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Resveratrol prevents pulmonary trunk remodeling but not right ventricular hypertrophy in monocrotaline-induced pulmonary hypertension

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ABSTRACT

Pulmonary hypertension (PAH) is characterized by abnormal vascular remodeling and increased pulmonary artery pressure which lead to right ventricular (RV) hypertrophy and heart failure. Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), a dietary polyphenol found in the skins and seeds of grapes, has been found to have antioxidant, anti-proliferative and anti-fibrotic effects. This study examined the effects of resveratrol on cardiac and pulmonary trunk remodeling, and common plasma markers of vascular function in rats with PAH was induced in male Sprague-Dawley rats by a single subcutaneous injection of monocrotaline (MCT, 60 mg/kg). Rats were treated with resveratrol (25 mg/kg/day) by oral gavage daily for 21 days. PAH was confirmed by the presence of increased RV/LV + septum weight, RV and lung weight. In MCT rats, total heart surface area and RV lumen area were increased without corresponding increases in total muscle area, indicating a dilation of the lumen. Pulmonary truck lumen area and thickness of the tunica media were increased by 43% and 44%, respectively, by MCT. Resveratrol had no significant effect on remodeling, although decreases of 12% and 27% were observed for overall heart area and pulmonary truck area, respectively. However, resveratrol significantly reduced the thickness of the pulmonary trunk tunica media. Plasma levels of angiotensin II, aldosterone, C-reactive protein and endothelin-1 were not altered with resveratrol. Our results indicate that daily treatment with resveratrol does not inhibit the abnormal remodeling of the RV induced by MCT, but attenuates the development of medial hypertrophy in the pulmonary trunk.

1. Introduction

Pulmonary hypertension (PAH) is a syndrome characterized by an elevated pulmonary artery pressure [1]. Although the etiology of PAH remains largely unknown, several studies indicate that PAH is the result of abnormal vascular remodeling, including hypertrophy of the pulmonary vasculature, proliferation in the intima and media, activation of inflammatory cytokines, and endothelium dysfunction [2]. This leads to increased pulmonary vascular resistance and right ventricular (RV) afterload, eventually leading to RV hypertrophy and failure [3].

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a naturally occurring polyphenol that is synthesized in human food products such as grapes, peanuts, and berries. Resveratrol exerts significant anti-oxidant, anti-inflammatory, and robust protective effects on endothelial function [4]. Epidemiological studies have shown that moderate consumption of red wine, which contains resveratrol, is associated with decreased mortality from coronary artery disease, thus describing the “French paradox” [5]. Experimental studies show that resveratrol prevents the development of PAH by delaying the proliferation of vascular smooth muscle cells in intrapulmonary resistance vessels, and inhibits oxidative stress [6]. Resveratrol treatment in rats with PAH also improves endothelial function, resulting in decreased RV and arterial pressures [6]. In the ischemic heart, resveratrol inhibits upregulation of pro-apoptotic genes from occurring, improving contractile reperfusion recovery and the incidence of ventricular dysrhythmias [7,8]. The hyper-

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trophic response of angiotensin II in cardiomyocytes is inhibited by resveratrol as well as the onset of LV hypertrophy in aortic-banded rats [9,10], suggesting that resveratrol can be effective in preventing abnormal remodeling of the ventricles.

Interestingly, most studies investigating the effects of resveratrol treatment in rat models of PAH have focused primarily on the remodeling occurring in small to intermediate size vessels located in the pulmonary system where they are subjected to lower pressures [6,11,12]. With the exception of one study showing beneficial effects of a red wine polyphenolic extract on vascular function in second- and third-order pulmonary arteries and thoracic aorta [13], this study was performed in non-treated and disease-free rats. Indeed, the beneficial effects on relaxation parameters were demonstrated in an isolated system in which function was measured following the acute addition of the extract in the absence of physiological workload conditions [13]. To our knowledge, no information exists regarding the effects of resveratrol treatment on remodeling of the pulmonary trunk, a vessel exposed to increased pressures in the presence of PAH and also closely mimic peak RV systolic pressures. Based on these studies describing the benefits of this polyphenol on vascular remodeling, it seems reasonable to hypothesize that resveratrol treatment diminishes the extent of abnormal hypertrophy that is expected to occur in the pulmonary trunk with PAH.

To test this hypothesis, we used the monocrotaline (MCT) model of PAH. MCT is a pyrrolizidine alkaloid derived from the plant *Crotalaria spectabilis* and has been used widely to induce and study PAH in rats [14]. Rats develop PAH and hypertrophy of the RV after a single injection of MCT as a result of endothelial damage followed by pulmonary vascular remodeling, increased oxidative stress and expression of pro-inflammatory cytokines [15–18].

2. Materials and methods

2.1. Animal model of PAH and treatment protocol

This study was approved by the Midwestern University Institutional Animal Care and Use Committee. Animals used were cared for in accordance with the recommendations in The Guide for the Care and Use of Laboratory Animals, NIH, Publ. No. 85-23, 1986. Male Sprague-Dawley rats (Charles River Laboratories, MA), weighing 260 ± 9.6 g, were randomly assigned into three groups: (n = 12) control group, MCT-treated (n = 12), and MCT + resveratrol-treated (n = 12). Rats in the MCT group received a single subcutaneous injection of MCT (60 mg/kg) in the nape of the neck. MCT (Sigma-Aldrich, MO) was dissolved in 0.5 N HCl and neutralized with 0.5 N sodium hydroxide and added to isotonic saline for injection. This concentration of MCT is known to increase peak RV systolic pressures above 30 mmHg [6,11,17]. Rats in the control group received an equivalent injection (0.6 ml) of isotonic saline solution. Resveratrol (*Trans*-resveratrol, Enzo, Life Sciences, NY) was mixed with a 1% solution of methylcellulose (Sigma-Aldrich, MO), viscosity 25 cP, to form a colloid and administered to rats by oral gavage [19]. The resveratrol concentration in the colloid was adjusted to 10.0 mg/mL and given to rats at a concentration of 25 mg/kg daily for a period of 21 days. To avoid oxidation of resveratrol, the final concentrated solution was wrapped in foil and kept under dark conditions. Rats were observed daily for signs of distress, such as respiratory dysfunction and cardiac failure, which included dramatic weight loss, dyspnea with increased respiratory efforts, cyanotic ears, and decreased activity level. Rats were housed in pairs in a temperature-controlled room with a 12:12 h light-dark cycle and fed a standard rat diet *ad libitum* and water.

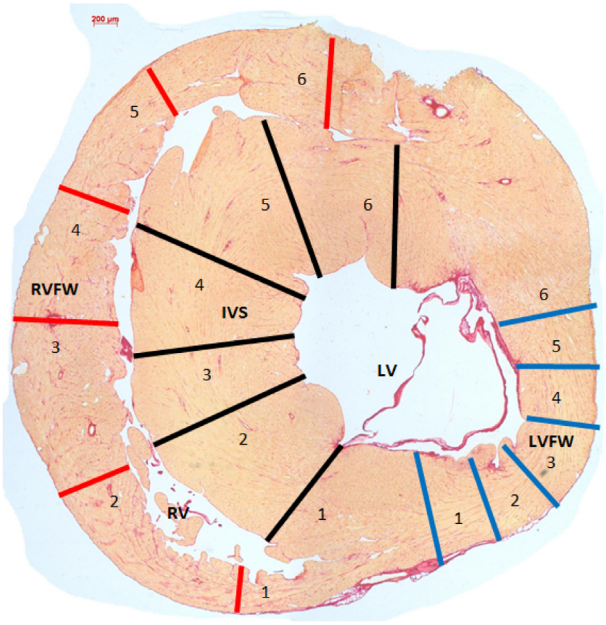

Fig. 1. Representative image of a transverse section of a whole heart from a control rat. Transverse sections were taken just inferior to the atrial-ventricular septum along the coronary sinus displaying the circular left ventricle (LV) and half-moon-shaped right ventricle (RV). Septum measurements were taken as shown for the interventricular septum (IVS) in black, right ventricle free wall (RVFW) in red, and left ventricle free wall (LVFW) in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Tissue sampling and preparation

After the treatment protocol, rats were euthanized by anesthetization in a CO_2 chamber followed by decapitation. The hearts and lungs were carefully extracted and weighed. The heart was dissected into the RV, LV, septum and both atria. Each section was individually weighed then frozen with tongs precooled to the temperature of liquid nitrogen. The ratio of RV to LV plus septum weight (RV/LV + septum) was used as an index of RV hypertrophy. In another series of rats (n = 8 per group), heart and pulmonary trunk were removed and embedded in OCT (Optimal Cutting Temperature compound, TissueTek, CA), frozen using dry ice, and stored at −80° C until used. Heart and pulmonary trunk were sectioned (10 μM thickness) on a cryostat microtome at −28 °C and samples placed on positively charged microscope slides for staining and morphological measurements.

2.3. Tissue staining and morphological measurements

Tissue slides were either stained with hematoxylin and eosin or picrosirius red stain for measurements of lumen area, wall thickness, or fibrotic tissue accumulation, respectively. Transverse sections of whole heart to expose ventricular lumen and walls were taken just inferior to the atrial-ventricular septum along the coronary sinus (Fig. 1). These sections were used to determine RV and LV luminal area, thickness of ventricular and septum wall. To determine luminal area and arterial medial wall thickness of the pulmonary artery, transverse sections of the upper pulmonary artery were performed just proximal to the pulmonary trunk bifurcation. Segments of ~0.3 cm in length of the pulmonary trunk were initially cut and embedded in OCT for cryosectioning. For the actual analysis, three 6 μM thick sections were randomly taken from each sample. All measurements were performed in triplicate for precision using two imaging software programs, AxioVision 4.8 (Carl Zeiss, NY) and an open source version of Image J, Fiji (NIH, General