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Differential cardiotoxicity in response to chronic doxorubicin treatment in male spontaneous hypertension-heart failure (SHHF), spontaneously hypertensive (SHR), and Wistar Kyoto (WKY) rats

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ABSTRACT

Life threatening complications from chemotherapy occur frequently in cancer survivors, however little is known about genetic risk factors. We treated male normotensive rats (WKY) and strains with hypertension (SHR) and hypertension with cardiomyopathy (SHHF) with 8 weekly doses of doxorubicin (DOX) followed by 12 weeks of observation to test the hypothesis that genetic cardiovascular disease would worsen delayed cardiotoxicity. Compared with WKY, SHR demonstrated weight loss, decreased systolic blood pressure, increased kidney weights, greater cardiac and renal histopathologic lesions and greater mortality. SHHF showed growth restriction, increased kidney weights and renal histopathology but no effect on systolic blood pressure or mortality. SHHF had less severe cardiac lesions than SHR. We evaluated cardiac soluble epoxide hydrolase (sEH) content and arachidonic acid metabolites after acute DOX exposure as potential mediators of genetic risk. Before DOX, SHHF and SHR had significantly greater cardiac sEH and decreased epoxyeicosatrienoic acid (EET) (4 of 4 isomers in SHHF and 2 of 4 isomers in SHR) than WKY. After DOX, sEH was unchanged in all strains, but SHHF and SHR rats increased EETs to a level similar to WKY. Leukotriene D4 increased after treatment in SHR. Genetic predisposition to heart failure superimposed on genetic hypertension failed to generate greater toxicity compared with hypertension alone. The relative resistance of DOX-treated SHHF males to the cardiotoxic effects of DOX in the delayed phase despite progression of genetic disease was unexpected and a key finding. Strain differences in arachidonic acid metabolism may contribute to variation in response to DOX toxicity.

Introduction

Increased success of cancer treatment has resulted in a growing population of cancer survivors, due in part to chemotherapeutics (Ganz and Hahn, 2008). Some of the most commonly used chemotherapeutic drugs, however, result in delayed toxicities, causing disease years to decades after treatment. Of childhood cancer survivors, 2/3 will experience at least one late-onset complication, and 1/3 will experience a severe or life threatening effect from treatment with chemotherapy, radiation, or a combination of the two (Landier and Bhatia, 2008). Anthracyclines are often successful in treating hematopoietic and solid tumors, but result in delayed development of subclinical to severe complications in some patients (Longhi et al., 2012; Octavia et al., 2012; Smith et al., 2010). Multisystemic late effects after doxorubicin treatment include cardiomyopathy, congestive heart failure and nephropathy (Fumoleau et al., 2006; Ganz and Hahn, 2008; Hudson et al., 2007; Jones et al., 2008; Santin et al., 2007; Steinherz et al., 1991). Congestive heart failure is a common disabling health risk in long-term cancer survivors, with exposure to high doses (250 mg/m^2 or higher) of

Abbreviations: BW, body weight; cTnT, cardiac troponin T; CYP, cytochrome P450; DHET, dihydroxyeicosatrienoic acid; DOX, doxorubicin; EET, epoxyeicosatrienoic acid; HE, hematoxylin and eosin; HETE, hydroxyeicosatetraenoic acid; LTD4, leukotriene D4; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; %FS, left ventricular fractional shortening; SAL, saline; SBP, systolic blood pressure; sEH, soluble epoxide hydrolase; SHHF, spontaneous hypertension heart failure rat; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto rat.

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anthracyclines increasing the relative risk of congestive heart failure compared with untreated siblings by a factor of five (Mulrooney et al., 2009; Volkova and Russell, 2011). Therefore, there is significant interest in understanding the role of predisposing factors, especially those of cardiovascular origin.

Spontaneously hypertensive rats (SHR) have been used to evaluate effects of doxorubicin (DOX) in hypertension (Hazari et al., 2009; Herman et al., 1985; Herman et al., 1988; Herman et al., 1998; Zhang et al., 1996). Hypertension exacerbates cardiac and renal toxicity in studies in which measurements are collected during the treatment period. Progressive cardiac damage after exposure to DOX or its derivatives is observed in normotensive rats (Chugun et al., 2000; Cirillo et al., 2000; Lebrecht et al., 2003; Lebrecht et al., 2006); however, studies of delayed toxicity in strains with genetic cardiovascular disease such as the SHR and the spontaneously hypertensive heart failure rat (SHHF) are lacking. SHHF exhibit progressive systolic and diastolic dysfunction characterized by initial hypertension and compensated left ventricular hypertrophy followed by deleterious cardiac remodeling culminating in congestive heart failure (Heyen et al., 2002). While precise mechanisms for cardiomyopathy are not entirely known, SHHF rats develop characteristic histopathology (myocardial degeneration/necrosis, fibrosis, mononuclear inflammatory infiltrates) and increased systemic and cardiac expression of inflammatory mediators.

Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites that are products of cytochrome P-450 (CYP) epoxygenase enzymes (See Supplemental Fig. 1). EETs promote vasodilation, angiogenesis, and cardiac myocyte contraction, and inhibit inflammation, platelet aggregation, and cardiac hypertrophy (Imig, 2012). Soluble epoxide hydrolase (sEH) is the primary catabolic enzyme that degrades EETs into less cardioprotective dihydroxyeicosatrienoic acid isoforms (DHETs) (Imig, 2012). Acute DOX exposure modifies the cardiac expression of CYP and sEH enzymes in male Sprague–Dawley rats, thus reducing EETs and increasing DHETs, which may be a novel mechanism of DOX cardiotoxicity (Zordoky et al., 2010). Alterations in EETs and sEH activity in the central nervous system of SHR are implicated in the development of hypertension (Sellers et al., 2005). Linkage analysis and genome-wide expression profiling demonstrated a mutation in the sEH gene of SHHF, causing up-regulation of transcription suggested to be associated with progression to heart failure (Monti et al., 2008).

The purpose of our study was to examine the role of genetic predisposition to cardiovascular disease on late-onset DOX toxicity. Our hypothesis was that genetic hypertension would worsen late-onset DOX toxicity, and that hypertension and propensity to heart failure together would further accelerate progression. We compared the delayed toxic effect 12 weeks after the cessation of DOX treatment in normotensive rats (Wistar Kyoto, WKY), genetically predisposed hypertensive rats (SHR) and genetically predisposed hypertension and cardiomyopathy rats (SHHF). Although our data showed enhanced delayed toxic effects as compared to WKY, there was clearly an attenuated delayed toxic response in SHHF as compared to the SHR. Given the suggested role of EETs and sEH in DOX cardiotoxicity and in the development of hypertension and heart failure in the SHHF rat, we suspected that alterations in arachidonic acid metabolism may contribute to the observed strain differences in response to DOX. As proof of principle for the potential role of this mechanism in strain differences in response to DOX, we subsequently examined cardiac sEH content and arachidonic acid metabolite production by electrospray mass spectroscopy in response to acute DOX in the 3 strains of rats. As expected, cardiac sEH activity was higher and cardiac EETs were lower in untreated SHR and SHHF than WKY, however, paradoxically, SHHF and SHR rats significantly increased cardiac EETs in response to DOX with no significant change in cardiac sEH activity or DHETs. This suggests alternative mechanisms for EET metabolism and subsequent response to DOX in genetic disease but does not explain the observed differences in sensitivity between SHHF and SHR. DOX significantly increased cardiac levels of leukotriene D4 (LTD4) in SHR but not in WKY and SHHF, suggesting a potential role for this leukotriene in DOX sensitivity in SHR. These data demonstrate the potential for a significant and complex role for arachidonic acid metabolism in influencing the interaction between the effects of DOX and genetic predisposition to cardiovascular disease that should be explored further in longer-term studies.

Methods

Assessment of delayed post-DOX toxicity

Thirteen 8 to 10-week old SHR and WKY male rats and fourteen 8 to 10-week old phenotypically lean SHHF male rats were obtained from Charles River Laboratories. Male rats were chosen for this investigation due to the existing data documenting the response to DOX in short-term studies and because of earlier expression of hypertension. All animals were housed in an AAALAC accredited facility according to NIH guidelines for the care and use of laboratory animals; protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee. After a one week acclimation period, DOX rats received 8 weekly doses of pharmacologic grade doxorubicin (Bedford Laboratories, Bedford, OH 44146) at a dose of 2 mg/kg by subcutaneous injection ($n = 7$ for each strain). Saline treated control rats (SAL) received an equivalent volume of sterile saline ($n = 7$ for SHHF, $n = 6$ for SHR and WKY). Injection sites were rotated to avoid repeated use of a single site. An 8 dose protocol was determined based on preliminary studies to optimize the protocol. During the 12 weeks following the final DOX injection, rats were monitored for general health as well as signs of heart failure (cyanosis, tachypnea and increased respiratory effort, edema, or body cavity effusions). Body weights (BWs), systolic blood pressures (SBPs), and echocardiography studies were performed at 1 and 12 weeks after the last dose of DOX or saline (designated week 1 and week 12) to evaluate the progression of delayed toxicity. Blood samples for the determination of serum cardiac troponin T (cTnT) were collected at 1 and 12 weeks after the last dose of DOX or saline and frozen at −80 °C until analysis. Unless euthanasia was indicated earlier for humane reasons, rats were humanely euthanized 12 weeks after the final DOX injection by isoflurane anesthesia followed by CO_2. Necropsies were performed to document gross lesions, obtain organ weights for heart and kidneys, and to collect heart and renal tissue for histopathologic studies.

Systolic blood pressure measurement (SBP)

Rats were acclimated to the tail cuff blood pressure method. Briefly, rats were gently restrained in a warmed environment. Tail cuff measurements were taken using a BP-2000 Blood Pressure Analysis System™ for mice and rats (Visitech Systems, Inc., Apex, NC). The average of 3 stable readings was recorded.

Echocardiographic studies

Echocardiography was performed by a board certified veterinary cardiologist (AT) who was blinded to strain and treatment group. Anesthesia was induced with 5% isoflurane in a chamber until movement ceased. Anesthesia was then maintained with isoflurane administered via face mask with concentration reduced to 1–2% titrated to the lightest anesthetic plane that eliminated movement and retraction of limbs during restraint for echocardiography. Echocardiography was performed using an ATL 5000CV ultrasound system (Philips Medical Systems, Maplewood, MN) and a 12 to 5 MHz multifrequency transducer. All images were captured digitally for off-line analysis. Right parasternal echocardiography was performed to obtain both long- and short axis two-dimensional imaging planes, followed by routine M-mode echocardiography. M-mode measurements (to the nearest 0.1 mm) included left ventricular internal diameter in diastole (LVIDd), left ventricular internal diameter in systole (LVIDs), aortic root diameter, and left atrial