control animals received the same volume of vehicle (phosphate buffer solution). The diaphragm was excised for analysis 72 hrs after injection. The total number of animals studied was 88. From each animal one hemi-diaphragm was used for functional studies (contractile function, cytosolic oxidant activity) and the other hemi-diaphragm was frozen for histologic and biochemical analysis (H&E, Evans Blue, MPO, oxidative protein markers).

Contractile function

Mice were anesthetized with isoflurane and euthanized by cervical dislocation. The diaphragm muscle was excised and placed in Krebs-Ringer solution (in mM: 137 NaCl, 5 KCl, 1 MgSO₄, 1 NaH₂PO₄, 24 NaHCO₃, 2 CaCl₂) equilibrated with 95% O₂-5% CO₂ (pH~7.4). A fiber bundle was isolated from the costal diaphragm with its associated rib and central tendon. The bundle was attached to a force transducer (BG Series 100g, Kulite, Leonia, NJ, USA) using 4-0 silk suture. The force transducer was mounted on a micrometer used to adjust muscle length. The diaphragm bundle was placed in a temperature-controlled organ bath between platinum wire stimulating electrodes and stimulated to contract

isometrically using electrical field stimulation (Grass S48, Quincy, MA, USA). The output of the force transducer was recorded using an oscilloscope (546601B; Hewlett-Packard, Palo Alto, CA, USA) and computer software (Axoscope 9.2, Molecular Devices, Sunnyvale, CA, USA). In each experiment, the muscle was adjusted to the length where twitch force was maximal (optimal length, L_0) at room temperature, and L_0 was measured using an electronic caliper (CD-6" CS, Mitutoyo America Corp, Aurora, IL, USA). The bath temperature was then increased to 37°C, followed by an equilibration period of 30 minutes. One minute prior to stimulation, 25 μ M (+)-tubocurarine chloride hydrate (Sigma, Saint Louis, MO, USA) was added to the organ bath. The force-frequency relationship was determined using contractions evoked at 2-min intervals using stimulus frequencies of 1 (twitch stimulus), 15, 30, 50, 80, 120, 150, 250, and 300 Hz. Pulse and train durations were 0.3 and 250 ms. Time-to-peak twitch force (TPT) and twitch half-relaxation time (1/2 RT) were also measured. Following each experiment, the muscle was removed, blotted dry, and weighed. Cross-sectional area was determined as described by Close (20). Specific forces were expressed as N/cm².

Cytosolic oxidant activity

The fluorochrome probe 2',7'-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, OR, USA) was used to measure oxidant activity (21). Hemidiaphragms were loaded with DCFH-DA (20 μ M) in Krebs-Ringer solution at 37°C for 60 mins. Accumulation of the oxidized derivative (DCF, 480 nm excitation, 520 nm emissions) was measured by use of an epifluorescence microscope (Labophot-2, Nikon Instruments, Melville, NY, USA), a CCD camera (Series 72, Dage-MTI Inc., Michigan City, IN, USA), and a computer-controlled shutter in the excitation light pathway. DCF emissions for muscles and solution background were acquired by 10 ms exposure to excitation light (480 nm). Records were stored to the computer for determination of emission intensity using commercial data software (Metamorph 6.2R6, Molecular Devices, Downingtown, PA, USA).

Histology

Cross sections of diaphragm bundles were cut on a cryostat (6 μ m) and stored at -80°C. Sections were fixed in methanol and stained with hematoxylin and eosin (22). After staining, slides were dehydrated in an ethanol series (85, 95, 100%), cleared with xylene, and mounted in Permount (Fisher Scientific, Pittsburgh, PA, USA). Slides were viewed with a Nikon E600 microscope (Melville, NY, USA). Images were captured with a Spot RT digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) and a PowerMac G4 computer (Apple, Cupertino, CA, USA) equipped with Spot RT software, version 4.0 (Diagnostic Instruments).

Sarcolemmal integrity

The vital dye Evans blue (i.p. 50 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) was injected into doxorubicin-treated and vehicle-treated mice to assess sarcolemmal integrity (23,24). Mice were euthanized 18 hrs post-injection (the 72 hr time point following doxorubicin or vehicle exposure). Muscles were frozen and sectioned as described above. Unfixed muscle sections were mounted with ProLong Gold antifade reagent with DAPI (Invitrogen, Carlsbad, CA, USA) and examined by fluorescence microscopy for myofiber dye incorporation.

Myofibrillar protein isolation

The method for purification of myofibrils was adapted from Solaro et al. (25). Hemidiaphragms were homogenized in relax buffer (in mM: 75 KCL, 20 MOPS pH 7, 2 MgCl₂, 2 EGTA, 1 NaN₃, 4 phosphocreatine, 1 ATP, 1 DTT, 1 benzamidine) plus 10 mM EDTA,

1% Triton, and protease and phosphatase inhibitors (30mM sodium fluoride, in mM: 200 PMSF, 1 leupeptin, 1 pepstatin A, 400 EDTA, 200 sodium orthovanadate). The homogenate was pelleted at 16,100 g (4°C) for 5 min and washed 3x by resuspension in standard rigor buffer (in mM: 75 KCL, 20 MOPS pH 7, 2 MgCl₂, 2 EGTA, 1 NaN₃) plus Triton and inhibitors, followed by 5 min on ice and centrifugation at 16,100 g (4°C) for 5 min. The pellet was rinsed in standard rigor buffer and pelleted at 1,500 g (4°C) for 1 min. The supernatant was decanted, and the pellet was re-suspended in K-60 solution (in mM: 60 KCl, 20 MOPS, 2 MgCl₂ – pH 7.0) plus bovine serum albumin (BSA, 1 mg/ml) and inhibitors as before. The supernatant was removed, and the pellet was re-suspended in 100 µl of K-60, BSA, and inhibitors plus 100 µl of 2 mM DTT in K60 mixed 1:1 with glycerol and stored at -80°C.

Western blot analysis

To detect changes in nitrotyrosine and 4HNE residues, myofibrillar protein samples were thawed and centrifuged at 1,500 g for 2 min at 4°C. The pellet was resuspended in 200 µl of urea sample buffer (in M: 8 urea, 0.05 Tris pH 6.8, and 0.075 DTT plus 0.05% bromophenol blue) and heated at 98°C for 3 min. Whole muscle homogenates were used for myeloperoxidase (MPO) content. Diaphragm muscles were homogenized in 2X lysis buffer (20 mM Tris pH 7.2, 2% SDS) then diluted 1:1 in 2X sample loading buffer (120 mM Tris pH 7.5, 200 mM DTT, 20% glycerol, 4% SDS and 0.002% bromophenol blue).

Proteins were fractionated on 15% SDS-polyacrylamide gels (Criterion pre-cast gels; Bio-Rad, Hercules, CA, USA) and transferred to reduced-fluorescence PVDF membrane (Immobilon-FL, Millipore, Bedford, MA, USA). Membranes with transferred proteins were blocked for 1 hr at room temperature in Odyssey blocking buffer (LI-COR, Lincoln, NE, USA). Membranes were incubated with primary antibodies overnight at room temperature in Odyssey blocking buffer mixed 1:1 with PBS plus 0.2% Tween. Secondary antibodies were incubated with the membrane for 45 min in Odyssey/PBS/0.2% Tween plus 0.01% SDS. MPO and nitrotyrosine antibodies were purchased from Millipore (Bedford, MA, USA). The HNE antibody was purchased from Abcam Inc. (Cambridge, MA, USA). Fluorescent secondary antibodies were used for detection (goat anti-mouse Alexa-680, Molecular Probes-Invitrogen; goat anti-rabbit IRD800, Rockland Immunochemicals, Gilbertsville, PA, USA). Fluorescence was imaged and results were quantified using the Odyssey Infrared Imaging System (LI-COR). Results were normalized for total protein using Simply Blue stain (Invitrogen, Carlsbad, CA, USA).

Statistical analyses

A power analysis was used to calculate the sample size required to detect a difference between means with 95% power (Prizm 5.0a, Graphpad Stat Mat, La Jolla, CA, USA). Force-frequency curves were analyzed using two-way repeated measures ANOVA with post-hoc Bonferroni tests. Diaphragm muscle characteristics, cytosolic oxidant activity, MPO content, and modifications of myofibrillar proteins were analyzed using Student's t-tests. Statistical calculations were performed using commercial software (Prizm 5.0a, Graphpad Software Inc., La Jolla, CA, USA; Microsoft Excel, Redmond, WA, USA). Statistical significance was accepted at the $p < 0.05$ level. Results are reported as means $\pm$ SE.

RESULTS

Doxorubicin weakens the diaphragm

Intravenous doxorubicin administration depressed body weight by 14 % after 72 hrs (doxorubicin 21 ± 1 g vs. vehicle 25 ± 1 g, $p < 0.05$). This did not affect the physical

dimensions of diaphragm fiber bundles. We saw no differences between groups in fiber bundle weight (doxorubicin 2.8 ± 0.1 mg vs. vehicle 2.7 ± 0.2 mg, $p > 0.9$) or cross-sectional area (doxorubicin $30 \pm 2 \mu\text{m}^2$ vs. vehicle $28 \pm 2 \mu\text{m}^2$, $p > 0.5$).

Isometric contractile function of the diaphragm was depressed following i.v. administration of doxorubicin. A modest decline in absolute force was not significant (Fig.1A) but specific force was decreased by approximately one-fourth, a loss of function that was evident across most of the stimulus frequency range (Fig.1B). The relative force-frequency curve was shifted leftward after i.v. infusion (Fig.1C), which is evidence of altered fiber activation at physiological stimulus frequencies.

Changes in twitch characteristics were consistent with the force-frequency shift. The twitch:tetanus ratio was significantly increased (doxorubicin 0.33 ± 0.01 vs. vehicle 0.26 ± 0.01 ; $p < 0.05$) without prolongation of twitch contractions. A 10 % increase in TPT was not statistically significant (doxorubicin 20 ± 1 ms vs. vehicle 18 ± 1 ms, $p > 0.07$), and $\frac{1}{2}$ RT was unaltered ($p > 0.3$).

Diaphragm weakness is exacerbated by i.p. administration

We previously showed that i.p. administration of doxorubicin causes body weight to decline over three days (18), similar to the loss seen after i.v. infusion (above). Unlike i.v. infusion, i.p. injection of doxorubicin altered the dimensions of diaphragm fiber bundles. Bundle weight was 14 % less than vehicle-injected controls (doxorubicin 2.8 ± 0.1 mg vs. vehicle 3.2 ± 0.2 mg, $p < 0.01$), and cross-sectional area was diminished (doxorubicin $32 \pm 1 \mu\text{m}^2$ vs. vehicle $35 \pm 1 \mu\text{m}^2$, $p < 0.05$).

Doxorubicin injection i.p. exacerbated diaphragm dysfunction. This is most obvious in measurements of absolute force (Fig.2A) which plummeted by 50-60 % across a range of stimulus frequencies. The fall in absolute force was not fully explained by loss of muscle mass. After normalizing for bundle cross-section, specific force was still depressed by one-third (Fig.2B). The relative force-frequency relationship was also shifted leftward (Fig.2C).

Twitch characteristics were altered substantially. We saw an increase in twitch:tetanus ratio (doxorubicin 0.36 ± 0.03 vs. vehicle 0.25 ± 0.02 ; $p < 0.05$) and prolongation of TPT (doxorubicin 22 ± 1 ms vs. vehicle 18 ± 1 ms, $p < 0.01$). A small increase in $\frac{1}{2}$ RT was not statistically significant (doxorubicin 17 ± 1 ms vs. vehicle 15 ± 1 ms, $p > 0.1$).

The decline in force was not associated with widespread loss of myofibrillar proteins. Comparing total protein content between doxorubicin and vehicle injected animals we observed a 20 ± 5 % decrease in myosin light chain ($p < 0.05$), with no change in myosin heavy chain or other myofibrillar proteins including actin, desmin, tropomyosin, or troponin (data not shown). There were no changes in myofibrillar protein levels following i.v. doxorubicin (data not shown).

Intraperitoneal injection causes diaphragm inflammation

Doxorubicin i.p. caused oxidative stress in the diaphragm. Cytosolic oxidant activity was increased in diaphragm muscle fibers by almost 200 % (Fig.3). We also detected oxidative modifications to myofibrillar proteins (Fig.4). Nitrotyrosine residues were elevated on desmin and tropomyosin, and 4-hydroxynonenal (HNE) adducts were increased on troponin T and myosin light chain.

Consistent with oxidative stress, we observed inflammatory injury following i.p. doxorubicin administration. H&E staining revealed tissue damage and accumulation of basophilic structures with evidence of neutrophil invasion (Fig.5A). Myeloperoxidase

(MPO) level is a separate indicator of neutrophil content that was also elevated (Fig.5A); we saw increases in both the MPO precursor protein (92 kDa; 106 ± 15 %) and active MPO enzyme (60 kDa; 584 ± 54 %). Sarcolemmal disruption was detected in a subset of muscle fibers by Evans blue staining (Fig.5B). These fibers were localized on or near the abdominal surface of the diaphragm. No staining was seen after vehicle injection i.p.

In contrast, i.v. infusion caused no detectable inflammation. We observed no structural changes or basophilic infiltrates, and MPO levels were not elevated (Fig.6A). Cytosolic oxidant activity was unchanged (doxorubicin 133 ± 10 arbitrary units vs. vehicle 122 ± 10 arbitrary units, $n = 11/\text{group}$, $p > 0.2$), as were nitrotyrosine and HNE residues on sarcomeric proteins (data not shown). We saw no evidence that i.v. infusion disrupted sarcolemmal integrity (Fig.6B).

DISCUSSION

Our study supports the hypothesis that systemic doxorubicin exposure causes respiratory muscle dysfunction. Diaphragm weakness was evident, regardless of the administration route, suggesting a possible mechanism for the dyspnea observed in clinical settings. Functional losses were exaggerated by i.p. administration which also caused tissue inflammation and injury. The latter effects were not seen after i.v. administration, demonstrating differences between the two models of chemotherapy.

Specific force of the diaphragm – force normalized for cross-sectional area – was consistently depressed by doxorubicin administration to intact animals. In contrast, superfusion of isolated single fibers with doxorubicin does not depress specific force (18). This suggests the weakness that occurred in vivo was a delayed response, was indirectly mediated, or both. Altered crossbridge dynamics is one potential mechanism by which doxorubicin could cause respiratory muscle weakness. In a rodent model of i.v. doxorubicin treatment, skinned cardiac muscle fibers showed impaired actin-myosin interactions without alterations in sarcoplasmic reticulum function (26). This was due to a decrease in the crossbridge cycling rate, both detachment and attachment processes (27). Our data show no net losses in myofibrillar protein levels or covalent modifications via HNE or NT residues. However, these findings do not rule out other posttranslational modifications, such as phosphorylation or sulfhydryl oxidation, that might also depress myofibrillar function.

Decrements in specific force were greater after i.p. than i.v. administration. Doxorubicin i.p. was associated with diaphragm inflammation and injury, effects previously seen in murine hindlimb muscles (28) and brain (12). In our study the loss of sarcolemmal integrity occurred in approximately 10 % of diaphragm fibers. This does not fully account for the 50-60% loss of specific force, suggesting contractile dysfunction in fibers that retained sarcolemmal integrity. We have previously shown that myofibrillar protein function in intact muscle fibers can be depressed by inflammatory cytokines (29,30) or oxidants (31). This is consistent with the loss of function observed following i.p. doxorubicin administration.

Doxorubicin i.p. also decreased the weight and cross-section of diaphragm fiber bundles. Loss of muscle mass confirms prior reports in the literature. Direct injection of doxorubicin into skeletal muscle causes loss of myofibers in both humans and rodents (32,33). Injection of doxorubicin into the peritoneal cavity diminishes murine limb muscle mass and causes a systemic inflammatory response (18).

The latter finding suggests a role for drug-induced peritonitis. In the current study, diaphragm histology after doxorubicin i.p. indicated localized inflammation with an abdominal source. Sarcolemmal disruption was primarily localized on or near the abdominal surface of the muscle. Also, the intensity of basophilic staining had an abdominal-to-

thoracic gradient that suggests neutrophil invasion from the abdomen. This model fits with several other observations. Doxorubicin i.p. increased tissue MPO, a marker of activated neutrophils and an enzymatic source of reactive oxygen species (34). Tissue oxidant activity rose accordingly. Elevated oxidant levels stimulate post-translational modifications to myofibrillar proteins (35) that contribute to contractile dysfunction (31). Our data confirm myofibrillar oxidation via two independent markers:

Nitrotyrosine residues are the hallmark of protein modification by peroxynitrite (36). Our data document increases in nitrotyrosine residues on desmin and tropomyosin following i.p. doxorubicin. Desmin is an intermediate filament responsible for stabilizing the sarcomere and providing connections to adjacent z lines and the subsarcolemmal cytoskeleton (37). The loss of desmin causes muscles to be weaker and more susceptible to damage. In patients, abnormal desmin distribution and accumulation occur in the gastrocnemius following isolated limb perfusion with doxorubicin (38). Nitrosylation of desmin can be found in myofibrillar myopathies such as myotilinopathies and desminopathies (39). Tropomyosin is associated with the thin filament in the sarcomere and involved in stabilization of the actin and troponin complex (40). Tropomyosin is also susceptible to nitrosylation which alters protein function (41).

HNE is a highly reactive, toxic aldehyde byproduct of lipid peroxidation that can form adducts with proteins (42). Our data show an increase in HNE residues on troponin T (TnT) and myosin light chain (MLC) in the diaphragm following i.p. doxorubicin exposure. Troponin regulates calcium activation of myofibrils during skeletal muscle contraction and is composed of three subunits: TnI, TnT, and TnC (40). Mutations or modifications of troponin alter skeletal muscle function (43). Modifications of the TnT subunit could affect the interaction of troponin with tropomyosin, altering crossbridge formation and resulting in contractile dysfunction. Similarly, MLC modulates the force of contraction, and mutations or modifications can cause dysfunction (44).

In conclusion, this study provides the first evidence that a cancer chemotherapeutic agent can cause respiratory muscle dysfunction. Our findings suggest a novel and potentially-serious side effect of cancer chemotherapy that could compromise physical performance (1) and quality of life in patients (45). Further research is needed to define the translational relevance of this observation and identify interventions to preserve respiratory muscle function in patients who receive chemotherapy.

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ABBREVIATIONS

i.p.	intraperitoneal
i.v.	intravenous
½ RT	twitch half-relaxation time
TPT	time-to-peak twitch force
HNE	4-hydroxynonenal
MPO	myeloperoxidase

Figure 1.

Intravenous (i.v.) doxorubicin administration causes contractile dysfunction of the diaphragm 72 hrs following injection. Panels show changes in (A) absolute force, (B) specific force, and (C) relative force. Data are means $\pm$ SE; $n = 9$ /group; for specific and relative force panels, $p < 0.01$ for overall difference by repeated-measures ANOVA; $*p < 0.01$ by Bonferroni test.

Figure 2.

Doxorubicin depresses diaphragm contractile function 72 hrs following intraperitoneal (i.p.) injection: (A) absolute force, (B) specific force, and (C) relative force. Data are means $\pm$ SE; $n = 11/\text{group}$; for all panels, $p < 0.01$ for overall difference by repeated-measures ANOVA; * $p < 0.01$ by Bonferroni test.

Figure 3. Doxorubicin increases DCFH oxidation in diaphragm fibers following i.p. injection. (A) Fluorescence images of vehicle (top) and doxorubicin (bottom) diaphragms 72 hrs following injection. (B) Averaged data for DCF fluorescence. Data are means $\pm$ SE; n = 13/group; *p<0.05 by Student's t-test.

Figure 4.

Doxorubicin i.p. increases nitrotyrosine and 4-hydroxynonenal (HNE) residues on myofibrillar proteins. Myofibrillar proteins isolated from diaphragms exposed to i.p. doxorubicin or vehicle. For original blots, left panels are stained for total protein, and right panels are stained for nitrotyrosine (A) or HNE (B). Averaged densitometry data depict nitrotyrosine- or HNE-conjugated protein-to-total-protein ratios. Data are means $\pm$ SE; $n = 3$ /group; * $p < 0.05$ by Student's t -test.

Figure 5.

Doxorubicin i.p. increases myeloperoxidase (MPO) content and disrupts sarcolemmal integrity. In histologic sections, arrows denote abdominal surface of diaphragm. (A) H & E stain of murine diaphragm 72 hrs after i.p. injection of vehicle or doxorubicin (left). Averaged data for MPO protein to total protein in diaphragm (right). Data are means $\pm$ SE; $n = 4/\text{group}$; $*p < 0.05$ by Student's t -test. Scale bar = 20 μm . (B) Evans blue (red) following i.p. injection 72 hrs post vehicle (left) or doxorubicin (right); DAPI used to stain nuclei (blue).

Figure 6.

Doxorubicin i.v. does not alter MPO content or sarcolemmal integrity. In histologic sections, arrows denote abdominal surface of diaphragm. (A) H & E stain of murine diaphragm 72 hrs after i.v. injection of vehicle or doxorubicin. Averaged data for MPO-protein-to-total-protein in diaphragm (right). Data are means $\pm$ SE; $n = 4$ /group. Scale bar = 20 μ m. (B) Evans blue (red) following i.v. injection 72 hrs post vehicle (left) or doxorubicin (right); nuclei stained using DAPI (blue).