Effect of 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione, isolated from Averrhoa carambola L. (Oxalidaceae) roots, on advanced glycation end-product-mediated renal injury in type 2 diabetic KKAY mice

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HIGHLIGHTS

- DMDM attenuates AGEs expression in renal tissue.
- DMDM blocks NF-κB/TGF-β1 pathway in renal tissue.
- DMDM relieves renal damage in KKAY mice.
- DMDM plays an antihyperglycemic effect.
- DMDM exerts renoprotection in KKAY mice.

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ABSTRACT

The roots of Averrhoa carambola L. (Oxalidaceae) have a long history of medical use in traditional Chinese medicine for treating diabetes and diabetic nephropathy. 2-Dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione (DMDDM) was isolated from the tuberous roots of A. carambola. The purpose of this study was to investigate the beneficial effect of DMDDM on the advanced glycation end-product-mediated renal injury in type 2 diabetic KKAY mice with regard to prove its efficacy by local traditional practitioners in the treatment of kidney frailties in diabetes. KKAY mice were orally administrated DMDDM (12.5, 25, 50 mg/kg body weight/d) or aminoguanidine (200 mg/kg body weight/d) for 8 weeks. Hyperglycemia, renal AGE formation, and the expression of related proteins, such as the AGE receptor, nuclear factor-κB, transforming growth factor-β1, and NF-κB (carboxymethyl)lysine, were markedly decreased by DMDDM. Diabetes-dependent alterations in proteinuria, serum creatinine, creatinine clearance, and serum urea-N and glomerular mesangial matrix expansion were attenuated by treatment with DMDDM for 8 weeks. The activities of superoxide dismutase and glutathione peroxidase, which are reduced in the kidneys of KKAY mice, were enhanced by DMDDM. These findings suggest that DMDDM may inhibit the progression of diabetic nephropathy and may be a therapeutic agent for regulating several pharmacological targets to treat or prevent of diabetic nephropathy.

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89. Introduction

Diabetes is a disorder of chronic hyperglycemia, and glucose participates in diabetic complications such as atherosclerosis, cardiac dysfunction, and nephropathy (Cooper, 2004). Among diabetic complications, diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in developed countries and is a major cause of morbidity and mortality in patients with diabetes. DN is a major microvascular complication of both type 1 and
type 2 diabetes mellitus, and its associated risk factors are high blood glucose, high blood pressure, and high cholesterol levels. Therefore, metabolic and hemodynamic factors should be controlled to prevent the occurrence of DN (Cooper, 2001).

DN is characterized histologically by glomerular basement thickening and mesangial expansion related to the loss of renal function and clinically progressive albuminuria followed by a gradual decline in renal function (Mauer et al., 1984). Measures to prevent the appearance and progression of diabetic nephropathy should therefore be instituted as early as possible. Although adequate control of blood glucose levels may prevent the development of complications, it is difficult to achieve strict glucose control; thus, there is a yearly increase in the number of patients with diabetes (Leboith and Rayfield, 2007). In addition to glucose, other metabolic factors, and in particular advanced glycation end products (AGEs), have been shown to be involved in the development of diabetic kidney disease. AGEs promote the progression of extracellular matrix (ECM) in the glomeruli via the receptor for advanced glycation end products and abnormal glomerular remodeling in the kidney (Horii et al., 1999; Rapis and Viber, 2001; Vlassara et al., 1994). The interaction between AGEs and the receptor for advanced glycation end products (RAGE) is important in the pathogenesis of DN because RAGE upregulation and activation might be involved in the accumulation of AGEs (Yonemura et al., 2005). Moreover, AGEs or high levels of glucose lead to increased production of extracellular matrix (ECM) proteins such as collagen IV via transforming growth factor-β (TGF-β1) (Allemano et al., 2006). TGF-β1 takes part in the uptake of albumin by renal proximal tubule cells and in the development of albuminuria (Russo et al., 2007). TGF-β1 also stimulates the synthesis of matrix components such as collagen IV (Tahara et al., 2008), which is thought to be the major collagenous component of GBM and ECM (Dong et al., 2004). To prevent the development and progression of DN, effective therapies directed toward key molecular targets are required (Sharma and Srinivasan, 2009).

Natural products derived from plants have long been used in folk medicine, making the compounds derived from these plants good candidates for new therapeutic strategies (Cordell and Colvard, 2005; Kobayashi et al., 2010; Padmanabhan et al., 2005). Averrhoa carambola L. commonly known as star fruit or carambola, is a plant originally from Asia that has become acclimatized to many tropical countries (Moreno et al., 2012). This plant is a small bush that can grow to 4–6 m in height (Vasconcelos et al., 2008). The leaves and fruits of A. carambola are commonly used to treat headaches, vomiting, coughing and hangovers (Caroline et al., 2005). Furthermore, it is used as an appetite stimulant, a diuretic, and as an antidiarrheal and febrifugal agent. Additionally, the extract obtained by decocting the leaves of A. carambola has been used in the treatment of diabetes (Pereira et al., 2008). The dry root of A. carambola L., known as Yang Tao Gen in China, has also been used in traditional Chinese medicine for diabetic mellitus and its complications because it invigorates the kidney and reinforces yang. In a previous study, we reported that the alcoholic extract of the A. carambola L. root (ACR), has a therapeutic potential in diabetes (Huan and Huan, 2009). Thus, we hypothesized that diodecyl-6-methoxychromone-2,5-dione-1,4-dione (DMDD), which can be isolated from ACR, may have a beneficial effect on the progression of DN. To test our knowledge, the mechanisms of DMDD against glucose-associated metabolic disorders in diabetes have yet to be explored. KKAy mice are considered to be a good animal model for the early pathological changes associated with diabetes (Chen et al., 2002). To determine whether DMDD has a strong effect on AGE formation in diabetes and/or diabetic nephropathy, we examined the effect of DMDD on type 2 diabetic KKAy mice and compared it with the effect of aminoguanidine, an inhibitor of AGE formation.

2. Materials and methods

2.1. Plant material and preparation of extract

DMDD were prepared as previously described (Wen et al., 2012) and contained particulate impurities. A powder from air-dried roots of A. carambola L. (12 kg) was extracted with 60% EtOH under reflux for 3 times (3 × 6 h, 1 h for each) and the ethanol solution was concentrated under vacuum conditions to yield a syrup-like extract, which was suspended in H2O and was extracted with cyclohexane (3 × 20 l), EtOAc (3 × 20 l), and n-BuOH (3 × 20 l). The cyclohexane extract (30 g) was subjected to open silica gel CC (3 × 8 cm, 200–300 mesh) via successively fractions with a gradient of chloroform:EtOAc (100:0–10:100, each 200 ml) to afford 7 fractions (Fr. 1–7). Fr. 1 (5 g) was further separated by open silica gel CC (3 × 8 cm, 200–300 mesh) via successive elutions with a gradient of cyclohexane:EtOAc (100:0–10:100, each 100 ml) producing 4 sub-fractions (Fr. 1–4). Fr. 4 was re-crystallized with MeOH to yield 2-dodecyl-6-methoxychromone-2,5-dione-1,4-dione (2350 mg). This compound was identified by FABR spectroscopy using a SpectrumOne Perkin-Elmer spectrophotometer and 1H and 13C NMR analysis on Bruker AV 600: the results were further compared with the literature (Schultz et al., 1988). As a yellow-colored water-soluble powder, DMDD was dissolved in distilled water (2.5 mg/ml) before administration to the mice.

2.2. Animals

The experiment procedures and protocols used in this investigation were approved by the Institutional Committee for the Experimental Animals of Guangzhou Medical University (Guangzhou, China). Twelve-week-old male KKAy mice and age-matched C57BL/6 (C57BL) mice were used in the study. The Experimental Animal Center, Chinese Academy of Medical Science, Beijing, were housed in individual cages under controlled temperature (23 ± 1°C) and humidity (55 ± 5%) on a 12:12 light-dark cycle and were given standard rodent Chow and free access to water, unless otherwise noted.

2.3. Acute toxicity test

Acute toxicity test in mice was performed according to the revised IP and Down method (OECD, 2008). Ten male C57BL/6 mice were used for the test. The mice were fasted (16 h) overnight and the body weight (g) of each mouse was recorded prior to the test. A fixed dose of DMDD (5000 mg/kg body weight) was administered orally to each mouse and observed closely at 4 h initially, then every 6 h intervals for changes in behavioral (abstention, restlessness, irritability, rebellion, vomiting, and furiness), neurological (spontaneous convulsion, gait, bleeding orifices, and touch (pain response), autonomic (distortion and micritic) tuitions), and mortality (Whitish and Kohl, 2004). The volume of the extract was adjusted to contain 500 mg/ml, and any significant morbidity or mortality within 24–72 h was recorded.

2.4. Experimental design

The mice were divided into the following groups: Group I (n = 10): C57BL/6 mice administered distilled water: normal control. Group II (n = 10): C57BL/6 mice administered DMDD (50 mg/kg body weight) by oral gavage once a day for 8 weeks: DMDD control. Group III (n = 10): KKAy mice administered distilled water: normal control. Group IV (n = 10): KKAy mice administered DMDD (50 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + DMDD. Group V (n = 10): KKAy mice administered DMDD (25 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + DMDD25. Group VI (n = 10): KKAy mice administered DMDD (50 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + DMDD50. Group VII (n = 10): KKAy mice administered aminoguanidine (200 mg/kg body weight) by oral gavage once a day for 8 weeks: KKAy + AG. The concentration of the aminoguanidine was adjusted to 10 mg/ml before administration to the mice.

2.5. Collection of blood, urine, and tissues

Blood, urine, and the biochemical parameters were determined after 8 weeks of DMDD treatment. Blood samples were collected using metabolic cages, and whole blood was collected from the retro-orbital venous plexus with heparinized capillary tubes. The mice were killed humanely with an overdose of sodium pentobarbital (150 mg/kg), and the sera were immediately separated from the blood samples by centrifugation at 1000 × g for 10 min. The kidneys were surgically removed, washed with cold saline and stored at −80°C until further analysis.

2.6. Biochemical index assay

Blood glucose levels were assessed using a One-Touch Ultra blood glucose meter (Accu-check Performa, Roche, Germany). Blood urea nitrogen (BUN), albumin, total cholesterol, serum glycosylated protein, and other metabolic parameters were assayed using routine laboratory techniques.
serum creatinine (Cr), urinary Cr, and 24-h urinary protein excretion were analyzed by the laboratory of the First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China). Creatinine clearance (CrCl) was calculated using the following equation: 
CrCl = [(1.72 × Scr in serum) × (body weight) × (urine volume in ml)] / [(0.886 × Scr in urine) × (urea Cr in serum) × (urea Cr in urine) × (1000) × (min)]

2.7. Renal histology assays

The renal samples were fixed in 10% formalin, embedded with paraffin and cut into 4-μm-thick sections. The slides were stained with hematoxylin and counterstained with eosin (HE) and examined under light microscopy with a magnification of 400× by a histopathologist who was blind to the experimental profile.

2.8. Lipid peroxidation assays

MDA The renal cortex was prepared according to commercial kits (Jiancheng Institute of Biological Technology, Nanjing, China) according to the protocol and reagents were expressed as nmoles of protein.

2.9. Assays for renal antioxidant enzyme activity

Total superoxide dismutase (SOD, EC 1.15.1.1) activity in kidney homogenates was assayed using a commercial kit (Jiancheng Institute of Biological Technology, Nanjing, China) according to the protocol and reagents were expressed as units of protein.

2.10. Renal advanced glycation end product (AGE) assays

The renal AGE level was determined according to previously described method with slight modifications (Nakayama et al., 1998). Specifically, minced kidney tissue was digested with cholesteryl ester-reducing method (2:1, w/v) overnight. After washing, the tissue was homogenized in 10 mM NaOH, followed by centrifugation at 18000 × g for 15 min at 4°C. The amount of AGEs in tissue samples was determined by measuring the fluorescent intensity at an excitation wavelength of 480 nm and an emission wavelength of 520 nm using a fluorescence spectrophotometer (RF-5000, Hitachi, Japan). A native RSA preparation (1 mg/ml) in 0.1 N NaOH was used as a standard, and its fluorescence intensity was defined as one unit of fluorescence. The fluorescence of AGEs samples was measured at a concentration of 5 μg/ml and expressed in AUs compared with the fluorescence of the native RSA preparation.

2.11. Protein extraction and Western blot analysis

The mice from all the groups were sacrificed by cervical dislocation, and the kidneys were promptly removed. Protein extraction was performed as follows: The samples were homogenized in ice-cold lysis buffer (20 mM Tris-HCl, 137 mM NaCl, 1% Triton X-100, 150 mM sodium deoxycholate, and 0.5% sodium deoxycholate) containing proteinase inhibitors. After centrifugation at 12,000g for 10 min at 4°C, the supernatants were removed, and the concentration of each sample was determined by a protein assay reagent (bicinchoninic acid). The samples were then separated and transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 1 h, and incubated with primary antibodies against RAGE, NF-κBp65, TGF-β1, CML and β-actinovernight at 4°C. After the blots were washed, they were incubated with a goat and rabbit anti-rabbit and mouse horseradish peroxidase-conjugated secondary IgG (Pierce Biotechnology) for 60 min at room temperature. The bands were detected with a digital camera system (UVV) after staining with diamino benzidine and sodium sulfate. The intensity of the bands was quantified using Image J software. The band intensities were normalized against the protein content of each sample. Western blotting was performed at least three times. The results were expressed as means ± SD of three independent experiments.

3. Results

3.1. Acute toxicity test of DMDM in mice

Acute toxicity test of DMDM was evaluated in mice at a single large dose of 5000 mg/kg body weight. The oral 50% value of DMDM was greater than 5000 mg/kg body weight in mice and considered to be a practically non-toxic substance.

3.2. Body weight and blood glucose

The effects of DMDM on body weight and blood glucose levels in the animals are shown in Fig. 1. The body weight (Fig. 1A) of KK mice was significantly higher than that of C57BL/6 mice at 12 weeks of age, and the KK mice gained significantly more weight throughout the 8-week experimental period. Administration of DMDM to KK mice resulted in a significant decrease in weight gain. The effects were similar to those in AG group. Although the reduction in DMDM-treated KK mice was dose dependent, it was not statistically significant. Whereas the weight gain in the DMDM-treated C57BL/6 mice was not significantly different from the normal controls.

As shown in Fig. 1B, the fasting blood glucose (FBG) levels in C57BL/6 mice maintained constant during the experimental period.
and was significantly lower than KKAy mice before experiment at the beginning of the treatment. The degree of hyperglycemia in the KKAy mice increased significantly during the 8-week study period. DMDD treatment markedly attenuated the hyperglycemia in KKAy mice, and the effect appeared to be dose dependent. However, it did not affect the blood glucose level of the C57BL/6J mice.

3.3. Serum constituents

The effect of DMDD on the serum constituents of the mice are shown in Fig. 2. The levels of glycosylated protein and cholesterol in plasma were markedly higher and the albumin level was significantly lower in vehicle-treated KKAy mice than in C57BL/6J mice. After 8 weeks of administration, the levels of glycosylated protein in the plasma of the DMDD and aminoguanidine-treated KK mouse were significantly lower than those in the vehicle-treated KKAy group. The albumin levels in the KKAy mice treated with DMDD at 12.5 mg/kg body weight/day for 8 weeks were somewhat higher than the corresponding value for the vehicle-treated group, but this difference was not statistically significant; however, the albumin levels of the KKAy mice treated with DMDD at 25 and 50 mg/kg body weight/day were significantly higher than those of the vehicle-treated KKAy mice. The total cholesterol level in the plasma of the DMDD and aminoguanidine-treated KKAy mice were significantly lower than those in the vehicle-treated KKAy group. DMDD had no effect on the basal glycosylated protein, cholesterol, and the albumin.

3.4. Renal function

The serum levels of Urea-N, Cr and 24-h urine protein were significantly higher, and the Cr level was significantly lower in the KKAy mice than the C57BL/6J mice. The results showed that the serum levels of Urea-N, Cr and 24-h urine protein were obviously decreased after treatment with DMDD (Fig. 3). In addition, Cr level was markedly increased after treatment. There were no significant differences in the levels of all previously listed renal functional parameters between the DMDD control group and normal control group.

3.5. Oxidant/antioxidant enzyme levels in the renal cortex

Oxidative stress was observed in the vehicle-treated KKAy mice, as indicated by a significant increase in MDA and reduced SOD and GSH-px activity in the renal cortex. Administration of variable doses of DMDD (12.5, 25, 50 mg/kg body weight/d, respectively) for 8 weeks lead to dose dependent elevation of plasma antioxidant activity in comparison to vehicle-treated KKAy group (Fig. 4). There were no significant differences in the activities of MDA, SOD and GSH-px between the DMDD control group and normal control group.
3.6. Renal AGE levels

The renal AGE levels in the vehicle-treated KKAY mouse were significantly higher than in the C57BL/6J mice, but they were effectively lowered by aminoguanidine and by DMDD. Additionally, DMDD reduced the renal AGE levels in a dose-dependent manner (Fig. 5). However, it did not affect the renal AGE levels of the C57BL/6J mice.

3.7. Renal histopathology

H&E staining of the kidneys of KKAY mice revealed glomerular hypertrophy and expansion of the mesangial area and ECM. After the 8-week treatment with DMDD, glomerular hypertrophy and mesangial matrix accumulation in the KKAY mice were significantly inhibited (Fig. 6). In addition, the DMDD control group exhibited minimal variation in these histological changes in comparison to normal controls. In tandem, the histological studies of the kidneys of treated animals revealed less matrix expansion and glomerular basement membrane thickening compared to the KKAY mice.

3.8. Western blotting of renal cortex proteins

Fig. 7 depicts the RAGE, NF-κB, TGF-β1, and CML protein levels in the renal cortex. Based on the band densities, renal RAGE, NF-κB, TGF-β1 and CML were significantly elevated in the diabetic KKAY mice compared with the C57BL/6J mice. However, the altered expression of these proteins was significantly normalized in the KKAY mice by the oral administration of either DMDD or aminoguanidine. DMDD alone had no significant effect on the basal expression of RAGE, NF-κB, TGF-β1, and CML.

4. Discussion

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes and is the leading cause of end-stage renal disease (Seets and King, 2002). The pathogenesis of DN is multifactorial, and chronic hyperglycemia plays a crucial role (Kikdawat et al., 2003). During the course of diabetes, supraphysiological glucose increases the formation of AGEs and the production of free radicals by the mitochondria. Cell death and renal dysfunction occur as a result.

Consistent with an earlier report (Hayase et al., 1996), we found that the KKAY mice developed non-insulin-dependent diabetes mellitus spontaneously at 12 weeks of age. The KKAY mice were lighter and weighed significantly more than the C57BL/6J mice; the blood glucose level in the KKAY mice was significantly higher than in the C57BL/6J mice. In this study, we demonstrated that treatment with DMDD or aminoguanidine for 8 weeks significantly reduced hyperglycemia and body weight in type 2 diabetic KKAY mice. We conclude that DMDD has an anti-hyperglycemic effect that is correlated with a decrease in body weight.

Excretion of albumin in the urine has been demonstrated to be a good clinical predictor of renal lesions in DN (Parving et al., 2001; Viberi and Wittsford, 2002). Many studies have shown that urine protein levels are associated with a graded increase in the risk of progression to end-stage renal disease and cardiovascular events (Lea et al., 2005). We observed an increase in the concentration of urinary albumin in the KKAY mice in the present study. This increase corresponds to the degree of hyperglycemia. Additionally, serum Cr and BUN levels and creatinine clearance, which are generally considered to be markers of renal function, were higher in the type 2 diabetic KKAY mice than in the C57BL/6J mice, implying the presence of diabetic kidney disease with renal hyperfiltration. DMDD-treated KKAY mice showed significant improvements in renal function, as indicated by urine protein, serum Urea-N, and Cr levels. However, it did not affect all previously listed renal functional parameters of the C57BL/6J mice.

A number of studies by important authors have shown that oxidative stress is a key pathogenic factor in the development of diabetic complications, including nephropathy (Busch et al., 2010; Giacco and Brownlee, 2010). It has been suggested that severe glucose-induced renal damage is associated with excessive levels of reactive oxygen species produced under hyperglycemic conditions (Jiang et al., 2010). Oxidants have direct biological effects that are connected to diabetic nephropathy (Koya et al., 2003). Thus, strategies to reduce oxidative stress in diabetes mellitus may have a favorable effect on the progression of diabetic glomerulosclerosis (Koya et al., 2003; Prabhakar et al., 2007). MDA content is a good index of increased oxidative stress in the tissues, as it reflects enhanced peroxidation processes (Kedziora-Komotowska et al., 2002). Among the antioxidative enzymes, SOD catalyzes dismutation of the superoxide anion into hydrogen peroxide, whereas GSH-Px both detoxifies hydrogen peroxide and converts lipid hydroperoxides to nontoxic alcohols. In this study, the production of MDA was significantly enhanced, indicating an oxidative stress state in the type 2 diabetic KKAY mice. At the same time, SOD and GSH-Px activity were markedly reduced in the KKAY mice compared with the C57BL/6J mice. Furthermore, we found for the first time that this oxidative damage is suppressed by DMDD treatment in a dose-dependent manner. However, there were no significant differences in the activities of MDA, SOD and GSH-Px between the DMDD control group and normal control group. These changes were attenuated by DMDD, suggesting that DMDD could be used to protect renal tissues against oxidative damage in diabetic nephropathy.

Much attention has been focused on exploring the mechanisms related to the development of DN. At present, several growth factors have been proposed to be involved in mediating the development of diabetic renal hypertrophy; among them, the multifunctional cytokine TGF-β1 is known to be upregulated in diabetic kidneys (Reeves and Andreadi, 2000). TGF-β1 plays an important role in ECM metabolism. Several studies have demonstrated that stimuli such as hyperglycemia, AGEs, and oxidative stress increase TGF-β1 expression (Forbes et al., 2007). TGF-β1 is believed to have a prominent role in the proliferation of mesangial cells and ECM,
production, which are the major pathological changes in early diabetic nephropathy (Reeves and Andreoli, 2000). Thus, TGF-β1 has been considered as a therapeutic target in fibrotic diseases such as diabetic nephropathy and other chronic kidney diseases (McGowan et al., 2004). In this study, the expression of renal TGF-β1 was markedly inhibited by DMDD in KKAy mice. The accumulation of TGF-β1 in KKAy mice was inhibited by DMDD at 8 weeks after starting treatment. The finding that DMDD improved renal function in type 2 diabetic KKAy mice and that this beneficial effect was associated with an inhibitory effect of DMDD on kidney TGF-β1 overexpression is novel. Additionally, histological examination of the kidneys revealed that DMDD significantly attenuated diabetes-induced mesangial expansion.

Hyperglycemia, a chronic metabolic glucose disorder, results in irreversible tissue damage because of protein glycation, which leads to the formation of glycosylated protein and AGEs (Kanwar et al., 2008). The increase in glycosylated protein in the serum is caused by the reaction glucose and other reducing sugars such as ribose and fructose with the amino residues of proteins to form Amadori products such as glycosylated hemoglobin (HbA1c). Oxygen is also generated in the process of AGE formation (Darouich et al., 2010). AGE accumulation occurs earlier and at an accelerated rate in diabetes mellitus patients than it does in non-diabetic individuals (Schleicher et al., 1997). AGE formation appears to be synergistic with other pathogenic pathways in diabetes, including oxidative stress, hypertension, and activation of the renin-angiotensin system. Each of these pathways may be activated by AGEs, and each may promote the formation of AGEs (Thomas et al., 2005). AGEs can contribute to renal aging. Through AGE accumulation, in situ glycation and RAGE activation, glycation could enhance the
physiological and pathological effects of renal aging (Daroux et al., 2010). It has been reported that AGES trigger the activation of NF-kB via interaction with the ACE receptor (RAGE), leading to its translocation to the nucleus, where it induces transcription (Yan et al., 1994). Additionally, the promoter region of the RAGE gene contains NF-kB binding sites (Li and Schmidt, 1997), potentially producing a self-perpetuating pathway. Moreover, the ACE-RAGE interaction activates TGF-β1 signaling pathways and subsequently induces mesangial hypertrophy and glomerulosclerosis by ECM synthesis (Khowar et al., 2008). The interactions between AGES and RAGE induce the activation of oxidative stress and stimulate the production and release of cytokines, which amplify tissue damage (Wautier and Guillausseau, 2002). Therefore, ACE accumulation in the kidney has been regarded as an index of progressive renal damage and diabetic nephropathy. In the present study, not only the overexpression of AGES and RAGE but also the higher levels of NF-kB and TGF-β1 in the kidneys of type 2 diabetic KK mice were attenuated by DMDM treatment for 8 weeks. It seems that DMDM inhibited not only ACE-RAGE signaling but also the NF-κB-TGF-β1-dependent pathway to some extent, thus leading to an attenuation of renal damage caused by protein glycation.

CML, pentosidine, and methylglyoxal derivatives are among the well-characterized compounds that are commonly used as markers of AGE (Chapuy et al., 2003). CML is not only a glycation product but also a sensitive and specific marker of advanced glycation. CML remains sensitive to protein in the presence of protein (Frut et al., 1996). Therefore, CML could serve as a general biomarker of oxidative stress resulting from carbohydrate and lipid oxidation reactions. We found that treatment of type 2 diabetic KK mice with DMDM for 8 weeks lowered the level of CML but also decreased the accumulation of lipid peroxidation products in kidney. These results indicate that the beneficial effect of DMDM on diabetic nephropathy may be linked to the reduction of oxidative stress.

In conclusion, we discovered that 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione had an antidiabetic effect via a reduction in hyperglycemia, which attenuated AGE expression and downregulated the NF-κB-TGF-β1 pathway in diabetic glomeruli, consequently decreasing ECM deposition in renal tissues. Hence, DMDM could be used as dietary supplement in the management of renal impairment associated with chronic diabetes inmflmations.

Conflict of interest

The authors declare that there are no conflicts of interest.

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