Therapeutic Potential of (−)-Epigallocatechin 3-O-Gallate on Renal Damage in Diabetic Nephropathy Model Rats

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ABSTRACT

Previous investigations have demonstrated that green tea polyphenols and partially hydrolyzed guar gum as dietary fiber have antioxidative and hypolipidemic activity, respectively, supporting their reduction of risk factors in the course of diabetic nephropathy via a hypoglycemic effect and ameliorating the decline of renal function through their combined administration to rats with subtotal nephrectomy plus streptozotocin (STZ) injection. As a further study, we examined whether (−)-epigallocatechin 3-O-gallate (EGCg), the main polyphenolic compound, could ameliorate the development of diabetic nephropathy. Rats with subtotal nephrectomy plus STZ injection were orally administrated EGCg at doses of 25, 50, and 100 mg/kg body weight/day. After a 50-day administration period, EGCg-treated groups showed suppressed hyperglycemia, proteinuria, and lipid peroxidation, although there were only weak effects on the levels of serum creatinine and glycosylated protein. Furthermore, EGCg reduced renal advanced glycation end-product accumulation and its related protein expression in the kidney cortex as well as associated pathological conditions. These results suggest that EGCg ameliorates glucose toxicity and renal injury, thus alleviating renal damage caused by abnormal glucose metabolism-associated oxidative stress involved in renal lesions of diabetic nephropathy.

Diabetic nephropathy is one of the most serious complications in diabetes mellitus and has been the most common cause of end-stage renal failure among patients undergoing chronic hemodialysis therapy since 1998 (Nakai et al., 2005). At present, patients with diabetes mellitus in Japan are estimated to number above 7 million, and the number is increasing; however, maintaining prolonged dialysis therapy is a great burden on patients both mentally and physically, and social problems, including financial issues, have also been raised. Therefore, the daily consumption of food and drink containing effective agents for the management of on-set and/or progression of diabetic complications has been receiving attention to reduce the number of patients with end-stage renal failure.

In Japan, there is much debate over the health benefits of green tea extracts, and it is common to drink tea with a meal. In previous studies, we reported that green tea polyphenols have antioxidant properties, and green tea is a useful agent to protect against protein oxidation- and glycation-associated diseases (Yokozawa et al., 1996, 1997, 1998; Nakagawa and Yokozawa, 2002; Nakagawa et al., 2002). Green tea polyphenols were also indicated as beneficial agents to manage the development of diabetic nephropathy induced by subtotal nephrectomy plus streptozotocin (STZ) injection (Yokozawa et al., 2005). Alternatively, of the catechins, (−)-epigallocatechin 3-O-gallate (EGCg) is known to be the most abundant in green tea. Recently, we have reported that EGCg had an antioxidative effect on creatinine (Cr) oxidation in rats with chronic renal failure and thus inhibited methylguanidine production in an adenine-induced renal failure model (Nakagawa et al., 2004). Furthermore, there are some experimental reports of EGCg on diabetes mellitus, e.g., administration of EGCg reduced the level of mRNAs for glyconeogenesis enzymes (Koyama et al., 2004), and EGCg caused many similar effects to insulin, including repression of glucose production and phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression in cells (Waltner-Law et al., 2002); however, the mechanisms of EGCg action in diabetic nephropathy remain unclear.

Therefore, to evaluate the effect of EGCg as a representative polyphenol on diabetic nephropathy, we investigated the...
effect of EGCg on glucose-derived metabolic disorders such as glycation and oxidative stress in diabetic nephropathy induced by subtotal nephrectomy plus STZ injection.

Materials and Methods

Materials. The following reagents were purchased from Wako Pure Chemicals (Osaka, Japan): 4,6-dihydroxy-2-mercaptoxypiridine (2-thiobarbituric acid; TBA), Coomassie Brilliant Blue R-250, bovine serum albumin (BSA), 2-amino-2-hydroxymethyl-1,3-propadiol (tris(hydroxymethyl) aminomethane), Tween 20, glycerol, p-phenylenediamine, CBB R-250, and Coomassie Brilliant Blue R-250. The other reagents were purchased from Sigma-Aldrich (St. Louis, MO). Enhanced chemiluminescence Western blotting detection reagents were purchased from GE Healthcare (Piscataway, NJ).

Epigallocatechin 3-O-Gallate. One hundred grams of commercial green tea was boiled gently in 1000 ml of water for 1 h. The extract was then evaporated to dryness under reduced pressure. The yield of green tea was 14.7% by weight of the starting material. The green tea polyphenol was prepared from a hot water extract of green tea, as reported previously (Sakanaka et al., 1989). For purification of EGCg, recycling high-performance liquid chromatography was performed on a JAI-LC-908 high-performance liquid chromatograph (Japan Analytical Industry Co., Tokyo, Japan) equipped with JAI RI and JAI UV detectors, operating at 280 nm, as described previously (Sakanaka et al., 1989). A prepurified EPA HP-GPC column (JAI Gel GS-320, 50 × 2 cm i.d.) was used. Methanol was used as the eluting solvent at a flow rate of 3 ml/min. The isolated EGCg was identified by analysis on a gas chromatograph mass spectrometer (JMS-DX 903, JEOL, Tokyo, Japan) and an NMR apparatus (GA-400; JEOL). The chemical structure of this compound is illustrated in Fig. 1.

Experimental Design. The Guidelines for Animal Experimentation approved by the University of Toyama were followed in all experimental studies. Five-week-old male Wistar rats, weighing 120 to 130 g, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). They were kept in wire-bottomed cages and exposed to a 12:12-h light/dark cycle. The room temperature and humidity were maintained automatically at approximately 25°C and 60%, respectively. They were allowed free access to laboratory pellet chow (CLEA Biotechnology, Inc. (Santa Cruz, CA). Rabbit anti-human fibronectin monoclonal antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit anti-human factor Xa (NF-Xa) p65 polyclonal antibody, rabbit anti-human factor Xa binding protein (NF-Xa) p65 polyclonal antibody, goat anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody, and goat anti-mouse IgG HRP-conjugated secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit anti-human cytokation (Ghentra, Denmark). Anti-mouse β-actin antibody and STZ were purchased from Sigma-Aldrich (St. Louis, MO). Enhanced chemiluminescence Western blotting detection reagents were purchased from GE Healthcare (Piscataway, NJ).

Proteinuria. The remaining kidney was removed from each rat, and one part of the tissue was immersed in formalin for histological examination. The other part was frozen at −80°C until analysis.

Serum and Urine Constituent Levels. Serum levels of glucose, total protein, albumin, total cholesterol, triglyceride, urea nitrogen, and Cr were examined using commercial reagents (Glucose CII-Test Wako, A/G B-Test Wako, Cholesterol E-Test Wako, and Triglyceride E-Test Wako were obtained from Wako Pure Chemicals; BUN Kainos and CRE-EN Kainos were obtained from Kainos Laboratories, Inc., Tokyo, Japan). Serum TBA-reactive substance level was determined using the methods of Naito and Yamanaka (1978). Urine component levels were determined as follows: protein by the sulfosalicylic acid method (Sakagishi, 1968) and Cr using a commercial reagent (CRE-EN Kainos). The Cr clearance (Cr) was calculated on the basis of urinary Cr, serum Cr, urine volume, and body weight using the following equation: Cr (milliliters per minute per kilogram of body weight) = [urinary Cr (milligrams per deciliter) × urinary volume (milliliters)/serum Cr (milligrams per deciliter)] × [1000/body weight (grams)] × [1/1440 (minutes)].

Electrophoretic Pattern Analysis of Proteinuria. Equal amounts (0.5 μg) of urinary protein were loaded onto a 10% acrylamide gel, they were subjected to SDS-PAGE, and the gel was stained with CBB R-250 to visualize the protein bands and destained overnight in 7% acetic acid. The molecular masses of the urinary protein bands were estimated by comparison with those of the bands of standard proteins.

Advanced Glycation End-Product Contents in Kidney. Renal AGE contents were determined by the method of Nakayama et al. (1993). Mincing renal tissue was delipidated by shaking gently with chloroform and methanol [2:1 (v/v)] overnight. After washing, the tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000g for 15 min at 4°C. The amounts of AGEs in these alkalai-soluble samples were measured at an emission wavelength of 440 nm and excitation wavelength at 370 nm against a blank of 0.1 N NaOH solution using an RF/550 spectrofluorometric detector (Shib
A native BSA preparation (1 mg/ml in 0.1 N NaOH) was used as a reference, and its fluorescent intensity was defined as 1 unit of fluorescence. The fluorescence intensities of the samples at a protein concentration of 1 mg/ml were measured and expressed in arbitrary units (AU) compared with a native BSA preparation.

**Mitochondrial TBA-Reactive Substance Levels in Kidney.** Mitochondria were prepared from kidney homogenates by differential centrifugation (800 and 12,000 g, respectively) at 4°C, according to the methods of Johnson and Lardy (1967) and Jung and Pergande (1985), respectively, with slight modifications. The pellets were suspended in preparation medium and the TBA-reactive substance levels were determined by the method of Uchiyama and Mihara (1978). Protein levels were determined by the method of Izhaki and Gill (1964) with BSA as the standard.

**Protein Extraction and Western Blot Analyses.** Renal cortical sections were homogenized with ice-cold lysis buffer, pH 7.5, containing 137 mM NaCl, 20 mM Tris-HCl, 1% Tween 20, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail. Samples were then centrifuged at 2000 g for 10 min at 4°C. To ensure equal loading of the lanes, the protein concentration of each tissue was determined using a Bio-Rad protein assay kit with BSA as a standard, and then immunoblotting was carried out.

For determination of iNOS, COX-2, NF-κB p65, IκB-α, RAGE, TGF-β1, and fibronectin protein expression, each sample (30 μg of protein) was electrophoresed through 8, 12, and 15% SDS-PAGE. Separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 1 h, and separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 1 h, and then incubated with the corresponding primary anti-iNOS, COX-2, NF-κB p65, IκB-α, RAGE, TGF-β1, fibronectin, and β-actin antibody overnight at 4°C. After the blots were washed, they were incubated with goat anti-rabbit and/or goat anti-mouse IgG HRP-conjugated antibody complex was visualized using enhanced chemiluminescence with LAS-1000 Plus (Fujifilm, Tokyo, Japan). A native BSA preparation (1 mg/ml in 0.1 N NaOH) was used as a reference, and its fluorescent intensity was defined as 1 unit of fluorescence. The fluorescence intensities of the samples at a protein concentration of 1 mg/ml were measured and expressed in arbitrary units (AU) compared with a native BSA preparation.

**Western blotting detection reagents and detected by chemiluminescence with LAS-1000 Plus** (Fujifilm, Tokyo, Japan). A native BSA preparation (1 mg/ml in 0.1 N NaOH) was used as a reference, and its fluorescent intensity was defined as 1 unit of fluorescence. The fluorescence intensities of the samples at a protein concentration of 1 mg/ml were measured and expressed in arbitrary units (AU) compared with a native BSA preparation.

**Histopathological Evaluation.** Renal tissues were fixed in 10% neutral formalin solution, embedded in paraffin, and cut into 4-μm sections. The sections were stained with hematoxylin-eosin and periodic acid-Schiff’s reagent and then examined by light microscopy. The glomerular area was determined as the average area of a total of 30 glomeruli, cut at their vascular poles, by tracing along the outline of the capillary loop using a VH analyzer VH-H1A5 (Keyence, Osaka, Japan). Diffuse and exudative lesions were also examined by 100 or few glomeruli in each sample and scored histologically as 0, 0%; 1, 0 to 5%; 2, 5 to 10%; and 3, >10% of each glomerulus.

**Statistical Analysis.** The results are presented as the mean ± S.E.M. The effect of EGCg on each parameter was examined using one-way analysis of variance. Individual differences among groups were analyzed by Dunnett’s test, and significance was accepted at p < 0.05.

**Results**

**Body and Kidney Weight Changes.** Table 1 shows the effects on the changes in body and kidney weights of rats with diabetic nephropathy after the oral administration of EGCg. At the beginning, the body weight was lower in diabetic nephropathy rats than normal rats; however, oral administration of EGCg at 25, 50, and 100 mg/kg body weight/day for 50 days did not lead to any significant differences in body weight gain. Alternatively, the kidney weight in diabetic nephropathy control rats was 2.3 times larger than the normal value (from 0.31 to 0.70 g/100 g body weight), showing reduced enlargement via EGCg administration at 25, 50, and 100 mg dose-dependently (0.64, 0.62, and 0.59 g/100 g body weight, respectively).

**Serum Constituents.** The effects of EGCg on serum constituents of rats with diabetic nephropathy are shown in Table 2. The level of serum glucose was reduced by oral administration of 25, 50, and 100 mg of EGCg to 497, 487, and 460 mg/dl, respectively, whereas diabetic nephropathy control rats showed a value of 592 mg/dl, which was 3.1-fold higher than normal rats. The serum total protein and albumin levels of rats with diabetic nephropathy were significantly lower than those of normal rats; however, after EGCg administrations at 50 and 100 mg/kg body weight/day, these levels were significantly improved. We also examined the serum total cholesterol, triglyceride, and TBA-reactive substance levels (index of lipid peroxidation); as shown in Table 2, the condition of diabetic nephropathy led to 2.4-, 2.2-, and 2.4-fold increases in these parameters, respectively, compared with normal values, whereas EGCg decreased the levels of total cholesterol and TBA-reactive substance significantly but only showed a slight tendency to decrease the triglyceride level.

**Renal Functional Parameters.** Table 3 shows that the effects of EGCg on serum and urinary parameters of renal function. The serum urea nitrogen and Cr levels and urinary protein excretion rate were increased significantly (2.6-, 2.5-, and 4.3-fold, respectively) by diabetic nephropathy. After oral administration of EGCg, serum urea nitrogen declined significantly at all doses; however, there was only a significant decrease in serum Cr at a dose of 100 mg. In contrast, 50 and

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight</th>
<th>Kidney Weight</th>
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<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Normal</td>
<td>266.3 ± 5.2</td>
<td>356.5 ± 15.2</td>
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<tr>
<td>Diabetic nephropathy</td>
<td>245.3 ± 5.5**</td>
<td>269.5 ± 11.7**</td>
</tr>
<tr>
<td>Control</td>
<td>250.1 ± 6.9*</td>
<td>269.0 ± 11.6**</td>
</tr>
<tr>
<td>EGCg (25 mg/kg body weight/day)</td>
<td>242.5 ± 7.4**</td>
<td>268.0 ± 6.7**</td>
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<tr>
<td>EGCg (50 mg/kg body weight/day)</td>
<td>245.2 ± 7.2**</td>
<td>271.2 ± 6.6**</td>
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<tr>
<td>EGCg (100 mg/kg body weight/day)</td>
<td>245.2 ± 7.2**</td>
<td>271.2 ± 6.6**</td>
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* p < 0.05, ** p < 0.01 versus normal values, *p < 0.05, ** p < 0.01, *** p < 0.001 versus diabetic nephropathy control values.
100 mg of EGCg markedly decreased urinary protein excretion from 82.3 to 47.9 mg/day (42% decrease; *p < 0.001) and 40.6 mg/day (51% decrease; *p < 0.001), respectively. In addition, the decrease in Ccr from 7.20 to 3.35 ml/kg body weight/min was ameliorated at 100 mg of EGCg (4.09 ml/kg body weight/min) as well as the serum Cr level.

**Electrophoretic Patterns of Proteinuria.** The patterns of urinary protein in five groups, i.e., normal, untreated, and EGCg 25-, 50-, and 100-mg-treated diabetic nephropathy groups, are presented in Fig. 2. Electrophoretic separation of urinary protein showed a large albumin fraction (molecular mass of 67 kDa) and low- and high-molecular-mass proteins divided by albumin band in the control rats with diabetic nephropathy, but all of these proteins were absent in normal rats. In contrast, as the dose of EGCg increased, the albumin and low-molecular-weight of proteins were effectively decreased compared with the control group.

**Renal AGEs and TBA-Reactive Substance Levels.** The levels of AGEs and mitochondrial TBA-reactive substance in the kidney were both increased approximately 1.7-fold in diabetic nephropathy untreated rats compared with normal values (Table 4). Significant changes were observed from the lowest dose of 25 mg, and there was a dose-dependent decrease in AGE accumulation from 2.35 AU (control) to 2.13, 2.20, and 2.04 AU (EGCg 25, 50, and 100 mg), respectively. The rats given EGCg at 50 and 100 mg showed a decrease to the same degree in the TBA-reactive substance level (p < 0.001), whereas in rats treated with 25 mg, there was a slight but significant decrease compared with these two administration groups (p < 0.05).

**Expression of iNOS, COX-2, NF-κB p65, and IκB-α Proteins.** To elucidate the expression levels of inflammatory enzymes iNOS and COX-2 as well as the upstream regulators NF-κB p65 and IκB-α, we performed Western blot analyses of the renal cortex. The results are presented in Fig. 3. Diabetic nephropathy rats showed increases in the levels of iNOS and COX-2 protein expression to 1.60- and 4.65-times the normal level, respectively. The rats given EGCg at 50 and 100 mg showed a decrease to the same degree in the TBA-reactive substance level (p < 0.001), whereas in rats treated with 25 mg, there was a slight but significant decrease compared with these two administration groups (p < 0.05).
diabetic nephropathy rats showed a slight increase in RAGE (1.13 ± 0.07 versus 1.00 ± 0.09) (not significant), but they showed increased expressions of TGF-β1 and fibronectin in the kidneys of 3.20 ± 0.62 versus 1.00 ± 0.12 (p < 0.001) and 3.50 ± 0.42 versus 1.00 ± 0.28 (p < 0.001), respectively. The 50- and 100-mg administrations of EGCG intensely inhibited the induction of fibronectin protein relative to that of TGF-β1 protein, that is, the fibronectin protein levels were reduced to 1.98 ± 0.25 and 1.97 ± 0.33 (p < 0.001, respectively), and the TGF-β1 protein levels were reduced to 2.33 ± 0.27 (p < 0.05) and 2.09 ± 0.13 (p < 0.01), although there was no effect observed in diabetic nephropathy rats given 25 mg of EGCG. In contrast, only a slight tendency to decrease RAGE protein was noted in the EGCG-treated groups.

**Histopathological Changes.** Periodic acid-Schiff-stained specimens from the experimental rats revealed exudative lesions and a mild increase of mesangial matrix in the diabetic nephropathy control group (Fig. 5, B and C). The EGCG 50- and 100-mg-administered diabetic nephropathy groups showed minimal lesions (Fig. 5, E and F) compared with the control group, whereas no changes were observed in the 25-mg treated group (Fig. 5D). Table 5 summarizes the histopathological changes in the kidneys of the experimental groups. The total glomerular area, obtained by tracing along the outline of the capillary loop, showed a 2.2-fold increase in diabetic nephropathy in the control group compared with that in the normal group, but the EGCG 50- and 100-mg-administered groups showed significant ameliorations of this increase. There were significant increases in diffuse and exudative lesions between the diabetic nephropathy control rats and normal rats, from none to 2.60 ± 0.16 and none to 1.60 ± 0.27; however, the EGCG 50- and 100-mg-administered diabetic nephropathy rats showed significantly lower levels.

**Discussion**

Diabetic nephropathy results from an interaction between metabolic and hemodynamic factors. Glucose-dependent pathways are activated within the diabetic kidney, such as increasing oxidative stress, polyol formation, and AGE accumulation. Hemodynamic factors also participate in systemic and intraglomerular pressure and in the activation of various vasoactive hormone pathways. These hemodynamic pathways, alone and in conjunction with metabolic pathways, stimulate intracellular secondary messengers such as protein kinase C and mitogen-activated protein kinase; nuclear
transcription factors such as NF-κB; and various growth factors such as the prosclerotic cytokine TGF-β and the angiogenic permeability enhancing growth factor vascular endothelial growth factor. These pathways eventually result in increased renal albumin permeability and extracellular matrix (ECM) accumulation, which causes increasing proteinuria, glomerular sclerosis, and tubulointerstitial fibrosis. Therefore, therapeutic strategies targeting the management and prevention of diabetic nephropathy include agents for controlling hyperglycemia, glucose-derived oxidative stress, and renal damage.

Hyperglycemia is the principle factor responsible for structural alterations at the renal level, and The Diabetes Control and Complications Trial Research Group (1993) has elucidated that hyperglycemia is directly linked to diabetic microvascular complications, particularly in the kidney; therefore, glycemic control remains the main target of therapy. In this study, the glucose level of diabetic nephropathy rats showed approximately a 3-fold, significant increase; however, EGCg inhibited this increase dose-dependently. In addition, the typical pattern of serum constituents, that is, a decrease in total protein and albumin due to their excessive excretion via urine, and also an increase in lipids, e.g., total cholesterol and triglyceride, whose abnormal metabolism has been proven to play a role in the pathogenesis of diabetic nephropathy (Sun et al., 2002) and to enhance lipid peroxidation, were all improved by administration of the EGCg. Therefore, we feel that EGCg had a positive effect on serum glucose and lipid metabolic abnormalities.

A progressive decline in the glomerular filtration rate, reflecting serum Cr and Ccr levels, is the most common characteristic in the development of diabetic nephropathy, which causes proteinuria, leading to histological damage in the kidney. This decline is thought to be due to changes in renal hemodynamic initiated by the loss of functioning nephrons, which leads to an increased urinary flow rate, ammonia production, and oxygen consumption (Brenner et al., 1982). The results of the study presented here demonstrate that diabetic nephropathy rats showed significant increases in the serum urea nitrogen, Cr, and urinary protein excretion rate, whereas the Ccr level showed a significant decrease compared with normal rats, representing a decline in renal function. However, the EGCg treatment positively affected these parameters, especially in the group given 100 mg. For further investigation, we performed pattern analysis of proteinuria using SDS-PAGE, and the EGCg treatment showed obvious decrease at all parts of the molecule. These data suggest that not only improvement of proteinuria but also its individual fractions may, at least in part, ameliorate the development of glomerular and tubulointerstitial injury.

As mentioned above, EGCg may improve the typical parameters under the development of diabetic nephropathy; therefore, in this study, we also determined whether EGCg could affect glucose-dependent renal injury. In the state of diabetic nephropathy, there is increased glomerular basement membrane thickening and mesangial ECM deposition, followed by mesangial hypertrophy and diffuse and nodular glomerular sclerosis, and these structural changes may be directly influenced by AGEs through excessive cross-linking of matrix molecules in a receptor-independent way (Vlassara et al., 1992, 1994). Moreover, another pathway exists in the action of AGEs, i.e., receptor-dependent processes, whereby AGEs bind to their cognate cell surface receptor RAGE, resulting in the activation of postreceptor signaling, generation of intracellular oxygen free radicals, and activation of gene expression. Particularly, the RAGE promoter contains NF-κB binding sites, through which AGEs are involved in the activation of reactive oxygen species and NF-κB via AGE-RAGE interaction (Yan et al., 1994; Li and Schmidt, 1997; Wang et al., 1999), and in turn, up-regulation of RAGE ensures that sustained NF-κB activation is not only maintained but also amplified (Bierhaus et al., 2001). Furthermore,
AGE-RAGE and signaling pathways including NF-κB and mitogen-activated protein kinase modulate the activation of TGF-β with subsequent effects in inducing the accumulation of matrix in synergistic ways, i.e., TGF-β causes renal cell hypertrophy and promotes the production of ECM molecules including type I and IV collagen, fibronectin, and laminin while inhibiting their decomposition, and it induces the expression of receptors for the matrix protein integrin, resulting in renal sclerosis and fibrosis (Schiffer et al., 2000; Fukami et al., 2004). In contrast, activated NF-κB is known to not only play a critical role mediating immune and inflammatory responses involving transcription of target genes such as iNOS, COX-2, tumor necrosis factor-α, interleukin-1, and chemokines (Karin and Ben-Neriah, 2000; Nishikawa et al., 2000) but also to have a significant correlation with the degree of severity in albuminuria in diabetic patients with renal complications (Hofmann et al., 1999). Furthermore, COX-2 up-regulation was reported in a model of STZ-induced diabetes and 5/6 nephrectomy to participate in renal damage (Komers et al., 2001; Fujihara et al., 2003). In this study, we demonstrated that renal AGE accumulation observed in diabetic nephropathy rats was decreased by EGCg administration, although EGCg showed only a slight tendency to reduce renal RAGE expression in diabetic nephropathy rats. However, remarkable antioxidative activity of renal tissue was shown in the level of lipid peroxidation at 50- and 100-mg
doses of EGCg, resembling the results of iNOS, COX-2, NF-κB, and phosphorylated IκB-α, and the fibrogenic cytokines TGF-β1 and fibronectin protein expression in the renal cortex.

Lin and Lin (1997) have reported that EGCg decreases the activity and protein levels of iNOS by reducing the expression of iNOS mRNA, and this reduction could occur through the blocking of NF-κB binding to the iNOS promoter, thereby inhibiting the induction of iNOS transcription in mouse macrophages stimulated with lipopolysaccharide. Ahmed et al. (2002) also demonstrated the effect of EGCg in human chorocytes in an interleukin-1β-induced inflammatory study in which inhibition of NO and prostaglandin E2 production correlated with iNOS and COX-2 activity. In addition, EGCg was revealed to prevent the progression of insulin-dependent diabetes mellitus according to research on interleukin-1β and interferon-γ-induced β-cell destruction via reducing NO production and iNOS mRNA and protein levels, including the inhibition of NF-κB activation (Han, 2003); however, no investigation has been performed with EGCg treatment against the induction and/or regulation of iNOS and COX-2 expression using the diabetic nephropathy rat model. Hence, this study may provide new evidence of the effect of EGCg on the NF-κB-regulated transcriptional target genes iNOS and COX-2 and protein expression in the process of developing diabetic nephropathy.

Moreover, diabetic nephropathy rats used in the present study showed significant glomerular hypertrophy and diffuse and exudative lesions. Longitudinal hyperfiltration is associated with renal enlargement such as the increase in glomerular size, and diffuse lesion development is dependent on increased mesangial matrix and glomerular basement membrane thickening, because both are composed of ECM molecules, as in the case of the TGF-β system, and they also correlate with proteinuria. The other phenomenon, the exudative lesion called capsular drop and fibrin cap, is suggested to consist of plasma components, such as IgM, fibrinogen, and AGEs. According to the results of histopathological evaluation, although diabetic nephropathy rats showed a 2.2-fold increase in glomerular area, mild but significant increases in diffuse and exudative lesions, and a slight increase in the mesangial matrix, EGCg could affect glomerular hypertrophy and these lesions at 50- and 100-mg doses, reflecting the effects of AGEs, TGF-β1, and fibronectin levels. Hence, we may hypothesize that EGCg could be advantageous against diabetic kidney damage, which correlates with AGEs with or without a receptor-dependent pathway and their relating inflammatory responses, and then EGCg subsequently suppresses the induction of mesangial hypertrophy and fibronectin synthesis in diabetic nephropathy.

In conclusion, our observations presented here suggest that EGCg has a beneficial effect on diabetic nephropathy via suppressing hyperglycemia, AGEs, their related oxidative stress and cytokine activations, and also pathological states due to its synergistic effect. This study may provide original and strong supporting evidence for the efficacy of EGCg in the early stage of diabetic nephropathy, suggesting that it would be a superior aid for the management of patients with diabetic nephropathy.

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