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THE ROLE OF TGF-β1 IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY EXPERIMENTALLY INDUCED BY STREPTOZOTOCIN AND THE NEPHROPROTECTIVE EFFECTS OF CANDESARTAN

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Abstract

Diabetic nephropathy (DN) stands as a prevalent and severe complication of diabetes mellitus (DM), lacking adequate medical therapy. The molecular mechanisms contributing to glomerular membrane damage involve the overactivity of angiotensin II, heightened expression of nephrin, vascular endothelial growth factor (VEGF), and notably, intraglomerular transforming growth factor TGF-β1.

Pharmaceutical treatments targeting hemodynamic disturbances in diabetic nephropathy, such as ACE inhibitors and angiotensin receptor blockers (ARBs), hold promise for DN therapy.

This study aimed to assess the role of intraglomerular TGF-β expression in experimentally induced DN in rats and explore the nephroprotective effects of candesartan.

Diabetes mellitus was induced through a single intraperitoneal injection of streptozotocin (STZ) at 60 mg/kg, and DN was allowed to develop over four weeks. DM rats were randomly assigned to two groups: STZ (untreated) and STZ+CAN (treated with candesartan at 5 mg/kg/day from week 4 to week 12).

STZ administration led to a substantial increase in TGF-β1 expression in the glomeruli, exceeding control levels by 5-6 times. Candesartan treatment demonstrated a significant reduction in glomerular proliferation and subsequent expansion of the mesangial matrix, suggesting a potential mechanism by which these drugs achieve therapeutic effects.

Key words: Streptozotocin, Diabetic nephropathy, TGF-β1, Candesartan, Rats

Introduction

Diabetic nephropathy stands as one of the prevalent causes leading to end-stage renal disease, clinically characterized by proteinuria and a gradual decline in renal function. Initial research on the molecular mechanisms behind the development of diabetic nephropathy and proteinuria primarily centered around mesangial matrix expansion, a key pathology in diabetic glomerulopathy. Additionally, the thickening of the glomerular basement membrane (GBM) stands out as a prominent histological alteration in diabetic nephropathy. The expansion of the mesangial matrix correlates with both proteinuria and compromised renal function. Accumulation of the mesangial matrix has been demonstrated to diminish the capillary surface area available for filtration, contributing to the progressive loss of renal function [1]. Renal failure can also manifest due to tubulointerstitial fibrosis, further exacerbating proteinuria [2,3].

The genesis of proteinuria in diabetes cannot be solely attributed to the expansion of the mesangial matrix. It is highly probable that alterations in the glomerulofiltration barrier, consisting of the glomerular endothelium, glomerular basement membrane, and podocytes (glomerular visceral epithelial cells), play a crucial role.

Traditionally, podocyte damage was considered a consequence that occurs later in response to increased proteinuria in diabetic nephropathy. However, recent research indicates that podocyte damage is a primary molecular mechanism in the development of diabetic nephropathy and represents one of the earliest stages of glomerular damage. Numerous biopsy studies conducted in patients with diabetes

mellitus reveal that functional and structural damage to podocytes occurs very early in the progression of diabetic nephropathy [4,5,6,7,8].

Moreover, experimental studies conducted in induced diabetic nephropathy, particularly through the administration of streptozotocin, demonstrate that nephropathy actually commences with the impairment of podocytes and a reduction in their numbers [9,10,11,12].

The precise etiology of podocyte damage and loss in diabetic nephropathy remains poorly understood and speculative. However, two mechanisms are believed to underlie these processes: podocyte apoptosis (programmed cell death of podocytes) and detachment of podocytes from the glomerular basement membrane, leading to their elimination through urine (podocytopenia).

Literature findings suggest that the increased level and excessive activity of intraglomerular transforming growth factor (TGF-β), particularly TGF-β1 [13.14], may significantly contribute to podocyte detachment and apoptosis. TGF-β1 has been demonstrated to play a pivotal role in the pathogenesis of diabetic nephropathy [15].

This fibrogenic cytokine is activated in the diabetic state, resulting in an increased level in the kidney during diabetes and capable of inducing hypertrophic and sclerotic changes in diabetic nephropathy [16].

Furthermore, the heightened activity of TGF-β may exert a proapoptotic effect by stimulating podocyte apoptosis and/or their detachment, leading to podocytopenia and the development of progressive glomerular sclerosis.

Podocytopenia can accelerate the onset of proteinuria because the damaged glomerular basement membrane may come into contact with Bowman's capsule, leading to the formation of synechiae, this marks an initial step in the development of glomerulosclerosis [17].

Additionally, morphological abnormalities in podocytes are considered a contributing factor to proteinuria.

Understanding the pathophysiological mechanisms of diabetic nephropathy has led to the identification of various groups of drugs that are either currently in use or could potentially be employed in the prevention and treatment of diabetic nephropathy. Of particular interest are drugs that primarily target the synthesis of endothelin-1, blockers of ET-1 receptors, synthetic analogs of prostacyclins, as well as medications that positively influence the hemodynamic disturbances present in diabetic nephropathy, such as angiotensin-converting enzyme inhibitors (ACE) and angiotensin receptor blockers (ARBs).

Angiotensin II receptor blockers, such as candesartan, also known as angiotensin receptor blockers (ARBs), AT1 receptor antagonists, or sartans, belong to a class of drugs that exert their effects by modulating the renin-angiotensin-aldosterone system. Specifically, they achieve this by blocking the action of the potent vasoconstrictor angiotensin II.

Numerous experimental studies have been conducted, demonstrating that angiotensin receptor blockers (ARBs) used as monotherapy, while not entirely curative, significantly alleviate symptoms and signs of experimentally induced diabetic nephropathy. These studies have showcased various members of the angiotensin-blocking drug group, including losartan, valsartan, olmesartan, etc., slowing the progression of diabetic nephropathy and markedly reducing proteinuria in animals, often induced experimentally by streptozotocin [18,19,20,21].

Materials and Methods

Experimental Model

A total of 75 normotensive Wistar rats, comprising both male and female individuals, aged 9-11 weeks, and with a body weight ranging from 160-300 g, were used to perform for the experiments.

To minimize the impact of extrinsic factors on renal function, standardized animal care practices were implemented, and equivalent volumes of administration fluid were injected across the experimental cohort.

Induction of diabetes and diabetic nephropathy

To induce diabetes and diabetic nephropathy in rats, a single intraperitoneal administration of streptozotocin (Sigma-Aldrich, Chemie GmbH, Germany) was carried out at a dose of 60 mg/kg/t.t., dissolved in 0.1 M citrate buffer (pH 4.5). Diabetes development was verified 72 hours later by assessing blood glucose levels using a blood glucose monitor (Accu-Chek, Roche Diagnostic,

Germany). Blood samples for glucose determination were obtained by drawing blood from the tail. Rats with fasting (morning) blood glucose levels ≥ 11 mmol/L were included in the study. Subsequently, for the next 4 weeks, the animals were kept in a diabetic state without receiving any treatment to induce diabetic nephropathy.

To induce diabetic nephropathy (DN), the animals were maintained in a diabetic state without any treatment for the subsequent 4 weeks. The diabetic rats (n=50) were randomly assigned to two experimental groups: STZ and STZ+CAN. To evaluate the symptoms and signs of DN, the STZ group of rats (n=25) received no treatment over the following 8 weeks.

For assessing the effects of the AT1 antagonist candesartan (CAN) treatment, the STZ+CAN group of diabetic rats (n=25) received candesartan at a dose of 5 mg/kg/per day via gavage, administered from week 4 to week 12.

The control group (nondiabetic rats) (n=25) received saline in an equivalent volume and at the same time intervals as the groups of animals receiving the tested drugs

Apoptosis

Cell apoptosis, assessed through the apoptotic index, served as a highly specific parameter to quantify streptozotocin-induced apoptosis and its potential inhibition by candesartan. The ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit from CHEMICON International was employed for this purpose.

The ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit enables the detection and differentiation of apoptotic cells from necrotic cells by specifically identifying DNA chain breaks and chromatin condensation, which are accompanying phenomena of apoptosis. It is worth noting that in some instances, cells with necrotic morphology may exhibit bright restaining, or, in rare cases, induced apoptosis may result in the absence or incompleteness of DNA fragments. Therefore, the evaluation of ApopTag® staining results should consider morphological criteria.

Positive ApopTag® staining visualization should reveal focal in situ staining at an early stage of apoptotic nuclei and apoptotic bodies. This positive staining directly correlates with the more typical biochemical and morphological aspects of apoptosis.

The ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit operates on the principle of marking free 3'OH DNA ends in situ with chemically marked and unmarked nucleotides. The reaction buffer in the kit, containing nucleotides, is used for the enzymatic addition of nucleotides to DNA by terminal deoxynucleotide transferase (TdT). TdT catalyzes the framework-independent addition of nucleotide triphosphates to the 3'-OH ends of double-stranded or single-stranded DNA. The incorporated nucleotides form an oligomer composed of digoxigenin-conjugated nucleotides and unlabeled nucleotides in a randomized sequence. The marked/unmarked nucleotide ratio in the ApopTag® Plus Peroxidase Kit is optimized to facilitate antidigoxigenin-antibody binding. The exact length of the added oligomer has not been determined.

The digoxigenin nucleotide-marked DNA fragments then bind to an anti-digoxigenin antibody conjugated to a peroxidase reporter molecule. Linked peroxidase-antibody conjugates enzymatically generate permanent, intense, localized restaining from chromogenic substrates, enabling sensitive detection in immunohistochemistry or immunocytochemistry (e.g., in tissues or cells). This combined molecular biological-histochemical system allows for sensitive and specific staining of the very high concentrations of 3'-OH ends localized in apoptotic bodies.

The results of the semiquantitative analysis of kidney samples from the analyzed groups of animals are expressed as mean values of the number of apoptotic cells (nuclei) in one large field of view (x 400 GVP) out of 10 analyzed large visual fields (GVP).

For each time point of cell apoptosis analysis, kidney sections from a maximum of 7 rats per group were included. In other words, for each examined group, at least 60 large visual fields (x 400 GVP) were analyzed.

Cell proliferation

Cell proliferation was assessed as a specific parameter to evaluate the impact of the studied drug (candesartan) on the proliferation and regeneration of epithelial tubular cells, as well as proliferation in the interstitium and glomeruli.

The monoclonal antibody Ki-67 (clone Mib-5) was used for the determination of cell proliferation.

Ki-67 is characterized by strong nuclear expression throughout all active phases of the cell cycle. In this study, Ki-67 served as a marker for assessing proliferation. Immunostaining for Ki-67 was conducted using the LSAB immunoperoxidase procedure (Dako, Denmark). Kidney sections, prepared at a thickness of 4 μm, were placed on silanized glass slides and microwaved for 5 minutes in citrate buffer (pH = 6). Subsequently, sections were incubated for 5 minutes with a blocking reagent to minimize non-specific background staining. This was followed by a 1-hour incubation at room temperature with a specific antibody (Ki-67 Dako, Denmark) diluted in PBS (1:400). After a 10-minute rinse in PBS, sections underwent a 10-minute incubation at room temperature with a biotinylated secondary antibody, followed by an avidin/biotin/peroxidase complex. Visualization of the bound complex was achieved with 3,3' diaminobenzedine. The sections were then counterstained with hematoxylin and mounted in Entelan (Merck, Germany). Negative controls were established by replacing the specific antiserum with normal unimmunized serum; no labeling was observed in negative controls, indicating that the entire procedure and all reagents used resulted in specific labeling. Lymph node samples served as a positive control for Ki-67.

Control sections of representative tissues were prepared by either substituting the primary antibody with a diluent of normal mouse serum or by omitting the primary antibody altogether.

Counting of Ki-67 positive nuclei was performed using light microscopy. The results of the analysis of tissue samples from the studied groups, stained for Ki-67, are presented as the mean values of the number of cells with positive nuclei in one large field of view (x 400 GVP) out of 10 analyzed large visual fields (GVP).

For each time point in the analysis of the proliferative index, kidney sections from a maximum of 7 rats per group were included. In other words, for each examined group, at least 60 large visual fields (x 400 GVP) were analyzed.

TGF-β1 expression

The expression of TGF-β1, with its profibrotic and proapoptotic properties, in glomeruli and tubules was utilized as a specific immunohistochemical parameter to assess its role in the induction of glomerulosclerosis, the process of podocyte detachment, and its significance as a trigger for apoptosis.

TGF-β1expression was determined using a monoclonal antibody for TGF-β1 (NCL-TGFB) (Clone TGFB17) from Novocastra, Leica Biosystems, with Catalog number 123903. The overall procedure for preparing kidney sections, staining, and further final processing was the same as that for Ki-67.

A semiquantitative analysis of TGF-β1 expression was conducted using a scale, where the expression of TGF-β1 in the cytoplasm of cells in the glomeruli, tubular compartment, and interstitium was graded as follows:

Grade 0 = No TGF-β1 expression present;
Grade 1 = Slightly pronounced expression of TGF-β1;
Grade 2 = Moderate expression of TGF-β1;
Grade 3 = Marked expression of TGF-β1;
Grade 4 = Maximal expression of TGF-β1

The results of the semiquantitative analysis of kidney samples for the expression of TGF-β1 from the analyzed groups of animals are expressed as mean values of one large field of view (x 400 GVP) out of 10 analyzed large visual fields (GVP).

For each time point of TGF-β1expression analysis, kidney sections from a maximum of 7 rats per group were included. In other words, for each examined group, at least 60 large visual fields (x 400 GVP) were analyzed.

Results

The administration of streptozotocin induced severe hyperglycemia in 78.74% of the tested animals within 72 hours, and these animals were subsequently included in the study. This induced hyperglycemia was maintained throughout the trial, and after 4 weeks of streptozotocin administration, clinical symptoms and signs of kidney disorders, specifically diabetic nephropathy, were clearly expressed in all experimental animals.

Key characteristics of these kidney damages included increased serum values of urea and creatinine, polyuria, polydipsia, elevated diuresis, acidified urine, albuminuria, proteinuria, as well as a poor general condition of the experimental animals. Elevated serum concentrations of urea and creatinine, along with polyuria, served as non-specific markers indicating impairment of renal function induced by streptozotocin administration.

Apoptosis

As highly specific parameters for determining the mechanism of development of streptozotocin-induced diabetic nephropathy and the therapeutic effects of RAS blockade, the determination of the glomerular apoptotic index, i.e., the average number of positive nuclei (cells) in one large field of view (LFV) was employed.

In the STZ group of rats after 8 weeks of streptozotocin administration, the average apoptosis index, representing the number of positive nuclei (apoptotic cells) in one large visual field in the glomerular compartment of the kidneys, was more than twice as high (8.36 ± 2.68) compared to the control group ($p < 0.05$). In the STZ+CAN group, although somewhat less pronounced, a significant increase in the values of the apoptosis index in the glomeruli (7.28 ± 2.24) was also observed (Figure 1 and Table 1).

This suggests that streptozotocin-induced diabetic nephropathy leads to increased apoptosis in the glomerular compartment, and while candesartan treatment somewhat mitigates this effect, apoptosis remains elevated compared to the control group.

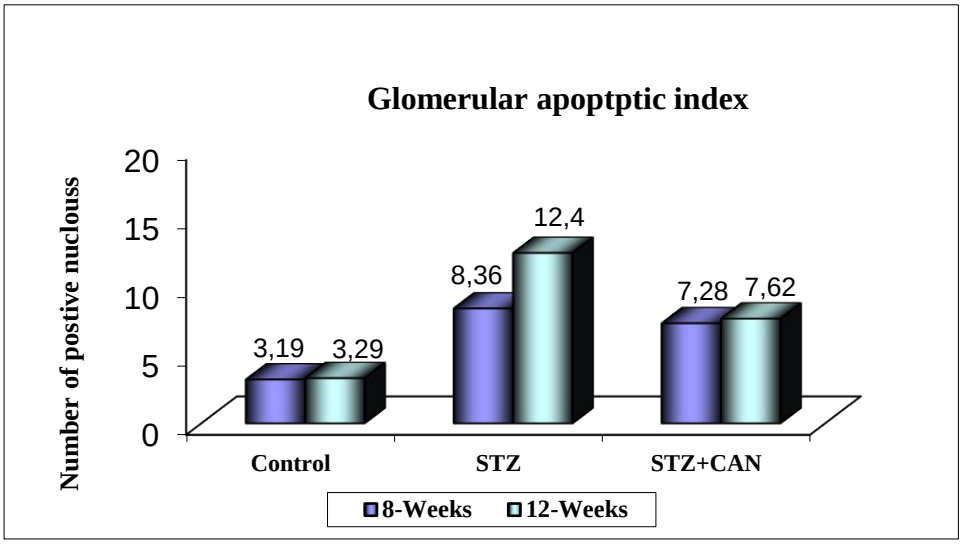

Figure. 1 Glomerular apoptotic index mean after 8 weeks and 12 weeks of streptozoticin administration

The analysis of kidney samples after 12 weeks revealed a progression in increasing the number of apoptotic cells in the glomeruli in the STZ group of rats, with the average values of the apoptosis index in the glomeruli being 12.4 ± 3.80. In the STZ+CAN group of rats, after 12 weeks of streptozotocin administration, stagnation was characteristic in relation to the increase in the values of the apoptosis index, with the average value being 7.62 ± 2.13, significantly lower than the average value in the STZ group of rats ($p < 0.05$) (Figure 1 and 2 and Table 1).

Table 1. Effects of treatment with candesartan on the values of Glomerular apoptotic index after 8 and 12 weeks.

Glomerular apoptotic index mean after 8 weeks and 12 weeks of streptozoticin administration						
	After 8-Weeks			**After 12-Weeks**		
	Control	**STZ**	**STZ+CAN**	**Control**	**STZ**	**STZ+CAN**
X	3.19	8.36[a]	7.28 [ab]	3.29	12.4a	7.62ab
SD	2.03	2.68	2.24	2.11	3.80	2.13
Min	0	2	1	0	6	3
Max	6	14	11	7	21	13
[a]<0.05 vs Control [b]<0.05 vs STZ						

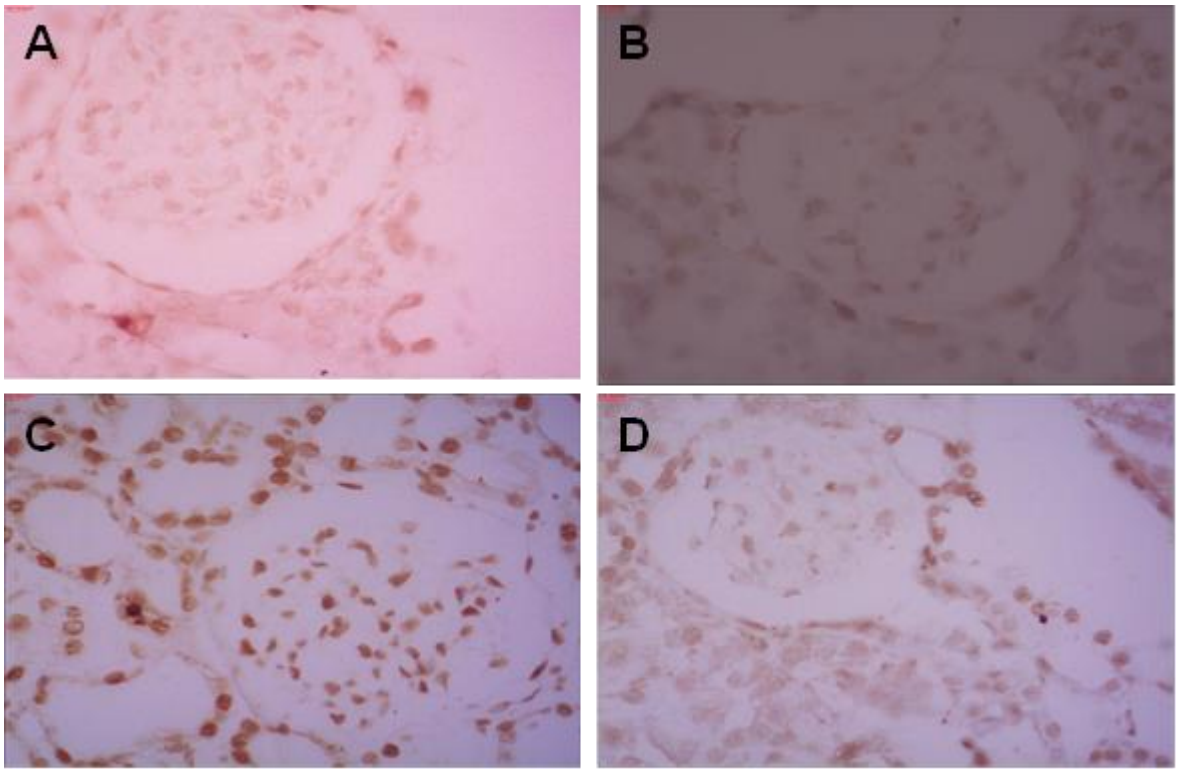

Figure 2. Glomerular apoptosis. (A) Control group; (B) STZ-group after 8 weeks; (C) STZ-group after 12 weeks; (D) STZ+CAN group after 12 weeks

Cell proliferation

Cell proliferation, as indicated by the proliferative index determined by Ki-67, was used to assess the influence of drugs on renal proliferation, particularly in the context of the experimental model of induced diabetic nephropathy.

In the STZ group of rats, the analysis of glomerular proliferation revealed an increase in the average values of the proliferative index after 8 weeks, reaching 9.25 ± 3.35. These values were significantly higher than the average values in the control group (4.30 ± 2.62), indicating an increased expansion of the mesangial matrix.

In the STZ+CAN group of rats, a significant increase in the glomerular proliferative index was also observed after 8 weeks of streptozotocin administration, although it was slightly less pronounced

than in the STZ group. The average values of the proliferative index in this group of rats after 8 weeks were 7.55 ± 3.05 (STZ+CAN) (Table 2 and Figure 3).

At the end of the study, after 12 weeks of streptozotocin administration, the proliferative index values in the STZ group of rats doubled (18.69 ± 6.60) compared to the values after 8 weeks ($p < 0.05$), indicating a marked progression of proliferation in the glomeruli. In contrast, in the STZ+CAN group of rats, no further progression of proliferation in the glomeruli was observed. The average values were similar to those registered after 8 weeks and were significantly lower ($p < 0.05$) compared to the STZ group (Table 2 and Figure 3).

Table 2. Glomerular cell proliferation values after 8 weeks and 12 weeks

Glomerular proliferative index after 8 weeks and 12 weeks of streptozoticin administration						
	After 8-Weeks			**After 12-Weeks**		
	Control	**STZ**	**STZ+CAN**	**Control**	**STZ**	**STZ+CAN**
X	4.30	9.25[a]	7.55 [ab]	3.53	18.69[a]	9.29[ab]
SD	2.62	3.35	3.05	1.96	6.60	4.47
Min	0	3	2	0	8	2
Max	8	16	13	6	25	15
[a]<0.05 vs Control; [b]<0.05 vs STZ						

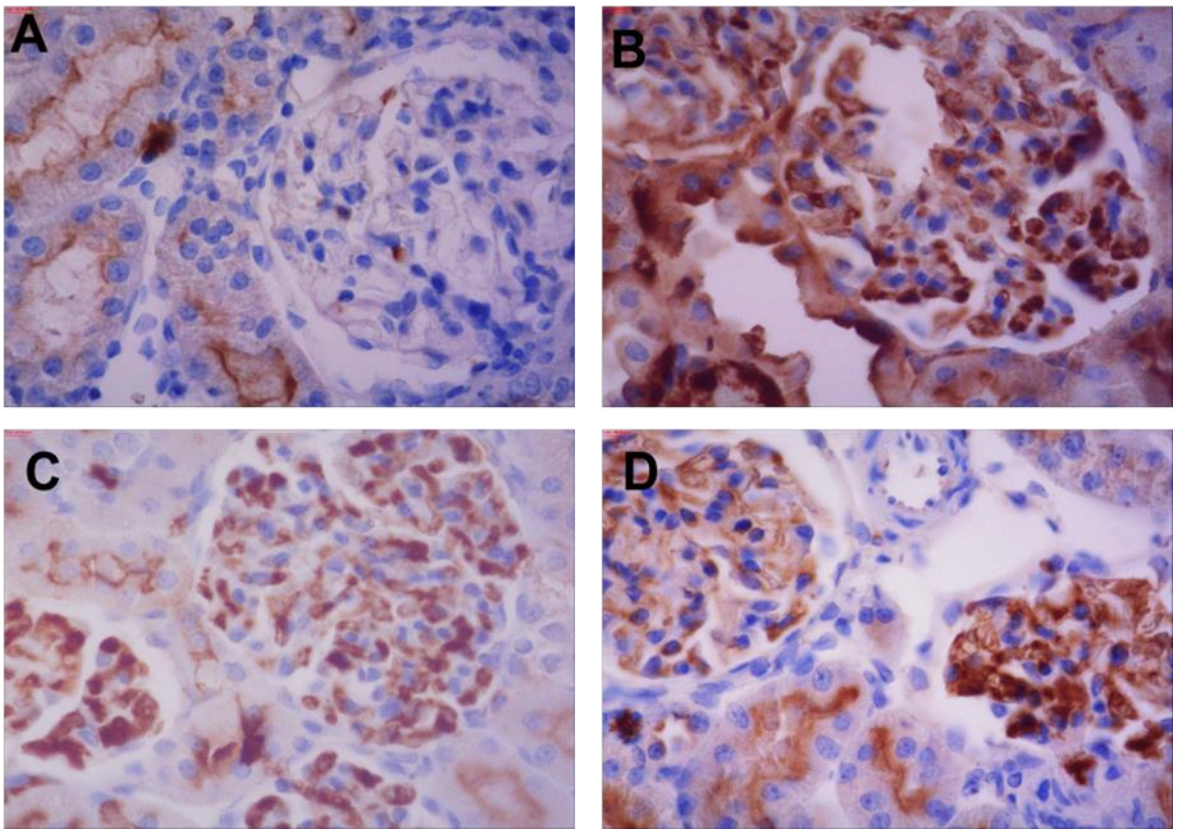

Figure 3. Glomerular cell proliferation (Ki-67). (A) Control group; (B) STZ group after 8 weeks; (C) STZ group after 12 weeks;; (D) STZ+CAN group after 12 weeks

TGF-β1 expression

Changes in glomerular TGF-β1 expression in the kidney, determined semiquantitative, were utilized as a highly specific marker for the development of diabetic nephropathy and to assess the effects of the study drugs on its progression.

After just 4 weeks of streptozotocin administration, there was a significant increase in the expression of TGF-β1 in the glomeruli, exceeding five times higher than that in the control group. In the STZ group of rats, the expression of TGF-β1 became even more pronounced as the study progressed, reaching approximately six times higher levels than in the control group by the end of the study (Figure 4 and Table 3).

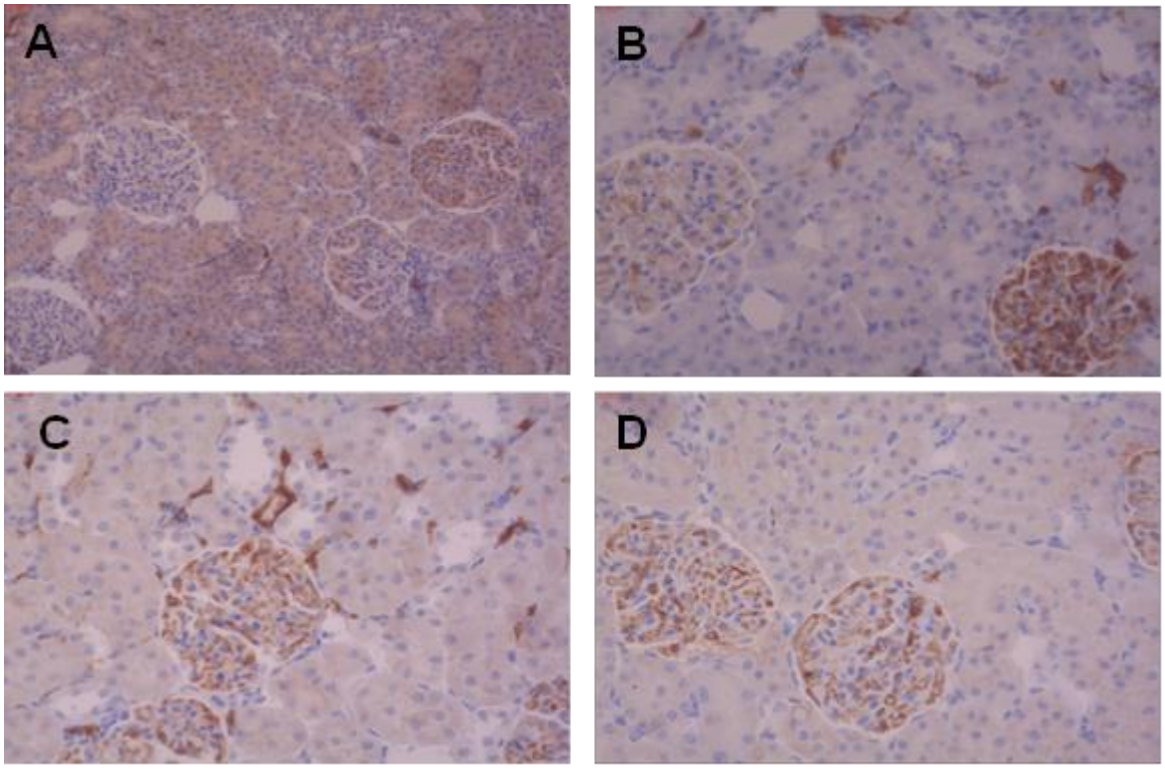

Figure 4. Glomerular expression of TGF-β1, determined semiquantitatively. (A) control group; (B) STZ group after 4 weeks; (C) STZ group after 8 weeks; (D) STZ group after 8 weeks.

Candesartan, administered as monotherapy after 4 weeks of streptozotocin administration, significantly reduced glomerular expression of TGF-β1, although not completely. By the end of the trial, the semiquantitative values of the glomerular expression of TGF-β1 were approximately 3 times higher than the control group (Figure 5 and Table 3) but approximately 2 times lower than the STZ group ($p < 0.05$).

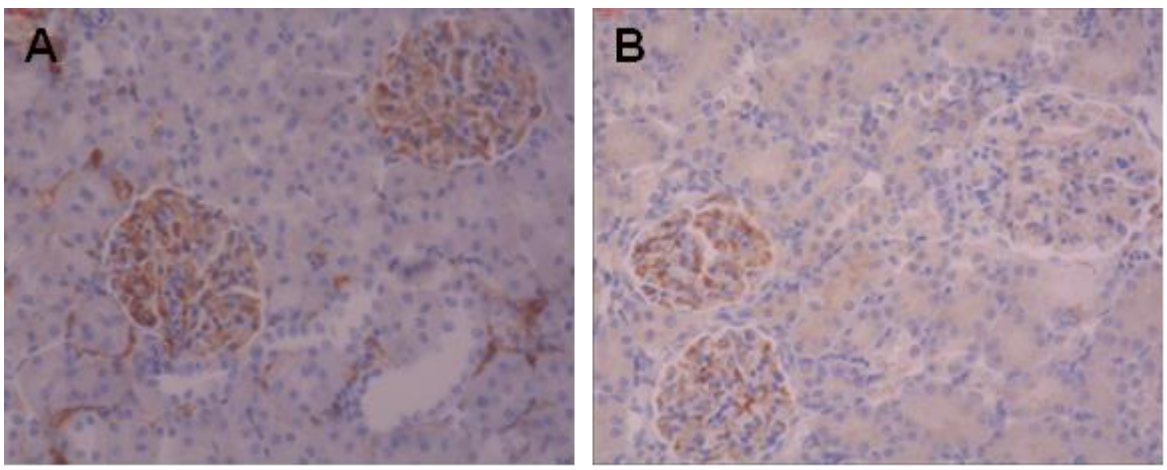

Figure 5. Glomerular expression of TGF-β1, determined semiquantitatively. (A) STZ group after 12 weeks; STZ+CAN group after 12 week

Table 3. Effects of streptozotocin and candesartan treatment on glomerular expression of TGF-β1 determined semiquantitatively throughout the trial.

	Expression of TGF-β1			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	0.537	0.569	0.473	0.513
SD	0.302	0.437	0.398	0.369
Min	0	0	0	0
Max	1	1	1	1
STZ				
X	0.462	2.351[a]	2.858[a]	3.225[a]
SD	0.294	0.883	0.881	0.942
Min	0	1	2	2
Max	1	4	4	4
STZ+CAN				
X	0.621	2.267[a]	1.975[a,b]	1.745[a,b]
SD	0.408	0.785	0.792	0.683
Min	0	1	1	1
Max	1	4	4	3

[a]<0.05 vs Control
[b]<0.05 vs STZ

Discusion

The primary goal of this study was to elucidate the molecular mechanisms underlying streptozotocin-induced diabetic nephropathy, as well as to understand the molecular mechanisms by which candesartan exerts its therapeutic effects.

The primary abnormalities in diabetic nephropathy, namely the enlargement of the mesangial matrix and thickening of the basement membrane, do not entirely account for the fundamental symptom of proteinuria. Recent investigations into the molecular mechanisms underlying the onset and progression of diabetic nephropathy have increasingly shifted their emphasis toward alterations in the filtration barrier. This barrier comprises the glomerular endothelium, glomerular basement membrane, and podocytes (glomerular visceral epithelial cells).

Podocytes serve as the ultimate barrier for plasma proteins, and their impairment is a pivotal factor in the progression of diabetic nephropathy [17].

Traditionally, podocyte damage was considered a late outcome of elevated proteinuria in individuals with diabetic nephropathy. Nevertheless, recent investigations indicate that podocyte damage is a fundamental molecular mechanism in the development of diabetic nephropathy, marking one of the earliest stages of glomerular injury [4,6,8,11,12].

For this reason, this study conducted specific histopathological examinations on the kidneys to assess the impact of damage to the filtration barrier in the glomeruli. A single administration of streptozotocin, inducing severe diabetes, consistently resulted in disruptions to the construction and structure of the glomeruli within a mere 4 weeks. Observable changes encompassed a substantial expansion of the mesangial matrix and thickening of the glomerular basement membrane due to deposits of basement-membranous sclerotic material. Moreover, noteworthy alterations were detected in the blood vessels of the renal parenchyma, characterized by medial hypertrophy and a mild degree of intimal thickening.

Confirmation of these findings involved assessing the expansion and proliferation of the mesangial matrix, coupled with inohistochemical examinations to determine the degree of cell

proliferation using the proliferative index, measured globally with the assistance of Ki-67. In the STZ group of rats, analysis of glomerular proliferation revealed a noteworthy increase in the average values of the proliferative index after 8 weeks, and this increase became even more pronounced after 12 weeks, reaching several times higher than the control group. These results unmistakably indicate heightened cell proliferation and mesangial matrix expansion as key characteristic findings in diabetic nephropathy. The impact of candesartan, characterized by a significant reduction in glomerular proliferation and, consequently, in the expansion of the mesangial matrix in the glomeruli, suggests that this could be one of the potential mechanisms through which the therapeutic effects of candesartan are achieved.

The precise cause of podocyte damage and depletion in diabetic nephropathy remains insufficiently understood and speculative. However, it is believed that two mechanisms may be at the core: podocyte apoptosis and detachment of podocytes from the glomerular basement membrane, leading to their elimination in the urine (podocytopenia).

For this reason, one of our objectives in exploring the molecular mechanisms of experimentally induced diabetic nephropathy was the immunohistochemical assessment and quantification of apoptosis in the glomerular compartment.

The findings of this study further validate that apoptosis can play a pivotal role in the onset and progression of diabetic nephropathy, serving as a significant contributor to the damage and subsequent loss of podocytes. The administration of streptozotocin to the STZ group of rats resulted in a more than twofold increase in the number of apoptotic cells in the glomeruli after 8 weeks. At the conclusion of the study, these values were approximately four times higher compared to baseline values and the control group.

Conversely, findings from an experimental regimen involving candesartan suggest that its positive effects in treating diabetic nephropathy may be attributed, in part, to the reduction of apoptosis associated with diabetic nephropathy. The precise molecular mechanisms that intensify apoptosis and lead to podocyte detachment from the basement membrane remain inadequately elucidated. However, it is believed that these mechanisms may involve the overactivity of angiotensin II, the expression of nephrin, the overexpression of vascular endothelial growth factor (VEGF), and notably, the overexpression and overactivity of intraglomerular transforming growth factor (TGF-β), particularly TGF-β1.

On the contrary, findings from an experimental regimen utilizing candesartan suggest that its positive effects in treating diabetic nephropathy may be attributed, in part, to the reduction of apoptosis associated with diabetic nephropathy. The molecular mechanisms underlying the intensification of apoptosis and podocyte detachment from the basement membrane remain insufficiently clarified. However, it is hypothesized that these mechanisms may encompass the overactivity of angiotensin II, the expression of nephrin, the overexpression of vascular endothelial growth factor (VEGF), and notably, the overexpression and overactivity of intraglomerular transforming growth factor (TGF-β), particularly TGF-β1.

In the context of understanding the molecular mechanisms underlying the development of diabetic nephropathy and the induction of apoptosis, the data indicating the pivotal role of elevated levels and excessive activity of intraglomerular transforming growth factor (TGF-β), especially TGF-β1, are particularly intriguing [13,14].

Given this information, the assessment of intraglomerular expression of TGF-β1 was established as a primary objective in this study, aiming to shed light on its potential significance.

The results derived from these specific investigations affirm the significance of TGF-β1 and underscore its primary role in the progression of diabetic nephropathy.

The administration of streptozotocin, leading to subsequent hyperglycemia and the onset of severe diabetes, resulted in a notably heightened expression of TGF-β1, reaching levels approximately fivefold higher than basal and control values. As the trial progressed, these values further increased in the untreated group, correlating with an escalation in apoptosis. Conversely, in the group of rats receiving candesartan, a substantial decrease in the intraglomerular expression of TGF-β1 was observed. These findings unequivocally affirm the significance of TGF-β1 in the pathogenesis of diabetic nephropathy while simultaneously suggesting potential mechanisms through which the investigated drugs achieve their therapeutic effects.

The obtained results align with findings from numerous other studies that have consistently demonstrated the integral role of TGF-β in the development of diabetic nephropathy [15]. This

fibrogenic cytokine is known to be stimulated in the diabetic state, and its presence in the kidney increases during diabetes [16]. Micropuncture techniques have revealed increased levels of TGF-β in glomeruli from streptozotocin-induced diabetic rats [22,23]. Additionally, inhibiting TGF-β with panselective neutralizing antibodies in diabetic mice has been shown to prevent diabetic renal hypertrophy, mesangial matrix expansion, and the development of renal failure [24]. In non-diabetic experimental models, the overexpression of active TGF-β1 in transgenic mice has been demonstrated to induce mesangial expansion, glomerulosclerosis, interstitial fibrosis, renal failure, and progressive proteinuria [25].

Of particular significance are the findings concerning the effects of TGF-β1 on podocytes. Evidence supports that podocytes are a target for the increased levels and expressed activity of TGF-β1 in the presence of diabetes. These studies have established that the heightened activity of TGF-β1 can lead to podocyte detachment, i.e., podocyte apoptosis [26,27].

Conclusion

The results of this experimental study affirm that disturbances in visceral epithelial cells, specifically podocyte apoptosis, may be a key factor in the molecular mechanisms underlying the onset of diabetic nephropathy. Concurrently, investigations into the mechanisms of podocyte apoptosis induction in diabetes and diabetic nephropathy indicate that excessive expression of TGF-β1 could be a crucial factor. The heightened expression of TGF-β1 appears to be among the most significant contributors to the development of diabetic nephropathy, playing a dual role. Firstly, with its prosclerotic potential, it significantly induces glomerulosclerosis, and secondly, with its proapoptotic potential, it stimulates the apoptosis of podocytes in the glomeruli. These findings underscore the integral role of TGF-β1 in the pathogenesis of diabetic nephropathy.

Exploring the molecular mechanisms of candesartan's action aligns with recent discoveries, suggesting that the favorable therapeutic effects of candesartan may stem from the inhibition of angiotensin II. At the molecular level, this inhibition results in the prevention of podocyte apoptosis and podocytopenia, the inhibition of mesangial proliferation, and a reduction in intraglomerular expression of TGF-β1.

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