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Mitochondrial aldehyde dehydrogenase protects against doxorubicin cardiotoxicity through a transient receptor potential channel vanilloid 1-mediated mechanism

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ABSTRACT

Cardiotoxicity is one of the major life-threatening effects encountered in cancer chemotherapy with doxorubicin and other anthracyclines. Mitochondrial aldehyde dehydrogenase (ALDH2) may alleviate doxorubicin toxicity although the mechanism remains elusive. This study was designed to evaluate the impact of ALDH2 overexpression on doxorubicin-induced myocardial damage with a focus on mitochondrial injury. Wild-type (WT) and transgenic mice overexpressing ALDH2 driven by chicken β-actin promoter were challenged with doxorubicin (15 mg/kg, single i.p. injection, for 6 days) and cardiac mechanical function was assessed using the echocardiographic and IonOptix systems. Western blot analysis was used to evaluate intracellular Ca^{2+} regulatory and mitochondrial proteins, PKA and its downstream signal eNOS. Doxorubicin challenge altered cardiac geometry and function evidenced by enlarged left ventricular end systolic and diastolic diameters, decreased factional shortening, cell shortening and intracellular Ca^{2+} rise, prolonged relengthening and intracellular Ca^{2+} decay, the effects of which were attenuated by ALDH2. Doxorubicin challenge compromised mitochondrial integrity and upregulated 4-HNE and UCP-2 levels while downregulating levels of TRPV1, SERCA2a and PGC-1α, the effects of which were alleviated by ALDH2. Doxorubicin-induced cardiac functional defect and apoptosis were reversed by the TRPV1 agonist SA13353 and the ALDH-2 agonist Alda-1 whereas the TRPV1 antagonist capsazepine nullified ALDH2/Alda-1-induced protection. Doxorubicin suppressed phosphorylation of PKA and eNOS, the effect of which was reversed by ALDH2. Moreover, 4-HNE mimicked doxorubicin-induced cardiomyocyte anomalies, the effect of which was ablated by SA13353. Taken together, our results suggested that ALDH2 may rescue against doxorubicin cardiac toxicity possibly through a TRPV1-mediated protection of mitochondrial integrity.

1. Introduction

Doxorubicin (daunorubicin) is one of the most widely employed anticancer anthracycline drugs [1]. Ample of clinical and experimental evidence has demonstrated that doxorubicin leads to the onset and development of cardiotoxicity, *en route* to congestive heart failure [1–3]. Several hypotheses have been put forward for doxorubicin cardiomyopathy [1], including mitochondrial damage [*e.g.*, accumulation of reactive oxygen species (ROS), lipid peroxidation], intracellular Ca^{2+} dysregulation, apoptosis and suppressed heart-specific gene expression [1]. Given the high risk of doxorubicin cardiotoxicity, effective pharmaceutical and non-pharmaceutical approaches targeting oxidative stress, apoptosis and iron-mediated cell injury are pertinent and have been demonstrated to mitigate doxorubicin-induced cardiac toxicity [1–4]. Recent findings from our own laboratory revealed a beneficial role of mitochondrial aldehyde dehydrogenase (ALDH2) against doxorubicin-induced cardiac injury [5]. ALDH2 serves as an essential mitochondrial enzyme to detoxify toxic reactive aldehydes including 4-hydroxy-2-nonenal (4-HNE) and to convert glyceryl trinitrate [GTN] to the biologically active 1,2-glyceryl dinitrate (1,2-GDN) [6–9]. A rather appealing cardioprotective property has been unveiled for ALDH2 in the settings of ischemic and reperfusion injury, diabetes mellitus and alcoholic cardiomyopathy possibly *via* regulation of autophagy, apoptosis and mitochondrial integrity [10–15]. To

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this end, our present study was designed to examine the role of mitochondrial integrity in ALDH2-offered protection against doxorubicin cardiotoxicity, if any, using our unique ALDH2 transgenic model. We also explored the role of transient receptor potential (TRP) channels in particular TRP vanilloid 1 (TRPV1), a channel opened by capsaicin, heat, protons and endovanilloids [16], in doxorubicin- and ALDH2-elicited functional and geometric changes, if any. TRP channels are implicated in the pathogenesis of cardiac anomalies through sensing a variety of stimuli including shear stress, cold, mechanical stretch, oxidative stress, hypertrophic and proinflammatory stimuli [17–19]. TRPV1 channels are known to govern mitochondrial integrity through their regulation of mitochondrial membrane depolarization in neurological and hepatic systems [20,21] although little is known for the heart. Given the pivotal role for the redox-activated protein kinases including cAMP-dependent protein kinase A (PKA) and its downstream target endothelial nitric oxide (eNOS) in cardiac homeostasis [22–24], we went on to evaluate the possible contribution of the PKA–eNOS cascade in doxorubicin- and ALDH2-elicited cardiac geometric and mechanical responses, if any.

2. Materials and methods

2.1. Experimental animals and doxorubicin challenge

All animal procedures were approved by our institutional Animal Care and Use Committees at the Fourth Military Medical University (Xi'an, China) and the University of Wyoming (Laramie, WY, USA). The ALDH2 transgenic line was produced in friendly virus B (FVB) background mice using chicken β-actin promoter as described [13]. In brief, five to six month-old adult male ALDH2 transgenic mice and FVB wild-type (WT) littermates were housed in a temperature-controlled room under a 12 h/12 h-light/dark cycle with access to tap water *ad libitum*. A cohort of WT and ALDH2 transgenic mice was challenged with doxorubicin (15 mg/kg, i.p. one single injection in total) or equal volume saline (i.p.) for 6 days, a well-established short-term model for cardiomyopathy [5,25–27]. A cohort of WT and ALDH2 transgenic mice was subject to doxorubicin treatment for 6 days while receiving concurrent daily administration of the TRPV1 agonist SA13353 (30 mg/kg/d, p.o) [28,29] or the TRPV1 antagonist capsazepine (5 mg/kg/d, i.p.) [29,30].

2.2. Echocardiographic assessment

Cardiac geometry and contractile function were evaluated in anesthetized (ketamine 80 mg/kg and xylazine 12 mg/kg, i.p.) mice using a 2-dimensional (2-D) guided M-mode echocardiography (Phillips Sonos 5500) equipped with a 15–6 MHz linear transducer (Phillips Medical Systems, Andover, MD, USA). Adequate depth of anesthesia was monitored using toe reflex. The heart was imaged in the 2-D mode in the parasternal long-axis view with a depth setting of 2 cm. The M-mode cursor was positioned perpendicular to interventricular septum and posterior wall of left ventricle (LV) at the level of papillary muscles from the 2-D mode. The sweep speed was 100 mm/s for the M-mode. Diastolic wall thickness, left ventricular (LV) end diastolic dimension (EDD) and LV end systolic dimension (ESD) were measured. All measurements were done from leading edge to leading edge in accordance with the Guidelines of the American Society of Echocardiography [31]. LV fractional shortening was calculated as [(EDD − ESD) / EDD] × 100. Estimated echocardiographically-derived LV mass was calculated as [(LVEDD + septal wall thickness + posterior wall thickness)^3 − LVEDD^3] × 1.055, where 1.055 (mg/mm^3) denotes the density of myocardium. Cardiac output was calculated using the following formula: (EDD^3 − ESD^3) × heart rate (averaged over 10 consecutive cycles) [32].

2.3. Cardiomyocyte isolation and in vitro drug treatment

After ketamine/xylazine (ketamine 80 mg/kg and xylazine 12 mg/kg, i.p.) sedation, hearts were removed and perfused with KHB buffer containing (in mM): 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 HEPES and 11.1 glucose. Hearts were digested with 223 U/ml Liberase Blendzyme 4 (Roche Diagnostics Inc. Indianapolis, IN) for 20 min. Left ventricles were removed and minced before being filtered. Cardiomyocyte yield was 75% which was unaffected by doxorubicin or ALDH2 transgene. Only rod-shaped cardiomyocytes with clear edges were used for mechanical evaluation [32]. To examine the effect of TRPV1 on doxorubicin or ALDH2-induced cardiac response, cardiomyocytes from WT mice were treated with doxorubicin (1 μM) [5] for 4 h in the absence or presence of the ALDH2 activator Alda-1 (20 μM) [5,33], the TRPV1 agonist SA13353 (1 μM) [29] or the TRPV1 inhibitor capsazepine (1 μM) [29] prior to biochemical and mechanical assessments. To assess the effect of the ALDH2 substrate 4-HNE on cardiomyocyte function, cardiomyocytes were treated with 4-HNE (20 μM) [10] for 4 h in the absence or presence of the TRPV1 agonist SA13353 (1 μM) [29] prior to mechanical assessment.

2.4. Cell shortening and relengthening

Mechanical properties of cardiomyocytes were evaluated utilizing the SoftEdge MyoCam® system (IonOptix Corporation, Westwood, MA, USA) [32]. Briefly, cardiomyocytes were visualized under an inverted microscope (Olympus Corporation, IX-70, Tokyo, Japan) and were stimulated with a voltage frequency of 0.5 Hz. The myocyte being observed was shown on a computer monitor using an IonOptix MyoCam camera. IonOptix SoftEdge software was utilized to capture cell shortening and relengthening changes. The indices considered were peak shortening amplitude (PS), time-to-peak shortening (TPS), time-to-90% relengthening (TR_{90}), maximal velocity of shortening and relengthening (±dL/dt).

2.5. Intracellular Ca^{2+} transients

A cohort of myocytes was loaded with fura-2/AM (0.5 μM, Molecular Probe, Eugene, OR, USA) for 10 min and fluorescence intensity was recorded with a dual-excitation fluorescence photomultiplier system (IonOptix). Myocytes were placed onto an Olympus IX-70 inverted microscope and imaged through a Fluor × 40 oil objective. Cells were exposed to light emitted by a 75 W lamp and passed through either a 360 or a 380 nm filter, while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm and qualitative change in fura-2 fluorescence intensity (FFI) was inferred from FFI ratio at the two wavelengths (360/380). Fluorescence decay time was calculated as an indicator of intracellular Ca^{2+} clearing [32].

2.6. Hematoxylin and eosin histological examination

Following anesthesia, hearts were excised and immediately placed in 10% neutral-buffered formalin at room temperature for 24 h after a brief rinse with PBS. The specimen were embedded in paraffin, cut in 5 μm sections and stained with hematoxylin and eosin (H&E). Cardiomyocyte cross-sectional areas (15–20 cells per heart) were calculated on a digital microscope (×400) using the Image J (version 1.34S) software [34].

2.7. Aconitase activity

Mitochondrial aconitase, an iron-sulfur enzyme located in citric acid cycle, is readily damaged by oxidative stress *via* removal of an iron from [4Fe–4S] cluster. Mitochondrial fractions prepared from whole heart homogenate were resuspended in 0.2 mM sodium citrate. Aconitase activity assay (Aconitase activity assay kit, Aconitase-340 assay™,