Continuous Electrocardiogram Reveals Differences in the Short-Term Cardiotoxic Response of Wistar-Kyoto and Spontaneously Hypertensive Rats to Doxorubicin

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Electrocardiography (ECG) is one of the standard technologies used to monitor and assess cardiac function, and provide insight into the mechanisms driving myocardial pathology. Increased understanding of the effects of cardiovascular disease on rat ECG may help make ECG assessments in rat toxicology studies routine, thus facilitating continuous measurement of functional decrements associated with cardiotoxicant exposure. These studies seek to test the hypothesis that hypertensive rats are more susceptible to the short-term cardiotoxic effects of doxorubicin (DOX) when compared with normotensive rats with respect to continuously measured ECG endpoints. Male Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats surgically implanted with radiotelemeters were treated once a week for three weeks with either vehicle, 1.25 (low), 2.5 (medium), or 5 (high) mg/kg DOX (i.p.). ECG, heart rate (HR), and core body temperature (Tco) were continuously monitored during the 1-week baseline and throughout the experimental period until rats were sacrificed 24 h after the third injection. DOX prevented normal body weight gain in both strains and significantly decreased diurnal HR and Tco of high DOX SH rats. In the ECG, SH rats had prolonged baseline PR intervals and QTc when compared with WKY rats. All DOX-treated WKY rats subsequently developed PR interval prolongation; however only those treated with high DOX had increased QTc. DOX caused an increase in ST interval in SH rats, and resulted in ECG morphology changes. The number of arrhythmias due to DOX was increased in both strains. In conclusion, ECG analysis can reveal underlying cardiovascular disease as a risk factor in the heart’s response to toxicant-induced injury in the rat; and be a valuable tool to evaluate baseline vulnerability and assess cardiotoxicity.

Key Words: electrocardiogram; doxorubicin; cardiotoxicity; hypertension.

Cardiovascular diseases, including hypertension and heart failure, compromise the body’s ability to deal with toxic insults. The response of the cardiovascular system to pharmacological and environmental toxicants is influenced by the physiological state of the heart, as determined by circulating vasoactive substances and hormones, myocardial perfusion, and neural input; and the electrical, mechanical, ionic, and energetic state of the myocardium itself. A systemic or cardiac disease may affect one or more of these factors deleteriously and thus lead to conditional susceptibility to a toxicant, such that when that toxicant is presented an exaggerated cardiovascular response occurs. These responses may be manifested as dysfunction in one or more cardiac responses including myocardial contractility, intracardiac pressure-volume relationships, cardiac output, systemic blood pressure, heart rate (HR), or cardiac rhythm.

Hypertension, which is a widespread clinical condition that predisposes to cardiac morbidity and mortality secondary to ischemic heart disease, heart failure and arrhythmia is thought to increase susceptibility to the adverse health effects of environmental pollutants and oxidant stress. The spontaneously hypertensive (SH) rat serves as an excellent model of human hypertensive heart disease and is characterized by progressive cardiac hypertrophy, molecular adaptations predisposing to arrhythmia and in its later stages heart failure and death (Choisy et al., 2007). Previous studies have established fundamental molecular changes in cardiac myocytes in the SH strain in comparison to the Wistar-Kyoto (WKY) and other strains of rat (Tang et al., 2008; Tribulova et al., 2003).

In this study, doxorubicin (DOX), an anti-neoplastic drug with known cardiotoxic effects, was administered to produce heart failure in rats and examine the utility of electrocardiogram (ECG) as an indicator of heart dysfunction. Although its detrimental effects may be due to multiple factors, DOX-mediated cell damage is likely due to generation of oxidative free radicals (Ferreira et al., 2008), or aberrant calcium signaling (Chacon et al., 1992), which could result in abnormal electrical conduction in the heart, arrhythmia and failure under chronic conditions. Previous studies in humans have shown an
The association between DOX and heart block and arrhythmias (Ali et al., 1979; Steinberg et al., 1987).

Investigators have studied some of the parameters of the ECG in humans, including cardiac arrhythmogenesis, which may be indicative of underlying disease and predictive of adverse effects upon exposure to toxicants (Peters et al., 2000; Wellenius et al., 2002; Zareba et al., 2001). Similar applications of ECG have been used in laboratory rodents (Chen et al., 2008), particularly the rat (Nadziejko et al., 2004; Watkinson et al., 1998; Wellenius et al., 2004). However, a thorough characterization of ECG signals and waveforms and how they change after injury are still necessary in the laboratory rat, particularly under conditions of underlying cardiovascular disease and heart failure. Using continuous monitoring, these studies seek to test the hypothesis that SH rats are more susceptible to the adverse effects of DOX when compared with an age-matched WKY rat strain with respect to electrocardiographic endpoints such as HR, rhythm, and ECG parameters representing intracardiac conduction and repolarization. Specifically, these experiments were designed to (1) examine the differential effects of DOX on HR and ECG parameters as determined by the extent of pre-existent cardiovascular disease, modeled by SH and WKY rats, and how they change following treatment with DOX; (2) classify and compare the types and frequency of cardiac arrhythmias in both strains; and (3) compare DOX-induced markers of inflammation and cardiac damage in both strains after treatment. This study provides a novel examination of ECG trends over an extended period of cardiac damage in conscious and unrestrained healthy, and hypertensive rats in response to DOX-induced cardiotoxicity.

MATERIALS AND METHODS

Animals. Eleven-week-old, male WKY and SH rats (Charles River, Wilmington, MA) weighing 300–400 g were studied. Upon arrival, animals were housed two per cage with food and water available ad libitum in an AAALAC-approved facility. All experimental protocols were approved by and in accordance with the guidelines of the Institutional Animal Care and Use Committee of the U.S. Environmental Protection Agency, Research Triangle Park, NC.

Groups and experimental protocol. Rats (n = 6/treatment group) from each strain were assigned to one of four treatment groups: (1) saline vehicle (v); (2) 1.25 mg/kg DOX (Sigma chemical, St Louis, MO) (low); (3) 2.5 mg/kg DOX (med); (4) 5 mg/kg DOX (high). Animals received one intraperitoneal injection of vehicle, 1.25, 2.5, or 5 mg/kg DOX per week for 3 weeks, for a total of three injections. Animals were sacrificed 24 h after the final injection. Animals were weighed during the baseline period (BL), prior to each injection (Pre-D1, Pre-D2, Pre-D3), and just before sacrifice (SAC). A separate group of SH rats were also treated with 5 mg/kg DOX once a week for three weeks, and were allowed to survive to assess the long-term effects of the drug.

Implantation of radiotelemetry. Radiotelemeters were implanted in all animals as previously described (Campen et al., 2000; Watkinson et al., 1995). Briefly, animals were weighed and anesthetized with ketamine hydrochloride/xylazine hydrochloride solution (1 ml/kg, i.p. Sigma-Aldrich, St Louis, MO). With use of aseptic technique, each animal was implanted with a radiotelemetry transmitter (Model TA11CTA-F40, Data Sciences International, St Paul, MN).

The transmitter module was placed in the abdominal cavity through a small incision. The electrode leads were guided through the abdominal musculature via separate stab wounds and tunneled subcutaneously across the lateral ventral thorax; the distal portions of the leads were secured in positions that approximated those of the lead II of a standard ECG. Body heat was maintained both during and immediately following the surgery. Animals were given food and water and were housed individually. All animals were allowed 7–10 days to recover from the surgery and reestablish circadian rhythms. Radiotelemetry methodology was used thereafter to continuously monitor and record ECG, HR, and core body temperature (T<sub>c</sub>) for the duration of the experimental period (7–30 days).

Radiotelemetry data acquisition and analysis. Radiotelemetry methodology (Data Sciences International, Inc.) was used in this study to track changes in cardiovascular function by monitoring ECG and HR. This methodology provided continuous monitoring and collection of physiologic data from unrestrained, un-anesthetized rats. Data signals were transmitted from implanted radiotelemeters to remote receivers positioned underneath home cages (DataART2.1: Data Sciences International). Sixty-second segments of ECG waveforms were acquired and saved at 15-min intervals from surgical recovery through sacrifice; values were obtained sequentially by animal. HR was obtained from the ECG. Baseline data permitted each animal to serve as its own control, whereas matched group animals treated with vehicle provided time-paired control data.

Electrocardiogram analysis. ECGAuto software (EMKA technologies USA, Falls Church, VA) was used to visualize individual ECG signals, analyze and quantify ECG segment durations, and for identification and enumeration of cardiac arrhythmic events. Using ECGAuto, P-wave, QRS complex, and T wave were identified for individual ECG waveforms and compiled into a library. Analysis of all experimental ECG traces was then based on established libraries. The following parameters were determined for each ECG waveform: PR interval, QRS duration, which was measured as the beginning of the R-wave (nadir of Q-wave) until the S-wave reached the isoelectric line because Q-waves were not clearly discernable in many traces, QT interval, QT corrected for HR (QTc) using Bazett’s formula, and ST interval, which was measured from the nadir of the S-wave until the end of the T-wave or when it reached the isoelectric line. The Lambeth (1988) conventions were used as guidelines for the identification of cardiac arrhythmic events in rats. Arrhythmias were identified as either atrial premature beats (APBs), ventricular premature beats (VPBs), nonconducted P-waves (NCP), or ventricular tachycardia (VT). Figure 5 shows examples of arrhythmias typically found in the animals in this study. Arrhythmias are, in general, infrequent, and were therefore quantified over the 1-week baseline period (BL), the first week (W1), and the second week (W2) of the treatment period. Arrhythmias in SH rats treated with 5 mg/kg DOX and allowed to survive were quantified in the week after the final treatment (post-1 week) as well. A modified scoring system (Curtis and Walker, 1988) was adopted to quantitatively compare arrhythmias. Table 1 describes the criteria for scoring arrhythmias in each animal; the principles were based on (1) ventricular arrhythmias being more severe than atrial arrhythmias; (2) the increasing severity of ventricular arrhythmias being occasional VPB, frequent VPB, and VT; (3) the longer the duration of the arrhythmias the greater the severity.

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Necropsy, blood collection, and bronchoalveolar lavage. Rats were deeply anesthetized with Euthasol (200 mg/kg sodium pentobarbital and 25 mg/kg phenytoin i.p.; Virbac Animal Health, Ft Worth, TX). Blood samples were collected from the abdominal aorta and renal artery, and fractionated into serum and plasma. The trachea was cannulated and the lungs were lavaged with a total volume of 35 ml/kg of Ca²⁺, Mg²⁺, and phenol red-free Hank’s balanced salt solution divided into two equal aliquots (HBSS; Life Technologies, Bethesda, MD). The BAL fluid was processed and cells were prepared and stained with Wright-Giemsa Stain Pack (Fisher Diagnostics, Middleton, VA) for determination of cell differentials. Assays for markers of cardiac and lung injury, and oxidant stress were performed on an aliquot of BAL supernatant, and serum was analyzed for creatine kinase (CK), alpha-hydroxybutyrate dehydrogenase (α-HBDH), and glutathione peroxidase (GPX) using a Konelab 30 spectrophotometer (Konelab 30, Espoo, Finland). Euthanasia was achieved by exsanguination after transection of the aorta.

Heart histopathology. After fixation in 10% acetate-buffered formalin, hearts were trimmed and processed to paraffin blocks, sectioned and stained with hematoxylin and eosin for microscopic examination or Barbeito-Lopez trichrome stain for routine diagnosis of myocardial degeneration or necrosis. A semiquantitative grading scheme was used to evaluate the extent of the cardiomyopathy in the heart section as follows: minimal (grade 1) involved 1–10% of the section; mild (grade 2), 11–40%; moderate (grade 3), 41–80%; and marked (grade 4), 81–100%.

Statistics. The statistical analyses for all the data in this study were performed using SAS version 9.1.3 software (SAS Institute, Inc., Cary, NC). PROC MIXED, PROC GLIMMIX, and GENMOD were used to analyze all parameters, PR interval, QTc, ST interval, and QRS for each group. This includes data for the baseline period, after the first injection to 37.6 ± 0.1°C). However, when compared with baseline, $T_{co}$ decreased significantly in SH rats treated with 5 mg/kg DOX 1 week after the third injection to 34.8 ± 2.2°C.

HR Effects

Figure 2 shows the average changes in HR of each group during a segment of the baseline and over the experimental period. All WKY and SH rat groups had a typical diurnal HR pattern with peaks during daytime and nadir during the night. The HR decreased in WKY rats treated with 5 mg/kg DOX relative to vehicle-treated rats after the second injection (Fig. 2A). All DOX-treated SH rats had a decrease in HR relative to vehicle-treated animals after the first injection. SH rats treated with 5 mg/kg DOX had the greatest decrease in HR, which was approximately 40 bpm lower than the control group; this decrease persisted through the final injection and during the week after the final injection when the average HR dropped significantly and the magnitude of the diurnal rate changes were attenuated (Fig. 2B).

Changes in Electrocardiogram Parameters and Morphology

Figure 3 shows the durations of the electrocardiographic parameters, PR interval, QTc, ST interval, and QRS for each group. This includes data for the baseline period, after the first
and second injections, and 24 h after the third injection. The figure also includes the 1-week post-treatment period for SH rats treated with 5 mg/kg DOX. PR interval increased significantly after the first injection in WKY rats treated with 2.5 and 5 mg/kg DOX, and after the second injection in rats treated with 1.25 mg/kg DOX. SH rats had longer baseline PR intervals than WKY rats, which did not change after treatment with DOX. On the other hand, DOX caused a dose-dependent increase in QTc in WKY rats during the treatment period. This was significantly higher on the day of sacrifice in WKY rats treated with 5 mg/kg DOX when compared with vehicle controls. SH rats had higher baseline QTc values than WKY rats, and were highest in 5 mg/kg DOX-treated SH rats at the end of 1-week post-treatment. ST interval duration increased steadily in WKY rats treated with 5 mg/kg DOX and was significantly longer than the baseline values on the day of sacrifice. SH rats exhibited longer baseline ST intervals than WKY rats, this grew significantly longer in 5 mg/kg DOX-treated SH rats at the end of 1-week post-treatment. QRS duration increased significantly in WKY rats treated with 5 mg/kg DOX after the first injection when compared with vehicle controls; however, there was no change in QRS duration in SH rats in response to DOX at any dose. SH rats treated with 2.5 and 5 mg/kg DOX developed persistent ST depression 3–5 days after receiving the first injection. Repeated episodes of inverted T-waves were observed in four of the six SH rats treated with 5 mg/kg DOX after the last injection until the end of the 1-week post-treatment period. Figure 4 shows typical 2-s ECG traces from an SH rat during baseline (A), with post-DOX ST depression (B) and with post-DOX inverted T-waves (C).
before and during the development of DOX-induced cardiac toxicity. ECG analysis indicated not only baseline differences in strain, but also differences in the cardiotoxic response to DOX; suggesting there may be prognostic value for ECG analysis in rat cardiotoxicity studies that presages the development of histological evidence of toxicity. Here, the goals were to

FIG. 3. DOX causes ECG changes in WKY and SH rats indicative of heart failure. Low, med. and high DOX caused a significant increase in the PR interval of WKY rats (A, top row), whereas there was no change in SH rats (B, top row), which had longer pre-existing PR interval than control WKY rats. High DOX caused a significant increase in QTc when compared with vehicle in WKY rats (A, second row); QTc increased significantly in SH rats 1-week post-high DOX when compared with vehicle-treated SH rats immediately after the third DOX treatment (SAC) (B, second row). High DOX caused a significant increase in ST interval duration when compared with vehicle in WKY rats (A, third row); ST interval was significantly increased in SH rats 1-week post-high DOX when compared with baseline and vehicle-treated SH rats immediately after the third DOX treatment (SAC) (B, third row). High DOX caused a significant increase in QRS duration in WKY rats when compared with baseline and vehicle-treated WKY rats (A, last row); med. and high DOX caused minor increases in QRS duration in SH rats (B, last row). *Significantly different from baseline, $p < 0.05$; §significantly different from vehicle, $p < 0.05$ ($n = 6–7$).
establish an ECG foundation for assessments in rats challenged with toxicant stressors such as air pollution, and to determine the features of the ECG in a cardiac susceptible model which would predict and reveal biomarkers of sensitivity.

SH rats had greater DOX-induced decrease in HR than WKY rats. Previous studies found no difference (Kelishomi et al., 2008; Sacco et al., 2001) or an increase (Rossi et al., 2008) in HR between DOX-treated animals and controls several weeks after treatment. In contrast, other studies have shown decreases in HR and contractility with repeated DOX treatments, likely due to cumulative free radical cardiac damage (Boucek et al., 1997; Villani et al., 1990; Yoshikawa et al., 1994). It has been demonstrated that sympa-ho-excitatory neuronal failure occurs locally in the heart following treatment with DOX (Yoshikawa et al., 1994). Hence, DOX-induced reduction in sympathetic influence may be partly to blame for reduced HR, however it has also been shown that β-adrenergic stimulation is inherently reduced in the failing human myocardium and in spontaneously hypertensive heart failure (SHHF) rats (Anderson et al., 1999). Therefore the reason for the large decline in HR during treatment and eventual failure observed in our SH rats may be due to a synergistic effect between the pre-existing β-adrenergic dysfunction and hypertension, and DOX-mediated decrease in sympathetic influence. Although DOX-treated animals appeared fatigued, they could not be “diagnosed” as having true clinical heart failure due to the lack of correlating CK levels and histopathology, and the absence of other causes (diabetes, lung disease, or cardiomyopathy). Clearly these rats had failing hearts as indicated by the changes in HR and ECG, however this condition can only be verified with functional measurements of cardiac mechanics, which were not performed.

We observed baseline differences in the ECG of healthy WKY rats and SH rats. Clearly, ECG parameters have been found to vary among strains of rats, even in the absence of any underlying cardiovascular disease. In one study, Long Evans rats were found to have comparable QRS complex and QT interval duration to our WKY rats (Jalili et al., 1996), whereas the durations were shorter in Sprague-Dawley and Wistar rats (Dragojevich-Simic et al., 2004; Kelishomi et al., 2008). Although, these variations are probably due to differences in electrical potential across the body surface, baseline ECG differences between the WKY and genetically derived SH rats in our study may also indicate a functional divergence, which is further manifested during the cardiotoxic response to DOX. WKY rats exposed to DOX experienced significant increases in PR interval, however only those animals treated with high DOX experienced increased QTc, ST interval duration and QRS complex duration. Previous findings in the Sprague-Dawley rat found similar increases in ST segment and QRS complex duration at comparable doses of DOX (Danesi et al., 1986; Kelishomi et al., 2008; Xin et al., 2007), but did not have any increase in PR interval. The lengthening of PR interval in the WKY rat, which has been demonstrated by others (Puri et al., 2005), may be due to unique characteristics that are not present in other strains. Interestingly, all our SH rat groups had increased PR intervals, which were comparable to

![FIG. 4. Treatment with DOX causes ST depression and T-wave inversion in SH rats. Traces A, B, and C. show typical ECG segments from the baseline period of a SH rat, post-DOX ST depression, and post-DOX ST depression and T-wave inversion, respectively.](image-url)
the DOX-treated WKY rats. Lengthening of the PR interval might be related to slowing of intra-atrial propagation, or more likely slowing of conduction in the atrioventricular (AV) node because there was no change in P-wave length. Collectively, these data suggest that PR interval may be a sensitive indicator of developing cardiomyopathy in the WKY strain and that the derived strain of naïve SH rats have baseline cardiomyopathy and elevated PR intervals which ostensibly are unaffected by DOX.

Lengthening of the duration of the QRS complex, ST interval, and QTc in the WKY rats treated with DOX were seen at baseline in all SH rat groups, and thus may reflect a preexisting pathology in the SH rat. The increased QTc in SH rats was largely accounted for by ST interval lengthening, indicating differences in the repolarization characteristics of both strains. QTc and ST interval duration progressively increased in the high dose DOX SH rats post-treatment; however, no change was observed in QRS duration, suggesting a “heterogeneity of repolarization,” which can often be mediated by changes in $I_{TO}$ K+ or $I_{UR}$ K+ currents (European Society of Cardiology, 2001). In fact, during this period, two of the eight SH rats had higher QTc and ST interval durations than other animals in the group and died prematurely. The lengthening of QTc and ST interval durations appear consistent with that seen in humans (Hombach, 2002) and may be predictive of mortality in rats as in patients with advanced heart failure (Vrtovec et al., 2003). The results of this study indicate that the strain of rat is a major determinant of the changes observed in ECG. Moreover, as in this study, the presence of underlying cardiovascular disease associated with hypertension has been shown to exacerbate DOX-induced cardiotoxicity (Rossi et al., 1994). The PR interval and QRS complex changes, which could be secondary to DOX-induced effects on calcium current, likely point to the initial stages of cardiomyopathy in the healthy rat strain. In contrast, ST interval and QTc lengthening, which are likely due to oxidant stress-induced repolarization changes, may be an indicator of more severe damage and thus enhanced cardiac susceptibility to cardiotoxicants in animals with underlying disease.

All SH rats treated with DOX developed ST depression, a response that was only present in some (3/6) of the high DOX WKY rats. However, many of the SH rats exhibited transient baseline ST depression, indicating that SH rats may have had underlying pathology which was clearly worsened by DOX exposure. The development of depression in the ST segment represents a delay in the repolarization of the ventricular myocardium, which could have been generated by the effects of DOX. However, this phenomenon may also have simply been due to slowed conduction, as evidenced by prolonged QRS complex duration, which changes direction and forms a notch or bump in the ECG trace at the J-point and thereafter forms the ST segment. Although the normal rat ECG does not contain an ST segment (Johnston et al., 1981; Osborne, 1981; Spear, 1981), the same conditions that cause ST segment changes in human ECG, ischemia, for example, also cause corresponding changes in rat ECG. ST segment changes are often associated with ischemia, oxidant stress or ventricular hypertrophy in humans. However, given none of the rats, WKY or SH, had any form of ventricular hypertrophy or fibrosis, and that persistent ischemia leads to cardiac injury and elevated CK levels, it is likely the ST segment depression observed in this study was due to DOX-induced oxidant stress and its deleterious electrophysiological effects. This may be due in part to calcium overload and impaired regulation of autonomic control of the heart (Danesi et al., 1986; Hrdina et al., 2000; van Acker et al., 1996; Villani et al., 1990).

SH rats treated with high DOX also had repeated and consistent inverted T-waves post-treatment. Although the T-wave is considered to be the most labile ECG component, in the current study, such anomalies were not observed in any other group or at any other time point. ST depression and T-wave inversion are acute changes that may be indicative of

FIG. 5. Typical arrhythmias observed in WKY and SH rats. (A) APB; (B) VPB; (C) nonconducted P-wave; (D) VT; (E) severe atrioventricular block.
ischemia and increased risk of myocardial infarction (Alpert et al., 2008). In humans, T-wave inversion may be normal, hence it is only indicative of ischemia when observed with certain anomalies in the other ECG leads (Channer and Morris, 2002). Very few studies have reported T-wave inversion or flattening in rodents (Berkowitz et al., 1988; Dragojevic-Simic et al., 2004). Interpretation of DOX-induced T-wave inversions in the present study are tempered given the limitations of the lead II signal in the conscious rat. Further studies with other lead signals are required to determine whether the DOX-induced T-wave inversions were in fact due to myocardial ischemia or other cardiac dysfunction.

SH rats, particularly those treated with high DOX, were clearly more susceptible to developing VT than WKY rats. However, the number of VT episodes seen in high DOX SH rats did not progress post-treatment; instead there was a significant increase in APBs. APBs occur commonly in healthy people and most go unnoticed; however their frequency is increased in people with ischemic heart disease or congestive heart failure. These arrhythmias can be dangerous because they are capable of eliciting atrial tachycardia and/or fibrillation (Chung, 1983). Hypertension is responsible for more atrial fibrillation than any other risk factor and it may be one of the causes of supraVPBs (Aidietis et al., 2007). Although blood pressure was not measured in this study, it has been established that SH rats have significantly higher mean arterial pressure relative to their background strain (Yamori et al., 1979). However, other factors may also contribute to arrhythmogenesis in heart failure; the effects of DOX, which are known to affect these factors, particularly ion flux, coupled with pre-existing cardiac lesions in the SH strain likely account for the arrhythmia observations in this study.

Treatment with DOX eventually caused the appearance of nonconducted P-waves in approximately half the WKY rats and 90% of SH rats. Aside from three high DOX WKY rats with ECGs indicative of second-degree AV block Mobitz type I, all other WKY rats and all SH rats presenting with nonconducting P-waves without prolongation indicated second-degree AV

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<th>Group</th>
<th>APB (BL)</th>
<th>W1</th>
<th>W2</th>
<th>VPB (BL)</th>
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<th>W2</th>
<th>VT (BL)</th>
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<th>W2</th>
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<td>0</td>
<td>0.2 ± 0.2</td>
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<td>1.2 ± 0.9</td>
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<td>0.2 ± 0.2</td>
<td>0</td>
<td>1.6 ± 0.8*</td>
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<td>BL Post-1 week</td>
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*Significantly different from baseline.
§significantly different from vehicle; p < 0.05.

FIG. 6. Medium and high doses of DOX increase cardiac arrhythmias in WKY and SH rats. Treatment with medium (2.5 mg/kg) or high (5 mg/kg) dose of DOX increased the arrhythmia scores of WKY rats when compared with vehicle-treated animals (A). SH rats treated with vehicle had more arrhythmias during the 2-week treatment period than all of the WKY groups, this effect was highest in the SH rats treated with high dose of DOX (B). Arrhythmia scores were even higher during the 1 week after the final injection in the high dose group (B, high’).
block Mobitz type II. In man, the designation of Mobitz type I or type II implies the mechanism of block. In Mobitz type I block occurs in AV node, whereas in the Mobitz type II the His bundle and bundle branches are often involved (Scheidt, 1986). In the least, WKY rats treated with DOX may have developed first degree AV block, which is a minor AV conduction defect, as evidenced by the prolongation of PR intervals after the first injection. Such a condition may already exist in naïve SH rats given the prolonged baseline PR interval.

It is uncertain whether these patterns of AV block has the same meaning, and thus further work is required to ascertain the origin of these arrhythmias. Because nonconducted P-waves were present in vehicle-treated animals, DOX may have only served to exacerbate the problem, particularly because the number of episodes in the DOX-treated animals was higher. Therefore the preceding may be an important pathological progression in the WKY rat, which when made hypertensive, as in the case of the SH strain, develops Mobitz type II second-degree AV block more readily. The progression of this block was better appreciated post-treatment when two of the high DOX SH rats manifested what appeared to be “high-grade” incomplete heart block or third-degree AV block, and a brief period of asystole. Therefore, the WKY strain may be prone to this form of conduction irregularity, or disturbances which occur due to lesions that are below the AV node, and become worsened in animals with hypertension and treatments that result in cardiotoxicity. It is apparent from these findings and the arrhythmia scores that SH rats have a greater baseline propensity for arrhythmogenesis than WKY rats, which was worsened by the DOX treatment. This predisposition is important in the characterization of both strains and identifying the cardiac susceptibilities.

An unexpected difference between strains was that DOX caused a greater decrease in body weight in WKY rats than in SH rats. Reduction in body weight gain due to DOX has been attributed to decreased food intake and inhibition of protein synthesis (Li and Singal, 2000; Tong et al., 1991), yet water retention and ascites in the SH rats, which was present at necropsy, may mask the actual loss in body mass. This suggests an obvious strain difference in the ability of rats to withstand stress and continue normal growth, which may be valuable when applied to the assessment of systemic toxicity. The significant drop in \( T_{co} \) in the high DOX SH rats observed post-treatment, which was also the period of most severe cardiac toxicity, likely indicated such systemic toxicity. Conversely, this response to DOX may have been different if the animals were housed closer to 30°C (thermoneutrality) rather than 22°C, which is typical for toxicological testing. Studies have indicated that there is a positive correlation between temperature and sensitivity to chemicals toxicants, particularly when considering pathophysiological effects in the heart and other sensitive organs (see review Gordon, 2003), and therefore we may not have attained as robust a response as may have been seen at thermoneutrality. Lastly, despite the DOX-induced toxicity to the heart, which has previously been shown as increased cardiac stress markers such as CK and HBDH (Singh et al., 2008); the levels were dose-dependently decreased in this study. This result was likely due to a single blood sampling at the end of the experimental period; at which point DOX-induced decreases in protein synthesis may have reflected depression of most proteins.

Adaptation of the heart to stress often results in electrical “remodeling,” which may occur due to changes in ion flux or perturbations in autonomic control. These factors can lead to enhancement of “heterogeneity of repolarization” or alterations in the directionality of cardiac activation, and trigger arrhythmia (European Society of Cardiology, 2001). Previous studies have examined the effects of DOX-induced cardiotoxicity, however most were limited by having to perform in-vivo observations in anesthetized animals at the end of a treatment period, or using a Langendorff isolated perfused heart system (Dragojevic-Simic et al., 2004; Puri et al., 2005; Kelishomi et al., 2008; Sacco et al., 2001; van Acker et al., 1996; Rossi et al., 2008; Wonders et al., 2008). This is the first study to
compare continuous ECG in two conscious, unrestrained rat strains before and after exposure to DOX and demonstrated that pre-existing cardiovascular disease has a substantial impact on the long-term manifestation of electrocardiographic parameters in conscious rats. The elimination of anesthesia and restraint seemingly unveiled subtle baseline difference in cardiac function and revealed clear difference in functional pathophysiology as cardiotoxicity developed. These findings have two implications, not only that pre-existing cardiovascular disease exerts a great influence on both the quality and magnitude of responsiveness to cardiotoxicants, but that this condition can be modeled in rats. The use of conscious, unrestrained ECG measures in studies of toxicants is relevant to human outcomes. The measures themselves are sensitive and sophisticated and can be readily used to discern both short and long-term dose-dependent effects of cardiotoxicants.

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