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Red Mold Dioscorea Has a Greater Antihypertensive Effect than Traditional Red Mold Rice in Spontaneously Hypertensive Rats

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The aim of this study is to investigate the antihypertensive effects of red mold rice (RMR) and red mold dioscorea (RMD) by low-dose oral administration to spontaneously hypertensive rats (SHRs). A single oral dose of 1-fold RMD (150 mg/kg) significantly ($p < 0.05$) decreased systolic blood pressure (SBP) and diastolic blood pressure (DBP) after 8 h of administration, but RMR showed no significant effect. During the chronic oral administration of 1-fold RMR (150 mg/kg), 0.5-fold RMD, 1-fold RMD, and 5-fold RMD to SHRs for 8 weeks, the increase of blood pressure was slowed significantly. The results indicated that only a 0.5-fold dose of RMD was able to significantly decrease both SBP and DBP. A 1-fold RMD showed a greater antihypertensive effect than 1-fold RMR, and both RMR and RMD can improve the vascular elastin structure remodeling. In comparison to RMR, RMD contained a higher amount of γ-aminobutyric acid (GABA) and anti-inflammatory yellow pigments (monascin and ankaflavin). Moreover, RMD also exhibited higher angiotensin-I-converting enzyme (ACE) inhibitory activity than RMR. These results suggest that RMD has greater antihypertensive bioavailability.

KEYWORDS: *Monascus*; Red mold rice; red mold dioscorea; systolic blood pressure; diastolic blood pressure; antihypertensive effect; γ-aminobutyric acid; angiotensin-I-converting enzyme; spontaneously hypertensive rats

INTRODUCTION

Hypertension is one critical factor of metabolic syndromes, such as dyslipidemia. Therapeutic control of blood pressure carries out a fundamental role in cardiovascular prevention (*1*). The *Monascus* species have been used as a traditional food fungus in Eastern Asia for several centuries. Because the worthful secondary metabolite monacolin K was proven as the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in the cholesterol biosynthesis pathway, *Monascus*-fermented rice known as red mold rice (RMR) was gradually developed as a popular functional food for hypolipidemia (*2*). The γ-aminobutyric acid (GABA) is known to be one of the major inhibitory neurotransmitters in the sympathetic nervous system and to play an important role in cardiovascular function. GABA has been reported to decrease blood pressure in experimental animals and in humans after oral as well as systemic administration. Our previous study found that red mold dioscorea (RMD) had greater hypolipidemic and antiatherosclerotic effects than tranditional RMR and unfermented dioscorea (*3*). In addition, RMD had more monascin, a *Monascus* yellow pigment with anti-inflammatory potential, which is significantly formed and substituted for the red pigment (monascorubramine and rubropunctamine) as the major pigment of RMD (*4*). Inflammation may activate the rennin−angiotensin system (RAS) and further contribute to vascular remodeling and hypertension (*5*). The anti-inflammatory ability is also proven to prevent the occurrence of hypertension.

RMD comprises a dioscorea root substance besides *Monascus* metabolite. The dioscorea root is regarded as a functional food or a worthful herb because of the inclusion of many functional ingredients for the prevention of various diseases (*6*). Dioscorin, polysaccharides, flavones, vitamin C, polyphenol, and sporamin of dioscorea are proven to exhibit great antioxidative ability (*7*), which should be of great benefit to blockade oxidation of nitric oxide (*8*). In addition, dioscin and diosgenin of dioscorea are proven to have anti-inflammatory and hypolipidemic abilities (*7*). Therefore, the function of dioscorea should strengthen the hypolipidemic and antiatherosclerotic effects of RMD.

This research focused on the effects of oral administration of a small amount of RMD and RMD fermented by *Monascus purpureus* NTU 568 for hypertensive rats on systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and aorta thin section. This study examined the liver somatic index, kidney index, muscle index, and plasma electrocytes to investigate the safety of *Monascus* powder.

MATERIALS AND METHODS

Preparation of RMD and RMR. The *M. purpureus* NTU 568 fermented RMR product has been proven to perform a potent hypolipidemic effect in our previous study (*2*). The culture strain was maintained

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on potato dextrose agar (PDA, Difco Co., Detroit, MI) slanted at 10 °C and transferred monthly. The dioscorea root (*Dioscorea batatas* Dence) purchased from a local supermarket in Taiwan was used to produce RMD using the method of solid-state culture (*4*). The preparation of RMR was carried out under the substrate of long-grain rice (*Ipomoea batatas*) purchased from a local supermarket in Taiwan using the method of solid-state culture (*9*). Briefly, a 500 g substrate was soaked in deionized water for 8 h. After that, excess water was removed with a sieve. The substrate was autoclaved for 20 min at 121 °C in a "koji dish" (the koji dish was made of wood with the dimensions of 30 × 20 × 5 cm). After having been cooled, the substrate was inoculated with a 5% (v/w) spore suspension. The inoculated substrate was cultivated at 30 °C for 10 days. During the culturing stage, 100 mL of water was added daily to the substrate from the second day to the fifth day. At the end of cultivation, the crushed and dried product with the mold was used for the experiments (*9*).

Determination of the GABA Concentration. RMR and RMD (0.2 g) was extracted respectively with 4 mL of deionized water at 60 °C for 30 min. The suspension was then filtered and analyzed by high-performance liquid chromatography (HPLC). The analysis method was described in a previous study (*10*) in triplicate. The chromatographic eluent pump (PU2089 plus, Jssco Co., Tokyo, Japan), injector (7725i, Rheodyne Co., Robert Park, CA), column (C_{18}, 25 cm × 4.6 mm inner diameter, 5 μm, Discovery, Supelco, Inc., Bellefonte, PA), and fluorescence detector (FL-1, Rainin Co., Tokyo, Japan) were used in GABA (Sigma Chemical Co., St. Louis, MO) analysis.

Determination of Monsacin and Ankaflavin Concentration. RMR and RMD (1 g) were extracted respectively with 10 mL of ethanol at 60 °C for 30 min. The extracts (10%, w/v) were further filtered with 0.45 μm pore size filter and analyzed by HPLC. HPLC was performed according to the method described previously (*11*) in triplicate. Monascin and ankaflavin were detected using a UV detector UV2075 plus (Jasco Co.) set at 231 nm.

Determination of Angiotensin-I-Converting Enzyme (ACE) Inhibitory Activity. Angiotensin converting enzyme inhibitor (ACEI) activity was measured by the assay method of Cushman and Cheung (*12*) with some modification in triplicate. The dried powder of RMR and RMD was extracted with water at 37 °C for 24 h and then filtered. The filtrate was freeze-dried and prepared for water extract. Each water extract was incubated with 45 μL of 0.1 M borate buffer (pH 8.3) containing 0.3 M NaCl and 0.033 unit/mL ACE (Sigma Chemical Co.) at 37 °C for 10 min. A total of 45 μL of 3 mM hippuic acid−histidine−leucine (HHL, Sigma Chemical Co.) was added, and the reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped with 150 μL of 1 N HCl. The reaction solution was further filtered with 0.45 μm pore size filter and analyzed by HPLC. A Discovery column, C_{18}, 25 cm × 4.6 mm inner diameter, 5 μm, was used as the analytical column. The mobile phase consisting of 50% methanol and 50% water was eluented as a flow rate of 0.6 mL/min. The HHL and hippuric acid were detected using a UV detector UV2075 plus (Jasco Co.) set at 228 nm. The calculation of the IC_{50} was performed according to the method described previously (*13*).

Animals and Diets. A total of 56 male spontaneous hypertensive rats (SHRs) weighing 290−320 g were housed in individual plastic cages and subjected to a 12 h light/dark cycle with a maintained relative humidity of 60% and a temperature at 25 °C. The animals were given free access to regular rodent chow and water for 5 weeks to adapt to the new environment. SHRs were weighed and randomly assigned to 7 groups of 8 animals each before the commencement of the animal experiment.

Dose and Grouping. The dose of RMD powder was calculated in accordance with Boyd's formula of body surface area as recommended by the Food and Drug Administration (FDA) (*14*). The daily dietary dose of commercial *Monascus* product is usually recommended and used at 1.0−2.0 g for an adult (*15*). Using 2 g of RMR as the reference dose of an adult to calculate the SHRs dose (150 mg kg^{-1} day^{-1} including 0.0245 mg of GABA) has been proven to exhibit a hypolipidemic effect in our previous study (*2*).

GABA was used as a positive hypotensive substance that has a minimal effective dose of 0.2445 mg kg^{-1} day^{-1} in our pre-experiment (data not shown). Therefore, RMD, RMR, and unfermented dioscorea are used as the reference dose at 2 g for an adult with a weight of 65 kg and a height of 170 cm. These dosages were used as a frame of reference for the conversion of the dose into a SHR model. Therefore, feeding a SHR with a 0.5-fold dose of RMD per day corresponds to supplementing the daily diet with 1 g of RMD for an adult. After the prebreeding stage, all test samples were suspended respectively in 1 mL of water and orally administrated to the SHRs using a stomach tube everyday. The food intake was recorded daily, and animals were weighed weekly. The animals were allowed free access to a standard laboratory chow diet (Ralston Purina, St. Louis, MO) and water.

All groups were fed with a normal chow diet, and RMD-0.5X, RMD-1X, and RMD-5X groups were orally given a 0.5-fold dosage of RMD (75 mg/kg BW per day including 0.0122 mg of GABA), a 1-fold dose of RMD (150 mg/kg BW per day including 0.0245 g of GABA), and a 5-fold dose of RMD (750 mg/kg BW per day including 0.1225 mg of GABA), respectively. In addition, the GABA group, a positive control group, was given orally a 10-fold RMD-1X dose of GABA (0.245 mg/kg BW per day).

To investigate and clarify whether the effect of RMD on hypotension is better than that of RMR and unfermented dioscorea, the same doses of RMD, RMR, and unfermented dioscorea were fed to the SHRs, respectively. The 1X-RMR group was fed with a 1-fold dose of RMR (150 mg/kg BW per day including 0.0197 mg of GABA) and the 1X-D group was fed with a 1-fold dose of dioscorea (150 mg/kg BW per day). After the prebreeding stage for 5 weeks, all test samples were suspended respectively in 1 mL of water and orally administrated to the SHRs using a stomach tube for 8 weeks. The food intake was recorded daily, and animals were weighed weekly.

At 24 h before sacrifice, all food was removed. Animals were anesthetized and sacrificed by carbon dioxide inhalation, and whole blood, plasma, and serum samples were collected, prepared, and then stored at −80 °C. The aorta was excised, rinsed frequently with a 0.8% sodium chloride solution to eliminate any blood, placed in buffered formalin (10%), and fixed. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taiwan University.

Blood Pressure Measurement. SBP, DBP, and HR were measured by tail cuff plethysmography with a photoelectric system (Visitech BP-2000, Napa Place, NC), which was controlled with a personal computer. A mean value from at least five consecutive readings was used for calculations. At 0, 4, 8, and 24 h after a single oral administration of the sample solutions, individual rats were gently placed in a constant temperature holder at 37 ± 1 °C for a few minutes, and then SBP, DBP, and HR were measured by the tail-cuff method using a blood pressure analyzer (Visitech BP-2000) connected to a personal computer. To assess the hypotensive effects of different samples alone, SBP, DBP, and HR were measured 0 and 4 h after a single administration. During chronic administration, SBP, DBP, and HR were measured before giving the sample once per week.

Plasma Liver and Kidney Index Analysis. Plasma glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), and creatinine (Cr) levels were measured in triplicate using an automatic biochemical analyzer (Beckman-700, Fullerton, CA).

Plasma Electrolytes and Creatine Phosphokinase (CPK) Analysis. Plasma electrolytes and CPK levels were measured in triplicate using an automatic biochemical analyzer (Beckman-700).

Statistical Analysis. Data are expressed as the mean ± standard deviation (SD). The statistical significance in the behavioral and biochemical effects was determined by one-way analysis of variance (ANOVA) using the general linear model procedure of SPSS software (SPSS Institute, Inc., Chicago, IL), followed by ANOVA with the Duncan's test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Change of Body Weight and Daily Intake by SHRs. The average body weights of SHRs were shown in **Table 1**. There was not a significant difference in body weight. The daily intake of the SHRs increased normally and had no difference among the various groups during the period of the practical experiment (data not shown). The externals and health of all experimental animals had a normal expression.

Table 1. Body Weight of Experimental SHR[a]

	body weight (g)				
group	0th week	2nd week	4th week	6th week	8th week
control	317.8 ± 14.1	324.3 ± 16.2	333.5 ± 18.7	343.5 ± 17.6	350.8 ± 15.7
GABA-1X	317.6 ± 19.5	323.7 ± 19.9	328.0 ± 17.6	335.0 ± 17.9	344.1 ± 24.0
D-1X	309.7 ± 18.3	319.7 ± 16.3	331.6 ± 14.4	334.0 ± 19.6	337.2 ± 17.3
RMR-1X	311.0 ± 17.9	323.9 ± 20.4	327.8 ± 19.1	353.3 ± 15.6	354.6 ± 15.8
RMD-0.5X	325.0 ± 25.9	334.6 ± 27.5	341.2 ± 26.8	350.4 ± 28.1	355.8 ± 28.6
RMD-1X	310.5 ± 13.2	321.8 ± 16.0	331.1 ± 19.5	340.6 ± 17.7	348.1 ± 13.1
RMD-5X	317.0 ± 11.8	329.3 ± 14.1	334.5 ± 16.9	346.8 ± 12.1	349.5 ± 14.5

[a] One groups of the SHRs were fed a normal diet without the administration of test materials (control group). The other SHRs were administrated with a l-fold dose of unfermented dioscorea (150 mg kg^{-1} day^{-1}) (D-1X group), GABA (0.245 mg kg^{-1} day^{-1}) (GABA-1X group), a l-fold dose of RMR (150 mg kg^{-1} day^{-1} including 0.0197 mg of GABA) (RMR-1X group), a 0.5-fold dose of RMD (75 mg kg^{-1} day^{-1} including 0.0122 mg of GABA) (RMD-0.5X group), a 1-fold dose of RMD (150 mg kg^{-1} day^{-1} including 0.0245 mg of GABA) (RMD-1X group), and a 5-fold dose of RMD (750 mg kg^{-1} day^{-1} including 0.1225 mg of GABA) (RMD-5X group). (∗) $p < 0.05$ versus the control group ($n = 8$).

Effect of Single Oral Administration of *Monascus*-Fermented Products on the Blood Pressure in SHRs. Before dosing, the average resting SBP in SHRs was shown in **Figure 1** at 0 h. There was a significant difference in SBP among SHRs between the control and RMD-treated groups ($p < 0.05$) by 4 and 8 h after dosing. The effects of a single oral dose of 1-fold RMR (150 mg/kg), 1-fold RMD (150 mg/kg), 1-fold unfermented dioscorea (150 mg/kg), and GABA (0.24 mg/kg) on SBP are shown in **Figure 1A**. In SBP (**Figure 1A**), one-way ANOVA showed independent effects of time ($p < 0.05$): SBP (mmHg) 4 h after administration were 182 ± 4 for the control group (water), 181 ± 4 for the D-1X, 178 ± 5 for the GABA, 174 ± 6 for the RMR-1X group, and 169 ± 3 ($p < 0.05$) for the RMD-1X group; SBP (mmHg) 8 h after administration were 180 ± 4, 177 ± 5, 175 ± 9, 172 ± 3, and 155 ± 9 ($p < 0.05$), respectively. SBP returned to the baseline value by 24 h after dosing. In DBP (**Figure 1B**), one-way ANOVA also showed independent effects of time ($p < 0.05$): DBP (mmHg) 4 h after administration were 154 ± 11 for the control group (water), 151 ± 5 for the D-1X, 147 ± 8 for the GABA, 145 ± 10 for the RMR-1X group, and 140 ± 6 ($p < 0.05$) for the RMD-1X group; DBP (mmHg) 8 h after administration were 152 ± 6, 149 ± 8, 144 ± 9, 143 ± 6, and 135 ± 5 ($p < 0.05$), respectively. DBP returned to the baseline value by 24 h after dosing. Only RMD-1X has a hypotensive effect by 8 h after dosing.

GABA Content, Monascin, Ankaflavin, and ACEI Activity of RMR and RMD. Table 2 shows that RMD contained more GABA than RMR. GABA has been reported to have an antihypertensive effect. In comparison to RMR, RMD also contained a higher level of both anti-inflammatory yellow pigments monascin and ankaflavin. It implies that RMD might have higher contents of functional compounds to improve the inflammation of the vascular wall. In addition, the aqua extracts of RMR and RMD both showed ACE inhibitory activity, and RMD exhibited highter ACEI activity than RMR.

Chronic Administration of *Monascus*-Fermented Products. The time course of SBP during the experiment is shown in **Figure 2**. The SBP increased gradually with age and reached > 200 mmHg at 8 weeks of the test administration period in the control group. A significantly slower increase in SBP compared to the control group was observed 4 or 6 weeks after the start (at 13 weeks of age) of feeding with *Monascus*-fermented product, respectively ($p < 0.05$), and this difference was maintained throughout the period of feeding. After 8 weeks of feeding the experimental diets, SBP values (**Figure 2A**) in control (C), unfermented dioscorea (D-1X), GABA, and 1-fold RMR (RMR-1X) groups were 208 ± 6, 204 ± 8, 189 ± 8 ($p < 0.05$), and 186 ± 6 ($p < 0.05$). SBP values (mmHg) in 0.5-fold RMD (RMD-0.5X), 1-fold RMD, and 5-fold RMD groups were 185 ± 10 ($p < 0.05$), 181 ± 10 ($p < 0.05$), and 178 ± 5 ($p < 0.05$). The DBP values (**Figure 2B**) in control (C), unfermented dioscorea (D-1X), GABA, and 1-fold RMR (RMR-1X) groups were 177 ± 7, 176 ± 7, 164 ± 7 ($p < 0.05$), and 164 ± 8 ($p < 0.05$). DBP values (mmHg) in 0.5-fold RMD (RMD-0.5X), 1-fold RMD, and 5-fold RMD groups were 158 ± 8 ($p < 0.05$), 155 ± 10 ($p < 0.05$), and 147 ± 4 ($p < 0.05$), respectively.

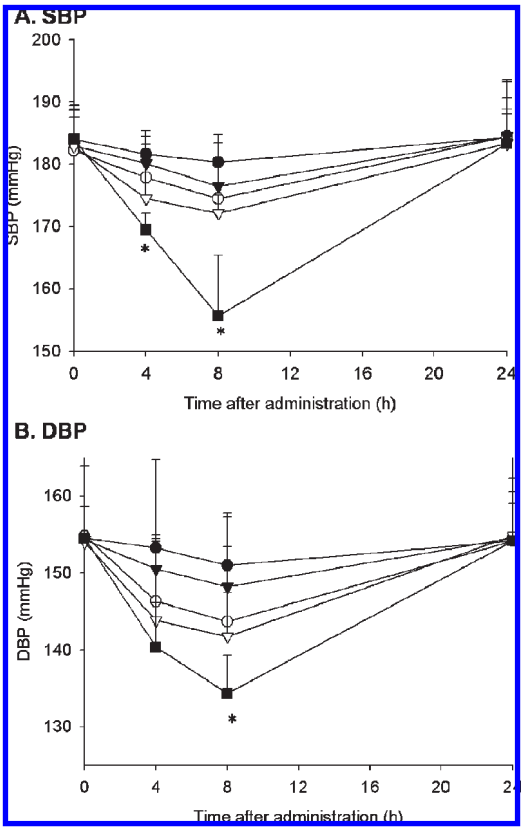

Figure 1. Effect of single oral administration of *Monascus*-fermented products on (A) SBP and (B) DBP in SHR. One groups of the SHRs were fed a normal diet without the administration of test materials (the control group; ●). The other SHRs were administrated with a l-fold dose of unfermented dioscorea (150 mg kg^{-1} day^{-1}) (the D-1X group; ▼), GABA (0.245 mg kg^{-1} day^{-1}) (the GABA-1X group; ○), a l-fold dose of RMR (150 mg kg^{-1} day^{-1} including 0.0197 mg of GABA) (the RMR-1X group; ▽), and a 1-fold dose of RMD (150 mg kg^{-1} day^{-1} including 0.0245 mg of GABA) (the RMD-1X group; ■). (∗) $p < 0.05$ versus the control group ($n = 6$).

Table 2. GABA, Monascin, Ankaflavin, and ACEI Activity in RMD and RMR

	Monascus secondary metabolite			IC_{50} of ACE inhibition
sample	GABA (mg/kg)	monascin (mg/kg)	ankaflavin (mg/kg)	water extract (mg/mL)
RMR	131.22 ± 7.68	16.87 ± 0.49	5.17 ± 0.19	20.20
RMD	163.08 ± 4.67	34.45 ± 0.84	7.08 ± 0.67	12.24

Effect of *Monascus*-Fermented Products on the Heart Rate. The result of the heart rate by single oral administration is shown in **Figure 3A**, and chronic oral administration is shown in **Figure 3B**. The heart rate by single oral administration decreased from 0 to 8 h and then increased from 8 to 24 h. The heart rate showed no difference among the various groups. The heart rate by chronic

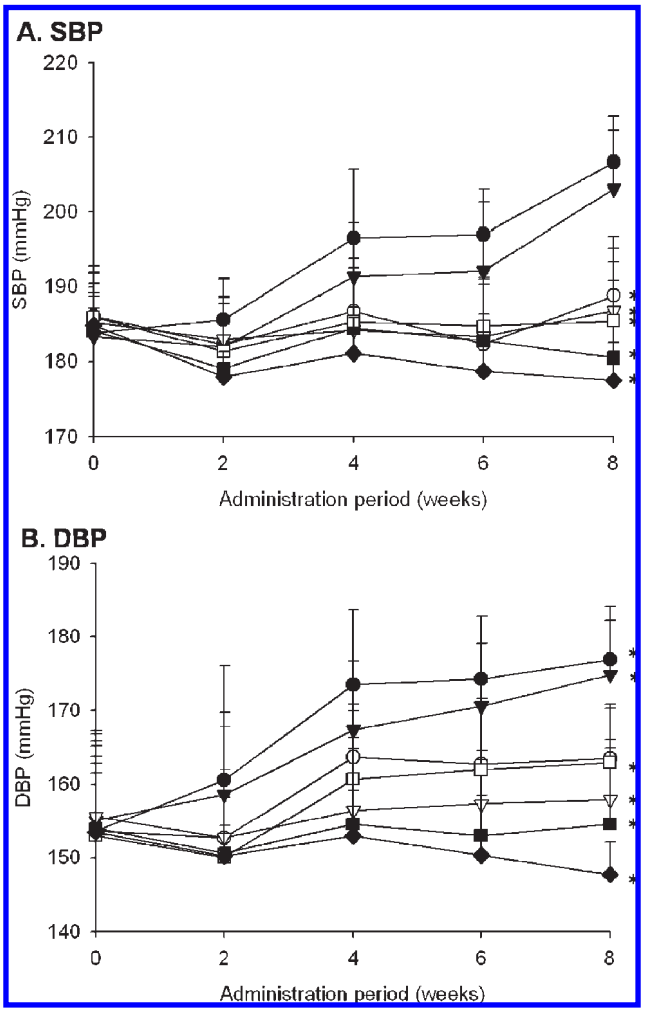

Figure 2. Effect of chronic administration of *Monascus*-fermented products on (A) SBP and (B) DBP in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (the C group; ●). Other groups of SHRs were administrated with a l-fold dose of unfermented dioscorea (150 mg kg^{-1} day^{-1}) (the D-1X group; ▼), GABA (0.245 mg kg^{-1} day^{-1}) (the GABA-1X group; ○), a l-fold dose of RMR (150 mg kg^{-1} day^{-1} including 0.0197 mg of GABA) (the RMR-1X group; ▽), a 0.5-fold dose of RMD (75 mg kg^{-1} day^{-1} including 0.0122 mg of GABA) (the RMD-0.5X group; □), a 1-fold dose of RMD (150 mg kg^{-1} day^{-1} including 0.0245 mg of GABA) (the RMD-1X group; ■), and a 5-fold dose of RMD (750 mg kg^{-1} day^{-1} including 0.1225 mg of GABA) (the RMD-5X group; ◆), respectively. (∗) $p < 0.05$ versus the control group ($n = 8$).

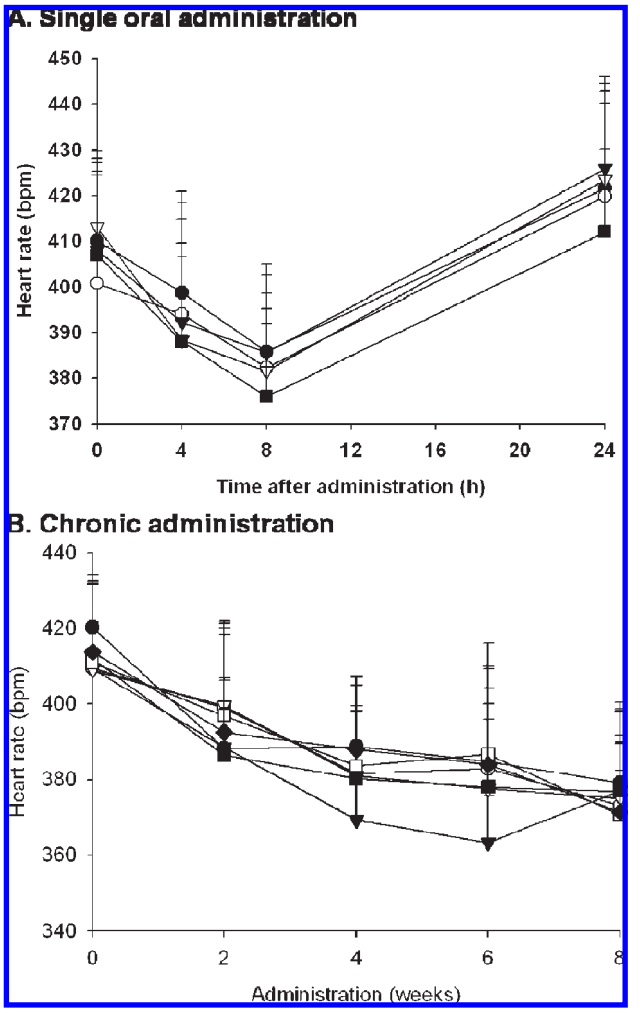

Figure 3. Effect of (A) single oral and (B) chronic administration of *Monascus*-fermented products on the heart rate in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (the control group; ●). The other SHRs were administrated with a l-fold dose of unfermented dioscorea (150 mg kg^{-1} day^{-1}) (the D-1X group; ▼), GABA (0.245 mg kg^{-1} day^{-1}) (the GABA-1X group; ○), a l-fold dose of RMR (150 mg kg^{-1} day^{-1} including 0.0197 mg of GABA) (the RMR-1X group; ▽), and a 1-fold dose of RMD (150 mg kg^{-1} day^{-1} including 0.0245 mg of GABA) (the RMD-1X group; ■). (∗) $p < 0.05$ versus the control group ($n = 6$).

oral administration decreased gradually with age from 410 to 375 bpm and also showed no difference among the various groups. These results showed that unfermented dioscorea, GABA, and *Monascus*-fermented products did not affect the heart rate in SHRs.

Effect of *Monascus*-Fermented Products on the Liver Index, Kidney Index, Muscle Index, and Electrolyte Balance. The results of the liver index, kidney index, muscle index, and plasma electrolyte balance are shown in **Tables 3** and **4**. The results indicate that the liver index and kidney index of the SHRs increased normally. The results also indicate that the muscle index and plasma electrolyte of SHRs had no difference among the various groups during the period of the practical experiment.

Effect of *Monascus*-Fermented Products on Vascular Histology. Vascular remodeling in aorta caused by hypertension is the vascular lesions in vascular disease. The SHRs were sacrificed after 8 weeks. The aorta tissue of SHRs was removed and collected, and then a thin section was performed. The photo obtained by microscopic examination during the aorta thin section (**Figure 4**) showed that the elastin fibers in the aorta of *Monascus*-fermented product-treated SHRs were significantly straighter than that of controls.

Table 3. Effect of the *Monascus* Product on Experimental SHR Performance Serum GPT and GOT Levels[a]

	liver		kidney	
group	GOT (units/L)	GPT (units/L)	BUN (mg/dL)	Cr (mg/dL)
control	135.5 ± 11.4	100.1 ± 5.2	22.5 ± 3.1	0.4 ± 0.1
GABA-1X	145.6 ± 20.0	104.3 ± 8.0	25.4 ± 2.2	0.3 ± 0.0
D-1X	124.5 ± 11.9	84.6 ± 7.9	21.7 ± 2.2	0.3 ± 0.0
RMR-1X	127.8 ± 12.3	89.3 ± 6.2	21.6 ± 2.1	0.3 ± 0.1
RMD-0.5X	122.3 ± 10.9	92.3 ± 8.0	21.9 ± 2.3	0.3 ± 0.1
RMD-1X	138.1 ± 19.9	93.0 ± 5.4	22.7 ± 2.9	0.4 ± 0.0
RMD-5X	136.7 ± 17.4	91.2 ± 14.0	22.9 ± 2.2	0.3 ± 0.1

[a] One group of the SHRs was fed a normal diet without the administration of test materials (control group). The other SHRs were administrated with various test material described in **Table 1**. (∗) $p < 0.05$ versus the control group ($n = 8$).

Table 4. Effect of the *Monascus* Product on Experimental SHR Performance Serum CPK and Electrolyte Levels[a]

	muscle	electrolyte				
group	CPK[b] (units/L)	Na (mequiv/L)	K (mequiv/L)	Cl (mequiv/L)	Ca (mequiv/L)	Mg (mequiv/L)
control	107.0 ± 16.3	149.2 ± 2.8	7.9 ± 0.5	95.4 ± 2.0	12.3 ± 0.7	3.5 ± 0.4
GABA-1X	124.0 ± 33.6	147.7 ± 0.9	7.8 ± 1.5	95.0 ± 1.0	12.1 ± 0.4	3.2 ± 0.4
D-1X	112.2 ± 21.2	149.0 ± 0.9	7.4 ± 0.9	95.8 ± 0.9	12.1 ± 0.6	3.5 ± 0.4
RMR-1X	113.0 ± 29.0	148.1 ± 1.1	7.7 ± 0.6	94.6 ± 1.2	12.4 ± 0.5	3.4 ± 0.2
RMD-0.5X	114.3 ± 30.0	148.3 ± 0.8	7.7 ± 1.1	95.7 ± 1.0	12.3 ± 0.4	3.4 ± 0.3
RMD-1X	116.3 ± 31.3	148.4 ± 1.0	7.4 ± 0.7	94.5 ± 1.8	12.0 ± 0.4	3.3 ± 0.3
RMD-5X	94.6 ± 15.8	148.5 ± 1.0	7.4 ± 0.7	95.6 ± 1.1	12.1 ± 0.6	3.3 ± 0.3

[a] One group of the SHRs was fed a normal diet without the administration of test materials (control group). The other SHRs were administrated with various test material described in **Table 1**. (*) $p < 0.05$ versus the control group ($n = 8$). [b] CPK = creatine phosphokinase.

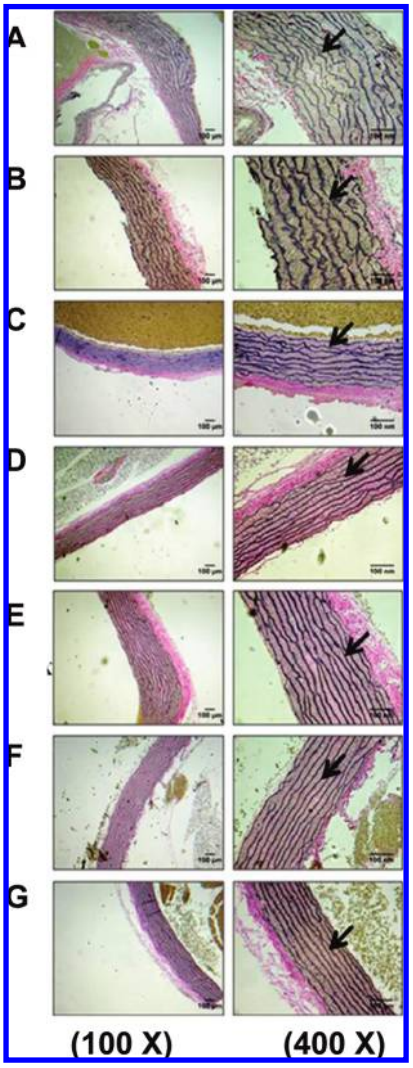

Figure 4. Microscopic examination (100× and 400×) of aorta thin section on experimental SHRs: (A) control group, (B) D-1X group, (C) GABA-1X group, (D) RMR-1X group, (E) RMD-0.5X group, (F) RMD-1X group, and (G) RMD-5X group.

DISCUSSION

Hypertension is a risk factor of coronary heart disease, stroke, and cardiac failure. Clinical trials have shown that, in hypertensive subjects, lowering the blood pressure reduces the risk of cardiovascular diseases (*16*). Dioscorea is considered to be the best substrate for *Monascus* species to produce monacolin K and monascin (*4*). RMD has greater hypolipidemic and antiatherosclerotic effects than traditional RMR and unfermented dioscorea (*3*). Therefore, we continue to study the other metabolites and antihypertensive activity in RMD. After 8 weeks of chronic oral administration, both a 1-fold dose of RMR and RMD performed a statistically significant antihypertensive effect on SBP and DBP and a 1-fold RMD had better antihypertensive effect than 1-fold RMR. In chronic oral administration, RMR and RMD could prevent genetically increased blood pressure. Although RMD has been proven to exhibit a greater hypotensive effect in this study, the dose−response was not obvious until 8 weeks of oral admistration. According to the final chronic administration result, the hypotensive effect is more potent, while the treated dose was increased from half- to 5-fold. But it just has a little dose−response relationship before the final weeks. The *in vivo* bioavailability of the natural complex, such as fermented food and herb, might have a milder effect, and these materials were possible to have less side effects.

The arterial and cardiopulmonary baroreceptors are the two most important neural reflex arches associated with the regulation of blood pressure (*17*). Aortic baroreceptors lower blood pressure through parasympathetic activation and sympathetic inhibition. GABA is the chief inhibitory neurotransmitter in the mammalian central nervous system. When the afferent signals from baroreceptors enter the vasomotor center in the medulla of the brain, the efferent signals are transferred via sympathetic nerves to the heart, vasculature, and kidneys. In this present study, *Monascus*-fermented products and GABA had a similar magnitude of antihypertensive effect by single-dose and chronic administration to SHRs. It has long been known that intravenously or orally administered GABA significantly lowers the blood pressure in animals and humans (*18*). Moreover, there is a positive relationship between resting blood pressure and the amplitude of the GABA-induced reduction in SBP. After a single GABA (about 7 mg/kg) was given to rats (body weight of 350 g), the plasma GABA concentration peaked (1.4 μg/mL) after 1 h, then decreased gradually to 0.8 μg/mL after 6 h, and disappeared after 24 h (*19*). It has been reported that a single oral administration of GABA (0.5 mg/kg) significantly lowered SBP in SHRs. GABA inhibits not only systemic blood pressure but also a perivascular nerve-stimulation-induced increase in perfusion pressure and noradrenaline release from sympathetic nerves in the mensenteric arterial bed via presynaptic $GABA_B$ receptors (G-protein-coupled receptors) (*20*).

The antihypertensive function of *Monascus*-fermented RMR has been reported previously (*21*). Kohama et al. isolated and identified hypotensive pure compound in RMR. They found that GABA and acetylcholine chloride were the major compounds that can lower blood pressure (*22*). Acetylcholine acts on muscarinic receptor of endotherial cells to stimulate the release of a substance that causes relaxation of vascular smooth muscle, resulting in systemic hypotension (*22*). However, acetylcholine only acts transiently, and it is easily hydrolyzed by cholinesterase in the body (*22*). Tsuji et al. indicated beni-food

(*Monascus*-fermented food), such as beni-bread, beni-miso, beni-soy sauce, and beni-somen, has a hypotensive effect (*23*). Moreover, a water-soluble extract of *Monascus*-fermented product displayed blood pressure lowering ability (*23*). They also indicated SHRs fed with a diet containing 3−10% beni-koji (*Monascus pilosus*) showed a better hypotensive effect than that with koji (*Aspergillus oryzae*) (*23*). The effect of *Monascus* mycelial weight on hypotensive activity of beni-koji was examined. The fermented products containing a high concentration of glucosamine also revealed good hypotensive ability, while the product after 90 °C heating for 20 min showed no effects on hypotension (*24*). The *Monascus* pigment was also reported to have hypotensive ability (*25*). In this study, we found RMD have a better hypotensive effect than RMR. In addition, it has a greater effect on both long- and short-term hypotension.

The rennin−angiotensin system is an important regulator of blood pressure for fluid and electrocyte balance (*21*). ACE is a key enzyme that produces the very potent vasoconstrictor octapeptide angiotensin II (Ang II) from the weak vasoconstrictor Ang I. In our study, we found that RMR and RMD exhibit ACE inhibitory activity. In single oral administration, a 1-fold dose of RMR performed a mild hypotensive effect but no statistically significant difference. In contrast, the same dose of RMD had a statistically significant hypotensive effect on SBP and DBP compared to the C group after 4 h of administration. It could lower blood pressure until 8 h and reached maximum hypotensive activity. After 24 h of administration, blood pressure returned to the initial blood pressure. In normal conditions, the central nervous system acts primarily as a short-term regulator of blood pressure. The central nervous system modulates blood pressure by controlling cardiac output and peripheral resistance. In the plasma, the rennin−angiotensin system is determined as a circulating hormone system, which regulates acute change in the cardiovascular system. This single administration hypotensive effect might activate the systemic neuron by inhibitory neurotransmitter GABA or by inhibiting ACE that converts angiotensin I to hypertensive peptide angiotensin II.

The kidneys play a central role in the long-term regulation of blood pressure (*26*), and in tissues, the rennin−angiotensin system also regulates long-term changes. This chronic administration hypotensive effect might also inhibit ACE activity. In our study, we used the animal models that were not fed with high-salt diets and water because we would like to figure out the relationship between the *Monascus*-fermented product and genetic hypertension. Importantly, we found that the *Monascus*-fermented product could prevent not merely age-induced genetic hypertension but high-salt diets and age-induced genetic hypertension.

A more potent antihypertensive effect performance by RMD should be attributed to the contribution of GABA and ACEI activity. Other *Monascus* metabolites might contribute to the antihypertensive effect. In most animal models of hypertension, the endothelium-dependent relaxation to acetylcholine (and other agonists) is impaired. The endothelial dysfunction is associated with decreased production of NO and/or increased production of endothelial-contracting factors, which destruct the production of oxidative stress, such as reactive oxygen species, free radicals, and peroxynitrite. *Monascus*-fermented products have many antioxidant compounds, such as dimerumic acid, tannin, and polyphenol. Inflammation might contribute to the acceleration of vascular damage and hypertension and activate the rennin−angiotensin system. *Monascus* yellow pigments, such as monascin and ankaflavin, have an anti-inflammatory ability, which may contribute to the repression of vascular inflammation and pro-inflammatory molecules in the wall, such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM), and E-selectin. Dioscorea, a substrate of RMD, has been widely used as a functional food and Chinese herb (*27*). Some functional compounds of dioscorea, including resistant starch, polysaccharides, flavones, polyphenol, sporamin, and vitamin C, are a performance of the antioxidative ability (*6*, *7*). In addition, diosgenin and dioscin of dioscorea are proven to repress an inflammatory response (*7*). These healthy functions of dioscorea and *Monascus* metabolite should augment the effect of RMD on the antihypertensive effect.

It is well-established that chronic hypertension is associated with structural changes in the resistance vasculature (*28*). The histopathological changes, known as "remodeling", are considered to be a complex process that might increase (hypertrophy), decrease (hypotrophy), or rearrange (eutrophy) the vascular wall (*28*). Elasin fiber is an important determinant of arterial distensibility (*29*). Several models of genetic hypertension have reported abnormalities of large arteries in elastin content or structure (*30*). RMD can prevent the rearrangement of the vascular wall; therefore, it may have a hypotensive effect depending upon the decrease of the peripheral vascular resistance.

In conclusion, RMD in our present results was compared to the antihypertensive effect of RMD, RMR, and unfermented dioscorea. A half-fold of RMD was able to significantly prevent the blood pressure from increasing and improve vascular elastin remodeling. We consider that the greater antihypertensive effect of RMD depends upon GABA and ACE inhibitory activity. There is no effect in the liver index and kidney index, and it does not affect electrolyte balance and myocyte. Therefore, it suggests that RMD has a greater antihypertensive effect than traditional RMR.

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