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Enhanced nitric oxide bioavailability in coronary arteries prevents the onset of heart failure in rats with myocardial infarction

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ABSTRACT

Aim: The endothelium, mainly via nitric oxide (NO) release, adjusts the coronary flow. Cardiac function is closely linked to blood flow; thus, we tested the hypothesis that NO modulation in coronary arteries could be differentially adjusted after myocardial infarction (MI) in the presence or absence of heart failure (HF).
Methods and results: Four weeks after coronary occlusion, the infarcted rats were subdivided into rats without (MI) or with HF signs according to haemodynamic parameters. The septal coronary arteries were subsequently used to perform functional and molecular experiments. Acetylcholine (ACh)-induced relaxation was decreased in the coronary arteries following HF, whereas it was enhanced in the arteries of the MI compared with those of SHAM-operated (SO) rats. The relaxation induced by the NO donor was similar among the groups. NO production, which was evaluated by 4,5-diaminofluorescein diacetate, was reduced in the coronary arteries of the HF group and increased in the arteries with MI after ACh-induced stimulation. HF coronary arteries exhibited oxidative stress, which was evaluated via ethidium bromide-positive nuclei, whereas it was decreased in MI. To evaluate the mechanisms involved in the enhanced ACh-induced relaxation in the arteries following MI, certain septal coronary arteries were pre-incubated with L-NAME (a nonselective NO synthase (NOS) inhibitor), 7-NI (a selective neuronal NOS (nNOS) inhibitor) or LY294002 (a PI3-kinase inhibitor). L-NAME and LY294002 reduced ACh-induced relaxation in the MI and SO rats; however, these effects were greater in the MI arteries. 7-NI reduced only the ACh-relaxation in MI. In addition, the eNOS, nNOS, Akt, and superoxide dismutase isoform protein expressions were greater in the coronary arteries of the MI than in those of the SO groups.
Conclusion: Our data suggested that endothelial function was closely related to cardiac function after coronary occlusion. The coronary arteries from the HF rats exhibited reduced NO bioavailability, whereas the MI rats exhibited increased NO bioavailability because of increased eNOS/nNOS/PI3-kinase/Akt pathway and a reduction in ROS generation. These results suggest that enhanced NO modulation can prevent the onset of HF.

1. Introduction

The coronary circulation provides oxygen and other nutrients to the heart. Therefore, blood flow must be regulated by the metabolic requirements of the myocardium. There are several mechanisms that control the coronary flow, such as myogenic tonus and the local production of metabolites, neurohumoral factors and endothelium. It is well established that the endothelium regulates the coronary vascular tone via the release of vasoactive agents, and nitric oxide (NO) appears to be a major endothelium-derived relaxing factor [1]. The role of NO in coronary blood flow regulation has been investigated in several pathological conditions. Thus, the basal or agonist-mediated NO release is reduced in the coronary arteries of hypertensive [2–4] and heart failure patients, as well as in experimental models of these diseases [5–11]. Cardiac function is closely linked to coronary blood flow; thus, there is an association between coronary endothelial dysfunction and heart failure [7–9].

Ischaemic heart diseases often lead to myocardial infarction and heart failure. The left coronary occlusion in rats is the most frequent experimental model used to reproduce the progression of human myocardial infarction to heart failure [12–15]. Despite well-established patterns for coronary occlusion, there is substantial variability in cardiac performance following myocardial infarction in rats. Consistent with this view, only rats with large myocardial infarctions exhibited congestive heart failure with increased right and left ventricular filling pressures and reduced cardiac output [12]. The development of heart failure, however, appears to depend on other factors beyond the infarct size. Thus, studies in rats have demonstrated that despite similar infarcted areas, groups of animals can be divided by the presence or

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absence of signs of heart failure, such as pulmonary congestion and increased left ventricular end diastolic pressure [13–15]. The exact mechanisms that contribute to the progression from mild left ventricle dysfunction to congestive heart failure are not completely understood. Cardiac function is closely linked to coronary blood flow; thus, the impairment of oxygen and substrate supply to the non-infarcted myocardial area could be involved in the cardiac dysfunction after myocardial infarction.

It has been demonstrated that endothelial dysfunction is present in arteries, such as the aorta, in heart failure rats. In these arteries, endothelial dysfunction is associated with a decrease in NO synthesis because of reduced endothelial nitric oxide synthase (eNOS) expression and/or NO bioavailability because of enhanced superoxide anion levels [16–18]. However, the presence of peripheral endothelial dysfunction after myocardial infarction in rats is not a consensus and appears to depend on the vascular bed investigated [19], extension of myocardial infarction [20], and time-course of remodelling [21], as well as on cardiac performance [13]. Although the majority of studies on endothelial function have been conducted in peripheral arteries, few studies have attempted to investigate the endothelial function in the coronary arteries of rats with myocardial infarction. Moreover, the data obtained are conflicting because the coronary endothelial function appears to be reduced [10,22] or unaltered [23] in heart failure rats.

The coronary flow to the non-infarcted myocardial area is pivotal for the maintenance of cardiac function after infarction. Thus, we hypothesized that coronary arteries can be differentially adjusted after myocardial infarction in the presence or absence of heart failure. The aim of the present study was to assess the endothelial function in coronary arteries from infarcted rats with or without heart failure, focusing on the possible role NO for heart failure development.

2. Material and methods

2.1. Experimental model

All experimental procedures were approved by the Animal Care and Use Committee of the Institute of Biomedical Sciences of the University of Sao Paulo and conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Male Wistar rats (230–280 g) were anesthetised (ketamine and xylazine, 90 and 10 mg/kg, respectively; i.p.) and submitted to myocardial infarction by permanent occlusion of the left coronary artery, as previously described [24]. In brief, the heart was exposed via a left-sided thoracotomy, and the anterior descending branches of the left coronary artery were occluded using 6-0 mononylon thread. The heart was rapidly returned to the chest cavity, and the thoracotomy was closed. The sham operated (SO) animals underwent the same surgical procedure with the exception of the coronary occlusion.

2.2. Haemodynamic study

After 4 weeks of coronary occlusion or sham-operation, the animals were anesthetised with urethane (1.2 g/kg; i.p; Sigma-Aldrich, Saint Louis, MO, USA) and allowed to spontaneously breathe room air. A polyethylene catheter (PE-50, 10 cm, filled with heparinised saline) was connected to a pressure transducer (TRA 021, Panlab Harvard Apparatus, Barcelona, Spain) and introduced into the right carotid artery to measure the systolic (SBP) and diastolic blood pressure (DBP). The heart rate was determined from the inter-beat intervals. The catheter was then advanced into the left ventricle, and the following data were acquired and analysed (PowerLab, ADInstruments, Bella Vista, New South Wales, Australia): the left ventricular systolic pressure (LVSP), the left ventricular end diastolic pressure (LVEDP) and their first time derivatives (positive and negative, dP/dt_{max} and dP/dt_{min}, respectively). According to the haemodynamic parameters, the infarcted rats were subdivided in two groups: myocardial infarction (MI) or heart failure (HF). Presence of HF was characterized by a LVEDP greater than 20 mm Hg. The haemodynamic parameters were recorded using a sample rate of 1 KHz.

2.3. Morphometric study

After measurement of the haemodynamic parameters, the animals were euthanized by exsanguination, and the heart and lungs were removed. First, the septal coronary artery was isolated from the ventricular septum. The right ventricle and the left ventricle plus the septum of the heart were subsequently weighed and normalised to the tibia length. This ratio was used as an index of ventricular hypertrophy. To evaluate the degree of pulmonary congestion, the lungs were also weighed and normalised to the tibia length.

The infarct size was determined by planimetry as described by Mill et al. [24]. Briefly, the scar tissue was separated from the remaining left ventricle muscle, and the infarct area was expressed as the percentage of left ventricle surface covered by the scar tissue. Only hearts with infarcts that covered 30 to 50% of the left ventricular surface were included in this study.

2.4. Coronary vascular reactivity

Septal coronary arteries (250–350 μm in diameter) were carefully isolated and placed in freshly prepared ice-cold Krebs–Henseleit solution (KHS) that contained (mM): NaCl 118; KCl 4.7; $NaHCO_3$ 25; $CaCl_2 \cdot 2H_2O$ 2.5; KH_2PO_4 1.2; $MgSO_4 \cdot 7H_2O$ 1.2; EDTA 0.01; and glucose 11. The septal coronary artery was cut into segments (2 mm in length) and mounted in a small vessel myography chamber (Danish Myo Technology, model 610 M, Aarhus N, Denmark) for isometric tension recordings. Briefly, two steel wires (40 μm diameter) were introduced through the lumen of the segments and mounted in a small vessel myograph chamber. After a 15 min equilibration period in oxygenated KHS at 37 °C and pH 7.4, the segments were stretched to their optimal lumen diameter for active tension development. The optimal lumen diameter was determined by the internal circumference/wall tension ratio of the segments by setting the internal circumference, L0, to 90% of the value that the vessels would have if they were exposed to a passive tension equivalent that produced by a transmural pressure of 100 mm Hg (L100) [25]. The optimal lumen diameter was determined using specific software for the normalisation of the resistance arteries (DMT Normalization Module; ADInstruments, Bella Vista, New South Wales, Australia). The segments were washed with KHS and allowed to equilibrate for 30 min. The vessel contractility was then tested by exposure to a high-K^+ (120 mM) solution. The contraction induced by the high-K^+ solution was similar among the groups (SO: 1.8 ± 0.1 ($n = 37$) vs. MI: 1.9 ± 0.1 ($n = 25$) vs. HF: 1.7 ± 0.2 mN/mm ($n = 6$); $p > 0.05$; one-way ANOVA).

After 30 min of stabilization, the endothelium-dependent relaxation induced by acetylcholine (0.1 ηmol/L–100 μmol/L; Sigma-Aldrich, Saint Louis, MO, USA) or endothelium-independent relaxation induced by the NO donor sodium nitroprusside (0.1 ηmol/L–100 μmol/L; Sigma-Aldrich, Saint Louis, MO, USA) were assessed in thromboxane mimetic 9,11-dideoxy-11α,9α-epoxy methanoprostaglandin (U46619, at a concentration that produced 50–70% of the contraction induced by a high-K^+ solution; Sigma-Aldrich, Saint Louis, MO, USA) pre-contracted rings. In certain rings, the effects of the nonselective NOS inhibitor L-NAME (100 μmol/L; Sigma-Aldrich, Saint Louis, MO, USA), the selective neuronal NOS inhibitor 7-Nitroindazole (7-NI, 100 μmol/L; Sigma-Aldrich, Saint Louis, MO, USA) or the PI3-kinase inhibitor LY294002 (50 μmol/L; Sigma-Aldrich, Saint Louis, MO, USA) on the concentration–response curve for acetylcholine were investigated. All drugs were added 30 min prior to the generation of the concentration–response curve.