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A novel adipocytokine, omentin, inhibits monocrotaline-induced pulmonary arterial hypertension in rats

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ABSTRACT

Omentin is a novel adipocytokine mainly expressed in visceral rather than subcutaneous adipose tissue. Several epidemiological studies demonstrated the negative relationship between blood omentin level and occurrence of obesity, type 2 diabetes and hypertension. Increases of inflammatory responses, contractile reactivity and structural remodeling of vascular wall contribute to hypertension development. Our in vitro studies previously demonstrated that omentin inhibited those hypertension-related pathological processes. In addition, our in vivo study demonstrated that intravenously injected omentin acutely inhibited agonists-induced increases of blood pressure in rats. However, the chronic effects of omentin on hypertension development are not determined. In the present study, we tested the hypothesis that chronic omentin treatment may inhibit pulmonary arterial (PA) hypertension (PAH). PAH was induced by a single intraperitoneal injection of monocrotaline (MCT: 60 mg/kg) to rats. Omentin (18 μg/kg/day) was intraperitoneally treated for 14 days. Chronic omentin treatment inhibited MCT-induced increases in PA pressure. Omentin inhibited MCT-induced right ventricular hypertrophy as well as increase of lung to body weight ratio. Histologically, omentin inhibited MCT-induced PA hyperplasia. Further, omentin inhibited the impairment of both endothelium-dependent and -independent relaxations mediated by acetylcholine and sodium nitroprusside, respectively. In conclusion, we for the first time demonstrate that chronic omentin treatment inhibits MCT-induced PAH in rats via inhibiting vascular structural remodeling and abnormal contractile reactivity.

1. Introduction

Pulmonary arterial (PA) hypertension (PAH) is a disease that is characterized by increases in PA resistance and PA pressure. Increases of inflammatory responses, contractile reactivity and vascular structural remodeling contribute to the increases in pulmonary vascular resistance, finally leading to right heart failure (RHF) through PAH. There are currently several reports demonstrating that a tyrosine kinase inhibitor, imatinib could inhibit PAH via inhibiting platelet-derived growth factor-induced vascular structural remodeling [1,2]. However, there is a report demonstrating that a long-term imatinib treatment has several side effects [3]. As other therapeutic drugs for PAH, prostacyclin, endothelin receptor blocker and phosphodiesterase-5 inhibitor are known [4]. Since these drugs have different sites of actions and molecular mechanisms, they are often given together. Nonetheless, more attractive and effective drugs for PAH are urgently demanded.

Obesity-induced adipocyte hypertrophy causes an increase or decrease in the production and secretion of adipocyte-derived cytokines, named adipocytokine. Adipocytokine can control progression of obesity-related cardiovascular diseases including hypertension [5]. Omentin, also referred as intelectin-1 and intestinal lactoferrin receptor, is an adipocytokine originally discovered in omental fat [6,7]. Omentin is mainly expressed in visceral rather than subcutaneous adipose tissue [7]. Several epidemiological reports demonstrated the negative relationship between blood omentin level and occurrence of obesity, type 2 diabetes and hypertension [8,9]. In addition, another report demonstrated that serum omentin level decreases in patients with obstructive sleep apnea syndrome (OSAS), ultimately inducing PAH and RHF [10].

Abbreviations: PA, pulmonary arterial; PAH, pulmonary arterial hypertension; MCT, monocrotaline; RHF, right heart failure; OSAS, obstructive sleep apnea syndrome; SMCs, smooth muscle cells; NO, nitric oxide; BP, blood pressure; IPAs, intrapulmonary arteries; ACh, acetylcholine; SNP, sodium nitroprusside; NA, noradrenaline; RV, right ventricular; NOX, NADPH oxidase; LV, left ventricular.

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Our previous in vitro studies demonstrated that omentin inhibited vascular inflammatory responses and smooth muscle cells (SMCs) migration [11,12], which are important components for the development of vascular structural remodeling. In addition, we demonstrated that omentin induced vasodilation of isolated blood vessel through endothelium-derived nitric oxide (NO) [13]. Moreover, our in vivo study demonstrated that intravenously injected omentin acutely inhibited agonists-induced increases of blood pressure (BP) in rats [14]. Thus, we hypothesized that chronic omentin treatment may inhibit PAH. To test the hypothesis, we examined the effects of chronic omentin treatment on monocrotaline (MCT)-induced PAH in rats.

2. Materials and methods

2.1. Animal experiments

Animal care and treatment were conducted in conformity with institutional guidelines of The Kitasato University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Wistar rats (Clea Japan, Tokyo, Japan) were maintained on a standard laboratory diet and tap water, and exposed to a 12 h/12 h light–dark cycle at 23 ± 2 °C. Rats (5-week-old) were randomly divided into three groups; control group (Control, $n = 8$), MCT-injected group (MCT, $n = 10$) and omentin-treated MCT-injected group (+Omentin, $n = 8$). PAH was induced by a single intraperitoneal injection of MCT (60 mg/kg) as previously described [15]. The rats in the Control were once intraperitoneally injected with saline. Saline (MCT) or recombinant omentin (+Omentin; 18 μg/kg/day) was intraperitoneally treated once daily for 14 days.

2.2. Mean PA pressure measurement

At the end of the treatment, PA pressure was measured under urethane (1.5 g/kg, i.p.) anesthesia as described previously [15]. The catheter filled with a heparin-saline solution was inserted into the pulmonary artery via the right external jugular vein as described previously [16]. Catheter was connected to MLT0670 BP transducer (ADInstruments Colorado Springs, CO, USA). Mean PA pressure was measured and digitally recorded using ML117 BP Amp (ADInstruments), ML825 PowerLab 2/25 (ADInstruments) system and Chart 5 software (ADInstruments).

2.3. Histological analysis

After PA pressure measurement, rats were euthanized by exsanguination under deep urethane (1.5 g/kg, i.p.) anesthesia, and hearts and lungs were isolated for histological examinations. The hearts were separated into right and left atrial or ventricular tissues. After measurement of isolated ventricular tissue and lung weight, a part of each tissue was fixed in 4% paraformaldehyde. Thin paraffin sections (4 μm) were made and stained with hematoxylin and eosin as described previously [12,17]. The images were obtained using a light microscope (BX-51, Olympus, Tokyo, Japan). Cross sectional area of cardiomyocytes (μm^2) was calculated using Image J software. Vascular structural remodeling was evaluated by calculating luminal to vessel area ratio (%) in the intrapulmonary arteries (IPAs) (diameter; <100 μm) using Image J software.

2.4. Measurement of isometric contraction

The intrapulmonary arterial rings (diameter; <1 mm) were placed in normal physiological salt solution, which contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, glucose 5.5 and EDTA 0.001. The high KCl solution was prepared by replacing NaCl with equimolar KCl. These solutions were saturated with a 95% O_2–5% CO_2 mixture at 37 °C and pH 7.4. Smooth muscle contractility was recorded isometrically with a force–displacement transducer (Nihon Kohden, Tokyo, Japan) as described previously [18]. Each arterial ring was attached to a holder under a resting tension of 0.5 g. After equilibration for 30 min in a 3 ml organ bath, each ring was repeatedly exposed to 72 mM KCl solution, until the responses became stable. Concentration–responses curves were obtained by the cumulative application of acetylcholine (ACh; 1 nM–30 μM) or sodium nitroprusside (SNP; 100 pM–3 μM) to the artery precontracted equally by 100 nM noradrenaline (NA).

2.5. Materials

Recombinant omentin (BioVendor, Candler, NC, USA); MCT (Wako Pure Chemical, Osaka, Japan); ACh (Daiichi-Sankyo, Tokyo, Japan); NA and SNP (Sigma–Aldrich, St. Louis, MO, USA).

2.6. Statistical analysis

Data were shown as mean + SEM. Statistical evaluations were performed by one-way ANOVA followed by Bonferroni's test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of chronic omentin treatment on MCT-induced increases in mean PA pressure

We first examined the effects of chronic omentin (18 μg/kg/day, 14 days) treatment on MCT-induced increases in PA pressure of rats. Omentin significantly inhibited MCT (60 mg/kg)-induced increases in PA pressure (from 27.2 ± 2.6 to 18.7 ± 1.4 mmHg, $n = 8$–10, $p < 0.05$, Fig. 1).

3.2. Effects of omentin on MCT-induced increases in right ventricular (RV) hypertrophy

Increases of PA pressure contribute to RV hypertrophy. We next examined the effects of omentin on MCT-induced RV hypertrophy. Omentin significantly inhibited both RV to left ventricular weight ratio (from 0.36 ± 0.02 to 0.28 ± 0.02 g/g, $n = 8$, $p < 0.01$, Fig. 2A)

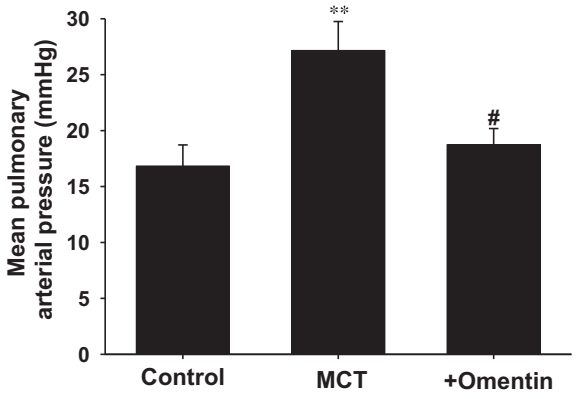

Fig. 1. Effects of chronic omentin treatment on monocrotaline (MCT)-induced increases in mean pulmonary arterial (PA) pressure of rats. After saline (Control, $n = 8$) or MCT (60 mg/kg) was intraperitoneally injected to rats (5-week-old), saline (MCT, $n = 10$) or omentin (+Omentin; 18 μg/kg/day, $n = 8$) was intraperitoneally treated everyday. After 14 days, mean PA pressure was directly measured by a cannulation method. $^{**}p < 0.01$ vs. Control; $^{\#}p < 0.05$ vs. MCT.