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Research Paper

Effects of morroniside isolated from Corni Fructus on renal lipids and inflammation in type 2 diabetic mice

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Abstract

Objectives The effects of morroniside isolated from Corni Fructus on renal lipids and inflammation provoked by hyperglycaemia were investigated using type 2 diabetic mice.
Methods Morroniside was administered orally to *db*/*db* mice at 20 or 100 mg/kg daily for 8 weeks, and its effects were compared with those in vehicle-treated *db*/*db* and *m*/*m* (non-diabetic) mice. Serum and renal biochemical factors and protein expression related to lipid homeostasis and inflammation were measured.
Key findings Morroniside produced significant dose-dependent reductions in serum triglyceride and renal glucose and lipid levels. Morroniside altered the abnormal protein expression of sterol regulatory element binding proteins (SREBP-1 and SREBP-2). In addition, the formation of reactive oxygen species and lipid peroxidation were inhibited in the morroniside-treated *db*/*db* mouse group, and the ratio of reduced glutathione to the oxidised form was significantly elevated. These results suggest that morroniside alleviated oxidative stress in the kidneys of *db*/*db* mice. Furthermore, 100 mg/kg morroniside down-regulated the expression of nuclear factor-κBp65, cyclooxygenase-2 and inducible nitric oxide synthase augmented in *db/db* mice.
Conclusions Morroniside may inhibit abnormal lipid metabolism and inflammation due to reactive oxygen species in the kidneys in type 2 diabetes.
Keywords inflammation; kidney; morroniside; oxidative stress; SREBP; type 2 diabetes

Introduction

Diabetes is a progressive metabolic abnormality associated with high blood glucose levels, caused by a defect in cellular consumption and/or excessive production. It is also associated with elevated lipid production, resulting in lipid accumulation in major organs such as the heart, liver and kidneys. In particular, renal lipid deposits are responsible for the pathogenesis of diabetic kidney disease.[1,2] In the kidney, abnormal metabolism and an accumulation of lipids is proposed to play a role in the pathogenesis of diabetic nephropathy.[3,4] A recent study demonstrated that, in diabetes, hyperglycaemia *per se* directly up-regulates renal expression of the transcriptional factors sterol regulatory element binding proteins (SREBP-1 and SREBP-2), which caused increased synthesis of fatty acids and cholesterol as well as the accumulation of lipids in the kidney.[5] Another transcriptional factor related to lipid metabolism, peroxisome proliferator activated receptor α (PPARα), was also down-regulated in the kidneys of animals with experimental diabetes.[6,7] An increase in PPARα expression is related to an anti-obesity effect through the transcription of regulatory enzymes of fatty acid oxidation.[8]

Accumulating evidence suggests that oxidative stress and inflammatory processes are crucial in the pathogenesis of metabolic disorders, including obesity and diabetes.[9] Oxidative stress partially results from increased generation of reactive oxygen species (ROS) and the reduced regeneration of an important cellular antioxidant, reduced glutathione (GSH) from the oxidised form (GSSG). In type 2 diabetes, the stress-sensitive intracellular signalling pathway is altered.[10] One major intracellular target of hyperglycaemia and oxidative stress is the nuclear κB transcription factor (NF-κB). Moreover, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) may accelerate inflammation-mediated insulin resistance.[11] Accordingly, the attenuation of oxidative stress and regulation of

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inflammatory responses have been considered as ways to alleviate diabetes and its complications.[12,13]

Corni Fructus (*Cornus officinalis* Sieb. *et* Zucc.) is a traditional medicinal plant which is a rich source of iridoid glycosides, loganin and morroniside, which exhibit powerful antioxidant activity.[14,15] In our previous studies, we discovered that Corni Fructus exerted beneficial effects on hyperglycaemia and related renal damage in rats with streptozotocin-induced type 1 diabetes.[16] Moreover, the administration of morroniside isolated from Corni Fructus could ameliorate hyperglycaemia, oxidative stress and inflammation in the kidneys of diabetic rats.[17] We also found that morroniside could prevent diabetic nephropathy by inhibiting the formation of advanced glycation endproduct (AGE) and suppressing expression of AGE receptors (RAGE).[17] However, the effects of morroniside on the kidneys in type 2 diabetes, one of the most susceptible tissues in the diabetic condition, have yet to be determined. We therefore investigated the effect of morroniside on metabolic parameters associated with the development of diabetic damage in the kidneys of C57BLKS/J *db/db* mice. These mice are characterised by obesity, sustained hyperglycaemia, hyperlipidaemia and hyperinsulinaemia as a result of destroyed leptin receptors with the C57BLKS/J background.[18,19] We examined the effects of morroniside on renal lipids and inflammation provoked by hyperglycaemia in *db/db* mice.

Materials and Methods

Materials

Morroniside was isolated from Corni Fructus, as described previously.[17] Protease inhibitor mixture solution, 4,6-dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid, TBA), EDTA, GSH and GSSG were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was purchased from Molecular Probes (Eugene, OR, USA). The Bio-Rad protein assay kit and pure nitrocellulose membrane were purchased from Bio-Rad Laboratories (Tokyo, Japan). β-Actin, *o*-phthalaldehyde, and *N*-ethylmaleimide were purchased from Sigma Chemical Co. (St Louis, MO, USA). Rabbit polyclonal antibodies against NF-κBp65, SREBP-1, SREBP-2 and PPARα, mouse monoclonal antibodies against COX-2 and iNOS, and goat anti-rabbit and goat anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). ECL Western blotting detection reagents were purchased from Amersham Bioscience (Piscataway, NJ, USA).

Experimental animals and treatment

The Guidelines for Animal Experimentation approved by the Ethics Committee of the University of Toyama were followed in this study (Registration no. S-2006 INM-22).

Five-week-old male C57BLKS/J *db/db* and *m/m* mice were purchased from Japan SLC Inc. (Hamamatsu, Japan) and housed with free access to laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan) and water. They were maintained in a controlled environment (22 ± 2°C, 50 ± 5% humidity, 12-h light–dark cycle starting at 7:00 am).

After adaptation, glucose levels in blood taken from the tail vein of *db/db* mice were 443–446 mg/dl and body weight was 41–42 g.

The *db/db* mice were divided into three groups (n = 10 per group), treated orally with vehicle (water), morroniside 20 mg/kg or morroniside 100 mg/kg daily for 8 weeks. Food and water intake was determined daily.

Non-diabetic *m/m* mice (n = 6) as a normal group were compared with the diabetic groups.

At the end of the 8 weeks' treatment, blood samples were collected from the inferior vena cava of anaesthetised mice. Serum was separated immediately after centrifugation. Subsequently, each mouse was perfused with ice-cold physiological saline, and the kidneys were harvested, snap-frozen in liquid nitrogen and stored at −80°C until analysis.

Analysis of serum samples

Serum glucose, triglyceride, total cholesterol and creatinine levels were measured using commercial kits (Glucose CII-Test, Triglyceride E-Test and Cholesterol E-Test from Wako Pure Chemical Industries, Ltd; CRE-EN Kainos from Kainos Laboratory Inc., Tokyo, Japan).

Determination of renal glucose and lipid contents

To measure the glucose concentration in the kidney, tissue was homogenised with 0.9% NaCl supplemented with 0.15 mol/l $Ba(OH)_2$ and 5% $ZnSO_4$, and then centrifuged at 1400*g* for 15 min at 4°C.[20] The glucose concentration in the supernatant was measured using a commercial kit (Glucose CII-Test, Wako Pure Chemical Industries). Total renal lipids were extracted with a mixture of chloroform and methanol (2 : 1 v/v) according to the method of Folch *et al.*[21] Levels of triglyceride and total cholesterol were measured using the commercial kits described above.

Assay of TBA-reactive substance levels and ROS generation

Levels of TBA-reactive substance (TBARS) were estimated according to the method of Mihara and Uchiyama.[22] ROS generation was measured by the method of Ali *et al.*[23] Renal tissue was homogenised on ice with 1 mmol/l EDTA, 50 mmol/l sodium phosphate buffer (pH 7.4). In brief, 25 mmol/l DCFH-DA was added to homogenates, and, after 30 min, changes in fluorescence were determined at an excitation wavelength of 486 nm and emission wavelength of 530 nm. ROS generation was calculated as ROS generation (%) = ([final value − initial value]/initial value) × 100.

Determination of GSH and GSSG levels

GSH and GSSG were measured using the method of Hissin and Hilf.[24] Renal tissue was homogenised on ice with 1 mmol/l EDTA, 100 mmol/l sodium phosphate buffer (pH 8.0), followed by the addition of 25% meta-phosphoric acid to precipitate the protein. The homogenate was centrifuged at 100 000*g* for 30 min at 4°C.

For the GSH assay, the supernatant was diluted with buffer followed by *o*-phthalaldehyde. For determination of the GSSG

concentration, after preincubation with *N*-ethylmaleimide for 20 min, 0.1 mol/l NaOH was used instead of sodium phosphate buffer (pH 8.0). After 15 min at room temperature, fluorescence was estimated at an excitation wavelength of 360 nm and emission wavelength of 460 nm.

The protein concentration was measured according to the method of Itzhaki and Gill[25] using bovine serum albumin (BSA) as the standard.

Preparation of nuclear and post-nuclear fractions

To prepare nuclear fractions, the kidney was homogenised with ice-cold lysis buffer containing 5 mmol/l Tris-HCl (pH 7.5), 2 mmol/l $MgCl_2$, 15 mmol/l $CaCl_2$ and 1.5 mol/l sucrose; 0.1 mol/l dithiothreitol (DTT) and protease inhibitor cocktail were then added. Samples were centrifuged at 10 500*g* for 20 min at 4°C and the pellet suspended in nuclear extraction buffer (20 mmol/l 2[4-(2-hydroxyethyl)-1-piperazyl] ethanesulfonic acid (pH 7.9), 1.5 mmol/l $MgCl_2$, 0.42 mol/l NaCl, 0.2 mmol/l EDTA, 25% (v/v) glycerol, 0.1 mol/l DTT and protease inhibitor cocktail). The mixture was placed on ice for 30 min and then centrifuged at 20 500*g* for 5 min at 4°C to prepare the nuclear fraction.

The post-nuclear fraction was extracted from the kidney of each mouse as follows. In brief, renal tissue was homogenised with ice-cold lysis buffer (pH 7.4) containing 137 mmol/l NaCl, 20 mmol/l Tris-HCl, 1% Tween 20, 10% glycerol, 1 mmol/l phenylmethylsulfonyl fluoride and protease inhibitor mixture. The homogenate was centrifuged at 2000*g* for 10 min at 4°C.

The protein concentration of each fraction was determined using the Bio-Rad protein kit, with BSA as the standard.

Western blot analysis

Post-nuclear protein (30 μg) for determination of iNOS and COX-2 expression and nuclear protein (30 μg) for NF-κBp65, SREBP-1, SREBP-2 and PPARα were electrophoresed in 8% sodium dodecyl sulfate-polyacrylamide gel. Separated proteins were transferred to a pure nitrocellulose membrane, blocked with 5% skimmed milk solution for 1 h, and then incubated with primary antibodies overnight at 4°C. After washing, the membrane was incubated with goat anti-rabbit or goat anti-mouse IgG HRP-conjugated secondary antibody for 1 h at room temperature. Antigen–antibody complexes were visualised using ECL Western blotting detection reagents and chemiluminescence (LAS-1000 plus; Fujifilm, Tokyo, Japan). Band densities were calculated using an image analyser (Atto, Tokyo, Japan) and normalised to β-actin.

Statistical analysis

All results are expressed as the mean ± SEM. Individual differences between groups were analysed using one-way analysis of variance followed by Dunnett's test; $P < 0.05$ was considered significant.

Results

Body weight and food and water intake

The food and water intake and body weight after 8 weeks were significantly higher in *db/db* mice than age-matched *m/m* mice. The administration of morroniside did not affect the food intake or body weight but significantly decreased water intake by 25% compared with control *db/db* mice (Table 1).

Serum glucose, lipids and creatinine

Serum glucose, triglyceride and total cholesterol levels were significantly higher in the control *db/db* than in the *m/m* mice, while the creatinine concentration was lower. The administration of morroniside did not alter the concentrations of serum glucose, total cholesterol or creatinine. However, it dose-dependently reversed the rise in serum triglyceride level compared with the control *db/db* mice (Table 2).

Renal weight and glucose and lipid levels

Renal weight expressed relative to body weight was lower in the three groups of *db/db* mice than in the *m/m* mice. The glucose level in the kidneys of *db/db* vehicle mice was increased more than 3.3-fold compared with that of *m/m* mice. There was a 1.8-fold increase in the triglyceride content and a 2.1-fold increase in the cholesterol content in the kidneys of *db/db* vehicle mice compared with *m/m* mice. These increases in renal glucose, triglyceride and cholesterol levels were significantly reduced by administration of 20 or 100 mg morroniside, as shown in Table 3.

Biomarkers associated with oxidative stress in the kidney

Renal TBARS levels were increased by 1.5 times in the *db/db* vehicle mice compared with *m/m* mice (1.90 vs 1.24 nmol/mg tissue, $P < 0.05$). However, this elevation in renal TBARS levels was significantly reduced in morroniside-treated *db/db*

Table 1 Change in body weight over 8 weeks and food and water intake

Group	Body weight (g)			Food intake (g/day)	Water intake (ml/day)
	Initial	Final	Gain		
m/m mice	18.6 ± 1.8*	25.4 ± 0.9*	6.4 ± 0.1*	2.7 ± 0.2*	4.1 ± 0.2*
db/db mice					
Vehicle	41.4 ± 0.3	55.2 ± 2.4	13.8 ± 1.2	7.0 ± 0.2	15.4 ± 1.2
Morroniside 20 mg/kg	42.5 ± 0.7	57.9 ± 1.3	15.4 ± 0.7	7.4 ± 0.1	15.4 ± 0.5
Morroniside 100 mg/kg	41.6 ± 0.4	57.5 ± 1.3	15.9 ± 0.5	6.6 ± 0.2	11.6 ± 0.9*

*$P < 0.05$ vs *db/db* vehicle-treated mice.

Table 2 Analysis of serum glucose, lipids and creatinine

Group	Glucose (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	Creatinine (mg/dl)
m/m mice	219.7 ± 12.0*	89.6 ± 5.7*	74.0 ± 1.3*	0.40 ± 0.05*
db/db mice				
Vehicle	765.2 ± 44.8	298.7 ± 24.6	168.5 ± 8.3	0.26 ± 0.02
Morroniside 20 mg/kg	753.8 ± 34.2	229.8 ± 24.8*	160.4 ± 11.2	0.27 ± 0.02
Morroniside 100 mg/kg	713.6 ± 32.3	175.4 ± 19.1*	173.8 ± 4.2	0.27 ± 0.04

*$P < 0.05$ vs *db/db* vehicle-treated mice.

Table 3 Kidney weight and renal glucose, triglyceride and total cholesterol contents

Group	Kidney weight		Glucose	Triglyceride	Total cholesterol
	(g)	(g/100 g body weight)			
m/m mice	0.35 ± 0.02*	1.39 ± 0.07*	97.2 ± 7.7*	26.1 ± 1.1*	20.7 ± 1.1*
db/db mice					
Vehicle	0.49 ± 0.01	0.91 ± 0.11	320.7 ± 16.0	45.9 ± 3.8	43.2 ± 4.7
Morroniside 20 mg/kg	0.52 ± 0.01	0.91 ± 0.02	230.6 ± 22.6*	31.7 ± 2.8*	25.7 ± 1.3*
Morroniside 100 mg/kg	0.48 ± 0.01	0.85 ± 0.03	220.1 ± 18.2*	26.4 ± 1.8*	24.5 ± 0.8*

Glucose and lipid concentrations are given in mg/kidney per 100 g body weight. *$P < 0.05$ vs *db/db* vehicle-treated mice.

Table 4 Biomarkers associated with oxidative stress in the kidney

	TBARS (nmol/mg protein)	ROS generation (%)	GSH concn (μmol/mg protein)	GSSG concn (μmol/mg protein)	GSH/GSSG ratio
m/m mice	1.24 ± 0.03*	154.9 ± 2.4*	7.44 ± 0.25*	6.29 ± 0.43*	1.20 ± 0.05*
db/db mice					
Vehicle	1.90 ± 0.09	220.4 ± 13.2	4.45 ± 0.15	5.12 ± 0.31	0.89 ± 0.05
Morroniside 20 mg/kg	1.54 ± 0.07*	144.0 ± 6.6*	6.61 ± 0.30*	6.24 ± 0.19*	1.07 ± 0.06*
Morroniside 100 mg/kg	1.30 ± 0.06*	126.4 ± 3.8*	7.41 ± 0.26*	6.33 ± 0.10*	1.17 ± 0.03*

GSH, reduced glutathione; GSSG, oxidised glutathione; ROS, reactive oxygen species; TBARS, 2-thiobarbituric acid reactive substance. *$P < 0.05$ vs *db/db* vehicle-treated mice.

mouse groups, and almost to the levels of *m/m* mice by 100 mg/kg morroniside.

In the morroniside-treated groups, elevated ROS generation in *db/db* vehicle mice was suppressed to almost the level of the *m/m* group.

In addition, the *db/db* vehicle group showed a significant reduction in the GSH/GSSG ratio compared with the *m/m* group, which resulted from the marked decrease in the GSH concentration in the kidney. This reduction in the renal GSH/GSSG ratio in *db/db* vehicle mice was recovered to nearly the level of *m/m* mice with 100 mg/kg morroniside (Table 4).

Protein expression related to lipid metabolism in the kidney

The renal expression of SREBP-1 and SREBP-2 proteins was significantly higher in *db/db* mice than *m/m* mice. *db/db* mice given 100 mg/kg morroniside showed significantly reduced expression of SREBP-1 and SREBP-2 proteins compared with the *db/db* vehicle mice (Figure 1a and b).

There were no significant differences between the experimental groups in the renal expression of PPARα protein (Figure 1c).

Expression of inflammatory proteins in the kidney

Expression of NF-κBp65, COX-2 and iNOS proteins were significantly up-regulated in the *db/db* vehicle group compared with the *m/m* group (Figure 2). These increases in the expression of proteins related to inflammation and ROS generation in the kidney were markedly reduced by the administration of morroniside, which indicates the deactivation of NF-κB and down-regulation of NF-κB-related COX-2 and iNOS.

Discussion

Corni Fructus has many biological constituents, such as polyphenols and iridoid glycosides, loganin and morroniside.[14] Among them, morroniside has been reported as the main active constituent of Corni Fructus, with several bioactivities such as antioxidant,[15] neuroprotective[26] and anti-inflammatory effects.[27] Furthermore, morroniside has proven effective in preventing diabetic complications such as diabetic angiopathy and the early stages of diabetic nephropathy by regulating renal mesangial cell growth through

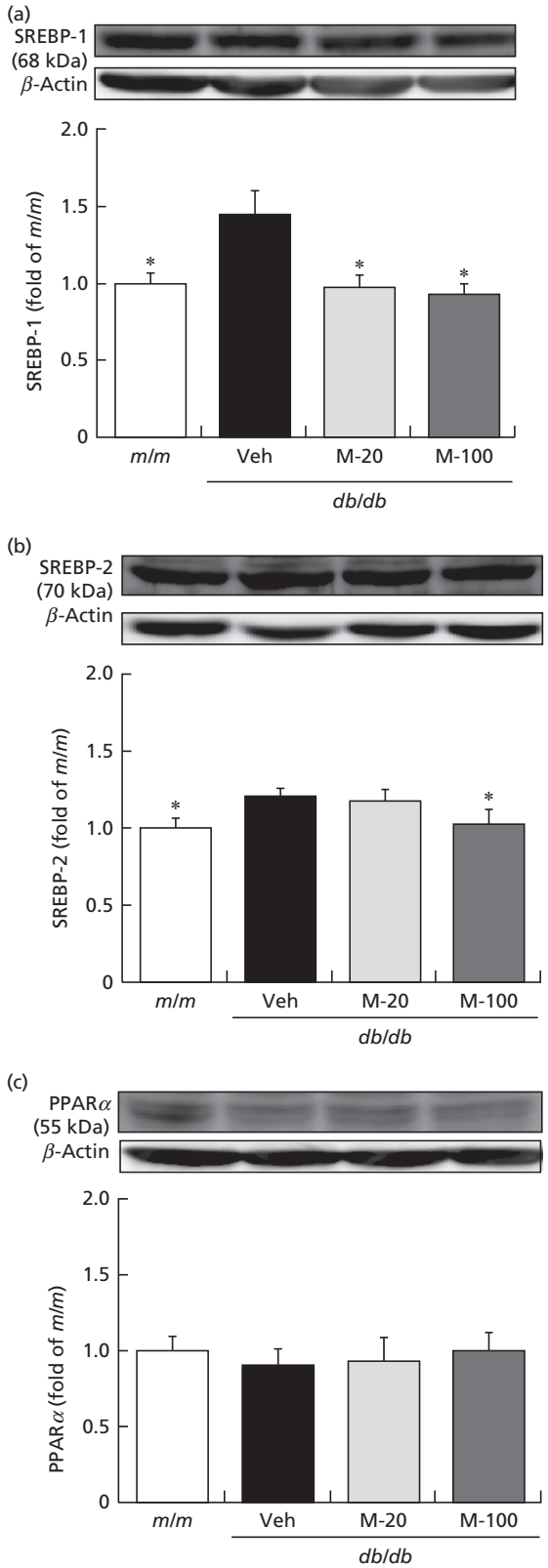

Figure 1 Expression of (a) SREBP-1, (b) SREBP-2 and (c) PPARα in the kidneys of *m/m* and *db/db* mice. Veh, vehicle; M-20, morroniside 20 mg/kg; M-100, morroniside 100 mg/kg. Values are means ± SE (n = 6 or 10). $^{*}P < 0.05$ vs *db/db* vehicle-treated mice

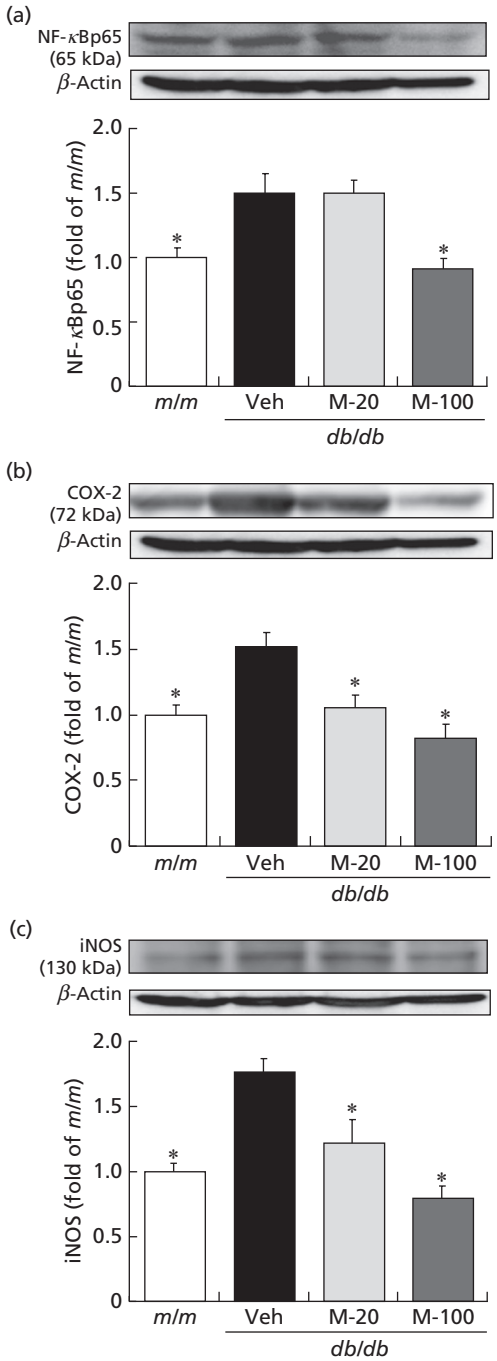

Figure 2 Expression of (a) NF-κBp65, (b) COX-2 and (c) iNOS in the kidneys of *m/m* and *db/db* mice. Veh, vehicle; M-20, morroniside 20 mg/kg; M-100, morroniside 100 mg/kg. Values are means ± SE (n = 6 or 10). $^{*}P < 0.05$ vs *db/db* vehicle-treated mice

inhibiting oxidative stress.[28,29] Our previous studies showed that Corni Fructus exerted protective effects against hyperglycaemia and its related renal damage in rats with streptozotocin-induced diabetes.[16] Moreover, we confirmed that morroniside isolated from Corni Fructus could improve hyperglycaemia and diabetic nephropathy through preventing AGE formation and down-regulating RAGE expression.[17]

However, the effects of morroniside on the kidney in type 2 diabetes, one of the tissues most vulnerable to dyslipidaemia and inflammation, have not yet been determined. Therefore, we investigated the protective effect of morroniside on renal lipid and inflammation provoked by hyperglycaemia and oxidative stress in C57BLKS/J *db/db* mice.

The diabetes (*db/db*) genotype mutation induces a hyperglycaemic/hyperinsulinaemic endometabolic state in C57BLKS/J mice, manifesting a type 2 diabetes/obesity syndrome associated with intrinsic leptin receptor expression defects.[30] As the onset of diabetes occurs within 2 months in *db/db* mice, their renal changes are characterised by glomerular hypertrophy and thickening of the glomerular basement membrane, albuminuria and mesangial matrix.[19]

Abnormal renal lipid metabolism is a major symptom of type 2 diabetes[4] and glucose uptake is markedly increased.[31] This could explain the accumulation of glycogen found in diabetic kidneys, and may play a role in the development of diabetic nephropathy.[31] Sun *et al.*[5] reported that SREBP-1 expression was increased in the kidney cortex, resulting in the up-regulation of enzymes responsible for fatty acid synthesis and a high renal triglyceride content as a consequence, which was associated with mesangial expansion and glomerulosclerosis. The transcriptional activation of SREBP-1 can be up-regulated by insulin,[32] glucose[33] and liver X receptor.[34]

Morroniside treatment led to significant reductions in renal glucose, triglyceride and total cholesterol contents in *db/db* mice, which suggested that morroniside effectively prevented the excessive glucose supply and abnormal lipid accumulation in the kidney. Furthermore, morroniside significantly lowered the expression of proteins associated with lipid homeostasis – SREBP-1 and SREBP-2 – in the kidneys of *db/db* mice. Thus, the hypolipidaemic effect of morroniside in the kidneys of *db/db* mice seems to be mediated through suppression of SREBP protein expression. These results demonstrate that morroniside has modulatory effects on abnormal renal lipid metabolism in this experimental model of type 2 diabetes.

Excessive fat accumulation in the kidney may cause marked ROS generation, leading to increased lipid peroxidation.[35] GSH is considered pivotal for the detoxification of cellular oxidative stress. Recent evidence indicates that the depletion of cellular GSH results in the accumulation of ROS and loss of mitochondrial function.[36] Increased oxidative stress can potentially impair cellular glucose metabolism via a variety of mechanisms, including redox imbalance and insulin resistance.[37] Increased formation of TBARS and oxidative stress induced by ROS production and a reduced GSH/GSSG ratio are known to decrease insulin sensitivity and increase renal inflammation.[37] In the present study, TBARS levels also increased markedly in *db/db* vehicle mice, and were significantly reduced by oral administration of morroniside, in a dose-dependent manner. In particular, renal TBARS was reduced by morroniside to levels comparable to those of normal mice. In the vehicle-treated *db/db* mice, both GSH and GSSG levels decreased compared with *m/m* mice (unlike in the general oxidative stress condition). Others have reported similar reductions in GSH and GSSG in diabetic animals and patients with chronic diseases, including diabetes mellitus.[38–40] The decrease in GSH and GSSG may be related to a reduction in GSH synthesis, a rise in its degradation or increased utilisation by other linked systems. In our research, the extent of GSSG reduction was less than that of the GSH content. As a result, the *db/db* control group showed a reduction in the GSH/GSSG ratio, which is a useful barometer of the oxidative stress imposed on a cellular system. On the other hand, the decreased GSH and GSSG levels in *db/db* mice were elevated on morroniside administration. Although morroniside-treated groups showed a higher GSSG level than vehicle-treated *db/db* mice, the GSH/GSSG ratio was significantly increased through a marked rise in the kidney GSH level. These results suggest that morroniside can alleviate oxidative stress under diabetic pathological conditions through inhibition of lipid peroxidation and augmentation of cellular antioxidants.

The activation of inflammatory processes may contribute to the development of type 2 diabetes. Hyperglycaemia is widely recognised as the pathogenesis of diabetes and diabetic complications, and enhances oxidative stress as well as inflammatory responses related to NF-κB activation.[9] In addition, inflammation appears to be a major mechanism responsible for vascular damage, leading to the well-recognised complications of diabetes. In our Western blot analysis, experimental type 2 diabetes induced by obesity also resulted in increased expression of NF-κBp65, iNOS and COX-2 associated with oxidative stress, while the expression of these three proteins was markedly reduced by morroniside administration, in a dose-dependent manner. Of note, the expression of NF-κBp65, iNOS and COX-2 proteins was fully recovered by 100 mg/kg morroniside to the expression levels of *m/m* mice. Moreover, we could confirm that inhibition of NF-κB translocation by morroniside led, in part, to a decrease in the induction of iNOS and COX-2 expression in the kidney. These results show that the anti-inflammatory effects of morroniside in the kidneys of mice with type 2 diabetes may be related to reduced ROS levels and the down-regulation of iNOS and COX-2, followed by the inhibition of NF-κB transcription.

The major challenge in diabetes research is not only to define the cause–effect relationship between various risk factors and complications, but also to clarify the effects of therapeutic agents that are beneficial in the management of diabetic complications.[41] The present study helped reveal the beneficial effects of a medicinal plant and its major component, as well as the possibility of applying specific therapeutic agents for diabetic complications. Further studies on the safety and toxicity of morroniside are required to support its clinical application.

Conclusions

We have shown that morroniside improves abnormal renal metabolism by decreasing the augmented concentrations of glucose, triglyceride and cholesterol via the down-regulation of SREBP-1 and SREBP-2 proteins. Morroniside also ameliorated oxidative stress and its related inflammation in the kidneys of mice with type 2 diabetes. This improvement was facilitated by the inhibition of renal ROS production and elevation of the GSH/GSSG ratio, which may, in part, have an

influence on the down-regulation of NF-κB, iNOS and COX-2 protein expression. Accordingly, morroniside, a major component of Corni Fructus, may reduce the risk of type 2 diabetes through the amelioration of metabolic disorders, including dyslipidaemia, and oxidative stress, as well as the inflammatory response.

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