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Gallic acid attenuates pulmonary fibrosis in a mouse model of transverse aortic contraction-induced heart failure

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ABSTRACT

Gallic acid, a trihydroxybenzoic acid found in tea and other plants, attenuates cardiac hypertrophy, fibrosis, and hypertension in animal models. However, the role of gallic acid in heart failure remains unknown. In this study, we show that gallic acid administration prevents heart failure-induced pulmonary fibrosis. Heart failure induced in mice, 8 weeks after transverse aortic constriction (TAC) surgery, was confirmed by echocardiography. Treatment for 2 weeks with gallic acid but not furosemide prevented cardiac dysfunction in mice. Gallic acid significantly inhibited TAC-induced pathological changes in the lungs, such as increased lung mass, pulmonary fibrosis, and damaged alveolar morphology. It also decreased the expression of fibrosis-related genes, including collagen types I and III, fibronectin, connective tissue growth factor (CTGF), and phosphorylated Smad3. Further, it inhibited the expression of epithelial-mesenchymal transition (EMT)-related genes, such as N-cadherin, vimentin, E-cadherin, SNAI1, and TWIST1. We suggest that gallic acid has therapeutic potential for the treatment of heart failure-induced pulmonary fibrosis.

1. Introduction

Heart failure is a fatal condition that leads to cardiac dysfunction. Transverse aortic constriction (TAC) is used to experimentally induce cardiac hypertrophy and heart failure in animals [1]. TAC induces left ventricular hypertrophy and cardiac dysfunction after 6 weeks [2]. Chronic TAC causes severe pulmonary fibrosis, inflammation, and alveolar remodeling [3]. The weight of the lungs increases in TAC-induced heart failure. These reports suggest that TAC induces severe pulmonary diseases. The TAC animal model mimics pulmonary hypertension due to left heart disease. Pulmonary hypertension is classified into five categories: pulmonary artery hypertension, pulmonary hypertension due to left heart disease, pulmonary hypertension due to lung diseases or hypoxia or both, chronic thromboembolic pulmonary hypertension, and pulmonary hypertension with unclear multifactorial mechanisms [4]. Several studies have reported that the prevalence of pulmonary hypertension caused by left heart diseases is approximately 70%, and it was measured by using echocardiography [5–7]. Pulmonary fibrosis is associated with pulmonary hypertension [8,9]. The phosphodiesterase 5 inhibitor sildenafil and the soluble guanylate cyclase stimulator riociguat ameliorated pulmonary hypertension due to left heart diseases in a TAC mouse model [2]. The antioxidant peptide SS-31 attenuated TAC-induced pulmonary arterial hypertension in mice [10].

Many drugs clinically used to treat or delay heart failure, such as angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), beta-blockers, aldosterone antagonists, and vasodilators [11], have been proven to be effective in regulating heart failure-induced remodeling [12].

Despite significant advancement in the development of drugs for heart failure, the mortality rate is relatively high. Recently, therapy by neurohormonal inhibition has been proven effective against chronic heart failure [13,14]. Additionally, dietary phytochemicals have been reported to protect against a variety of pathological diseases [15]. Resveratrol found in grape wine protects against pressure overload-induced heart failure [16]. Imperatorin, a dietary furanocoumarin,

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prevents cardiac hypertrophy and the transition to heart failure in a TAC mouse model [17]. The natural compound, curcumin has shown potential as a novel therapeutic agent against heart failure [18].

Gallic acid, a trihydroxybenzoic acid found in tea and other plants, attenuates vascular calcification [19], cardiac hypertrophy [20], cardiac fibrosis [20], and hypertension. However, the effect of gallic acid on heart failure is not determined. Furthermore, given that heart failure accompanies pulmonary edema, we also investigated the effect of furosemide (a diuretic) on TAC-induced pulmonary fibrosis.

The aim of this study was to evaluate the effect of gallic acid on pulmonary fibrosis and pathological remodeling in TAC-induced heart failure. In this study, we show for the first time that gallic acid inhibits pulmonary fibrosis, expression of related marker genes, and epithelial-mesenchymal transition (EMT). We observed that gallic acid attenuated heart failure-induced pathological changes in the pulmonary tissues and heart dysfunction. Our findings suggest that gallic acid could be used as a potential therapeutic agent for the treatment of pathological pulmonary fibrosis.

2. Materials and methods

2.1. TAC and administration of gallic acid or furosemide

All animal procedures were approved by the Animal Experimental Committee of Chonnam National University Medical School (CNU IACUC-H-2015-52) and carried out according to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health Publications, 8th edition, 2011). CD-1 male mice (8-week-old, 30–35 g) were anesthetized by an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (14 mg/kg). The surgical procedure was performed as reported previously [21]. After endotracheal intubation, the transverse aortic arch was ligated (7–0 silk suture) between the brachiocephalic and left common carotid arteries with an overlaying 27 G needle. Mice in the sham group underwent the same procedure except for TAC. Gallic acid (100 mg/kg/day), furosemide (3 mg/kg/day), or vehicle (DMSO) was intraperitoneally administered daily to TAC mice for 2 weeks. The induction of TAC-induced heart failure after 8 weeks was confirmed by echocardiography that was carried out every 2 weeks. After induction, we evaluated heart function every week using echocardiography.

2.2. Histology and Masson's trichrome staining

The paraffin-embedded tissues were sectioned 3 μm thick, deparaffinized with xylene, and then rehydrated with different grades of alcohol. Hematoxylin and eosin (H & E) staining was performed as described [22]. The arterial wall thickness was measured using NIS Elements Software (Nikon, Japan). Masson's trichrome staining was performed as described previously [23].

2.3. Echocardiography

Echocardiography was performed using a Vivid S5 echocardiography system (Vivid S5, GE Healthcare, USA) with a 13 MHz linear array transducer. The procedures were carried out on mice after anesthetizing them with tribromoethanol (Avertin, 114 mg/kg intraperitoneal injection). M-mode (2-D guided) images and recordings were acquired from the long axis view of the left ventricle at the level of the papillary muscles. The thickness of the anterior and posterior wall was measured from the images whereas the left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) were measured from the M-mode recordings. Fractional shortening (FS) was calculated as FS (%) = (LVEDD − LVESD) × 100/LVEDD.

Table 1
Primers for reverse transcription-polymerase chain reaction (RT-PCR).

Gene (mouse)	Primer sequence (5′ to 3′)
GAPDH	F: GCATGGCCTTCCGTGTTCCT
	R: CCCTGTTGCTGTAGCCGTATTCAT
Collagen type I	F: GAGCGGAGAGTACTGGATCG
	R: GCTTCTTTTCCTTGGGGTTC
Collagen type III	F: TGATGGAAAACCAGGACCTC
	R: CAGTCTCCCCATTCTTTCCA
Fibronectin	F: GATGCACCGATTGTCAACAG
	R: TGATCAGCATGGACCACTTC
CTGF	F: CAAAGCAGCTGCAAATACCA
	R: GGCCAAATGTGTCTTCCAGT
Smad3	F: CACAGCCACCATGAATTACG
	R: GTGTTCTCGGGAATGGAATG
N-cadherin	F: AGTTTCTGCACCAGGTTTGG
	R: TGATGATGTCCCCAGTCTCA
Vimentin	F: AAGGAAGAGATGGCTCGTCA
	R: TTGAGTGGGTGTCAACCAGA
E-cadherin	F: CGGAGAGGAGAGTCGAAGTG
	R: CATGCTCAGCGTCTTCTCTG
SNAI1	F: GAGGACAGTGGCAAAAGCTC
	R: CCAGGCTGAGGTACTCCTTG
TWIST1	F: CAGCGGGTCATGGCTAAC
	R: CAGCTTGCCATCTTGGAGTC

2.4. Reagents

Gallic acid (G7384) and furosemide (S1603) were purchased from Sigma (Billerica, MA, USA) and Selleckchem (Houston, TX, USA), respectively.

2.5. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from the aortic tissue was isolated with the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, USA), and 1 μg of RNA was used for the reverse transcription reaction with TOPscript RT DryMIX (Enzynomics, South Korea). mRNA levels were quantified with the SYBR Green PCR kit (Enzynomics). The PCR primers used in this study are shown in Table 1.

2.6. Western blot

Total protein from lung tissues was extracted in a lysis buffer (RIPA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 50 mM Tris-HCl pH 7.5, 2 mM EDTA, 1 mM PMSF, 1 mM DTT, 1 mM Na_3VO_4, and 5 mM NaF) containing a protease inhibitor cocktail (Calbiochem, EMD Millipore, MA, USA). Proteins were subjected to SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% skim milk in TBST buffer (20 mM Tris, 200 mM NaCl, and 0.04% Tween 20) for 1 h at 25 °C. The membranes were incubated overnight at 4 °C with primary antibodies against collagen types I and III, fibronectin, connective tissue growth factor (CTGF), p-Smad3, Smad3, N-cadherin, E-cadherin, SNAI1, and β-actin. They were then incubated with the anti-rabbit or anti-mouse horseradish-peroxidase-conjugated secondary antibodies (1:5000) for 1 h at room temperature. Protein bands were visualized using Immobilon Western detection reagents (EMD Millipore, Billerica, MA, USA). The Bio-ID software was used to quantify protein expression (Vilber Lourmat, Eberhardzell, Germany).

2.7. Statistical analysis

All data are expressed as means ± standard errors (SE). Differences between data were analyzed by one-way analysis of variance (ANOVA) with the Bonferroni post hoc test using GraphPad Prism, version 5 and a value of $P < 0.05$ was considered significant.

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