ELSEVIER

Nutrition 25 (2009) 964–972

NUTRITION

www.nutritionjrnl.com

Basic nutritional investigation

Curcumin prevents diabetes-associated abnormalities in the kidneys by inhibiting p300 and nuclear factor-κB

Jane Chiu, M.Sc., Zia A. Khan, Ph.D., Hana Farhangkhoee, M.Sc., and Subrata Chakrabarti, M.D., Ph.D.*

Department of Pathology, University of Western Ontario, London, Ontario, Canada

Manuscript received August 8, 2008; accepted December 21, 2008.

Abstract

Objective: Diabetic nephropathy is a debilitating disease that leads to end-stage renal failure in the Western world. Hyperglycemia is the initiating factor in several chronic diabetic complications which mediates increased oxidative stress and eventually the increased production of vasoactive factors and extracellular matrix proteins. We hypothesized that curcumin, a potent antioxidant, might be beneficial in preventing the development of diabetic nephropathy because this compound has been shown to inhibit p300, a histone acetyltransferase that plays a role in regulating gene expression through its interaction with the transcription factor nuclear factor-κB.

Methods: To test this hypothesis, male Sprague-Dawley rats were injected with streptozotocin to induce diabetes. These animals were subsequently treated with curcumin for a period of 1 mo.

Results: Real-time reverse transcriptase polymerase chain reaction analyses showed that diabetes-induced upregulation of vasoactive factors (endothelial nitric oxide synthase and enothelin-1), transforming growth factor-β1 and extracellular matrix proteins (fibronectin and extradomain-B–containing fibronectin) in the kidneys. These changes were associated with increased oxidative stress, mesangial expansion, and p300 and nuclear factor-κB activity that were prevented with curcumin treatment.

Conclusion: These beneficial effects of curcumin were mediated through the inhibition of p300 and nuclear factor-κB.

Keywords: Diabetes; Mesangial expansion; Curcumin; p300; Nuclear factor-κB

Introduction

Diabetic nephropathy is a major cause of morbidity in diabetic patients. A structural hallmark of this disease is thickening of the glomerular basement membrane and mesangial matrix expansion. Biochemically, such lesions are characterized by increased production of extracellular matrix (ECM) proteins [1]. Hyperglycemia is the key initiating factor in the development of all chronic diabetic complications including diabetic nephropathy [2,3]. It has been hypothesized that an increase in oxidative stress, as a result of chronic hyperglycemia, activates several signaling pathways that alter gene expression [4]. We and others previously demonstrated that high levels of glucose increase ECM proteins [5–7] in vitro and in vivo. Furthermore, we reported endothelin-1 (ET-1)–mediated expression of ECM proteins, fibronectin (FN), and extradomain-B–containing FN (EDB^+ FN) in all organs affected in chronic diabetes [5,6,8]. EDB^+ FN is a splice variant of FN that is absent in mature adult tissue and is upregulated in all target organs of diabetes, including the kidneys [8]. We also found increased serum levels of EDB^+ FN in patients with diabetic nephropathy [8]. Using cultured endothelial cells, we showed that EDB^+ FN may provide outside-in signaling leading to cell damage in diabetes [9]. Previous studies have indicated that ECM protein expression is dependent on transforming growth factor (TGF)-β1 [10] and that significant cross-talk occurs between TGF-β and transcription factor nuclear factor-κB (NF-κB) in the kidneys [11,12]. Consistent with this

This work was supported by grants from the Canadian Diabetes Association, the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Ontario.

* Corresponding author. Tel.: +519-685-8500, ext. 36350; fax: 519-661-3370.

E-mail address: Subrata.Chakrabarti@schulich.uwo.ca (S. Chakrabarti).

0899-9007/09/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.nut.2008.12.007

notion, we and others demonstrated significantly upregulated expression of TGF-β1 in the kidneys in diabetes [8,10,13]. We further showed that transcriptional coactivator p300, known to interact with NF-κB to regulate gene expression [13,14], may be involved in mediating FN expression [15].

Curcumin, a powerful antioxidant, is a component of turmeric found in the *Curcuma longa* plant and has been used for centuries in treating inflammatory ailments and conditions [16]. Its anti-inflammatory properties have been attributed to the main components of turmeric, the curcuminoids including curcumin [17]. Curcumin is a potent scavenger of reactive oxygen and nitrogen species such as hydroxyl radicals and nitrogen dioxide radicals [18,19]. We recently reported that curcumin is effective in preventing glucose-induced oxidative stress in the endothelial cells and in the heart of diabetic animals [20]. It has also been shown that short-term treatment of diabetic rats with curcumin prevents diabetes-induced decreased antioxidant enzyme levels and kidney dysfunction [21]. p300 is known to associate with the p65 subunit of NF-κB to regulate gene expression [14]. These changes may be mediated by the inhibition of histone acetyltransferase p300 [22]. In this study, we investigated whether curcumin prevents the development of structural lesions characteristic of diabetic nephropathy. To gain further insight into the effects of curcumin, we examined the possible mechanism of action and relevant ECM protein molecules such as FN and EDB$^+$ FN and vasoactive factors, endothelial nitric oxide synthase (eNOS) and ET-1, and TGF-β1. We further expanded the study to investigate whether the action of curcumin is mediated through p300 and NF-κB.

Materials and methods

Animal studies

Male Sprague-Dawley rats weighing 200–250g were obtained from Charles River (Montreal, QC, Canada) and randomly divided into three groups: control (CO), diabetic (DM), or diabetic treated with curcumin (DM-CUR). Diabetes was induced by a single intravenous injection of streptozotocin (65 mg/kg in citrate buffer, pH 5.6) [23,24]. CO animals were injected with the same volume of citrate buffer. Curcumin, reconstituted in dimethylsulfoxide and diluted in ethanol, was administered (150 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}, Sigma-Aldrich, Oakville, ON, Canada) intraperitoneally [20,25,26]. The diabetic animals were implanted with insulin implants that released small doses of insulin to prevent ketonuria (2 U/d, Linshin Canada Inc., Toronto, ON, Canada). The animals were monitored through the regular assessment of body weight and blood glucose concentrations. After 4 wk of treatment, the animals were sacrificed and the kidney tissues were snap-frozen for gene expression analysis or placed in 10% formalin for paraffin embedding. All animals were cared for according to the Guiding Principle in the Care and Use of Animals. All experiments were approved by the University of Western Ontario council on animal care committee.

RNA extraction and real-time reverse transcriptase polymerase chain reaction analysis

RNA was isolated from rat kidney tissues as previously described [5,27,28]. Complementary DNA was subsequently synthesized from the total RNA. The mRNA levels of FN, EDB$^+$ FN, eNOS, ET-1, TGF-β1, heme oxygenase-1 (HO-1), and p300 were quantified using LightCycler (Roche Diagnostics Canada, Laval, QC, Canada). The reaction mixture (total volume 20 μL) consisted of the following reagents: 10 μL of SYBR Green Taq Ready Mix (Sigma-Aldrich), 1.6 μL of 25 mmol/L of $MgCl_2$, 1 μL of each forward and reverse 10-μmol/L primers, 5.4 μL of H_2O, and 1 μL of cDNA template. The data were normalized to housekeeping gene β-actin to account for reverse transcription efficiencies. The data were expressed relative to CO. The primer sequences have previously been published [15,29,30].

Immunohistochemistry

Formalin-fixed tissues embedded in paraffin were sectioned at 5 μm thickness on positively charged slides. The

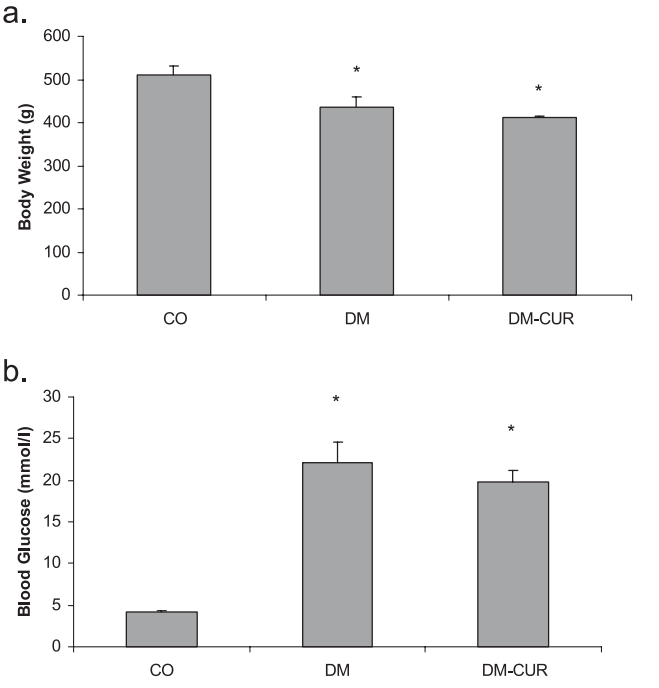

Fig. 1. Diabetic animals showed (a) decreased body weight gain and (b) increased blood glucose levels compared with the CO group at the time of sacrifice. Curcumin treatment had no effects on these parameters ($^*P < 0.05$ versus CO, $n = 5$/group). CO, control rats; DM, diabetic rats; DM-CUR, diabetic rats treated with curcumin.

sections were stained with hematoxylin and eosin and periodic acid-Schiff.

The kidney tissues were also analyzed for 8-hydroxy-2′-deoxyguanosine (8-OHdG; Chemicon International Inc., Hercules, CA, USA), a sensitive marker for oxidative DNA damage [31], nitrotyrosine (NT; Cayman Chemical, Ann Arbor, MI, USA), a marker for oxidative protein damage [32], and p300 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The slides were stained using a Vectastain Elite Kit for 8-OHdG and EnVision kits for NT. The chromogen 3,3′ diamino benzine (Sigma-Aldrich) was used for detection. Non-immune horse serum was used as a negative control. Ten random fields were examined by two investigators unaware of the experimental treatment. 8-OHdG and p300 immunoreactivities were assessed by the presence of positively stained nuclei in the glomeruli, and NT was evaluated by comparing the relative staining intensity in the cytoplasm.

Nuclear factor-κB was investigated by using a monoclonal NF-κB p65 antibody (1:200, Santa Cruz Biotechnology). An AlexaFluor 488-labelled goat anti-mouse secondary antibody (Invitrogen Canada Inc., Burlington, ON, Canada) was used for detection using a fluorescent microscope (Olympus BX51, Olympus Canada Inc., Markham, ON, Canada) and Northern Eclipse software (Empix Inc., Buffalo Grove, IL, USA). The slides were counterstained with Hoechst 33342 dye (1 μg/mL, Invitrogen Canada Inc.).

Statistical analysis

The data are expressed as mean ± standard error of the mean. Statistical significance was determined by analysis of variance followed by the Bonferroni-Dunn test. Differences were considered to be statistically significant at values of $P < 0.05$.

Results

Induction of diabetes and clinical monitoring

Diabetic dysmetabolism in the animals was assessed through the monitoring of body weight gain and blood glucose levels. The diabetic animals showed a significantly decreased body weight gain (CO 510 ± 20g, DM 435 ± 25g, $P = 0.047$) and increased blood glucose levels (CO 4.2 ±

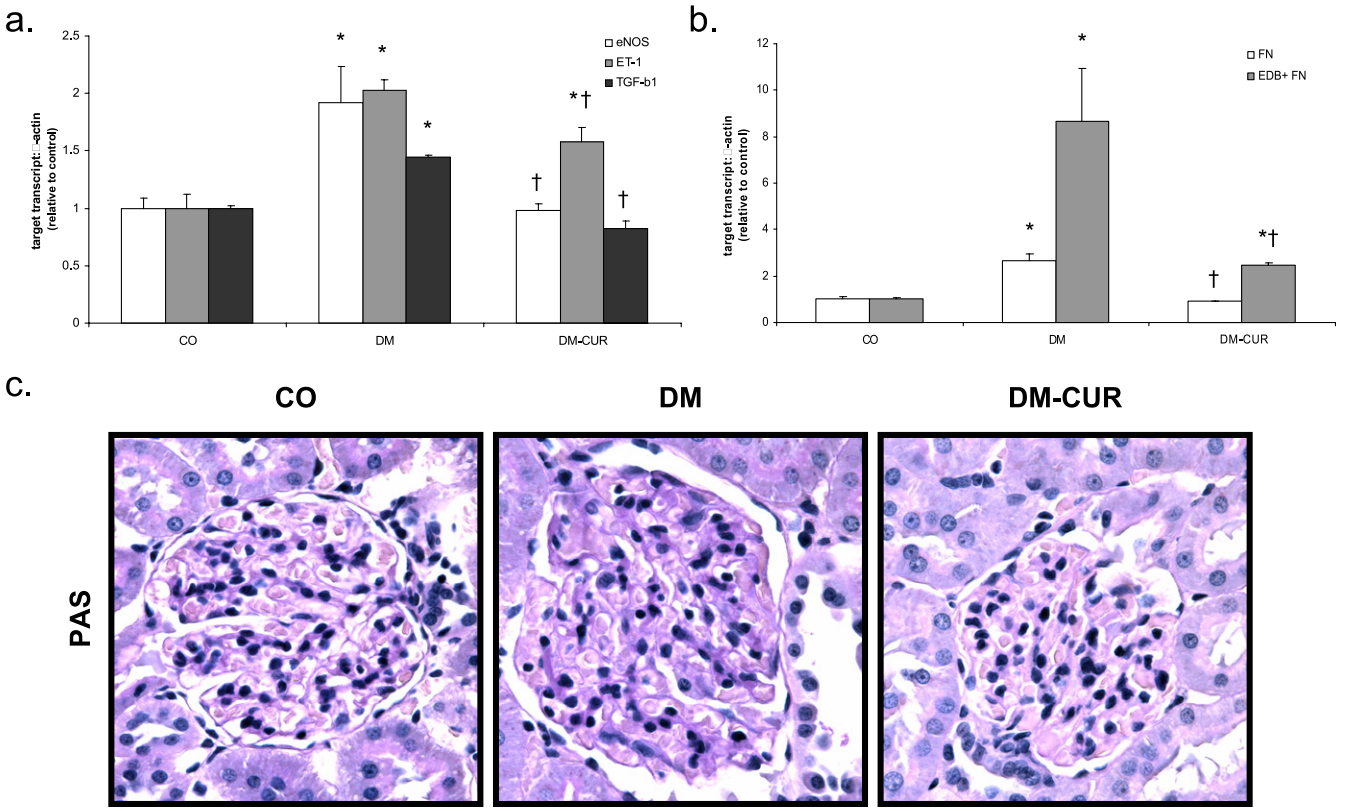

Fig. 2. Real-time reverse transcriptase polymerase chain reaction analyses of vasoactive factors eNOS and ET-1 (a) and extracellular matrix proteins FN and EDB$^+$ FN (b) showing that diabetes-induced upregulation of these transcripts are prevented with curcumin treatment. (c) Histologic analysis utilizing PAS stain verified the molecular findings indicating that diabetes-induced mesangial expansion is prevented with curcumin ($^*P < 0.05$ versus CO, $^\dagger P < 0.05$ versus DM, magnification 60× for all micrographs, $n = 5$/group). CO, control rats; DM, diabetic rats; DM-CUR, diabetic rats treated with curcumin; EDB$^+$ FN, extradomain-B–containing fibronectin; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; FN, fibronectin; PAS, periodic acid-Schiff; TGF-b1, transforming growth factor-β1.

0.2 mmol/1, DM 22.1 ± 2.5 mmol/1, $P = 0.0001$) when compared with the non-diabetic CO animals (Fig. 1). Treatment with curcumin did not affect these parameters (DM-CUR 413 ± 2g, $P = 0.001$ versus CO for body weight, and DM-CUR 19.8 ± 1.4 mmol/1, $P = 0.0001$ versus CO for blood glucose levels; Fig. 1).

Diabetes-induced abnormalities in the kidneys are prevented by curcumin

The kidney tissues from the diabetic animals showed increased mRNA expression of vasoactive factors eNOS (CO 1 ± 0.086, DM 1.920 ± 0.313, $P = 0.022$) and ET-1 (CO 1 ± 0.125, DM 2.027 ± 0.094, $P = 0.0001$) and for TGF-β1 (CO 1 ± 0.022, DM 1.446 ± 0.0125, $P = 0.00001$) when compared with the non-diabetic CO group (Fig. 2a). Curcumin treatment completely prevented eNOS (DM-CUR 0.979 ± 0.058, $P = 0.022$ versus DM) and TGF-β1 (DM-CUR 0.820 ± 0.067, $P = 0.002$ versus DM) upregulation and significantly decreased ET-1 (DM-CUR 1.575 ± 0.129, $P = 0.008$ versus CO and $P = 0.022$ versus DM). Similar results were obtained when we analyzed ECM proteins FN (CO 1 ± 0.125, DM 2.651 ± 0.308, $P = 0.020$) and its splice variant EDB$^+$ FN (CO 1 ± 0.080, DM 8.674 ± 2.246, $P = 0.019$). As with the vasoactive factors and TGF-β1, this diabetes-induced effect was prevented with curcumin treatment in FN (DM-CUR 0.897 ± 0.026, $P = 0.029$ versus DM) and EDB$^+$ FN (DM-CUR 2.466 ±

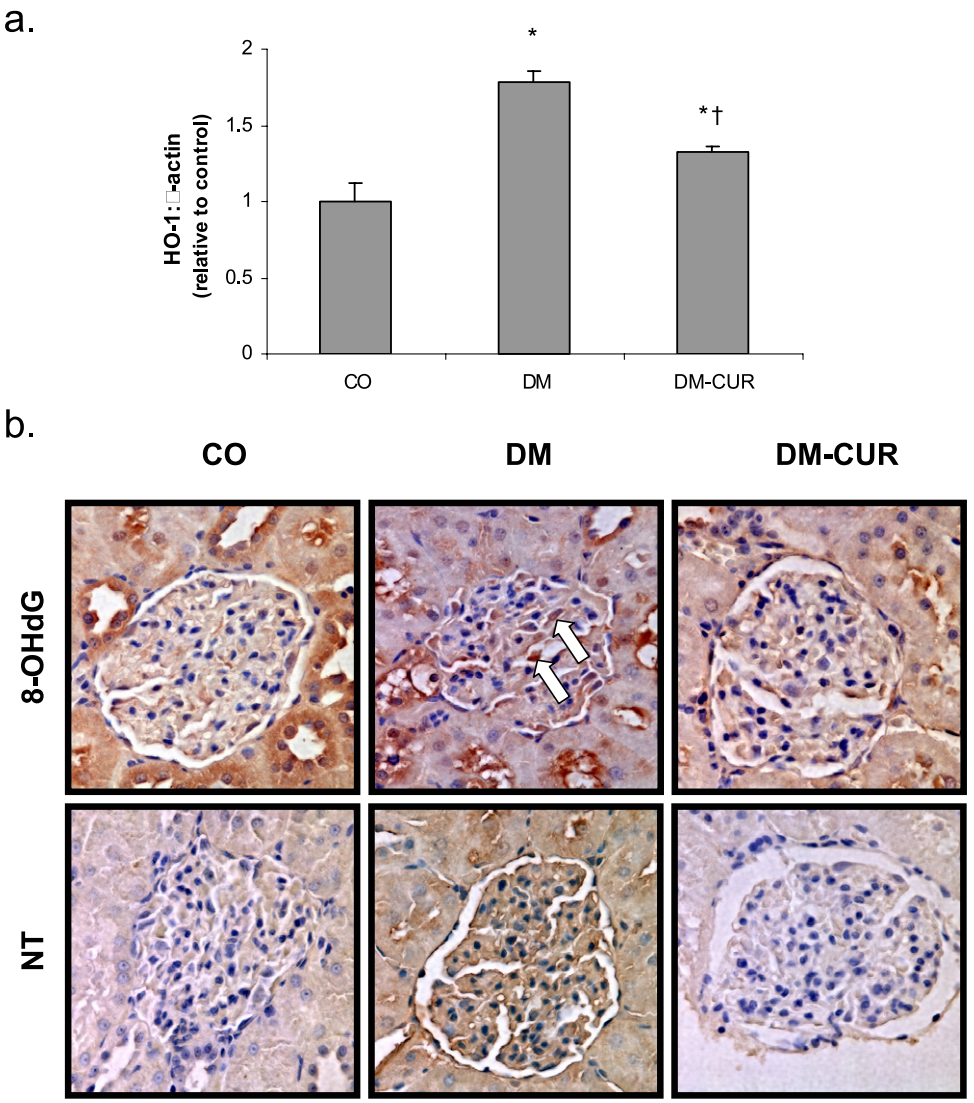

Fig. 3. (a) Real-time reverse transcriptase polymerase chain reaction analysis of HO-1, a molecular marker of oxidative stress, showed that diabetes-induced increased mRNA is prevented with curcumin. (b) 8-OHdG and NT showing diabetes-induced increased positivity in the glomeruli and the effects of curcumin ($^*P < 0.05$ versus CO, $^\dagger P < 0.05$ versus DM, magnification 60× for all micrographs, arrows indicate positive nuclei, $n = 5$/group). 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CO, control rats; DM, diabetic rats; DM-CUR, diabetic rats treated with curcumin; HO-1, heme oxygenase-1; NT, nitrotyrosine.

0.120, $P = 0.00008$ versus CO and $P = 0.040$ versus DM; Fig. 2b). To determine whether these molecular alterations yielded structural changes at the level of the organ, histologic analysis of the mesangium in the kidneys through periodic acid-Schiff stain was undertaken. These stains revealed mesangial expansion in the diabetic rat kidneys that was not evident in the CO or DM-CUR rats (Fig. 2c).

Curcumin decreases diabetes-induced oxidative stress in the kidneys

We investigated oxidative stress using several methods because glucose-induced oxidative stress has been postulated to be a key mechanism in chronic diabetic complications. Diabetic animals exhibited increased HO-1 transcript levels (CO 1 ± 0.115, DM 1.788 ± 0.064, $P = 0.0009$), a molecular marker of oxidative stress (Fig. 3a). One month of curcumin treatment reduced HO-1 upregulation (DM-CUR 1.319 ± 0.0394, $P = 0.0492$ versus CO and $P = 0.005$ versus DM; Fig. 3a). Furthermore, immunohistochemical examination of the kidney tissues showed increased nuclear staining for 8-OHdG (Fig. 3b, upper panel) and cytoplasmic staining for NT (Fig. 3b, lower panel), respectively, in the glomeruli of the diabetic animals. In complete accordance with the molecular changes and periodic acid-Schiff staining, curcumin reduced 8-OHdG and NT-positive cells (Fig. 3b).

Curcumin may exert its effect through transcriptional coactivator p300 and transcriptional factor NF-κB in kidneys of diabetic rats

We previously reported that diabetes-associated ECM protein expression is mediated through p300 induction in the heart [15]. Therefore, we tested whether the beneficial

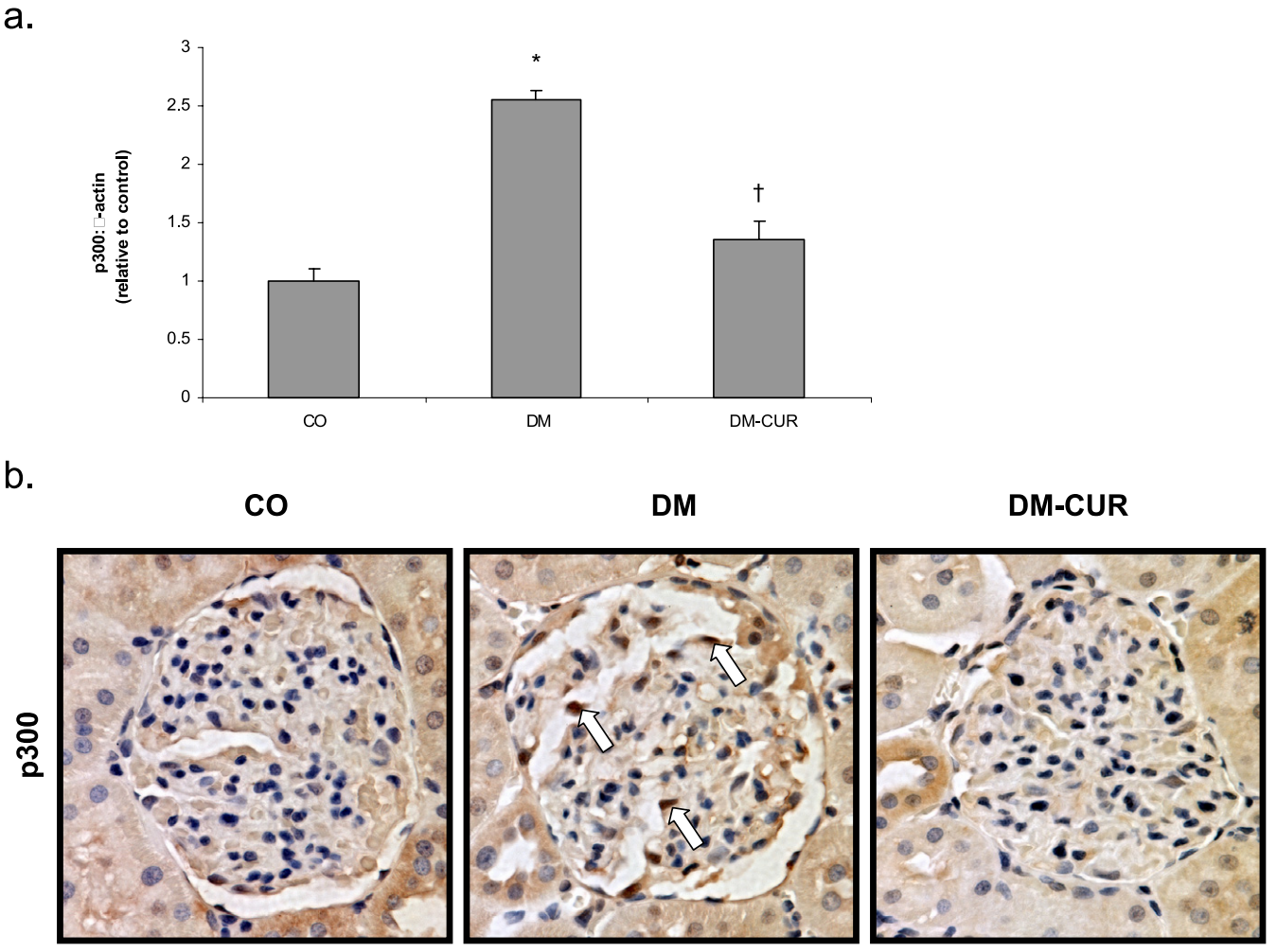

Fig. 4. p300 mRNA and protein expression as assessed through real-time reverse transcriptase polymerase chain reaction and immunohistochemical analyses, respectively, revealed that diabetes-induced increases in p300 mRNA and protein are prevented with curcumin ($^{*}P < 0.05$ versus CO, $^{\dagger}P < 0.05$ versus DM, magnification 60× for all micrographs, arrows indicate positive nuclei, $n = 5$/group). CO, control rats; DM, diabetic rats; DM-CUR, diabetic rats treated with curcumin.

effects of curcumin are mediated by p300 inhibition. Analysis of transcriptional coactivator p300 mRNA expression in the diabetic animals exhibited increased levels compared with the non-diabetic controls (CO 1 ± 0.111, DM 2.549 ± 0.077, $P < 0.00001$; Fig. 4a). This diabetes-induced upregulation of p300 was also prevented in the curcumin-treated animals (DM-CUR 1.360 ± 0.155, $P = 0.00004$ versus DM; Fig. 4a). Immunohistochemical analysis showed increased p300 nuclear positivity in the glomeruli of diabetic rats that were not seen in the diabetic rats treated with curcumin (Fig. 4b).

We used immunofluorescence to determine NF-κB p65 translocation because a limited amount of tissues was available for multiple studies. Staining for NF-κB p65 revealed increased nuclear protein in the glomeruli of diabetic animals (Fig. 5). This was not seen in the curcumin-treated animals because their staining was similar to that of the non-diabetic controls (Fig. 5).

Discussion

The diabetic animals displayed diabetic dysmetabolism and renal lesions consistent with diabetic nephropathy such as mesangial matrix expansion. Vasoactive factors eNOS and ET-1 were upregulated in diabetes in association with TGF-β1 and increased ECM proteins in the kidneys. These alterations, which are characteristic in the kidneys in diabetes, were prevented with curcumin. Furthermore, we demonstrated diabetes-induced oxidative damage to the kidneys

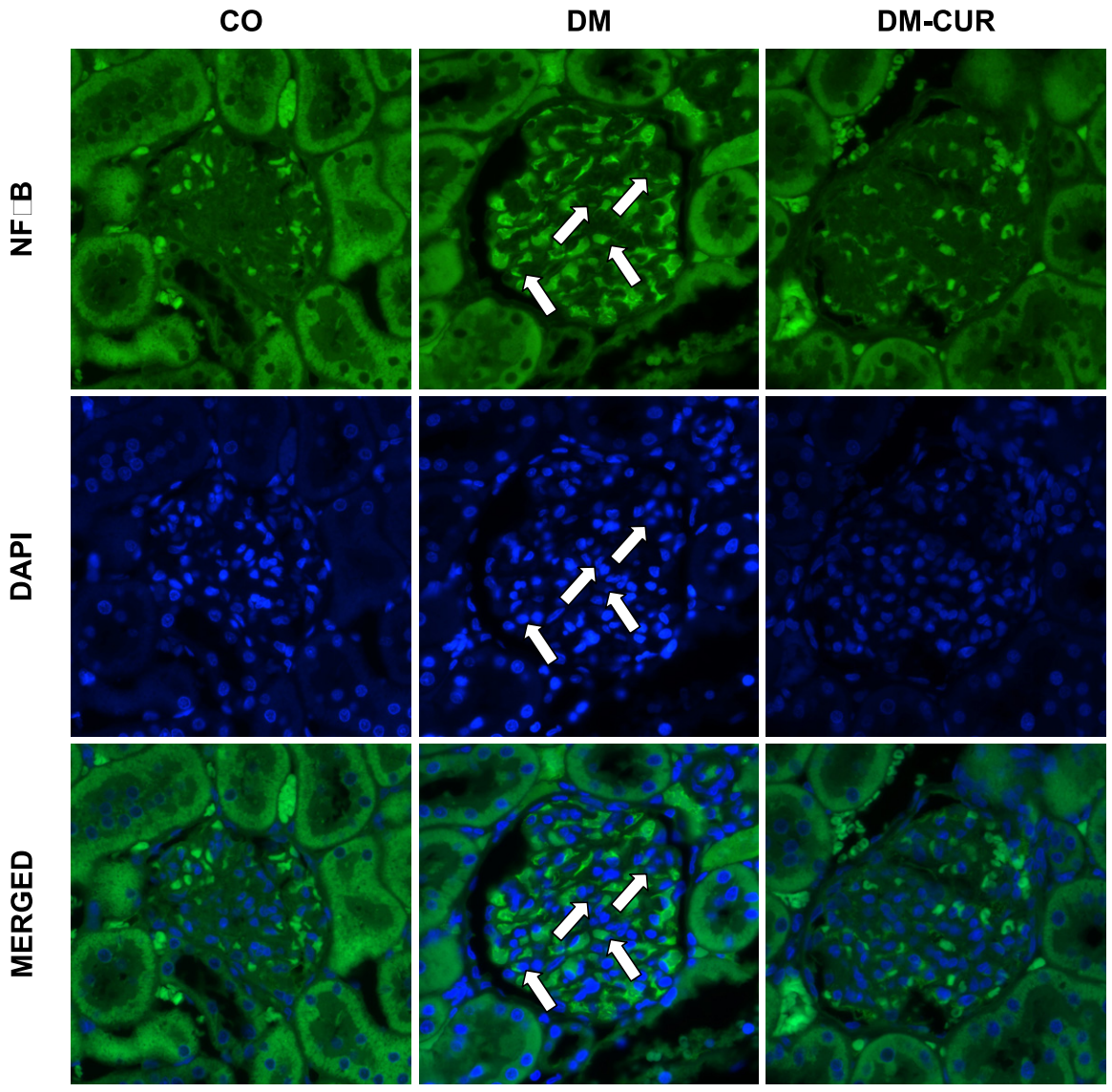

Fig. 5. Immunofluorescent micrographs depicting increased NFκB protein and nuclear translocation in the glomeruli of the diabetic rats compared with the controls that were not seen in the diabetic animals treated with curcumin (arrows indicate positive nuclei, magnification 60× for all micrographs, $n = 3$/group). CO, control rats; DAPI, 4,6-diamidino-2-phenylindoe; DM, diabetic rats; DM-CUR, diabetic rats treated with curcumin; NFκB, nuclear factor-κB.

as evidenced by increased HO-1, NT, and 8-OHdG levels in the glomeruli, suggesting that oxidative stress may play a key role in diabetic nephropathy. And at the transcription factor level, p300 and NF-κB was increased in diabetes. All such abnormalities were attenuated by curcumin treatment.

Curcumin has been shown to prevent oxidative stress in several cell types including endothelial cells and in several malignant cell types [33–35]. This compound is a potent antioxidant that has been reported to scavenge oxidative and nitrosative radicals [18,19,36,37]. The results from this study corroborate previous findings because we have now found that curcumin prevented diabetes-induced HO-1 upregulation and 8-OHdG and NT formation in the kidneys, with 8-OHdG being a marker for oxidative DNA damage and NT being a marker for nitrosative protein damage [31,32]. We also previously reported that curcumin prevents diabetes-induced oxidative stress in the heart [20]. Oxidative stress has been demonstrated to be a key factor in the development of diabetic complications, including diabetic nephropathy [38–40]. It is of interest to note that in our study curcumin treatment did not significantly affect the blood glucose level or body weight gain of the diabetic rats. It has previously been reported that curcumin may have antihyperglycemic properties [21,41,42]. However, numerous studies have also shown that curcumin does not significantly alter blood glucose levels [43–45]. Further studies are necessary to determine whether this discrepancy is due to experimental conditions.

Diabetic nephropathy is characterized structurally by glomerular basement membrane thickening and mesangial expansion due to the accumulation of ECM proteins including FN. Previous studies have found that TGF-β1 mediates FN synthesis in mesangial cells under high glucose conditions [46]. Furthermore, our laboratory has shown that vasoactive factors such as ET-1 may act as an upstream mediator of FN expression in diabetes by transcription factor NF-κB [5,6]. It has been demonstrated that curcumin exerts one of its beneficial effects through the inhibition of NF-κB [47–50]. It has also been suggested that curcumin may act by influencing p300 [20]. We previously demonstrated that p300 may be one of the key factors influencing glucose-induced FN upregulation and activation of transcription factors in the heart and retina [15]. In keeping with such studies, we have demonstrated that curcumin may also be effective in preventing renal damage in diabetes. The present study has shown curcumin to be effective in attenuating p300 transcript and protein levels in the diabetic kidney and preventing nuclear translocation of NF-κB. Recently, researchers have shown that TGF-β1 enhanced the acetylation of the p65 subunit of NF-κB by p300 in HeLa cells [51]. Taken together, these findings suggest that p300 and NF-κB may be important in mediating the development and progression of diabetic nephropathy, possibly through the upregulation of vasoactive factors and ECM proteins. Furthermore, increased expression of TGF-β1 in diabetes may further exacerbate this pathologic process through p300 and NF-κB. Thus, curcumin may act on gene expression through its interaction with p300 and NF-κB to attenuate the upregulation of ECM proteins. A schematic representation of the possible pathway is illustrated in Figure 6. In keeping with previous studies, the findings from this study exemplified the beneficial effects of curcumin treatment on diabetes-induced renal lesions [52].

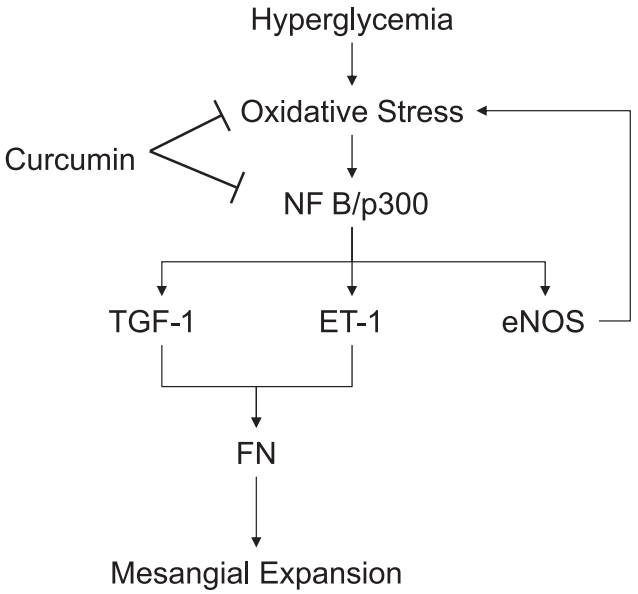

Fig. 6. Schematic representation of the points of curcumin blockade in hyperglycemia-induced mesangial expansion in diabetic nephropathy. eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; FN, fibronectin; NFκB, nuclear factor-κB; TGF-β1, transforming growth factor-β1.

Conclusions

This study shows, for the first time, that a specific mechanism involving transcriptional coactivator p300 and NF-κB may mediate diabetes-induced increased expression of ECM proteins in the kidneys. These data, in conjunction with studies from other investigators, suggest that curcumin may be beneficial in patients with chronic diabetic complications.

References

[1] Schena FP, Gesualdo L. Pathogenetic mechanisms of diabetic nephropathy. J Am Soc Nephrol 2005;16(suppl 1):S30–3.

[2] Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.

[3] United Kingdom Prospective Diabetic Study. Lancet 1998;352: 837–53.
[4] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.
[5] Chen S, Mukherjee S, Chakraborty C, Chakrabarti S. High glucose-induced, endothelin-dependent fibronectin synthesis is mediated via NF-kappa B and AP-1. Am J Physiol Cell Physiol 2003;284: C263–72.
[6] Chen S, Khan ZA, Cukiernik M, Chakrabarti S. Differential activation of NF-kappa B and AP-1 in increased fibronectin synthesis in target organs of diabetic complications. Am J Physiol Endocrinol Metab 2003;284:E1089–97.
[7] Pugliese G, Pricci F, Pugliese F, Mene P, Lenti L, Andreani D, et al. Mechanisms of glucose-enhanced extracellular matrix accumulation in the rat glomerular mesangial cells. Diabetes 1994;43: 478–90.
[8] Khan ZA, Farhangkhoee H, Mahon JL, Bere L, Gonder JR, Chan BM, et al. Endothelins: regulators of extracellular matrix protein production in diabetes. Exp Biol Med (Maywood) 2006;231: 1022–9.
[9] Khan ZA, Chan BM, Uniyal S, Barbin YP, Farhangkhoee H, Chen S, Chakrabarti S. EDB fibronectin and angiogenesis—a novel mechanistic pathway. Angiogenesis 2005;8:183–96.
[10] Sharma K, Jin Y, Guo J, Ziyadeh FN. Neutralization of TGF-beta by anti–TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. Diabetes 1996;45:522–30.
[11] Nagarajan RP, Chen F, Li W, Vig E, Harrington MA, Nakshatri H, et al. Repression of transforming-growth-factor-beta–mediated transcription by nuclear factor kappaB. Biochem J 2000;348: 591–6.
[12] Wang W, Huang XR, Li AG, Liu F, Li JH, Truong LD, et al. Signaling mechanism of TGF-beta1 in prevention of renal inflammation: role of Smad7. J Am Soc Nephrol 2005;16:1371–83.
[13] Shankland SJ, Scholey JW, Ly H, Thai K. Expression of transforming growth factor-beta 1 during diabetic renal hypertrophy. Kidney Int 1994;46:430–42.
[14] Gerritsen ME, Willams AJ, Neish AS, Moore S, Shi Y, Collins T. CREB-binding protein/p300 are transcriptional coactivators of p65. Proc Natl Acad Sci U S A 1997;94:2927–32.
[15] Kaur H, Chen S, Xin X, Chiu J, Khan ZA, Chakrabarti S. Diabetes-induced extracellular matrix protein expression is mediated by transcription coactivator p300. Diabetes 2006;55:3104–11.
[16] Ammon HP, Wahl MA. Pharmacology of Curcuma longa. Planta Med 1991;57:1–7.
[17] Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci 2006;78: 2081–7.
[18] Reddy AC, Lokesh BR. Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol Cell Biochem 1994;137:1–8.
[19] Sreehayan, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997;49:105–7.
[20] Farhangkhoee H, Khan ZA, Chen S, Chakrabarti S. Differential effects of curcumin on vasoactive factors in the diabetic rat heart. Nutr Metab 2006;3:27–34.
[21] Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric (Curcuma longa), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol 2006;33:940–5.
[22] Balasubramanyam K, Varier RA, Altaf M, Swaminathan V, Siddappa NB, Ranga U, et al. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. J Biol Chem 2004;279:51163–71.
[23] Cai L, Chen S, Evans T, Cherian MG, Chakrabarti S. Endothelin-1–mediated alteration of metallothionein and trace metals in the liver and kidneys of chronically diabetic rats. Int J Exp Diabetes Res 2002;3:193–8.
[24] Chen S, Evans T, Deng D, Cukiernik M, Chakrabarti S. Hyperhexosemia induced functional and structural changes in the kidneys: role of endothelins. Nephron 2002;90:86–94.
[25] Srimal RC, Dhawan BN. Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. J Pharm Pharmacol 1973;25:447–52.
[26] Arora RB, Kapoor V, Basu N, Jain AP. Antiinflammatory studies on Curcuma longa (turmeric). Indian J Med Res 1971;59:1289–95.
[27] Chen S, Apostolova MD, Cherian MG, Chakrabarti S. Interaction of endothelin-1 with vasoactive factors in mediating glucose-induced increased permeability in endothelial cells. Lab Invest 2000;80: 1311–21.
[28] Khan ZA, Cukiernik M, Gonder JR, Chakrabarti S. Oncofetal fibronectin in diabetic retinopathy. Invest Ophthalmol Vis Sci 2004; 45:287–95.
[29] Fukuda G, Khan ZA, Barbin YP, Farhangkhoee H, Tilton RG, Chakrabarti S. Endothelin-mediated remodeling in aortas of diabetic rats. Diabetes Metab Res Rev 2005;21:367–75.
[30] Xu B, Chiu J, Feng B, Chen S, Chakrabarti S. PARP activation and the alteration of vasoactive factors and extracellular matrix protein in the retina and kidneys in diabetes. Diabetes Metab Res Rev 2008;24: 404–12.
[31] Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic Res Commun 1986;1:163–72.
[32] Beal MF. Oxidatively modified proteins in aging and disease. Free Radic Biol Med 2002;32:797–803.
[33] Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic Biol Med 2000; 28:1303–12.
[34] Chan WH, Wu CC, Yu JS. Curcumin inhibits UV irradiation-induced oxidative stress and apoptotic biochemical changes in human epidermoid carcinoma A431 cells. J Cell Biochem 2003; 90:327–38.
[35] Chan WH, We HJ, Hsuuw YD. Curcumin inhibits ROS formation and apoptosis in methylglyoxal-treated human hepatoma G2 cells. Ann N Y Acad Sci 2005;1042:372–8.
[36] Toda S, Miyase T, Arichi H, Tanizawa H, Takino Y. Natural antioxidants. III. Antioxidative components isolated from rhizome of Curcuma longa L. Chem Pharm Bull 2005;33:1725–8.
[37] Unnikrishnan MK, Rao MN. Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. Mol Cell Biochem 1995;146: 35–7.
[38] Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19:257–67.
[39] Agardh CD, Stenram U, Torffvit O, Agardh E. Effects of inhibition of glycation and oxidative stress on the development of diabetic nephropathy in rats. J Diabetes Complications 2002;16: 395–400.
[40] Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R, Haneda M. Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. J Am Soc Nephrol 2003;14:S250–3.
[41] Arun N, Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr 2002;57: 41–52.
[42] Seo KI, Choi MS, Jung UJ, Kim HJ, Yeo J, Jeon SM, Lee MK. Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. Mol Nutr Food Res 2008;52:995–1004.
[43] Babu PS, Srinivasan K. Influence of dietary curcumin and cholesterol on the progression of experimentally induced diabetes in albino rat. Mol Cell Biochem 1995;152:13–21.

[44] Majithiya JB, Balaraman R. Time-dependent changes in antioxidant enzymes and vascular reactivity of aorta in streptozotocin-induced diabetic rats treated with curcumin. J Cardiovasc Pharmacol 2005;46:697–705.
[45] Suryanarayana P, Saraswat M, Mrudula T, Krishna TP, Krishnasway K, Reddy GB. Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci 2005;46:2092–9.
[46] Oh JH, Ha H, Yu MR, Lee HB. Sequential effects of high glucose on mesangial cell transforming growth factor-beta 1 and fibronectin synthesis. Kidney Int 1998;54:1872–8.
[47] Chan MM. Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem Pharmacol 1995;49:1551–6.
[48] Singh S, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) (corrected). J Biol Chem 1995;270:24995–5000.
[49] Bierhaus A, Zhang Y, Quehenberger P, Luther T, Haase M, Muller M, et al. The dietary pigment curcumin reduces endothelial tissue factor gene expression by inhibiting binding of AP-1 to the DNA and activation of NF-kappa B. Thromb Haemost 1997;77:772–82.
[50] Pendurthi UR, Williams JT, Rao LV. Inhibition of tissue factor gene activation in cultured endothelial cells by curcumin. Suppression of activation of transcription factors Egr-1, AP-1, and NF-kapp B. Arterioscler Thromb Vasc Biol 1997;17:3406–13.
[51] Ishinaga H, Jono H, Lim JH, Kweon SM, Xu H, Ha UH, et al. TGF-beta induces p65 acetylation to enhance bacteria-induced NF-kappaB activation. EMBO J 2007;26:1150–62.
[52] Suresh BP, Srinivasan K. Amelioration of renal lesions associated with diabetes by dietary curcumin in streptozotocin diabetic rats. Mol Cell Biochem 1998;181:87–96.