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Resveratrol is equipotent to perindopril in attenuating post-infarct cardiac remodeling and contractile dysfunction in rats

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Abstract

Background: Angiotensin-converting enzyme (ACE) inhibitors improve prognosis in patients with post-myocardial infarction (MI) related cardiac dysfunction. Resveratrol is a polyphenol that has been reported to be beneficial in hypertension, ischemic heart disease, and cardiotoxicity in preclinical studies. Accordingly, we investigated the comparative and combinatorial efficacy of resveratrol and perindopril (ACE inhibitor) treatment on MI-related cardiac remodeling and contractile dysfunction.

Methods: Left anterior descending artery-ligated and sham-operated male Sprague–Dawley rats were gavaged with vehicle, resveratrol, perindopril, and combination of resveratrol+perindopril (2.5 mg/kg bodyweight/day) for 8 weeks (starting immediately after acute MI). Echocardiography was performed to assess cardiac structure and function at baseline and 8 weeks.

Results: At 8 weeks, vehicle-MI rats had a significantly lower left ventricular ejection fraction (LVEF) and increased LV dilatation compared to vehicle-sham rats. MI rats treated with resveratrol, perindopril and a combination of both had significantly improved LVEF and reduced LV dilatation. Vehicle-treated MI rats also had increased level of lipid peroxidation product- malondialdehyde (MDA), proinflammatory protein- tumor necrosis factor-alpha (TNF-α) and cardiac fibrosis marker- collagen and decreased enzymatic activity of superoxide dismutase and catalase compared to vehicle-sham rats. Resveratrol, perindopril and combination of both significantly prevented the /ed to determine systolic functional parameter increase in MDA, TNF-α and collagen and improved the activity of superoxide dismutase and catalase in MI rats compared to vehicle-MI rats.

Conclusion: Treatment with resveratrol or perindopril was equivalent in significantly improving remodeling and attenuation of contractile dysfunction in MI rats. Combination treatment also attenuated the cardiac abnormalities. The improvement in cardiac abnormalities may partly be through reducing oxidative stress by preventing the decrease in the activity of superoxide dismutase and catalase, and decreasing cardiac inflammation and fibrosis.

Keywords: Acute myocardial infarction; Cardiac remodeling; Cardiac dysfunction; Resveratrol; Perindopril

1. Introduction

Heart failure is a complex clinical syndrome characterized by the inability of myocardium to efficiently fill or eject blood due to cardiac abnormities [1]. Heart failure has been growing in prevalence with more than 23 million worldwide and deemed an epidemic as it is posing an increased risk of morbidity and mortality among the affected populations [1]. Myocardial infarction (MI) is a leading cause of systolic dysfunction and the development of heart failure [2]. Well-timed reperfusion can rescue ischemic myocardium and improve the prognosis by limiting cardiomyocyte death [3]. However, this modality may be detrimental due to reperfusion injury mediated by oxidative stress [3]. Injured myocardium triggers various immune cells and cardiac fibroblasts to act in a coordinated way to replace the damaged cardiomyocytes with additional extracellular matrix [3]. Nevertheless, uninterrupted oxidative stress, inflammatory response and fibrosis could worsen the cardiac dysfunction and affect prognosis in MI patients [3]. Sympathetic and renin–angiotensin–aldosterone systems adversely affect the recovery of myocardium after the ischemic injury by contributing to pathological cardiac remodeling [4]. Accordingly, angiotensin-converting enzyme (ACE) inhibitors, β-adrenergic, mineralocorticoid and angiotensin receptors' antagonists form the major successful treatment options in MI patients with cardiac dysfunction and heart failure [4]. Even though these

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interventions are effective, new alternative therapeutic strategies are needed to prevent or manage heart failure [5].

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenol produced by plants to fight environmental stress and infections [5]. We and others reported that resveratrol prevents or reverses cardiac remodeling and dysfunction in animal models of cardiovascular disease [6,7]. Preclinical evidence from rodent and swine models of permanent ischemia or ischemia/reperfusion suggests that resveratrol could be beneficial in the setting of ischemic heart disease [5]. Interestingly, administration of resveratrol at a very low dose (0.1 mg/kg bodyweight/day), a low dose (5 mg/kg bodyweight/day), and a moderately high dose (17 mg/kg bodyweight/day) in MI-induced rats and mice, respectively, has been reported to have no cardioprotective effects [8–10]. While there are reports showing the standalone cardioprotective effect of resveratrol, it is clinically imperative to evaluate the efficacy of resveratrol in comparison and in combination with clinically proven drug therapies as well as examine the possible drug interactions thereof. In this regard, no study has examined the effects of a combination of resveratrol and a first-line post-MI heart failure medication on cardiac structure and function in the setting of acute MI. ACE inhibitor treatment improves morbidity and mortality in post-MI patients [4]. Whether resveratrol could be synergistic or antagonistic to a front-line medication such as ACE inhibitor is currently not known. The comparative efficacy of resveratrol is also yet to be investigated alongside a major post-MI drug like ACE inhibitor. In this study, we have addressed this interesting question of clinical value by examining the effects of resveratrol alongside perindopril (ACE inhibitor) and a combination of perindopril+resveratrol on cardiac structure and function, and correlating it with oxidative stress and inflammation status in left anterior descending coronary artery (LAD) ligated rats.

2. Materials and methods

2.1. Experimental design

This study protocol was approved by the University of Manitoba Office of Research Ethics & Compliance and Animal Care Committee and was done in accordance with the guidelines by Canadian Council for Animal Care. Three-week-old male Sprague–Dawley rats (175–215 g) were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle (Charles River Laboratories, Quebec). Rats were anesthetized with 1%–5% isoflurane with oxygen at a flow rate of 2 L/min and kept in surgical plane on anesthesia with 2% isoflurane during surgery and subjected to permanent ligation of LAD to induce MI or sham surgery after baseline echocardiographic examination. A left thoracotomy was done, and the heart was gently exposed from the pericardial sac through the incision. The LAD was located and occluded with 6-0 polypropylene silk suture at about 2 mm from aortic root. The suture was tied, and the ligation was estimated to be successful when the anterior wall of the left ventricle turned pale. The heart was repositioned, the chest was compressed to remove any air from the cavity, and the incision was closed using a purse string suture. Sham-operated animals that served as normal control were subjected to the similar surgical procedures except that the LAD was not ligated. Buprenorphine 0.05 mg/kg was administered pre- and postsurgery (two times a day for 2 days) subcutaneously as an analgesic agent to all rats. All surviving sham and MI rats were assigned to following four treatment groups: (a) vehicle (50% ethanol 2.5 mL/kg bodyweight/day); (b) resveratrol (2.5 mg/kg bodyweight/day, trans-resveratrol, ≥99%, Sigma-Aldrich, Ltd., Ontario); (c) perindopril (2.5 mg/kg bodyweight/day, Servier Inc., Quebec); (d) resveratrol+perindopril (both 2.5 mg/kg bodyweight/day). All four groups consisted of sham (n=6–9) and MI (n=10–13) rats and received the treatments by oral gavage for 8 weeks. The dose for the present study was chosen based on previous studies [6,11,12]. Animals were regularly weighed and evaluated for well-being.

2.2. Transthoracic echocardiography

All experimental rats were weighed and anesthetized with 3% isoflurane in a chamber and then kept under 1.5%–2% isoflurane throughout the procedure. Echocardiogram was obtained at baseline and at 8 weeks of treatment by two-dimensional guided M-mode and tissue Doppler imaging (TDI) modalities with a 13-MHz probe (Vivid 7; GE Medical Systems, Milwaukee, WI, USA) by a procedure described elsewhere [2]. Two-dimensional M-mode parasternal long-axis view images were obtained to determine systolic functional parameter such as left ventricular ejection fraction (LVEF) from end-systolic and end-diastolic volumes. The cardiac structural parameters such as interventricular septal wall thickness (IVSTd), and LV posterior wall thickness (LVPWTd) at diastole and LV internal dimension (LVIDd and LVIDs) at diastole and systole were determined from parasternal short-axis view images. All echocardiographic images were analyzed to calculate the listed parameters using EchoPAC software (GE Medical Systems, Milwaukee, WI, USA). The values obtained for the mentioned parameters in three consecutive cardiac cycles were averaged to obtain final data [13].

2.3. Blood and tissue collection

All animals were anesthetized with ketamine/xylazine 9.0 mg/100 g and 0.9 mg/100 g im. Depth of anesthesia was assessed by pedal withdrawal reflex. Blood sample was collected from the inferior vena cava by opening the thoracic cavity, and the heart was immediately excised. Whole heart was rinsed in phosphate-buffered saline, and atria, right and LVs, septum and fibrotic scar tissue were separated, weighed and flash frozen in liquid nitrogen.

2.4. Infarct size (scar size) and lung wet/dry weight determination

Percentage of infarcted (scarred/fibrotic) LV tissue was calculated by dividing the weight of scarred LV tissue by whole weight of LV tissue as described previously [14]. Evidence of heart failure was assessed by checking the presence of ascites and by calculating the lung wet-to-dry weight ratio in both vehicle and investigational compounds received MI rats.

2.5. Oxidative stress marker and antioxidant enzyme activity assays

In order to determine MI-associated oxidative stress, the level of the lipid peroxidation product, malondialdehyde (MDA), was assessed using the Oxiselect MDA quantification kit (Cell Biolabs, San Diego, CA, USA) following the manufacturer's instructions [6]. Antioxidant enzyme status was also determined by measuring the activity of major endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) by following manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI, USA) [15].

2.6. Proinflammatory and cardiac fibrosis marker assays

Tumor necrosis factor-alpha (TNF-α) protein level in heart tissue was measured by enzyme-linked immunosorbent assay following the manufacturer's instructions (Thermo Scientific, IL, USA). Hydroxyproline level in the heart was also assayed following the manufacturer's instructions (Sigma-Aldrich, Ltd., Ontario). Collagen concentration was calculated by multiplying hydroxyproline level by a factor of 7.46 as the interstitial collagen contains an approximately 13.4% hydroxyproline by a procedure described elsewhere [14].

2.7. Statistical analysis

All values are expressed as means±SEM. One-way analysis of variance was used to analyze variations between the means of the groups. Significant values are defined as P<.05. When significance was obtained, one-way analysis of variance was followed by Newman–Kuels *post hoc* test.

3. Results

3.1. General characteristics

Postsurgery, at 8 weeks, bodyweight was comparable between sham and MI rats in all groups (Fig. 1A). However, perindopril- and perindopril+resveratrol-treated sham and MI rats had significantly lower bodyweight compared to vehicle-treated sham and MI rats, respectively (Fig. 1A). Resveratrol treatment alone did not alter the bodyweight in sham and MI rats in comparison to vehicle-treated sham rats and vehicle-treated MI rats (Fig. 1A). Heart-to-bodyweight ratio was also comparable between sham and MI rats in all groups (Fig. 1B). Perindopril- and perindopril+resveratrol-treated sham rats had significantly lower heart-to-bodyweight ratio compared to vehicle-treated sham rats as well as resveratrol-treated sham rats. Perindopril- and perindopril+resveratrol-treated MI rats also had lower heart-to-bodyweight ratio compared to vehicle-treated MI rats as well as resveratrol-treated MI rats (Fig. 1B). No signs of overt heart failure were observed in MI rats as pleural and abdominal cavities were devoid of effusions or ascites at 8 weeks. There was no significant difference in lung wet-to-dry weight ratio in MI rats, which further confirmed the absence of overt heart failure (Fig. 1C). All MI rats included in the study had well-defined scarred LV tissue at the anterior region due to the large anterior infarct that resulted from LAD ligation.