The effect of aminoguanidine on the kidney of diabetic albino Balb/c mice

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Abstract

The aim of this study is to find out how activated inducible nitric oxide synthase (iNOS) and nitric oxide (NO) affect kidney tissue in streptozotocin (STZ)-induced diabetic mice and whether its influence can be prevented by aminoguanidine (AG), a specific iNOS inhibitor. Twenty-four male mice were divided into four study groups (n=6) receiving a daily dose of 100 mg.kg^{-1} AG for 90 days (Group AG), a single dose of 150 mg.kg^{-1} STZ (Group STZ), a single dose of 150 mg.kg^{-1} STZ followed by daily administration of 100 mg.kg^{-1} AG for 90 days (Group STZ-AG), and intraperitoneally injections of saline only (Group Control) for 90 days. Dispersion of NADPH-diaphorase (NADPH-d) was stronger in the kidney sections of STZ-treated animals compared with the controls. STZ treatment caused disruption of continuity of the brush borders in proximal tubules, glomerular endothelial damage, and considerable renin granules in the juxtaglomerular cells. AG administration following STZ treatment partly prevented histological and cytological changes in kidney cortex, and renin dispersion was similar to that in control animals. We found that increased inducible nitric oxide (iNO) caused kidney tissue degeneration that could be prevented to some extent by AG treatment. There is a possible relationship between increased iNOS and dispersion of renin granules in juxtaglomerular cells in diabetes.

Keywords: Nitric Oxide Synthase (NOS), Kidney, Aminoguanidine, *Diabetes mellitus*
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Diyabetik albino Balb/c farelerde aminoguanidinin böbrek üzerindeki etkisi

Özet

Bu çalışmanın amacı, uyarılabilir nitrik oksit sentaz (iNOS) aktivasyonunun ve nitrik oksidin streptozotosin (STZ) ile uyarılmış diyabetik farelerde böbrek dokusunu nasıl etkilediğini ve etkisinin spesifik bir iNOS inhibitörü olan aminoguanidin (AG) ile önlenip önlenemediğini öğrenmektir. Yirmi dört erkek fare, 90 gün boyunca günlük 100 mg.kg^{-1} AG (AG Grubu), tek doz 150 mg.kg^{-1} STZ (STZ Grubu), tek doz 150 mg.kg^{-1} STZ'yi takiben 90 gün boyunca günlük 100 mg.kg^{-1} AG (STZ-AG Grubu) ve sadece intraperitonal fizyolojik tuzlu su (Kontrol Grubu) alan dört gruba ayrılmıştır. NADPH-diaforez (NADPH-d) dağılımı, STZ uygulanmış hayvanların böbrek kesitlerinde kontrolle karşılaştırıldığında daha fazlaydı. STZ uygulaması proksimal tübüllerde fırça kenarların devamlılığının bozulmasına, glomerulus endotelinde hasara ve jukstaglomerular hücrelerde renin granüllerinin daha fazla olmasına yol açmıştır. STZ uygulamasını takiben verilen AG, böbrek korteksindeki histolojik ve sitolojik değişiklikleri kısmen önlemiştir ve renin dağılımı kontrol hayvanlardakine benzer şekilde olmuştur. Uyarılabilir nitrik oksit (iNO) artışıyla böbrekte meydana gelen bozulmanın AG uygulamasıyla kısmen önlenebildiği bulunmuştur. Diyabette, artan iNOS ile jukstaglomerular hücrelerde renin granülleri dağılımı arasında olası bir ilişki vardır.

Anahtar Kelimeler: Nitrik Oksit Sentaz (NOS), Böbrek, Aminoguanidin, *Diabetes mellitus*

Introduction

Since 1987, nitric oxide (NO) was found to be synthesized in many cells, where it acts as a multifunctional messenger molecule (Bogdan et al. 2000). It is a lipophilic, low molecular weight, fast diffusing gas with a half-life of a few seconds (Ignarro 1991). Having a single unpaired electron, it acts as a free radical, reacting with many substrates including nucleic acids, proteins, low molecular weight thiols and reactive O_2-messengers (Bogdan 2001; Smutzer 2001).

The signal transmission mechanism of NO occurs by linking to iron-containing proteins. Under normal conditions, small amounts of NO are synthesized and released in cells, but if produced in larger amounts, the excess has a negative effect (Schmidt et al. 1993; Thiemermann 1997; Lane and Gross 1999; Bogdan 2001). Thus, NO plays a major role in many pathophysiological changes occurring in diseases such as diabetes, septic shock, hypertension, and atherosclerosis (Marin and Rodrigues-Martinez 1997; Maxwell et al. 1998).

Three isoforms of nitric oxide synthase (NOS) are produced in normal rat kidney. The endothelial NO synthase (eNOS) has been found in kidney arteries, glomerulus and afferent- and efferent arterioles, proximal tubules and collecting ducts (Bachmann et al. 1995; Tojo et al. 1997). Neuronal NO synthase (nNOS) immunoreactivity has been determined in macula densa and in cortical collecting ducts (Terada et al. 1992; Wilcox and Welch 1998) and inducible NO synthase (iNOS) is normally present in the kidney tubules, and in the thicker side of Henle's loop (Furusu et al. 1998).

Under normal conditions NO produced in the kidney controls the glomerular filtration rate (GFR), blood flow, sodium (Na^+) cation transport, and production of enzymes such as renin. NO also plays role in tonus of the glomerular mesangial cells, inhibiting platelet accumulation and adhesion, and preventing excessive venous stenosis, discharging of Na^+ and water (Forstermann et al. 1994; Raij and Baylis 1995).

Renin-producing juxtaglomerular (JG) cells are affected by factors from cells in their vicinity capable of producing NO: smooth muscle cells in the afferent arterioles, endothelial cells, intraglomerular mesangial cells, and macula densa cells (Bachmann et al. 1995). As a result, various experiments were carried out to determine if NO had some influence on renin synthesis and release (Wagner et al. 1998; Kurtz 2011). To this date, this relationship has not been clearly established.

NO is involved in changes of the kidney tissue caused by diabetes, but the role of increased NO production is not completely understood. Studies of Tanaka et al. (1995) point out that NO production may, at least in part, be responsible for the STZ-induced damage of pancreatic beta-cells of the rodents. In early studies on experimental diabetes, increased NO was found to induce hyperfiltration. At the same time it was established that NO played a major role in the increase of renal blood flow and GFR, and that the levels of nitrite/nitrate, which are NO indicators, are higher in the plasma and urinary fluids of diabetic rats in comparison to healthy controls (De Vriese et al. 2001). The eNOS expression in pre-glomerular veins and glomerular endothelial cells is increased in diabetic rats, as are NO, iNOS and messenger ribonucleic acid (mRNA) produced by macrophages (Stevens et al. 1997; De Vriese et al. 2001).

Vein hypertrophy and albumin elevation were decreased in diabetic rats after administration of aminoguanidine (AG), a specific iNOS inhibitor. Other complications in kidneys and peripheral nerves were also improved by administration of AG (Ellis and Good 1991; Soulis et al. 1999; Yavuz et al. 2001; Azal et al. 2002; Atasayar et al. 2009). AG is an inhibitor of advanced glycation end products (AGE) (Rao et al. 2002). It prevents protein cross-linking and is employed as protective agent used in cases of diabetes,

atherosclerosis, renal diseases, and for improving aging factors (Soulis et al. 1997; Brown 1999; Heidland et al. 2001).

In the light of this knowledge we decided to study the histochemistry of NOS dispersion in the kidneys of STZ-induced diabetic rats aiming to define how increased production of NO affects renal tissue, and to establish if there is any relationship between this effect and iNOS in the presence and in the absence of aminoguanidine, a specific iNOS inhibitor. At the same time, we desired to establish if AG prevents *diabetes mellitus*, and if increased levels of NO had any influence on renin-secreting JG cells.

Material and methods

Experimental animals

All experiments were performed at the Istanbul University Experimental Medicine Research Institute following the recommendations of the Experimental Animal Care and Use Commitee. Twenty-four albino balb/c male mice, 2-3 months old and weighing between 25 to 30 g, were selected for the study. They were kept in plastic cages and fed a commercial pellet feed and allowed to drink water *ad libitum*. The animals were divided into four groups, and received the following treatments for 90 days:

1. Group AG (n=6) Received a daily intraperitoneal (i.p.) dose of 100 $mg.kg^{-1}$ AG for 90 days.
2. Group STZ (n=6) Received a single i.p. dose of 150 $mg.kg^{-1}$ STZ at the beginning of the study.
3. Group STZ-AG (n=6) Received a single i.p. dose of 150 $mg.kg^{-1}$ STZ at the beginning of the study followed by daily administration (i.p) of 100 $mg.kg^{-1}$ AG for 90 days.
4. Group Control (n=6) Received i.p. injections of saline instead of the treatment solutions.

The blood glucose levels were measured before and one week after administration of STZ. Mice having blood glucose levels >180 $mg.dl^{-1}$ were accepted as diabetics. At the end of 90 days the animals were anesthetized with chloroform, the kidneys were removed and kidney sections were placed in 10% formalin for light microscopy, 4% paraformaldehyde for histochemical observa-tions and 2% glutaraldehyde for electron microscopy.

Light microscopy

The kidney sections were dehydrated in ethanol and embedded in paraffin. Sections, 4-5 μm thick, were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS) and with the Bowie technique (Pitcock and Hartroft 1958).

PAS-reaction intensity in brush border of proximal tubules was examined light microscopically from 5 different cortical areas in all groups. Additionally, 5 different afferent arterioles of kidney cortex of each animal were evaluated for distribution pattern of renin granules. A semi-quantitative score of PAS staining intensity and distribution of renin granules were graded using a scoring system [0=none; + (less) = 0-25%; ++ (moderate) = 25-50%; +++ (strong) = >50%]. The scoring system was modified according to the method of Fujii et al. (2006).

Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) histochemistry

The kidney tissues were fixed with 4% paraformaldehyde in 0.1 M pH 7.4-phosphate buffer for 2 hours, and then cryoprotected overnight in 20% sucrose in the same buffer at 4 ^{o}C. The next day, 8 μm thick sections were obtained and mounted on slides. The sections were incubated with 1 mM β-NADPH (Sigma), 0.5 mM nitroblue tetrazolium (NBT, also from Sigma) and 2% Triton X-100 in 50 mM pH 8.0 Tris-HCl at 37 ^{o}C for 45 min and then rinsed with 0.1 M phosphate buffer and distilled water. Finally, they were mounted in glycergel. The catalytic activity of NOS was demonstrated by enzymatic reduction of NBT in the presence of NADPH by the NADPH-diaphorase reaction

(Hope et al. 1991). NOS dispersion was evaluated with the same scoring system as PAS staining.

Electron microscopy

Kidney sections were fixed in 2% glutaraldehyde prepared with pH 7.4 Sorensen phosphate buffer overnight at 4°C and 1% osmium (VIII) oxide for one hour. After washing in phosphate buffer for two hours the samples were dehydrated with alcohol series and propylene oxide. Embedded in Epon 812, sections of 30-50 nm thickness were stained with 5% uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) and then examined on a Zeiss EM 9S2 electron microscope.

Statistical Analysis

The blood glucose results were expressed as mean ± SE. The statistical significance was established by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using GraphPad Prism (GraphPad Prism Version 5 Software Program, San Diego, CA). A p value $P < 0.05$ was considered statistically significant.

Results

Initial blood glucose levels of all groups were in the range of 100±10 $mg.dl^{-1}$ to 104±4 $mg.dl^{-1}$. Blood glucose levels of STZ injected animals increased during first week. STZ and STZ+AG groups showed increased values between 300±25 $mg.dl^{-1}$ and 312±26 $mg.dl^{-1}$ at the end of 90 days (Table 1).

Table 1. The initial and final glucose levels of all groups.

Groups	Initial blood glucose levels (before STZ application) (mg/dl)	Final blood glucose levels (90 days after STZ application) (mg/dl)
Control (n=6)	101 ± 6	100 ± 6
STZ (n=6)	100 ± 10	300 ± 25***
STZ+AG (n=6)	102 ± 7	312 ± 26***
AG (n=6)	104 ± 4	103 ± 3

***: p <0.001 vs the control group and AG group.

Light microscopy

The appearance of kidney tissue from the healthy controls was shown in Fig. 1A. However, the kidneys from the STZ-treated mice showed severe changes, Fig. 1B. The proximal tubule brush border lost its continuity, the glomeruli were damaged, especially glomerular endothelial damage was marked, and numerous erythrocytes were present in the peritubular area and capsular area. The mice in the STZ-AG group showed a tighter brush border, but some areas of their kidneys were equally damaged as in the STZ group, Fig. 1C. In the AG group a healthy brush border was seen in the proximal tubule. The glomeruli were similar to controls. Capsular areas were narrower than those of the STZ group and there were no signs of hemorrhagic areas, Fig. 1D. The mice in the STZ group showed severe vessel damages, compared to all other groups. The parietal layer of Bowman's capsule was also damaged and many erythrocytes were seen in the peritubular area and capsular area, Figs. 2A and 2B.

Damage of the brush borders and basal membrane were also detected by the periodic acid-Schiff (PAS) reaction. STZ treatment caused disruption of continuity of the brush borders in proximal tubules, Fig. 3B. In the STZ-AG group the intensity of PAS was close to controls, Figs. 3C and 3A. Application of AG alone resulted in increased PAS in the brush borders and basal membrane, which were thicker when compared with all other groups, Fig. 3D. Intensity of PAS reaction in brush borders were shown in Table 2.

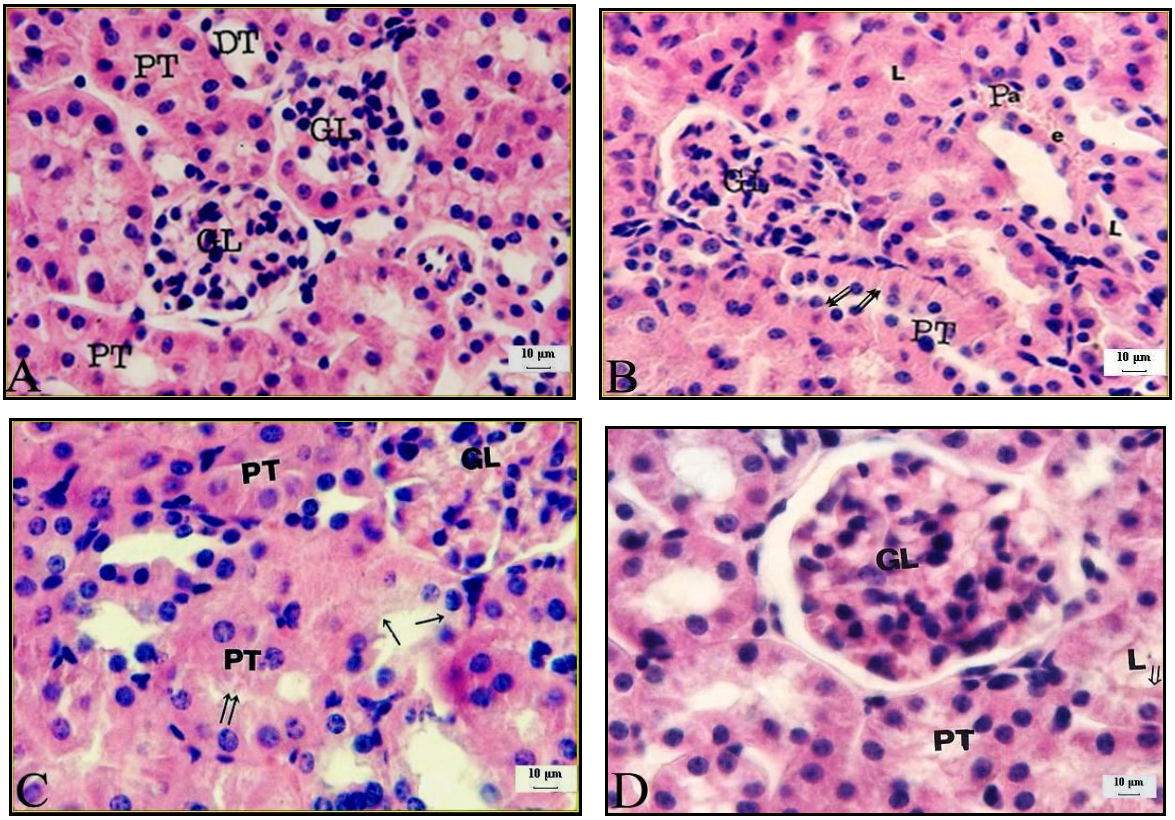

Figure 1A-D. Histological sections from cortex of kidney tissue of the experimental groups. A) Control group, B) STZ-group, C) STZ-AG-group, D) AG-group; glomerulus (GL), proximal tubules (PT), distal tubules (DT), intact brush border (↑), damaged brush border (↑↑), tubular lumen (L), peritubular area (Pa), erythrocyte (e). HE, scale bar: 10 µm.

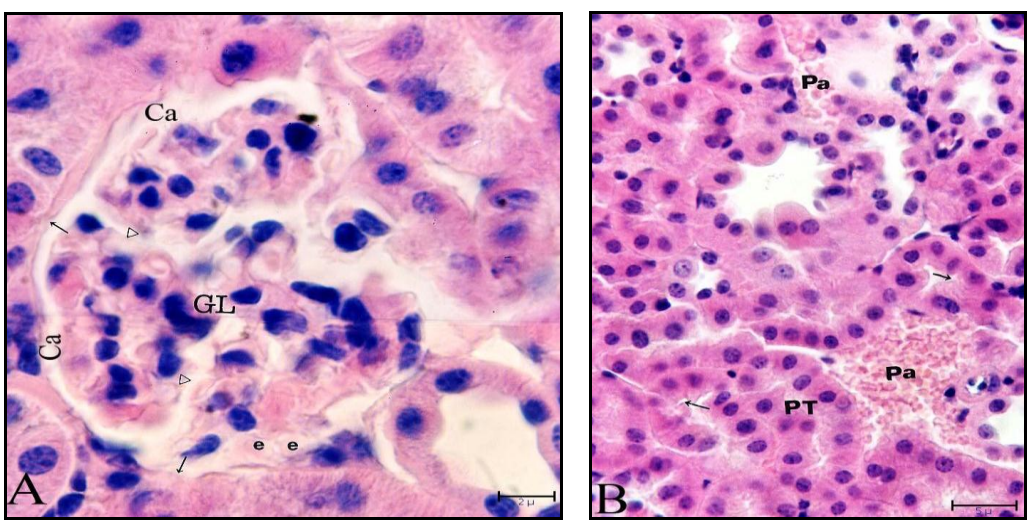

Figure 2A-B. Histological sections from cortex of kidney tissue of the STZ-group. A) Glomerulus (GL), irregular capillaries (Δ), capsular area (Ca), damaged parietal (↑) layer of Bowman's capsule, erythrocytes (e) in Bowman's capsule. HE, scale bar: 2 µm. B) Proximal tubules (PT), damaged brush border (↑), peritubular area (Pa). HE, scale bar: 5 µm.

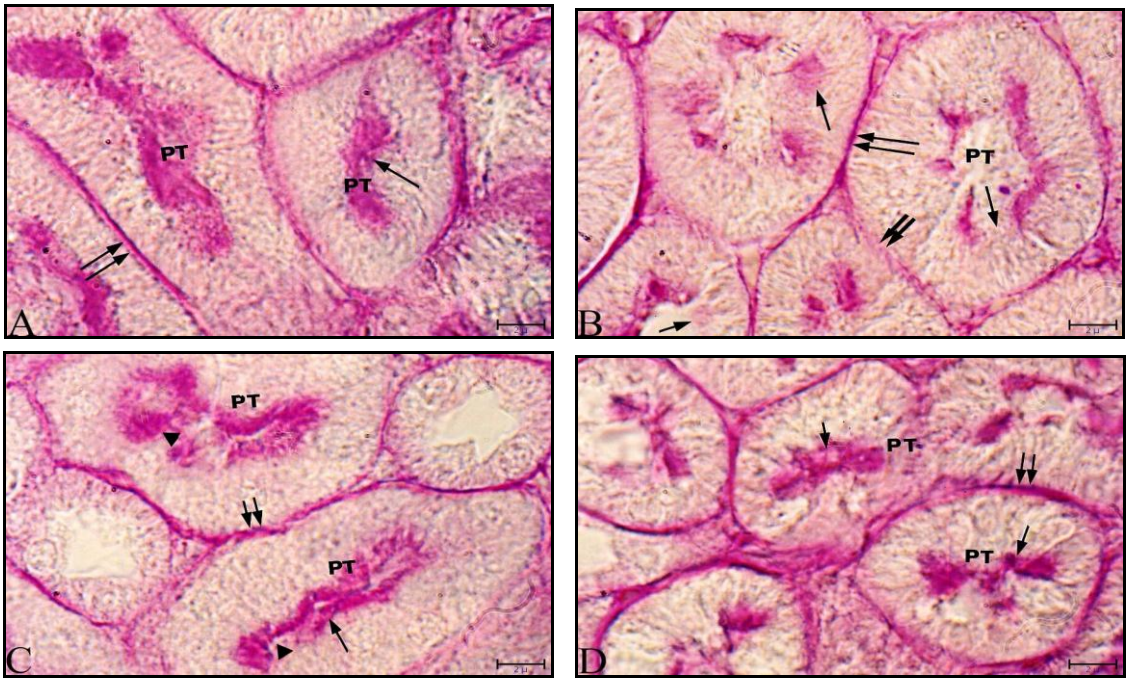

Figure 3A-D. Histological sections from cortex of kidney tissue of the experimental groups. A) PAS reaction in brush borders (↑) of proximal tubules (PT), and basal membranes (↑↑) in the cortex of kidney of control group animals. B) Proximal tubules (PT) with damaged brush borders (↑) which lost their continuity and indicated less PAS reaction in comparison with controls, and damaged basal membranes (↑↑) in the cortex of kidney of STZ-group animals. C) Proximal tubule (PT) brush borders (↑) which indicated PAS reaction similar to controls, irregular brush borders (▲) similar to those in STZ-group, and basal membranes (↑↑) in the cortex of kidney of STZ-AG-group animals. D) Proximal tubule (PT) brush borders (↑) which indicated stronger PAS reaction when compared to controls, and basal membranes (↑↑) in the cortex of kidney of AG-group animals. PAS, scale bar: 2 μm.

Table 2. Intensity of periodic acid-Schiff (PAS) reaction in brush border of proximal tubules.

	Control	STZ	STZ-AG	AG
Intensity of PAS reaction in brush border	++	+	++	+++

[0= none; + (less)= 0-25%; ++ (moderate)= 25-50%; +++ (strong)= >50%]

As to distribution of NOS in the cortex which was examined by NADPH-d histochemistry, STZ mice showed stronger NOS immunoreactivity in the proximal tubule than controls, Figs. 4B and 4A. The macula densa indicated also stronger NOS immunoreactivity, comparing with all other groups, Fig. 4B. STZ-AG group displayed features similar to controls, with stronger immunoreactivity than that in the AG group, Figs. 4C and 4D; Table 3. There was no NOS immunoreactivity in the glomeruli of all groups.

The distribution of renin granules in the JG cells also showed differences between the experimental groups. Granules in the STZ group were denser and numerous, in comparison with all other groups except the AG group, Figs. 5B and 5D. The controls and the STZ-AG animals showed almost the same distribution, Figs. 5A and 5C; Table 4.

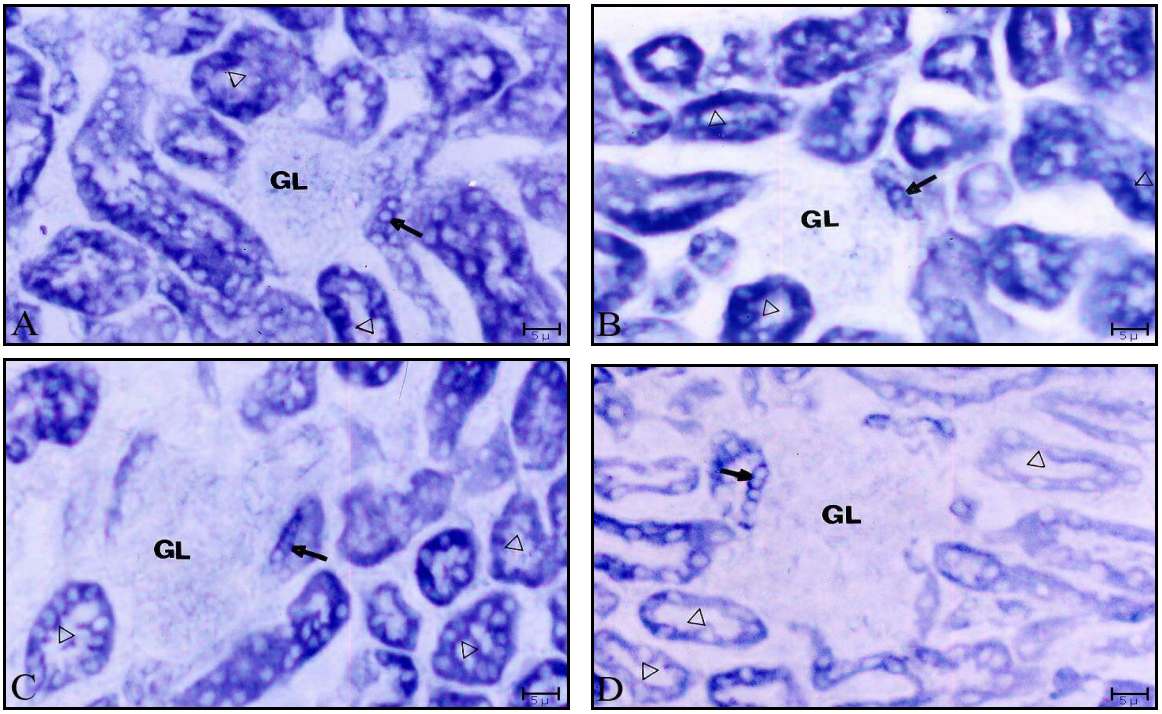

Figure 4A-D. NADPH-d reaction in the cortex of kidney sections of experimental groups. A) Control group, B) STZ-group, C) STZ-AG-group, D) AG-group; glomerulus (GL), cortical tubules (Δ), NADPH-d reaction in macula densa (↑). Scale bar: 5 µm.

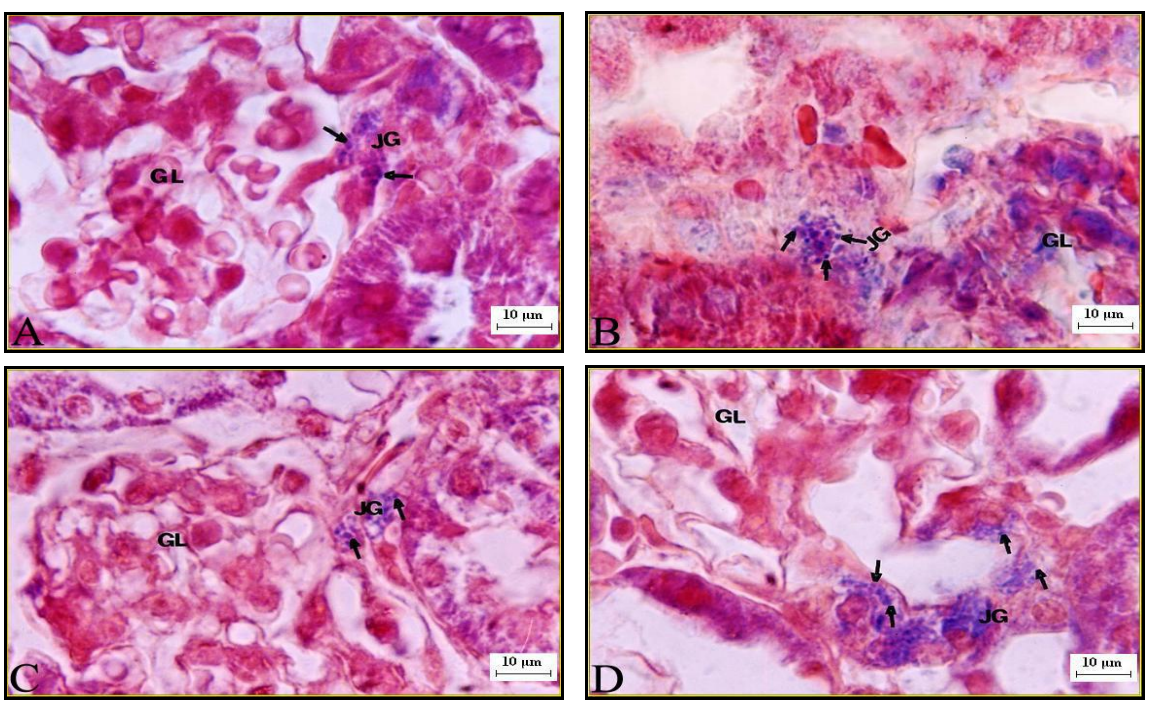

Figure 5A-D. Histological sections in JG cells of kidney tissue of the experimental groups. A) Control group, B) STZ-group, C) STZ-AG-group, D) AG-group; glomerulus (GL), renin granules (↑), JG cells (JG). Bowie technique, scale bar: 10 µm.

Table 3. Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) reaction of cortical tubules.

	Control	**STZ**	**STZ-AG**	**AG**
NADPH-d reaction of cortical tubules	++	+++	++	+

[0= none; + (less)= 0-25%; ++ (moderate)= 25-50%; +++ (strong)= >50%]

Table 4. Distribution of renin granules in juxtaglomerular cells

	Control	STZ	STZ-AG	AG
Distribution of renin granules	+	++	+	++

[0= none; + (less)= 0-25%; ++ (moderate)= 25-50%; +++ (strong)= >50%]

Electron microscopy

Intact glomeruli with normal capillary endothelial cells and podocytes were observed in controls, Fig. 6A. Proximal tubule had also unaffected membranes and organelles, Fig. 7A. However, enlarged lumen and damaged endothelial cells in the glomeruli were seen in the STZ diabetic mice, Fig. 6B. Moreover, glomerular basement membrane thickening, podocytes and fenestration changing were marked, so that podocytes showed foot process effacement as seen in Fig. 6B. There were differences in the mitochondrial distribution of proximal tubule between controls and diabetics. Many autophagic vacuoles were seen along with enlargement of the peritubular areas (Fig. 7B). Many blood cells were present in this area of the diabetic cortex. Damage was partly prevented by AG in the diabetic mice. The capillary lumens were narrower than those of the STZ group. Podocytes were similar to controls. Damage to endothelial cells was not as severe as in the STZ group, Fig. 6C. The microvilli of the proximal tubules were similar to controls. Enlarged granular endoplasmic reticulum (GER) was seen both in STZ and STZ-AG groups, Fig. 7C. In the proximal tubules of the STZ-AG group GER, polisomes and mitochondria were increased when compared to STZ mice. Application of AG did not completely prevent endothelial damage. There were still blood cells in the peritubular area. Basal lamina was thick in the AG, a finding which was not observed in other groups, Fig. 6D. All other ultrastructural features were the same as the controls, Fig. 7D.

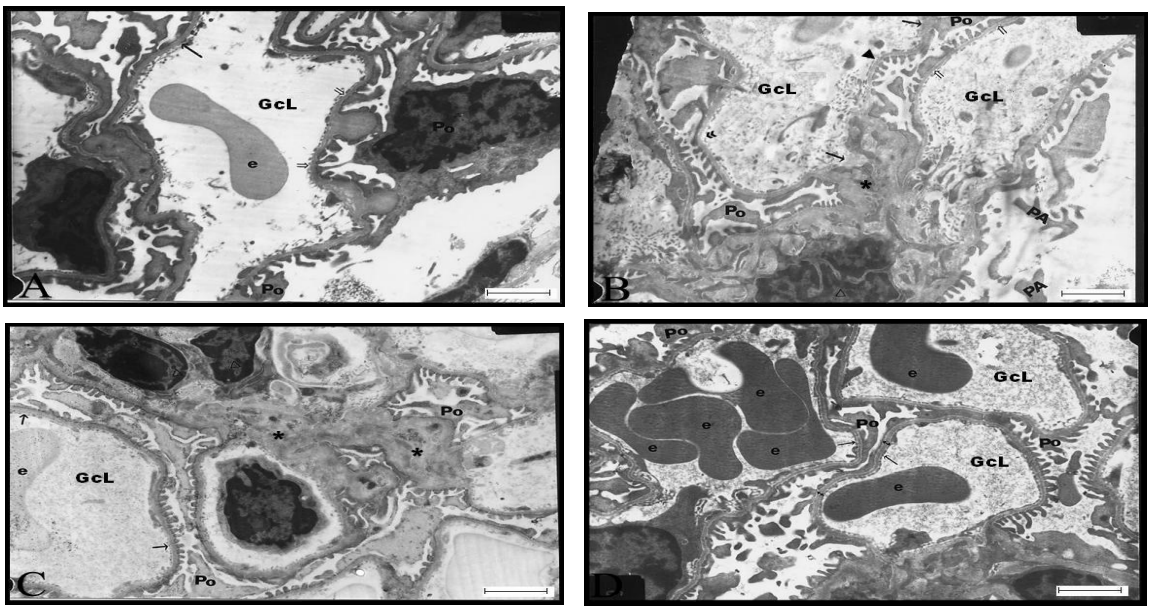

Figure 6A-D. Electron microscopic sections from glomerulus of kidney tissue of the experimental groups. A) Control group, B) STZ-group, C) STZ-AG-group, D) AG-group; glomerular endothelium (↑), glomerular lumen (GcL), erythrocytes (e), podocytes (Po), podocyte foots processes (PA), fenestra (⇑), mesangial cell nucleus (Δ), basal lamina (↕). Glomerular basement membrane thickening (*), damaged endothelial cells (▲), and changes in fenestration («) were marked in STZ-group. Scale bar: 1 μm.

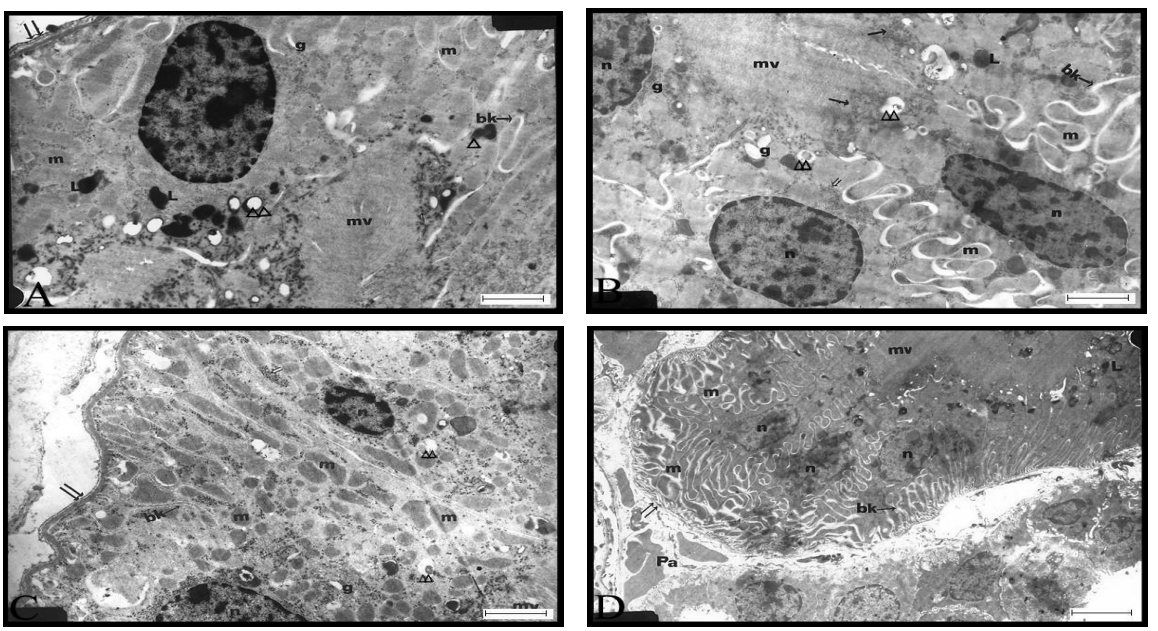

Figure 7A-D. Electron microscopic sections from proximal tubules of kidney tissue of the experimental groups. A) Control group B) STZ-group, C) STZ-AG-group, D) AG-group; nucleus (n), mitochondrium (m), lysosome (L), microvillus (mv), autophagic vacuole (ΔΔ), granular endoplasmic reticulum (g), peroxisome (Δ), basal lamina (↑↑), polisome (⇑), basal foldings (bk→), peritubular area (Pa). Scale bar: 1 μm.

Discussion

It is known that after STZ administration to rats and mice, there is an increase in nitrite and nitrate, the end products of NO metabolism (Sugimoto et al. 1999). Administration of STZ also causes leukocyte accumulation, endothelial adhesions in the venules, capillary obstruction, degeneration of the endothelial layer, and migration of the leukocytes to the intercellular area and, related to all these changes, hemorrhage (Mauer et al. 1984).

In diabetes induced by a single high dose of STZ in mice, cytokines stimulate macrophage formation and increments of iNO synthesis (Stevens et al. 1997). It also results in irregular shape of glomeruli, overextension, and obstruction of the capillaries and veins, and in leukocyte and erythrocyte accumulation. As a result, the blood pressure increases and endothelial NO (eNO) then softens the arterioles' smooth muscles through cyclic guanosine monophosphate (cGMP) (Ignarro, 1996). This physiologically affects the glomerular filtration rate and material transfer into Bowman's space (Veelken et al. 2000).

Electron microscopy of the kidney tissues of diabetic mice showed degeneration of the basal laminae and foldings of the proximal tubule, just as seen in the PAS stained sections. The basal foldings were expanded and mitochondria were damaged. Enlarged basal foldings prove that the ion transport between cells and peritubular areas is highly affected. Degeneration of mitochondria results in reduced ATP synthesis, necessary for cellular energy. The endothelial layer and basal laminae were also damaged, and lost their durability, their capillary lumens were excessively enlarged, and podocyte foot processes were irregular. The degeneration seen in the edge of the proximal tubular brush borders and basal foldings implies that the tubules lost their resorptive functions. The increment in autophagic vacualization and presence of lysosomes in the proximal tubule cells show that those cells and tubules were degenerated.

The PAS reactions in the STZ-AG group indicate basal laminas and brush borders that were similar to controls, implying that quenching of iNO synthesis partially prevents the physiological and pathological consequences of STZ and points to the significance of iNO production in diabetes. In the electron micrographs of this group, increased polyribosomes and GER vesicles in the proximal tubule cells indicate that the protein synthesis elevated within those cells. Electron microscopy examination of the kidney cortex reveals that the microvilli and basal foldings are similar to the controls, again suggesting that AG partially prevents the cellular damage caused by STZ and iNO. The extensive appearance of the mitochondria similar to that in the diabetic group may be related to the fact that the cell tries to ensure necessary ATP energy supply to prevent the damage.

Within the AG group, the proximal tubule brush borders are more defined and regular, similar to those in the controls, suggesting that AG prevents NO synthesis in these tubules by increasing the synthesis of some cell surface membranes. The mice of AG group possess regular glomerular and capillary structures and low leukocyte levels in the peritubular area, indicating that long-term AG does not have any toxic effect on kidney tissue. To date, there are no studies showing that a single AG application has any cellular effect. There are, however, studies in which AG may be used against diabetes and as an anti-aging antioxidant agent (Rao et al. 2002; Azal et al. 2002; Atasayar et al. 2009). For this reason, the cellular effect of the long term AG application remains under question.

When NOS dispersion is examined, NADPH-d reaction was stronger in the STZ group, showing that there was an increase in NO. Conversely, in the STZ-AG group the reaction is decreased in proximal tubules, confirming that elevated NO plays an important role in cellular changes in diabetes. The occurrence of NADPH-d reaction in the controls shows the presence of NOS in normal kidney tissue. In the AG group, reaction after iNOS inhibition suggests that not only iNOS but other NOS enzymes are found under normal

conditions and that all contribute to this reaction as cofactors.

The renin-angiotensin system plays a major role for normal function of kidneys. Electron microscopy demonstrates that STZ and iNO are highly effective in inducing changes in kidneys. The glomerular filtration rate is controlled by changes in the diameter of afferent and efferent arterioles. In pathological situations, however, the blood pressure changes because of an increase in NO, thus affecting kidney cell functions (Wagner et al. 1998; Birrell et al. 2002). In this regard, the production of renin by JG cells needs to be controlled. Any decrease in blood pressure triggers the synthesis and release of renin (Wagner et al. 1998; Bucher et al. 2001). Stimulation of muscle and mesangial cells results in additional renin synthesis in order to maintain a normal glomerulus pressure (Skøtt and Jensen 2000).

The fact that, compared to controls, the number of renin granules in JG cells on the afferent arteriole walls is greater in the STZ but not in the STZ-AG group suggests stimulation of these cells. It is interesting to note that long term AG application increases renin granules, implying that NO has a definite role in renin synthesis and secretion.

In the sections where NADPH-d reaction was used to determine whether there is a connection between NO synthesized in macula densa cells and JG cells, NOS dispersion in macula densa cells was more significant in the STZ group in contrast to controls and other groups, leading us to think that NO production was intensified within the macula densa cells, thus stimulating the elevation of renin in JG cells.

In conclusion, we have demonstrated that NOS isoforms and iNOS are increased in diabetes, causing significant damage to the kidney cortex. Application of AG as iNOS inhibitor may partially prevent this damage. In addition, renin granules within JG increased, returning to normal as soon as iNOS production is quenched.

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References

Atasayar S., Gürer-Orhan H., Orhan H., Gürel B., Girgin G. and Ozgüneş H. (2009) Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in rats. *Exp. Toxicol. Pathol.*, 61 (1): 23-32.

Azal O., Yönem A., Güler S., Cakir B., Baydar A., Corakçi A. and Kutlu M. (2002) Effects of aminoguanidine and tolrestat on the development of ocular and renal structural changes in experimental diabetic rats. *Diabetes Obes. Metab.*, 4 (1): 75-79.

Bachmann S., Bosse H.M. and Mundel P. (1995) Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. *Am. J. Physiol*., 268: F885-F898.

Birrell A.M., Heffernan S.J., Kirwan P., McLennan S., Gillin A.G. and Yue D.K. (2002) The effects of aminoguanidine on renal changes in a baboon model of Type 1 diabetes. *J. Diabetes Complications*, 16 (4): 301-309.

Bogdan C., Rollinghoff M. and Diefenbach, A. (2000) The role of nitric oxide in innate immunity. *Immunol. Rev*., 173: 17-26.

Bogdan C. (2001) Nitric oxide and the immune response. *Nat. Immunol*., 2 (10): 907-916.

Brown G.C. (1999) Nitric oxide and mitochondrial respiration. *Biochim. Biophys. Acta*, 1411 (2-3): 351-369.

Bucher M., Ittner K.P., Hobbhahn J., Taeger K. and Kurtz A. (2001) Downregulation of angiotensin II type 1 receptors during sepsis. *Hypertension*, 38 (2): 177-182.

De Vriese A.S., Stoenoiu M.S., Elger M., Devuyst O., Vanholder R., Kriz W. and Lameire N.H. (2001) Diabetes-induced microvascular dysfunction in the hydronephrotic kidney: Role of nitric oxide. *Kidney Int*., 60 (1): 202-210.

Ellis E.N. and Good B.H. (1991) Prevention of glomerular basement membrane thickening by aminoguanidine in experimental *diabetes mellitus. Metabolism*, 40(10): 1016-1019.

Forstermann U., Closs E.L., Pollock J.S., Nakane M., Schwarz P., Gath I. and Kleinert H. (1994) Nitric oxide synthase isozymes. Characterization,

purification, molecular cloning, and functions. *Hypertension*, 23: 1121-1131.

Fujii H., Nakamura S., Kuroda S., Yoshihara F., Nakahama H., Inenaga T., Ueda-Ishibashi H., Yutani C. and Kawano Y. (2006) Relationship between renal artery stenosis and intrarenal damage in autopsy subjects with stroke. *Nephrol Dial Transplant*., 21 (1): 113-119.

Furusu A., Miyazaki M., Abe K., Tsukasaki S., Shioshita K., Sasaki O., Miyazaki K., Ozono Y., Koji T., Harada T., Sakai H. and Kohno S. (1998) Expression of endothelial and inducible nitric oxide synthase in human glomerulonephritis. *Kidney Int.*, 53 (6): 1760-1768.

Heidland A., Sebekova K. and Schinzel R. (2001) Advanced glycation end products and the progressive course of renal disease. *Am. J. Kidney Dis*., 38 (1): 100-106.

Hope B.T., Michael G.J., Knigge K.M. and Vincent S.R. (1991) Neuronal NADPH diaphorase is a nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A*., 88: 2811-2814.

Ignarro L.J. (1991) Signal transduction mechanisms involving nitric oxide. *Biochem. Pharmacol*., 15; 41 (4): 485-490.

Ignarro L.J. (1996) Physiology and pathophysiology of nitric oxide. *Kidney Int. Suppl*., 55: 2-5.

Kurtz A. (2011) Renin release: sites, mechanisms, and control. Annu. Rev. Physiol., 17(73): 377-399.

Lane P. and Gross S.S. (1999). Cell signaling by nitric oxide. *Semin. Nephrol*., 19: 215-229.

Marin J. and Rodrigues-Martinez M.A. (1997) Role of vascular nitric oxide in physiological and pathological conditions. *Pharmacology and Therapeutics*, 75: 111-134.

Mauer S., Steffes M., Ellis E., Sutherland D., Brown D. and Goetz F. (1984) Structural-functional relationships in diabetic nephropathy. *J. Clin. Invest*., 74: 1143-1155.

Maxwell A.J., Tsao P.S. and Cooke J.P. (1998) Modulation of the nitric oxide synthase pathway in atherosclerosis. *Exp. Physiol*., 83(5): 573-584.

Pitcock J.A. and Hartroft P.M. (1958) The juxtaglomerular cells in man and their relationship to the level of plasma sodium and to the zona glomerulosa of the adrenal cortex. *Am. J. Path*., 34 (5): 863-864.

Rao V.S., Santos F.A., Silva R.M. and Teixiera M.G. (2002) Effects of nitric oxide synthase inhibitors and melatonin on the hyperglycemic response to streptozotocin in rats. *Vascul. Pharmacol*,. 2002 Mar;38(3): 127-130.

Raij L. and Baylis C. (1995) Glomerular actions of nitric oxide. *Kidney Int*., 48(1): 20-32.

Reynolds E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol*., 17: 208-212.

Schmidt H.H., Lohmann S.M. and Walter U. (1993) The nitric oxide and cGMP signal transduction system: Regulation and mechanism of action. *Biochem. Biophys. Acta*, 1178: 153-175.

Skøtt O. and Jensen B.L. (2000) Cellular and renal control of renin secretion. Odense, Southern Denmark University. http://www.uninet.edu/cin2000/conferences/OleS/OleS.html

Smutzer G. (2001) Research tools for nitric oxide: A wide variety of reagent are available for nitric oxide research. *The Scientist*, 15 (6): 23-29.

Soulis T., Cooper M.E., Sastra S., Thallas V., Panagiotopoulos S., Bjerrum O.J. and Jerums G. (1997) Relative contributions of advanced glycation and nitric oxide synthase inhibition to aminoguanidine-mediated renoprotection in diabetic rats. *Diabetologia*, 40(10): 1141-1151.

Soulis T., Sastra S., Thalla V., Mortensen S.B., Wilken M., Clausen J.T., Bjerrum O.J., Petersen H., Lau J., Jerums G., Boel E. and Cooper M.E. (1999) A novel inhibitor of advanced glycation end-product formation inhibits mesenteric vascular hypertrophy in experimental diabetes. *Diabetologia*, 42(4): 472-479.

Stevens R.B., Sutherland D.E., Ansite J.D., Saxena M., Rossini T.J., Levay-Young B.K., Hering B.J. and Mills C.D. (1997) Insulin down-regulates the inducible nitric oxide synthase pathway: Nitric oxide as cause and effect of diabetes? *J. Immunol*., 1; 159 (112): 5329-5335.

Sugimoto H., Shikata K., Wada J., Horiuchi S. and Makino H. (1999) Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: aminoguanidine ameliorates the overexpression of tumour necrosis factor-alpha and inducible nitric oxide synthase in diabetic rat glomeruli. *Diabetologia*, 42(7): 878-886.

Tanaka Y., Shimizu H., Sato N., Mori M. and Shimomura Y. (1995) Involvement of spontaneous nitric oxide production in the diabetogenic action of streptozotocin. *Pharmacology*, 50(2): 69-73.

Terada Y., Tomita K., Nonoguchi H. and Marumo F. (1992) Polymerase chain reaction localization of

constitutive nitric oxide synthase and soluble guanylate cyclase messenger RNAs in microdissected rat nephron segments. *J. Clin. Invest*., 90: 665-669.

Thiemermann C. (1997) Nitric oxide and septic shock. *Gen Pharmacol*., 29(2): 159-166.

Tojo A., Welch W.J., Bremer V., Kimoto M., Kimura K., Omata M., Ogawa T., Vallance P. and Wilcox C.S. (1997) Colocalization of demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. *Kidney Int*., 52(6): 1593-1601.

Veelken R., Hilgers K.F., Hartner A., Haas A., Böhmer K.P. and Sterzel B. (2000) Nitric oxide synthase isoforms and glomerular hyperfiltration in early diabetic nephropathy. *J. Am. Soc. Nephrol*., 11: 71-79.

Wagner C., Jensen B.L., Kramer B.K. and Kurtz A. (1998) Control of the renin system by local factors. *Kidney Int*., 67: 78-83.

Watson M.L. (1958) Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol*., 4: 475-478.

Wilcox C.S. and Welch W.J. (1998) Macula densa nitric oxide synthase: Expression, regulation, and function. *Kidney Int*., 54 (67): 53-57.

Yavuz D.G., Ersöz H.O., Tuncel M., Sargon M.F., Küçükkaya B., Ahiskali R. and Akalin S. (2001) Effects of aminoguanidine on glomerular basement membrane thickness and anionic charge in a diabetic rat model. *Int. J. Exp. Diabetes Res.*, 2 (3): 225-232.