HHS Public Access

Author manuscript

Hypertension. Author manuscript; available in PMC 2022 May 05.

Published in final edited form as:

Hypertension. 2021 May 05; 77(5): 1581–1590. doi:10.1161/HYPERTENSIONAHA.120.16759.

Tumor necrosis factor alpha-mediated inflammation and remodeling of the extracellular matrix underlies aortic stiffening induced by the common chemotherapeutic agent doxorubicin

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Abstract

Aortic stiffening is a major independent risk factor for cardiovascular diseases, kidney dysfunction and cognitive impairment. Doxorubicin (DOXO) chemotherapy-treated cancer survivors have greater aortic stiffness relative to healthy controls, but the mechanisms by which DOXO induces arterial stiffening are unknown. We tested the hypothesis that DOXO increases aortic stiffness by increasing intrinsic mechanical wall stiffness due to pro-inflammatory signaling-induced adverse structural changes, including collagen deposition (fibrosis), elastin fragmentation and/or formation of advanced glycation end products (AGEs). *In vivo* aortic stiffness (assessed via aortic pulse-wave velocity [PWV]), aortic intrinsic wall stiffness (*ex vivo* assessment of elastic modulus [EM]) and potential underlying mechanisms were assessed 4 weeks after administration of DOXO (10 mg/kg) or vehicle (saline) in young adult male C57BL6/J mice. Aortic PWV increased by ~30% following DOXO (Pre: 341±18 vs. Post: 431±28 cm/s, mean±SEM, P=0.001) and aortic EM was ~100% higher following DOXO (5438±445 kPa) vs. vehicle (2659±433 kPa) (P=0.003). These effects of DOXO were associated with an ~3-fold greater formation of AGEs (P=0.01) and an ~50% reduction in elastin (P=0.01), whereas collagen deposition was unaffected. DOXO increased aortic pro-inflammatory cytokines (P=0.03) without a compensatory increase in the anti-inflammatory cytokine interleukin-10. Direct *ex vivo* exposure of aorta rings to DOXO mimicked the increase in aortic EM observed *in vivo* with DOXO, whereas tumor necrosis factorα (TNFα) inhibition prevented this response. DOXO induces aortic stiffening *in vivo* due in part to an increase in intrinsic wall stiffness associated with elastin degradation and AGES formation and mediated by TNFα-dependent vascular inflammation.

Summary

We show that doxorubicin-induced aortic stiffening is mediated by an increase in intrinsic mechanical wall stiffness associated with elastin degradation and accelerated formation of

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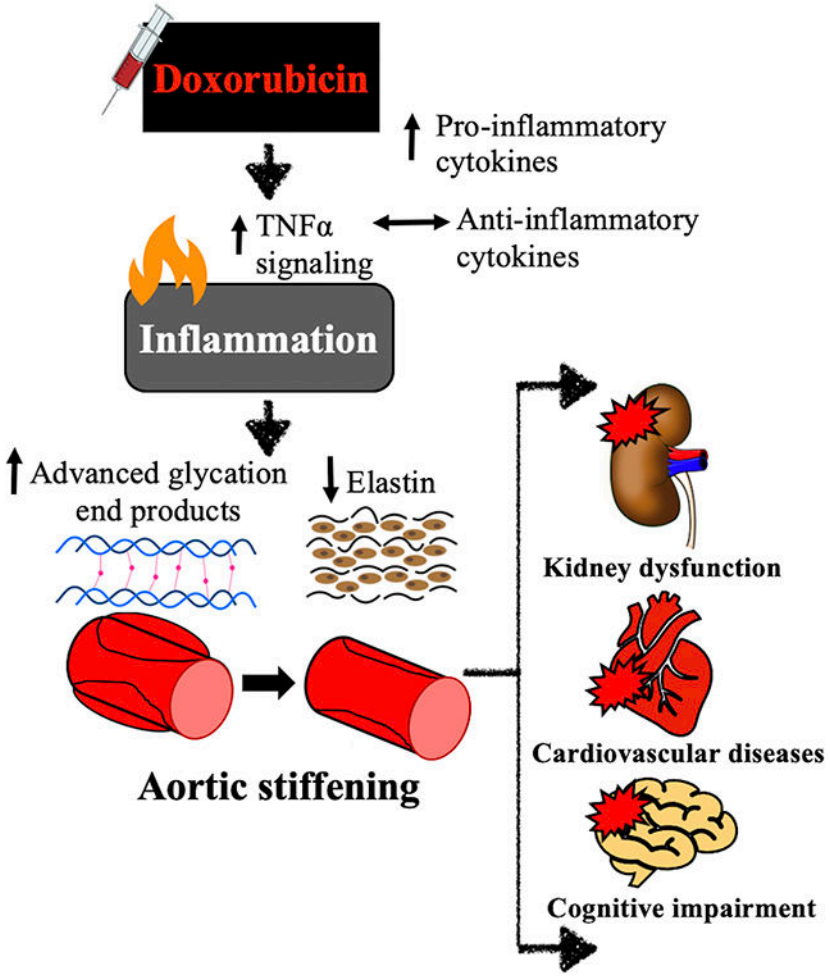
Disclosures: Authors have no conflicts of interest.

advanced glycation end products linked to tumor necrosis factor alpha-related vascular inflammation. Our results identify several potential therapeutic targets for reducing cardiovascular risk associated with aortic stiffening in cancer patients treated with anthracycline-based chemotherapy.

Graphical Abstract



Keywords

anthracyclines; Adriamycin; aortic pulse-wave velocity; elastic modulus

INTRODUCTION

The National Cancer Institute estimates 2 million new cancer cases and 600,000 cancer-related deaths in the United States in 2021[1]. Approximately 650,000 people undergo chemotherapy treatment annually[2]. In many cases, these treatments are effective at treating the cancer, but damage the cardiovascular system of the surviving patients[3]. As a result, cardiovascular diseases (CVD) are a leading cause of later morbidity and mortality among

chemotherapy-treated cancer survivors[4]. A major cause of chemotherapy-associated increases in CVD risk in cancer survivors stems from use of anthracyclines[5]. Anthracyclines are a class of chemotherapeutics used to treat several common cancers, including leukemias and lymphomas, that elicit particularly toxic effects on the CV system[6, 7]. Doxorubicin (DOXO) is the most commonly administered anthracycline[8], and its use markedly increases the risk of subsequent CVD[9].

Much of the CVD-related mortality associated with DOXO is linked to clinical atherosclerotic diseases and stroke[10–13], and the major antecedent of these CV disorders is arterial dysfunction[14]. A key feature of arterial dysfunction is, in turn, aortic stiffening[15, 16], which is also a major risk factor for cognitive dysfunction, Alzheimer's Disease, chronic kidney disease and many other chronic disorders[17–19]. Aortic stiffness is determined in part by the intrinsic stiffness of the arterial wall, which is influenced by the composition of the extracellular matrix, including the abundance of the main structural proteins -- collagen and elastin -- and the presence or absence of advanced glycation end products (AGEs), which form cross-links between structural proteins[20–22]. Cellular processes such as chronic low-grade inflammation can stimulate changes in these determinants of arterial stiffness[23]. DOXO-treated cancer survivors develop greater aortic stiffness compared with age-matched healthy controls, as indicated by higher carotid-femoral pulse wave velocity (PWV)[5, 24–26]. However, the mechanisms by which DOXO induces aortic stiffening have not been systematically investigated.

Here we use complementary experimental approaches to investigate the cellular and molecular mechanisms underlying DOXO treatment-evoked aortic stiffening. We first determined the temporal development of aortic stiffening following DOXO treatment *in vivo* by measuring aortic PWV in young adult mice. Using this model, we established that DOXO induces aortic stiffening within 4 weeks of administration compared with vehicle-treated controls. Next, we used an *ex vivo* stress-strain model to show that the increase in aortic PWV with DOXO is accompanied by a corresponding increase in intrinsic mechanical wall stiffness, and that direct incubation of aorta rings from untreated animals with DOXO induces a similar effect. We then show that these increases in aortic stiffness are associated with changes in the extracellular matrix of the aortic wall featuring elastin degradation and increases in the formation of AGEs. Finally, we demonstrate that the aortas of DOXO-treated mice show increased abundance of pro-inflammatory cytokines, most predominantly tumor necrosis factor α (TNFα), in the absence of compensatory induction of anti-inflammatory cytokines, and that inhibition of TNFα prevents DOXO-stimulated increases in intrinsic mechanical wall stiffness. Overall, our results extend current insight into the pathophysiological mechanisms by which DOXO administration induces aortic stiffening and identify potential therapeutic targets for preventing arterial stiffness in DOXO-treated cancer survivors.

METHODS

All data presented in this article and in the Online Supplement will be made available upon reasonable request to the corresponding author.

Animals

All animal protocols were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee (protocol #2618) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 61 male C57BL/6 J mice were obtained for these studies from Jackson Laboratories (mice received at 3 months of age). Male mice were selected as male C57BL/6 mice are an established model or arterial stiffness in response to various stressors (e.g. aging[27, 28] and Western-style diet[29]). All mice were housed in a conventional facility on a 12-hour light/dark cycle, given *ad libitum* access to rodent chow and drinking water, and allowed to acclimate to our facility for 4 weeks before the start of any testing. Mice that were assigned to receive *in vivo* injections of vehicle or DOXO were single housed for the duration of the study to prevent indirect exposure of DOXO, as DOXO and its primary metabolites are secreted in the urine and feces for up to ~96 hours following injection[30]. Food (Envigo 7917) and water intake were assessed every other day for the duration of the study and averaged. Energy intake was calculated my multiplying food intake (g) by kcal/g of the diet.

All mice were euthanized by exsanguination while maintained under anesthesia (inhaled isoflurane). The thoracic aorta was excised, dissected free of surrounding tissue, sectioned and stored appropriately for later stress-strain testing to assess aortic intrinsic mechanical stiffness, protein abundance by WES capillary electrophoresis-based immunoblotting (Protein Simple, San Jose, CA) and concentrations of pro-inflammatory cytokines. Investigators were blinded to treatment group for data collection and biochemical analyses. Details on all procedures, antibodies, and kits used are provided in the Online Supplement.

Aortic pulse wave velocity and blood pressure

Aortic PWV was assessed using Doppler ultrasound, as we have previously described[27, 28, 31, 32]. Briefly, mice were anesthetized via inhaled isoflurane (2.5–3%) and positioned supine on a warmed platform with paws secured to electrocardiogram leads. Doppler probes were placed at the transverse aortic arch and abdominal aorta to detect pulse waves. Three consecutive 2-second recordings were made for each animal and used to determine the time delay between the electrocardiogram R-wave and the foot of the Doppler signal for each site ($\Delta time_{abdominal}$ and $\Delta time_{transverse}$). Aortic PWV was then calculated as: aortic PWV = (physical distance between the two probes)/($\Delta time_{abdominal}$ minus $\Delta time_{transverse}$) and reported in centimeters per second. *In vivo* blood pressure was measured in the second cohort of mice at baseline and 4 weeks following injection of DOXO or vehicle on three consecutive days using a non-invasive tail-cuff method (CODA; Kent Scientifc, Torrington, CT), as we have previously described[27, 28, 31, 32].

DOXO administration

At 4 months of age, mice received a single injection of DOXO (10 mg/kg intraperitoneal injection; n = 14) or vehicle (intraperitoneal injection of saline; n = 10). Groups were matched for baseline body weight and aortic PWV. This method of administration causes cardiac dysfunction in 4-month-old male C57BL/6 J mice[33]. Estrogen may be protective against DOXO-induced cardiac dysfunction[34]; thus, only male mice were used in the present study to determine the mechanism by which DOXO causes aortic stiffening without the

confounding effects of female sex hormones. All mice were sacrificed 4 weeks following treatment, with the exception of the pilot longitudinal cohort of animals.

Statistical analyses

Detailed descriptions of all statistical analyses performed are provided in the Data Supplement. Data are presented as mean ± SEM in text, figures, and tables unless specified otherwise. Statistical significance was set to α=0.05. All statistical analyses were performed using Prism, version 8 (GraphPad Software, Inc, La Jolla, CA).

RESULTS

Animals

Four-month old C57BL/6J mice were administered DOXO (single 10 mg/kg intraperitoneal injection; in sterile saline) or vehicle (body weight to volume-matched intraperitoneal injection of sterile saline). In C57BL/6J mice, this age is equivalent to ~25 years of age in humans[35], which falls within the adolescent and young adult (AYA) age range for cancer (15–39 years of age) – a common age range for diagnosis of lymphoma and leukemia[36, 37] and subsequent treatment with DOXO[37].

After 4 weeks post injection, mice that received DOXO had lower body mass, energy intake, water intake, and quadricep skeletal muscle and epididymal white adipose mass as observed previously in rodent models[38, 39] and similar to the effects of DOXO chemotherapy in humans[40, 41]. DOXO did not alter spleen weight, aortic diameter or blood pressure (Table S1).

Aortic stiffness

Patients who receive DOXO have higher aortic stiffness (carotid-femoral PWV) relative to age-matched untreated controls[24–26], but the underlying mechanisms are unknown. First, we first conducted a pilot study to determine the temporal response of aortic stiffening after DOXO treatment assessed *in vivo* using serial non-invasive measurements of aortic pulse wave velocity [PWV] and found that aortic PWV peaks 4 weeks post-injection, and remains elevated, in young adult (5 mo) male C57BL/6 J mice [Figure S1]).

Guided by the results of this pilot study, in a second cohort of young adult (4 months of age) male C57BL/6 J mice, we next determined aortic PWV prior to and 4 weeks following a single injection of DOXO or vehicle and assessed several potential mechanisms of action. Prior to the injections (Pre) there were no differences in aortic PWV between the DOXO- and vehicle-treated groups. However, 4 weeks following the injections (Post), mice that received DOXO had a 30% increase (P = 0.004) in aortic PWV, while mice that received the vehicle showed no change (Figure 1). These data are consistent with observations in humans that DOXO chemotherapy increases carotid-femoral PWV by ~30% at 4 weeks following treatment[5].

Intrinsic stiffness of the aortic wall

To investigate whether increased aortic PWV caused by DOXO is associated with increased intrinsic mechanical stiffness of aortic wall, we measured the elastic modulus of aorta rings isolated from mice treated with vehicle or DOXO. Elastic modulus is the strain on the arterial wall in response to a given stress (stretch), which is indicative of the intrinsic mechanical stiffness of the aorta[27, 28, 31, 32]. Figure 2 shows representative stress-strain curves and group average aortic elastic moduli from vehicle- and DOXO-treated mice. The aortic elastic modulus was ~100% greater ($P = 0.003$) in DOXO- vs vehicle-treated mice, indicating that DOXO increases the intrinsic stiffness of the aortic wall.

Direct effect of DOXO on aortic stiffening

To determine whether DOXO directly affects aortic stiffening, i.e., independent of changes in the circulating plasma and endovascular milieu, thoracic aorta rings (1 mm in length) from young adult (4 months of age) untreated C57BL6/J male mice were incubated with = medium (DMEM + 10% fetal calf serum + 1% penicillin-streptomycin) containing vehicle or DOXO (1 μM) for 72 hours (2 rings for each condition per mouse), an experimental paradigm previously utilized by our laboratory to systemically interrogate mechanisms underlying changes in aortic stiffness in response to various treatments[42, 43]. 1 μM was previously shown to be the peak concentration of DOXO in plasma following administration in humans[44]. Intrinsic mechanical wall stiffness was assessed after the 72-hour incubation period. Similar to *in vivo* treatment with DOXO, *ex vivo* DOXO exposure increased the aortic elastic modulus by ~2-fold ($P = 0.03$ vs. vehicle) (Figure 3), suggesting that DOXO directly increases aortic stiffening independent of potential changes in circulating factors or other *in vivo* processes that might have occurred with *in vivo* treatment.

Effects of DOXO on arterial wall structural proteins and advanced glycation end products

Aortic stiffening is increased by collagen deposition[31] and/or cross-linking by advanced glycation end products (AGEs)[43], and the latter can occur independent of changes in collagen abundance[45]. Elastin degradation (reduced elastin abundance) also contributes to aortic stiffening in various settings as this is the structural protein that confers elasticity to the arterial wall[46]. Therefore, we assessed the abundance of collagen, AGEs and elastin in aortic lysates from DOXO- and vehicle-treated mice by immunoblotting. Aortic collagen was unchanged after DOXO treatment ($P = 0.89$) (Figure 4A). In contrast, aortic AGEs were ~3-fold higher ($P = 0.012$) (Figure 4B) and aortic elastin was ~2.5-fold lower ($P = 0.011$) (Figure 4C) after DOXO treatment. Next, we aimed to determine whether the DOXO-mediated elastin degradation and increased formation of AGEs were associated with the corresponding DOXO-associated increases in aortic PWV and elastic modulus. Linear regression analyses revealed that DOXO-induced aortic elastin degradation was indeed related to the increases in aortic PWV ($P = 0.007$; Figure S2A) and aortic elastic modulus ($P = 0.003$, Figure S2C), as were the DOXO-associated increases in aortic AGEs ($P = 0.002$ vs. change in aortic PWV, Figure S2B; $P = 0.0007$ vs. change in elastic modulus, Figure S2D). These results suggest that DOXO induces aortic stiffening, at least in part, by degrading elastin and increasing AGEs-related cross-linking.

Effect of DOXO on aortic inflammatory cytokines

We have previously demonstrated an association between higher aortic inflammatory cytokine abundance and aortic stiffening[28, 47, 48]. Furthermore, DOXO stimulates inflammation systemically[39] and in specific tissues, including CV tissues[49]. Therefore, we asked if higher levels of inflammatory cytokines are associated with DOXO-induced aortic stiffening. To address this, we assessed pro- and anti-inflammatory cytokines in aortic lysates from DOXO- and vehicle-treated mice by multiplex ELISA. Indeed, aortas from mice that received DOXO had higher protein concentrations of the pro-inflammatory mediators IFNγ ($P = 0.04$) (Figure 5A), IL-1β ($P = 0.03$) (Figure 5B), IL-2 ($P = 0.03$) (Figure 5C), IL-6 ($P = 0.04$) (Figure 5D), and TNFα ($P = 0.04$) (Figure 5E), without a compensatory upregulation in the anti-inflammatory cytokine, IL-10 (Figure 5F). Among the pro-inflammatory cytokines, TNFα appeared to be most strongly stimulated by DOXO treatment.

Direct effect of TNFα on DOXO-induced arterial stiffening

Our interrogation into DOXO-induced changes in aortic inflammation demonstrated that TNFα was most influenced by DOXO. Accordingly, we next asked whether TNFα is a major regulator of aortic stiffening by DOXO. We incubated aorta rings (1 mm in length) from a separate cohort of intervention-naïve young adult (5 mo old) C57BL/6 J male mice in the following conditions: 1) vehicle (in culture medium); 2) vehicle + the TNFα inhibitor C87[50, 51] (20 μM); 3) DOXO (1 μM in culture medium); and 4) DOXO + C87. Notably, C87 directly binds to TNF and prevents its interaction with the TNF receptor[52]. After 72 hours, we assessed the intrinsic mechanical stiffness of the aortic wall as described above. Similar to our previous mouse cohort, DOXO increased ($P = 0.004$) the elastic modulus by ~2-fold and C87 completely prevented this increase ($P = 0.002$, DOXO vs. DOXO + C87; $P = 0.41$ vehicle vs. DOXO + C87). There were no differences ($P = 0.41$) between vehicle and vehicle + C87, suggesting there were no off-target effects of C87 (Figure 6).

DISCUSSION

Aortic stiffening is a key antecedent to the development of clinical CVD, including hypertension[53], atherosclerotic occlusive diseases[54] and stroke[55], and is an independent predictor of CV-related mortality[56]. Aortic stiffening is also a major risk factor for chronic kidney disease[19] and is strongly implicated in impaired cognitive function and risk of Alzheimer's disease and other dementias[17, 18]. DOXO-treated cancer survivors have greater aortic stiffness relative to age-, sex- and CV risk factor-matched healthy individuals and demonstrate higher prevalence of the above-mentioned arterial stiffening-related chronic disorders with advancing age[24–26]. As such, it is clinically important that we understand the mechanisms of DOXO treatment-associated aortic stiffening in order to identify the processes involved that can be targeted therapeutically. However, to date, the mechanisms underlying DOXO-induced aortic stiffening have not been systemically investigated.

In the present study, we used complementary *in vivo* and *ex vivo* translational models of aortic stiffness to gain insight into these issues. Our major findings are that DOXO induces aortic stiffening *in vivo* at least in part by increasing intrinsic mechanical wall stiffness. The

latter is, in turn, associated with a marked increase in AGEs formation and elastin degradation, as well as aortic inflammation in the absence of an obvious compensatory anti-inflammatory response. Finally, the increase in intrinsic wall stiffness with DOXO is prevented by inhibition of TNFα. Overall, these findings provide new evidence regarding the mechanisms by which DOXO increases aortic stiffness and identify several possible therapeutic targets for future treatment strategies.

Implications for management and treatment of aortic stiffening after DOXO chemotherapy

At this time, there are no guidelines in place to monitor aortic stiffness during or following DOXO chemotherapy treatment. However, a recent meta-analysis on the topic of DOXO chemotherapy and aortic stiffness concluded that that non-invasive approaches, including assessment of carotid-femoral PWV, should be used to monitor changes in vascular health during treatment and throughout survival to better understand the risk of CVD and other chronic disorders associated with DOXO[25].

The present results address current research gaps by providing experimental evidence for potential targets of future therapies aimed at preventing/treating aortic stiffening with DOXO. Specifically, our results suggest that lifestyle and pharmacological strategies that inhibit AGEs formation, elastin degradation and TNFα signaling-dependent vascular inflammation are among the candidate targets for mitigating DOXO treatment-associated aortic stiffening. Of these therapeutic targets, TNFα inhibitors may be the most promising, as etanercept, adalimumab and infliximab all are presently in clinical use and have shown efficacy for reducing arterial stiffness in other groups, including patients with chronic inflammatory disorders and estrogen-deficient postmenopausal women free of clinical disease[57–60]. However, it should be noted that in a preclinical model of atherosclerosis, the TNFα inhibitor CNTO5048 was reported to paradoxically exacerbate vascular inflammation[61].

Other lifestyle and pharmacological interventions aimed at reducing vascular inflammation, including voluntary aerobic exercise[62], energy restriction[63, 64], salicylate treatment[65, 66], statin therapy[67], and dietary supplementation with both mitochondrial antioxidants[31, 68] and NAD^+ boosting compounds[69, 70] reduce arterial stiffness in healthy older adults and also may be effective for mitigating DOXO-mediated aortic stiffening, which may be viewed as a condition of accelerated arterial aging. Inhibition of AGEs formation and crosslinking are effective in reducing arterial stiffness in preclinical models[71–73] but these compounds are not used clinically at this time. Taken together, there are currently a variety of lifestyle and pharmacological strategies that may be effective for reducing arterial stiffening in patients that have undergone DOXO chemotherapy treatment. Establishing the efficacy of these approaches represents an important future goal in cardiovascular-oncology research.

The mouse as a translational model of DOXO-induced aortic stiffening

In general, assessment of aortic stiffness using aortic PWV in C57BL/6 mice is a well-established translational model of studies of carotid-femoral PWV in humans[27, 74]. The results of the present study extend the use of this model to establish DOXO-treated young adult mice as a translational model of DOXO-induced aortic stiffening in clinical settings.

Carotid-femoral PWV is reported to be ~30% higher several weeks following DOXO administration in cancer patients[5], i.e., the same magnitude of increase we observed in aortic PWV in the present study. Thus, our findings also provide the first evidence supporting the validity of C57BL/6 mice as a translational model for studying the underlying mechanisms of DOXO-induced aortic stiffness clinically. Further refinement of this mouse model to facilitate more clinically relevant translation to cancer patients include the use of a tumor-bearing mice to model human cancer and the administration of DOXO intravenously in smaller consecutive doses over time, as is used medically[25].

Perspectives

Here, we demonstrate for the first time that DOXO-induced aortic stiffening is mediated, at least in part, by enhanced TNFα-dependent pro-inflammatory signaling without an obvious compensatory anti-inflammatory response. These events trigger pathophysiological changes to the extracellular matrix of the aortic wall featuring a marked increase in the formation of AGEs and accelerated degradation of elastin in the absence of augmented collagen deposition. Collectively, these changes substantially increase the intrinsic mechanical stiffness of the wall of the aorta and result in a physiologically and clinically meaningful aortic stiffening *in vivo*, as indicated by an increase in aortic PWV similar to that observed in DOXO-treated cancer patients. Lifestyle strategies or targeted pharmacological therapies that suppress pro-inflammatory signaling in arteries may be effective strategies for preventing and/or treating DOXO chemotherapy-induced aortic stiffening, which could ultimately decrease the risk for CVD, kidney dysfunction and cognitive impairment in this patient population.

Future preclinical studies should consider other experimental approaches to facilitate translation to patient populations including: 1) studying female animals; 2) administering DOXO intravenously in smaller consecutive doses over time to more closely mimic clinical therapy; 3) assessing cardiac function in parallel with aortic stiffness; and 4) extending the age-range to older animals given the common use of DOXO in middle-aged and older patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Authors thank Marissa Burnsed-Torres, Jill Miyamato-Ditmon, Nicholas VanDongen and Nathan Greenberg for assistance with data collection, and Dr. Brad Fleenor for consultations regarding the assessment of aortic elastic modulus.

Sources of Funding: R01 AG055822 (D.R.S.; J.C.; and S.M.); T32 DK007135 (Z.S.C.); and F32 HL151022 (Z.S.C.).

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Novelty and Significance

What Is New?

- Using complementary *in vivo* and *ex vivo* translational models, we systematically identified key mechanisms underlying doxorubicin-mediated aortic stiffening.
- We demonstrate a clear role of increased intrinsic wall stiffness associated with remodeling of the extracellular matrix and tumor necrosis factor alpha-related increases in vascular inflammation as key mechanisms driving aortic stiffening with doxorubicin.

What Is Relevant?

- Aortic stiffening is a major antecedent and key initiating step in the development of cardiovascular diseases, including hypertension.
- Doxorubicin chemotherapy treatment causes aortic stiffening, but the underlying mechanisms have not been systematically investigated.
- This study provides evidence for increased tumor necrosis factor alpha signaling as a potentially important upstream mechanism in doxorubicin-induced aortic stiffening that could be targeted to improve vascular health and reduce cardiovascular disease risk in anthracycline-treated cancer survivors.

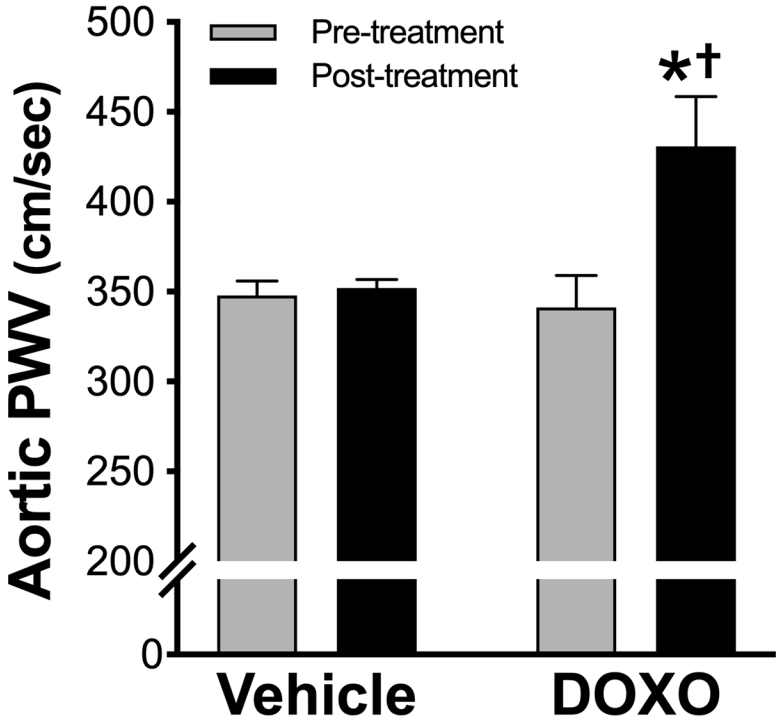

Figure 1. ***In vivo*** **aortic pulse-wave velocity (PWV) is increased in young mice four weeks following a single injection of Doxorubicin.**

n = 8/group. Data are expressed as mean ± SEM. *$P < 0.05$ vs. vehicle; $^{\dagger}P < 0.05$ vs. Pre-treatment within group.

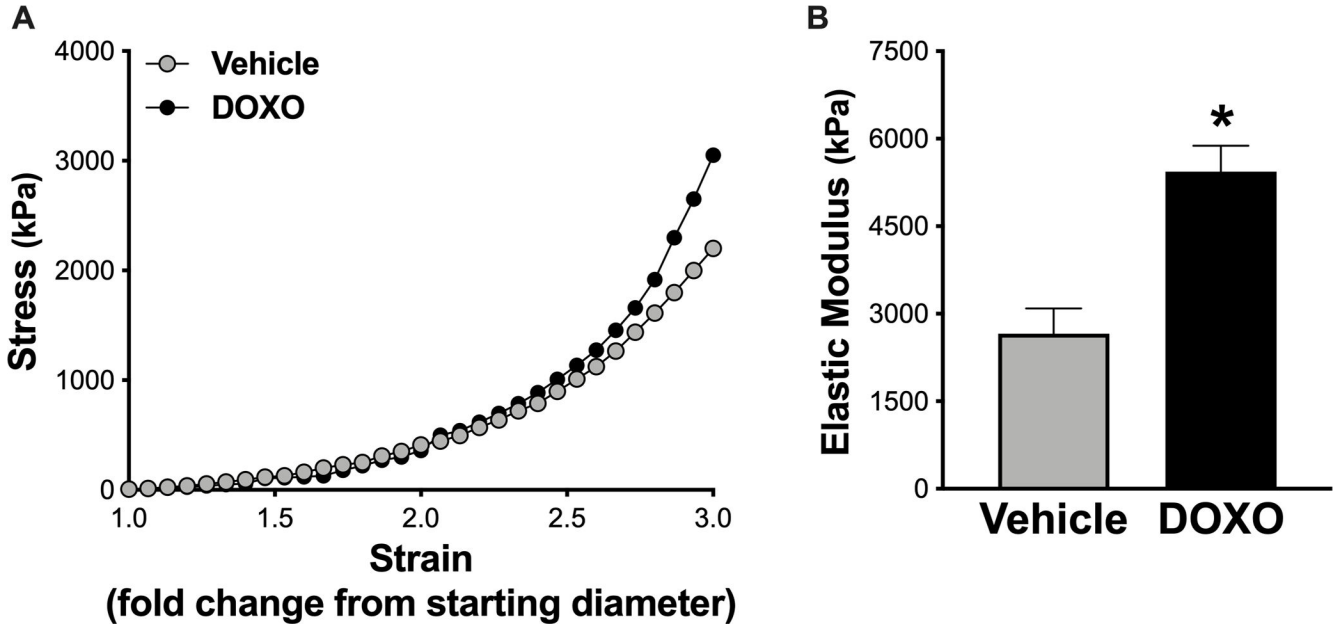

Figure 2. ***Ex*** **vivo aortic elastic modulus (intrinsic mechanical stiffness) is higher in mice treated with Doxorubicin (DOXO).**

A) Representative stress-strain curve of an aortic ring from vehicle- and DOXO-treated mice for determination of *ex vivo* elastic modulus. **B)** Aortic elastic modulus in vehicle and DOXO-treated mice. n = 8/group. Data are expressed as mean ± SEM. **P* < 0.05 vs. vehicle.

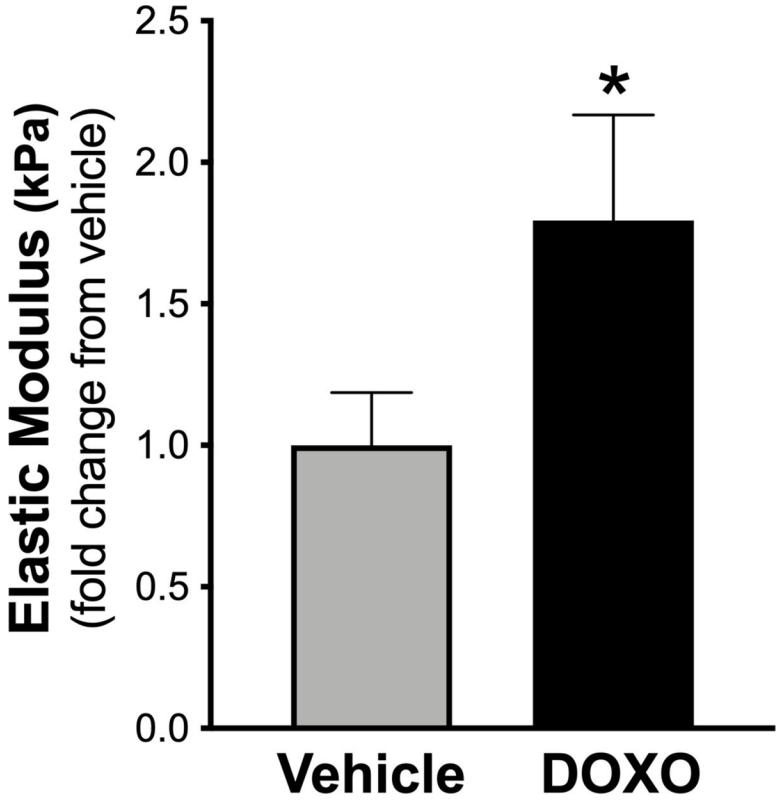

Figure 3. Direct exposure of aorta rings with Doxorubicin (DOXO) increases the elastic modulus (intrinsic mechanical stiffness).

Direct exposure of aorta rings with vehicle (DMEM + 10% fetal calf serum + 1% Penicillin-Streptomycin) or 1 μM DOXO (in vehicle). n = 10/condition. Data (mean ± SEM) in the DOXO condition is expressed as fold-change relative to vehicle. *$P < 0.05$ vs. vehicle.

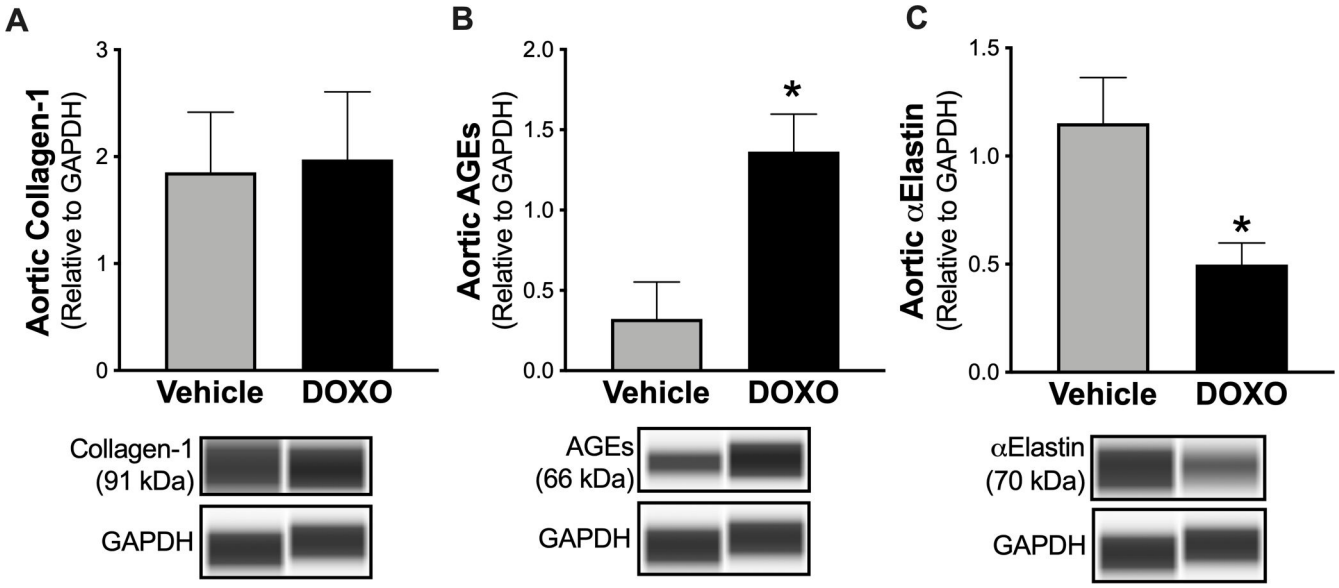

Figure 4. ***In vivo*** **administration of Doxorubicin (DOXO) results in higher advanced glycation end products (AGEs), and lower elastin abundance in the aorta.**

Aortic abundance of **A)** collagen-1, **B)** AGEs, and **C)** α-elastin in mice treated with vehicle or DOXO. n = 10/group. Data are expressed as mean ± SEM. **P* < 0.05 vs. vehicle.

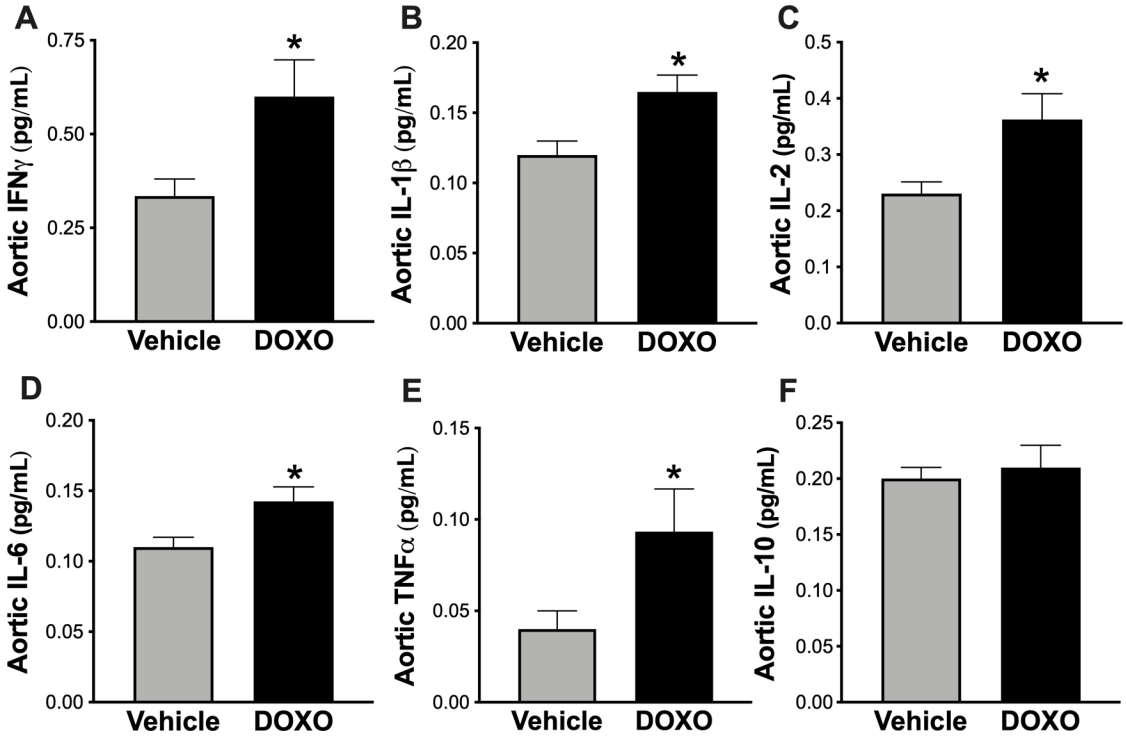

Figure 5. Greater abundance of pro-inflammatory, but not anti-inflammatory cytokines in the aorta of Doxorubicin (DOXO) treated mice.

Aortic abundance of **A)** interferon-gamma (IFNγ), **B)** interleukin-1β (IL-1β), **C)** interleukin-2 (IL2), **D)** interleukin-6 (IL-6), **E)** tumor necrosis factorα (TNFα), and **F)** interleukin-10 (IL10) in mice treated with vehicle or DOXO. n = 8/group. Data are expressed as mean ± SEM. *$P < 0.05$ vs. vehicle.

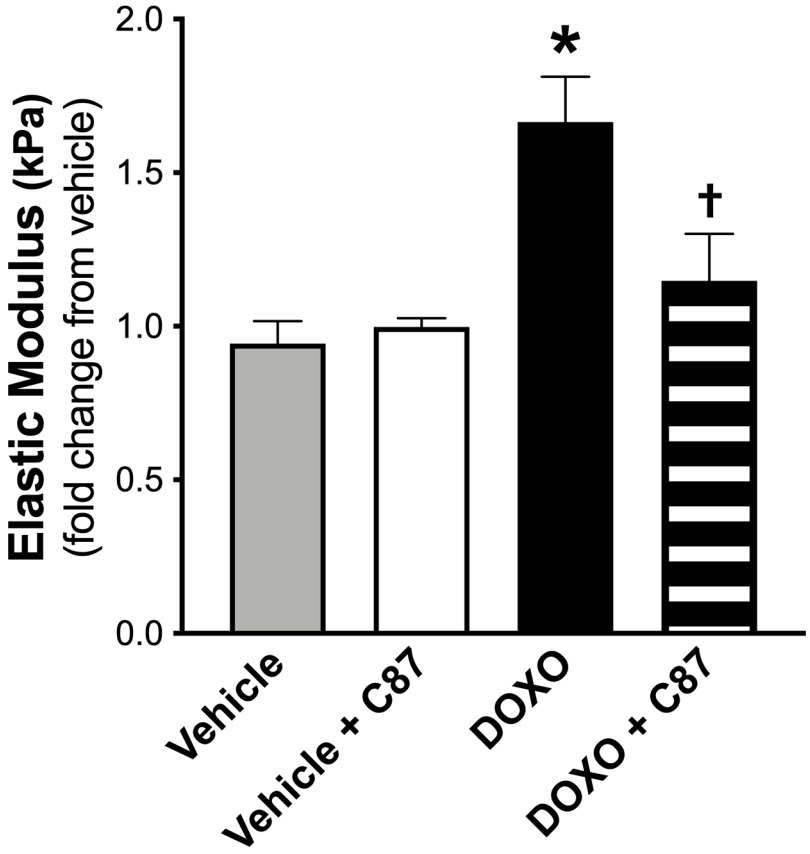

Figure 6. Direct exposure of aorta rings with the tumor necrosis factorα (TNFα) inhibitor C87 prevents Doxorubicin (DOXO)-mediated aortic stiffening.

Direct exposure of aorta rings with vehicle (DMEM + 10% fetal calf serum + 1% Penicillin-Streptomycin); vehicle + C87 (20 μM); DOXO (1 μM DOXO); and DOXO + C87. n = 10/condition. Data are the mean ± SEM expressed as fold-change relative to vehicle. $^{*}P < 0.05$ vs. vehicle; $^{\dagger}P < 0.05$ vs. DOXO.