Doxorubicin acts through tumor necrosis factor receptor subtype 1 to cause dysfunction of murine skeletal muscle

Laura A. A. Gilliam,1 Leonardo F. Ferreira,1 Joseph D. Bruton,2 Jennifer S. Moylan,1 Häkan Westerblad,2 Daret K. St. Clair,3 and Michael B. Reid1

1Department of Physiology, 2Graduate Center for Toxicology, University of Kentucky, Lexington, Kentucky; 3Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

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Doxorubicin acts through tumor necrosis factor receptor subtype 1 (TNFR1) to cause dysfunction of murine skeletal muscle. J Appl Physiol 107: 1935–1942, 2009. First published September 24, 2009; doi:10.1152/japplphysiol.00776.2009.–Cancer patients receiving doxorubicin chemotherapy experience both muscle weakness and fatigue. One postulated mediator of the muscle dysfunction is an increase in tumor necrosis factor-α (TNF), a proinflammatory cytokine that mediates limb muscle contractile dysfunction through the TNF receptor subtype 1 (TNFR1). Our main hypothesis was that systemic doxorubicin administration would cause muscle weakness and fatigue. Systemic doxorubicin administration (20 mg/kg) depressed maximal force of the extensor digitorum longus (EDL; P < 0.01), accelerated EDL fatigue (P < 0.01), and elevated serum TNF levels (P < 0.05) 72 h postinjection. Genetic TNFR1 deficiency prevented the fall in specific force caused by systemic doxorubicin, without protecting against fatigue (P < 0.01). These results demonstrate that clinical doxorubicin concentrations disrupt limb muscle function in a TNFR1-dependent manner.

Address for reprint requests and other correspondence: M. B. Reid, Dept. of Physiology, Univ. of Kentucky, 800 Rose St., MS-508, Lexington, KY 40536-0298 (e-mail: michael.reid@uky.edu).

DOXORUBICIN (ADRIAMYCIN) is an anthracycline antibiotic commonly prescribed for malignant cancers. Cardiotoxicity is a fatal side effect of doxorubicin-based chemotherapy that is minimized by limiting the dosage. Despite this precaution, patients undergoing doxorubicin chemotherapy experience both limb muscle weakness and fatigue (42). These debilitating symptoms can delay treatment, reducing the effectiveness of chemotherapy and increasing morbidity (11, 35).

Clinical data suggest that limb muscle may be weakened by doxorubicin. In patients who receive doxorubicin, maximal handgrip strength is depressed, indicating weakness, and the rate of handgrip fatigue is increased (42). In theory, such findings could reflect a change in neural activation, peripheral muscle dysfunction, or both. Muscle function after systemic doxorubicin treatment has not been tested directly.

One possible contributor to muscle dysfunction is the capacity of doxorubicin to elevate serum tumor necrosis factor-α (TNF) levels. TNF is a proinflammatory cytokine produced by many cell types, including cardiac and skeletal myocytes (18, 36). TNF causes contractile dysfunction of limb muscle (1, 38) by activating the TNF receptor subtype 1, or TNFR1 (21). Circulating TNF levels are elevated in patients with cancer; doxorubicin chemotherapy exacerbates this response (6, 41). Animals treated with doxorubicin show a similar increase in circulating TNF levels (44, 47). Thus the rise in circulating TNF stimulated by doxorubicin could promote muscle dysfunction through TNFR1.

Experiments using isolated muscle preparations have yielded paradoxical results. In skinned muscle fibers, short-term doxorubicin exposure (<1 h) increases calcium sensitivity of myofilaments (9) and stimulates calcium release from isolated sarcoplasmic reticulum terminal cisternae (51). These responses predict that doxorubicin will increase submaximal force, contradicting clinical reports.

The objective of this research was to define doxorubicin effects on skeletal muscle function. We hypothesized that systemic doxorubicin administration would mimic the observed clinical effects, causing weakness and fatigue in skeletal muscle. We tested this hypothesis in mice using a clinical dose of doxorubicin and measuring force of limb skeletal muscle in vitro. We observed a depression in specific force of unfatigued muscle following doxorubicin administration and a decrease in the force developed during repetitive, fatiguing contractions. To investigate whether TNF/TNFR1 signaling mediates the systemic response, we conducted the same experiments using mice deficient in TNFR1. Our results show that TNFR1 deficiency abolishes the depression in specific force of unfatigued muscle.

In contrast to the systemic model, we hypothesized that direct, short-term exposure to doxorubicin would increase tetanic force in isolated muscle preparations. We isolated limb muscle fibers, and measured tetanic [Ca2+]i and force following direct exposure to a concentration of doxorubicin that occurs in patients. We found direct doxorubicin exposure in vitro increases intracellular calcium transients, but does not change tetanic force.

MATERIALS AND METHODS

Overview of experimental design. In brief, systemic studies were conducted in mice given an intraperitoneal (ip) injection of doxorubicin (20 mg/kg), then functional measurements were made on hindlimb skeletal muscle 72 h postinjection. Isolated limb muscle fibers were used to assess the effects of direct doxorubicin (2 μM) exposure for 1 h: tetanic force and cytosolic calcium concentrations, using the fluorescence indicator indo-1, were measured. Specific details of the experiments are outlined below.

Animal care. Studies of systemic doxorubicin administration were conducted at the University of Kentucky using 6- to 8-wk-old male TNFR1 receptor-deficient mice (TNFR1−/−; B6.129-Tnfrs1aΔtm1Mak; The Jackson Laboratory, Bar Harbor, ME) and the background strain C57BL/6 as wild types. Animals were maintained in the Division of Laboratory Animal Resources facility on a 12:12-h dark/white cycle and provided food and water ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee.

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Muscles were isolated from adult male NMRI mice to study the effect of direct doxorubicin exposure on force and free cytosolic calcium concentrations ([Ca$^{2+}$]) at the Karolinska Institutet. Animals were maintained in the animal facility at the Department of Physiology and Pharmacology on a 12:12 h dark/light cycle and provided food and water ad libitum. All experiments were approved by the Stockholm North local ethical committee.

**Doxorubicin treatment.** Mice were given an intraperitoneal injection of doxorubicin (20 mg/kg; Bedford Laboratories, Bedford, OH). This dose is equivalent to doxorubicin chemotherapy given to patients with small cell lung cancer (7). The amount of doxorubicin was based on the conversion factor established by Freireich (16), which is derived from the relationship between weight and surface area of the animal. Control animals received the same volume of vehicle (phosphate buffer solution). The extensor digitorum longus (EDL) muscle was excised for functional studies 72 h after injection. Measurements of TNF in serum and muscle were made at 24-h time points following doxorubicin administration (24–72 h). To examine the direct effects of doxorubicin exposure, muscles were incubated in vitro for 1 h in Krebs-Ringer solution containing 2 µM doxorubicin, a dose that corresponds to serum levels in patients undergoing doxorubicin chemotherapy (37).

**EDL contractile function and fatigue.** Mice were anesthetized with isoflurane and killed by cervical dislocation. EDL muscles were excised and placed in Krebs-Ringer solution (in mM: 137 NaCl, 5 KCl, 1 MgSO$_4$, 1 NaH$_2$PO$_4$, 24 NaHCO$_3$, 2 CaCl$_2$) equilibrated with isoflurane and killed by cervical dislocation. EDL muscles were homogenized in 2% SDS and 0.002% Bromphenol Blue. Proteins were fractionated on a 4%–20% SDS polyacrylamide gel (Criterion pre-cast gels, Bio-Rad, Hercules, CA) and transferred to reduced-fluorescence PVDF membranes (Hercules, CA) and transferred to reduced-fluorescence PVDF membranes (Immobilon-FL, Millipore, Bedford, MA). Membranes with transferred proteins were blocked for 1 h at room temperature in Odyssey Blocking Buffer (LI-COR, Lincoln, NE). Primary antibodies were incubated overnight at room temperature in Odyssey Blocking Buffer mixed 1:1 with PBS plus 0.2% Tween. Secondary antibodies were incubated for 45 min in Odyssey/PBS/0.2% Tween plus 0.01% SDS. TNF antibody was purchased from Chemicon (Millipore, Billerica, MA). Fluorescent secondary antibodies were used for detection (goat anti-mouse Alexa-680, Molecular Probes-Invitrogen; goat anti-rabbit IRD800, Rockland Immunocchemicals, Gilbertsville, PA). 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).

**Western blot analysis.** EDL muscles were homogenized in 2× lysis buffer (20 mM Tris pH 7.2, 2% SDS) and diluted 1:1 in 2× sample buffer (120 mM Tris pH 7.5, 200 mM DTT, 20% glycerol, 4% SDS and 0.002% Bromphenol Blue). Proteins were fractionated on 15% SDS-polyacrylamide gels (Criterion pre-cast gels, Bio-Rad, Hercules, CA) and transferred to reduced-fluorescence PVDF membrane (Immobilon-FL, Millipore, Bedford, MA). Membranes with transferred proteins were blocked for 1 h at room temperature in Odyssey Blocking Buffer (LI-COR, Lincoln, NE). Primary antibodies were incubated overnight at room temperature in Odyssey Blocking Buffer mixed 1:1 with PBS plus 0.2% Tween. Secondary antibodies were incubated for 45 min in Odyssey/PBS/0.2% Tween plus 0.01% SDS. TNF antibody was purchased from Chemicon (Millipore, Billerica, MA). Fluorescent secondary antibodies were used for detection (goat anti-mouse Alexa-680, Molecular Probes-Invitrogen; goat anti-rabbit IRD800, Rockland Immunocchemicals, Gilbertsville, PA). 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).

**Intact flexor digitorum brevis fibers.** Mice were deeply anesthetized and killed by cervical disarticulation. Intact single fibers were mechanically dissected from the FDB muscle of the hindlimb as described previously (27). The isolated fiber was mounted between an Akers AE801 force transducer and adjustable holder in the perfusion channel of a muscle bath placed on the stage of an inverted microscope. Intact fibers were superfused with Tyrode solution (in mM: 121 NaCl, 5.0 KCl, 1.8 CaCl$_2$, 0.5 MgCl$_2$, 0.4 NaH$_2$PO$_4$, 24.0 NaHCO$_3$, 0.1 EDTA, and 5.5 glucose) and bubbled with 5% CO$_2$–95% O$_2$, giving an extracellular pH of 7.4. Indo-1 (10 mM, Molecular Probes Europe) was pressure injected into fibers and used to measure [Ca$^{2+}$], during tetanic contractions as previously described (3). This was
accomplished using a system consisting of a xenon lamp, a monochromator, and two photomultiplier tubes (PTI, Wedel, Germany). Indo-1 was excited at 360 ± 5 nm and emissions were measured at 405 ± 5 nm and 495 ± 5 nm. The ratio of the light emitted at 405 nm to that at 495 nm (R) was converted to [Ca²⁺], using the equation by Grynkiewicz (20):

\[
[Ca^{2+}] = \frac{K_d \beta (R - R_{\text{min}})/(R_{\text{max}} - R)}
\]

where \( K_d \) is the dissociation constant of Indo-1, \( \beta \) is the ratio of the 495 nm signals at very low and saturating [Ca²⁺], \( R_{\text{min}} \) is the ratio of very low [Ca²⁺], and \( R_{\text{max}} \) is the ratio of saturating [Ca²⁺]. The force-[Ca²⁺] relationship was established by stimulating the fiber at 1-min intervals using stimulus frequencies of 15, 20, 30, 40, 50, 70, 100, and 150 Hz, and train duration of 350 ms. The fiber was first stimulated in the absence of doxorubicin and a second time after exposure to 2 μM doxorubicin for 30 min at 24°C. Fluorescence and force signals were stored on a desktop computer system for subsequent data analysis (Felix 1.4, Photon Technology). Force data were fitted to the Hill equation:

\[
\%F_{\text{max}} = \frac{100[Ca^{2+}]^n}{[Ca^{2+}]^n + (C_{A0})^n}
\]

using computer software (SigmaPlot, San Rafael, CA) where \( C_{A0} \) is the intracellular calcium concentration required to produce half-maximal activation and \( n \) is the Hill coefficient.

Statistical analyses. Physical characteristics of intact EDL, and twitch properties were analyzed using unpaired Student’s t-tests. Differences in body weight, food and water consumption, and force-frequency curves were analyzed using two-way repeated measures ANOVA with post hoc Tukey tests. Differences in TNF measurements were analyzed using a one-way ANOVA. [Ca²⁺], values obtained at different frequencies were analyzed using Student’s t-test. Statistical calculations were performed using commercial software (SigmaStat, SPSS, Chicago, IL; Microsoft Excel, Redmond, WA).
Statistical significance was accepted when \( P \leq 0.05 \). Results are reported as means \( \pm \) SE.

RESULTS

Systemic effects of doxorubicin. Clinical doses equivalent to treatment for patients with small cell lung cancer (7) were used for in vivo studies. Systemic doxorubicin decreased body weight over 3 days (Fig. 1A). Water and food consumption were also reduced (Fig. 1, B and C). EDL muscle weight was 20% less than vehicle-injected controls (doxorubicin 7.9 \( \pm \) 0.3 vs. vehicle 9.6 \( \pm \) 0.3 mg, \( P < 0.05 \)) and estimated cross-sectional area was decreased (doxorubicin 0.56 \( \pm \) 0.03 vs. vehicle 0.66 \( \pm \) 0.02 mm\(^2\), \( P < 0.05 \)) with no obvious injury or alterations of muscle fiber size (Fig. 2). Neither wet-to-dry weight ratio (doxorubicin 4.1 \( \pm \) 0.1 vs. vehicle 4.2 \( \pm \) 0.1) nor \( L_o \) (doxorubicin 136 \( \pm \) 4 vs. vehicle 139 \( \pm \) 2 mm) was different between groups.

Function of EDL following systemic doxorubicin exposure. Figure 3A depicts absolute force of EDL muscles isolated 72 h post doxorubicin injection. Maximal absolute force was depressed 40 \( \pm \) 2% compared with controls. When normalized for cross-sectional area, specific force remained depressed at stimulus frequencies >120 Hz (Fig. 3B). Maximal specific force was decreased 28 \( \pm \) 5% in the doxorubicin-treated group. Figure 3C depicts the relative force-frequency relationships. Doxorubicin treatment shifted the curve leftward. During twitch contractions, half-relaxation time (1/2 RT) was extended in the doxorubicin-treated group (doxorubicin 10.3 \( \pm \) 1.5 vs. vehicle 7.2 \( \pm \) 1.7 ms, \( P < 0.01 \)). Time to peak twitch tension (TPT) was not altered (\( P > 0.6 \)). Stability of the muscle, reflected by a decline in \( P_o \), was not different between groups (doxorubicin 0.72 \( \pm \) 0.16 vs. vehicle 0.91 \( \pm \) 0.22 %/min, \( P > 0.4 \)).

Figure 4 depicts fatigue characteristics of muscles from doxorubicin-treated mice. Specific force fell by 59 \( \pm \) 5% within the first 30 s of the fatigue protocol and remained depressed relative to control through the first half of the protocol (Fig. 4A). Similarly, unstimulated force sustained by doxorubicin-treated muscle was depressed during the final 90 s of the fatigue protocol (Fig. 4B).

TNF/TNFR1 signaling. Circulating TNF was undetectable in control mice and elevated in doxorubicin-treated animals 24 and 72 h following doxorubicin administration (24 h: 6.2 \( \pm \) 2.7 pg/ml; 72 h: 5.1 \( \pm \) 1.2 pg/ml, \( P < 0.05 \)). Low levels of TNF

Fig. 3. Systemic doxorubicin alters contractile function of unfatigued muscle 72 h following injection. A, absolute force; B, specific force; C, relative force. Data are means \( \pm \) SE; \( n = 5 \) (vehicle) or 6 (doxorubicin); for all panels, \( P < 0.05 \) for overall difference by repeated-measures ANOVA; *\( P < 0.01 \) by Tukey test.

Fig. 4. Systemic doxorubicin increases muscle fatigue 72 h following injection. A, stimulated force; B, unstimulated force normalized for cross-sectional area. Data are means \( \pm \) SE; \( n = 5 \) (vehicle) or 4 (doxorubicin); for both panels, \( P < 0.05 \) for overall difference by repeated-measures ANOVA; *\( P < 0.01 \) by Tukey test.
mRNA and TNF precursor protein were detected in control EDL muscles but neither was altered 24, 48, or 72 h following doxorubicin administration \((P < 0.01)\). The depression in specific force caused by doxorubicin was abolished (Fig. 5B). The leftward shift of the relative force-frequency curve persisted (Fig. 5C), but prolongation of 1/2 RT was not statistically significant (doxorubicin 11.2 ± 0.9 vs. vehicle 9.7 ± 0.8 ms, \(P > 0.2\)) and TPT remained unaltered (\(P > 0.4\)). Doxorubicin accelerated fatigue of TNFR1 \(^{-/-}\) muscle (Fig. 6A) without affecting unstimulated force (Fig. 6B).

**Doxorubicin exposure in vitro.** Therapeutic concentrations of doxorubicin were used during in vitro experiments, matching serum levels in patients undergoing doxorubicin chemotherapy (10, 37). We used intact single fibers from FDB muscles to measure tetanic \([Ca^{2+}]_i\), and force following direct doxorubicin exposure (Fig. 7A). Neither tetanic \([Ca^{2+}]_i\) nor force was altered at stimulation frequencies between 15 and 100 Hz (Fig. 7, B and C). Doxorubicin did increase tetanic \([Ca^{2+}]_i\) by 37% at 150 Hz (Fig. 7C). This is a supramaximal stimulus frequency; accordingly, higher tetanic \([Ca^{2+}]_i\) transients in doxorubicin-treated fibers did not increase force (Fig. 7B). Doxorubicin had no effect on myofibrillar \(Ca^{2+}\) sensitivity (Ca\(_{50}\): doxorubicin 0.76 ± 0.11 vs. control 0.71 ± 0.13 \(\mu M\)). Similar results were obtained in studies of single fibers isolated from mouse EDL (data not shown). Finally, incubation of intact EDL muscles with doxorubicin did not alter specific force (\(P_0\): doxorubicin 63.3 ± 1.5 vs. control 60.2 ± 1.1 N/cm\(^2\)) or fatigue characteristics (data not shown).

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**Fig. 5.** Doxorubicin acts via TNF receptor subtype 1 (TNFR1) to depress specific force. Functional studies performed 72 h following injection in TNFR1-deficient (TNFR1 \(^{-/-}\) ) mice. A, absolute force; B, specific force; C, relative force. Data are means ± SE; \(n = 8/group\); for A and C, \(P < 0.01\) for overall differences by repeated-measures ANOVA; \(*P < 0.001\) by Tukey test.

**Fig. 6.** TNFR1 deficiency does not protect EDL against accelerated fatigue induced by doxorubicin. Functional studies performed 72 h following injection. A, stimulated force; B, unstimulated force normalized for cross-sectional area. Data are means ± SE; \(n = 8/group\); for A, \(P < 0.05\) for overall differences by repeated-measures ANOVA; \(*P < 0.01\) by Tukey test.
DISCUSSION

Muscle weakness and fatigue are common symptoms in cancer patients undergoing chemotherapy. Clinical reports show that patients with advanced cancer develop less limb muscle force compared with controls (42) and have impaired physical performance when undergoing doxorubicin-based chemotherapy (14). Impaired muscle function is commonly attributed to the loss of muscle mass, since cancer cachexia and muscle wasting are known clinical problems (25).

Doxorubicin caused our mice to lose weight over a 3-day period following a single injection, as confirmed by other reports (40). This same phenomenon occurs in the clinic. Tozer and colleagues (45) documented weight loss in lung cancer patients undergoing doxorubicin-based chemotherapy. One contributor to the loss in body weight is a decrease in food and water consumption by patients (15), as seen in our rodent model. This decrease in sustenance can promote catabolism and muscle wasting, both of which occur during chemotherapy. Decrements in femoral quadriceps muscle thickness are detectable by ultrasound within the first 4 wk of chemotherapy (24). A similar loss of muscle mass was evident in EDL muscles of our mice and contributed to the drop in absolute force. One possible cause of doxorubicin-induced dysfunction is tissue edema, which depresses specific force by increasing muscle cross section. Others have reported that systemic doxorubicin administration causes interstitial edema in murine gastrocnemius muscle (12). However, we observed no changes in wet-to-dry weight ratio or fiber cross section in the EDL muscle, arguing against significant edema in our model. An alternative mechanism is loss of contractile regulation secondary to TNFR1 activation. In the absence of doxorubicin, TNF/TNFR1 signaling has been shown to depress specific force (21), a response that closely resembles doxorubicin-stimulated dysfunction in the current study.

Doxorubicin increases circulating TNF in patients undergoing chemotherapy (6) and rodent models of chemotherapy (29, 43) and also stimulates TNF expression by cardiac muscle (36). We found that doxorubicin increases circulating TNF without altering TNF expression by skeletal muscle. This argues against an autocrine or paracrine action of muscle-derived TNF. Instead, circulating TNF appears to function as an endocrine mediator.

TNF exerts the majority of its biological actions through the TNFR1 receptor subtype. TNF binding activates the receptor’s apoptotic death domain to stimulate inflammatory and stress responses. These include increased protein turnover during cancer cachexia (31) and depression of specific force in skeletal muscle (21). TNF also stimulates oxidant production by skeletal muscle (28, 32) via a TNFR1-dependent mechanism (21). Antioxidant pretreatment opposes TNF-stimulated dysfunction (21, 28), identifying muscle-derived oxidants as essential second messengers in this response.

Oxidants can disrupt the contractile process by affecting myofibrillar proteins (4, 26) or calcium homeostasis (46, 49). Direct exposure of intact single fibers to TNF depresses spe-
cific force without altering tetanic calcium transients or resting calcium concentrations (38). This finding suggests TNF-stimulated oxidants act downstream of the calcium signal, i.e., on the myofibrillar lattice. A myofibrillar target has been confirmed by experiments using permeabilized muscle fibers from TNF-treated animals; functional studies showed that calcium-activated force was depressed with no change in calcium sensitivity or cross-bridge cycling rate (21). Similar events may mediate the TNFRI-dependent weakness stimulated by doxorubicin. Studies of permeabilized fibers from doxorubicin-treated animals is a logical next step to define the underlying mechanism.

Loss of specific force does not appear to be a direct response to doxorubicin at clinically relevant concentrations. Our in vitro experiments showed that direct doxorubicin exposure increases [Ca²⁺], during tetanic stimulation. This increase was only evident at supramaximal stimulus frequencies and therefore had no effect on tetanic force. van Norren and colleagues (48) observed a significant depression in absolute force of the EDL following in vitro exposure to doxorubicin. These results were obtained using bath concentrations 35- to 75-fold higher than therapeutic serum levels in patients (10, 37) and must be interpreted with caution.

Fatigue is a debilitating symptom of cancer chemotherapy (35, 42). In our murine model, doxorubicin promoted an early onset of fatigue in electrically stimulated muscle that was not prevented by TNFR1 deficiency. Specific force fell by ~60% within the first 30 s in doxorubicin-treated mice. One possible mechanism behind this initial drop in force is inhibition of skeletal muscle myosin light chain kinase (skMLCK). skMLCK phosphorylates myosin regulatory light chain, increasing the rate at which cross bridges enter a force-producing state and contributing to post-tetanic potentiation (50). Accordingly, genetic deficiency of skMLCK abolishes post-tetanic potentiation following repeated contractions (50). In cardiac muscle, doxorubicin treatment decreases activity of the smooth muscle isoform of MLCK (13). An analogous action in skeletal muscle, decreasing skMLCK activity, could account for early fatigue of the EDL. However, fatigue is a complex process mediated by multiple, concurrent mechanisms (2). Systematic research will be required to define the cause of premature fatigue induced by doxorubicin.

The doxorubicin dose administered to mice in our experiment is based on clinical concentrations (7). It was calculated using a standard conversion factor derived from the relationship between weight and surface area of the animal (16). The current dose is designed to achieve circulating doxorubicin levels similar to those found in patients. Johansen (22) measured circulating levels of doxorubicin in mice after a 12 mg/kg intraperitoneal injection. They found levels similar to standard clinical values: ~1.25 μg/ml in mice vs. 1 μg/ml in patients (37). Extrapolating from our 20 mg/kg dose, serum levels in our animals are expected to be comparable, i.e., ~2 μg/ml. At clinical doses, doxorubicin causes muscle weakness (42) that is not selective for individual muscles or fiber types. In both animals and patients, doxorubicin treatment results in loss of muscle mass that is independent of fiber type. Rats that received unilateral intramuscular injections of doxorubicin exhibited marked fiber atrophy but no differences in fiber type composition compared with noninjected contralateral muscle (33). In 60% of patients, limb muscles exposed to doxorubicin by isolated limb perfusion showed a significant decrease in diameter of both types I and II muscle fibers compared with pretreatment measurements (5).

In summary, this study is the first to show that systemic administration of doxorubicin at clinical doses depresses specific force of skeletal muscle. Our results show that doxorubicin causes muscle weakness and fatigue, independent of atrophy and that TNFR1 deficiency protects against doxorubicin-induced weakness. Currently there is no clinical treatment to prevent weakness and fatigue caused by cancer chemotherapy, a limitation in daily life (14). The present findings identify TNF/TNFRI signaling as a novel target for future interventions to improve the quality of life for patients and increase the effectiveness of cancer treatment.

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