Effects of Morin on Blood Pressure and Metabolic Changes in Fructose-Induced Hypertensive Rats

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High fructose (HF) feeding induces a moderate increase in blood pressure in rats, which is associated with insulin resistance, hyperinsulinemia, and hypertriglyceridemia. In the present study, we examined the chronic effect of morin, a flavonoid isolated from medicinal plants, on blood pressure, lipid profiles, and serum insulin and glucose in HF-induced hypertensive rats. Rats were divided into control group and HF-fed group during the first three weeks of experiments. Then, rats were further divided into four groups and treated for 4 more weeks as follows: 1) control group; 2) morin-treated (intraperitoneal 5 mg/kg/d) control group; 3) HF-fed group; 4) morin-treated, HF-fed group ($n=8$, each group). Morin-treated HF-fed group showed lower systolic blood pressure (SBP) (132.0±2.5 mmHg *vs.* 142.8±2.2 mmHg, $p<0.05$), lower serum insulin level (1.21±0.27 *vs.* 2.73±0.30 μIU/dl, $p<0.05$), and lower plasma triglycerides (47.8±5.0 *vs.* 65.5±5.0 mg/dl, $p<0.05$) than those of HF-fed group. Morin treatment also suppressed mRNA expression of endothelin-1 (ET-1) in the thoracic aorta from HF-induced hypertensive rats. Moreover, decreased renal sodium excretion in HF-induced hypertensive rats was ameliorated by morin treatment. In conclusion, the results of this study demonstrate that morin has an anti-hypertensive effect in HF-induced hypertensive rats. This effect of morin may be associated with the suppression of serum insulin and plasma triglyceride level, with the down-regulation of ET-1 in the thoracic aorta, and with the partial amelioration of renal dysfunctions in HF-induced hypertensive rats.

Key words morin; high fructose; hypertension; hyperinsulinemia

Insulin resistance has long been recognized to be associated with hypertension, hyperinsulinemia and dyslipidemia.[1,2] These are also critical components of recently re-defined metabolic syndrome.[3—5] Insulin resistance and hyperinsulinemia have been demonstrated in not only genetic, but also nongenetic rat models of hypertension, such as the spontaneously hypertensive,[6] Dahl salt-sensitive,[7] Milan hypertensive,[8] and fructose-fed rat,[1,9] suggesting that mechanism leading to hypertension may be shared by these experimental models. Fructose-induced hypertension in rats represents an acquired model of insulin resistance such as metabolic syndrome (also known as syndrome X).[10] Chronic fructose treatment in rats has been shown to elevate blood pressure in association with insulin resistance, hyperinsulinemia, and hypertriglyceridemia. Although the mechanisms underlying fructose-induced hypertension are not completely established, it has been proposed that increase in blood pressure in fructose-fed rats is secondary to the development of insulin resistance and hyperinsulinemia.[1,9] The flavonoids are a large group of plant-derived compounds that are known to exhibit a lot of biological effects including reducing plasma levels of low density lipoprotein,[11] scavenging reactive oxygen species,[12] inhibiting platelet aggregation,[13,14] and inhibiting carcinogenesis.[15] In addition, a number of flavonoids have been reported to dilate vascular smooth muscle and then reduce blood pressure in various animal models of hypertension.[16—18] Morin (3,5,7,2′,4′-pentahydroxyflavone; Fig. 1) is a kind of flavonoid found in the fig and other Moraceae, which are used as herbal medicines.[19,20] A very wide range of biological actions of morin including antioxidant properties,[21] inhibitory activity of oxidative modification of low density lipoprotein,[11] and vasorelaxant effect[22] has been reported. Such beneficial effects of morin could be expected to work in human or animal models of various diseases including metabolic syndrome, cardiovascular disease, and diabetes. However, there is little information about *in vivo* effects of morin in animal models or patients with above diseases. Therefore, in the present study, we examined the effects of chronic administration of morin on the blood pressure, renal function, and lipid metabolites in rats with HF-induced hypertension.

MATERIALS AND METHODS

Experimental Design The animal procedures were in strict accordance with the National Institutes of Healthy Guidelines for the Care and Use of laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee. Male Sprague–Dawley rats (200—220 g) were purchased from Korean Experimental Animals Co. (Daejeon, Korea). The rats were housed in metabolic cages to collect 24-h urine samples in the animal room with an automatic temperature (22 °C) and lighting (12 h light–dark cycle) control. An adaptation period of 1 week for vehicle (tap water) administration and blood pressure measurements was allowed before initiation of the experimental protocol. A total of 7 weeks of experiments were planned. The rats were allocated to normal rat chow diet with water alone (control group) or with additional 10% fructose in water (HF-fed group) during the first three weeks of experiments. Then, rats

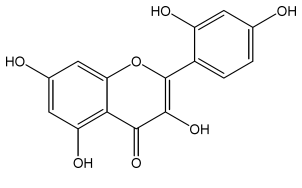

Fig. 1. Chemical Structure of Morin

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were further divided into four groups and treated for 4 more weeks as follows: 1) control group; 2) morin-treated (intraperitoneal 5 mg/kg/d) control group; 3) HF-fed group; 4) morin-treated, HF-fed group ($n=8$, each group). Morin was dissolved in saline solution before each injection. And, saline only was administered to the control and the HF-fed rats.

SBP was measured weekly in conscious rats by tail-cuff plethysmography. At least six determinations were made in every session and the mean of the three measurements that had variations within the range of 5 mmHg was taken as the SBP level. On the experiment day, the trunk blood was collected by decapitation without anesthesia in a prechilled tube containing 1 mg/ml ethylenediaminetetraacetic acid (EDTA) or not for the biochemical analyses of plasma or serum. The thoracic aorta was removed and immediately frozen in liquid nitrogen, and stored at −72 °C until assayed.

Renal Function Monitoring Each group of rats was maintained in separate metabolic cages, allowing quantitative urine collections and measurements of water intake. On a day before sacrifice, 24-h urine samples were collected (between 09:00 and 10:00 am) for the determinations of the levels of creatinine, sodium ion, potassium ion, osmolality, and other parameters of renal function. Plasma sodium, potassium, osmolality, and creatinine were also measured. Urine and plasma levels of sodium and potassium were measured using an electrolyte analyzer (NOVA 4, Biochemical, Waltham, MA, U.S.A.). Urine and plasma osmolalities were measured using an osmometer (Model 3900, Advanced Instruments Inc., Norwood, MS, U.S.A.). Creatinine concentrations in plasma and urine were measured by colorimetric methods using a spectrophotometer (Milton Roy, Rochester, NY, U.S.A.). Solute-free water reabsorption (T^cH_2O) was calculated by the following formula: $T^cH_2O=V\times(Uosmol/Posmol-1)$, where V is urine volume, Uosmol is urine osmolality, and Posmol is plasma osmolality.

Reverse Transcription–Polymerase Chain Reaction Total RNA was extracted from the aorta as described by Chomczynski and Sacchi[23] using a commercial solution (Biotecx Laboratories, Houston, TX, U.S.A.). Reverse transcription (RT) followed by polymerase chain reaction (PCR) was then performed. Avian myeloblastosis virus reverse transcriptase (16 U per reaction; Promega, Madison, WI) was used for RT, along with the reaction mixture recommended by the enzyme manufacturer, in a volume of 20 μl using 1.25 μmol/l downstream primer. The PCR was then performed with 2 μl of the resulting cDNA using the upstream and downstream primers at 1.25 μmol/l each. Each PCR mixture contained 5 U *Taq* polymerase (Takara, Japan) and 60 μmol/l dNTP. cDNA for endothelin-1 (ET-1) mRNA was synthesized with specific primers [upstream 5′-ATG GAT TAT TTT CCC GTG AT-3′ (1 to 20) and downstream 5′-GGG AGT GTT GAC CCA GAT GA-3′ (212 to 231)]. The PCR with these primers yielded 231 base pairs. In a preliminary study, we found that 35 PCR cycles for ET-1 were necessary to obtain a visible product on agarose gel, and the quantity of the product was in proportion to the amount of cDNA used. After an initial denaturation step at 94 °C for 2 min, cycles of annealing at 56 °C for 45 s, elongation at 72 °C for 1.5 min, and denaturation at 94 °C for 45 s were performed with cDNA (2 μl). The RT-PCR product of the gene encoding for β-actin served as a control. The upstream primer sequence 5′-GAC TAC CTC ATG AAG ATC CTG ACC-3′ used to amplify β-actin mRNA corresponds to positions 210 to 217, and the downstream primer 5′-TGA TCT TCA TGG TGC TAGG AGC C-3′ corresponds to positions 320 to 327. They yielded a 423-base pair DNA fragment. The PCR products were size-fractionated by 1.5% agarose gel electrophoresis and visualized under UV light with ethidium bromide staining. cDNAs ET-1 and β-actin were quantified by Imager analyzer (Bioneer, Seoul, Korea). ET-1 cDNA was normalized by comparison to β-actin cDNA.

Plasma Glucose, Triglycerides and Cholesterol and Serum Insulin Plasma glucose concentration was determined by the glucose oxidase method. Plasma triglycerides and cholesterol levels were assayed enzymatically by an automated commercial method (Behringer Mannheim, Marburg, Germany). Serum insulin level was measured by radioimmunoassay (Behringer Mannheim, Marburg, Germany). Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured using commercial kit reagent (Behringer Mannheim, Marburg, Germany).

Chemicals Morin and fructose were purchased from Sigma (St. Louis, MO, U.S.A.). All other unstated chemicals and reagents were analytical grade.

Statistical Analysis The statistical significance of differences between four groups means was determined using one-way ANOVA. Student's *t*-test was used to determine significance between two group means, if applicable, $p<0.05$ was considered statistically significant.

RESULTS

Systolic Blood Pressure Changes Figure 2 illustrates SBP in four experimental groups over the experimental period in response to treatment of morin. At the starting point of experiment, SBPs of four experimental groups ware comparable (101±5.0 mmHg). One week administration of 10% fructose solution increased SBP to 124±3.9 mmHg. In two weeks, SBP in HF-fed rats reached plateau (140±4.2 mmHg), which was maintained thereafter. Morin therapy in HF-fed rats lowered the elevated SBP significantly and the therapeutic effects of morin were maintained throughout the experimental period. There were no differ-

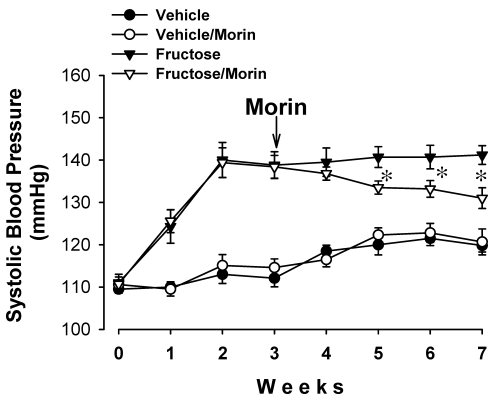

Fig. 2. Effect of Morin on Systolic Blood Pressure in Different Experimental Rats

There were eight experiments in each group. Data are means±S.E. of the three values had small deviation within 5 mmHg range. $*p<0.05$ ($n=8$, each group) compared with HF group.

Table 1. Body Weight and Biochemical Data of the Experimental Groups

Parameters	Experimental groups			
	V	V+M	HF	HF+M
Body weight (g)	390±7.1	362±5.5	383±11.6	361±3.1
Total cholesterol (mg/dl)	65.5±9.5	62.0±7.4	70.3±4.4	61.8±5.1
HDL-cholesterol (mg/dl)	18.8±3.6	17.2±2.5	19.3±1.9	17.0±2.0
LDL-cholesterol (mg/dl)	28.0±1.7	28.2±1.8	26.7±1.0	25.4±1.0
Triglyceride (mg/dl)	57.7±7.6	49.7±7.2	75.5±3.5*	59.8±5.0#
AST (U/l)	92.8±15.3	87.1±7.8	153.6±20.9*	112.4±12.7#
ALT (U/l)	51.6±8.0	49.8±4.4	68.9±8.4*	44.1±3.8#

Values are means±S.E. (n=6, each group). *p<0.05, compared with vehicle group, #p<0.05 compared with HF group. V, vehicle; V+M, morin-treated vehicle; HF, high fructose-fed; HF+M, morin-treated, high fructose-fed group.

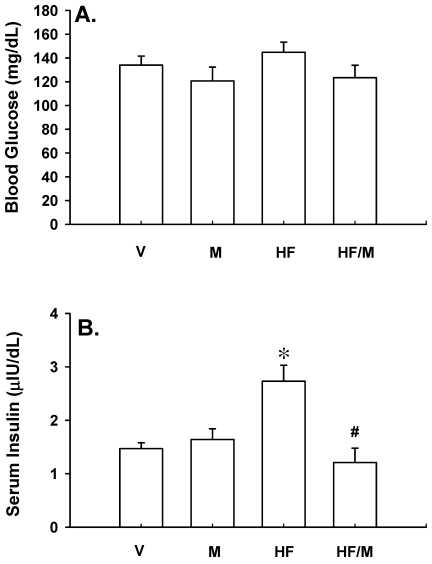

Fig. 3. Effect of Morin on Plasma Glucose (A) and Serum Insulin Concentration (B) in HF-Induced Hypertensive Rats

V, vehicle; M, morin; HF, high fructose; HF/M, morin-treated group in HF during the last 4 weeks. *p<0.01 (n=6, each group), compared with vehicle; #p<0.01 (n=6, each group), compared with HF group.

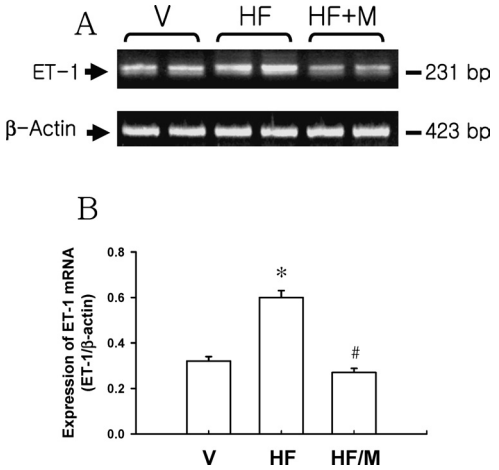

Fig. 4. Effect of Morin on the mRNA Expression of Endothelin-1 in Aortic Tissues from HF-Induced Hypertensive Rats

Representative RT-PCR products (A) and quantification (B) of ET-1 mRNA expression in aortic tissues. *p<0.01 (n=4, each group), compared with vehicle; #p<0.01 (n=4, each group), compared with HF group.

ences in SBP between control and morin-treated control group during the entire experimental period.

General Characteristics Table 1 illustrates body weight, plasma cholesterols and triglyceride concentrations, and the levels of AST and ALT at the end of experiment. Although final body weights of each morin-treated group were slightly lower than those of their control rats, the differences did not reach statistical significances. Plasma levels of total cholesterol, HDL-cholesterol, and LDL-cholesterol were not different among the four experimental groups. However, plasma triglyceride concentrations were found to be higher in HF-fed rats than in control rats (p<0.01). Morin treatment prevented triglyceride elevations in HF-fed rats (p<0.01 compared with HF group). Administration of HF caused elevations of AST and ALT levels (p<0.05, compared with control group, respectively), but morin treatment prevented such changes. Morin treatment did not affect the hepatic functions of control rats.

Plasma Glucose and Serum Insulin Concentrations Plasma glucose and serum insulin concentrations in each experimental group are shown in Fig. 3. There were no significant differences in plasma glucose concentrations among the four experimental groups. However, HF-administered rats showed a significant increase in serum insulin compared to control rats (p<0.05), which were completely prevented by co-treatment with morin (p<0.05, compared with HF-administered rats).

Aortic ET-1 mRNA Expression As is shown in Fig. 4, ET-1 mRNA expression, determined by RT-PCR, in the aortic tissues from HF-administered rats was significantly increased compared to that of control rats (p<0.05). But, such an increase did not occur in morin-treated HF-fed rats (p<0.05, compared with HF-administered rats).

Renal Functional Parameters Table 2 illustrates renal functional parameters in the different experimental groups at the end of experiment. Compared with the control, HF-administered rats showed a higher 24 h urinary volume (UV) (p<0.05), which was not altered by treatment with morin. Renal sodium excretion (UNaV) was decreased in HF-administered group, along with decrease in urinary osmolality (Uosmol) and solute-free reabsorption (T^cH_2O). Among these parameters, UNaV was partially restored by treatment with morin (p<0.05), but other parameters were not influenced by treatment with morin. Morin treatment increased creatinine clearance (Ccr) significantly in both control group and HF-administered group (p<0.05, respectively).

Table 2. Effects of Morin Treatment on the Parameters of Renal Function in Fructose-Induced Hypertensive Rats

Parameters	Experimental groups			
	V	V+M	HF	HF+M
UV (ml/kg/d)	33.6±3.1	30.1±1.4	55.9±12.4*	55.2±9.3*
UNaV (mEq/min/kg)	3.19±0.17	3.49±0.18	2.37±0.49*	2.76±0.15*,#
UKV (mEq/min/kg)	5.91±0.33	6.02±0.12	5.22±0.84	4.96±0.75
Uosmol (mosmol/kg H_2O)	1469±160.6	1670±85.6	1087±97.3*	1074±89.6*
Ccr (ml/min/kg)	2.07±0.15	2.95±0.22*	2.13±0.12	2.56±0.13*,#
T^cH_2O (ml/min/kg)	100.3±14.6	103.1±8.2	67.0±4.9*	74.6±6.7*

Values are means±S.E. (n=6, each group). *p<0.05, compared with control group; #p<0.05 compared with fructose-induced hypertensive group. UV, urinary volume; UNaV, urinary sodium excretion; UKV, urinary potassium excretion; Uosmol, urinary osmolality; Ccr, creatinine clearance; T^cH_2O, solute-free water reabsorption. V, vehicle; V+M, morin-treated vehicle; HF, high fructose; HF+M, morin-treated high fructose group.

DISCUSSION

Insulin resistance and hyperinsulinemia have been correlated with hypertension, dyslipidemia, glucose intolerance, and obesity, as well as with cardiac hypertrophy and atherosclerotic cardiovascular disease.[1)] The present study showed that oral administration of 10% fructose solution to rats caused hypertension associated with increases in serum insulin and triglycerides, which are in line with previous reports.[1,2)] The present study clearly demonstrated that treatment with the morin, a flavonoid from medicinal plants, prevented such elevations of blood pressure and of serum insulin and triglyceride concentrations in HF-administered rats. Previous data suggest a strong relationship between hyperinsulinemia and hypertension in this hypertensive rat model.[1)] Although the mechanisms underlying fructose-induced hypertension are not completely clarified, it has been proposed that elevation of blood pressure in fructose-fed rats is secondary to the development of insulin resistance and hyperinsulinemia. That is, compensatory hyperinsulinemia has been thought to be a cause of hypertension because insulin could cause sodium retention, sympathetic nerve activation, and vascular smooth muscle cell proliferation.[9,24)]

ET-1 is also thought to be an important vasoactive peptide contributing to the development of hypertension in this experimental model.[9,25)] ET-1 is mostly produced in endothelial cells (EC) and acts on various tissues in an endocrine, paracrine, or autocrine manner as a strong endogenous vasoconstrictor and as a growth factor for vascular smooth muscle cells.[26,27)] Thus, ET-1 may promote vascular hypertrophy and atherosclerosis.[27)] In addition, it has been reported that insulin could stimulate the production of ET-1 in EC both *in vitro* and *in vivo*.[28,29)] Insulin is not only a growth factor for vascular cells, but also an inducer of a vasoactive substance including ET-1 in vascular cells. ET-1 induced by insulin may affect blood pressure and involve in atherosclerotic process in subjects with hyperinsulinemia and insulin resistance. The over-expression of ET-1 has been reported to involve in the pathogenesis of this disease model, as it could cause blood pressure elevation and vascular dysfunction.[30)] Conversely, the blood pressure elevation is attenuated by long-term ET-1 antagonist treatment in fructose-fed rats.[31,32)] Therefore, ET-1 may play a pivotal role in the development of hypertension in metabolic syndrome. In the present study, both mRNA expression of ET-1 in the aortic tissues and serum insulin were significantly increased in HF-administered rats, which were prevented by chronic morin treatment. Thus, morin could exert its beneficial effects on blood pressure by improving insulin sensitivity and by reducing ET-1 production in fructose-fed hypertensive rats.

The kidney plays a central role in the regulation of the balance of body salt and water, and then disordered regulation of renal functions is responsible for the altered balance of salt and water in pathophysiological states including some experimental models of hypertension.[33)] Administration of HF solution for 7 weeks significantly increased urinary volume compared to that of control group, which was not altered by treatment with morin. Renal sodium excretion was decreased in rats with HF-administered group along with the decrease in urinary osmolality and solute-free reabsorption. Among these parameters, only UNaV was partially restored by treatment with morin. Morin treatment increased creatinine clearance in both control and HF-administered groups. These findings suggest that renal dysfunction could be partially ameliorated by treatment of morin in rats with HF-induced hypertension, which also may be beneficial in the control of blood pressure. To the best our knowledge, this is the first report that reveals the salutary biologic actions of morin in the animal model of metabolic syndrome. Further *in vivo* studies are necessary to establish a role of morin in the management of metabolic syndrome or other cardiovascular diseases.

In conclusion, the results of this study demonstrate that morin has an anti-hypertensive effect in HF-induced hypertensive rats. This effect of morin may be associated with the suppression of hyperinsulinemia and hypertriglyceridemia, with the down-regulation of ET-1 in the thoracic aorta, and with the partial amelioration of renal dysfunctions in HF-induced hypertensive rats.

Acknowledgements This study was supported by the Brain Korea 21 Project (2003) and grants of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea (03-PJ9-PG6-SO02-0001).

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