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Adenosine prevents isoprenaline-induced cardiac contractile and electrophysiological dysfunction

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ABSTRACT

Excessive levels of catecholamines are believed to contribute to cardiac dysfunction in a variety of disease states, including myocardial infarction and heart failure, and are particularly implicated in stress-induced cardiomyopathy, an increasingly recognized cardiomyopathy associated with significant morbidity and mortality. We have previously shown that a high dose of isoprenaline induces reversible regional dysfunction of the left ventricle in mice. We now hypothesize that adenosine can prevent cardiac dysfunction in this mouse model of stress-induced cardiomyopathy. Hundred male C57BL/6 mice were injected with 400 mg/kg isoprenaline and then randomized to either 400 mg/kg adenosine or saline. Cardiac function was evaluated by echocardiography at baseline and 2, 24, 48, 72, 96 and 120 min post isoprenaline. Myocardial fibrosis was quantified after 10 days. Intracellular lipid accumulation was quantified after 2 and 24 h. Electrophysiological parameters and degree of lipid accumulation were evaluated in cultured HL1 cardiomyocytes. Two hours post isoprenaline treatment, echocardiographic parameters of global and posterior wall regional function were significantly better in adenosine-treated mice ($P < 0.05$). This difference persisted at 24 h, but saline-treated mice gradually recovered over the next 96 h. Intracellular lipid accumulation was also significantly lower in adenosine mice. We found no sign of fibrosis in the adenosine mice, whereas the extent of fibrosis in isoprenaline mice was 1.3% ($P < 0.05$). Furthermore, adenosine-treated HL1 cells showed preserved electrophysiological function and displayed less severe intracellular lipid accumulation in response to isoprenaline. In conclusion, adenosine attenuates isoprenaline-induced cardiac dysfunction in mice and cells.

1. Introduction

Sympathetically-induced catecholamine toxicity is of major importance in several cardiovascular disease states ranging from acute myocardial infarction to chronic heart failure, and catecholamines are hypothesized to play a central role in stress-induced cardiomyopathy (Lopez-Sendon et al., 2004), (Hurst et al., 2010). Plasma catecholamine levels are greatly increased in stress-induced cardiomyopathy and are believed to trigger severe but transient cardiac dysfunction (Wittstein et al., 2005). Although physiological levels of catecholamines induce positive inotropic and chronotropic changes in cardiomyocytes, several important homeostatic processes may be disrupted by extensive stimulation of adrenergic receptors (Ellison et al., 2007). Catecholamines have a great influence over myocardial energy metabolism, and the catecholamine-induced accumulation of intracellular lipids is a putative detrimental process that can lead to cardiac dysfunction (Mohan and Bloom, 1999). We have previously induced stress-induced cardiomyopathy-like regional myocardial dysfunction in mice by intraperitoneally administering a high dose of isoprenaline (Shao et al., 2013b). Administration of catecholamine caused intramyocardial lipid accumulation, which was associated with stress-induced cardiomyopathy-like cardiac dysfunction (Chappel et al., 1959; Soltysinska et al., 2011).

Adenosine is an endogenous cardioprotective molecule with proven anti-catecholaminergic effects (Dobson Jr., 1978; Headrick et al., 2011a; Mustafa et al., 2009; Shao et al., 2013b). Adenosine has been widely used in the clinic for many years, both as a therapeutic regimen and as a diagnostic tool, and has an established safety profile and a wide therapeutic window (Karamitsos et al., 2009). Adenosine has been shown to be cardioprotective and decreases infarct size in humans and animals (Liu et al., 1991; Ross et al., 2005). We hypothesize that adenosine is protective in the setting of severe catecholamine overstimulation. The aim of

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this study was therefore to investigate whether adenosine would counteract catecholamine-induced perturbation of cardiac lipid metabolism and prevent the deterioration of cardiac function in the mouse model of isoprenaline-induced cardiotoxicity.

2. Material and methods

2.1. Mice

Fourteen-week-old C57BL/6 mice ($n=100$) were used in this study. The study protocol was approved by the Animal Ethics Committee at Gothenburg University, and all mice were handled in accordance with the NIH guidelines for use of experimental animals. Housing was in a temperature-controlled (25 °C) facility with a 12 h light/dark cycle, and the mice was given free access to food and water. The mice were randomized to intraperitoneal injections of either isoprenaline (400 mg/kg) or saline. Isoprenaline-injected mice were further randomized to treatment with either adenosine (400 mg/kg) or saline (untreated).

A pilot study including 20 mice was performed to determine the appropriate dose of adenosine, i.e. a dose that was well tolerated and appeared to prevent isoprenaline-induced cardiac dysfunction. The isoprenaline dose had been determined previously (Shao et al., 2013b). Baseline echocardiographic indices of cardiac function in mice treated only with 400 mg/kg adenosine were compared with those in untreated mice.

2.2. Cells

HL-1 cardiomyocytes were obtained from Dr. William Claycomb (Louisiana State University Medical Center, New Orleans, LA, USA). This cell line displays biological characteristics similar to those of adult cardiomyocytes. The cells were grown in Claycomb medium (JRH Biosciences, KS, USA) supplemented with 10% fetal bovine serum (JRH Biosciences), 2 mM L-glutamine, 100 μM noradrenaline, 100 U/ml penicillin and 100 μg/ml streptomycin on fibronectin (BD Biosciences, PA, USA) pre-coated flasks. The medium was changed every 24 h. Experiments were performed when HL-1 cells had grown into mono-layer confluence after a 3-day culture.

2.3. Echocardiography

The mice were anesthetized with isoflurane (1%) and echocardiography was performed using a VisualSonics 770 VEVO imaging station, which includes an integrated rail system for consistent positioning of the ultrasound probe. The chest hair was removed with an electric clipper and a hair removal gel before the examination. The mice were placed on a heating pad and connected to an ECG and the rectal temperature was monitored to maintain body temperature between 36 and 38 °C. A 45 MHz linear transducer (RMV 707) was used for imaging. Optimal parasternal long axis cine loops (i.e. visualization of both the mitral and aortic valves, and maximum distance between the aortic valve and the cardiac apex) of >1000 frames/s were acquired using the ECG-gated kilohertz visualization technique. The probe was then rotated 90° and parasternal short-axis cine loops of >1000 frames/s were acquired at exactly 3 mm below the mitral annulus. The echocardiographic protocol was repeated 2 h post isoprenaline injection. The extent of akinesia was traced in the short axis and expressed as percentage of total LV endocardial length. Fractional area change (FAC) was calculated in the long axis cine loop using FAC=(EDA−ESA)/EDA, where EDA and ESA are end-diastolic and end-systolic areas, respectively. For assessment of regional myocardial function, the heart was divided into six segments (anterolateral, lateral, posterolateral, posteroseptal, septal and anteroseptal), and segmental fractional wall thickening was calculated as the average of the ratio between the local myocardial transmural thickness at the end-systole and end-diastole at three equally spaced points along each segment. Fractional wall thickness was considered representative of the transmural end-systolic radial wall strain (systolic wall strain). Daily echocardiographic assessment of the surviving mice was continued for 10 days post isoprenaline, after which they were sacrificed.

2.4. Histology

Mice were sacrificed 2 h, 24 h or 10 days post isoprenaline, and cardiac tissue was collected for further analysis. Masson's trichrome stain was used to detect the degree of fibrosis. The extent of fibrosis was calculated planimetrically in a short axis mid-myocardial slice and expressed as percentage of the total myocardial area.

Intracellular lipid content was quantified, as previously described, in mouse cardiac slices (at 2 h and at 24 h post isoprenaline) and in HL1 cells (Kim et al., 2010). Briefly, hearts were harvested 2 h and 24 h post isoprenaline, frozen and cryosectioned into 8 μm-thick slices. The prepared cardiac slices were fixed with 2% formaldehyde for 1 min and rinsed in phosphate buffered saline. The preparations were then treated with 20% isopropanol for 1 min and incubated with 3% (w/v) oil red O (Sigma) solution in 60% isopropanol for 20 min. Images were obtained using a stereoscope (ScanScope CS, Aperio, Olympus), and lipid contents were evaluated with BioPix iQ 2.1.8 and expressed as the lipid area normalized to the total investigated tissue area.

2.5. Plasma lipid measurement:

Blood samples were collected from mice 2 h and 1 week post isoprenaline with heparinised syringes and were immediately centrifuged at 4 °C and stored at −80 °C. Plasma free fatty acid, triglycerides and cholesterol were measured with the NEFA-HR kit (Nordic Biolabs).

2.6. Gene expression profiles

Total RNA was extracted from mouse ventricular tissue and HL-1 cardiomyocytes using the Qiagen RNeasy Mini Kit according to the manufacturer's recommendations. Briefly, 1 μg of RNA was reversely transcribed using the TaqMan High capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). The cycling parameters are as follows: 25 °C for 10 min, 37 °C for 2 h and 85 °C for 5 min. A quantitative real-time polymerase chain reaction was performed using the TaqMan Assay-on-Demand on ABI 7700 Sequence Detection System (ABI), according to the manufacturer's recommendations. Primers were designed to detect mouse microsomal triglyceride transfer protein (MTTP), very low density lipoprotein receptor (VLDLr), CD36, fatty acid transporter1 (FATP1), peroxisome proliferated-activated receptor gamma (PPARγ), peroxisome proliferated-activated receptor alpha (PPARα), long-chain acyl-coenzyme A dehydrogenase (Acad1), muscle carnitine palmitoyltransferase 1b (Cpt1b), hypoxia-inducible factor 1 alpha (HIF1α) and mitochondrial transcription factor A (Tfam). The reactions were analyzed in duplicate, and the relative expression levels were calculated according to the comparative ΔCT method. The data were normalized to an endogenous control, murine ribosomal S18 (18S).

2.7. Measurement of oxidation products

Mouse heart tissue was minced in 0.5 ml distilled H2O containing 0.1 mM butylated hydroxytoluene (BHT) by using the Tissue