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Fermented garlic extract ameliorates monocrotaline-induced pulmonary hypertension in rats

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ABSTRACT

This study investigated the effect of fermented garlic extract (FGE) on pulmonary hypertension in monocrotaline (MCT)-treated rats. Sprague-Dawley rats were subcutaneously injected with either 50 mg/kg MCT or a vehicle 3 days after the FGE feeding (0.97 mg nitrite/ml/day) was initiated. MCT treatment increased the weight, systolic pressure, and atrial natriuretic peptide concentration in the right ventricle but not in the left ventricle. FGE feeding attenuated these effects as well as the endothelial damage and medial hypertrophy of the pulmonary arterioles and the pulmonary fibrosis induced by MCT. These FGE effects were blocked by a soluble guanylyl cyclase (sGC) inhibitor. Increases in VCAM-1 and MMP-9 protein expressions, and decreases in PKG and eNOS protein expressions in the lung of MCT rats were attenuated by FGE feeding. These findings suggest that FGE ameliorates MCT-induced pulmonary hypertension by decreasing the inflammatory reaction via the NO–sGC–PKG pathway.

1. Introduction

Pulmonary arterial hypertension is a fatal disease that is characterized by vascular proliferation and remodeling followed by a progressive elevation of pulmonary vascular resistance. These remodeled and obstructed vessels persistently limit blood flow through the pulmonary arteries, resulting in an increased afterload on the right ventricle (RV) and subsequently hypertrophy and heart failure (Rabinovitch, 2008). Pulmonary arterial hypertension can either be idiopathic or secondary, originating from cardiovascular diseases. However, regardless of the cause, pulmonary arterial hypertension is closely associated with endothelial dysfunction, which is an early component of the pulmonary hypertensive process, leading to an imbalance between vasodilator and vasoconstrictor substances (Morrell et al., 2009). Although its exact pathophysiology remains controversial, many reports suggest that inflammation plays an important role in primary pulmonary arterial hypertension (Crosswhite & Sun, 2010; Wang et al., 2013). Therefore, inflammation-induced pulmonary vascular remodeling has become one of the key targets for therapy (Cohen-Kaminsky, Hautefort, Price, Humbert, & Perros, 2014; El Chami & Hassoun, 2011). Current therapies improve the symptoms and hemodynamics of pulmonary arterial hypertension, but true reversal of pulmonary vascular remodeling is rarely achieved. Therefore, further studies are required to develop more effective therapies.

Garlic (*Allium sativum* L.) has been widely used as an important medicine in many ancient civilizations (Pittler & Ernst, 2007). It contains many bioactive compounds with medicinal value, although manufacturing processes of garlic preparations affect its chemical constituents. The major organosulfur bioactive compounds are allicin in raw garlic extract (RGE) (Iberl, Winkler, Muller, & Knobloch, 1990) and S-allyl-L-cysteine (SAC) in aged garlic extract (AGE) (Harauma & Moriguchi, 2006; Kim, Chang, Kim, & Chun, 2006). Garlic has a range of beneficial effects on the cardiovascular system (Fleischauer & Arab, 2001; Ide & Lau, 2001; Jalal, Bagheri, Moghimi, & Rasuli, 2007). In particular, numerous studies have investigated the anti-hypertensive effects of RGE, AGE, and garlic-based preparations using various hypertensive models (Al-Qattan, Alnaqeeb, & Ali, 1999; Ashraf, Khan, Ashraf, & Qureshi, 2013; Harauma & Moriguchi, 2006; Kim et al., 2006; Pedraza-Chaverri, Tapia, Medina-Campos, de los Angeles Granados, & Franco, 1998; Ried, Frank, & Stocks, 2013), including pulmonary arterial hypertension (Fallon et al., 1998; Sun & Ku, 2006). These

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studies have found that different garlic preparations have different anti-hypertensive activities, possibly due to differences in the stability and toxicity of the bioactive compounds they contain. To address these issues, Chun (2014) developed a fermentation method that increases the stability and nitrite content in RGE. We recently reported that fermented garlic extract (FGE) has a potent anti-hypertensive activity in spontaneously hypertensive rats (Park, Cha, Han, & Kim, 2014). Therefore, the aim of the present study was to investigate the effect of FGE on pulmonary arterial hypertension and to determine the molecular mechanisms of action using monocrotaline (MCT)-treated rats.

2. Materials and methods

2.1. Rats and diet

Eight-week-old male Sprague-Dawley (SD) rats were obtained from Daehan biolink (Eumsung, Republic of Korea) and housed in individual cages in a temperature-controlled room with 12-h light/12-h dark cycle. The rats were allowed open access to standard laboratory chow (Seoul Feed, Republic of Korea) and water for 1 week to acclimate prior to the experiment. The approximate composition of the diet was as follows: moisture, 10.0%; crude protein, 20.8%; crude fat, 3.9%; crude fiber, 4.3%; crude ash, 6.3%; and nitrogen-free extract, 53% (w/w). All animal experiments described in this study were conducted in accordance with the Guiding Principles in the Care and Use of Animals by the American Physiological Society, and all animal experimental protocols were approved by the Institutional Animal Care and Use Committee of the Chonbuk National University.

2.2. Preparation of FGE

The FGE used in this study was manufactured by HtO Life Co., Ltd. (Wanju-gun, Republic of Korea) following Chun (2014), as previously described (Park et al., 2014). Briefly, fresh garlic purchased from the local market was peeled, and the cloves were soaked and sterilized with ozonated water for 20 h. The garlic cloves were then crushed using a crusher, which was incubated with *Bacillus subtilis* pre-activated in water in a 1:9 (w/v) ratio at 37 °C for 1 month under aerobic conditions. The supernatant was separated by ultrafiltration and sterilized.

Nitrite (NO_2^-) and nitrate (NO_3^-) levels in the preparation were analyzed using the standard method of the American Public Health Association (APHA) at the Korea Testing & Research Institute (Gwacheon-si, Republic of Korea). SAC, alliin, and allicin contents were analyzed using the Thermo ACCELA high performance liquid chromatography and TSQ Quantum Access Max system (Thermo Fisher Scientific Inc., Waltham, MA, USA) at the Namhae Garlic Research Institute (Namhae, Republic of Korea) (Jung et al., 2014; Park et al., 2014). As previously reported (Park et al., 2014), FGE contained higher level of nitrite (9.75 ± 1.18 mg/g of garlic, 0.97 mg/ml of FGE, $p < 0.01$) and lower level of alliin (0.008 ± 0.008 mg/g of garlic, $p < 0.01$) and allicin (not detected) compared with raw garlic (nitrite, 0.043 ± 0.040 mg/g of garlic; alliin, 10.650 ± 0.030 mg/g of garlic; allicin, 1.450 ± 0.010 mg/g of garlic) (Table 1). Therefore, nitrite was used as a marker compound for the standardization of FGE. FGE used in this study contained 0.97 mg nitrite/ml.

2.3. Experimental protocols

Adult male SD rats received a single subcutaneous injection of 50 mg/kg MCT (Sigma-Aldrich, St Louis, MO, USA) (Gao et al., 2010) to induce pulmonary arterial hypertension, while sham rats were injected with saline. The sham rats were randomly divided into two groups: group 1 was freely fed water (sham-vehicle group, n = 10) and group 2 was freely fed FGE (containing 0.97 mg nitrite/ml) for 21 days (sham-FGE group, n = 8). MCT-treated rats were randomly divided into three groups: group 3 was freely fed water (MCT-vehicle group, n = 12), group 4 was freely fed FGE for 21 days (MCT-FGE group, n = 14), and group 5 was freely fed FGE in the presence of 1H-[1,2,4] oxadiazolo [4,3,-α] quinoxalin-1-one (ODQ; Enzo Life Science, Plymouth Meeting, PA, USA), which inhibits soluble guanylyl cyclase (sGC) (MCT-ODQ group, n = 8). The MCT-ODQ group was pretreated with ODQ 2 days before FGE feeding, and this continued for 23 days at a dose of 2 mg/kg/day. The ODQ was infused via a mini-osmotic pump (Alzet 2002, Cupertino, CA, USA) that was implanted subcutaneously between the scapula (Park et al., 2014).

Table 1
Comparison of components of fermented and raw garlic extracts.

	Fermented garlic extracts (n = 4)	Raw garlic (n = 3)
NO_2^- (mg/g garlic)	9.745 ± 1.177**	0.043 ± 0.040
NO_3^- (mg/g garlic)	0.067 ± 0.078	0.043 ± 0.020
Alliin (mg/g garlic)	0.008 ± 0.008**	10.650 ± 0.030
Allicin (mg/g garlic)	ND	1.450 ± 0.010
SAC (mg/g garlic)	ND	0.030 ± 0.000

Values are the mean ± SE. SAC, S-allyl-L-cysteine; ND, not detected.
** vs raw garlic extract, $p < 0.01$ (Park et al., 2014).

Body weight was measured every 4 days and changes in body weight were expressed as the accumulated gain. Rats were sacrificed 21 days after MCT injection, before which the body weight, systolic blood pressure (SBP), and right ventricular systolic pressure (RVSP) were measured. Blood was collected into vials containing 50 μl of 0.1 M ethylenediamine- tetraacetic acid and centrifuged at 4 °C at 10,000*g* for 15 min. The plasma was kept at −70 °C for the hormone assay, as described below. Following the measurement of the left ventricular and septal weight (LV + S), and the RV weight, tissues including the lungs were quickly removed and kept at −70 °C.

2.4. Measurement of SBP and RVSP

One day before sacrifice, SBP was measured indirectly using tail cuff plethysmography (Power Lab 2/20, AD Instruments Pvt. Ltd, Australia). RVSP was measured 21 days after MCT injection, by anesthetizing each rat with ketamine (Yuhan, Republic of Korea) and xylazine (Bayer Korea, Republic of Korea), and inserting a polyethylene tube connected to Power lab (ML-820, AD Instruments) via a pressure transducer (Statham P23Db, Oxnard, CA, USA) into the RV via the right jugular vein (Gao et al., 2010). Following stabilization, RVSP was measured for 10 min and blood was collected from the abdominal aorta.

2.5. Radioimmunoassay of ANP, renin, and cGMP

Plasma atrial natriuretic peptide (ANP) was extracted using a Sep-Pak C_{18} cartridge and the concentration was then measured using specific radioimmunoassay (RIA), as described previously (Cho et al., 1989). The plasma renin concentration (PRC) (Cho et al., 1989) and cGMP level were also measured using RIA, as described previously (Lee et al., 2000).

2.6. Histological analysis

Left upper lung tissues were sliced, fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at a thickness of 4 μm. The lung tissue sections were then stained with hematoxylin and

eosin (H&E). To determine the extent of collagen deposition in the pulmonary arteries, sections were stained with sirius red and masson trichrome (MTS), and the amount of collagen was quantified using a modified Ashcroft scoring system (Shah, Oh, Lee, Lim, & Kim, 2012).

For immunohistochemistry, the tissue sections were blocked with peroxidase blocking agent for 5 min and washed with Tris-HCl with Tween (TBST), following which they were blocked with protein blocking serum free buffer for 5 min. Sections were then incubated with anti-α smooth muscle actin (α-SMA, Sigma-Aldrich) or anti-von Willebrand Factor (vWF) (Millipore, Temecula, CA, USA) overnight at 4 °C, and then washed with TBST for 5 min. A labeled polymer conjugated with secondary antibodies (Dako, Carpinteria, CA, USA) was applied to the slides for 30 min, following which they were washed with TBST for 5 min. Peroxidase activity was detected with the ready-to-use AEC + substrate chromogen (Dako). At least 20 arteries per rat ranging from 15 to 100 μm were evaluated on the α-SMA stained slides through a light microscope (Imager M1, Carl Zeiss, Jena, Germany) at ×400 magnification and were quantified using image J software. The medial wall thickness was calculated as follows:

$$\text{Wall thickness} = (\text{total area of artery} - \text{lumen area of artery})/\text{total area of artery}$$

2.7. Western blotting

Total protein was extracted from the lung. The tissue samples were placed in lysis buffer (M-PER, Thermo, Rockford, IL, USA) containing protease inhibitor, homogenized, incubated on ice for 30 min, and then centrifuged at 16,000*g* for 15 min. After determining the protein concentrations in the supernatant using a modified Bradford assay, 30 μg of total protein was boiled in a loading buffer for 5 min and loaded onto gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels. Following electrophoresis, the proteins were transferred to an immobilon-polyvinylidene fluoride membrane and blocked with TBST containing 5% skim milk at room temperature for 1 h. The membrane was then incubated with primary antibodies against vascular cell adhesion molecule (VCAM)–1, matrix metalloprotease-9 (MMP-9), protein kinase G (PKG), α-SMA (all from Abcam, Cambridge, MA, USA), and endothelial nitric oxide synthase (eNOS; Enzo Life Science). Proteins were detected by incubating them in horseradish peroxidase conjugated secondary antibodies (Enzo Life Science) at room temperature for 1 h. Immunoreactivity was detected by chemiluminescence (Park, Gao, Cha, Park, & Kim, 2013).

2.8. Statistical analysis

The results are presented as means ± SEM. Statistical differences were assessed using one-way analysis of variance followed by the Duncan's multiple range test (GraphPad Prism 4). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Changes in SBP, RVSP, and RV weight in FGE-fed MCT rats

Sham and MCT rats were allowed to freely drink water or FGE for 21 days, with their body weight measured every 4 days. Changes in body weight, expressed as the accumulated gain, are shown in Fig. 1A. The MCT treatment resulted in a lower body weight gain than was observed in the sham-vehicle rats, and this was not affected by FGE feeding or the ODQ pretreatment. Both SBP (which was measured in a conscious condition 1 day before sacrifice) and RVSP (which was measured in an anesthetized condition the next day) were higher in MCT-vehicle rats than in sham-vehicle rats (Fig. 1B and C). FGE feeding reduced these increases in SBP and RVSP in MCT-vehicle rats, but these effects were attenuated by the pretreatment with ODQ, a sGC inhibitor.

The ratio of the RV to LV + S weight was significantly higher in MCT-vehicle rats than in sham-vehicle rats, but this effect was reduced by FGE feeding (Fig. 1D). The attenuation of RV hypertrophy by FGE feeding was blocked by the pretreatment with ODQ (Fig. 1D). There were no significant differences in the weight of the heart and the LV + S between the five groups (Fig. 1D). The ANP concentration was higher in the RV (but not the LV) of MCT-vehicle rats and MCT-FGE rats (Fig. 1E).

3.2. Changes in plasma renin, ANP, and cGMP levels in FGE-fed MCT rats

PRC was higher in MCT-vehicle rats than in sham-vehicle rats (Fig. 2A) and FGE feeding reduced this effect, although, again, pretreatment with ODQ blocked this effect. The plasma level of ANP was higher in MCT-FGE rats and pretreatment with ODQ attenuated this effect (Fig. 2B). There was no difference in the plasma cGMP levels between the five treatment groups (Fig. 2C).

3.3. Improvement of MCT-induced lung damage by FGE feeding

Fig. 3A shows a representative image of a pulmonary arteriole stained with vWF and α-SMA antibodies, which are markers of endothelial damage and medial hypertrophy, respectively (Veit et al., 2013). There was a markedly higher degree of vWF and α-SMA staining in the pulmonary arterioles of MCT-vehicle rats, while FGE feeding attenuated the expression of vWF and α-SMA. However, this effect was blunted by the ODQ pretreatment. The immunohistochemical analysis showed that the medial thickness of the pulmonary arterioles was greater in MCT-vehicle rats and that FGE feeding attenuated this medial hypertrophy (Fig. 3Ba), although the beneficial effect of FGE on the pulmonary arterioles was blunted by the ODQ pretreatment.

In the MCT-vehicle group, most of the lung structures were damaged and more blue colored collagen deposition was seen compared with the sham-vehicle group (Fig. 3A). In both the perivascular region and the pulmonary interstitium, the alveolar septal walls were severely thickened and confluent fibrotic masses were evident in the MCT-vehicle rats. However, FGE feeding decreased this adventitial fibrosis and collagen deposition, with no fibrotic masses present, although these effects were attenuated by the ODQ pretreatment (Fig. 3Bb).

3.4. Changes in inflammation-related protein expressions in FGE-fed MCT rats

To determine whether FGE feeding affects inflammation-related protein expressions, the expression levels of VCAM-1, MMP-9, PKG, α-SMA, and eNOS proteins were measured in the lung of sham and MCT rats. Fig. 4A shows a representative western blot for the inflammation-related proteins in the lung for the five groups, while the quantitative analysis is shown in Fig. 4B. The VCAM-1 and MMP-9 proteins were highly expressed in the MCT-vehicle group, and this effect was attenuated in the MCT-FGE group (Fig. 4B). The change in α-SMA protein expression was similar to MMP-9. By contrast, the expression of eNOS and PKG proteins was higher in the MCT-FGE group than in the MCT-vehicle group (Fig. 4B). The effects of FGE feeding on the eNOS, PKG, and inflammatory protein expressions tended to be attenuated by the pretreatment with ODQ.

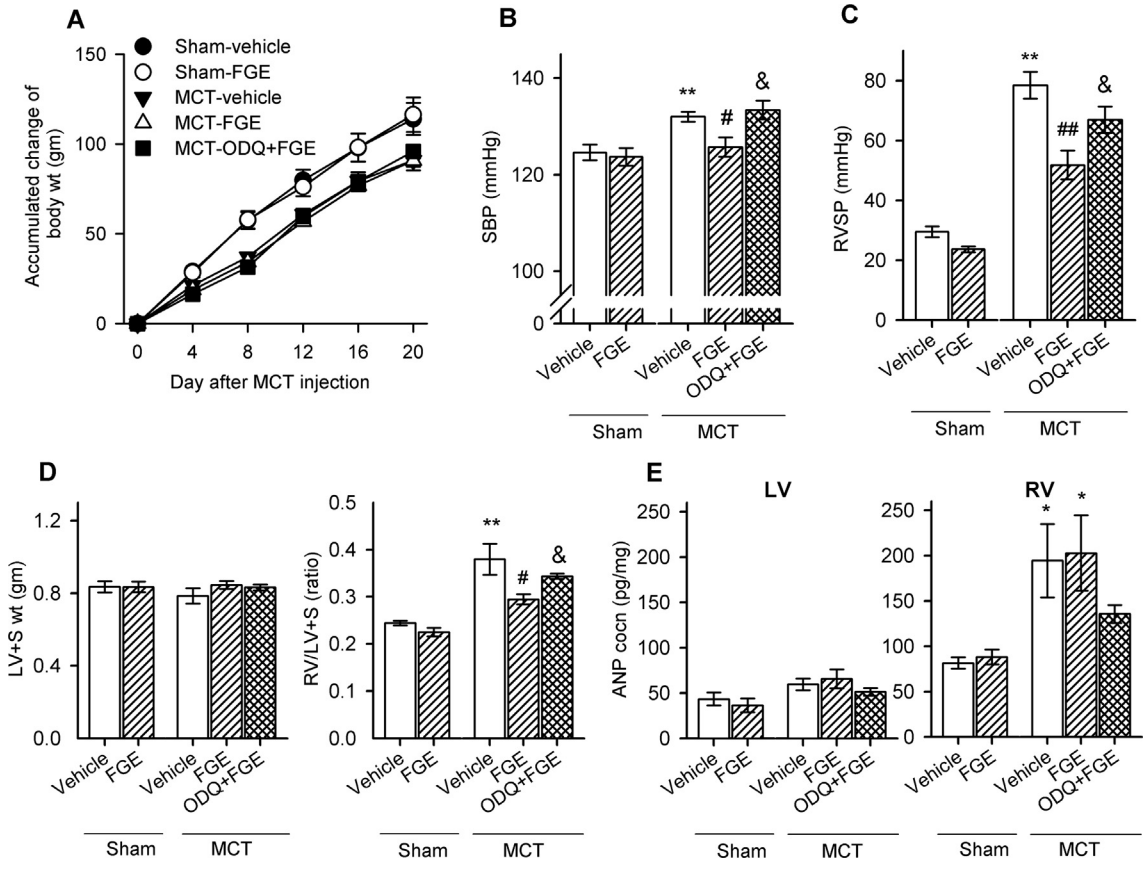

Fig. 1. Changes in body weight (A), systolic blood pressure (SBP; B), right ventricular systolic pressure (RVSP; C), left and right ventricular weight (D), and atrial natriuretic peptide concentration (ANP conc; E) in sham and monocrotaline (MCT) rats fed fermented garlic extract (FGE). Following injection with 50 mg/kg MCT or saline, rats received either water or FGE (containing 0.97 mg nitrite/ml) for 21 days with or without a soluble guanylyl cyclase (sGC) inhibitor. Values are expressed as means ± SEM (n = 8–14 rats per group). Sham-vehicle, sham rats fed a vehicle; Sham-FGE, sham rats fed FGE; MCT-vehicle, MCT rats fed a vehicle; MCT-FGE, MCT rats fed FGE; MCT-ODQ + FGE, MCT rats fed FGE in the presence of the sGC inhibitor 1 H-[1,2,4] oxadiazolo [4,3,-α] quinoxalin-1-one (ODQ); LV, left ventricle; RV, right ventricle. *vs sham-vehicle rats: *p < 0.05, **p < 0.01; #vs MCT-vehicle rats: #p < 0.05, ##p < 0.01; & vs MCT-FGE rats: & p < 0.05.

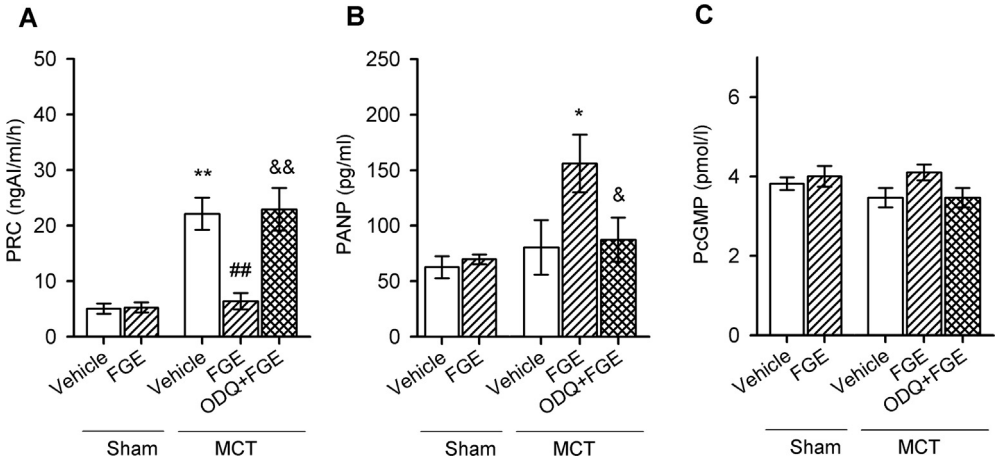

Fig. 2. Changes in the plasma renin concentration (PRC; A), atrial natriuretic peptide level (PANP; B), and cGMP level in the plasma (PcGMP; C) in sham and monocrotaline (MCT) rats fed fermented garlic extract (FGE). Values are expressed as means ± SEM (n = 8–12 rats per group). *vs sham-vehicle rats: *p < 0.05, **p < 0.01; #vs MCT-vehicle rats: #p < 0.01; & vs MCT-FGE rats: & p < 0.01.

4. Discussion

In this study, we demonstrated that FGE has a protective effect against pulmonary arterial hypertension, and also causes increases in PKG and eNOS protein expression in MCT rats. However, these FGE effects were blocked by a sGC inhibitor. The expression of inflammatory proteins in the lung of MCT rats was also attenuated by FGE feeding. These results suggest that FGE exhibits an anti-pulmonary hypertensive effect via the NO-sGC-PKG pathway in MCT rats, and that the major compound with anti-pulmonary hypertensive activity in FGE may be nitrite-related substances.

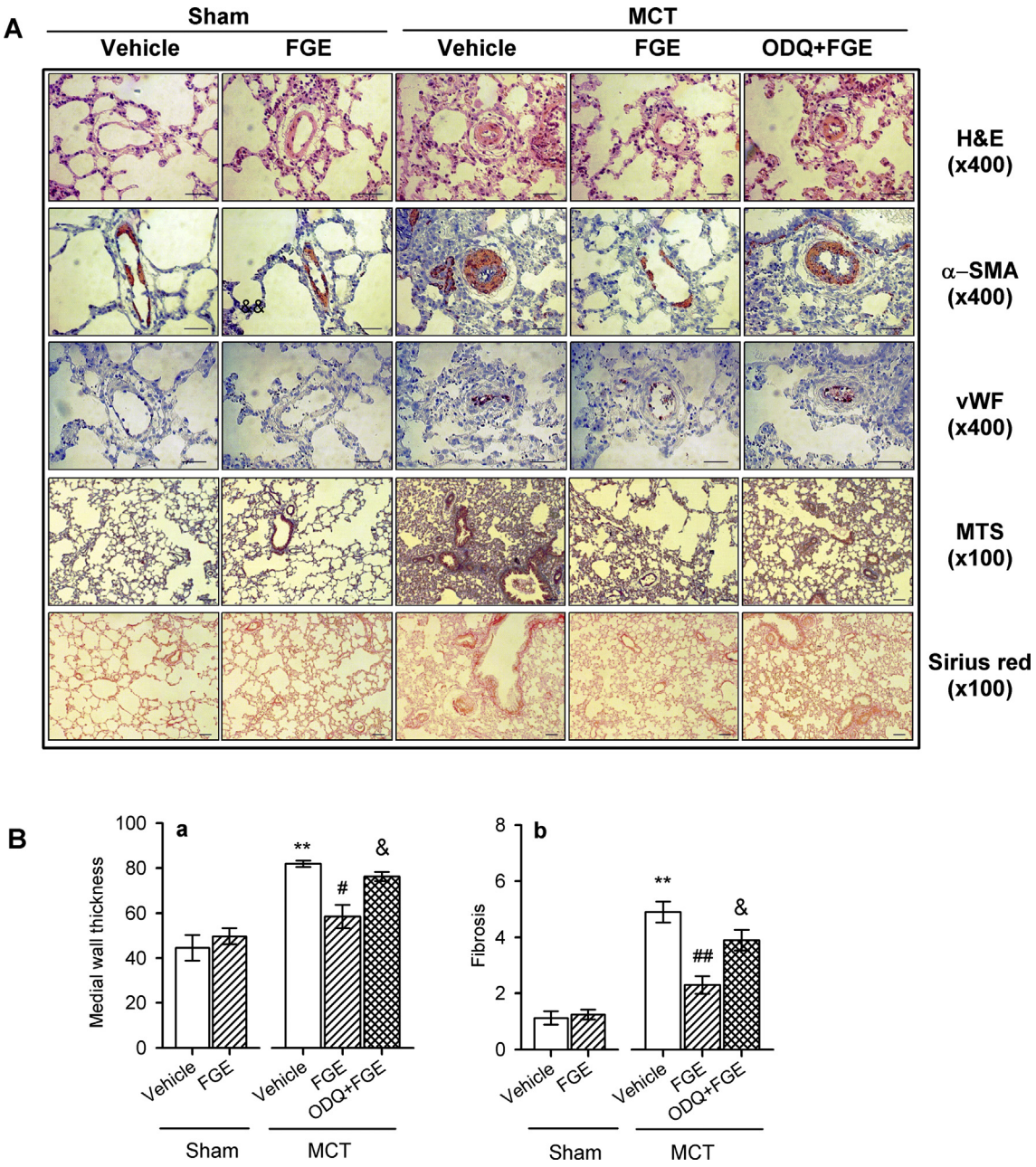

Fig. 3. Changes in the medial wall thickness, endothelial damage, and fibrosis of the pulmonary arteriole in sham and monocrotaline (MCT) rats fed fermented garlic extract (FGE). (A) Representative lung tissues stained with hematoxylin and eosin (bar = 100 μM), anti-α-smooth muscle actin (α-SMA) and anti-von Willebrand Factor (vWF) antibodies (bar = 50 μM), and masson trichrome (MTS) and sirius red (bar = 50 μM). (B) Quantification of the medial hypertrophy in pulmonary arterioles sized 15–100 μm over ~20 arterioles per rat (Ba) and fibrosis using a modified Ashcroft scoring system for pulmonary arteries stained with sirius red and MTS (Bb). Values are expressed as means ± SEM (n = 5 rats per group). *vs sham-vehicle rats: **$p < 0.01$; #vs MCT-vehicle rats: #$p < 0.05$, ##$p < 0.01$; & vs MCT-FGE rats: & $p < 0.05$.

Numerous studies have investigated the effects of garlic extracts on various hypertensive models (Al-Qattan et al., 1999; Fallon et al., 1998; Harauma & Moriguchi, 2006; Kim et al., 2006; Pedraza-Chaverri et al., 1998; Sun & Ku, 2006; Ashraf et al., 2013; Ried et al., 2013). However, inconsistent and controversial results (Duda, Sulibursk, & Pupek-Musialik, 2008) as well as some adverse effects (Nakagawa, Masamoto, Sumiyoshi, Kunihiro, & Fuwa, 1980; Shashikanth, Basappa, & Sreenivasa Murthy, 1984) have been reported. However, FGE that is produced using a new fermentation method (Chun, 2014) exerts a potent anti-hypertensive effect in SHR via the NO-sGC-cGMP pathway (Park et al., 2014). Therefore, in this study, we evaluated the anti-pulmonary hypertensive effect of FGE with the same dose as the previous study (Park et al., 2014) in a well-established animal model of pulmonary arterial hypertension (Huxtable, 1990; Ito, Sato, Ushijima, Nakai, & Ito, 2000; Rosenberg & Rabinovitch, 1988).

MCT treatment caused intimal proliferation of the pulmonary arteriole followed by pulmonary arterial hypertension and RV hypertrophy. However, FGE feeding significantly attenuated MCT-induced increases in the weight, SP, and ANP concentration in the RV. In addition, FGE feeding reduced the MCT-induced increases in the medial wall thickness and fibrosis of the pulmonary arteriole, and the increases in the expression of inflammation-related proteins such as MMP-9 and VCAM-1 in the lung. These results are partly consistent with other reports (Kim-Park & Ku, 2000; Ku et al., 2002; Sun & Ku, 2006).

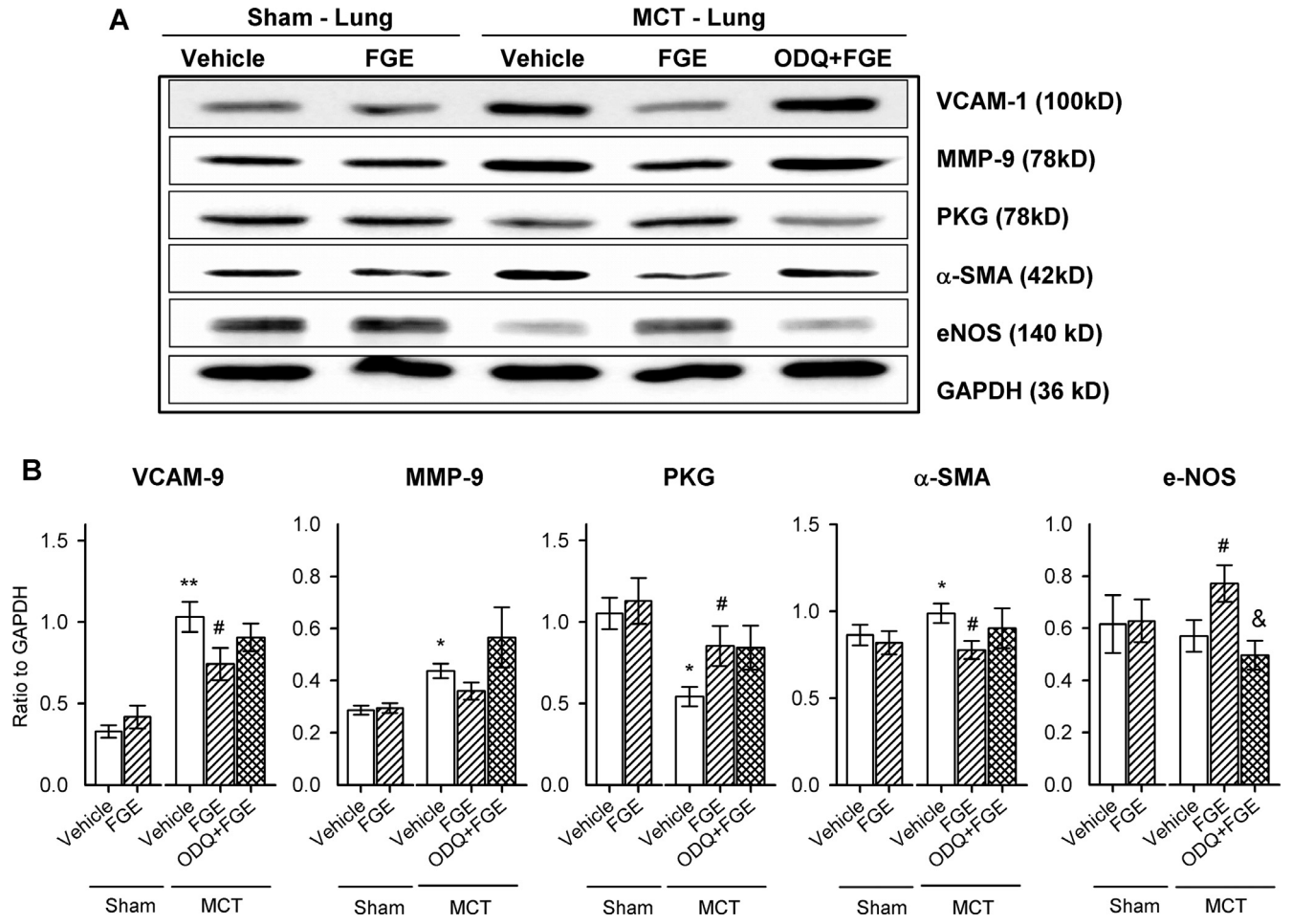

Fig. 4. Representative western blot (A) and densitometric (B) analysis of VCAM-9, MMP-9, PKG, α-SMA, and eNOS protein expressions in the lung tissue of sham and monocrotaline (MCT) rats. Values are means ± SEM ($n = 6$ rats per group). *vs sham-vehicle rats: *$p < 0.05$; #vs MCT-vehicle rats: #$p < 0.05$; & vs MCT-FGE rats: & $p < 0.05$.

Sun and Ku (2006) previously observed that RGE inhibits the development of RVSP and RVH in MCT rats, but that boiled garlic extract or AGE, which do not contain the active allicin metabolite, are ineffective. Thus, they suggested that the protective effect of garlic in MCT rats is mediated via the action of allicin on coronary endothelial function and vasoreactivity (Sun & Ku, 2006). Garlic and the active metabolite allicin are also capable of eliciting a nitric oxide (NO)-dependent relaxation in the right pulmonary artery, and this response is likely to be mediated via garlic activation of NO formation rather than its stabilization (Ku et al., 2002). Thus, different garlic preparation methods may yield different outcomes due to differences in the amounts of biologically active compounds present. AGE contains more water-soluble organic sulfur-containing compounds, such as SAC (Kim et al., 2006) and S-allyl-mercaptocysteine, and RGE contains more alliin (Jung et al., 2014), whereas the FGE used in the present study contains high levels of stable nitrite and less alliin and allicin compared with RGE. Therefore, these results suggest that factors other than allicin may be involved in FGE-induced anti-pulmonary arterial hypertensive effects.

In general, unstable, naturally formed nitrite is converted to nitrate (Habermeyer et al., 2015), which is rapidly absorbed in the upper gastrointestinal tract and reaches the salivary glands. Salivary nitrate can then be reduced to nitrite by commensal bacteria, thereby reentering the gastrointestinal tract (Cosby et al., 2003; Dejam et al., 2007). Saliva-derived nitrite also contributes to NO formation under acidic conditions in the stomach (Modin et al., 2001). Therefore, nitrite and nitrate have been considered as an alternative source for endogenous NO (Habermeyer et al., 2015). Garlic exerts its anti-hypertensive activity by increasing NO (Al-Qattan et al., 2006; Maslin, Brown, Das, & Zhang, 1997) and hydrogen sulfide production (Benavides et al., 2007). Therefore, in the present study, some of the FGE-fed rats were pretreated with an inhibitor of sGC to identify the molecules involved in FGE-induced anti-pulmonary hypertension. This sGC inhibitor treatment attenuated the decreases in RVSP and RV hypertrophy induced by FGE, as well as FGE-induced improvements in the medial wall thickening and fibrosis of the pulmonary arteriole. The treatment with sGC inhibitor tended to increase the expression of inflammation-related proteins. Additionally, MCT-FGE rats exhibited an increased expression of eNOS and PKG proteins in the lung. Together, these findings suggest that nitrite-related substance is one of the functional molecules in FGE, exerting anti-pulmonary arterial hypertensive effects via the NO–sGC–PKG pathway in MCT rats. However, it is not clear whether nitrite in FGE exists in free or bound form. To find out the molecular characteristics of effective molecule in FGE is under investigation with Fourier transform infrared spectroscopy and LC-MS/MS method. Even though it seems that nitrite exists as R-NO form in FGE (unpublished data), related studies are ongoing.

5. Conclusions

The FGE used in the present study contained a high level of stable and natural nitrite that was produced during the fermentation process and was shown to have a protective effect against

pulmonary hypertension, suggesting that FGE could become a functional food. The finding that FGE-induced an anti- pulmonary hypertensive effect by suppressing inflammation-related protein blockade via the sGC inhibitor suggests that FGE improves MCT-induced pulmonary hypertension by decreasing the inflammatory reaction via nitrite-related substance and the sGC-cGMP-PKG pathway.

Conflict of interest

None.

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