RESEARCH

Dibenzoylmethane ameliorates lipid-induced inflammation and oxidative injury in diabetic nephropathy

Eun Soo Lee¹, Mi-Hye Kwon², Hong Min Kim¹, Nami Kim³, You Mi Kim¹, Hyeon Soo Kim³, Eun Young Lee⁴ and Choon Hee Chung¹

¹Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea

²The East Coast Research Institute of Life Science, Gangneung-Wonju National University, Gangneung, Korea

³Department of Anatomy, Korea University College of Medicine, Seoul, Korea

⁴Department of Internal Medicine and Institute of Tissue Regeneration, Soonchunhyang University, Cheonan, Korea

Correspondence should be addressed to C H Chung: cchung@yonsei.ac.kr

Abstract

Dibenzoylmethane (DBM) is a beta-diketone analog of curcumin. Numerous studies have shown the beneficial effects of curcumin on diabetes, obesity and diabetic complications including diabetic nephropathy. Recently, we investigated the beneficial metabolic effects of DBM on high-fat diet-induced obesity. However, the effects and mechanisms of action of DBM in the kidney are currently unknown. To investigate the renoprotective effects of DBM in type 2 diabetes, we administered DBM (100 mg/kg) orally for 12 weeks to high-fat diet-induced diabetic model mice. We used mouse renal mesangial (MES13) and macrophage (RAW 264.7) cells to examine the mechanism of action of DBM (20 μ M). After DBM treatment, the albumin-to-creatinine ratio was significantly decreased compared to that of the high-fat-diet group. Moreover, damaged renal ultra-structures and functions including increased glomerular volume, glomerular basement membrane thickness and inflammatory signals were ameliorated after DBM treatment. Stimulation of MES13 and RAW264.7 cells by palmitate or high-dose glucose with lipopolysaccharides increased inflammatory signals and macrophage migration. However, these changes were reversed by DBM treatment. In addition, DBM inhibited NADPH oxidase 2 and 4 expression and oxidative DNA damage. Collectively, these data suggested that DBM prevented diabetes-induced renal injury through its anti-inflammatory and antioxidant effects.

Key Words

- ▶ diabetic nephropathy
- ▶ dibenzoylmethane
- ▶ lipotoxicity
- ▶ inflammation
- ▶ oxidative stress

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Introduction

The rapid onset of obesity is one of the main causes of insulin resistance and diabetic complications, such as retinopathy, neuropathy, cardiovascular disease and diabetic nephropathy (DN), which are associated with elevated glucotoxicity, lipotoxicity, inflammation and cellular apoptosis (Serrano Rios 1998). Among these complications, DN is a leading cause of end-stage kidney

diseases and is associated with increased morbidity and mortality in diabetes. Under obesity-induced excessive cellular stress conditions, NADPH oxidase (NOX)-derived reactive oxygen species (ROS) are increased and accelerate macrophage polarization to proinflammatory macrophage M1 and activation. In particular, activated macrophages are recognized

as prominent inflammatory cells that secrete many proinflammatory cytokines and chemokines, as well as pro-fibrotic and anti-angiogenic factors that contribute to the pathogenesis of DN. Continuous macrophages accumulation is correlated with proteinuria and renal fibrosis and is inversely correlated with renal function in humans (Nguyen *et al.* 2006). Thus, suppressing the infiltration of inflammatory macrophages alleviated insulin resistance and DN (Nistala *et al.* 2014, Eun Lee *et al.* 2016). Studies by Kodera *et al.* (2014) and Seok *et al.* (2013) showed that the administration of dipeptidyl peptidase 4 (DPP4) or C-C chemokine receptor type 2 (CCR2) inhibitors to diabetic animal models ameliorates DN through the inhibition of macrophage infiltration and inflammation (Seok *et al.* 2013, Kodera *et al.* 2014).

Curcumin, a polyphenolic compound derived from dietary turmeric, is known to have anti-inflammatory, antioxidant and antiangiogenic effects (Anand *et al.* 2007). In obesity or diabetes, curcumin improves insulin resistance and fatty liver by increasing fatty oxidation and decreasing lipid synthesis (Zhao *et al.* 2018). Furthermore, curcumin also ameliorates DN by inhibiting macrophage infiltration and inflammatory signaling including nuclear factor (NF)- κ B p65 and antioxidant effects (Soetikno *et al.* 2011). Many studies have investigated the protective effects of curcumin and our previous study reported that curcumin ameliorates DN by regulating renal lipid metabolism and oxidative stress (Trujillo *et al.* 2013, Pan *et al.* 2013a, Kim *et al.* 2016, Lu *et al.* 2017). In addition to curcumin its analogs such as C66 and B06 were also identified to have DN therapeutic effects by the inhibition of inflammation and macrophage infiltration (Pan *et al.* 2013b, Wu *et al.* 2016).

Dibenzoylmethane (DBM), a beta-diketone analog of curcumin, has been reported to have anticarcinoma (Khor *et al.* 2009), antioxidant and cellular protective effects against endoplasmic reticulum stress (Takano *et al.* 2007). Moreover, our previous study showed the beneficial metabolic effects of DBM, which regulates glucose uptake via AMP-activated protein kinase (AMPK) activation in skeletal muscle and inhibits fatty acid synthesis in pre-adipocytes (Kim *et al.* 2015). However, the effects and action mechanisms of DBM on DN have not yet been studied.

Using a high-fat diet (HFD)-induced obesity mouse model, we sought to investigate the potential protective effects of DBM against DN and clarify its mechanisms of action.

Materials and methods

Animal study

Four-week-old male C57BL/6 mice (15 g) were purchased from Dae Han Bio Link Co. (Chungbuk, Korea). The animal experiments were conducted as described in a previous study (Kim *et al.* 2015). In brief, animals were housed in cages placed in a room on a 12-h light/darkness cycle and ambient temperature (22–24°C). At 8 weeks, the mice were randomly divided into the following three groups of ten each: (i) normal diet fed (ND), (ii) HFD fed (HFD, 60% kcal from fat) and (iii) DBM-treated (HFD+DBM 100 mg/kg) groups. DBM, an analog of curcumin (Fig. 1), was administered by mixing with the chow. Food intake and body weights were recorded weekly. After 12 weeks, the animals were anesthetized, and the serum and tissues were collected and stored for further studies. All experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University at the Wonju Campus (YWC-121030-1).

Assessment of urine albumin and creatinine

Urine was collected using a metabolic cage for 24 h and the urinary albumin and creatinine levels were measured using the Albuwell M and Creatinine Companion Kits in accordance with the manufacturer's instructions (Exocell, Inc., Philadelphia, PA, USA).

Figure 1
Chemical structures of curcumin and dibenzoylmethane (DBM).

Measurement of renal triglycerides (TGs)

The renal TG content was examined in kidney tissues. Briefly, ethanolic potassium hydroxide was added to the tissue and the mixture was incubated at 55°C overnight. Then, 50% ethanol was added to the tube, which was centrifuged for 5 min. The supernatant was transferred to a new tube, 50% ethanol was added and the mixture was vortexed. The supernatant was transferred to a new tube, 1 M magnesium chloride was added and then the mixture was kept on ice for 10 min. The tube was centrifuged for 5 min and the supernatant was transferred to a new tube. To determine the glycerol content, reconstituted glycerol reagent (Sigma-Aldrich) was added according to the kit instructions. The mixture was incubated for 15 min and the absorbance was measured at 540 nm. Renal TG levels were calculated using the following formula: TG (mg/g tissue) = cuvette triolein equivalent glycerol concentration (mg/dL) $\times$ (10/30) $\times$ (415/200) $\times$ 0.012 (dL)/tissue weight (g).

Transmission electron microscopy

Slit pore numbers and the glomerular basement membrane (GBM) thickness were measured using a transmission electron microscope (JEM-1200EX II, JEOL, Ltd, Tokyo, Japan). Electron micrographs were captured at 20,000 $\times$ magnification for each sample.

Estimation of glomerular volume and adipocyte size

Paraffin-embedded kidney and epididymal fat tissues were stained with hematoxylin and eosin (H&E). The tissue sections were examined using an optical microscope equipped with a charge-coupled device camera (Pulnix, Sunnyvale, CA, USA).

Immunohistochemistry of glomeruli

Paraffin-embedded kidney tissues were stained for monocyte chemoattractant protein-1 (MCP1), inflammatory macrophage markers (F4/80 and CD11c), and oxidative stress marker (8-hydroxy-2'-deoxyguanosine (8-OHdG)) using a commercial immunohistochemistry staining kit in accordance with the manufacturer's instructions (Thermo Scientific). Positively stained area and cells were counted with ImageJ program (National Institute of Mental Health, Bethesda, MD, USA).

RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

Total RNA from the kidney was isolated using TRIzol (Sigma-Aldrich) according to a standard protocol. cDNA was prepared from 1.0 μ g total RNA of each sample using a commercially available kit (QuantiTect Reverse Transcription Kit; Promega, Hilden, Germany). qPCR was performed using a SYBR Green PCR master mix (Applied Biosystems) using an ABI PRISM 7900HT sequence detection system (Applied Biosystems). The following primers were used: mMcp1: sense, 5'-CTT GCC TAA TCC ACA GAC TG-3' and antisense, 5'-GCC TGA ACA GCA CCA CTA-3'; interleukin (IL)-1 β : sense, 5'-GGG CTG CTT CCA AAC CTT TG-3' and antisense, 5'-GGG CTG CTT CCA AAC CTT TG-3'; mNox2: sense, 5'-AAC TCC GCT CTT TCA CCA GG-3' and antisense, 5'-GTG i ACA CCC AGA GTC AG-3'; mNox4: sense, 5'-TGT TGG GCC TAG GAT TGT GTT-3' and antisense, 5'-GGC TAC ATG CAC ACC TGA GA-3' and glyceraldehyde 3-phosphate dehydrogenase (GAPDH): sense, 5'-CTG GAG AAA CCT GCC AAG TA-3' and antisense 5-AGT GGG AGT TGC TGT TGA AG-3'.

Western blot analysis

Kidney tissues and cultured cells were homogenized in the PRO-PREP protein extraction solution (iNtRON Biotechnology, Korea) containing proteinase and phosphatase inhibitors (GenDEPOT, Barker, TX, USA). The protein concentration in the supernatant was measured using a bicinchoninic acid protein assay kit (Pierce). The protein samples were electrophoresed on 8–12% SDS-PAGE and the resolved proteins were transferred to PVDF membranes. The blots were incubated with the following primary antibodies: anti-arginase 2, anti-cluster of differentiation 68 (CD68), anti-nuclear factor, erythroid 2 like 2 (NRF2), anti-heme oxygenase1 (HO1), anti-NOX2, and anti-NOX4 (Santa Cruz Biotechnology), anti- β -actin, anti-nephrin (Abcam), anti-phospho (p)-AMPK, anti-p-P38, anti-p38, anti-p-inhibitor of NF- κ B (I κ B) kinase (IKK) β , anti-IKK β , anti-I κ B, anti-p-NF κ B p65 and anti-NF κ B p65 (Cell Signaling Technology) antibodies. The blots were visualized using a chemiluminescence UVP BioSpectrum 600 imaging system and quantified with ImageJ program (National Institute of Mental Health).

Cell culture

Mouse mesangial (MES-13) cells were cultured at 37.5°C in Dulbecco's modified Eagle's medium (DMEM)

containing 5.5 mM glucose, 1% antibiotics and 10% fetal bovine serum (FBS). The cultured cells were starved for 24 h and treated with low glucose (5.5 mM) and palmitate (PA, 250 μ M) with or without DBM (20 μ M). Mouse macrophages Raw 264.7 cells were cultured at 37.5°C in DMEM containing 25.5 mM glucose, 1% antibiotics and 5% FBS. To prepare the conditioned medium (CM), Raw 264.7 cells were stimulated with lipopolysaccharide (LPS, 100 ng/mL), in the presence or absence of DBM (20 μ M) for 24 h. The collected CM was stored at -80°C until further use.

Migration assay

To determine whether DBM inhibited the migration of RAW 264.7 cells, an LPS stimulation migration assay was performed in a 96-well chemotaxis chamber (ChemoTx; Neuro Probe, Gaithersburg, MD, USA). The lower chambers were filled with 30 μ L medium in the presence or absence of LPS or DBM. Cells ($1.4 \times 10^4/50 \mu$ L) were seeded in the upper chambers and incubated for 4 h at 37°C in an atmosphere of 5% CO₂. Cells that had migrated to the bottom side were fixed for 5 min in methanol and the nuclei were stained with hematoxylin for 5 min. The cytoplasm was stained with an eosin

solution for 1 min, and the cells were washed with tap water to remove the background stain. The stained cells were then photographed and counted using an image analysis system (Olympus SIS).

NOX2 and NOX4 immunofluorescence staining

MES-13 cells were seeded into chamber slides and incubated in starvation medium for 12 h and the cells were transferred to high-glucose (HG, 30 mM) medium with LPS in the presence or absence of DBM. After 24 h, the culture medium was removed and the cells were fixed in 4% paraformaldehyde solution for 15 min at room temperature. Then, the cells were stained for NOX2 and NOX4 using a commercial immunohistochemistry kit in accordance with the manufacturer's instructions (Thermo Scientific).

Statistics

All data are expressed as the means $\pm$ standard error of the mean. A Student's *t*-test was used for experiments with only two groups. Differences were considered statistically significant when $P < 0.05$.

Figure 2

Changes in number of high-fat diet (HFD)-induced crown-like structures in white adipose tissue and levels of renal triglycerides caused by dibenzoylmethane (DBM) administration. (A) H&E staining of white adipose tissue. (B) Measurement of adipocyte sizes. (C) Renal TG levels were measured by converting TG to glycerol. (D) Renal p-AMPK and p-ACC levels were analyzed using western blotting. Arrow, crown-like structures; DBM, HFD plus dibenzoylmethane; H&E, hematoxylin and eosin; HFD, high-fat diet group; ND, normal diet group; TG, triglycerides; WAT, white adipose tissue. $aP < 0.05$ compared with the ND group; $bP < 0.05$ compared with HFD group. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0206>.

Results

DBM reduces adipocyte size and number of crown-like structures in epididymal fat

The HFD-induced diabetic mouse model of obesity exhibits adipocyte hypertrophy and crown-like structures in fat tissues (Sun *et al.* 2011). In the HFD group, crown-like structure formation and adipocyte size were increased compared with that of the ND group (Fig. 2A and B). However, these changes were reversed by DBM administration.

DBM inhibits renal fatty acid synthesis by activating AMPK

A high level of TG is a risk factor for the progression of renal disease in humans (Trevisan *et al.* 2006). To measure the levels in the kidneys, TG was hydrolyzed to glycerol. HFD increased renal glycerol levels, but DBM administration to the HFD group did not significantly increase the levels compared with those of the ND group (ND, 0.33 ± 0.1 ; HFD, 0.63 ± 0.21 , and HFD+DBM; 0.47 ± 0.15 mg/g, Fig. 2C). In the HFD group, renal p-AMPK levels were significantly decreased, but acetyl-CoA carboxylase

(ACC) phosphorylation did not change, whereas DBM treatment activated AMPK and ACC phosphorylation (Fig. 2D). These findings suggest that DBM regulates renal lipid metabolism.

DBM improves damaged renal structure and urinary albumin-to-creatinine ratio (ACR)

The urinary ACR is a marker of impaired renal function and the level sharply increased in the HFD group, but DBM reversed the decreased urinary ACR (Fig. 3A). Moreover, the renal glomerular volume increased in the HFD group, but was similarly restored by DBM (Fig. 3B and C). Structurally, increased renal GBM thickness, podocyte effacement and slit diaphragm loss occurred in the HFD group, but these changes were alleviated by DBM (Fig. 3D, E and F). These data indicate that DBM exerts protective effects against renal injury induced by diabetes.

DBM inhibits macrophage infiltration and inflammation in DN

Using western blotting assays, we determined that the expression of renal nephrin protein was lower in

Figure 3

Effects of dibenzoylmethane (DBM) on high-fat diet (HFD)-induced diabetic nephropathy (DN). (A) Albumin-to-creatinine ratio (ACR) was measured in urine over a 24-h collection period. (B) Glomerular expansion was analyzed using H&E staining of renal glomeruli and renal ultrastructure was analyzed using transmission electron microscopy (TEM). Changes in glomerular volumes (C), slit pore numbers (D and E) and GBM thickness (D and F) were determined. ACR, albumin/creatinine ratio; DBM, HFD plus dibenzoylmethane; GBM, glomerular basement membrane; HFD, high-fat diet group; ND, normal diet group. ^a $P < 0.05$ compared with the ND group; ^b $P < 0.05$ compared with HFD group. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0206>.

Figure 4

Effects of dibenzoylmethane (DBM) on macrophage infiltration and inflammatory signals in the kidney. Renal nephrin, CD68, and arginase 2 levels were analyzed using western blotting and quantified (A). Renal MCP1 mRNA level was quantified using qPCR (B). Immunohistochemical staining of the MCP1 (C), F4/80 and CD11c (D). p-AMPK and inflammatory signals, including p-p38 and p-IKK β , were detected by western blotting in the kidney and quantified (E). AMPK, AMP-activated protein kinase; DBM, HFD plus dibenzoylmethane; HFD, high-fat diet group; ND, normal diet group; qPCR, quantitative polymerase chain reaction. ^a $P < 0.05$ compared with the ND group; ^b $P < 0.05$ compared with HFD group. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0206>.

the HFD group than in the ND group. DBM restored the level of nephrin expression. In the DBM group, both the protein levels of HFD-induced inflammation-related genes such as CD68 and arginase 2 and the mRNA levels of inflammatory cytokines such as MCP1 were significantly decreased by DBM (Fig. 4A and B). Moreover, MCP1, total macrophages (F4/80) and inflammatory macrophage (CD11c) markers were also strongly stained in the glomeruli of the HFD group compared to those of the ND group, but these effects were reduced by DBM administration (Fig. 4C and D). Furthermore, increased p-p38 and p-IKK kinase β expression levels

in the HFD group were significantly decreased by DBM administration (Fig. 4E). These findings suggest that DBM suppressed macrophage infiltration to the glomeruli and inflammation.

DBM activates AMPK more than curcumin in MES-13 cells

DBM activation of AMPK was stronger than that of curcumin, and AMPK phosphorylation increased in a dose-dependent manner in MES-13 cells (Fig. 5A and B). The HG-induced reduction of AMPK phosphorylation was

restored by DBM treatment. This change was inhibited by the AMPK inhibitor compound C (Fig. 5C). Thus, these findings suggested that DBM was a more potent AMPK activator than curcumin.

DBM inhibits inflammatory signal activation and macrophage migration

PA stimulation activated inflammatory signals, such as I κ B degradation and NF κ B p65 phosphorylation, which were ameliorated by DBM treatment (Fig. 5D). Moreover, DBM treatment with LPS suppressed RAW264.7 cell migration (Fig. 5E). In the CM, IKK activation, NF κ B p65 phosphorylation and I κ B degradation were observed in

the renal proximal tubule cells, but these effects were also ameliorated by DBM treatment (Fig. 5F). These data indicated that DBM inhibited macrophage activity and inflammation.

DBM exerts antioxidant effects on DN

In mesangial cells, HG and LPS stimulation increased the expression of both NOX2 and NOX4. With these changes, antioxidant pathways such as Nrf2/HO-1 expression were also activated. However, DBM treatment reduced these changes (Fig. 6A, B and C) and inhibited HFD-induced 8-OHdG expression in the glomeruli (Fig. 6D). These findings suggested that DBM exerted renoprotective effects through antioxidant activity.

Figure 5

AMP-activated protein kinase (AMPK) activation by dibenzoylmethane (DBM) and curcumin. (A) Comparison of effects of curcumin and DBM on AMPK activation in NRK-52E cells. (B) DBM dose-dependently activated p-AMPK. (C) Changes in p-AMPK expression induced by high-glucose (HG), compound C, or both in the presence or absence of DBM. (D) PA-induced inflammatory signals (I κ B and p-NF κ B p65) in MES-13 cells caused by DBM treatment. (E) LPS-induced macrophage migration was affected by DBM treatment. (F) p-IKK, I κ B, and p-NF κ B p65 levels were changed by CM and changes were reversed by DBM treatment. CM, Raw 264.7 cultured medium with LPS; DBM, dibenzoylmethane; HG, high-glucose, 30 mM glucose in DMEM; LG, low-glucose, 5.5 mM glucose in DMEM; LPS, lipopolysaccharide; PA, palmitate. ^a $P < 0.05$ compared with LG; ^b $P < 0.05$ compared with HG. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0206>.

Figure 6

Antioxidant effects of dibenzoylmethane (DBM). (A) Oxidative DNA damage marker, 8-OHdG, showed staining in glomeruli. (B) Changes in Nrf2/HO-1 expression by DBM in HG and LPS-stimulated MES-13 cells. (C and D) NOX2 and NOX4 protein and mRNA expressions change by DBM treatment in HG with LPS-stimulated MES-13. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; DBM, dibenzoylmethane; HG, high-glucose, 30 mM glucose in DMEM; LG, low-glucose, 5.5 mM glucose in DMEM; LPS, lipopolysaccharide. * $P < 0.05$ compared with LG; # $P < 0.05$ compared with HG. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0206>.

Discussion

Obesity induces cellular stress concomitantly with metabolic syndromes, including insulin resistance, glucose intolerance, dyslipidemia and hypertension, as well as the induction of DN by increased cellular inflammation mediated by the interaction between oxidative stress induction and macrophage interaction, which exacerbates cellular damage to lipids, proteins and DNA (Keaney *et al.* 2003, Qatanani & Lazar 2007). Metabolic inflammation is intensified in obesity by the infiltration of activated macrophages into tissues and secretion of inflammatory cytokines such as tumor necrosis factor (TNF)- α , IL-1 β and IL-6 (McArdle *et al.* 2013).

NOXs have been regarded as the major contributors to ROS generation. They consist of seven subunits, NOX1 to 5 and dual oxidase 1 (DUOX1) and 2. Among them, NOX2 and 4 are predominantly expressed in DN (Sedeek *et al.* 2010, You *et al.* 2013). Excessive ROS generation accelerates macrophage infiltration, and numerous

studies have provided strong evidence that macrophages are the cause of renal disease. Macrophage infiltration into the kidney in diabetes, which is a characteristic feature of DN, is one of the central mediators of renal vascular inflammation (Sassy-Prigent *et al.* 2000). MCP1 or chemokine (C-C motif) ligand 2 (CCL2) is one of the key chemokines that regulates the infiltration of monocytes/macrophages (Deshmane *et al.* 2009). Moreover, urinary MCP1/CCL2 levels are significantly increased in patients with DN and are correlated with the infiltration of CD68-positive macrophages into the renal interstitium. NF κ B signaling, activated by obesity, also increases proinflammatory chemokine production in macrophages (Mezzano *et al.* 2004, Eardley *et al.* 2006). In our previous study, the blockade of the MCP1 receptor by a CCR2 inhibitor ameliorated DN by preventing macrophage infiltration and inflammation in type 2 diabetic mice (Seok *et al.* 2013). Based on these data and the suppression of oxidative stress, the inhibition of inflammation is also considered to be an effective target

for DN treatment. In this study, DBM treatment reduced both macrophages infiltration and MCP1 expression, resulting in reduced oxidative-induced DNA damage of the glomeruli.

AMPK is a ubiquitous heterotrimeric kinase, which consists of a catalytic α -subunit and regulatory β - and γ -subunits. The enzyme, which is highly expressed in the kidneys, has various physiological functions and is involved in pathological processes, including ion transport, podocyte function and diabetic renal hypertrophy (Hallows *et al.* 2010). In diabetes, increase in numerous intracellular factors such as glucose, vascular endothelial growth factor MAPJ and angiotensin II lead to the inhibition of AMPK activation and induction of DN. Moreover, reduced AMPK activation associated with autophagy deficiency and cellular apoptosis subsequently induces diabetic complications including renal injury (Yao *et al.* 2016). Infiltrating macrophages secrete inflammatory cytokines, such as TNF- α , and induce apoptosis of proximal tubule cells (Wang *et al.* 2015). Conversely, an AMPK activator was shown to inhibit the proinflammatory responses by decreasing inflammatory stimuli induced by cellular ROS production and mitogen-activated protein kinase activation in macrophages (Jeong *et al.* 2009).

Curcumin, the active ingredient of turmeric, has been extensively investigated for its beneficial effects on health such as anticancer (Zhang *et al.* 2015), antioxidant (Xiong *et al.* 2015), antidiabetic (He *et al.* 2012) and anti-inflammatory in HFD obese rats (Maithilikarpagaselvi *et al.* 2016), which were shown to be mediated by AMPK activation. The renoprotective effects of curcumin have already been investigated in many studies (Soetikno *et al.* 2013, Ho *et al.* 2016). Our previous study also reported that curcumin ameliorates DN in type 2 diabetic rats through its antioxidant effects and regulation of renal lipid metabolism (Kim *et al.* 2016).

DBM, a β -diketone structural analog of curcumin (Fig. 1), has also received much attention owing to its biological properties. DBM has been reported to show anti-inflammatory effects (Anand *et al.* 2011), cell cycle regulation (Jackson *et al.* 2002) and anti-mutagenic effects (Shishu *et al.* 2003). We previously demonstrated the beneficial metabolic effects of DBM; in summary, DBM increased the phosphorylation of AMPK, stimulated glucose uptake in skeletal muscle cells and reduced the expression of fatty acid synthesis-related genes in preadipocytes. Moreover, DBM suppressed HFD-induced weight gain and fat accumulation in the liver (Kim *et al.* 2015). Although DBM exhibits antidiabetic effects,

its beneficial effects on DN have not been studied. Therefore, we studied the effects of DBM on DN and found that the compound reduced the number of HFD-induced crown-like structures, which consisted of dead or dying adipocytes surrounded by macrophages, in white adipose tissue. To evaluate the effect of DBM on lipid metabolism, we determined a renal TG level by hydrolyzing TGs to glycerol. The renal glycerol level was significantly increased by HFD, whereas DBM administration slightly reduced this level; however, the differences were not significant. The infiltration of inflammatory macrophage into the renal glomeruli and inflammatory signals, which were increased by HFD, were inhibited by DBM administration.

In this study, both *in vivo* and *in vitro* experiments indicated that renal AMPK activation decreased in the HFD group, whereas inflammatory signals such as p38, c-Jun N-terminal kinase, and IKK were increased. LPS stimulation increased Raw 264.7 cell migration and Raw 264.7 CM-induced inflammatory signals in NRK-52E cells; however, these changes were alleviated by DBM treatment. A study by Miller *et al.* (2008) showed that the macrophage migration inhibitory factor modulated AMPK activation, as well as functionally linked inflammation and metabolism in the heart (Miller *et al.* 2008) and induced cardiomyocyte apoptosis. Moreover, the administration of metformin, an AMPK activator, suppressed the LPS-induced inflammatory response of Raw 264.7 cells (Kim *et al.* 2014). However, we did not investigate the mechanisms of DBM action on macrophages.

Figure 7
Schematic presentation of effects of dibenzoylmethane (DBM) on diabetic nephropathy (DN) in mouse model of high-fat diet (HFD)-induced diabetes. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0206>.

Therefore, further studies are needed to elucidate how DBM directly regulates macrophage activation.

Collectively, as indicated in Fig. 7, the current study demonstrated the renoprotective effects of DBM against HFD-induced renal injury, which were mediated by the reduction of oxidative stress, attenuation of inflammatory macrophage infiltration into the kidneys, suppression of inflammatory signaling and stimulation of AMPK activation (Fig. 7). Therefore, DBM is a promising agent for the treatment of DN.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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