JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 3945−3950 **3945**

Aqueous Extract of *Monascus purpureus* M9011 Prevents and Reverses Fructose-Induced Hypertension in Rats

PO-SHIUAN HSIEH* AND YUEH-HUA TAI

Department of Physiology and Biophysics, National Defense Medical Center, National Defense University, Taipei, Taiwan, Republic of China

This study aimed to determine the antihypertensive and metabolic effects of an aqueous extract of *Monascus purpureus* M9011 on fructose-induced hypertensive rats. After dietary feeding of fructose for 2 weeks, the rats exhibited significantly higher systolic blood pressure (SBP), mean arterial pressure (MAP), and plasma insulin and triglyceride levels, but lower insulin sensitivity than those in control rats on regular diet. The intragastric loading of fructose-fed rats with M9011 containing γ-aminobutyric acid (GABA, 1 mg·kg^{-1}·day^{-1}) prevented the development of fructose-induced hypertension. After fructose-induced hypertension had been established, intragastric loading of M9011 reversed the elevated blood pressure to normal level. Administration of pure GABA at the same dose as that contained in M9011 failed to prevent or reverse hypertension due to fructose consumption. Chronic M9011 treatment significantly suppressed the fructose-induced elevation in total cholesterol levels and enhanced the recovery of high-density lipoprotein cholesterol/total cholesterol ratio. However, M9011 treatment did not alter insulin sensitivity or the plasma levels of insulin, glucose, and triglyceride in fructose-fed and control rats. The present results suggest that M9011 is a novel, potent, food-based antihypertensive agent with the capability to improve long-term control of cholesterol metabolism in rats and may be of importance in clinical application for the hypertensive diabetic population.

KEYWORDS: ***Monascus purpureus*** **M9011; fructose; hypertension; hypertriglyceridemia; dyslipidemia; hyperinsulinemia; insulin resistance; GABA; rats**

INTRODUCTION

Insulin resistance-associated syndromes (hyperinsulinemia and hypertriglyceridemia) have been demonstrated frequently in hypertensive patients (*1*). It has also been shown that hypertension in diabetic patients is observed more often than in nondiabetic individuals (*2*−*5*). These associations carry a significant increase in mortality and morbidity due to atherosclerosis and microvascular diabetic complications such as nephropathy and retinopathy (*6*−*8*). It is recognized that the management of hypertension especially in the diabetic patient is complicated by multiple factors related to the metabolic state, that is, glucose and lipid levels and insulin resistance. The metabolic effects of the antihypertensive regimens, therefore, should be carefully evaluated before therapy is started.

The association between insulin resistance and hypertension has also been documented in the fructose-induced hypertensive rat model (*9*, *10*). Fructose feeding induces hypertension associated with hyperinsulinemia/insulin resistance and hypertriglyceridemia (*9*, *10*). Although the precise mechanism has not been clearly elucidated, it has been proposed that hypertension in fructose-fed rats is secondary to the development of insulin resistance syndromes. Thus, the fructose-induced hypertensive rat is an appropriate animal model for evaluating the possible therapeutic effects of antihypertensive regimens on the hypertensive diabetic population.

Red-mold rice prepared by use of *Monascus* fungi was first mentioned in the ancient Chinese pharmacopoeia, *Pen Chow Kang Mu*, published during the Ming Dynasty in the 14th−17th centuries (*11*). In this text, red-mold rice is characterized as a mild and useful agent for improving blood circulation. *Monascus purpureus*, *Monascus pilosus*, and *Monascus anka* are the representatives of the *Monascus* fungi traditionally used in southern China, Taiwan, Japan, Korea, and Hong Kong as a source of food colorants. Recently, two active hypotensive components, γ-aminobutyric acid (GABA) and acetylcholine chloride, have been extracted from *Monascus pilosus* IFO4520 (*12*), the only antihypertensive *Monascus* species being published so far. Daily ingestion of beni-koji (BK) aqueous extracts prepared from *M. pilosus* produced a mild to moderate reduction in blood pressure in spontaneously hypertensive rats (SHRs) (*13*) and essential hypertensive volunteers (*14*). However, the effects of BK aqueous extract on insulin-resistance syndromes (hyperinsulinemia/insulin resistance and hypertriglyceridemia) have not been evaluated yet. Thus, it is important to cautiously assess the metabolic effects of this aqueous extract before its clinical application, especially in hypertensive diabetic patients.

* Address correspondence to this author at 161, Sec. 6 Min-Chuan East Rd., National Defense Medical Center, Department of Physiology and Biophysics, Taipei, Taiwan 114 (telephone 886-2-87923100, ext. 18622; fax 886-2-87924827; e-mail pshsieh@hotmail.com).

10.1021/jf026157n CCC: $25.00
Published on Web 05/31/2003

The aim of the present study was therefore to evaluate the antihypertensive and metabolic effects of *M. purpureus* M9011, the novel GABA-enriched *Monascus* species, on fructose-induced hypertension associated with insulin resistance/hyperinsulinemia and hypertriglyceridemia in rats. The effective component of the antihypertensive action was also examined.

MATERIALS AND METHODS

Chemicals. Aqueous extract of *M. purpureus* M9011 was produced and kindly provided by the Food Industrial Research Institute (FIRI), Taiwan. The levels of GABA (10 mg/mL) were measured according to the enzymatic colometric method (*15*), and the levels of monacolins (trace) and citrinin (trace) in the M9011 aqueous extract were measured by HPLC. Identification and characterization of GABA, monacolins, and citrinin in *Monascus* sp. have been demonstrated in previous papers (*12, 16*−*19*).

Animals and Treatments. Male Sprague−Dawley (SD) rats (5−6 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The rats were housed in individual cages in an animal room with a constant temperature of 22 ± 1 °C and a fixed 12 h light−dark cycle. The rats were allowed free access to regular laboratory rat chow and water before the study. Thereafter, the rats were divided into seven groups: control (C, $N = 9$), M9011-treated alone from week 0 (CM0, $N = 9$), fructose-treated alone (F, $N = 9$), fructose and M9011-treated from week 0 (FM0, $N = 9$), fructose and M9011-treated from the end of week 6 (FM6, $N = 9$), fructose and GABA-treated from week 0 (FG0, $N = 9$), and fructose and GABA-treated from the end of week 6 (FG6, $N = 9$).

Starting at week 0, group C was continuously on regular diet. Group F was fed a high-fructose diet. Groups CM0 and FM0 were, respectively, fed regular or fructose diets (60% fructose, 12% fat, 22% protein, TD89247, Teklad Primer Laboratory, Madison, WI) combined with intragastric administration of M9011 aqueous extract (containing GABA at 1 mg·kg^{-1}·day^{-1}) from week 0. Group FM6 was fed a fructose diet from week 0 and then administered M9011 from the end of week 6. Group FG0 received the fructose diet and GABA (1 mg·kg^{-1}·day^{-1}) from week 0. Group FG6 was given the fructose diet from week 0 and then was administered with the same dose of GABA at the end of week 6. The systolic blood pressure (SBP), mean arterial pressure (MAP), and heart rate (HR) were measured weekly in all groups. Plasma levels of glucose, insulin, triglyceride, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured every other week in all groups except those with GABA treatment alone. In addition, insulin sensitivity was determined by comparing the insulin/glucose ratio at weeks 0, 2, 4, 6, and 8.

Blood Pressure (BP) Measurements. BP measurements were performed in conscious rats by indirect tail-cuff method (volume-oscillometric method) using a fully automatic blood pressure monitoring system (UR-5000, UEDA) as described previously (*20, 21*). At least 10 BP measurements were carried out in each animal. An average of the six consistent readings of SBP and MAP was taken as the individual SBP and MAP. To validate readings obtained by the indirect tail-cuff method, the MAP was also measured directly by intra-arterial cannulation in 14 rats randomly selected from all groups at the end of the study. Comparison of the mean values of the direct and indirect MAP measurements (107 ± 4 vs 109 ± 3 mmHg) showed a high correlation of 98.5% in these rats.

Chemical Analysis. Blood samples (1 mL) from overnight-fasted rats were collected into heparinized tubes by the tail-bleeding method and were centrifuged for 15 min at 3000*g* to separate plasma. Plasma levels of glucose, triglyceride, total cholesterol, and HDL-C were determined by using appropriate enzymatic colorimetric methods (Roche Mira plus). Plasma insulin levels were measured by solid phase two-site enzyme immunoassay techniques using a commercially available kit provided by ALPCO (rat insulin ELISA kit).

Statistical Analysis. Statistical analysis was performed according to the repeated measurements of one-way analysis of variance (ANOVA) followed by the Bonferroni test. A possibility of $p < 0.05$ was taken to indicate a significant difference between means. Values are expressed as means ± SEM.

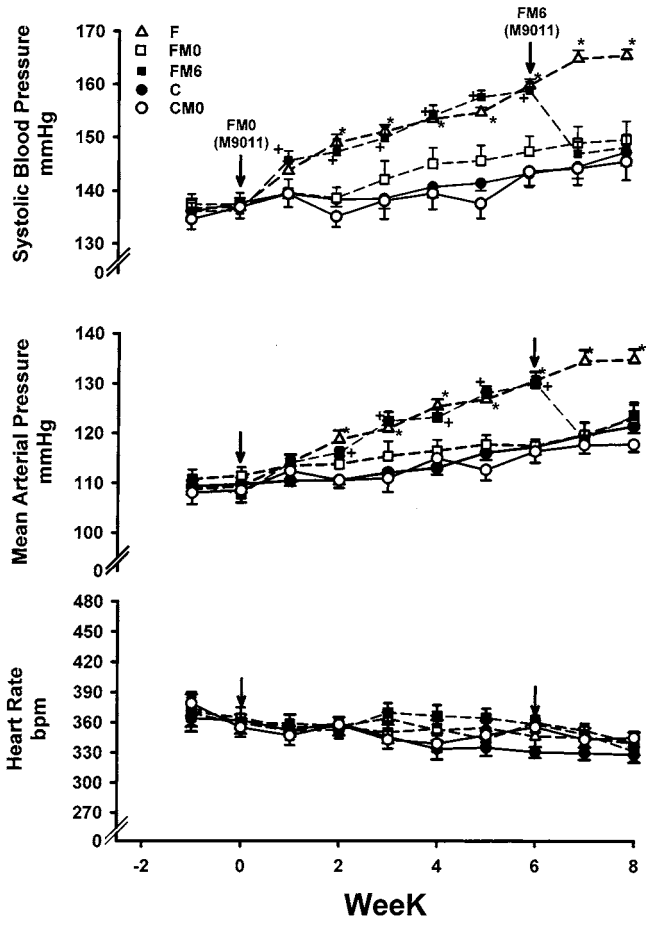

Figure 1. Changes in systolic blood pressure, mean arterial pressure, and heart rate in control (C, ●); control, M9011-treated from week 0 (CM0, ○); fructose (F, △); fructose, M9011-treated from week 0 (FM0, □); and fructose, M9011-treated from week 6 (FM6, ■) during the study period. $N = 9$/group. *, +: $p < 0.05$ versus group C in the corresponding time point.

RESULTS

Effects of M9011 and GABA on SBP, MAP, and HR (Figures 1 and 2). After fructose feeding for 2 weeks, group F exhibited significantly higher SBP and MAP than group C throughout the study. In group FM0, fructose feeding combined with daily administration of M9011 aqueous extract completely prevented the development of fructose-induced hypertension. In group FM6, when hypertension had been established after 6 weeks of fructose feeding, superimposed treatment with M9011 rapidly reversed the elevated blood pressure toward normal level within 1 week. Thereafter, the normalized blood pressure was sustained to the end of the study. However, in group CM0, treatment of the control rats with M9011 from week 0 did not significantly change SBP, MAP, and HR as compared to the same treatment in group C (**Figure 1**).

In group FG0, fructose feeding combined with intragastric loading of pure GABA as the same dose as that used in the M9011-treated groups (1 mg·kg^{-1}·day^{-1}) from week 0 only transiently suppressed the rise of blood pressure for the first 2 weeks. The blood pressure then progressively elevated to a significantly higher level than that seen in group C throughout the remaining period of experiments. However, the magnitude of the elevation of blood pressure in group FG0 was still significantly lower than that obtained in group F after fructose feeding. In group FG6, treatment of fructose-fed rats with pure GABA at a dose of 1 mg·kg^{-1}·day^{-1} from the end of week 6

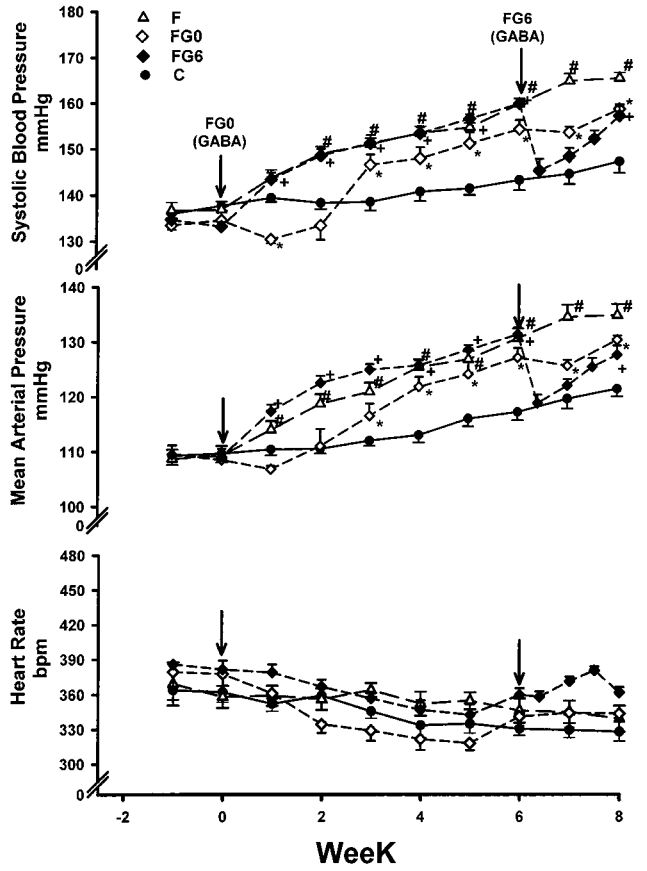

Figure 2. Changes in systolic blood pressure, mean arterial pressure, and heart rate in control (C, ●); fructose (F, △); fructose, GABA-treated from week 0 (FG0, ◇); and fructose, GABA-treated from week 6 (FG6, ◆) during the study period. $N = 9$/group. *, +, #: $p < 0.05$ versus group C in the corresponding time point.

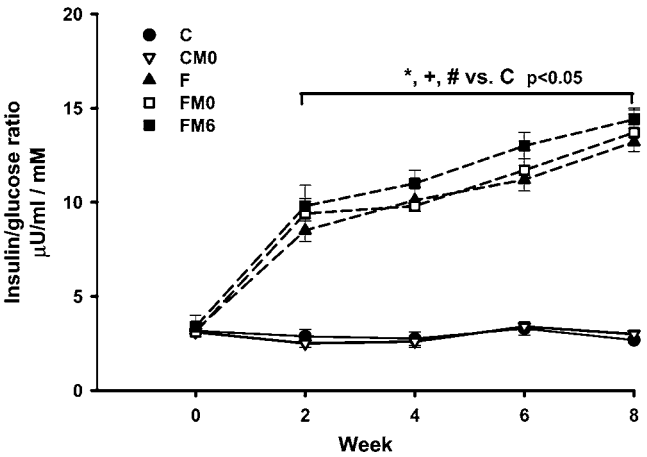

Figure 3. Changes in insulin sensitivity in control (C, ●); control, M9011-treated from week 0 (CM0, △); fructose (F, ▲); fructose, M9011-treated from week 0 (FM0, □); and fructose, M9011-treated from week 6 (FM6, ■), at weeks 0, 2, 4, 6, and 8. $N = 9$/group. *, +, #: $p < 0.05$ versus group C in the corresponding time point.

significantly reduced the elevated blood pressure only for 1 week, and the blood pressure increased significantly thereafter. However, their SBP and MAP levels were still lower than those in group F at the corresponding time points (**Figure 2**).

Effects of M9011 on Insulin Sensitivity, Plasma Insulin and Glucose Levels, and Body Weight (Figure 3; Table 1). During the fructose-feeding period, the insulin/glucose ratios in the fructose-fed groups were significantly higher than those in control groups on regular diet. The elevated insulin/glucose ratio reflected a reduction in insulin sensitivity in the fructose-fed groups. However, M9011 treatment did not affect the insulin sensitivity in control and fructose-fed rats.

Plasma insulin levels were significantly increased in the fructose-fed groups with or without M9011 treatment as compared with those in group C. There were no significant differences in plasma insulin concentration among fructose-fed groups at the corresponding time point. Moreover, M9011 treatment alone did not cause any change in the plasma insulin levels (group CM0).

Plasma glucose levels and body weight were not significantly different among all groups throughout the study. Furthermore, treatment of fructose-fed rats with GABA (1 $mg \cdot kg^{-1} \cdot day^{-1}$) did not affect the plasma insulin and glucose levels, body weight, and insulin sensitivity in group F (data not shown).

Effects of M9011 on the Plasma Levels of Triglyceride, Total Cholesterol, and HDL-C and HDL-C/Total Cholesterol Ratio (Figure 4; Table 2). The plasma triglyceride levels were significantly higher in fructose-fed groups with or without M9011 treatment than in control rats but did not differ significantly among fructose-treated groups. M9011 treatment in control rats did not change the plasma triglyceride levels.

The plasma total cholesterol levels were significantly increased at the 6th and 8th weeks of fructose feeding in group F. Treatment of fructose-fed rats with M9011 completely suppressed the rise of total cholesterol levels in group FM0 throughout the study and also significantly attenuated the total cholesterol levels in group FM6 after M9011 treatment for 2 weeks (**Table 2**).

The plasma HDL-C levels were significantly decreased in groups F and FM6 at weeks 2, 4, 6, and 8 as compared with those in the same group at week 0. Although the HDL-C levels of group FM0 were reduced significantly at weeks 2, 4, and 6, they were returned to a level similar to that of the control period at the end of the study. Furthermore, plasma HDL-C levels were not different between the control rats with and without M9011 treatment throughout the study (group CM0 versus group C).

After fructose feeding for 2 and 4 weeks, the HDL-C/total cholesterol ratios reduced significantly in the fructose-fed groups as compared with those of group C but did not differ among the three fructose-fed groups with or without M9011 treatment in the same period. However, after the combined treatment of M9011 for 6 weeks, the HDL-C/total cholesterol ratio of group FM0 gradually increased and did not differ significantly from that of group C at weeks 6 and 8.

DISCUSSION

The present results demonstrated that treatment with M9011 effectively prevented and reversed fructose-induced hypertension but did not affect the blood pressure levels of the normotensive rats fed regular diet. Although earlier studies reported that two hypotensive components (acetylcholine chloride and GABA) were isolated from *M. pilosus*, only GABA but not acetylcholine has been considered to be the important antihypertensive substance in the *Monascus* species. This is because the action of acetylcholine is easily hydrolyzed by cholinesterase in the body (*22*) and peripherally administered GABA produced comparatively prolonged hypotension (*23*). There are no studies available to quantify the hypotensive effect of the GABA component in *Monascus* species. In our present study, GABA-enriched *M. purpureus* M9011 was selected and the GABA concentration in its water extract was used as a biomarker. We found that M9011 contained a potent antihypertensive substance

Table 1. Effects of Aqueous Extract of M9011 on Plasma Insulin and Glucose Levels and Body Weight[a]

	week/group	C	CM0	F	FM0	FM6
insulin (μU/mL)	0	25 ± 2	23 ± 1	25 ± 1	24 ± 2	25 ± 4
	2	22 ± 3	18 ± 2	65 ± 4*	73 ± 11*	77 ± 3*
	4	22 ± 3	19 ± 2	78 ± 6*	72 ± 5*	85 ± 5*
	6	26 ± 3	25 ± 2	87 ± 11*	88 ± 8*	98 ± 5*
	8	20 ± 1	21 ± 1	103 ± 16*	96 ± 7*	106 ± 5*
glucose (mM)	0	7.8 ± 0.4	7.5 ± 0.2	7.8 ± 0.3	7.5 ± 0.2	7.5 ± 0.3
	2	7.8 ± 0.4	7.4 ± 0.2	7.7 ± 0.3	7.8 ± 0.1	8.0 ± 0.3
	4	7.8 ± 0.3	7.4 ± 0.1	7.6 ± 0.2	7.4 ± 0.2	7.8 ± 0.2
	6	7.8 ± 0.2	7.4 ± 0.2	7.6 ± 0.2	7.5 ± 0.2	7.8 ± 0.2
	8	7.6 ± 0.3	7.1 ± 0.3	7.1 ± 0.2	7.0 ± 0.2	7.4 ± 0.1
BW (g)	0	263 ± 10	250 ± 10	259 ± 7	262 ± 6	266 ± 6
	2	388 ± 8	402 ± 14	388 ± 13	414 ± 6	424 ± 11
	4	463 ± 9	464 ± 15	476 ± 14	476 ± 6	496 ± 15
	6	520 ± 11	507 ± 19	522 ± 12	527 ± 8	551 ± 16
	8	544 ± 8	525 ± 22	559 ± 14	544 ± 9	574 ± 19

[a] Data are presented as the mean ± SE from nine rats per group. Group C, on regular diet; group CM0, on regular diet with M9011 from week 0; group F, on 60% fructose diet; group FM0, on 60% fructose diet with M9011 from week 0; group FM6, on 60% fructose diet and start M9011 from the end of week 6. The dose of M9011 was calculated as the GABA content (1 mg·kg^{-1}·day^{-1}) in aqueous extract of M9011. *, $p < 0.05$ versus the values of group C in the corresponding time period.

Table 2. Effects of Aqueous Extract of Plasma Triglyceride, Total Cholesterol, and HDL-C Levels[a]

	week/group	C	CM0	F	FM0	FM6
TG (mg/dL)	0	133 ± 18	133 ± 9	125 ± 12	111 ± 4	118 ± 10
	2	169 ± 14	144 ± 7	532 ± 51*	556 ± 30*	552 ± 33*
	4	166 ± 13	150 ± 15	589 ± 59*	604 ± 63*	607 ± 59*
	6	122 ± 5	136 ± 15	549 ± 38*	640 ± 82*	627 ± 67*
	8	132 ± 8	124 ± 13	503 ± 50*	498 ± 30*	479 ± 40*
total cholesterol (mg/dL)	0	73 ± 1	78 ± 2	78 ± 2	74 ± 4	73 ± 3
	2	72 ± 2	74 ± 4	79 ± 3	78 ± 5	76 ± 3
	4	73 ± 2	81 ± 5	73 ± 4	76 ± 4	84 ± 3
	6	75 ± 2	80 ± 4	93 ± 8*,+	80 ± 3	96 ± 2*,+
	8	78 ± 1	72 ± 3	94 ± 9*,+	76 ± 4#	89 ± 2+
HDL-C (mg/dL)	0	53 ± 1	51 ± 2	51 ± 2	53 ± 1	51 ± 2
	2	47 ± 3	42 ± 3	21 ± 2*,+	14 ± 1*,+,#	15 ± 3*,+
	4	45 ± 4	51 ± 4	32 ± 4*,+	27 ± 3*,+	27 ± 5*,+
	6	43 ± 4	46 ± 5	35 ± 5*,+	31 ± 5*,+	33 ± 7*,+
	8	39 ± 3	42 ± 2	37 ± 4*,+	48 ± 7	29 ± 4*,+

[a] Data are presented as the mean ± SE from nine rats per group. Group C, on regular diet; group CM0, on regular diet with M9011 from week 0; group F, on 60% fructose diet; group FM0, on 60% fructose diet with M9011 from week 0; group FM6, on 60% fructose diet and start M9011 from the end of week 6. The dose of M9011 was calculated as the GABA content (1 mg·kg^{-1}·day^{-1}) in aqueous extract of M9011. *, $p < 0.05$ versus the values of group C in the corresponding time point; +, $p < 0.05$ versus the control values of the same group; #, $p < 0.05$ versus the values of group F in the corresponding time point.

that not only completely prevented the genesis of fructose-induced hypertension but also effectively reversed the elevated blood pressure in rats on fructose-enriched diet for 6 weeks. However, in the fructose-fed rats at the same dose of GABA administration as those contained in M9011 the development of fructose-induced hypertension was only partially suppressed and the elevated blood pressure was attenuated but not reversed. The present results clearly demonstrate that, in addition to GABA, other active antihypertensive component(s) may exist in the aqueous extract of M9011.

The aqueous extract of M9011 has a high level of GABA and other components but no monacolin, a potent inhibitor of cholesterol biosynthesis, which has been applied in clinical therapy. The present data show that the water extract of M9011 can prevent the rise of total cholesterol and accelerate the increase of HDL-C in chronically fructose-fed rats. This observation implies that other cholesterol-lowering agent(s) besides monacolins may be present in the aqueous extract of M9011. Recently, Wang et al. (*24*) compared the anti-hyperlipidemic effects of anka (a fermented rice product of another *Monascus* species) with that of lovastatin (monacolin K, an inhibitor of 3-hydro-3-methylglutaryl CoA reductase, the rate-limiting enzyme in cholesterol synthesis) on rats fed a 30% high-fructose diets for 6 months. Their results demonstrated that anka treatment not only lowered total cholesterol levels as lovastatin did but also significantly decreased the VLDL-C levels while slightly increasing the HDL-C levels. Their results were consistent with our present observations. Both studies suggest that an additional hypocholesterolemic substance(s) besides monacolin may exist in *Monascus* species and that the hypocholesterolemic action is not mediated by the same mechanism as monacolin. The cholesterol-lowering action remains to be further investigated.

Multicenter studies have suggested that an efficacious antihypertensive therapy can prevent the occurrence of stroke, congestive heart failure, and renal failure in the essential hypertension population (*25*−*27*), but none of the studies has focused on the diabetic population. The results of the present study may be of clinical implication in the diabetic population. It has been recommended that caution should be exercised in the

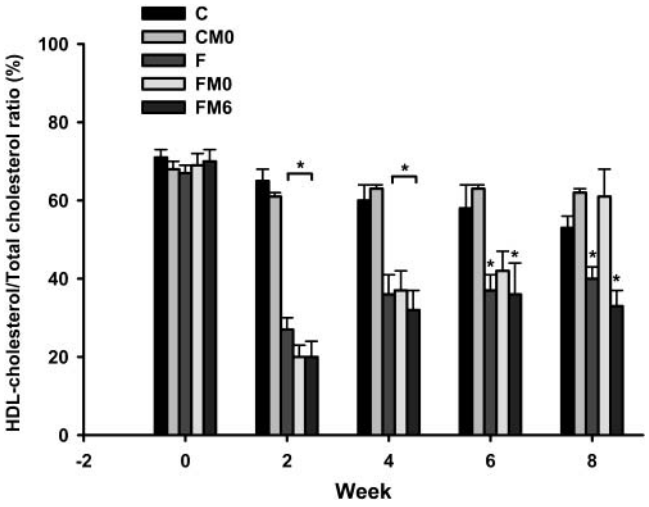

Figure 4. Changes in HDL cholesterol/total cholesterol ratio of the control (C); control, M9011-treated from week 0 (CM0); fructose (F); fructose, M9011-treated from week 0 (FM0); and fructose, M9011-treated from week 6 (FM6) at weeks 0, 2, 4, 6, and 8. $N = 9$/group. *: $p < 0.05$ versus group C in the corresponding time point.

evaluation of the effects of antihypertensive regiments on metabolic parameters. This is because metabolic changes can potentially counteract the beneficial effect of blood pressure reduction, especially in diabetic patients (*28*−*31*). For example, beta-blockers have metabolic effects that may increase the cardiovascular risk, such as elevating serum very low-density lipoprotein levels and decreasing HDL cholesterol levels (*29*, *30*). Diuretic agents are also effective antihypertensive drugs, but their negative effects on glucose control and the plasma lipid profile make them a less desirable choice in the treatment of diabetic individuals (*31*). The aqueous extract of M9011 derived from *M. purpureus* showed a strong antihypertensive effect but did not affect insulin sensitivity or plasma insulin, glucose, and triglyceride levels in fructose-fed and control rats. These results imply that M9011 may be applicable to the primary or secondary prevention of hypertension, especially in diabetic patients.

Monascus fungi have been demonstrated to have several valuable therapeutic activities in different *Monascus* species under variable periods of culture time and conditions. For instance, the monacolin groups have been found in the second metabolites of *Monascus* rubber (*16*−*18*). Moreover, two hypotensive compounds, acetylcholine chloride and GABA, were isolated and identified from red-mold rice prepared with *M. pilosus* IFO4520. These two active agents account, at least in part, for the hypotensive action of the red-mold rice (*13*) in SHR and essential hypertensive volunteers, although *M. pilosus* IFO4520 has only a mild to moderate antihypertensive effect (*14*, *15*). The present study strongly suggests that M9011 may contain potent antihypertensive substance(s) other than GABA and hypocholesterolemic compounds except monacolins.

The sodium and potassium intakes from the aqueous extracts of M9011 were trivial (about 10^{-1}% and 10^{-3}%, respectively) as compared with those in the daily diet in rats and humans. Thus, their impact on blood pressure in the experimental rats can be ignored. On the other hand, another known component, citrinin, does exist in the *Monascus* metabolites and is a mycotoxin with nephrotoxic and hepatotoxic effects (*32*). It is thus essential that the production of *Monascus* species should avoid or reduce the content of citrinin. The citrinin level in M9011 was not detectable. In our preliminary studies, we treated the normal rats with a daily ingestion of M9011 (10 $mg \cdot kg^{-1} \cdot day^{-1}$) that was a 10-fold higher dose than that used in the present study. We did not show any changes in biochemical analysis of renal and hepatic functions and in morphological examination with H−E stain under optical microscope. Furthermore, the present result demonstrated that the control rats with or without M9011 treatment did not show any change in BP and insulin/lipid metabolism, which indirectly suggests that the possible trivial presence of citrinin in M9011 is unable to affect BP and insulin/lipid metabolism. In this study, M9011 treatment did not affect the growth rate of rats in either the fructose-fed or control group throughout the study.

In summary, we have demonstrated that the GABA-enriched aqueous extract prepared from new antihypertensive *Monascus* species (*M. purpureus* M9011) can prevent and reverse fructose-induced hypertension without affecting fructose-induced hyperinsulinemia/insulin resistance and hypertriglyceridemia in rats. However, administration of the same dose of GABA alone as that contained in M9011 caused a significantly less antihypertensive effect than that produced by M9011. Moreover, chronic treatment with M9011 can significantly suppress the increase in the total cholesterol levels and promote the recovery of the HDL-C levels in fructose-fed rats. These data suggest that there are antihypertensive components other than GABA and hypocholesterolemic components other than monacolin in the M9011 aqueous extract. The present study also suggests that M9011 may be a new, novel, and potent food-based antihypertensive agent that can be used not only for lowering blood pressure but also for improving cardiovascular function in the hypertensive and diabetic population.

ACKNOWLEDGMENT

We appreciate the support from the Food Industry Research and Development Institute, Taiwan, especially from Dr. Gwo-Fang Yuan and Aeen-Lin Chen in culture collection and research center of FIRDI. We thank Dr. Wann-Chu Huang for reviewing the manuscript.

LITERATURE CITED

(1) DeFronzo, R. A.; Ferrannini, E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. *Diabetes Care* **1991**, *14*, 173−194.

(2) U.K. Prospective Diabetes Study. III. Prevalence of hypertension and hypotensive therapy in patients with newly diagnosed diabetes. A multicenter study. *Hypertension* **1985**, *7* (6 pt 2), II8−13.

(3) Drury, P. L. Diabetes and arterial hypertension. *Diabetologia* **1983**, *23*, 1−9.

(4) Fuller, J. H. Epidemiology of hypertension associated with diabetes mellitus. *Hypertension* **1985**, *7* (6 Pt 2), II3−7.

(5) Parving, H. H.; Andersen, A. R.; Smidt, U. M.; Hommel E.; Mathiesen, E. R.; Svendsen, P. A. Effect of antihypertensive treatment on kidney function in diabetic nephropathy. *Br. Med. J. Clin. Res. Ed.* **1987**, *294* (6585), 1443−1447.

(6) Hasslacher, C.; Stech, W.; Wahl, P.; Ritz, E. Blood pressure and metabolic control as risk factors for nephropathy in type 1 (insulin-dependent) diabetes. *Diabetologia* **1985**, *28* (1), 6−11.

(7) Hypertension in Diabetes Study (HDS) II. Increased risk of cardiovascular complications in hypertensive type 2 diabetic patients. *J. Hypertens.* **1993**, *11* (3), 319−325.

(8) Janka, H. U.; Warram, J. H.; Rand, L. I.; Krolewski, A. S. Risk factors for progression of background retinopathy in long-standing IDDM. *Diabetes* **1989**, *38* (4), 460−464.

(9) Hwang, I. S.; Ho, H.; Hoffman, B. B.; Reaven, G. M. Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **1987**, *10*, 512−516.

(10) Hwang, I. S.; Huang, W. C.; Wu, J. N.; Shieh, L. R.; Reaven, G. M. Effect of fructose-induced hypertension on the renin-angiotensin-aldosterone system and atrial natriuretic factor. *Am. J. Hypertens.* **1989**, *2*, 424−427.

(11) Ying, S. S.; T'ien Jung K'ai, Wu. *Chinese Technology in the Seventeenth Century*; Sun, E.-T., Sun, S.-C., transl.; Pennsylvania State University Press: State College, PA, 1966; pp 291−294.

(12) Kohama, Y.; Matsumoto, S.; Mimura, T.; Tanabe, N.; Inada, A.; Nakanishi, T. Isolation and identification of hypotensive principles in red-mold rice. *Chem. Pharm. Bull.* **1987**, *35* (6), 2484−2489.

(13) Tsuji, K.; Ichikawa, T.; Tanabe, N.; Abe, S.; Tarui, S.; Nakagawa, Y. Antihypertensive activity of beni-koji extracts and γ-aminobutyric acid in spontaneously hypertensive rats. *Jpn. J. Nutr.* **1992**, *50* (5), 285−291.

(14) Kushiro, T.; Hashida, J.; Kawamura, H.; Mitsubayashi, H.; Saito, T.; Suzuki, Y.; Takahashi, N.; Ishii, T.; Kimura, T.; Tsuji, K.; Tanabe, N.; Asano, K.; Abe, S.; Tarui, S. Clinical effects of beni-koji in mild essential hypertension—a multicenter double-blind comparison with placebo. *Jpn. J. Nephrol.* **1996**, *38*, 625−633.

(15) Zhang, G.; Bown, A. W. The rapid determination of γ-aminobutyric acid. *Phytochemistry* **1997**, *44*, 1007−1009.

(16) Endo, A.; Monacolin, K. A new hypocholesterolemic agent produced by a *Monascus* species. *J. Antibiot.* **1979**, *32*, 852−854.

(17) Endo, A.; Monacolin, K. A new hypocholesterolemic agent that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Antibiot.* **1980**, *33*, 334−336.

(18) Endo, A.; Hasumi, K.; Nakamura, T.; Kunishima, M.; Masuda, M. Dihydromonacolin L and monacolin Y new metabolites those inhibit cholesterol biosynthesis. *J. Antibiot.* **1985**, *38*, 321−327.

(19) Blanc, P. J.; Laussac, J. P.; Bars, J.; LeBars, P.; LeLoret, M. D.; Pareilleux, A.; Prome, D.; Prome, J. C.; Santerre, A. L.; Goma, G. Characterization of monascidin A from *Monascus* as citrinin. *Int. J. Food Microbiol.* **1995**, *27*, 201−213.

(20) Hsieh, P. S.; Huang, W. C. Chemical sympathectomy attenuates hyperinsulinemia- induced hypertension in conscious rats. *Nutr. Metab. Cardiovasc. Dis.* **1993**, *3*, 173−178.

(21) Hsieh, P. S.; Huang, W. C. Neonatal chemical sympathectomy attenuates fructose-induced hypertriglyceridemia and hypertension in rats. *Chin. J. Physiol.* **2001**, *44* (1), 25−31.

(22) Furchgott, R. F.; Zawadzki, J.V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **1980**, *288* (5789), 373−376.

(23) Giuliani, S.; Maggi, C. A.; Meli, A. Differences in cardiovascular responses to peripherally administered GABA as influenced by basal conditions and type of anaesthesia. *Br. J. Pharmacol.* **1986**, *88* (3), 659−670.

(24) Wang, I. K.; Lin-Shiau, S.-Y.; Chen, P.-C.; Lin, J.-K. Hypotriglyceridemic effect of Anka (a fermented rice product of *Monascus* sp.) in rats. *J. Agric. Food Chem.* **2000**, *48*, 3183−3189.

(25) Smith, W. M. Treatment of mild hypertension: results of a ten-year intervention trial. *Circ. Res.* **1977**, *40* (5 Suppl. 1), I98−105.

(26) Veterans administration cooperative study group or antihypertensive agents. Effects of treatment on morbidity in hypertension: results in patients with diastolic blood pressure averaging 115 through 129 mmHg. *JAMA—J. Am. Med. Assoc.* **1967**, *202*, 1028.

(27) Veterans administration cooperative study group or antihypertensive agents: effects of treatment on morbidity in hypertension: results in patients with diastolic blood pressure averaging 90 through 114 mmHg. *JAMA—J. Am. Med. Assoc.* **1970**, *213*, 143.

(28) Hebert, P. R.; Marson, J.; Henrekers, C. H. Pharmacologic therapy of mild to moderate hypertension: possible generalizability to diabetes. *J. Am. Soc. Nephrol.* **1992**, *3*, S135.

(29) Weidmann, P.; Uehlinger, D. E.; Gerber, A. Antihypertensive treatment and serum lipoproteins. *J. Hypertens.* **1985**, *3*, 297.

(30) Weinberger, M. H. Antihypertensive therapy and lipids: evidence, mechanisms and implications. *Arch. Intern. Med.* **1985**, *145*, 1102.

(31) Warram, J. H.; Laffel, L. M.; Valsania, P.; Christlieb, A. R.; Krolewski, A. S. Excess mortality associated with diuretic therapy in diabetes mellitus. *Arch. Intern. Med.* **1991**, *151*, 1350.

(32) Bilgrami, K. S.; Sinha, S. P.; Jeswal, P. Nephrotoxic and hepatotoxic effects of citrinin in mice. *Proc. Indian Natl. Sci. Acad.* **1988**, *54*, 35−37.

Received for review November 24, 2002. Revised manuscript received March 24, 2003. Accepted April 23, 2003. This study was supported by the National Science Council of the ROC under Grants NSC-89-2320-B-016-086 and NSC-90-2316-B-016-001.

JF026157N