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Original Research

Doxorubicin Cardiotoxicity in the Rat: An In Vivo Characterization

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The chemotherapeutic agent doxorubicin (DOX) is associated with a dose-dependent cardiotoxicity that can eventuate into heart failure. This study aimed to characterize the onset and degree of cardiotoxicity in rats receiving 10 mg/kg DOX administered as a single intraperitoneal injection (DOX1), 10 daily intraperitoneal injections of 1 mg/kg (DOX2), or in 5 weekly intraperitoneal injections of 2 mg/kg (DOX3). Transthoracic echocardiography measurements were recorded every week to characterize the onset and degree of cardiotoxicity in the 3 groups. An 80% mortality rate was observed at day 28 in DOX1, whereas DOX2 and DOX3 reached 80% mortality at days 107 and 98, respectively. Fractional shortening decreased by 30% at week 2 in DOX1, 55% at week 13 in DOX2, and 42% at week 13 in DOX3. In addition, cardiac function clearly differed between DOX1 and DOX3, whereas DOX2 and DOX3 were similar. These findings indicate that administration of the dose over the course of days (DOX2) or weeks (DOX3) results in a better survival rate and more classic signs of DOX-induced dilated cardiomyopathy, albeit with later onset, as compared with a single 10 mg/kg bolus injection of DOX.

Abbreviations: DOX, doxorubicin; ET, ejection time; FS, fractional shortening; ICT, isovolumic contraction time; IVRT, isovolumic relaxation time; LV, left ventricle; LVDd, left ventricle end diastolic diameter; LVDs, left ventricle end systolic diameter; MPI, myocardial performance index; PWd, posterior wall thickness during diastole; PWs, posterior wall thickness during systole; RWT, relative wall thickness; SWd, septal wall thickness during diastole; SWs, septal wall thickness during systole; V_{cf}, velocity of circumferential shortening; V_{cfc}, heart rate corrected velocity of circumferential shortening; V_{max}, maximal flow velocity, V_{mean}, mean flow velocity

Doxorubicin (DOX) is one of the most potent antineoplastic agents used in the treatment of lymphoid malignancies and solid tumors in both adults and children. More widespread use of DOX has been limited by a dose-dependent cardiotoxicity that can subsequently lead to heart failure.[1,5,16,26,34,45] The past 20 y have witnessed numerous attempts to derive novel anthracyclines superior to DOX in terms of its cardiotoxic effects. This effort has resulted in the production of approximately 2000 analogs, with only a few reaching clinical development and approval (for example, epirubicin, idarubicin). Other strategies aimed at limiting DOX cardiotoxicity include the use of slow infusions,[4,21,22] antioxidants,[24,42] and iron chelators.[11,38] Overall, these strategies have, at best, provided only limited improvement in cardiac function. Even with the intense pursuit of a better anthracycline, DOX continues to be a staple in the treatment of many cancers, and DOX cardiotoxicity remains a clinical dilemma.

Numerous experimental models[9,14,23,35,51] and dosing schedules[6,12,20,35] have been used to investigate the mechanisms of DOX cardiotoxicity as well as possible experimental therapies to mitigate its cardiotoxic effects. For those models with an intact cardiovascular system, the assessment of cardiovascular function can provide important information regarding the therapeutic value of experimental treatments. With this continued interest in understanding DOX and its side effects, there is, understandably, an increasing interest in assessing in vivo cardiovascular function in laboratory animals treated with DOX.

Echocardiography is a well-established, noninvasive, reproducible, and accurate means to assess cardiac anatomy and function. Commercially available ultrasound imaging systems now offer software and transducer technology that provide a high degree of resolution and clarity which allows for cardiac imaging of standard small laboratory animals. In addition, commercial in vivo blood pressure systems are available that allow for repeated measures of blood pressure in conscious experimental animals. The value of noninvasive ultrasound imaging and blood pressure assessment in the rat is that it may serve as a potential replacement for more invasive terminal procedures and can effectively allow for serial measures on a single laboratory animal. Although one of the more common experimental models for investigating DOX cardiotoxicity is the rat, a comprehensive in vivo characterization of short- and long-term DOX-induced cardiovascular dysfunction in the rat has not been reported. With this background, the purpose of this study was to characterize the in vivo cardiovascular dysfunction in a rat model of DOX cardiotoxicity.

Materials and Methods

Principles of laboratory animal care were followed during this study, and all procedures were conducted in accordance with guidelines of the University of Northern Colorado's Institutional Animal Care and Use Committee. The University of Northern Colorado adheres to the policies and procedures detailed in the *Guide for the Care and Use of Laboratory Animals* as published by the US Department of Health and Human Services[33] and

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proclaimed in the Animal Welfare Act, and follows the rules, procedures, and recommendations for the care of laboratory animals as advocated by the American Association for Accreditation of Laboratory Animal Care International.

DOX treatment. Thirty male Sprague-Dawley (*Rattus norvegicus*, Hsd:SD) rats weighing 250 to 300 g were purchased from Harlan Laboratories (Indianapolis, IN), housed in large polycarbonate cages with pelleted paper bedding (Pelleted Paper Bedding 7084, Harlan Teklad, Madison, WI) in an environmentally controlled facility (22 °C, 45% to 50% relative humidity) with a 12:12-h light:dark cycle (lights on, 1030; lights off, 2230), and provided food (Global Rodent Diet 2016, Harlan-Teklad) and distilled water ad libitum. Health status reports obtained from the vendor certified that all animals were free of known viral, bacterial, and parasitic pathogens. After a 2-wk acclimation period, animals were randomly assigned to 1 of 3 experimental groups: DOX schedule 1 (DOX1, n = 10), DOX schedule 2 (DOX2, n = 10), or DOX schedule 3 (DOX3, n = 10). For all DOX treatment schedules, the cumulative dose of DOX was 10 mg/kg. Schedule 1 involved a single bolus intraperitoneal injection of DOX at 10 mg/kg. Schedule 2 involved 10 intraperitoneal injections of DOX at 1 mg/kg for 10 consecutive days. Schedule 3 involved 5 intraperitoneal injections of DOX at 2 mg/kg, once each week, for 5 wk. Immediately before the first DOX treatment and at weekly intervals after beginning DOX treatment, blood pressure and cardiac function were assessed in all surviving animals as long as there were at least 3 rats per group.

Blood pressure. Tail cuff blood pressure measurements were made in conscious rats by using a noninvasive system (CODA 6 Non-Invasive Blood Pressure System, Kent Scientific, Torrington, CT) according to the manufacturer's recommendations and as reported by others.[25,46] Animals were positioned in acrylic holders with tails exposed and placed on an infrared heating pad to maintain a tail temperature of 34 °C for a minimum of 15 min before measurements. Animals were monitored by using an infrared thermometer to ensure target temperatures. An occlusion cuff was placed proximally on the tail, and a volume pressure recording sensor cuff was placed just distally to the occlusion cuff. After 5 inflation–deflation cycles for acclimation of the rat, 25 inflation–deflation cycles were run for data collection. Additional cycles were conducted if insufficient data were obtained. We selected 5 to 10 similar systolic, diastolic, and mean arterial pressures (no more than 10 mm Hg difference) for inclusion into the data set, and the means of the selected similar pressures were used for each animal's blood pressure measurement.

Echocardiography. Transthoracic echocardiography was conducted on anesthetized rats by using a commercially available echocardiographic system (Nemio 30 with 10-MHz pediatric transducer, Toshiba, Tustin, CA). Animals were sedated with ketamine (40 mg/kg intraperitoneally), and echocardiography was completed within 10 to 15 min after the administration of sedation. Ketamine was chosen as the sedative because use of this drug elicits minimal cardiovascular depressive effects. Once each rat was sedated, the anterior and left lateral thoracic regions were shaved, and animals were placed in the left lateral decubitus position. Ultrasound transonic gel was placed on the thorax to optimize visibility. The probe was positioned to obtain short- and long-axis and 4-chamber views.

From the short-axis view, an M-mode tracing of the left ventricle (LV) was obtained for measures of septal wall thickness during systole (SWs) and diastole (SWd), posterior wall thickness during systole (PWs) and diastole (PWd), LV end-systolic diameter (LVDs), and LV end-diastolic diameter (LVDd). For all cardiac dimensions, we used a leading edge-to-leading edge technique, as described by the American Society of Echocardiography.[8] Aortic flow was assessed from the 4-chamber apical view by using pulsed-wave Doppler, with the smallest possible sample volume placed at the level of the aortic annulus. From a 4-chamber apical view, mitral flow was assessed using pulsed wave Doppler with the smallest possible sample volume placed at the tips of the mitral valve. LV mass was calculated as

$$1.04[(LVDd + PWd + SWd)^3 - LVDd^3],$$

relative wall thickness (RWT) was calculated as

$$(PWd + SWd)/LVDd,$$

and fractional shortening (FS) was calculated as

$$(LVDd - LVDs)/LVDd.$$

The velocity of circumferential shortening (V_{cf}) was calculated as

$$FS/ET,$$

where ET is ejection time; ET was obtained from Doppler measures of aortic flow and measured as the time between aortic valve opening and aortic valve closure. V_{cf} can be corrected to account for variability in heart rate (V_{cfc}), and was calculated as

$$V_{cf}/\sqrt{(HR)}.$$

The myocardial performance index (MPI), an index used to assess combined systolic and diastolic function as described by Tei and colleagues,[47] was calculated as

$$(ICT + IVRT)/ET,$$

where ICT is isovolumic contraction time, and IVRT is isovolumic relaxation time. From pulsed Doppler mitral and aortic flow images, the velocity–time integral, maximal flow velocity (V_{max}), and mean flow velocity (V_{mean}) were measured. Measures of filling time and ET were obtained from mitral and aortic Doppler flow images, respectively. For measures of cardiac dimensions, time intervals, and flow velocities, data from 3 consecutive cardiac cycles, when possible, were obtained and averaged for each rat.

Statistical analyses. Data are reported as mean ± standard error. Differences over time were determined by one-way analysis of variance. When differences over time occurred, Dunnett post-hoc analyses were conducted to compare time course values with their corresponding baseline values. Survival curves were analyzed by using the logrank test. The threshold for significance was a P value of 0.05 for all statistical analyses. All statistical analyses were conducted using GraphPad Prism version 4.0 for Windows (San Diego, CA).

Results

Survival. One purpose of the current investigation was to analyze the survival rates associated with various DOX dosing regimens. Figure 1 summarizes the survival of animals from DOX1, DOX2, and DOX3. DOX1 animals showed a substantial decrease in survival between 14 and 21 d. By 21 d, survival had dropped to 40% and by 28 d, survival was 20%. In contrast, animals in DOX2 and DOX3 groups demonstrated a more moderate decline in survival during this same time period, and this rate of decline appeared to be maintained throughout the 15-wk observation period. In these groups, survival exceeded 90% at day 21, and a 20% survival rate did not occur until day 98 for DOX3 and day 107 for DOX2. In addition, 50% survival rates occurred after 21, 51, and 57 d for animals in the DOX1, DOX2, and DOX3 groups, respectively.

Body mass. For all groups, body mass demonstrated an overall increase during the observation period (Figure 2). Initial body masses for all groups were similar to those of historical control animals (294 ± 4 g) of equivalent age in our laboratory (data not shown). DOX1 mean body mass declined at week 1, and there was a progressive increase in body mass during the

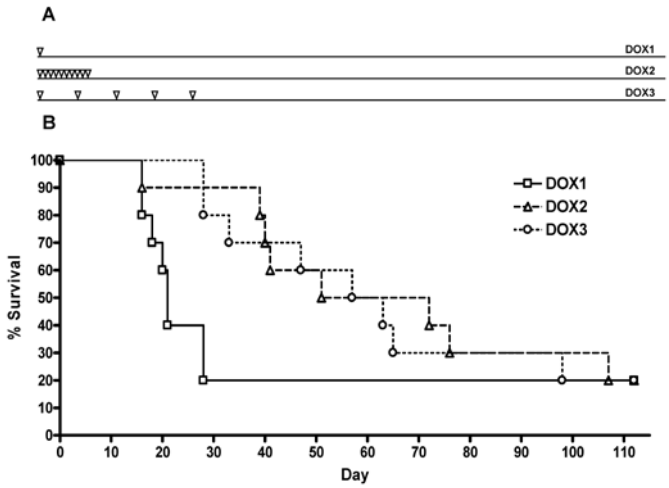

Figure 1. Treatment schedule and survival. (A) Animals were treated with a cumulative DOX dose of 10 mg/kg according to 1 of 3 treatment schedules. DOX1 animals received a single bolus injection of DOX at 10 mg/kg (triangle represents single dose at 10 mg/kg on day 0). DOX2 animals received DOX at 1 mg/kg on 10 consecutive days (each triangle represents 1 mg/kg). DOX3 animals received DOX at 2 mg/kg once weekly for 5 wk (each triangle represents 2 mg/kg). (B) Corresponding survival for each experimental group.

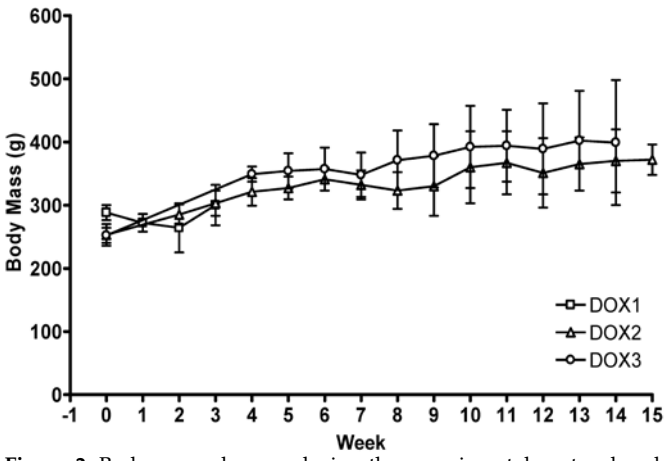

Figure 2. Body mass changes during the experimental protocol and observation period. DOX1, 10 mg/kg bolus; DOX2, 1 mg/kg daily for 10 consecutive days; DOX3, 2 mg/kg weekly for 5 wk.

2 subsequent weeks, with mean values approximately 10% below those of historical control animals. The high mortality in this group prevented any further analysis beyond the 3-wk interval. Although there were similarities between DOX2 and DOX3 growth curves, mean body masses for these 2 groups were consistently below those of historical controls. Throughout the observation period, DOX3 mean body mass was consistently 16% to 18% lower than that of historical controls, whereas DOX2 mean body mass was consistently 26% to 28% lower than that of historical controls. These data indicate that even with the overall increase in body mass during the observation period, growth curves for all groups were stunted in comparison with those of historical control animals, with more pronounced impairment in growth in DOX2 animals.

Blood pressure. Noninvasive blood pressure methods allow for repeated measurements with minimal stress to the animal, and systolic, diastolic, pulse, and mean arterial pressures at all time points are presented in Table 1. For DOX1 animals, there was a significant ($P < 0.05$) decline in systolic, diastolic, and mean arterial pressures at week 1. However, blood pressure values began to normalize toward baseline by week 2 and were not significantly different from baseline at either week 2 or week 3. For DOX2 and DOX3 groups, in vivo blood pressures did not change significantly throughout the entire 15-wk observation period.

Echocardiography. ***Cardiac geometry and geometry-derived measures.*** To asses the morphologic changes associated with various DOX dosing schemes, weekly echocardiographic measures were taken on all 3 groups. Representative images appear in Figure 3. A summary of cardiac dimensions obtained from echocardiography is presented in Table 2. Treatment with DOX resulted in a profound thinning of the septal and posterior ventricular walls during both systole and diastole, regardless of the treatment schedule. By week 3, there was a decrease of 35%, 27%, 37%, and 33% in SWs, PWs, SWd, and PWd, respectively, for DOX1 animals ($P < 0.05$ for each variable). Likewise, DOX2 and DOX3 rats showed a similar degree of wall thinning, yet in most instances the onset of this degree of wall thinning was not apparent until after the 9-wk interval. DOX1 appeared to develop a moderate dilation of the left ventricular chamber evidenced by an increase in mean LVDd at week 3 (6.56 ± 0.58 mm at week 0 versus 7.16 ± 0.52 mm at week 3, $P < 0.05$). This dilation was accompanied by a larger ventricular dimension at end systole, with left ventricular chamber dimension increasing from 3.31 $\pm$ 0.86 mm (week 0) to 4.55 ± 0.49 mm (week 3; $P < 0.05$). As was the case with left ventricular wall thicknesses, chamber dimensions showed a great deal of similarity in the pattern of left ventricular dilation, yet a comparable degree of dilation required approximately 8 to 11 wk for DOX2 and DOX3.

Cardiac geometry-derived variables are presented in Figure 4 and Table 3. Administration of a bolus dose of DOX at 10 mg/kg (DOX1) resulted in a significant ($P < 0.05$) and rapid loss of left ventricular mass as calculated by using echocardiography. At the 2-wk interval there was a 46% decrease in left ventricular mass. The effect of DOX treatment in the DOX2 and DOX3 groups was apparent but less severe and had a longer latency period, with the greatest loss of mass being 13% for DOX2 (weeks 7, 9, and 10) and 33% for DOX3 (week 14). Although the loss in mass for these groups was not significantly different from baseline values, LV mass in these animals was significantly ($P < 0.05$) less than that calculated by using echocardiography for control animals, indicating that DOX treatment in these groups impaired normal cardiac growth and development. RWT declined in a linear fashion for DOX1 from weeks 1 to 3, with the nadir reaching $0.27 \pm .02$ mm ($P < 0.05$). For DOX2 the onset of decline in RWT began at week 3, and a steady downward trend occurred between weeks 2 and 10. RWT remained fairly stable between weeks 8 and 14, with values ranging between 0.30 and 0.39 mm ($P < 0.05$ versus baseline at all time points). In contrast, DOX3 animals did not demonstrate a downward trend in RWT until the completion of the DOX regimen (week 5). DOX3 showed a steady downward trend in RWT between weeks 5 and 10, followed by relatively stable values between weeks 10 and 15, ranging between 0.32 and 0.42 mm ($P < 0.05$ versus baseline at weeks 12, 13, and 14).

Understandably, due to their common derivation, FS, V_{cf}, and V_{cfc} for all groups demonstrated a similar response to that of RWT. For each of these variables, DOX1 showed a rapid decline during weeks 1 to 3. FS (baseline, $50\% \pm 10\%$), V_{cf}, and V_{cfc} declined 30%, 32%, and 33%, respectively at week 2 ($P < 0.05$). In stark contrast to these data, FS remained relatively unchanged during weeks 1 through 3 for both DOX2 and DOX3, after which both groups showed a steady downward trend throughout the

Table 1. Noninvasive blood pressure

	Systolic (mm Hg)	Diastolic (mm Hg)	Pulse (mm Hg)	Mean arterial (mm Hg)
Baseline				
DOX1 (n = 10)	150 ± 6	108 ± 5	41 ± 2	122 ± 5
DOX2 (n = 10)	153 ± 7	110 ± 6	42 ± 1	125 ± 6
DOX3 (n = 10)	150 ± 4	110 ± 3	40 ± 1	123 ± 3
Week 1				
DOX1 (n = 10)	103 ± 10[a]	71 ± 8[a]	33 ± 4	81 ± 9[a]
DOX2 (n = 10)	164 ± 4	118 ± 3	47 ± 2	133 ± 3
DOX3 (n = 10)	148 ± 5	107 ± 5	41 ± 2	120 ± 5
Week 2				
DOX1 (n = 7)	131 ± 9	105 ± 10	26 ± 6	113 ± 9
DOX2 (n = 10)	158 ± 6	115 ± 5	43 ± 2	129 ± 5
DOX3 (n = 10)	153 ± 6	110 ± 5	43 ± 3	124 ± 5
Week 3				
DOX1 (n = 4)	149 ± 7	107 ± 9	42 ± 2	121 ± 8
DOX2 (n = 9)	161 ± 5	116 ± 4	45 ± 2	131 ± 4
DOX3 (n = 10)	152 ± 6	111 ± 5	41 ± 1	124 ± 5
Week 4				
DOX2 (n = 9)	147 ± 5	102 ± 4	45 ± 5	117 ± 4
DOX3 (n = 10)	153 ± 4	113 ± 5	40 ± 3	126 ± 5
Week 5				
DOX2 (n = 9)	141 ± 4	99 ± 3	42 ± 2	113 ± 3
DOX3 (n = 7)	154 ± 5	116 ± 7	38 ± 3	128 ± 6
Week 6				
DOX2 (n = 6)	149 ± 6	109 ± 6	40 ± 3	122 ± 6
DOX3 (n = 7)	161 ± 3	121 ± 5	40 ± 4	134 ± 4
Week 7				
DOX2 (n = 6)	138 ± 6	102 ± 4	35 ± 2	113 ± 4
DOX3 (n = 7)	160 ± 5	118 ± 7	42 ± 3	132 ± 6
Week 8				
DOX2 (n = 5)	146 ± 11	111 ± 11	35 ± 4	122 ± 11
DOX3 (n = 6)	169 ± 2	130 ± 2	40 ± 1	142 ± 2
Week 9				
DOX2 (n = 5)	133 ± 7	93 ± 9	40 ± 6	106 ± 7
DOX3 (n = 6)	156 ± 8	127 ± 7	29 ± 2	136 ± 7
Week 10				
DOX2 (n = 5)	128 ± 13	103 ± 7	25 ± 6	111 ± 9
DOX3 (n = 4)	157 ± 7	124 ± 5	34 ± 2	134 ± 5
Week 11				
DOX2 (n = 4)	146 ± 10	108 ± 10	38 ± 2	120 ± 10
DOX3 (n = 4)	132 ± 8	91 ± 8	42 ± 4	104 ± 8
Week 12				
DOX2 (n = 3)	161 ± 3	129 ± 9	33 ± 7	139 ± 7
DOX3 (n = 3)	158 ± 4	128 ± 5	30 ± 5	138 ± 4
Week 13				
DOX2 (n = 3)	151 ± 18	101 ± 13	50 ± 5	117 ± 15
DOX3 (n = 3)	131 ±15	87 ± 14	44 ± 6	102 ± 14
Week 14				
DOX2 (n = 3)	114 ± 11	89 ± 9	25 ± 3	97 ± 10
DOX3 (n = 3)	119 ± 16	93 ± 15	26 ± 6	101 ± 15
Week 15				
DOX2 (n = 3)	118 ± 14	78 ± 16	39 ± 5	91 ± 15

[a]$P < 0.05$ versus baseline value.

remainder of the observation period. For DOX2, FS dropped to 25% ± 9% at week 13 ($P < 0.05$), equaling a 55% decline (baseline, 56% ± 7%); for DOX3, FS dropped to 31% ± 13% also at week 13 ($P < 0.05$), equaling a 42% decline (baseline, 54% ± 6%). A similar pattern and magnitude of decline was observed for V_{cf}, and V_{cfc} for both DOX2 and DOX3.

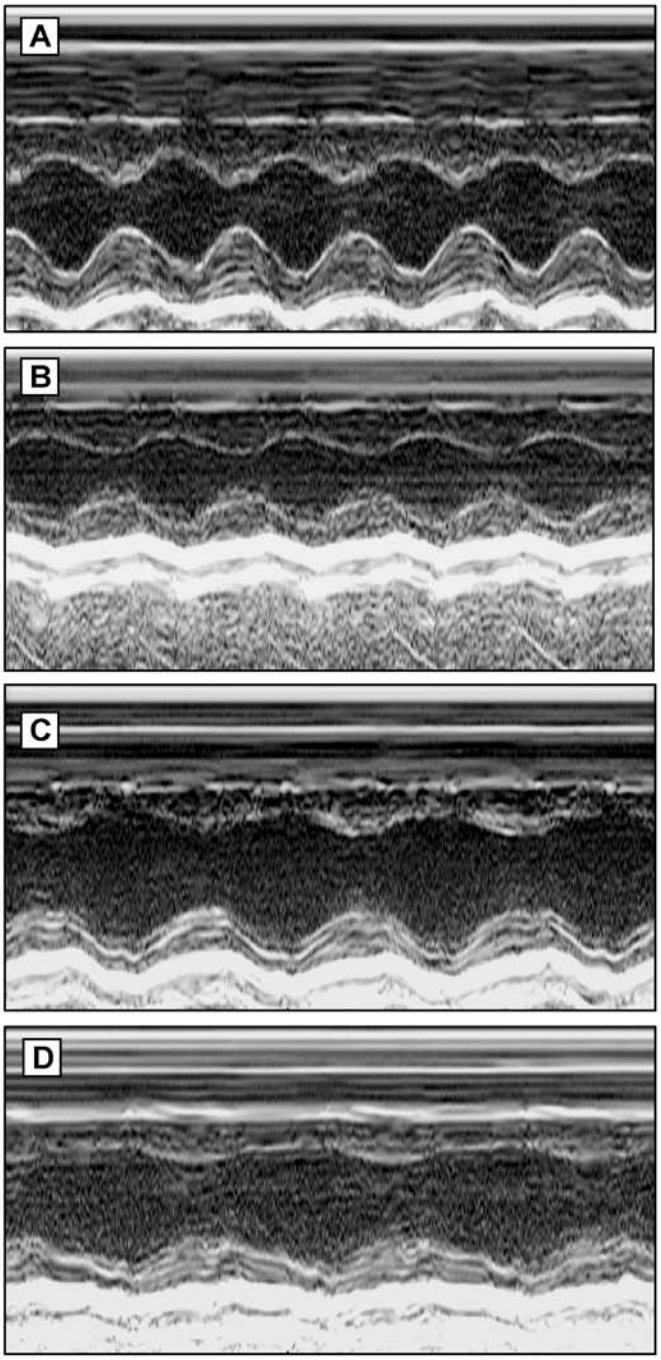

Figure 3. Representative M-mode images obtained at baseline (A) and after the onset of cardiac dysfunction in (B) DOX1, (C) DOX2, and (D) DOX3 animals. DOX1, 10 mg/kg bolus; DOX2, 1 mg/kg daily for 10 consecutive days; DOX3, 2 mg/kg weekly for 5 wk.

Doppler. Mitral and aortic Doppler blood flow measurements were taken weekly to assess the LV functional changes throughout the experimental period. Tables 4 and 5 provide a summary of echocardiographic Doppler measures obtained from the mitral and aortic valves, and representative images appear in Figure 5. Mitral (TVI_M, V_{maxM}, V_{meanM}) and aortic (TVI_A, V_{maxA}, V_{meanA}) Doppler measures followed a consistent, distinct pattern for DOX1. Each of these variables demonstrated a progressive

Table 2. Cardiac geometry

	SWs (mm)	SWd (mm)	PWs (mm)	PWd (mm)	LVDs (mm)	LVDd (mm)
Baseline						
DOX1 (n = 10)	2.68 ± 0.11	1.44 ± 0.11	2.79 ± 0.11	1.58 ± 0.09	3.31 ± 0.27	6.56 ± 0.18
DOX2 (n = 10)	2.86 ± 0.13	1.60 ± 0.11	2.82 ± 0.12	1.56 ± 0.10	2.57 ± 0.17	5.76 ± 0.18
DOX3 (n = 10)	2.73 ± 0.08	1.63 ± 0.07	2.93 ± 0.10	1.58 ± 0.07	2.87 ± 0.13	6.27 ± 0.15
Week 1						
DOX1 (n = 10)	2.15 ± 0.15[a]	1.15 ± 0.09	2.28 ± 0.16[a]	1.25 ± 0.11[a]	3.62 ± 0.30	5.95 ± 0.33
DOX2 (n = 10)	2.88 ± 0.13	1.56 ± 0.11	2.86 ± 0.12	1.64 ± 0.10	2.89 ± 0.17	5.65 ± 0.18
DOX3 (n = 10)	2.83 ± 0.08	1.57 ± 0.08	3.19 ± 0.10	1.68 ± 0.08	2.75 ± 0.16	6.07 ± 0.17
Week 2						
DOX1 (n = 7)	1.77 ± 0.15[a]	0.95 ± 0.08[a]	1.84 ± 0.14[a]	1.11 ± 0.09[a]	3.90 ± 0.16	6.07 ± 0.19
DOX2 (n = 9)	2.78 ± 0.10	1.61 ± 0.10	2.79 ± 0.11	1.57 ± 0.11	3.02 ± 0.20	6.32 ± 0.20
DOX3 (n = 10)	2.94 ± 0.12	1.61 ± 0.06	3.15 ± 0.13	1.73 ± 0.10	2.93 ± 0.24	6.08 ± 0.26
Week 3						
DOX1 (n = 4)	1.79 ± 0.15[a]	0.89 ± 0.09[a]	2.08 ± 0.11[a]	1.05 ± 0.07[a]	4.55 ± 0.25[a]	7.16 ± 0.26[a]
DOX2 (n = 9)	2.86 ± 0.13	1.58 ± 0.11	2.96 ± 0.11	1.58 ± 0.07	3.11 ± 0.17	6.34 ± 0.14
DOX3 (n = 10)	2.89 ± 0.07	1.60 ± 0.07	2.94 ± 0.12	1.56 ± 0.07	2.97 ± 0.17	6.22 ± 0.17
Week 4						
DOX2 (n = 9)	2.62 ± 0.10	1.27 ± 0.09	2.81 ± 0.10	1.54 ± 0.07	3.46 ± 0.15[a]	6.31 ± 0.10
DOX3 (n = 9)	2.92 ± 0.11	1.60 ± 0.08	3.08 ± 0.12	1.76 ± 0.10	2.80 ± 0.20	6.02 ± 0.16
Week 5						
DOX2 (n = 9)	2.48 ± 0.10	1.29 ± 0.09	2.46 ± 0.11	1.32 ± 0.06	3.77 ± 0.14[a]	6.60 ± 0.18
DOX3 (n = 7)	2.74 ± 0.12	1.61 ± 0.13	2.98 ± 0.15	1.73 ± 0.11	3.19 ± 0.25	6.19 ± 0.37
Week 6						
DOX2 (n = 6)	2.31 ± 0.16	1.23 ± 0.10	2.36 ± 0.10	1.36 ± 0.08	3.86 ± 0.20[a]	6.32 ± 0.23
DOX3 (n = 7)	2.62 ± 0.14	1.57 ± 0.12	2.75 ± 0.23	1.55 ± 0.17	3.76 ± 0.45	6.73 ± 0.36
Week 7						
DOX2 (n = 5)	2.44 ± 0.20	1.23 ± 0.12	2.54 ± 0.09	1.51 ± 0.10	3.57 ± 0.27[a]	6.02 ± 0.22
DOX3 (n = 7)	2.62 ± 0.08	1.52 ± 0.08	2.63 ± 0.13	1.28 ± 0.07	3.92 ± 0.29	6.96 ± 0.21
Week 8						
DOX2 (n = 5)	1.99 ± 0.13	1.18 ± 0.09	2.11 ± 0.16[a]	1.35 ± 0.10	4.70 ± 0.42[a]	6.89 ± 0.45[a]
DOX3 (n = 7)	2.47 ± 0.18	1.32 ± 0.08	2.44 ± 0.14	1.28 ± 0.09	3.93 ± 0.30[a]	6.74 ± 0.35
Week 9						
DOX2 (n = 5)	2.15 ± 0.26[a]	1.18 ± 0.11	2.11 ± 0.24[a]	1.08 ± 0.16[a]	4.46 ± 0.20[a]	6.94 ± 0.23[a]
DOX3 (n = 5)	2.55 ± 0.16	1.30 ± 0.09	2.71 ± 0.18	1.28 ± 0.16	3.55 ± 0.35	6.72 ± 0.16
Week 10						
DOX2 (n = 5)	1.78 ± 0.21[a]	0.98 ± 0.07[a]	1.85± 0.15[a]	1.19 ± 0.15	4.89 ± 0.16[a]	7.27 ± 0.10[a]
DOX3 (n = 4)	2.40 ± 0.10	1.29 ± 0.11	2.34 ± 0.11	1.15 ± 0.09	4.25 ± 0.25[a]	7.05 ± 0.38
Week 11						
DOX2 (n = 3)	1.87 ± 0.26[a]	1.31 ± 0.14	1.98 ± 0.09[a]	1.35 ± 0.18	5.19 ± 0.48[a]	7.31 ± 0.34[a]
DOX3 (n = 3)	2.04 ± 0.28[a]	1.40 ± 0.20	2.33 ± 0.32	1.20 ± 0.21	4.54 ± 0.76[a]	6.73 ± 0.85
Week 12						
DOX2 (n = 3)	1.96 ± 0.19[a]	1.25 ± 0.13	2.23 ± 0.21	1.33 ± 0.12	4.78 ± 0.14[a]	7.26 ± 0.27[a]
DOX3 (n = 3)	2.21 ± 0.13[a]	1.09 ± 0.12[a]	2.32 ± 0.19	1.17 ± 0.12	4.56 ± 0.21[a]	7.17 ± 0.31
Week 13						
DOX2 (n = 3)	1.76 ± 0.11[a]	1.27 ± 0.10	2.11 ± 0.12[a]	1.16 ± 0.25	5.22 ± 0.24[a]	7.14 ± 0.45[a]
DOX3 (n = 3)	2.03 ± 0.19[a]	1.14 ± 0.11[a]	2.14 ± 0.29	1.13 ± 0.11[a]	5.23 ± 0.96[a]	7.42 ± 0.73
Week 14						
DOX2 (n = 3)	1.81 ± 0.16[a]	1.04 ± 0.13[a]	1.88 ± 0.23[a]	1.42 ± 0.14	5.07 ± 0.34[a]	6.82 ± 0.18
DOX3 (n = 3)	2.01 ± 0.17[a]	1.12 ± 0.08[a]	2.21 ± 0.15	1.02 ± 0.09	4.55 ± 0.67[a]	6.73 ± 0.66
Week 15						
DOX2 (n = 3)	1.70 ± 0.28[a]	1.10 ± 0.20	1.72 ± 0.13[a]	1.17 ± 0.18	5.19 ± 0.29[a]	7.29 ± 0.57[a]

LVDd, left ventricle end diastolic diameter; LVDs, left ventricle end systolic diameter; PWd, posterior wall thickness during diastole; PWs, posterior wall thickness during systole; SWd, septal wall thickness during diastole; SWs, septal wall thickness during systole.
[a]$P < 0.05$ versus baseline value.

decline from week 0 to week 2, with declines ranging from 20% to 30%. This decline was followed by a rapid increase in all variables from week 2 to week 3, with most variables approximating or exceeding baseline values.

DOX2 and DOX3 showed a definitive downward trend in TVI_M during weeks 1 to 8, and these decreased values were maintained throughout the remainder of the observation period. After the 9-wk observation point, declines in mean TVI_M ranged from 18% to 33% for DOX3 ($P < 0.05$ versus baseline at all time points), whereas declines ranged from 32% to 47% for DOX2 ($P < 0.05$ versus baseline at all time points). V_{maxM} and V_{meanM} progressively declined for both DOX2 and DOX3 through week

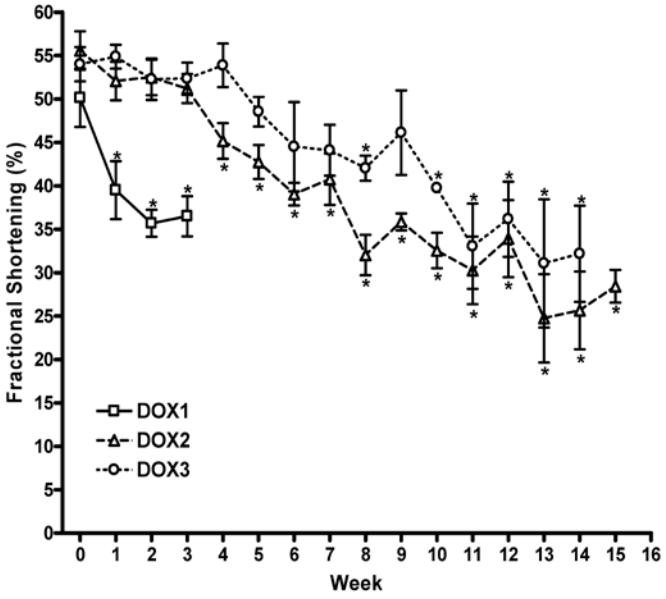

Figure 4. Fractional shortening. DOX1, 10 mg/kg bolus; DOX2, 1 mg/kg daily for 10 consecutive days; DOX3, 2 mg/kg weekly for 5 wk. *, $P < 0.05$ versus value for week 0.

10 and then remained relatively stable for the balance of the observation period. However, as was the case for TVI_M, the severity of decline for all mitral Doppler variables appeared to be much greater for DOX2 than for DOX3. Specifically, between weeks 10 to 14, mean V_{maxM} values ranged from 55 to 83 cm/s for DOX3, whereas between weeks 10 to 15 mean V_{maxM} values ranged from 40 to 63 cm/s for DOX2. A similar time course and magnitude of change was observed in V_{meanM} for DOX2 and DOX3 groups.

Mean TVI_A values were roughly at or above baseline values through week 13 for DOX2. A similar pattern was observed for DOX3, with mean TVI_A values declining no more than 15% at week 5, and mean values at or above baseline values at weeks 1, 3, 6, 7, 9, 10, and 11. V_{maxA} remained fairly stable through week 7 for both DOX2 and DOX3. V_{maxA} fluctuated slightly during weeks 8 to 15 but with an overall downward trend for both groups. By week 14 the overall decrease in V_{maxA} was 35% for DOX2 ($P < 0.05$), and by week 12 the overall decrease in V_{maxA} was 39% for DOX3 ($P < 0.05$). The time course and magnitude of change for V_{meanA} mirrored that of V_{maxA} for both DOX2 and DOX3 groups.

IVRT, as measured from Doppler images, rapidly increased in DOX1 rats, increasing by over 10 ms by week 2 (20.4 ± 2.4 ms at baseline versus 30.5 ± 8.2 ms at week 2, $P < 0.05$). For DOX2 and DOX3 animals, IVRT steadily progressively increased until week 11. During this interval IVRT had increased 2-fold for DOX2 ($P < 0.05$) and 2.2-fold for DOX3 ($P < 0.05$). After week 11, mean IVRT values for DOX2 maintained the 2-fold increase while fluctuating between 32 to 42 ms. For DOX3, mean IVRT values declined from weeks 11 to 14, with a mean value of 23.6 ± 3 ms obtained at week 14. MPI values progressively increased from week 0 to week 2 for DOX1 ($P < 0.05$). MPI differed between the DOX2 and DOX3 groups ($P < 0.05$) during weeks 8 to 15. When MPI was expressed as a function of FS, all groups showed significant ($P < 0.05$) within-group differences. When compared with baseline, DOX1 FS/MPI was significantly ($P < 0.05$) lower at weeks 1 to 3 ($P < 0.05$). DOX2 FS/MPI was significantly different from baseline at weeks 3, 5, 6, and 8 to 15 ($P < 0.05$), and for DOX3 FS/MPI was significantly ($P < 0.05$) lower than baseline during weeks 2 to 13 ($P < 0.05$).

Discussion

The rat has proven to be a well-accepted, reliable, cost-effective model for investigating the pharmacology of DOX and possible treatments to alleviate its cardiotoxicity. The purpose of this research was to characterize the long-term in vivo cardiovascular response of the rat to DOX treatment using 3 different dosing regimens. Various DOX dosing regimens have been reported in the literature, with several using single doses ranging from 5 mg/kg[15] to 45 mg/kg,[35,36] whereas others incorporate multiple injections of 1 to 8 mg/kg, given daily or weekly, over periods ranging from 2 d[28] to 10 wk.[17,53] Numerous studies using the rat model have incorporated cumulative doses of 5 to 8 mg/kg,[12,15,39] 10 to 15 mg/kg,[6,7,57] and 16 to 45 mg/kg.[10,20,23,32,36,44,49]

We selected a cumulative dose of 10 mg/kg for 2 primary reasons. First, this represents a dose that induces cardiac dysfunction without the degree of sickliness observed with doses exceeding 15 mg/kg. In our laboratory, although a cumulative dose of 15 mg/kg or higher results in significant cardiac dysfunction, it is accompanied by severe diarrhea, large volumes of ascites, a high mortality rate within the first 10 to 14 d post-treatment, and a subjectively observed high level of discomfort. In contrast, with the 10 mg/kg dose, we observe a similar degree of cardiac dysfunction as that of the 15 mg/kg dose but significantly reduced severity of diarrhea, less ascites, enhanced survival rates, greater daily voluntary running distances, and in general, a more healthy-looking rat.

Second, we have selected a dose of 10 mg/kg because this dose represents a clinically relevant cumulative dose. For example, most patients undergoing DOX treatment will not exceed a cumulative dose of 550 mg/m^2. Assuming a mass of 175 pounds (79.5 kg), height of 6 ft (72 in.), and a resulting body surface area of 2.0, this example patient would receive a total of 1100 mg of DOX, for a cumulative dose of 13.8 mg/kg.

Animals in the DOX1 group demonstrated many of the physical characteristics associated with DOX treatment, such as scruffy fur, patches of the coat with a reddish/orange tinge, reddish exudate around the eyes, and lethargy. At week 2, 40% of animals in the DOX1 group were noticeably ascitic, as determined by a grossly distended abdomen and later confirmed during necropsy. All animals in DOX1 with ascites died within 1 wk of the onset of noticeable abdominal distension. Ascites has been reported to be a characteristic of DOX-induced heart failure.[27,30,54] Some researchers have dismissed the accumulation of ascitic fluid as evidence of DOX-mediated heart failure,[19] whereas others have attempted to assess the degree of heart failure according to the volume of ascites.[43] Although ascites is associated with the development of heart failure, the possibility of DOX-mediated peritonitis can not be ruled out. Although animals in DOX1 exhibited overt signs of DOX exposure, the severity of all symptoms was noticeably less than that we typically observe with a bolus injection of DOX at 15 mg/kg.[6,7]

In contrast to what was observed with DOX1, animals in the DOX2 and DOX3 groups exhibited no changes in the coat and eyes, were more active, and lacked noticeable abdominal distention, even during the days immediately prior to death. These observations suggest that spreading the administration of DOX at the same cumulative dose of 10 mg/kg over the course of 10 d (DOX2) to 5 wk (DOX3) noticeably improves the general health and appearance of treated animals. In addition, these observations suggest that the ascites associated with DOX treatment at this dose may be more directly related to drug-induced peritonitis, given that neither DOX2 nor DOX3 animals had noticeable ascitic fluid accumulation.

Table 3. Variables derived from echocardiographic geometry

	LV mass (mg)	RWT (mg)	V_{cf} (mm)	V_{cfc} (cm·s^1)
Baseline				
DOX1 (n = 10)	628 ± 48	0.46 ± 0.03	0.79 ± 0.06	0.065 ± 0.005
DOX2 (n = 10)	540 ± 33	0.55 ± 0.03	0.84 ± 0.03	0.072 ± 0.003
DOX3 (n = 10)	630 ± 20	0.51 ± 0.02	0.88 ± 0.06	0.075 ± 0.006
Week 1				
DOX1 (n = 10)	389 ± 41[a]	0.42 ± 0.05	0.64 ± 0.06	0.053 ± 0.005
DOX2 (n = 10)	535 ± 33	0.58 ± 0.03	0.75 ± 0.03	0.062 ± 0.003
DOX3 (n = 10)	615 ± 41	0.54 ± 0.03	0.92 ± 0.04	0.079 ± 0.005
Week 2				
DOX1 (n = 7)	334 ± 37[a]	0.34 ± 0.02[a]	0.54 ± 0.04[a]	0.043 ± 0.004[a]
DOX2 (n = 10)	627 ± 16	0.51 ± 0.03	0.73 ± 0.05	0.061 ± 0.005
DOX3 (n = 10)	633 ± 24	0.57 ± 0.04	0.89 ± 0.05	0.073 ± 0.005
Week 3				
DOX1 (n = 4)	401 ± 16[a]	0.27 ± 0.02[a]	0.61 ± 0.05	0.051 ± 0.005
DOX2 (n = 9)	626 ± 28	0.50 ± 0.03	0.80 ± 0.05	0.067 ± 0.005
DOX3 (n = 10)	615 ± 40	0.51 ± 0.02	0.81 ± 0.04	0.068 ± 0.004
Week 4				
DOX2 (n = 9)	530 ± 29	0.45 ± 0.02	0.69 ± 0.05	0.057 ± 0.004
DOX3 (n = 10)	633 ± 31	0.57 ± 0.03	0.89 ± 0.08	0.073 ± 0.008
Week 5				
DOX2 (n = 9)	514 ± 23	0.40 ± 0.01[a]	0.59 ± 0.06[a]	0.047 ± 0.006[a]
DOX3 (n = 7)	652 ± 37	0.56 ± 0.07	0.65 ± 0.14	0.051 ± 0.011
Week 6				
DOX2 (n = 6)	475 ± 25	0.41 ± 0.02[a]	0.61 ± 0.04[a]	0.052 ± 0.004
DOX3 (n = 7)	671 ± 35	0.48 ± 0.06	0.73 ± 0.09	0.058 ± 0.008
Week 7				
DOX2 (n = 6)	470 ± 12	0.46 ± 0.04	0.52 ± 0.05[a]	0.039 ± 0.005[a]
DOX3 (n = 7)	619 ± 37	0.40 ± 0.01	0.67 ± 0.07	0.054 ± 0.007[a]
Week 8				
DOX2 (n = 5)	529 ± 40	0.38 ± 0.04[a]	0.45 ± 0.07[a]	0.035 ± 0.008[a]
DOX3 (n = 6)	524 ± 22	0.40 ± 0.04	0.61 ± 0.07	0.046 ± 0.008[a]
Week 9				
DOX2 (n = 5)	470 ± 69	0.33 ± 0.04[a]	0.44 ± 0.06[a]	0.032 ± 0.007[a]
DOX3 (n = 6)	517 ± 52	0.38 ± 0.04	0.62 ± 0.12	0.049 ± 0.012[a]
Week 10				
DOX2 (n = 5)	470 ± 45	0.30 ± 0.03[a]	0.33 ± 0.03[a]	0.020 ± 0.002[a]
DOX3 (n = 4)	529 ± 55	0.35 ± 0.03	0.54 ± 0.01[a]	0.042 ± 0.001[a]
Week 11				
DOX2 (n = 4)	605 ± 27	0.37 ± 0.03[a]	0.36 ± 0.08[a]	0.025 ± 0.008[a]
DOX3 (n = 3)	532 ± 78	0.43 ± 0.10	0.43 ± 0.10[a]	0.028 ± 0.008[a]
Week 12				
DOX2 (n = 3)	592 ± 55	0.39 ± 0.06	0.46 ± 0.07[a]	0.033 ± 0.006[a]
DOX3 (n = 3)	485 ± 30	0.32 ± 0.05[a]	0.57 ± 0.13[a]	0.040 ± 0.012[a]
Week 13				
DOX2 (n = 3)	532 ± 39	0.37 ± 0.09[a]	0.29 ± 0.06[a]	0.016 ± 0.004[a]
DOX3 (n = 3)	514 ± 42	0.32 ± 0.07[a]	0.39 ± 0.08[a]	0.021 ± 0.005[a]
Week 14				
DOX2 (n = 3)	502 ± 33	0.36 ± 0.04[a]	0.35 ± 0.07[a]	0.024 ± 0.004[a]
DOX3 (n = 2)	417 ± 85	0.32 ± 0.03[a]	0.57 ± 0.10[a]	0.046 ± 0.008[a]
Week 15				
DOX2 (n = 3)	510 ± 181	0.32 ± 0.13[a]	0.35 ± 0.05[a]	0.023 ± 0.007[a]

LV, left ventricle; RWT, relative wall thickness; V_{cf}, velocity of circumferential shortening; V_{cfc}, heart rate corrected velocity of circumferential shortening.
[a]$P < 0.05$ versus baseline value.

Loss of body mass consistently has been shown to be associated with DOX treatment and can be attributed to reduction in food intake and inhibition of protein synthesis due to the drug's antineoplastic effect. However, the pattern and degree of loss varies markedly depending on the cumulative dose and time course of treatment. For this study, overall mean body mass values from all groups increased during the observation period. DOX1 was the only group to show a decrease in body

Table 4. Aortic and mitral Doppler measures

	Aortic			Mitral		
	TVI_A (cm)	V_{meanA} (cm/sec)	V_{maxA} (cm/sec)	TVI_M (cm)	V_{meanM} (cm/sec)	V_{maxM} (cm/sec)
Baseline						
DOX1 (n = 10)	4.3 ± 0.2	68 ± 4	106 ± 7	4.1 ± 0.2	64 ± 4	96 ± 6
DOX2 (n = 10)	4.1 ± 0.2	63 ± 3	97 ± 4	4.3 ± 0.1	69 ± 3	104 ± 4
DOX3 (n = 10)	4.2 ± 0.2	67 ± 4	102 ± 5	4.0 ± 0.2	72 ± 5	102 ± 7
Week 1						
DOX1 (n = 10)	2.9 ± 0.3[a]	48 ± 6[a]	73 ± 7[a]	3.2 ± 0.2[a]	58 ± 4	89 ± 6
DOX2 (n = 10)	3.6 ± 0.2	51 ± 3	81 ± 4	4.4 ± 0.1	62 ± 3	96 ± 4
DOX3 (n = 10)	4.3 ± 0.3	72 ± 5	109 ± 6	4.2 ± 0.2	72 ± 4	109 ± 6
Week 2						
DOX1 (n = 7)	2.9 ± 0.4[a]	46 ± 6[a]	72 ± 10[a]	2.8 ± 0.3[a]	50 ± 5[a]	79 ± 8
DOX2 (n = 10)	4.6 ± 0.4	63 ± 5	103 ± 8	3.6 ± 0.2	60 ± 4	97 ± 6
DOX3 (n = 10)	3.8 ± 0.3	61 ± 4	94 ± 5	4.1 ± 0.2	69 ± 5	103 ± 7
Week 3						
DOX1 (n = 4)	3.9 ± 0.3	65 ± 4	103 ± 7	3.9 ± 0.2	72 ± 5	106 ± 7
DOX2 (n = 9)	4.0 ± 0.4	62 ± 5	99 ± 8	3.9 ± 0.2	67 ± 4	105 ± 7
DOX3 (n = 10)	4.4 ± 0.3	66 ± 4	109 ± 6	3.7 ± 0.1	69 ± 3	101 ± 5
Week 4						
DOX2 (n = 9)	4.6 ± 0.3	67 ± 4	105 ± 9	3.6 ± 0.1	63 ± 2	98 ± 3
DOX3 (n = 10)	3.9 ± 0.3	62 ± 3	100 ± 5	4.2 ± 0.1	68 ± 4	100 ± 7
Week 5						
DOX2 (n = 9)	4.9 ± 0.3	68 ± 5	101 ± 5	4.2 ± 2.7	53 ± 5	84 ± 7
DOX3 (n = 7)	3.6 ± 0.3	59 ± 5	90 ± 8	3.1 ± 0.2	52 ± 5	68 ± 8
Week 6						
DOX2 (n = 6)	4.3 ± 0.5	65 ± 7	96 ± 9	3.5 ± 0.3	66 ± 4	100 ± 6
DOX3 (n = 7)	4.5 ± 0.4	66 ± 4	102 ± 6	3.8 ± 0.2	63 ± 4	93 ± 8
Week 7						
DOX2 (n = 6)	4.9 ± 0.3	61 ± 3	94 ± 3	3.5 ± 0.4	48 ± 4[a]	75 ± 6[a]
DOX3 (n = 7)	4.2 ± 0.2	62 ± 2	95 ± 4	2.9 ± 0.2[a]	55 ± 6	82 ± 9
Week 8						
DOX2 (n = 5)	3.9 ± 0.5	54 ± 8	82 ± 11	2.7 ± 0.3[a]	47 ± 10[a]	71 ± 13[a]
DOX3 (n = 6)	3.7 ± 0.2	52 ± 3	81 ± 5	3.6 ± 0.1	55 ± 10	86 ± 14
Week 9						
DOX2 (n = 5)	3.8 ± 0.4	47 ± 8	70 ± 11	2.7 ± 0.4[a]	42 ± 10[a]	64 ± 14[a]
DOX3 (n = 6)	4.1 ± 0.3	54 ± 7	82 ± 12	2.8 ± 0.7[a]	50 ± 12	75 ± 19
Week 10						
DOX2 (n = 5)	4.5 ± 0.3	46 ± 4[a]	69 ± 6[a]	2.4 ± 0.4[a]	27 ± 1[a]	47 ± 3[a]
DOX3 (n = 4)	4.6 ± 0.2	62 ± 3	99 ± 4	3.0 ± 0.2[a]	49 ± 6	76 ± 8
Week 11						
DOX2 (n = 4)	4.9 ± 0.3	57 ± 5	93 ± 8	2.6 ± 0.4[a]	39 ± 12[a]	59 ± 14[a]
DOX3 (n = 4)	4.2 ± 0.6	53 ± 4	78 ± 6	2.7 ± 0.2[a]	38 ± 5[a]	55 ± 5[a]
Week 12						
DOX2 (n = 3)	4.1 ± 0.3	53 ± 3	85 ± 4	2.7 ± 0.2[a]	41 ± 5[a]	59 ± 8[a]
DOX3 (n = 3)	2.9 ± 0.7	42 ± 7[a]	62 ± 9[a]	2.7 ± 0.1[a]	43 ± 5[a]	66 ± 14[a]
Week 13						
DOX2 (n = 3)	3.9 ± 0.3	45 ± 3[a]	73 ± 5	2.3 ± 0.5[a]	25 ± 3[a]	40 ± 5[a]
DOX3 (n = 3)	3.8 ± 0.6	49 ± 8[a]	74 ± 11[a]	3.3 ± 0.2[a]	37 ± 8[a]	66 ± 9[a]
Week 14						
DOX2 (n = 3)	2.9 ± 0.3	39 ± 2[a]	63 ± 5[a]	2.8 ± 0.7[a]	44 ± 9[a]	63 ± 12[a]
DOX3 (n = 3)	3.3 ± 0.5	51 ± 8	84 ± 7	3.0 ± 0.6[a]	51 ± 5[a]	83 ± 8
Week 15						
DOX2 (n = 3)	3.7 ± 1.0	45 ± 8[a]	64 ± 13[a]	3.0 ± 0.2[a]	36 ± 2[a]	51 ± 4[a]

A, aortic; M, mitral; V_{max}, maximal flow velocity, V_{mean}, mean flow velocity; VTI, velocity–time integral.
[a]$P < 0.05$ versus baseline value.

mass during the first 3 wk, and this effect is likely due to the bolus administration of DOX and the sickliness associated with bolus injections. After the initial loss of mass, these animals demonstrated a progressive gain in mass over the next 2 wk, although mean body mass remained below historical control levels. Body mass increases for DOX2 and DOX3 followed the

Table 5. Other echocardiographic measures

	IVRT (ms)	MPI	FS/MPI
Baseline			
DOX1 (n = 10)	20.0 ± 0.7	0.35 ± 0.04	165 ± 26
DOX2 (n = 10)	16.2 ± 1.0	0.25 ± 0.04	248 ± 32
DOX3 (n = 10)	17.3 ± 1.0	0.27 ± 0.05	238 ± 29
Week 1			
DOX1 (n = 10)	26.3 ± 2.4	0.56 ± 0.09	83 ± 14[a]
DOX2 (n = 10)	20.2 ± 1.0	0.29 ± 0.04	227 ± 32
DOX3 (n = 10)	19.3 ± 1.2	0.36 ± 0.05	196 ± 43[a]
Week 2			
DOX1 (n = 7)	30.5 ± 2.6[a]	0.60 ± 0.08[a]	68 ± 9[a]
DOX2 (n = 10)	20.8 ± 1.1	0.25 ± 0.04	263 ± 61
DOX3 (n = 10)	19.4 ± 1.0	0.52 ± 0.03	104 ± 9[a]
Week 3			
DOX1 (n = 4)	17.8 ± 0.8	0.43 ± 0.03	87 ± 9[a]
DOX2 (n = 9)	20.4 ± 0.7	0.42 ± 0.05	134 ± 14[a]
DOX3 (n = 10)	21.1 ± 1.0	0.41 ± 0.03	138 ± 12[a]
Week 4			
DOX2 (n = 9)	22.9 ± 1.3	0.36 ± 0.04	144 ± 22
DOX3 (n = 10)	22.3 ± 1.6	0.51 ± 0.09	125 ± 17[a]
Week 5			
DOX2 (n = 9)	28.7 ± 1.8[a]	0.38 ± 0.05	125 ± 17[a]
DOX3 (n = 7)	24.9 ± 3.2[a]	0.61 ± 0.09	72 ± 18[a]
Week 6			
DOX2 (n = 6)	22.2 ± 1.6	0.41 ± 0.04	100 ± 12[a]
DOX3 (n = 7)	25.2 ± 1.6[a]	0.41 ± 0.06	133 ± 23[a]
Week 7			
DOX2 (n = 6)	27.5 ± 2.4[a]	0.37 ± 0.04	120 ± 24
DOX3 (n = 7)	26.2 ± 0.9[a]	0.56 ± 0.06	84 ± 9[a]
Week 8			
DOX2 (n = 5)	26.7 ± 3.1[a]	0.55 ± 0.07	64 ± 13[a]
DOX3 (n = 6)	27.5 ± 3.3[a]	0.70 ± 0.18[a]	72 ± 10[a]
Week 9			
DOX2 (n = 5)	33.3 ± 4.9[a]	0.84 ± 0.37	67 ± 16[a]
DOX3 (n = 6)	27.7 ± 3.8[a]	0.74 ± 0.36[a]	113 ± 38[a]
Week 10			
DOX2 (n = 5)	37.3 ± 3.0[a]	1.02 ± 0.33[a]	51 ± 15[a]
DOX3 (n = 4)	28.7 ± 1.6[a]	0.45 ± 0.07	97 ± 21[a]
Week 11			
DOX2 (n = 4)	33.1 ± 3.7[a]	0.70 ± 0.24	51 ± 11[a]
DOX3 (n = 3)	37.8 ± 2.1[a]	1.19 ± 0.44[a]	36 ± 12[a]
Week 12			
DOX2 (n = 3)	33.3 ± 6.3[a]	0.69 ± 0.07	51 ± 11[a]
DOX3 (n = 3)	32.7 ± 1.9[a]	0.69 ± 0.12	50 ± 2[a]
Week 13			
DOX2 (n = 3)	40.3 ± 3.4[a]	1.67 ± 0.59[a]	26 ± 16[a]
DOX3 (n = 3)	30.0 ± 2.3[a]	0.60 ± 0.07	56 ± 21[a]
Week 14			
DOX2 (n = 3)	32.4 ± 2.4[a]	0.98 ± 0.35[a]	33 ± 10[a]
DOX3 (n = 3)	23.6 ± 2.1	0.61 ± 0.10	60 ± 1[a]
Week 15			
DOX2 (n = 3)	42.3 ± 5.7[a]	1.33 ± 0.66[a]	32 ± 12[a]

FS, fractional shortening; IVRT, isovolumetric relaxation time; MPI, myocardial performance index.
[a]$P < 0.05$ versus baseline value.

same pattern seen in historical controls. However, for the DOX2 and DOX3 groups, mean body masses were well below historical control values throughout the observation period, indicating a stunting of the growth process.

Although several studies have shown increases in blood pressure after treatment with DOX to a cumulative dose of 20 mg/kg,[29,55] most indicate that DOX treatment lowers blood pressure. Cumulative DOX doses of 13[37] and 15 mg/kg[27,42,43] have led to declines in systolic and diastolic blood pressures ranging from 7% to 20% 3 wk after completing the treatment regimen. Here, we show that blood pressure was significantly lowered in only 1 group at only 1 time interval. Specifically, at week 1 there was a reduction in systolic, diastolic, and mean arterial pressures in DOX1 when compared with baseline (decreases of 31%, 34%, and 34%, respectively). By week 2, blood pressure values were not significantly different from baseline and did not vary significantly from baseline for the remainder of the observation period. DOX2 and DOX3 animals exhibited blood pressure values similar to baseline throughout the observation period. Considering that many indices of systolic function were significantly affected by DOX treatment in all groups, the lack of sustained significant declines in blood pressure suggests the role of compensatory mechanisms in the maintenance of blood pressure in this model.

Although others have seen basal tachycardia in response to DOX treatment,[29,37,55] we did not. For DOX1, no significant differences in heart rate occurred between weeks 1 to 3, but heart rate was reduced consistently for DOX2 and DOX3 rats between weeks 8 to 15. These observations suggest that changes in peripheral resistance may have compensated for the decline in those determinants of cardiac output (that is, contractile function and HR) for as long as 15 wk after beginning treatment. This hypothesis is supported by the findings of Gorodetskaya and colleagues,[17] showing a 70% increase in total peripheral resistance in conscious animals after DOX treatment.

DOX has been shown to mediate measurable changes in left ventricular geometry, typically characterized by a thinning of the ventricular wall and a dilation of the ventricular chamber. As such, DOX treatment has been used to produce a model of dilated cardiomyopathy.[31,41,50] DOX1 animals did show chamber dilation during systole and diastole, but these enlargements in chamber size were not significantly different from baseline values. Although DOX2 and DOX3 rats showed thinning of both the septal and posterior left ventricular walls, significant changes did not occur until after week 8. Administering DOX at 10 mg/kg over the course of as few as 10 d (DOX2) to as many as 28 d (DOX3) produced ventricular wall thinning comparable to that observed with a bolus dose of DOX (DOX1) but more pronounced dilation of the left ventricular chamber. Although animals in the DOX1 group demonstrated a trend toward development of classic dilated cardiomyopathy, they typically died before the onset of significant ventricular morphologic aberrations. As such, DOX2 and DOX3 produced alterations in cardiac geometry that better resembled the dilated cardiomyopathy often associated with delayed-onset DOX cardiotoxicity.

The benchmarks for assessment of systolic function using transthoracic echocardiography have long been FS, RWT, and V_{cf}. According to these measures, animals in all groups exhibited signs of systolic dysfunction, with DOX1 rats showing evidence of dysfunction from weeks 1 to 3, and DOX2 and DOX3 animals showing delayed onset of dysfunction between weeks 3 to 5. In addition, the severity of systolic dysfunction as measured by FS was greater for DOX2 (55%) and DOX3 (42%) than for DOX1 (30%). Although animals in DOX1 may subsequently have developed more severe systolic dysfunction, they typically died before its onset. Large-bolus DOX doses have typically been used to induce acute myocardial oxidative stress[13,35,40] or

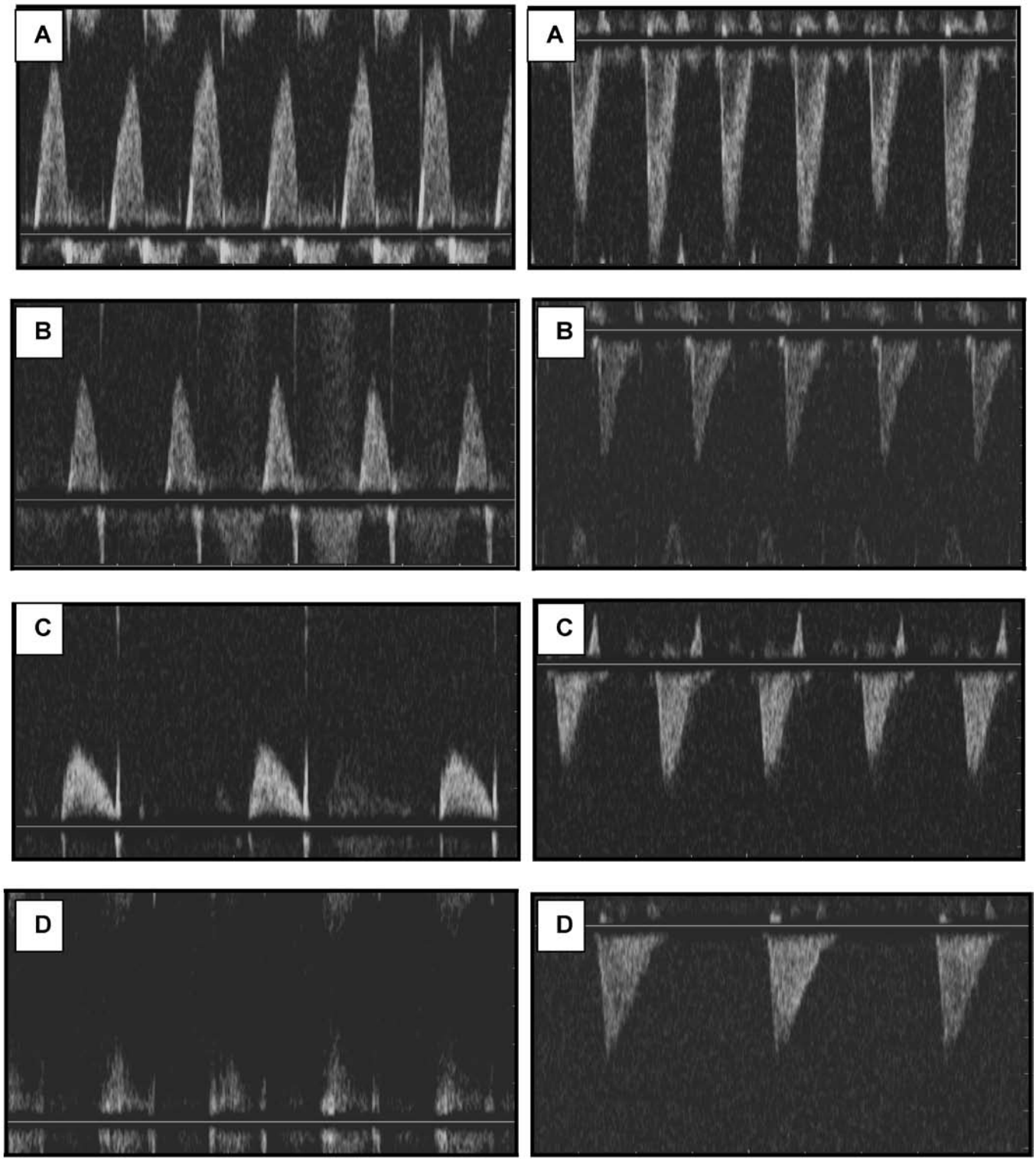

Figure 5. Representative mitral (left) and aortic (right) Doppler images obtained at baseline (A) and after the onset of cardiac dysfunction in (B) DOX1, (C) DOX2, and (D) DOX3 animals. DOX1, 10 mg/kg bolus; DOX2, 1 mg/kg daily for 10 consecutive days; DOX3, 2 mg/kg weekly for 5 wk.

acute cardiac dysfunction (for example, 2 to 10 d).[3,6,7,40] Lower quantities of DOX administered over time have been shown to alleviate the obvious signs of congestive heart failure,[48,56] and this approach is now being used by researchers with greater frequency. These studies, along with our data, highlight an important observation. Lower doses of DOX administered over time do indeed alleviate many of the overt signs of DOX-induced heart failure (that is, physical characteristics, ascites) and may have little effect on cardiac function when compared with those of animals treated with an equivalent cumulative dose at equivalent time intervals (for example, FS for DOX1 versus DOX2 at the 3-wk interval). However, even at cumulative doses of 10 mg/kg, as reported here, if given enough time, lower doses given over time (10 d or 5 wk) can result in systolic dysfunction exceeding that of a bolus dose without many of the negative side-effects of the drug.

There are a number of benchmarks used to assess diastolic function using transthoracic echocardiography, including IVRT,

TVI_M, E and A wave velocities, and the E/A ratio. One of the hallmarks of DOX is diastolic dysfunction, and it is often asserted that diastolic dysfunction precedes systolic dysfunction. In our study, this difference was not apparent. IVRT and TVI_M did show significant decreases during the observation period for all groups, yet the onset of these changes did not appear before changes in systolic function were manifested. Significant increases in IVRT did not occur until weeks 2, 5, and 7 for DOX1, DOX2, and DOX3, respectively, with each of these occurring after the onset of systolic dysfunction.

E and A wave velocities and the E/A ratio may be better indices of diastolic function, but assessment of these variables in this model presents several problems. The presence of E and A waves in the normal rat is a rate-dependent phenomenon. In our laboratory, when heart rates are approximately 300 beats per minute, no E or A waves are noted because these phases of left ventricular filling overlap one another and thus are indistinguishable. In contrast, at heart rates below approximately 250 beats per minute in the normal rat, E and A waves are seen clearly. Other laboratories have indicated that this cutoff point is approximately 300 beats per minute ,[52] whereas some studies lower HR to approximately 215 beats per minute in order to see E and A waves.[2] In DOX-treated animals we commonly see E and A waves, but again, this pattern is likely due to the depressive effects of DOX on heart rate. Although it is possible to artificially lower the heart rate via additional anesthesia such that E and A waves are present, this technique requires a different anesthesia protocol for animals that do versus those that do not present with these waveforms.

The MPI (that is, Tei Index), a relatively new variable for assessment of cardiac function, is gaining support as a standard noninvasive measure of cardiac function in animal models. MPI correlates strongly with several invasive measures of cardiac function, including the peak positive value of the time derivative of LV pressure (dP/dt_{max}), left ventricular end-diastolic pressure, end-diastolic volume, and end-systolic volume.[18,47] The MPI is dependent on contractility, preload, and afterload and is purported to be independent of cardiac geometry. If these characteristics are indeed valid, MPI is an attractive variable for the assessment of cardiac function in a model of DOX cardiotoxicity because of the significant changes in cardiac morphology that typically occur with DOX treatment. We noted early (weeks 1 to 3) increases in MPI for DOX1 animals and late (weeks 8 to 15) increases in the DOX2 and DOX3 groups, thus demonstrating similarity with other noninvasive measures of cardiac function in this model.

When designing these experiments, we hypothesized that there would be clear and observable differences between all 3 groups in terms of survival, physical characteristics, and cardiac function. Specifically, we hypothesized that within 3 wk of treatment with a single bolus injection of DOX at 10 mg/kg (DOX1), rats would have a low survival rate, exhibit left ventricular dilation, and demonstrate signs of overt heart failure as assessed by echocardiography. We further hypothesized that animals given the same cumulative dose of DOX (10 mg/kg) distributed over the course of 5 wk (2 mg/kg weekly, DOX3) would have higher survival rates and have less severe cardiac dysfunction than would animals in the DOX1 group. In addition, we hypothesized that animals treated with 10 mg/kg over the course of 10 d (1 mg/kg daily, DOX2) would demonstrate an intermediate level of survival and cardiac dysfunction.

Indeed, there were clear differences in physical characteristics and survival between DOX1 and DOX3 rats. Spreading the 10 mg/kg DOX over the course of 5 wk (DOX3) did not reduce the degree of cardiac dysfunction, with the severity of dysfunction equal to or more severe than the dysfunction noted in DOX1. This result likely was due to the fact that the DOX3 treatment regimen prolonged survival, thereby extending exposure to the drug and intensifying the degree of cardiac-specific toxicity. In addition, virtually all variables were remarkably similar between animals treated with 10 mg/kg DOX over the course of 10 d (DOX2) and those treated over the course of 5 wk (DOX3). This finding indicates that for studies investigating chronic DOX cardiotoxicity, small incremental doses of DOX could be administered over the course of several days, not several weeks, to derive the benefits of healthier experimental animals without sacrificing the degree of cardiotoxicity.

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