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Long-term administration of ranolazine attenuates diastolic dysfunction and adverse myocardial remodeling in a model of heart failure with preserved ejection fraction

Antonella De Angelis [1], Donato Cappetta [1], Elena Piegari [1], Barbara Rinaldi [1], Loreta Pia Ciuffreda [1], Grazia Esposito [1], Fiorella Angelica Valeria Ferraiolo [1], Alessia Rivellino [1], Rosa Russo [1], Maria Donniacuo [1], Francesco Rossi [1], Konrad Urbanek *[,1], Liberato Berrino [1]

Department of Experimental Medicine, Section of Pharmacology, Second University of Naples, Via Costantinopoli 16, 80138 Naples, Italy

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ABSTRACT

Background: To investigate the effects of chronic administration of ranolazine (RAN) on experimental model of heart failure with preserved ejection fraction.
Methods: Seven-weeks old Dahl salt-sensitive rats were fed a high salt diet for 5 weeks to induce hypertension. Afterwards, rats continued with a high salt diet and were administered either with vehicle or RAN (20 mg/kg/die, ip) for the following 8 weeks. Control rats were maintained on a low salt diet.
Results: While systolic parameters were not altered, diastolic parameters were changed in high salt animals. Hemodynamic analysis showed a decreased dP/dt min, increased LVEDP, longer time constant and steeper slope of the end-diastolic pressure–volume relationship. Treatment with RAN attenuated these alterations and determined a reduction in mortality. Additionally, the magnitude of myocardial hypertrophy and activation of PI3K/Akt pathway were reduced. Alteration in diastolic compliance as a consequence of elevated myocardial stiffness was confirmed by an increase of collagen deposition and activation of pro-fibrotic TGF-β/SMAD3/CTGF signaling. These effects were counteracted by RAN. High salt rats had a decrease in SERCA2 and an increase in Na^+/Ca^{2+} exchanger (NCX). Treatment with RAN reduced NCX expression and determined an increment of SERCA2. Moreover, the levels of nitrotyrosine and oxidized dyhydroethidium were higher in high salt rats. RAN induced a decrement of oxidative stress, supporting the concept that reduction in ROS may mediate beneficial effects.
Conclusions: Our findings support the possibility that diastolic dysfunction can be attenuated by RAN, indicating its ability to affect active relaxation and passive diastolic compliance.

1. Introduction

Approximately half of the patients with symptoms and signs of heart failure (HF) present with preserved ejection fraction (HFpEF), that was historically referred as diastolic HF. Up to now, the prevalence and the prognosis of HFpEF is comparable to that of heart failure with reduced ejection fraction (HFrEF), but it is predicted that in the next 10 years the prevalence will raise [1–4]. Although significant advances have been made in understanding the clinical characteristics of this complex clinical syndrome, a lot of information is still missing. This is one of the reasons why none of the pharmacological treatments has shown to be effective in reducing mortality in patients with HFpEF, and most clinical trials have failed or have disappointing results [5]. This can be partly explained by the lack of specific evidence-based treatment options [6] although a broad variety of pathophysiology-based approaches are under development with some being currently tested [5,7]. Moreover, HFpEF has proven further challenging due to its association with diabetes, atherosclerosis, hypertension, obesity, renal dysfunction, chronic obstructive lung disease etc., when systemic proinflammatory state and endothelial dysfunction significantly influence patient's cardiovascular profile, affecting cardiac structure and function as well as long-term prognosis.

There is a general agreement that extracellular matrix changes, myocyte hypertrophy and altered intracellular calcium homeostasis contribute to a diastolic dysfunction by impairing relaxation and increasing stiffness of the left ventricle [7,8].

One of the potential mechanisms involved in HFpEF pathophysiology is an increase late Na^+ current (I_{Na}) in cardiac myocytes. It leads to elevated intracellular Na^+ levels that stimulate reverse mode of Na^+/Ca^{2+} exchanger (NCX) with the consequent Ca^{2+} overload [9,10]. This raises diastolic tone of the heart, contributing to diastolic dysfunction.

* Corresponding author.
E-mail address: konrad.urbanek@unina2.it (K. Urbanek).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Based on this knowledge and following a need to investigate new strategies, the attention, among other targets, has been directed to ranolazine (RAN). RAN by selectively inhibiting late I_{Na} can decrease Na^+-dependent calcium accumulation [11–14] and is expected to promote Ca^{2+} extrusion through the Na^+/Ca^{2+} exchanger improving myocyte relaxation and diastolic tension. Data from animal models have shown that RAN leads to a reduction in late I_{Na} improving diastolic function è [14–16]. Furthermore, RAN has been shown to improve diastolic function in patients with an ischemic heart disease [17]. These results led to the proof-of-concept study conducted in 20 patients with HFpEF that showed positive effects on hemodynamic parameters but not relaxation [18]. Overall, interesting experimental and clinical data that suggest the beneficial role of RAN in diastolic dysfunction justify the need of additional studies. For this purpose, the effects of chronic administration of RAN were tested on Dahl salt-sensitive (Dahl SS) rats that represent one of the few accepted murine models of HFpEF [19].

2. Material and methods

2.1. Experimental animals, dietary and drug regimen

The present study conforms with the National ethical guidelines (Italian Ministry of Health; D.L.vo 26, March 4, 2014) and has been performed upon approval of Ministry of Health (protocol n. 582/2015-PR) and local ethics committee. Six weeks old male Dahl SS rats (Charles River Laboratories, Wilmington; MA, USA) were fed laboratory chow containing a high salt diet (8% NaCl) for 5 weeks to induce hypertension. Afterwards, rats continued with a high salt diet and were randomly divided into 2 groups: those treated with vehicle (n = 40; high salt group) and those treated with RAN (n = 30; high salt + RAN group; 20 mg/kg/die, i.p.; Gilead Sciences, Foster City, CA, USA) for the following 8 weeks. The posology resulted in a clinically relevant, average 24 h concentration of RAN in plasma of 1500 ng/ml [20]. Control rats (n = 10) were maintained on a low salt diet (0.3% NaCl, low salt group). At the end of the treatment (19 weeks of age), functional studies were performed and the hearts were collected from each experimental group. Body and organ weights were also determined.

To avoid inter-operator variability, every data analysis was conducted by a single operator. A schematic representation of the study protocol is shown in Fig. 1.

2.2. Blood pressure, echocardiographic and hemodynamic measurements

Mean blood pressure was measured weekly in conscious animals using the tail-cuff method. Echocardiography was performed with a high resolution Micro-Ultrasound System equipped with a 25-MHz linear transducer (Vevo 770, VisualSonics Inc., Ontario, Canada). Rats were anesthetized with an intramuscular injection of ketamine (100 mg/kg b.w., i.p.) and medetomidine (0.25 mg/kg) and body temperature was maintained at ~37 °C with a heating pad. Serial M-mode images were recorded along the minor axis at the level of the papillary muscles to measure diastolic left ventricle (LV) diameter and wall thickness and to calculate ejection fraction (EF) and fractional shortening (FS). To avoid inter-operator variability, a single investigator, blinded to the animal groups, performed all image acquisitions and offline measurements. Prior to sacrifice, hemodynamic parameters were collected. In anesthetized animals the right carotid artery was cannulated with a microtip pressure transducer (SPR-612, Millar Instruments, Houston, TX, USA) connected to an A/D converter (iWorx 214) and a computer system. The catheter was advanced into the LV cavity for the evaluation of LV pressures and + and − dP/dt in the closed-chest preparation.

2.3. Sample preparation

After hemodynamic measurements, the abdominal aorta was cannulated, the heart was arrested in diastole by injection of $CdCl_2$ (100 mM; Sigma-Aldrich, St. Louis, MO, USA), the thorax was opened and perfusion with phosphate-buffered formalin was started, as previously described [21,22]. After fixation, the heart was dissected and weighted. Tissue specimens were embedded in paraffin and histological sections of 5 μm thickness were cut. Alternatively, samples were placed in 30% sucrose solution overnight at 4% for cryoprotection, subsequently embedded in tissue freezing medium (OCT; Bio-Optica, Milan, Italy), frozen at −80 °C and processed for histological analysis. Tissue sections (10 μm thick) were generated using a Leica CM3050 S cryostat.

2.4. Histochemistry

Histological sections, 5 μm in thickness, were deparaffinized with xylene and rehydrated with aqueous solutions of decreasing ethanol concentrations. Masson's thricrome staining (Sigma-Aldrich) was used to detect tissue fibrosis. Interstitial fibrosis and myocyte cross-sectional area analysis was determined using ImagePro Plus software (Media Cybernetics, Rockville, MD, USA). Fluorescence immunolabeling and confocal microscopy were also performed. Cardiac fibroblasts were labeled with vimentin (Abcam, Cambridge, UK) and alpha-smooth muscle actin (α-SMA, Sigma-Aldrich), myocytes were identified by α-sarcomeric actin (Sigma-Aldrich). Oxidative stress was assessed by nitrotyrosine (Millipore, Milan, Italy). To measure superoxide generation, frozen sections, 10 μm in thickness, were incubated with 5 μM dyhydroethidium (DHE, Sigma-Aldrich). Nuclei were stained with DAPI (Sigma-Aldrich). Fluorescein isothiocyanate (FITC) conjugated

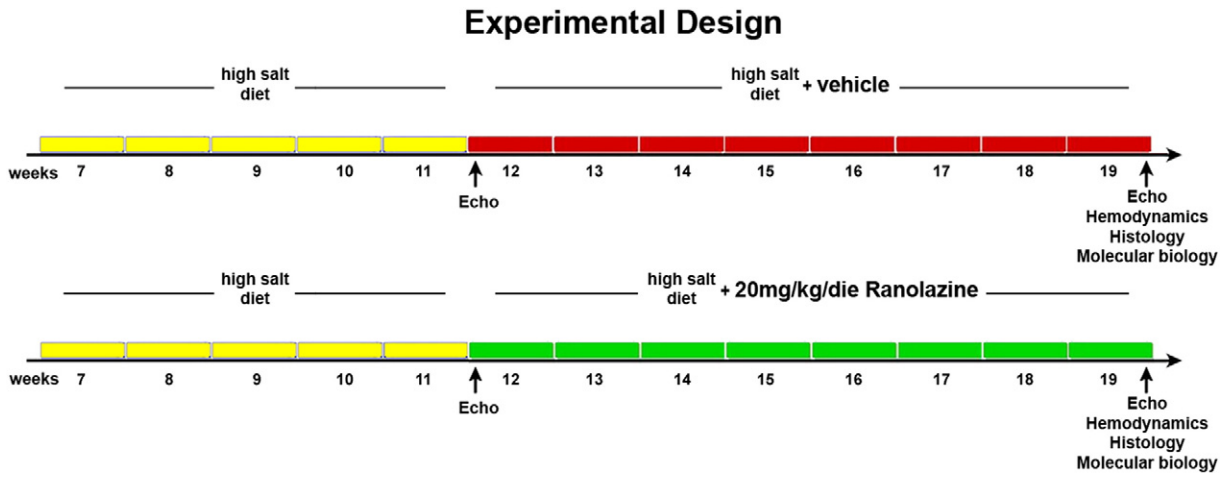

Fig. 1. Experimental Design. Scheme of *in vivo* experiments.