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Novel Effects of a Single Administration of Ferulic Acid on the Regulation of Blood Pressure and the Hepatic Lipid Metabolic Profile in Stroke-Prone Spontaneously Hypertensive Rats

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We studied the effects of a single oral administration of ferulic acid (FA) on the blood pressure (BP) and lipid profile in stroke-prone spontaneously hypertensive rats (SHRSP). Male 12-week-old SHRSP were administered FA (9.5 mg/kg of body weight) and distilled water as the control (C) (1 mL) via a gastric tube. The hypotensive effect of FA was observed at the lowest value after 2 h administration. A decrease in the angiotensin-1-converting enzyme (ACE) activity in the plasma corresponded well with the reduction of BP. Plasma total cholesterol and triglyceride levels were lower after 2 h administration. The mRNA expression of genes involved in lipid and drug metabolism was downregulated in the FA group. These results suggest that oral administration of FA appears beneficial in improving hypertension and hyperlipidemia.

KEYWORDS: Ferulic acid; blood pressure; lipid profile; hepatic gene expression

INTRODUCTION

High blood pressure (BP) or hypertension is the most important risk factor in stroke and metabolic factors such as glucose, lipid profile, and insulin resistance. The risk of developing cardiovascular diseases (CVD) is directly related to the BP level, and a 5 mmHg decrease in BP has been equated to a 16% decrease in CVD (*1*). Some strategies are currently recommended to regulate BP and other metabolic factors such as maintaining dietary intake and lifestyle, pharmacological treatment, and use of bioactive components in food to decrease the risk of CVD.

Due to the increasing consumer knowledge of the link between diet and health, there is an increased awareness and demand for functional food ingredients and nutraceuticals. This leads to a mindset of self-medication to avoid the undesirable side effects associated with consumption of synthesized drugs and also to avoid the increasing cost of drug therapy. The nutritional effects of phenolic compounds have been well established in their role of modulating specific physiological functions in rodents and human beings.

Ferulic acid (FA, 4-hydroxy-3-methoxycinnamic acid) is one of the phenolic compounds. FA is the most common phenolic acid in monocotyledonous cell walls (*2*). The FA dehydrodimers are important structural components in plant cell walls and serve to enhance their rigidity and strength (*3*). A large proportion of ferulate dimers are present in a range of plant materials, notably in wheat bran (5-8-BendiFA, 8-*O*-4-diFA, and 5-5-diFA) (*3*).

The antioxidant properties of two chemically synthesized ferulate dimers—5-5-diFA and 5-8-BendiFA—have been documented in an in vitro study (*4*). FA has high antioxidant potential due to its resonance-stabilized phenoxy radical structure. It is an effective scavenger of free radicals and an approved food additive in certain countries that is used to prevent lipid peroxidation (*5*). Moreover, FA is also reported to be a good candidate for use as a topical protective agent against UV-radiation-induced skin damage (*6*).

Our previous study documented that blood pressure (BP) significantly decreased in stroke-prone spontaneously hypertensive rats (SHRSP) 8 weeks after feeding with FA (*7*). Another study reported that when free FA (50 mg/kg) was orally administered to spontaneously hypertensive rats (SHRs), the BP was at the lowest after 1 h and the values returned to basal levels at 6 h (*8*). Although the FA antioxidant is well recognized, there have been only a few reports from short-term studies concerning FA as an antihypertensive and antihyperlipidemic agent. The present study was conducted to identify whether or not oral FA administration physiologically affected BP reduction and improvement in the lipid level in SHRSP, which are an

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animal model of hypertension-related disorders that are similar to human essential hypertension and hyperlipidemia.

MATERIALS AND METHODS

Materials. Reagent kits for measuring the total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), glucose, blood urea nitrogen (BUN), and albumin were purchased from Wako Pure Chemical Co. (Osaka, Japan). Hippuryl-L-histidyl-L-leucine (HHL) and FA were purchased from Sigma Chemical Co. (St. Louis, MO).

Antihypertensive and Antihyperlipidemic Effects by Oral Administration of Ferulic Acid. We used 6-week-old male rats of the inbred SHRSP/Izumo strain in this study. They were housed in individual stainless steel cages in a room maintained at 23 ± 2 °C with 50 ± 10% humidity and a 12 h light−dark cycle. The rats were given free access to a fresh diet based on an AIN-93 M formula and drinking water for 6 weeks. The composition of the diet (g/kg) was as follows: *tert*-butylhydroquinone (0.008), L-cystine (1.8), choline bitartrate (2.5), vitamin mixture (10), mineral mixture (35), soybean oil (40), cellulose (50), sucrose (100), casein (140), and corn starch (620.692). The rats ($n = 5$) were employed for the oral administration at 12 weeks of age after 16 h of fasting. FA dissolved in distilled water (9.5 mg/kg of body weight) or distilled water alone (control, C; 1 mL) was orally administered to SHRSP via a gastric tube. The BP was measured before administration and 1, 2, 4, and 6 h after the administration. The BP was measured by the tail-cuff method with a BP meter without warming (MK-2000, Muromachi Kikai, Tokyo, Japan). The rats were administered again using the same procedure 5 days later and sacrificed under light anesthesia with diethyl ether after 2 h oral administration, following which blood was collected from the abdominal aorta and the plasma was separated for future analysis.

The experimental plan for the present study was approved by the Animal Research−Animal Care Committee of the Graduate School of Agricultural Science, Tohoku University. The entire experiment was in accordance with the guidelines issued by this committee and the Japanese governmental legislation (1980). The same committee supervised the care and use of the rats used in the present study.

Since the oral administration of FA at 9.5 mg/kg of body weight decreased the BP after 2 h administration, we have performed additional experiment on 10- and 11-week-old rats. Male SHRSP/Izumo rats were given free access to a diet (F-2; Funabashi farm, Chiba, Japan) and drinking water for a 1 week acclimatization period. At 10 weeks (3 mg/kg of body weight of FA) and 11 weeks (30 mg/kg of body weight of FA), the rats were divided into two groups ($n = 4$): ones for the control group (C) and the others for the FA group. After SHRSP were fasted for 16 h, they were orally administered FA dissolved in distilled water and distilled water alone as a control (C) (1 mL) via a gastric tube. The BP was measured before and 1, 2, 4, and 6 h after the administration.

Biochemical Analysis. Plasma levels of BUN, albumin, TC, TG, and glucose and HDL-C levels were determined by enzymatic colorimetric methods (Wako Pure Chemical Co., Osaka, Japan). LDL-C was calculated according to the Friedewald formula: LDL-C = (TC − HDL-C) − (1/5 × TG). Total liver lipids were determined according to Folch et al. (*9*). Liver TC and TG concentrations were determined using the same kit as that used for plasma TC and TG, following extraction of liver samples with chloroform−methanol (2:1, v/v). Angiotensin-1-converting enzyme (ACE) activity in the plasma was determined according to the method of Chusman and Cheung (*10*) with slight modifications. We preincubated 150 μL of 5 mmol/L hippuryl-L-histidyl-L-leucine (HHL) in borate buffer (100 mmol/L borate and 100 mmol/L NaCl; pH 8.3) at 37 °C for 10 min, added 60 μL of sample, and incubated the mixture at 37 °C for 30 min. The content of hippuric acids liberated from HHL by the enzymatic reaction of ACE was spectrophotometrically measured at 228 nm after ethyl acetate extraction. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 mol of substrate per minute.

RNA Preparation and DNA Microarray Experiment. Total RNA was isolated from the liver of each experimental animal with the phenol/guanidine-isothiocyanate-based reagent Isogen (Nippon Gene Co., Toyama, Japan), as detailed in the instruction manual. In each experimental group ($n = 4$), equivalent amounts of RNA were pooled from individual animals, and 500 ng of RNA was labeled using the Agilent Fluorescent Linear Amplification kit (Palo Alto, CA). Labeled RNA was further purified using the Qiagen RNeasy Mini kit protocol for liquid samples (Valencia, CA) and hybridized to the Agilent Rat Oligo Array for more than 43628 spots. A pooled sample was amplified and labeled with two fluorescent dyes (Cy3 for control and Cy5 for the FA group). After hybridization, arrays were scanned in an Agilent DNA Microarray Scanner (Palo Alto, CA), and fluorescence intensities for each probe were recorded. Ratios of transcript abundance (experimental to control) were obtained following normalization by a signal ratio of the control group (Cy5/Cy3) of each probe and correction of the array intensity data. Gene expression data analysis was done with the Agilent Feature Extraction software (version 7.1) (Palo Alto, CA). For each gene sequence present on the microarray, statistical significance of differential gene expression by >1.5 compared to control.

Quantitative Reverse Transcriptase - PCR. We used 5 μg of total RNA as a template to synthesize the cDNA. The RNA was denaturated with oligo-dT/random primers, and 10 mmol/L dNTP (Amersham Biosciences, Piscataway, NJ) and distilled water were added. The mixture was incubated at 65 °C for 5 min. The RNA was then incubated in 50 mmol/L Tris-HCl buffer (pH 8.3) and 0.1 M dithiothreitol (DTT) containing 50 units of SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) and 20 units of RNaseOUT RNase inhibitor (Invitrogen, Carlsbad, CA) in 20 μL at 25 °C for 5 min, 50 °C for 60 min, and 70 °C for 15 min. Aliquots of the cDNA were used as templates for the following quantitative reverse transcriptase−polymerase chain reaction (RT−PCR) using Applied Biosystems Sequence Detection System 7000 according to the manufacturer's instructions. The relative expression levels of gene expression were normalized by the amount of eukaryotic elongation factor-1α1 (EF-1) mRNA as described previously (*7*). The genes listed in **Table 1** were amplified by cDNA-specific primers in a Takara Bio. Inc., Shiga, Japan.

Data Analysis. Values are presented as means ± SE. The differences between group means were evaluated by the Student's *t* test (SPSS statistical package, version 11.0). Differences were considered significant at $p < 0.05$.

RESULTS

Antihypertensive Activity. We investigated whether FA exhibited antihypertensive activities after oral administration at a dose of 9.5 mg/kg of body weight (**Figure 1**). The systolic BP value in SHRSP after administration of distilled water was almost constant for 6 h. When FA was administered to SHRSP, a hypotensive effect was observed at 1, 2, and 4 h that returned to the basal condition after 6 h, with the lowest value observed after 2 h administration. The maximum reduction of BP was approximately 34 mmHg after 2 h of the administration ($p < 0.05$) as compared to the control group. We found that there were significant decreased time courses of blood plasma from the tail vein regarding plasma total cholesterol (TC) and triglyceride (TG) after 6 h of administration ($p < 0.05$) (data not shown). Moreover, we observed the BP-lowering effect of FA with two other concentrations: BP was reduced at 3 and 30 mg/kg of body weight at 2 h after oral administration, and the reduction in BP was approximately 13 and 19 mmHg ($p < 0.05$), respectively (data not shown).

Biochemical Parameters. Table 2 summarizes the parameters of the lipid profile, ACE activity, BUN, albumin, and the glucose levels after 2 h oral FA administration. After the administration, the FA group revealed significantly lower plasma TC and TG levels than the C group ($p < 0.01$). However, there was no difference in the HDL-C, LDL-C, ratio of HDL-C/LDL-C, glucose, BUN, albumin, and liver lipid levels between the FA and C groups. With respect to the analysis of plasma ACE activity, oral FA administration exhibited a significantly lower

Table 1. Sequences of Primers for PCR Amplifications

gene[a]	forward primer	reverse primer
Actn4	GAAGATCTGTGACCAGTGGGATAA	CTGCGACTGTGGGTCAGAGA
Aldh1a4	AGGCTTCTTTGTCCAGCCTACA	CGCATCTCATCGGTCACATT
Cyp7a1	CTGCCGGTACTAGACAGCATCA	ACCGTCCTCAAGATGGAGAGTG
EF-1	GATGGCCCCAAATTCTTGAAG	GGACCATGTCAACAATTGCAG
G6Pase	TTGTGCATTTGCTAGGAAGAGAAG	ATCTAAAGACCCAGGCATAACTGAAG
Hmgcr	AATTGTGTGTGGCACTGTGATG	GATCTGTTGTGAACCATGTGACTTCT
Lfabp	AGTACCAAGTGCAGAGCCAAGAG	GCAGACCCATCGCCTTCA
Onecut1	CAGGAAGGGTTGCACCATATG	CTTAGAAATCCCACACCAGCTTG
Pcca	TATTGGCTATTGCATCATCCTTGT	GCTGTGCTCGTAGCTGGGA
Pepck	GAGGACATTGCCTGGATGAAGTTT	TGGGTTGATGGCCCTTAAGT
Slc25a25	AATGTGCCAACACTAAGCTGACTT	CGTCCCCTGCAGCATCC
Udpgtr2	TGTAGTT GCTTCTGCTCTTGCC	GGATCCACTTGTACAGCCGAGT
Vlacs	ACTCTTTCAGCACATCTCGGAGTA	CCGGTCATCTCAATGGTATCTTGTA

[a] *Actn4*, Actinin α 4; *Aldh1a4*, Aldehyde dehydrogenase family 1, subfamily A4; *Cyp7a1*, Cytochrome P450, family 7, subfamily a, polypeptide 1;*EF-1*, eukaryotic elongation factor-1α1; G6Pase, glucose-6-phosphatase; Hmgcr, 3-hydroxy-3-methylglutaryl-CoA reductase; *Lfabp*, liver fatty acid binding protein; *Onecut1*, one cut domain, family member 1; *Pcca*, propionyl-coenzyme A carboxylase α polypeptide; *Pepck*, phosphenolpyruvate carboxykinase; *Udpgtr2*, UDP-glucuronosyltransferase, phenobarbital-inducible form; *Slc25a25*, Solute carrier family 25, member 25; *Vlacs*, very long chain acyl-CoA synthetase.

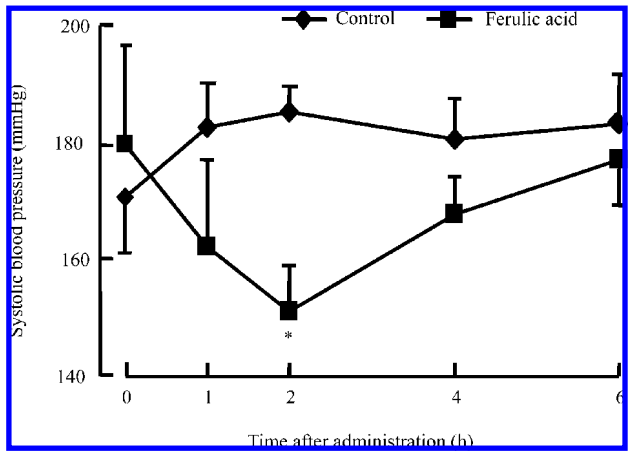

Figure 1. Effect of oral administration of ferulic acid (9.5 mg/kg of body weight) on blood pressure in SHRSP. Each point represents the mean ± SEM ($n = 5$). This is a significant difference from the control group ($p < 0.05$).

Table 2. Effect of FA on Plasma and Liver Biochemical Parameters after 2 h Administration[a]

biochemical parameter	control	ferulic acid
plasma		
total cholesterol (mg/dL)	85.6 ± 1.7	75.4 ± 2.8[b]
HDL cholesterol (mg/dL)	33.8 ± 1.4	32.4 ± 2.5
LDL cholesterol (mg/dL)	36.9 ± 1.3	33.3 ± 3.3
HDL-C/LDL-C	0.92 ± 0.06	1.01 ± 0.14
triglycerides (mg/dL)	74.6 ± 3.9	48.6 ± 3.6[b]
glucose (mg/dL)	150.6 ± 6.7	162.5 ± 9.0
ACE activity (mU/mL)	20.6 ± 1.0	16.9 ± 0.5[b]
BUN (mg/dL)	14.9 ± 1.1	14.9 ± 1.6
albumin (mg/dL)	3.9 ± 0.1	4.0 ± 0.1
liver		
total lipid (mg/g of liver)	52.1 ± 4.7	64.3 ± 5.2
total cholesterol (mg/g of liver)	4.3 ± 0.9	4.6 ± 1.5
triglycerides (mg/g of liver)	25.7 ± 2.7	23.8 ± 1.2

[a] Values are given as means ± SEM ($n = 5$). [b] Significant difference from the control group: ($p < 0.01$). FA was administered per oral (p.o.) at 9.5 mg/kg of body weight.

value than that of the C group ($p < 0.01$). FA administration at the 9.5 mg/kg of body weight dose lowered the ACE activity by 18%.

Hepatic Gene Expression Levels. We investigated the effect of a single-dose FA administration by using a DNA microarray to clarify its novel physiological effect in the liver. The gene expression profile of the ratio (FA/C groups) demonstrated that the genes downregulated (**Table 3**) and upregulated (**Table 4**) by FA were involved in lipid and drug metabolism. We attempted to confirm the DNA microarray data, and we further quantified the mRNA expression levels using the quantitative RT-PCR method (**Table 5**). There was a significant downregulation ($p < 0.05$) in the hepatic mRNA level of aldehyde dehydrogenase family 1 (*Aldh1a4*), solute carrier family 25, member 25 (*Slc25a25*), and a phenobarbital-inducible form of UDP-glucuronosyltransferase (*Udpgtr2*). However, there was no significant decrease in the mRNA expression levels of cytochrome P450, family 7, subfamily a, polypeptide 1 (*Cyp7a1*), one cut domain family member 1 (*Onecut1*), and propionyl-coenzyme A carboxylase α polypeptide (*Pcca*). In

Table 3. Downregulated Genes in the FA Group Determined by Gene Expression Profile Using DNA Microarray

gene symbol	ratio	name
Spetex-2F	0.08	spetex-2F protein
TC541991	0.09	Q7TQ12 (Q7TQ12) Aa1114, partial (9%)
L20998	0.16	T cell receptor mRNA
TC564206	0.19	unknown
AI072943	0.23	cDNA clone UI-R-Y0-mc-h-09–0-UI 3
AW143740	0.25	cDNA clone RGIBZ23 5′-end
Pcca	0.25	propionyl-coenzyme A carboxylase α polypeptide
Arih2	0.28	ariadne homologue 2 (Drosophila) (predicted)
Gadd45 g	0.51	growth arrest and DNA-damage-inducible 45 γ (predicted)
Actn4	0.51	actinin α 4
AW917767	0.53	cDNA clone RGIEH70 5′-end
LOC310395	0.55	carbon catabolite repression 4 protein homologue
Ca2	0.57	carbonic anhydrase 2
Hbb	0.57	hemoglobin beta chain complex
Aldh1a4	0.57	aldehyde dehydrogenase family 1, subfamily A4
Onecut1	0.59	one cut domain, family member 1
Cyp7a1	0.63	cytochrome P450, family 7, subfamily a, polypeptide 1
Nnmt	0.63	nicotinamide *N*-methyltransferase (predicted)
Slc38a2	0.65	solute carrier family 38, member 2
MGC109115	0.65	hypothetical protein FLJ13448
Udpgtr2	0.66	UDP-glucuronosyltransferase, phenobarbital-inducible form
Slc25a25	0.67	solute carrier family 25, member 25

Table 4. Upregulated Genes the in FA Group Determined by Gene Expression Profile Using DNA Microarray

gene symbol	ratio	name
M67496	1.50	kallikrein-binding protein
BG375381	1.67	cDNA clone UI-R-CV1-bsz-g-01–0-UI 3′
COLIA1	1.84	collagen type 1α1

Table 5. Hepatic mRNA Expression Level by Quantitative RT−PCR[a]

	group	
gene[c]	control	ferulic acid
Actn4	1.0 ± 0.2	0.9 ± 0.2
Aldh1a4	1.0 ± 0.1	0.5 ± 0.1[b]
Cyp7a1	1.0 ± 0.2	0.6 ± 0.2
G6pase	1.0 ± 0.2	1.2 ± 0.2
Hmgcr	1.0 ± 0.2	1.1 ± 0.4
Lfabp	1.0 ± 0.2	0.9 ± 0.2
Onecut1	1.0 ± 0.3	0.6 ± 0.3
Pcca	1.0 ± 0.1	0.8 ± 0.1
Pepck	1.0 ± 0.2	1.2 ± 0.2
Slc25a25	1.0 ± 0.2	0.5 ± 0.1[b]
Udpgtr2	1.0 ± 0.1	0.7 ± 0.2[b]
Vlacs	1.0 ± 0.1	0.9 ± 0.2

[a] Expression of mRNA (fold). Values are given as means ± SEM ($n = 4$). [b] Significant difference from the control group ($p < 0.05$). [c] *Actn4*, Actinin α 4; *Aldh1a4*, Aldehyde dehydrogenase family 1, subfamily A4; *Cyp7a1*, Cytochrome P450, family 7, subfamily a, polypeptide 1; *G6Pase*, glucose-6-phosphatase; *Hmgcr*, 3-hydroxy-3-methylglutaryl-CoA reductase; *Lfabp*, liver fatty acid binding protein; *Onecut1*, one cut domain family member 1; *Pcca*, propionyl-coenzyme A carboxylase α polypeptide; *Pepck*, phosphoenolpyruvate carboxykinase; *Udpgtr2*, phenobarbital-inducible form of UDP-glucuronosyltransferase; *Slc25a25*, Solute carrier family 25, member 25; *Vlacs*, very long chain acyl-CoA synthetase.

addition, we also measured several mRNA expression levels involved in glucose and lipid metabolism (**Table 5**); however, there was no significant difference in the glucose-6-phosphatase (*G6Pase*), 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), liver fatty acid binding protein (*Lfabp*), phosphenolpyruvate carboxykinase (*Pepck*), and very long chain acyl-CoA synthetase (*Vlacs*) expressions.

DISCUSSION

The present study aimed to investigate the effect of single oral FA administration on the regulation of BP and lipid profiles of SHRSP. In fact, we revealed that plasma ACE activity and hepatic gene expression involved in lipid and drug metabolism were decreased by FA administration. We also demonstrated that FA has hypotensive and hypolipidemic effects in SHRSP. Administration of FA rapidly decreased the BP after 2 h oral administration in SHRSP (**Figure 1**). Further, plasma TG, TC, and ACE activity significantly decreased after 2 h FA administration (**Table 2**). This is the first report to demonstrate the novel physiological effect of FA of decreasing high BP and lipid levels in SHRSP. We found that FA is a good candidate compound for the prevention of cardiovascular diseases and that its activity is related to the metabolic state, such as changes in lipid profiles, glucose metabolism, and the regulation of hypertension.

In this study, we used SHRSP as the animal model for hypertension-related disorders similar to human essential hypertension (*11*), hyperlipidemia (*12*), insulin resistance syndrome (*13*), and higher oxidative stress in the brain (*14*).

The single oral administration of FA was performed at the dose level of 9.5 mg/kg of body weight, and the antihypertensive effect was shown to be statistically significant after 2 h administration (**Figure 1**). The result of the present study was in good agreement with that of Suzuki et al. (*8*). According to their report, the dose of 50 mg/kg of body weight administered to spontaneously hypertensive rats (SHR) produced a BP lowering effect of −19, −21, −16, and −14 mmHg for 0.5, 1, 2, and 4 h after administration, respectively. In the present study, a statistically significant reduction of 13, 34, and 19 mmHg was observed after 2 h administration at the doses 3, 9.5, and 30 mg/kg of body weight, respectively. We suggested that the concentration of 3 mg of FA/kg of body weight is sufficient to improve BP, and 9.5 mg of FA/kg of body weight was the best concentration to improve BP in SHRSP. Therefore, we used 9.5 mg of FA/kg of body weight for further studies regarding ACE activities, lipid improving effect, and gene expression levels.

ACE can catalyze angiotensin I to angiotensin II, a potent vasoconstrictor, and it has been reported that the inhibition of ACE activity causes a decrease in BP. The present results demonstrated that the plasma ACE activity was reduced after 2 h oral administration (**Table 2**). This result corresponds to the reduction of BP after 2 h oral administration. Many studies have focused on the isolation of peptide ACE inhibitors from food protein (*15, 16*); however, there are few reports on nonpeptide ACE inhibitors present in foods. Recently, it was reported that the consumption of certain flavanol-rich foods, i.e., wines, chocolates, and tea, and purified flavonoids can mediate a reduction in BP. The inhibition of ACE activity was associated with both phenolic and flavanol content in the foods (*17*). From this viewpoint, the results of our study clearly demonstrated that single-dose FA administration has a hypotensive effect in SHRSP.

This is the first report for the potential of FA to decrease TC and TG in a single-dose administration (**Table 2**). These results suggest that the blood pressure-lowering effect of FA could correspond to the effect on lipid metabolism. Since FA has antioxidant properties, these results suggest that FA can be used to improve BP and hyperlipidemia in spontaneous hypertensive condition. It is well recognized that multiple factors related to the metabolic disease, such as changes in the glucose and lipid profiles and insulin resistance, complicate the management of hypertension, particularly under diabetic conditions. Therefore, any food component having the ability to reduce high BP is a potential candidate for the prevention or treatment of cardiovascular diseases. Consequently, in this study, we attempted to confirm that a decreased BP level has an effect on lipid metabolism. We demonstrated that at 2 h after the oral administration of FA there was a significant decrease in the level of BP and that this event was followed by improvements in two lipid parameters (plasma TC and TG) compared to the control group. The heart outcomes prevention evaluation study also indicated that use of ACE inhibitors may lower the incidence of hypertension and heart failure in the high-risk group of CVD (*18*). Therefore, we suggest that FA can act to improve BP and hyperlipidemia in spontaneous hypertensive conditions.

The exact mechanism by which FA lowers lipid levels remains unknown; however, there are some studies that reported the capacity of FA-related compounds to decrease the blood lipid level. First, the intravenous administrations of γ-oryzanol and cycloartenol ferulic acid ester decreased the level of blood lipids (*19*). γ-Oryzanol is a naturally occurring mixture of plant chemicals called sterols and ferulic acid esters. Second, FA is a potent antioxidant and prevents LDL oxidation induced by copper ions; hence it facilitates the uptake and degradation of cholesterol in the liver (*20*). Third, curcumin (active principle of turmeric) produces vanillin and ferulic acid on photoirradiation (*21*), and the hypocholesterolemic effect of curcumin is due to the increased HDL formation, which transports the excess cholesterol from extrahepatic tissues to the liver, where it is catabolized (*22*). Moreover, curcumin also increases the activity of 7α-hydroxylase, which is the main enzyme involved in the conversion of cholesterol to bile acid, and thus facilitates biliary cholesterol excretion (*23*).

Until now, there have been a limited number of studies to address the effect of FA on gene expression. Since the plasma TC and TG levels decreased after oral FA administration, the present study analyzed the gene expression profile by the DNA microarray technique to investigate the novel physiological effect of FA on hepatic gene expression. It was identified that FA affected the mRNA expression of 22 downregulated genes (**Table 3**) and 3 upregulated genes (**Table 4**). We next analyzed the abundance of mRNA by using the quantitative RT−PCR method to confirm the results of the DNA microarray method. We focused on the expression of 7 downregulated genes as the primary genes involved in lipid and drug metabolism, and this is the first study that demonstrates the effects of FA on lipid and drug metabolism. Furthermore, we also measured the expression of other genes involved in glucose and lipid metabolism, such as *G6Pase*, *Hmgcr*, *Lfabp*, *Pepck*, and *Vlacs* (**Table 5**).

As a result, the mRNA expression levels of *Slc25a25*, *Aldh1a4*, and *Udpgtr2* were significantly lower in the FA group as compared to the control group; moreover, the mRNA expression levels of *Cyp7a1*, *Onecut1*, and *Pcca* were lower in the FA group compared to the control group. The results obtained from quantitative RT−PCR almost correlated with those from DNA microarray analyses, suggesting that oral FA administration influences lipid and drug metabolism.

Slc25a25 (ATP-Mg/P_i carrier) plays an important role in regulating metabolic activities that have adenine nucleotide-dependent steps localized to the mitochondrial compartment, such as gluconeogenesis, urea synthesis, and oxidative phosphorylation (*24*). This transporter catalyzes an electroneutral exchange of ATP carrying two negative charges for divalent phosphate. Because matrix ATP concentration is in equilibrium with ADP and AMP concentrations, changes in ATP concentration due to net movement via the *Slc25a25* carrier result in proportional changes in the concentrations of other adenine nucleotides (*25*). We supposed that the decrease in the mRNA expression level of *Slc25a25* could maintain the cellular level of ATP and lipid synthesis. The downregulation of this transporter could contribute to the decrease in the TC and TG levels after 2 h oral administration. Furthermore, we also demonstrated that lower expression of the *Cyp7a1* and *Pcca* mRNA levels could contribute to the decrease in the lipid levels in the FA group. *Pcca* may be important in the development of fatty liver or in the expression of hyperlipidemia. *Pcca* is of interest due to its known function in ketogenesis, i.e., conversion of propionyl-CoA to succinyl-CoA in the citric acid cycle (*26*).

Aldh1a4 is related to pathways of bile acid biosynthesis (conversion of 3-α,7α-dihydroxy-5β-cholestan-26-α1 to 3-α,7α-dihydroxy-5β-cholestanoate) and fatty acid metabolism (conversion of fatty acid to aldehyde) (*27*). *Udpgtr2* is one of the intracellular membrane proteins that catalyzes the transfer of glucuronic acid from UDP-glucuronic acid to a lipophilic acceptor (*28*). Since FA has antioxidant properties that make it an important antiaging supplement and these properties also contribute to the other potential uses of FA, we suggested that oral FA administration can decrease the mRNA levels of *Aldh1a4* and U*dpgtr2*, the two genes that are involved in drug metabolism.

Our previous study demonstrated that oral administration of FA for 8 weeks in SHRSP resulted in the downregulation of the *Vlacs* mRNA level, upregulation of the *Hmgcr* and *Pepck* mRNA levels, and no significant difference in *G6pase* and *Lfabp* (*7*). *Vlacs* is one of the genes involved in TG metabolism and hepatic TG levels parallel to the ACS mRNA expression level (*29*). *Hmgcr* is a gene encode that catalyzes a rate-limiting step in cholesterol biosynthesis. The *Pepck* and *G6Pase* genes encode rate-controlling enzymes of hepatic gluconeogenesis. The physiological significance of the dysregulation of hepatic gluconeogenic genes is supported by the observation of the overexpression of both *Pepck* and *G6Pase* in the liver, and several groups have studied the metabolic and physiological consequences of altering either *Pepck* and *G6Pase* in vivo (*30–32*). The results from the present study revealed that there was no significant difference between these genes. Therefore, we assume that this discrepancy could be due to the different experiment models used. In a previous study, we fed SHRSP rats for 8 weeks on a diet supplemented with FA (chronic study). However, in the present study, we used a single oral administration of FA (acute experiment). We presume that the single oral administration was insufficient to cause a change in the hepatic mRNA levels of the above-mentioned genes.

The *Onecut1* gene is related to the glucocorticoid receptor, and it is also termed hepatocyte nuclear factor 6 (Hnf6). Hnf6 inhibits the glucocorticoid-induced stimulation of two genes coding for enzymes of liver glucose metabolism, namely, 6-phosphofructo-2 kinase and *Pepck*. Binding of Hnf6 to DNA is required for the inhibition of glucocorticoid receptor activity. Thus, in addition to its known function of stimulating the transcription of liver-expressed genes, Hnf6 can antagonize glucocorticoid-stimulated gene transcription (*33*). In this study, we determined that the *Pepck* and *G6pase* mRNA levels tended to be higher but not statistically significant. These two genes encode the rate-controlling enzymes in glucose metabolism.

In conclusion, we determined that a single oral dose of FA can decrease the BP and plasma lipid levels. Furthermore, we discovered the novel physiological action of FA in the regulation of BP and hyperlipidemia in spontaneous hypertensive conditions. The gene expression profiles determined by DNA microarray and quantitative RT−PCR resulted in FA-mediated downregulation of *Slc25a25* mRNA expression, which contributes to maintain cellular levels of ATP and lipid synthesis in lipid metabolism. In addition, FA could decrease the mRNA level of *Aldh1a4* and *Udpgtr2* that are involved in drug metabolism. Finally, further studies will increase our understanding of the relationship between FA and improvement of diseases at the level of gene expression.

ABREVIATIONS USED

ACE, angiotensin-1-converting enzyme; *Actn4*, actinin α 4; *Aldh1a4*, aldehyde dehydrogenase family 1, subfamily A4; BP, blood pressure; C, control; *Cyp7a1*, cytochrome P450, family 7, subfamily a, polypeptide 1; FA, ferulic acid; *Onecut1*, one cut domain, family member 1; *Pcca*, propionyl-coenzyme A carboxylase α polypeptide; quantitative RT−PCR, reverse transcriptase−polymerase chain reaction; SHRSP, stroke-prone spontaneously hypertensive rats; *Slc25a25*, solute carrier family 25, member 25; TG, triglycerides; TC, total cholesterol; *Udpgtr2*, UDP-glucuronosyltransferase, phenobarbital-inducible form.

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