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PERINDOPRIL TREATMENT IN STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATY

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Abstract
Diabetic nephropathy (DN) is one of the most common causes of terminal stadium damage to the kidneys. The angiotensin-converting enzyme (ACE) represents a significant risk factor for the progression of DN. ACE inhibitors are medications of particular interest knowing the role of angiotensin II in the development of DN. This study aimed to examine the effects of ACE inhibitor treatment perindopril (PER), administered to rats with streptozotocin (STZ) induced DN, that developed albuminuria, renal hypertrophy and mild glomerulussclerosis. DN was induced by a STZ (60 mg/kg ip) single injection to normotensive Wistar rats. The administration of STZ caused diabetes mellitus (DM) with symptoms and signs of DN including poor general condition, body-weight loss, kidney weight increase as well as increased values of BUN and serum creatinine, accompanied by increased diuresis as well as distinct albuminuria. The majority of these symptoms were manifested 4 weeks after, and even more distinctly 8 and 12 weeks after administering STZ. The perindopril treatment (6 mg/kg BW), starting 4 weeks after administering STZ, resulted in a significant improvement of all symptoms and signs of DN, significantly lowering the values of BUN and serum creatinine, albuminuria and diuresis. The histopathological examination of the renal samples at 8 and 12 weeks after the beginning of the study have shown that perindopril significantly lowers the progression of glomerulopathy, and significantly improves the glomerulosclerotic index, as well as the progression of renal histological abnormalities induced with STZ. Thus perindopril treatment ameliorates STZ-induced nephropathic changes in DM rats.

Key words: Diabetic Nephropathy, STZ, Perindopril, Glomerulosclerosis, Rats.

Introduction

Diabetic nephropathy (DN) is one of the most common causes of terminal stadium damage to the kidneys, clinically characterised with proteinuria, microalbuminuria and progressive renal insufficiency. The mechanisms causing this kidney damage have not been defined with certainty, however, the interactions between high glycaemia, vascular-endothelial growth factor, angiotensin II, endothelin, glycosylation end products and TGF-β, as well as the haemodynamic changes in the kidney microcirculation (glomerular hyperfiltration, increased intercapillary pressure in the glomerulus) and the structural changes in the glomerulus (increased extracellular matrix, thickening of the basal membrane, expansion of the mesangial tissue and fibrosis) are the most commonly referred to. The glomerular basement membrane (GBM) thickening is considered to be one of the main histopathological changes of the condition. The expansion of the mesangial matrix correlates with the proteinuria and impaired kidney function. It has been proven that the accumulation of the mesangial matrix reduces the capillary area available for filtration, thus contributing towards progressive renal insufficiency [1]. Renal insuffiency can also occur as a result of tubulointerstitial fibrosis, which for its part additionally intensifies the proteinuria [2, 3].

The angiotensin converting enzyme (ACE) represents a significant risk factor for the progression of DN [4]. ACE inhibitors are medications of

particular interest, knowing the role of angiotensin II in the development of diabetic nephropathy.

It is known that ACE inhibitors have been an effective therapy for treating both human and experimental DN [5–7]. It is considered that the renoprotection achieved with ACE inhibitors is not exclusively a consequence of decreased systemic blood pressure but also of decreased intraglomerular pressure [8]. Furthermore, it is considered that the blockage of the renin-angiotensin system (RAS) can suppress locally activated kidney RAS which results in lowering the proinflamatory and prosclerotic cytokines which have an important role in the progression of diabetic microvascular and tubulointerstitial conditions [9, 10]. The streptozotocin (STZ) induced DN in rats represents an excellent model for evaluation of the treatment with perindopril (ACE inhibitor), due to the progressive development of severe glomerular sclerosis and tubulointerstitial fibrosis in the STZ-induced diabetes mellitus (DM) rats, as well as the high similarity of the intrarenal enzyme distribution with that in humans.

The main aim of this study was to examine the effects of ACE inhibitor treatment perindopril (PER) (Alkaloid AD, Skopje), administered to rats with STZ induced DM, that developed albuminuria, renal hypertrophy and mild glomerulussclerosis.

Materials and methods

Animal model

A total of 75 male and female normotensive Wistar rats at the age of 9 to11 weeks, weighing 160–300 g were used in this investigation.

All the rats were housed in a temperature-controlled environment (temperature of 20 ± 2ºC, and 12-h light/dark cycle) with permanent access to fresh water and standard laboratory diet.

DM was induced by a single ip injection of STZ (Sigma-Aldrich, Chemie GmbH, Germany) at dosage of 60 mg/kg dissolved in 0.1 M citrate buffer (pH 4.5). The control group received an ip injection of citrate buffer alone (control nondiabetic rats).

72 hours after the STZ application, DM was confirmed by measurement of blood glucose levels using a blood glucose monitor (Accu-Chek, Roche Diagnostic, Germany). Blood samples for determination of glucose were obtained by tail bleeding.

Only the rats with blood glucose levels ≥ 11 mmol/L, under fasting conditions (morning blood samples) were included in the study. In order to develop DN the animals were left in DM condition without any treatment during the following 4 weeks. The diabetic rats (n = 50) were randomly assigned to the two experimental groups (STZ and STZ+PER). To estimate the symptoms and signs of diabetic nephropathy, the STZ group of rats (n = 25) was left without treatment in the following 8 weeks. For assessment of the effects of the ACE inhibitor perindopril (PER) treatment on rats with experimentally induced diabetic nephropathy, in the STZ+PER group of diabetic rats (n = 25), perindopril was administered at a dose of 6 mg/kg/per d by gavage, from week 4 to week 12.

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

Biochemical parameters

The level of blood glucose was determined before the beginning of the investigation, 72 hours after STZ application and after 4, 8 and 12 weeks from the beginning of the study.

Renal functional tests

During the study, the renal function in the examined animals was assessed by determination of serum creatinine and blood urea nitrogen (BUN) by autoanalyser (Cobas Integra 400 Plus; Roche Diagnostics, Germany), as well as determination of the of 24-hour urine volume. Urine albumin in 24-hour urine samples was determined by autoanalyser (Cobas Integra 400 Plus; Roche Diagnostics, Germany). These tests were performed prior to the beginning of the study (Day 0), and at 4, 8 and 12 weeks from the beginning of the study. In order to determine the serum levels of creatinine and BUN, blood was taken by venepuncture from the orbital sinus of the rats under light ether anaesthesia. Blood samples of 400 μl were taken for serum separation (200 μl). Metabolic cages were used for collecting 24-hour urine samples for determination of urinary albumin quantity.

Body weight

The body weight of the examined animals was measured during the whole study at weekly intervals.

The body weight/kidney weight ratio was determined at the end of the study after 12 weeks.

Histopathological examination and renal histology

At 8 and 12 weeks after STZ administration, the animals were euthanized by intraperitoneal injection of pentobarbital (50 mg/kg; Boehringer Ingelheim), the abdomen was opened by midline incision and both kidneys were removed. The kidneys were promptly bisected and fixed in 10% buffered formaldehyde and then embedded in paraffin; at least 6 sections were cut at 4–6 μm and stained with haematoxylin and eosin, periodic acid-Schif (PAS), silver methenamine Jones and trichrome Masson.

To evaluate the severity of glomerular sclerosis, 20 randomly non-overlapping fields per kidney were examined at a magnification of × 200 using a Nikon light microscope. Glomerular sclerosis was defined as glomerular basement membrane thickening, mesangial hypertrophy and capillary occlusion. The degree of glomerular damage was assessed using a semi-quantitative scoring method: grade 0, normal glomeruli; grade 1, sclerotic area up to 25% (minimal sclerosis); grade 2, sclerotic area 25 to 50% (moderate sclerosis); grade 3, sclerotic area 50 to 75% (moderate-severe sclerosis); grade 4, sclerotic area 75 to 100% (severe sclerosis). The glomerulosclerotic index (GSI) was calculated using the following formula: $GSI = (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)/n_0 + n_1 + n_2 + n_3 + n_4$, where n_x is the number of glomeruli in each grade of glomerular sclerosis [11]. This analysis was performed with the observer masked to the treatment groups.

Changes in kidney structures were analysed in a double-blinded manner. Glomeruli were carefully graded in a sequential manner, to avoid grading the same glomeruli twice.

The changes detected by light microscopy were analysed and confirmed by transmissive scanning microscopy (TEM).

Kidney tissue samples with a size of 1–2 mm^2 after deparafination and rehydration procedure were postfixed in 1% OsO4 for 1 hour and then processed for embedding in Durcupan resin. Semi-thin sections were stained with Toluidine blue, while ultrathin sections obtained from ultramicrotome (PT-PC PowerTome Ultramicrotomes-RMC Products), were contrasted in autostainer (QG-3100 Automated TEM Stainer-RMC Products) for ultrathin sections by Uranyl acetate and Lead citrate. Samples were then analysed on a transmission electron microscope (JEOL JEM 1400, JAPAN) attached to a digital camera (Veleta TEM Camera, Olympus, Germany) and controlled by iTEM software v. 5.2.

Statistical analyses

All data were expressed as mean ± SD. To test more than two groups, Kruskal-Wallis variance analysis was used, followed by the Mann-Whitney U-test to determine which groups were significantly different. P-values of less than 0.05 were considered as statistically significant.

Results

The overall condition of the STZ rats deteriorated and was accompanied by significant bodyweight loss as well as increased urine volume at 4, 8 and 12 weeks after drug administration compared to the control (nondiabetic) rats. Moreover, the kidney weight as well as the body/kidney weight ratio of the STZ rats was significantly increased by the end of the 12th week, compared to the control group (Table 1).

In the diabetic rats treated with perindopril in the period from week 4 to week 12, the body weight loss as well as the kidney/body weight ratio increase were less emphasised. Regardless of the significant difference of the kidney/body weight ratio compared to the control group (3.20 ± 0.29 vs 4.45 ± 0.39; $p < 0.05$) at the end of the study, this ratio was significantly lower than that in the STZ group (4, 45 ± 0.39 vs 5, 41 ± 0.69; $p < 0.05$) (Table 1).

Table 1

Effects of perindopril on body weight/renal weight (mg/g) ratio in rats with STZ induced DN

Body weight/renal weight mg/g (12 week)			
	Control	**STZ**	**PER**
X	3.20	5.41[a]	4.45[a,b]
SD	0.29	0.69	0.39
Min	2.81	4.40	3.90
Max	3.68	6.49	5.11

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

Renal function

Serum concentrations of creatinine, BUN and urinary excretion of albumins were used as basic parameters for the renal function.

After 8 weeks the serum concentrations of creatinine in the STZ group were increased twofold in comparison to the basal values ($p < 0.05$). At the end of the study, after 12 weeks, there was an additional increase in the serum creatinine values. With the group of animals receiving perindopril treatment there was only a mild increase of the serum concentrations of creatinine after 8 and 12 weeks (Figure 1).

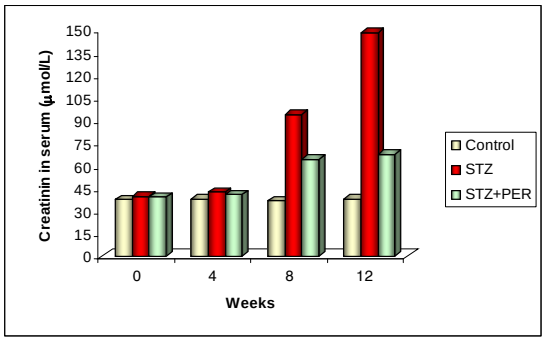

Figure 1 – Effects of perindopril on serum creatinine levels

Comparative statistical analysis of the serum creatinine levels of the STZ and STZ+PER groups

showed a significant difference after week 8 with a peak difference at week 12, when the serum concentrations of the STZ and STZ+PER groups were 148.32 ± 67.91 and 67.37 ± 19.41 μmol/L, respectively ($p < 0.05$) (Table 2).

Table 2

Effects of perindopril treatment on serum creatinine levels in rats with STZ induced DN

	Serum creatinine (μmol/L)			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	37.44	37.89	36.81	38.11
SD	3.18	3.78	3.53	3.37
Min	30.10	31.01	30.3	31.0
Max	41.9	46.05	44.2	43.5
STZ				
X	39.58	42.49	94.09[a]	148.32[a]
SD	5.43	7.37	41.63	67.91
Min	30.5	35.25	35.7	57.3
Max	49.6	58.14	158.3	253.8
STZ+PER				
X	39.18	40.93	64.34[ab]	67.37[ab]
SD	4.08	7.80	26.00	19.41
Min	34.02	31.24	34.7	37.6
Max	48.10	57.15	119.9	98.7

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

A significant increase of the BUN values was registered 4 weeks after the administration of STZ, whereas the serum concentrations of BUN in the STZ group increased additionally after 8 and 12 weeks. The treatment with perindopril resulted in a constant reduction of the BUN levels; however, the values remained on a significantly higher level compared to the control group ($p < 0.05$) (Figure 2).

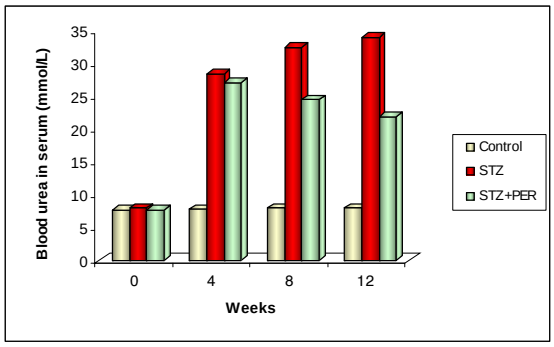

Figure 2 – Effects of perindopril on BUN levels

The comparison of the BUN levels after 8 and 12 weeks between the two experimental groups (STZ and STZ+PER) revealed a significant positive effect of perindopril on the reduction of BUN levels ($p < 0.05$) (Table 3).

Table 3

Effects of perindopril treatment on BUN levels in rats with STZ induced DN

	BUN (mmol/L)			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	7.67	7.79	8.01	7.96
SD	1.90	1.75	1.45	1.85
Min	4.30	5.30	5.40	5.20
Max	11.50	10.30	10.60	11.60
STZ				
X	8.03	28.41[a]	32.48[a]	34.05[a]
SD	1.79	12.48	16.45	20.63
Min	4.50	8.70	11.95	12.90
Max	11.20	53.50	77.60	85.40
STZ+PER				
X	7.72	27.08[ab]	24.52[ab]	21.91[ab]
SD	2.15	10.88	11.73	9.76
Min	4.50	10.50	10.82	11.34
Max	10.60	52.90	51.30	42.74

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

A significant increase in the urine albumin values were recorded 4 weeks after the administration of STZ. These values in the STZ group were additionally increased after 8 weeks and peaked significantly after 12 weeks (3.316 ± 0.822 mg/24h). The treatment with perindopril in (STZ+PER group) resulted in a significant decrease in the urine albumin values after 8 weeks which was even more pronounced after 12 weeks from the beginning of the study (1.309 ± 0.535 mg/24h) (Figure 3).

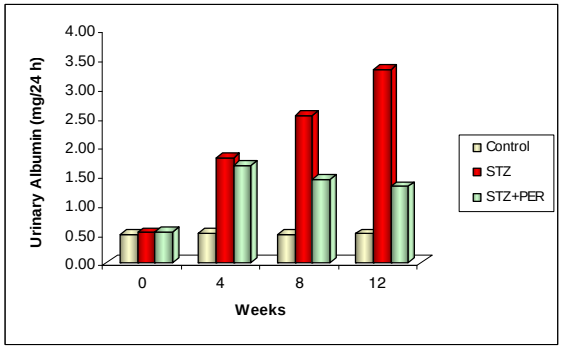

Figure 3 – Excretion of perindopril on urinary albumin levels

The treatment with perindopril resulted in significant ($p < 0.05$) decrease of urine albumin values in the STZ+PER group compared to the STZ group after 8 weeks with a peak difference at week 12 (Table 4).

Table 4

Effects of perindopril treatment on urinary albumin excretion in rats with STZ induced DN

	Urine albumine (mg/24h)			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	0.490	0.504	0.483	0.496
SD	0.118	0.120	0.109	0.116
Min	0.335	0.345	0.340	0.318
Max	0.714	0.756	0.652	0.707
STZ				
X	0.520	1.799[a]	2.523[a]	3.316[a]
SD	0.158	0.775	0.824	0.822
Min	0.295	0.765	0.953	1.696
Max	0.836	3.454	3.765	4.364
STZ+PER				
X	0.532	1.676[a,b]	1.434[a,b]	1.309[a,b]
SD	0.140	0.596	0.591	0.535
Min	0.305	0.654	0.2712	0.399
Max	0.857	2.896	2.325	2.318

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

Histopathological research

Macroscopic analysis of the renal parenchyma in the control group showed preserved corticomedullar construction and patent of calyces and renal pelvis.

The microscopic evaluation of the renal cortex of nondiabetic rats (control group) revealed a normal appearance in most of the glomeruli after 8 and 12 weeks, with normal glomerular basal membrane, without increased intra- and extracapillary cellular effusions or any other type of exudative changes. The tubular excretory ducts within the tubulointerstitium had normal morphology, whereas the interstitium was slightly altered (Figure 4A, Figure 5A).

Most of the changes in the renal tissue 4 weeks after administering STZ could be found in the glomerular compartment, and minor changes could be noticed in the tubular compartment of the corticomedular region, where both the proximal and the distal tubule were affected.

Macroscopic analysis of the kidneys in the STZ group showed a paler cortical region with pronounced discoloration of the medulla and lower kidney weight. Calyces and renal pelvis retained normal morphology.

The renal tissue examination of the STZ group under light microscopy 8 weeks after administration of STZ showed the presence of a moderate degree of glomerulopathy characterized with basement membrane thickening, expansion of the mesingial matrix, arteriolar hyalinosis and insudative protein deposits that obstruct some of the capillaries (Figure 4B).

Similar and even a more severe degree of glomerular damage was found in the same animal group 12 weeks after STZ administration (Figure 4C and D).

Moreover, histopathological examination of the renal samples 8 and 12 weeks after STZ administration showed signs of interstitial expansion with interstitial fibrosis and dilatation of the tubules with atrophy of the epithelium in the corticomedullar region. Some renal samples showed tubular epithelial vacuolization with the presence of tubular lumen dilatation and glycogen deposits.

The blood vessels in the renal parenchyma showed medial hypertrophy with a low level of intimal thickening.

The ultrastructure analysis identified uneven fusion of the podocytes as well as widening of the mesangial matrix with GBM thickening due to deposition of basalmembranaceous sclerotic material.

The macroscopic appearance of the kidney samples from the DM rats treated with perindopril (STZ + PER) did not show significant deviations compared to the morphology of the samples from the STZ group, with the exception of macroscopically visible reduction in the size of the kidneys.

In the STZ + PER group a light level of diabetic glomerulopathy was detected, in contrast to the STZ group. Basement membrane thickening, glomerulosclerosis and changes in tubulointerstitium were detected in both experimental groups, however, this was significantly less pronounced in the STZ + PER group compared to the STZ group (Figure 4E and F, Figure 5B).

Reduction in the thickness of the arterial wall in the affected vessels was also observed.

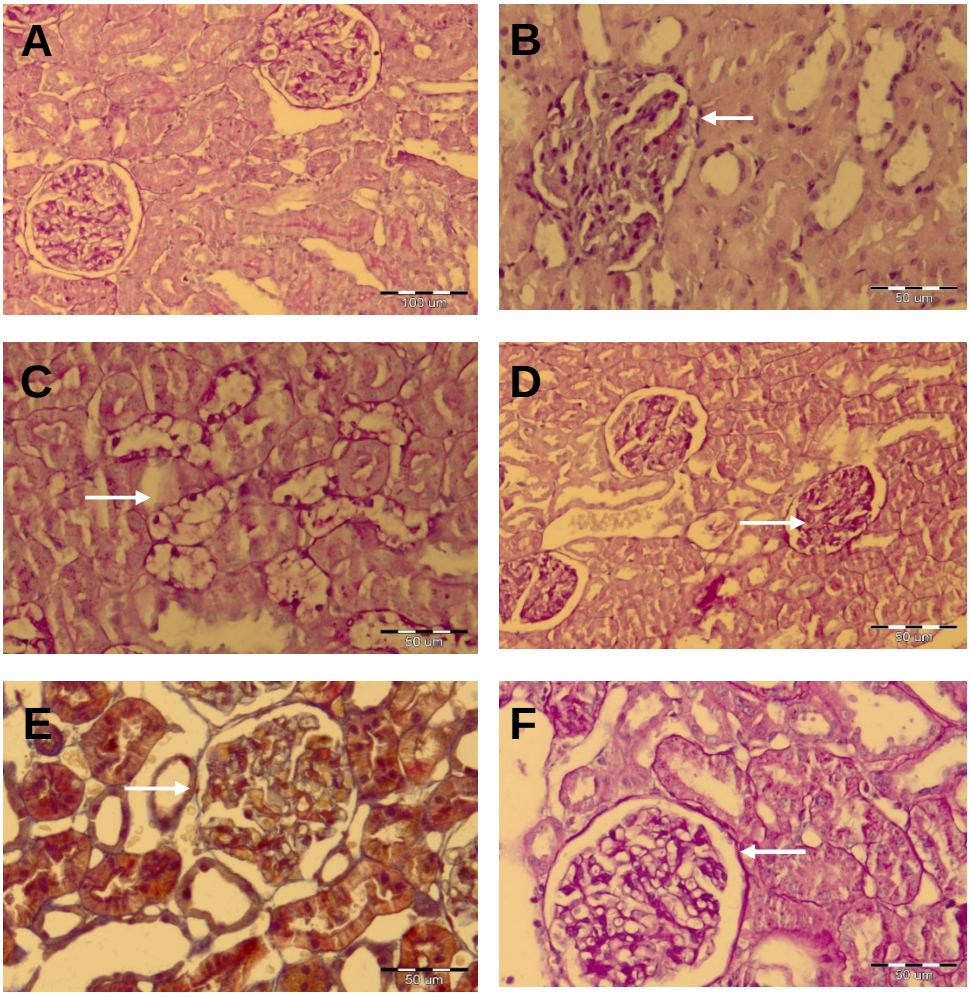

Figure 4 – Histopathological features of kidney from nondiabetic, from STZ-induced DN rats and STZ-induced DN rats treated with perindopril. Sections were stained with PAS reagent. Magnification × 100 and × 200. (A) Untreated nondiabetic rats at 12 wks. (B) Untreated diabetic rat at 8 wks. (C) Untreated diabetic rat at 12 wks. (D) Untreated diabetic rat at 12 wks. with damaged glomeruli, thickened GBM, altered tubular epithelium with clear cytoplasm due to intracellular glycogen accumulation and areas of partial tubular dilatation. (E) Diabetic rat treated with perindopril at 8 wks. (F) Diabetic rat treated with perindopril at 12 wks

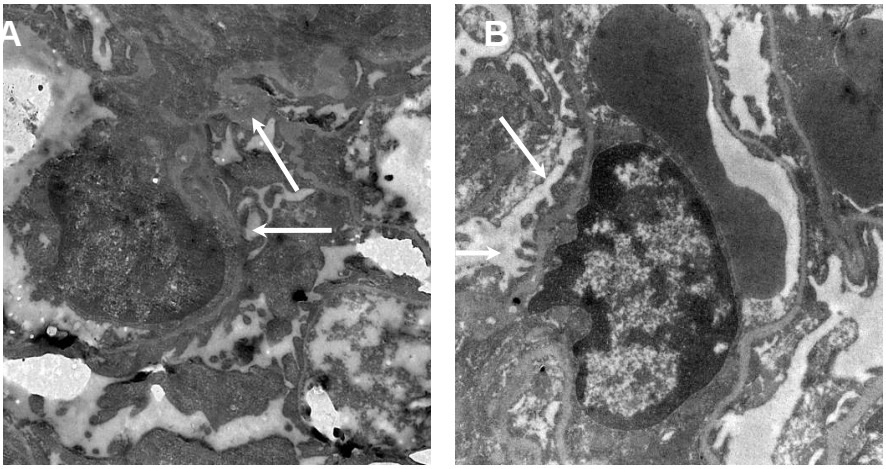

Figure 5 – An electron micrograph from STZ-induced DN rats and STZ-induced DN rats treated with perindopril. (A) Untreated diabetic rat at 12 wks., uneven fusion of the podocytes as well as widening of the mesangial matrix with sclerotic GBM thickening (magnification × 20,000). (B) Diabetic rat treated with perindopril at 12 wks., showing mild thickening of the GBM and regenerated podocyte (magnification × 15,000)

Glomerulosclerotic Index

The calculation of the glomerulosclerotic index shows a significant increase in the STZ group after 8 weeks (1.636 ± 0.674) which was even more distinct at the end of the study (2.077 ± 0.862). In contrast, a lower increase of the glomerulosclerotic index was registered with the STZ+PER group (Figure 6).

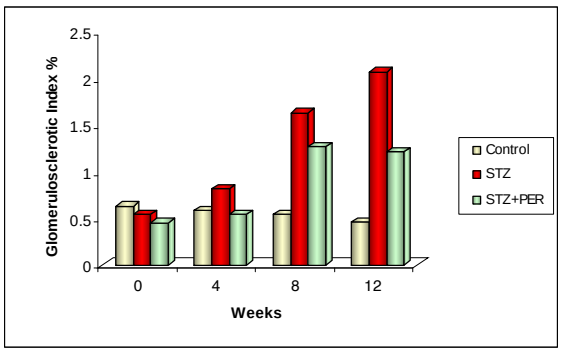

Figure 6 – Glomerulosclerotic index (GSI) of STZ and STZ+PER groups

Comparative statistical analysis of the GSI of the STZ and STZ+PER groups showed a significant difference after week 8 with a peak difference at week 12 (2.077 ± 0.862, and 1.214 ± 0.579, respectively) ($p < 0.05$) (Table 5).

Table 5

Glomerulosclerotic index in kidneys from nondiabetic, STZ induced DN rats and STZ induced DN rats treated with perindopril

	Glomerulosclerotic Index %			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	0.636	0.583	0.545	0.462
SD	0.505	0.515	0.522	0.519
Min	0	0	0	0
Max	1	1	1	1
STZ				
X	0.545	0.545	1.636[a]	2.077[a]
SD	0.522	0.522	0.674	0.862
Min	0	0	1	1
Max	1	1	3	3
STZ+PER				
X	0.455	0.455	1.273 [a,b]	1.214 [a,b]
SD	0.522	0.522	0.647	0.579
Min	0	0	0	0
Max	1	1	2	2

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

Discussion

DN is one of the most severe complications of DM and at the same time an important prognosis factor for diabetic patients.

For the time being there is no specific treatment for diabetic nephropathy. The best treatment is prevention, in the form of early detection and treatment of microalbuminuria.

Taking into account the known pharmacodynamics effects of the ACE inhibitors they stand out as potential medication for the treatment of diabetic nephropathy.

In this study, the efficiency of ACE inhibitor Perindopril was assessed, for the treatment of symptoms and signs of diabetic nephropathy. For that purpose an experimental model was used for the induction of DM and subsequently induced diabetic nephropathy.

The administration of STZ caused diabetes with distinct symptoms and signs of DN including poor general condition, body weight loss, kidney weight increase as well as increased values of BUN and serum creatinine, accompanied by increased diuresis as well as distinct albuminuria of the experimental animals. The majority of these symptoms were manifested 4 weeks after, and even more distinctly 8 and 12 weeks after administering STZ. The perindopril treatment, starting 4 weeks after administering STZ, at the dose of 6 mg/kg BW, resulted in significant improvement of all symptoms and signs of DN, significantly lowering the values of BUN and serum creatinine, albuminuria and diuresis. The results of this study undoubtedly point to the fact that ACE inhibitors such as perindopril, although not fully yet to a large extent, alleviate the functional renal disorder resulting from DN-induced experimentally with STZ.

ACE inhibitors such as perindopril represent a first line therapy in the treatment of diabetic patients, reducing the blood pressure and albuminuria and protecting the renal function [12–15].

The obtained results regarding albuminuria confirm the importance of this parameter in detecting DN induced by STZ as well as the therapeutic effects of perindopril. Microalbuminuria represents an independent predictor of the later progression of nephropathy and a risk of cardiovascular morbidity and mortality [16]. The increased permeability of the basal membrane during DN, caused by a decrease in the amount of proteoglycans as an integral part of the basal membrane, is a primary cause of albuminuria. The microalbuminuria occurs occasionally in the early and permanently in the later stages. With the advance of the condition, due to the glomerular sclerosis,

additional deterioration of the renal function can occur as well as the occurrence of distinct proteinuria and chronic renal insufficiency.

According to the glomerular-hyperfiltration theory, the increase of the blood osmotic pressure with hyperglycaemia and the increase of the circulating volume of blood stimulates hypersecretion of the atrial natriuretic peptide (ANP) which causes dilatation of the glomerular afferent arterioles. Moreover, hyperglycaemia causes the production of angiotensin II, which stimulates arteriolar constriction, but more emphasised is its effect on the glomerular efferent arterioles, which results in increased intraglomerular pressure and subsequent development and progression of DN [17, 18].

There are different theories and explanations of the beneficial effects of ACE inhibitors on diabetic nephropathy. Given that perindopril and the other ACE inhibitors do not affect the glucose level in the blood, the amelioration of DN symptoms cannot be linked to the improvement of the glycaemic control. It is known that ACE inhibitors cause dilatation of the efferent arterioles more selectively than of the afferent arterioles [19, 20] and inhibit the bradykinine degradation, which also dilatates the efferent arterioles [21]. In this way the intraglomeural pressure decreases and glomerular filtration improves.

The previous results corroborate the results of [22], proving that ACE inhibitors are efficient in the treatment of DN by means of control of the glomerular pressure, renal blood pressure and the system hypertension, which also results in decreased urinary volume.

The histopathological analysis of the renal samples taken 8 weeks after the administration of STZ undoubtedly confirmed the development and the progression of DN in the experimental animals. The histopathological examination, using light microscopy, of renal samples of diabetic rats undoubtedly showed the presence of glomerulopathy characterized by basement membrane thickening, expansion of the mesinginal matrix, and arteriolar hyalinises as well as insudative protein deposits that obstruct some capillaries. Similar but more severe were the histopathological renal changes within the same experimental group 12 weeks after administering STZ. The results of the gloumerosclerotic index, which had deteriorated significantly after 8 weeks and the same was even more distinct at the end of the study, confirm the progression of glomerulopathy. These findings corroborate the findings of Nevin E et al. (2004) [23], who concluded that glomerulopaty actually represents the most significant structural change of DN and is manifested with thickening of the glomerular basal membrane and mesangial expansion which results ina progressive decrease in the glomerular filtration surface.

Moreover, the histopathological examination of the kidneys showed signs of interstitial fibrosis and tubular dilatation, similar to the study of Huang et al. (2001) [24], who reported glomerular hypertrophy, sporadic interstitial fibrosis and tubular atrophy without overly distinct glomerular sclerosis in diabetic rats.

The findings of the histopathological examinations confirmed the renoprotective effects of perindopril. The histopathological examination of the renal samples at 8 and 12 weeks after the beginning of the study undoubtedly showed that perindopril significantly lowers the progression of glomerulopathy and significantly improves the glomerulosclerotic index, as well as the progression of histological abnormalities in the tubular compartment, interstitium and blood vessels of kidneys induced with STZ.

The results of the study of Wolff G et al. (1999) show that renal hypertrophy in Type I diabetic rats is a result of the increased glomerular expression of the inhibitor of cycline-dependan quinasis (p27, K_1P_1) and that ACE inhibitors (enalapril) decrease the glomerular expression of the cycline-dependent quinasis [25]. Probably perindopril, as well, can prevent renal hypertrophy in diabetic kidneys through the same mechanism. Moreover, changes in the glomerular permeability, loss of podocytes (apoptosis) and the albuminuric effect of endogenous atrial natriuretic peptide (ANP) can have a significant role in the pathogenesis of microalbuminury which is antagonised by ACE inhibitors [26, 27]. Thus through antagonising the effects of ANP, the changes in the glomerular permeability and apoptosis perindopril could decelerate the progress of diabetic nephropathy. Antioxidant antiatherosclerotic, antitrophic and other metabolic actions of ACE inhibitors are also important in delaying the progression of DN [26, 28].

Based on the results obtained from this study, it can be concluded that perindopril although not completely but to a great extent ameliorates functional renal disorder and significantly decreases the progression of glomerulopathy and histological abnormalities in the tubular compartment, interstitium and blood vessels of kidneys induced with STZ.

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Резиме

ТРЕТМАН СО ПЕРИНДОПРИЛ КАЈ СТРЕПТОЗОТОЦИН ИНДУЦИРАНА ДИЈАБЕТИЧНА НЕФРОПАТИЈА

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Дијабетичната нефропатија (ДН) е една од најчестите причини за развивање на бубрежна болест во краен стадиум. Ангиотензин конвертирачкиот ензим (АКЕ) претставува сигнификантен ризик фактор за прогресијата на ДН. Познавајќи го механизмот на настанувањето на ДН и знаејќи ја улогата на ангиотензин II во развојот на истата, како посебно интересни лекови се издвојуваат АКЕ инхибиторите. Основната цел на оваа студија беше да се испитаат ефектите од третманот со периндоприл (АКЕ инхибитор) администриран кај стаорци со стрептозоцин (СТЗ) индуцирана дијабетична нефропатија кај кои е евидентирана албуминурија, ренална хипертрофија и лесна гломерулосклероза. ДН беше индуцирана со СТЗ (60 mg/kg ip) еднократна администрација кај нормотензивни Wistar стаорци. Администрацијата на СТЗ предизвика дијабет, со изразити симптоми и знаци за ДН вклучувајќи лоша општа состојба, намалување на телесната тежина, зголемување на тежината на бубрезите, зголемени серумски вредности на уреа и креатинин, придружени со зголемена диуреза, како и изразена албуминурија. Најголем дел од овие симптоми беа манифестни по 4 недели, а уште поизразени по 8 односно 12 недели од администрацијата на СТЗ. Третманот со периндоприл (6 mg/kg т.т.), почнувајќи 4 недели по администрацијата на СТЗ, резултираше со сигнификантно подобрување на сите симптоми и знаци на ДН, намалувајќи ги сигнификантно серумските вредности на уреа и креатинин, албуминуријата и диурезата. Патохистолошките испитувања на бубрезите по 8 и 12 недели од почетокот на студијата покажуваат дека периндоприл значително ја намалува прогресијата на гломерулопатијата, сигнификантно го подобрува гломерулосклеротичниот индекс, како и прогресијата на бубрежните хистолошки нарушувања индуцирани со СТЗ. Третманот со периндоприл ги ублажува нефропатичните промени кај стаорците со ДН индуцирана со СТЗ.

Клучни зборови: дијабетична нефропатија, СТЗ, периндоприл, гломерулосклероза, стаорци.