The comparative effects of perindopril and catechin on mesangial matrix and podocytes in the streptozotocin induced diabetic rats

Salime Pelin Ertürkuner a,*, Murat Başar a, Matem Tunçdemir b, İsmail Seckin a

aDepartment of Histology and Embryology, Cerrahpasa Faculty of Medicine, Istanbul University, Turkey
bDepartment of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University, Turkey

Original research article

Introduction

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus that causes end-stage renal disease. Hyperglycemia, high glomerular capillary pressure, various cytokines, and oxidative stress play important roles in the development of DN [4,34,44,60]. The characteristic properties of DN in the early stages of diabetes are increased glomerular volume and kidney size, which are detected by microalbuminuria (Ma), glomerular extracellular matrix accumulation, and glomerulosclerosis [46,51]. Podocytes, terminally differentiated and highly specialized cells, are the main components of the selective glomerular filtration barrier. They have primary cytoplasmic processes, which originate from the cell body, and secondary cytoplasmic processes, called pedicels, which originate from the primary processes.
Pedicels are aligned under the glomerular basal membrane (GBM) [11,42]. GBM thickening, mesangial matrix expansion, and podocyte hypertrophy have been observed in many studies of diabetic nephropathy. According to these studies, the loss of podocytes and the abnormal function of slit pore membranes cause proteinuria [30,37,45].

High blood glucose levels and hyperglycemia are responsible for excessive ROS production, which causes oxidative stress. This is the major cellular event responsible for DN [22]. Angiotensin II (AII) causes ROS to accumulate in the vessel walls of hypertensive patients. The increase in ROS causes oxidative damage in the slit pore membrane, which can lead to the development of Ma [1,38].

In addition, hyperglycemia accelerates the formation of diacetylcylerol and activates protein kinase C, leading to the overexpression of the gene encoding transforming growth factor beta (TGF-beta) and thereby to the expansion of matrix components in mesangial cells and glomeruli [23]. TGF-beta 1, a fibrogenic growth factor produced locally in the kidney, is involved in the pathogenesis of kidney damage [8,39]. Locally increased AII in diabetes, increases NADPH oxidase by stimulating the production of superoxide. Superoxide reacts with nitric oxide (NO), and oxidized NO induces podocyte hypertrophy and mesangial cell proliferation. By stimulating the synthesis of collagen and fibronectin, TGF-beta 1 promotes the expansion of the mesangial matrix and the interstitial matrix [2]. Expression of TGF-beta 1 by podocytes stimulates sclerosis in the mesangial matrix, induces the apoptosis of glomerular cells, and causes hypertrophic and ultrastructural changes in podocytes [51,52].

Some studies have reported that traditional and new therapies administered separately or together can prevent or reverse the development of diabetic nephropathy. Podocytes were the target cells in these studies [30,37,45].

Green tea, prepared from the leaves of Camellia sinensis L., is popular worldwide [36]. Catechins in green tea bind ROS, free radicals, and peroxynitritides and thereby act as antioxidants. Their antioxidant effects are renoprotective [57]. Green tea polyphenols inhibit mesangial cell proliferation and prolong the survival of the kidney in experimental glomerulosclerosis.

It has been reported that ACEIs prevent mesangial proliferation in the kidney. According to some studies, perindopril, an ACEI, decreases the capillary blood pressure in the glomerulus, which reduces Ma [1]. Despite treatment of diabetic nephropathy patients with agents such as ACEIs, angiotensin II receptor blocker (AIIB-RB), and antihypertensives, large numbers of diabetic patients continue to suffer from nephropathic complications [3].

In this study, using ultrastructural, histological, and biochemical methods, we compared the renoprotective effects of catechin and perindopril, administered separately and together, on the mesangial matrix and podocyte damage.

**Materials and methods**

**Animals and protocols**

All experiments were approved by the ethics committee of Istanbul University Medical School of Cerrahpasa. Animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Male Wistar albino rats (180–200 g, 10–12 weeks old) were obtained from the Experimental Research Laboratory at Istanbul University, Cerrahpasa Faculty of Medicine. The blood glucose levels and body weights of all animals were measured at the beginning of the study. Forty rats were randomly assigned to 5 groups, each containing 8 rats. One group was used as the control group. All other groups received a single injection of streptozotocin (STZ; 60 mg/kg, ip; Sigma, St. Louis, MO, USA). The second day after the injection of STZ, the rats became diabetic. Of the 4 STZ-treated groups, one was used as an untreated diabetic group. A second was treated with perindopril (6 mg/kg/day, gavage; Servier, Istanbul, Turkey) for 30 days after STZ injection. A third was treated with catechin (20 mg/kg/day; C0567; Sigma, St. Louis, MO, USA) starting 7 days before the injection of STZ and continuing for 30 days after. The fourth was treated with catechin and perindopril as described for the individual treatments. All animals had free access to standard rat chow and drinking water. At the end of the experiment (day 31), the rats were anesthetized with ketamine (90 mg/kg, ip) and xylazine (10 mg/kg, ip) and sacrificed after both kidneys were excised.

**Blood glucose (BG)**

The glucose levels (mg/dl) in blood taken from the rat tail were measured using the Freestyle MediSense Optium Blood Glucose Monitoring System (Abbott Laboratories, Illinois, USA) at 3 different times: before the experiment, 48 h after STZ injection, and at the end of the experiment.

**Microalbuminuria (Ma)**

Rats were placed in metabolic cages and housed for 24 h. Urine samples were collected after 24 h on days 1, 15, and 30. Ma levels were measured by the Central Biochemistry Laboratory of the Cerrahpasa Faculty of Medicine.

**Kidney weight (KW)**

At the end of the experiment, rats were anesthetized and sacrificed. The kidneys were excised and washed with physiologic saline solution. The kidney weights were measured using a sensitive scale and recorded.

**Body weight (BW)**

On the first day of the experiment and before anesthetization on the last day of the experiment, the BW of each rat was measured using a scale and recorded.

**Immunohistochemistry**

The right kidney of each rat was excised, placed into 10% neutral formalin for fixation, embedded in paraffin, and cut into sections 5-μm thick. The sections were mounted on slides coated with poly-l-lysine (PLL; Sigma, St. Louis, MO, USA), deparaffinized with toluene (Merck, Germany), and rehydrated with a graded alcohol series (Merck, Darmstadt, Germany).

Immunoperoxidase staining was performed using the Histoscan-Plus Bulk Kit (Zymed LAB-SA Detection System, 85-9043) and a rabbit polyclonal TGF-beta 1 antibody (1:200; sc-146; Santa Cruz Biotechnology) according to the streptavidin–biotin-peroxidase method. Immunostaining procedures were carried out following the guidelines of the manufacturer. The sections were heated for 15 min in 10 mM citric acid buffer, pH 6 (DAKO, Glostrup, Denmark) in a microwave oven. Afterwards, to inactivate the endogenous peroxidase activity, the sections were washed with PBS, incubated with a horseradish peroxidase–streptavidin complex. Sections were counterstained with hematoxylin.

Some studies have reported that traditional and new therapies administered separately or together can prevent or reverse the development of diabetic nephropathy. Podocytes were the target cells in these studies [30,37,45].
To determine the specificity of immunostaining, sections were incubated following the procedure described above, but sections were incubated with control serum instead of the primary antibody.

**Semi-quantitative analysis of immunoperoxidase staining**

The immunoperoxidase staining of kidney tissues was analyzed and scored from 0 to +4 (0, no staining; +1, weak; +2, moderate; +3, strong; +4, very strong) using a double-blind, randomized mode of analysis. Each slide, 20 fields in which a glomerulus was visible were selected and assessed at 40× magnification. We used a modified version of the method described by Hill et al. [15].

**Staining specificity controls**

For negative controls, PBS was used instead of the primary antibody.

**Light microscopy analysis of the glomerular mesangial matrix ratio**

Kidney tissue sections were prepared as described above and stained with periodic acid-Schiff (PAS) for examination of the glomerular mesangial matrix ratio under a Leica DM LS microscope. Using a 40× ocular lens, 20 glomeruli were randomly chosen, and the mesangial matrix was assessed in a double-blind manner. The mesangial matrix ratio in each glomerulus was rated using a 0 to +4 scale:

- 0: Normal mesangial matrix ratio;
- +1: 25% increase in mesangial matrix ratio;
- +2: 50% increase in mesangial matrix ratio;
- +3: 75% increase in mesangial matrix ratio;
- +4: 100% increase in mesangial matrix ratio.

For this experiment, we used the method described by Raij et al. [44]. We used the method of Hill et al. to assess TGF-beta 1 staining [15].

**Ultrastructural imaging by electron microscopy (EM)**

The left kidney of each rat was excised, fixed in 4% glutaraldehyde (G5882; Sigma, St. Louis, MO, USA) in a 0.1 M phosphate buffer solution, post-fixed in 1% OsO4 prepared in the same buffer, dehydrated with graded ethanol (Merck, Darmstadt, Germany), and embedded in araldite (G4901; Sigma, St. Louis, MO, USA). The tissue was cut into ultra-thin sections 50-nm thick using an ultramicrotome (Reichert UM 2 and UM 3, Austria), which were positioned on copper grids (200 mesh). Tissue sections were stained with uranyl acetate and lead citrate and analyzed with a transmission electron microscope (JEM-1011, Jeol Tokyo, Japan) and a MegaView III Soft Imaging camera system (Tokyo, Japan).

**Counting of slit pores**

The methods of Powell et al. [43] and Seefeldt et al. [47] were modified for our study. We chose 3 glomerular areas and 10 glomerular basement membrane lines where the pedicles of podocytes were aligned. The total length of the line along which the slit pores were counted was 300 μm. Photographs were taken and printed to a size 10,000 times larger than EM, and the slit pores were counted along a length of 20 cm (10 μm in EM).

**Statistical analysis**

The ANOVA test was used to assess blood glucose levels, body weights, kidney weights, microalbuminuria levels, and glomerulosclerosis ratio. Dunn’s test was used when variances were not equal. Differences were considered significant when p < 0.05.

**Results**

**Blood glucose levels (mg/dl)**

The blood glucose levels in all rats administered STZ were significantly higher than the levels in the control rats (p < 0.01) 48 h after injection. These rats were therefore considered diabetic. At the end of the experiment, the BG levels in the untreated diabetic (414.5 ± 22.83), catechin-treated diabetic (396.0 ± 39.0), perindopril-treated diabetic (399.60 ± 63.6), and catechin + perindopril-treated diabetic (288.33 ± 27.14) groups were higher than the BG levels in the control group (100.6 ± 6.4; p < 0.001). The BG levels were significantly lower in the catechin + perindopril-treated group than in the untreated, catechin-treated, and perindopril-treated diabetic groups (p < 0.001) (Table 1).

**Microalbuminuria (mg/l)**

At the beginning of the experiment, there was no significant difference between the groups when Ma levels in the urine were assessed (p > 0.05). At day 15, the Ma levels in all STZ-treated diabetic groups were significantly lower than in the control group (p < 0.001). At the end of experiment, Ma levels were significantly lower in the catechin-treated, perindopril-treated, and catechin + perindopril-treated diabetic groups than in the untreated diabetic group (p < 0.001) (Table 1).

**Kidney weights (g)**

In both treated and untreated diabetic groups, the KW increased more than it did in the control group. The KW in the catechin + perindopril-treated diabetic group was significantly lower than the KW in the untreated diabetic group (p < 0.001) (Table 1).

**Body weights (g)**

At the end of the experiment, the BW in all of the diabetic groups was significantly lower than the BW in the control group (p < 0.01, p < 0.05) (Table 1). The BW in the catechin-treated and catechin + perindopril-treated diabetic groups was significantly higher than the BW in the perindopril-treated diabetic group (p < 0.01).

**Morphological diagnosis**

Under a light microscope, examination of PAS-stained kidney tissue sections from the untreated diabetic group showed

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Blood glucose (BG) levels, microalbuminuria (Ma) level, kidney weight (KW) at the end of the experiment.</td>
</tr>
<tr>
<td>Group (n=8)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Untreated diabetic</td>
</tr>
<tr>
<td>Catechin-treated diabetic</td>
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<tr>
<td>Perindopril-treated diabetic</td>
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<tr>
<td>Catechin + perindopril-treated</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

a p < 0.01 vs. control group.

b p < 0.01 vs. untreated, cat and per treated diabetic groups.

c p < 0.01 vs. untreated diabetic group.

d p < 0.01 vs. untreated, cat treated diabetic group.

e p < 0.01 vs. control group.

f p < 0.05 vs. untreated diabetic group.

z p < 0.05 vs. cat treated diabetic group.
increased thickness of the GBM, increased mesangial matrix ratio, hypertrophy and hyperplasia of the parietal layer of Bowman’s capsule, and hypertrophy of the whole glomerulus (Fig. 1B). The features were also observed in the kidney tissues from the treated diabetic groups, but they were less pronounced (Fig. 1C–E). The extent of mesangial matrix expansion in all groups was determined according to the method described by Raij et al. [44]. According to the statistical assessment of the results, the mesangial matrix expansion in the treated diabetic groups was significantly less than that in the untreated diabetic group ($p < 0.001$) (Table 2).

**Immunohistochemical analysis of TGF-beta 1**

Cells whose nucleus and cytoplasm were stained in immunohistochemical procedures with anti-TGF beta 1 antibody were considered TGF-beta 1-positive. In the control group, TGF-beta 1 staining was weak (Fig. 2A). The immunostaining increased markedly in the untreated diabetic group, compared with that in the control group ($p < 0.001$) (Fig. 2B). Immunostaining in the catechin-treated diabetic group and the perindopril-treated diabetic group was similar, but weaker than that in the untreated diabetic group ($p < 0.001$) (Fig. 2C and D). In the diabetic group treated with perindopril + catechin, immunostaining were significantly lower than in the groups treated with catechin or perindopril alone, but it was significantly higher than in the control group ($p < 0.001$) (Table 2) (Fig. 2E).

**Ultrastructural diagnosis by EM**

An EM image of kidney tissue from the control group is shown in Fig. 3A. In the untreated diabetic group, the pedicels which were

![Figure 1](image1.png) **Fig. 1.** PAS staining of kidney sections from the control group (A), untreated diabetic group (B), catechin-treated diabetic group (C), perindopril-treated diabetic group (D), and catechin + perindopril-treated diabetic group (E). Magnification: 40×.

<table>
<thead>
<tr>
<th>Group</th>
<th>TGF-beta 1</th>
<th>PAS</th>
<th>Slit pore score</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.21 ± 0.05</td>
<td>26.66 ± 7.58</td>
<td>29.09 ± 1.07</td>
</tr>
<tr>
<td>Untreated diabetic</td>
<td>2.63 ± 0.24</td>
<td>195.16 ± 42.23</td>
<td>14.06 ± 4.76</td>
</tr>
<tr>
<td>Catechin-treated diabetic</td>
<td>1.17 ± 0.16</td>
<td>136.23 ± 30.58</td>
<td>24.09 ± 2.91</td>
</tr>
<tr>
<td>Perindopril-treated diabetic</td>
<td>1.15 ± 0.20</td>
<td>133.66 ± 13.57</td>
<td>21.59 ± 1.04</td>
</tr>
<tr>
<td>Catechin + perindopril treated diabetic</td>
<td>0.84 ± 0.41</td>
<td>125.66 ± 5.57</td>
<td>24.40 ± 2.57</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

\[ a \ p < 0.001 \text{ vs. control group.} \]

\[ b \ p < 0.001 \text{ vs. untreated diabetic group.} \]
closed to the GBM had more microfilaments, and pedicel fusions and retractions were often observed. Thus, the number of slit pores decreased (Fig. 3B). Locally, the slit pore distances between the pedicels became narrower, and the membranes were at the apical part of these pores (Fig. 3B). Additionally, podocyte hypertrophy, enlargement of GER, and Golgi complex such as ultrastructural degeneration was observed (Fig. 3C). The glomerular basement membrane made subepithelial invaginations with the pedicels, and there were subepithelial deposits in the expanded mesangial matrix and the perimesangium (Fig. 3C). Locally, there were protein absorption granules (PAGs) in the cytoplasm of some hypertrophic podocytes (Fig. 3C). The podocytes touched the parietal layer of Bowman’s capsule in the renal corpuscles in some fields (Fig. 3C). The GBM became convoluted and thickened in some areas in the renal corpuscle (Fig. 3C). Locally, some parts of the GBM were bare because of the disappearance of the pedicels.

In the catechin-treated diabetic group, hypertrophy of podocytes and PAGs were absent, and the podocytes remained intact at the ultrastructural level. Additionally, there were some invaginations of the pedicels and the nucleus of the podocytes (Fig. 3D). The basement membranes became thicker, and some accumulation and duplications were visible in the intramembranous area. There were less microfilament in the pedicels than in the untreated diabetic group, and the pedicels were closed to each other.

In the perindopril-treated group, some hypertrophic podocytes were visible, but fewer than in the untreated diabetic group (Fig. 3E). Some podocyte bodies were thin and long, and lysosomes contained liquefied PAGs (Fig. 3E). The number of microfilaments in the pedicels was less than in the untreated diabetic group (Fig. 3E), and the local expanded GBMs were fewer. In addition, the glomerular capillary width and the thickness of the parietal layers were the same as in the control group. Some parts of the GBM had humps and convolutions. Apoptotic mesangial cells were present.

Ultrastructural pathologies were infrequently observed in the group treated with both perindopril and catechin. In particular, thicker GBM regions, fusion/contraction of pedicels, and degenerative podocytes were rarely seen (Fig. 3F).

Morphometric assessment

Slit pore counts

The number of the slit pores in the untreated diabetic group was significantly lower than in the control group ($p < 0.001$). The number of slit pores in the catechin + perindopril-treated group
was significantly higher than in untreated group. This increase was more than the untreated diabetic group ($p < 0.001$) (Table 2).

**Discussion**

Podocyte injury causes glomerulosclerosis, which leads to renal failure. According to studies of glomerular disease conducted in the last 20 years, podocyte changes fall into 3 main groups: effacement, apoptosis, and proliferation [11,16,25]. The diabetic condition, characterized by hyperglycemia, causes pedicel expansion and then effacement, resulting in the loss of podocytes [56]. In pathological situations, mitosis occurs in podocytes, but cytoplasmic division does not. Consequently, the podocytes cannot reduce their numbers, and the hypertrophy and the effacement of pedicels leaves the GBM bare. This compensatory hypertrophy causes glomerulosclerosis [35].

In recent years, the roles of free radicals, oxidative stress, and antioxidants in the mechanism of renal disease have been studied [31]. An increase in ROS plays major role in the development of diabetic nephropathy (DN) and affects the reduction of podocytes and albuminuria. Consequently, there is a relation between the reduction of podocytes and albuminuria [7,26,54]. In humans, podocyte loss causes progressive and persistent proteinuria. The number of pedicels decreases in all glomerular areas [41]. The slit pore reduction is associated with an increase in the number of podocytes and with the inability of existing podocytes to increase the number of pedicels to compensate for this loss [32].

Green tea contains antioxidant substances called catechins. Catechins bind to ROS, other free radicals, and peroxynitrite. ROS cause cell death through chemical damage to DNA, proteins, and unsaturated fats. Oleic acid, which has antioxidant effects, reduces the pro-inflammatory effects of the IKK-beta/NF-kappa-B pathway, thereby increasing antioxidant/reductant molecules, decreasing oxidative stress, and regenerating redox homeostasis at inflammation sites. In various cell lines, oleic acid increases cell proliferation and differentiation and inhibits apoptosis [46]. Treatment with resveratrol, a known antioxidant, reduced the enlargement of glomerular mesangial areas in STZ-treated diabetic rats [10]. Catechins are thought to inhibit NF-kappa-B- and AP-1-related gene expression [59]. According to Zaveri et al. [59], the catechin epigallocatechin-3-gallate directly inhibits the activity of the proteasome and inhibits NF-kappa-B inhibitory proteins, such
as inhibitor of kappa B (IkappaB) and Bax, as well as other proapoptotic proteins.

Hemodynamic pathways, which are activated by vasoactive hormones, and metabolic pathways stimulate protein kinase C, mitogen activated protein kinase (MAPK), NF-kappa-B, TGF-beta, and vascular endothelial growth factor (VEGF). As a result, the albumin permeability of the kidney and the extracellular matrix increases, causing proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis [57]. ACE enzyme activity, structural damage in diabetes mellitus, and albuminuria decreased when diabetic rats were treated with catechin [14]. Similarly, flavonoids inhibited ACE activity, and albumin excretion subsequently decreased [6,14,40].

In our study, the scores for Ma and glomerulosclerosis were lower in the catechin-treated diabetic group than in the untreated diabetic group. These findings are consistent with those in the studies described above. In addition, in the catechin-treated group, fewer apoptotic cells were observed by electron microscopy. The ultrastructure of podocytes was normal; podocyte hypertrophy and the number of slit pores were similar in the catechin-treated group and the control group. These findings suggested that ROS plays a significant role in DN and that catechin reduces damage in the kidney.

The renin–angiotensin–aldosterone system (RAAS) has a major role in the development and progression of diabetic nephropathy. RAAS blockade is considered the most effective strategy to reduce proteinuria and to slow the development of diabetic and non-diabetic nephropathy [46]. In diabetes mellitus, local RAS increase was seen in the proximal tubule cells, mesangial cells, and podocytes. Lerco et al. [25] observed a significant decrease in the numbers of slit pores and foot processes and a significant increase in Ma in STZ-treated diabetic rats. They hypothesized that the changes were caused by high glomerular capillary blood pressure.

Recent studies showed that the RAAS has a direct effect on the slit pore. All weakens zona occludens-1 junctions by activating biochemical changes; this can be reduced by ACE inhibitors. Macconi et al. [29] and Liebau et al. [27] showed the same effect with lisinopril. ACEI also reduces systemic and glomerular blood pressure, which reduces glomerular hyperfiltration. In this situation, the mechanical stress on the podocytes and glomerular basal membrane is reduced. Consequently, GBM thickening and the loss of foot processes are diminished [17].

Kriz et al. [24] reported that eliminating the effects of All reduces the tonsus of the podocyte, the rate of podocyte apoptosis, and protein leakage. Liu et al. [28] observed that cell proliferation and the expression of TGF-beta-1, VEGF, type IV collagen, and nephrin in the renal glomeruli decreased after treatment with All for 4 weeks to reduce All activity. According to Forbes et al. [12], ACEIs are potent inhibitors of AGE, which is responsible for diabetic complications, especially nephropathy.

In this study, we observed that GBM thickness, the width of capillaries in the glomerulus, and the slit pore count were similar in the kidneys of perindopril-treated rats and control rats. The slightly hypertrophic appearance of the podocytes and the partial reduction in pedicels were compatible with results from other studies, in which ACE inhibition with perindopril decreased the effects of All and minimized damage. In addition, in the diabetic groups treated with catechin and perindopril, separately or together, Ma values decreased significantly. For all groups, the EM results were consistent with the literature [43,47]. Also in accordance with the literature, the slit pore count in the untreated diabetic group was significantly lower than in the control group and other diabetic groups. In our study, especially in the untreated diabetic and perindopril-treated diabetic groups, various numbers and sizes of electron-dense PAGs were present in podocytes.

Some studies of rats with nephrosis have reported that protein intake occurs via podocytes endocytosis at the basement membrane close to the pedicels. The endocytosed proteins accumulate in vesicles within the cell. As the density of the vesicles decrease, they become PAGs. While the density decreases, PAGs merge with primary and secondary lysosomes, and their contents interact with lysosomal enzymes and melt. Subsequently, low-density heterogeneous residual bodies develop. These residual bodies are surrounded by dense microfilament packages, and their contents are emptied to the urinary field by exocytosis [9,33].

Currently, there is no evidence of endocytotic activity in podocytes of healthy rats [19,49]. On the other hand, endocytotic activity in podocytes was observed in diabetic animals [18]. AGEs induce inflammatory cytokines in macrophages, which increases the endocytotic activity. Activation in the cells is not dependent on receptors or on ROS production and oxidative stress, which are generated by glyco-oxidants in the cells [53].

Immunohistochemical studies have shown high levels of AGEs in the GBM, mesangium, podocyte, and renal tubular cells. In ultrastructural studies, AGEs in the glomerular structures were identified by immunogold. [13,53]. Related to these observations, hypertrophy and PAG were not observed in the podocytes of the catechin-treated diabetic group in this study. We conclude that catechin effectively eliminated ROS.

Studies of human chronic kidney disease and experimental animals have shown that TGF-beta accumulates in damaged kidneys [11]. AGEs increase TGF-beta mRNA expression in some cell lines [5,61]. Additionally, administering AGEs to healthy rats increases the mRNA levels of TGF-beta, collagen type I, and laminin [5,58]. The relationship between All and TGF-beta 1 expression in diabetic nephropathy has been extensively studied [21,55]. All acts as a local growth factor. All increases cellular hypertrophy and the expression of collagen type IV mRNA in proximal tubule cells and increases the expression of fibronectin and collagen type I mRNA in rat mesangial cells [20]. TGF-beta 1 mediates the growth function of All. Increases in TGF-beta 1 in mesangial cell cultures were observed by Branton et al. [5]. All stimulates tubular hypertrophy and mesangial matrix expression in the kidney by autocrine stimulation of TGF-beta 1 [48]. In addition, All acts like glucose, and TGF-beta 1 synthesis increases in mesangial cells. RAS increases locally in proximal tubule cells, mesangial cells, and podocyte cells in diabetes mellitus [55]. The expression of ATI receptor and the production of All increase in podocytes [51].

Hyperglycemia increases the formation of All in podocytes and thereby the expression of angiotensin [51]. Additionally, All, induced by hyperglycemia in the kidney, stimulates the expression of TGF-beta 1 and inhibits nephrin, resulting in the release of TGF-beta 1, VEGF, and other cytokines from the kidney [28]. In this study, we observed that TGF-beta 1 immunostaining was lowest in the perindopril + catechin-treated diabetic group. In this experimental model of diabetic nephropathy, the ACEI reduced the increase in TGF-beta 1 expression and thereby reduced structural and functional damage. We propose that catechin and perindopril inhibit damage formation in different ways and thus potentiate each other's effects.

In the early stages of the diabetic nephropathy, the major structural change is renal hypertrophy [51]. This is mainly a result of glomerular hypertrophy. KW is considered an indicator of renal hypertrophy. We observed that KW was higher in the untreated diabetic group than in the control group and treated diabetic groups. These results are consistent with those of similar studies of the first stages of DN [15,32,50,51].

In conclusion, co-administration of catechin and perindopril was more effective than separate administration in reducing nephropathy caused by streptozotocin-induced diabetes in rats.
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