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# Strain echocardiography combined with pharmacological stress test for early detection of anthracycline induced cardiomyopathy

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ABSTRACT

*Introduction:* Advances in echocardiography, including 2-D speckle tracking to quantitate myocardial strain and strain rate, have improved myocardial functional and mechanical evaluation and may provide a more sensitive assessment of cardiac functional and mechanical changes. Additionally, evaluating myocardial performance induced by a pharmacologic stress test (dobutamine infusion) may further improve the evaluation of potential changes in cardiac function. This study evaluates the use of 2-D speckle tracking strain echocardiography (2DSE) combined with a dobutamine stress test to detect doxorubicin induced cardiomyopathy in the rat.
*Methods:* Rats were dosed once per week with 2 mg/kg doxorubicin for 6 weeks. Echocardiography was performed weekly at rest and during dobutamine infusion (20 mcg/kg/min IV).
*Results:* Throughout the study there were no differences between control and doxorubicin treated groups at rest for radial strain, circumferential strain, fractional shortening (FS), or heart rate (HR). During dobutamine infusion, radial strain, circumferential strain, FS, and HR similarly increased significantly in both the control and doxorubicin treated groups at weeks 0, 1, and 2. At week 3 there was a significant attenuation of the increase in radial strain in the doxorubicin treated group, and at weeks 4 and 6 there was significant attenuation in radial strain and circumferential strain. No significant differences were detected in FS or HR between the two groups at any time points. Histology of the left ventricle at week 7 showed mild changes (mild cardiomyocyte vacuolation with minimal inflammation and no fibrosis) in the doxorubicin treated animals as compared to the control animals, which were consistent with mild doxorubicin induced injury.
*Discussion:* These data suggest that 2 D speckle tracking strain echocardiography combined with dobutamine stress test can detect early changes in myocardial function and may be useful tools in early detection of drug-induced cardiac dysfunction.



## 1. Introduction

Drug-related cardiovascular liabilities are a focus of significant concern in the pharmaceutical and biomedical communities. Accordingly, early detection of drug induced changes in cardiac function is vital to early selection of drug candidates with best opportunity for success and to ensure patient safety (Kola & Landis, 2004; Moreno & Pearson, 2013). This has become particularly true for a number of novel oncology compounds that have demonstrated great benefit to be ultimately tempered by cardiotoxicity and dysfunction in some patients (Bristow, Lopez, Mason, Billingham, & Winchester, 1982; Jiji, Kramer, & Salerno, 2012). Echocardiography is a useful tool for evaluation of myocardial function. Standard echocardiography techniques (i.e. 2 dimensional grey scale, M-mode, Doppler) have included measuring such functional parameters as fractional shortening (FS), ejection fraction, or cardiac output. Although these methods and parameters have been frequently used, there is evidence that they lack sensitivity for the early detection of myocardial dysfunction (Christian et al., 2012) and that potentially more sensitive techniques for the early detection of functional and mechanical changes are emerging (Christian et al., 2012). One such technique is 2-D speckle echocardiography (2DSE) which incorporates post image analysis of a standard 2 dimensional grey scale ultrasound image to track individual novel acoustic markers in the myocardium through the full cardiac cycle to derive strain and strain rate parameters both regionally and globally (Heimdal, Stoylen, Torp, & Skjaerpe, 1998; Leitman et al., 2004; Migrino et al., 2007; Piegari et al., 2008). Clinically 2DSE has been reported to improve detection of chemotherapeutic induced cardiac dysfunction (Geyer et al., 2010; Villarraga, Herrmann, & Nkomo, 2014).

Stress echocardiography, including dobutamine pharmacologic stress echocardiography, is widely used as diagnostic tool in human

*Abbreviations:* 2DSE, 2D speckle echocardiography; FS, fractional shortening; HR, heart rate; IP, intraperitoneal; LV, left ventricle; NCE, new chemical entity; REST, at rest.

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cardiology to aid in the detection of coronary artery disease and assesses cardiac functional (contractile) reserve (Armstrong, Pellikka, Ryan, Crouse, & Zoghbi, 1998; Pellikka et al., 1995). Dobutamine is a beta-1 adrenergic agonist that functions as a positive inotrope to increase myocardial contraction. It serves as a useful tool to increase cardiac work and function when a patient is unable to exercise to increase cardiac function. When used in a clinical setting, the dobutamine stress test may allow earlier detection of mild cardiac dysfunction in patients with normal cardiac function at rest by increasing contractile demand on the heart and assessing the associated response of cardiac functional reserve (Civelli et al., 2006; De Wolf et al., 1996; Hamada, Ohkubo, Maeda, & Ogawa, 2006). It has also been used in a similar manner for stress echocardiography in rodent species (Plante et al., 2005; Tontodonati, Fasdelli, Repeto, & Dorigatti, 2011).

Doxorubicin is an anthracycline chemotherapeutic agent with well documented and characterized cardiotoxic effects (Chugun et al., 2001; Olson & Capen, 1978; Schwarz et al., 1998; Takemura & Fujiwara, 2007). Despite the known cardiotoxic side effects, doxorubicin and other anthracyclines continue to be widely used in chemotherapeutic treatment of neoplastic disease (Volkova & Russell, 2011). It has also been shown to induce myocardial dysfunction in rats (Migrino et al., 2008) and therefore it was selected to induce myocardial functional changes in this study.

Previous studies investigating techniques for early detection of cardiomyopathy have demonstrated the ability of echocardiography using 2DSE to detect changes in myocardial function (Migrino et al., 2007; Piegari et al., 2008). The objective of this study is to determine if 2DSE is capable of detecting early myocardial functional changes in an established animal model of doxorubicin induced cardiomyopathy either alone or in combination with a pharmacological stress test, compared to standard echocardiography modalities.

## 2. Methods

### 2.1. Animal procedures

Twenty male Crl:CD(SD) rats (age: approximately 6 weeks, weight: 150–200 g) were randomized into two groups ($n = 10$), a control group and a doxorubicin treated group. Rats were pair housed with a member of the same treatment group in standard solid floor caging, had access to food (Lab Diet 5001/5002, St. Louis, MO, USA) and water ad libitum and were kept in a 12–12 light cycle. On day 0 of week 0 rats were weighed, anesthetized for echocardiography and then dosed with either saline or doxorubicin. The same procedure was performed at week 1, 2, 3, and 4. At week 5 rats were weighed and dosed, but not imaged; and at week 6 rats were weighed and anesthetized for final echocardiography, but not dosed. Following weighing and imaging (if performed), rats received either 2 mg/kg Doxorubicin (Doxorubicin HCl Injection, 10 mg/5 ml, Teva Pharmaceuticals USA) intraperitoneal (IP) (Olson & Capen, 1978), or comparable volume of saline IP, in weeks 0, 1, 2, 3, 4 and 5 for a total Doxorubicin dose of 12 mg/kg. All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee at GSK.

### 2.2. Echocardiograms

Echocardiography was performed prior to dosing on day 0 and then on the first day of weeks 1, 2, 3, 4, and 6. Rats were lightly anesthetized to the level of immobilization with Isoflurane (2–3%). Using a GE Vivid 7i™ ultrasound machine with a M12L linear probe and rodent software package (GE Healthcare, USA), images were acquired at 11 MHz and 125 frames per second (fps) by a single sonographer for the entire study. Simultaneous electrocardiograms (ECG) were recorded using subcutaneous ECG electrodes. A short axis view of the left ventricle (LV) taken from the left thorax with the rat in left dorsolateral recumbency was obtained at the level of the papillary muscles. Images were stored as 2D grey scale video loops and short axis M-mode images. The papillary muscles served as a landmark to ensure appropriate region of imaging. Echocardiograms were obtained initially under resting conditions and again during dobutamine infusion as a dobutamine stress test procedure (see below). In order to be able to complete post-imaging analysis for this study, it was necessary to ensure that the short axis view of the LV was consistently obtained from the same level of the LV, was not an oblique view of the LV, and showed the full thickness of the LV wall and clear endocardial lining.

### 2.3. Dobutamine stress echocardiography

Following imaging at rest, a catheter was placed in the lateral tail vein of the rat. Placement was confirmed by the ability to withdraw blood, prior to and upon completion of the infusion. As adapted from Plante et al., a continuous infusion of Dobutamine (Dobutamine Injection, 250 mg/20 ml, Hospira Inc, Lake Forest, IL, USA) was given at 20 mcg/kg/min (Plante et al., 2005). After 1 min of infusion echocardiography was performed acquiring the same protocol as described for at rest.

### 2.4. Post-mortem and histopathology

At week 7, following the completion of the imaging phase of the study, all rats were weighed before the terminal procedure and necropsies were performed. Hearts were removed and the LVs were isolated, weighed, and preserved in 10% buffered formalin for histopathology. Formalin-fixed hearts were bisected longitudinally and processed routinely for paraffin embedment and microtomy. Briefly, heart samples were dehydrated in a graded series of ethanol, cleared with xylene, and infiltrated with paraffin. The paraffin infiltrated samples were embedded in paraffin wax and sectioned at 5 microns for light microscopic examination. Replicate sections were stained with hematoxylin and eosin (H&E) or Masson's trichrome stain. H&E stained sections were examined for the cytoplasmic vacuolar change of doxorubicin cardiotoxicity and other evidence of cellular injury and inflammation. The vacuolar changes were qualitatively graded on a scale of 0 to 4+ where 0 represented no vacuolation, +1 represented an occasional vacuolated cell in fields scanned at 40×, +2 represented more frequently affected cells and so on (Herman, el-Hage, Ferrans, & Ardalan, 1985). Masson's trichrome stain differentially stains collagen and allows sensitive evaluation of interstitial collagen accumulation (fibrosis). The stained sections were evaluated light microscopically by a veterinary anatomic pathologist.

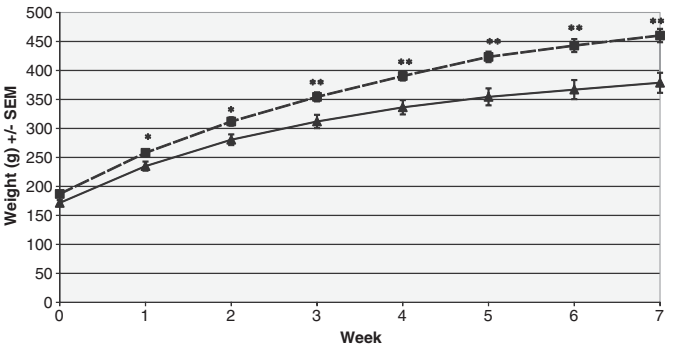


**Fig. 1.** Body weights for control (■) and doxorubicin (▲) rat groups. Rats in control group were larger than those in the doxorubicin group at time points of week 1 and beyond.*$p < 0.05$, **$p < 0.01$.