Abstract. – Objectives: Diabetes mellitus (DM) causes organ dysfunction and increases the sensitivity of organs to damages. To test this hypothesis, we used renal ischemia/reperfusion (I/R) experiment to evaluate the renoprotective activity of telmisartan versus pioglitazone on I/R induced renal damage in diabetic rats.

Materials and Methods: Renal I/R was performed in both normal and diabetic rats. The protocol comprised ischemia for 45 minutes followed by the reperfusion for 24 hours and a treatment period of two weeks before induction of ischemia.

Results: Renal I/R in both control and diabetic rats induced marked renal dysfunction associated with a significant increase in the arterial pressure, tumor necrosis factor alpha (TNF-α) levels, and the malondialdehyde formation (MDA). The activities of the anti-oxidant enzymes such as reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were found to be decreased significantly compared to control rats. Diabetic animals that underwent renal I/R exhibited a significant increase in all the studied parameters with a reduction in the anti-oxidant enzymes as compared to non-diabetic rats. Histopathological studies confirm these results. Treatment with pioglitazone or telmisartan demonstrated a significant improvement in the reperfusion-induced renal injury in comparison with diabetic I/R group, without difference between the two treated groups. Therefore, the treatment with pioglitazone or telmisartan have the same corrective effect.

Conclusions: Type 2 diabetes had exaggerated renal I/R injury in STZ-NAD induced diabetes. Telmisartan treatment is equieffective as pioglitazone in attenuating acute I/R-induced renal injury in diabetic rats by a modification in the oxidative stress and the inflammation.

Key Words: Renal ischemia reperfusion, Type 2 diabetes, Oxidative stress, Pioglitazone, Telmisartan.

Introduction

Ischemic injury to brain, heart, and kidney is associated with high morbidity and mortality. Improving the ability of these organs to tolerate ischemic injury would have important implications. Renal ischemia/reperfusion (I/R) injury is encountered in many clinical situations such as, kidney transplantation, partial nephrectomy, renal artery, angioplasty, aortic aneurysm surgery, and elective urological operations. In these conditions, I/R injury initiates a complex and interrelated sequence of events, resulting in injury to and the eventual death of renal cells during the reperfusion period.

Diabetes mellitus (DM), causes organ dysfunction and increases the sensitivity of organs to damages. Moreover, ischemic insults are often recurrent in diabetic patients. In the setting of loss of renal blood flow autoregulation that characterizes the post ischemic kidney, renal I/R injury is a major cause of acute renal failure (ARF). Diabetic patients may need renal transplantation in their later life due to diabetic nephropathy and I/R injury is one of the dangerous complications of this procedure.

Several factors have been implicated in the patho-physiological changes occurring while renal I/R injury including vascular or microvascular injury, endothelial dysfunction, accelerated cell necrosis, granulocyte activation, and modulation of nitric oxide/angiotensin II axis.

Angiotensin II is an important mediator in kidney injury. Accumulating evidence suggests that angiotensin II stimulates intracellular formation of reactive oxygen species (ROS) such as the superoxide anion and hydrogen peroxide, that are capable of reacting with lipids leading to lipid peroxidation of biological membranes and ultimately development of cell death.
Thiazolidinediones (TZDs, i.e. pioglitazone), which are synthetic PPAR-γ (peroxisome proliferator activator receptor-γ) agonists, act as insulin sensitizers and are used in the treatment of type 2 DM. In the last few years, it has become evident that the therapeutic effects of PPAR-γ ligands reach far beyond their use as insulin sensitizers. Recently, PPAR-γ has been implicated as a regulator of cellular inflammatory and ischemic responses. However, clinical use of TZDs is significantly limited by the occurrence of fluid retention, hemodilution, and heart failure in up to 15% of patients.

Recently, it has been demonstrated that telmisartan, a structurally unique angiotensin II receptor antagonist, approved for the treatment of hypertension, is also a partial agonist of PPAR. Whereas full agonists of PPAR, such as rosiglitazone and pioglitazone, promote weight gain while altering fat distribution and adipocyte differentiation, partial agonists (mixed agonists/antagonists) of PPAR may have the capacity to retard weight gain while promoting adipocyte differentiation. PPAR-γ has been assumed to be one of the targets for the metabolic effects of telmisartan, which is structurally similar to a PPAR-agonist.

So, the present study was conducted to study the protective effect of telmisartan on I/R induced renal injury in diabetic rats in comparison with the protective effect afforded by pioglitazone.

Materials and Methods

Experimental Animals

Forty eight (n=48) adult albino rats weighing 250 ± 10 g were used in this study. They were purchased from the Egyptian Organization for Biological Products and Vaccines (Egypt), and allowed free access to food and water ad libitum. They were kept under constant conditions with 12/12 h light/dark cycles and left for acclimatization for one week before the start of the study. The care and handling of the animals were in accordance with the guidelines of the National Institutes of Health (NIH).

Drugs

Pioglitazone HCl (Sigma Chemical Company, Cairo, Egypt) and Nicotinamide “NIC” (Sigma Chemical Company, Cairo, Egypt) were used. Pioglitazone and telmisartan were given orally once a day by gastric tube at doses of 10 mg/kg and 10 mg/kg respectively. Both pioglitazone and telmisartan were suspended in distilled water and given orally for diabetic rats for two weeks.

Experimental Induction of Type 2 Diabetes in Rats

T2 DM in rats was induced by the administration of nicotinamide (NIC) (230 mg/kg, i.p.) 15 min prior to a single intraperitoneal (i.p) injection of streptozotocin (50 mg/kg, STZ) dissolved in di-sodium citrate buffer (pH 4.5) in a dose volume of 1 ml/kg body weight. After one week following STZ and NIC administration, blood was collected from tail vein after overnight fasting rats for 16 h, and serum samples were analyzed for blood glucose. Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study.

Experimental Protocol

All rats were subjected to right nephrectomy; then, they were divided into six groups each consisting of eight animals:

Group 1: Sham operated control group: non diabetic rats were submitted to right nephrectomy and were used as normal control group.

Group 2: Diabetic sham operated control group: diabetic rats were submitted to right nephrectomy and were used as diabetic control group.

Group 3: I/R control group: After right nephrectomy, non diabetic rats received distilled water orally daily for two weeks; then, they were subjected to I/R.

Group 4: Diabetic I/R control group: After right nephrectomy, diabetic rats received distilled water orally daily for two weeks; then, they were subjected to I/R.

Group 5: Diabetic I/R + pioglitazone group: After right nephrectomy, diabetic rats received pioglitazone orally daily for two weeks; then, they were subjected to I/R.

Group 6: Diabetic I/R + telmisartan group: After right nephrectomy, diabetic rats received telmisartan orally daily for two weeks; then, they were subjected to I/R.

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Animal Model of Renal I/R for Both Normal and Diabetic Rats

Renal I/R was performed to both normal and diabetic animals in all groups except the two sham groups. Animals were given water but no food for 24 hours before the experiment. Rats were anaesthetized with intraperitoneal injection of urethane in a dose of 1.25 mg/kg. The renal I/R protocol was performed according to Kakadiya et al. Right nephrectomy was performed through a right flank incision. After right nephrectomy, several treatments were given as mentioned previously for two weeks. Then, ischemia was produced in the left kidney by performing left flank incision and dissecting the renal pedicle so as to expose the renal vessels. A thick cotton thread was passed over the renal vascular pedicle to induce ischemia. Both ends of the thread were exteriorized through the back of the rat and tied tightly above a length of latex tubing on the external side of the skin. Ischemia was confirmed visually by blanching of the kidney. The renal pedicle was released 45 minutes after occlusion by cutting the thread and pulling it out, and the wound was closed with 3-0 silk suture followed by 24 hours of reperfusion. Animals were allowed to recover from anesthesia without further interventions (Figure 1).

At the end urine samples were collected during the period of I/R using the metabolic cadges. Rats from each group were anaesthetized with (i.p.) urethane 1.25 mg/kg. Arterial pressure changes were measured through cannulation of the carotid artery. Blood samples were collected via the tail veins; then, rats were sacrificed.

Figure 1. Scheme showing experimental protocols employed. Each protocol comprised of the following phases: ischemia, 45 minutes; reperfusion, 24 hours; treatment period, 14 days before induction of ischemia.
**Measurement of Renal Function**
Renal dysfunction was evaluated by measuring serum levels of blood urea nitrogen and creatinine by standard urease assays and picric acid reactions by colorimetric (Boehringer, Ingelheim, Germany) methods\(^\text{18}\), using Bioclin kit (Santa Coloma, Spain).

**Determination of Blood Glucose Level**
Serum glucose level was determined enzymatically according to the principle of\(^\text{19}\) using Spin-react diagnostics Kits (Santa Coloma, Spain).

**Evaluation of TNF-\(\alpha\) in Kidney**
Kidneys were homogenized using 0.1 M phosphate buffer (pH 7.4) containing 0.05\% (wt/vol) sodium azide at 4\(^\circ\)C. Homogenates were sonicated SONICOR (Boehringer, Ingelheim, Germany) for 20 seconds and centrifuged (2000 g for 10 minutes at 4\(^\circ\)C). The resulting supernatants were used for assaying TNF-\(\alpha\) levels using ELISA (BioSource Europe S.A., Brussels, Belgium)\(^\text{20}\).

**Determination of Lipid Peroxides:**
Lipid peroxides (LP) in kidney tissues were determined spectrophotometrically (Optronik, Berlin, Germany) as thiobarbituric acid reactive substances (TBARS) according to the method of\(^\text{21}\). Tissue lipid peroxide levels were expressed as nanomoles of MDA formed per gram tissue weight.

**Determination of Glutathione**
Reduced glutathione (GSH) level in kidney tissues were measured enzymatically by the method of\(^\text{22}\). Tissue GSH levels were expressed as nanomoles per g tissue weight.

**Determination of Superoxide Dismutase and Catalase**
The activity of superoxide dismutase (SOD) was assessed as described by\(^\text{23}\), and catalase (CATA) activity was measured according to Aebi\(^\text{24}\).

**Statistical Analysis**
Results were collected and expressed as Mean ± SD. Results were analyzed using The Statistical Package for the Social Sciences, version 15 (SPSS Software, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Bonferroni’s post-hoc test were used to test the significance of the difference between quantitative variables. \(P\) value <0.05 was considered to be statistically significant.

**Results**
Reperfusion of ischemic kidney in both normal and diabetic rats, induced a significant elevation in serum levels of urea and creatinine with a significant reduction in urine volume \((p<0.05)\) compared with sham-operated group, suggesting a significant degree of glomerular dysfunction caused by renal I/R (Table I). These functional changes were associated with a significant elevation in arterial blood pressure in both normal and diabetic rats \((p<0.05)\) in comparison with the sham-operated group (Figures 2, 3a). Additionally, Table I showed a significant increase of the glycemia in diabetic rat with no further increase in the blood glucose by renal I/R injury. Renal I/R induced a marked elevation in the concentration of TNF-\(\alpha\) in both normal and dia-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>Diabetic Sham</th>
<th>IR control</th>
<th>Diabetic I/R</th>
<th>Diabetic I/R + pioglitazone</th>
<th>Diabetic I/R + telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>94.6 ± 8.4</td>
<td>373.1 ± 9.3**##</td>
<td>110.2 ± 7.6</td>
<td>380.6 ± 13.4**##</td>
<td>112.6 ± 10.2##</td>
<td>116.2 ± 9.6##</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.91 ± 0.05</td>
<td>1.15 ± 0.02*</td>
<td>1.33 ± 0.1**</td>
<td>2.25 ± 0.27**##</td>
<td>1.19 ± 0.11##</td>
<td>1.22 ± 0.14##</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>29.2 ± 3.6</td>
<td>39.0 ± 4.2*</td>
<td>41.1 ± 7.01*</td>
<td>65.8 ± 6.4**##</td>
<td>33.3 ± 3.2##</td>
<td>35.8 ± 2.0##</td>
</tr>
<tr>
<td>Urine volume</td>
<td>1.5 ± 0.17</td>
<td>1.28 ± 0.16*</td>
<td>1.09 ± 0.05**</td>
<td>0.7 ± 0.04**</td>
<td>1.4 ± 0.2##</td>
<td>1.3 ± 0.19##</td>
</tr>
</tbody>
</table>

\(n = 8.\) *Significantly different from sham group at \(p < 0.05);**Significantly different from sham group at \(p < 0.001);†Significantly different from IR group at \(p < 0.05);\) **Significantly different from I/R group at \(p < 0.001.\) §Significantly different from DM+IR group at \(p < 0.05,\) §§Significantly different from DM+IR group at \(p < 0.001;\) †Significantly different from pioglitazone group at \(p < 0.05.\)
Figure 2. Effect of pioglitazone and telmisartan on systolic and diastolic blood pressure (BP) (mm Hg) in both normal and diabetic rats exposed to renal I/R for 24 hours.

Figure 3. Effect of pioglitazone and telmisartan on (A) systolic and diastolic blood pressure (BP) (mm Hg). B, TNF–α level (pg/100 mg tissue) in both normal and diabetic rats exposed to renal I/R for 24 hours. Values are mean ± S.D. (n= 8), analyzed by one-way ANOVA followed by Bonferroni multiple comparisons test. *, †, \( P < 0.05 \); **, ††, \( P < 0.001 \). *Compared with normal control group; †Compared with IR group; ‡Compared with DM+IR group; ††Compared with pioglitazone group.
Renoprotective activity of telmisartan versus pioglitazone

Renoprotective activity of telmisartan versus pioglitazone (Figures 3b). Moreover, reperfusion of ischemic kidneys induced oxidative stress in both normal and diabetic kidneys in the form of a significant increase (p < 0.05) of malondialdehyde concentrations associated with significant (p < 0.05) reduction in superoxide dismutase, glutathione reductase activities, and catalase levels in comparison with the sham-operated group (Figure 4).

Diabetic rats that underwent renal I/R exhibited a significant increase in all the studied parameters with a reduction in the anti-oxidant enzymes as compared to nondiabetic rats (p < 0.05), suggesting a significant degree of kidney dysfunction caused by renal I/R in diabetes (Table I and Figures 2, 3 and 4).

These deleterious effects associated with renal I/R were improved by the treatment with pioglitazone and telmisartan in comparison with diabetic I/R group (p < 0.05), without a significant difference between the two treated groups. Therefore, the treatment with pioglitazone or telmisartan have the same corrective effect (Table I and Figures 2, 3 and 4).

As shown in Figure 5, histological examination of renal sections stained with H & E showed no histopathological changes in the kidney of Sham-operated group (A), (B) While kidney sections of diabetic group showed mild vacuolization and congestion with development of mild necrosis in tubular cells. (C) While kidney sections of I/R group showed moderate tubular cell vacuolization and necrosis. (D) Diabetic I/R group showed immunocompetent cell aggregation with severe congestion, vacuolization and necrosis. Treatment with pioglitazone was nearly similar to sham group except that there was a light edema of tubular cells (E), and telmisartan showed mild congestion (F).

Discussion

Diabetics are at a higher risk of an ischemic condition caused by the decreased blood flow. With increasing the duration and severity of ischemia, greater the cell damage can develop,

![Figure 4. Effect of pioglitazone and telmisartan on (A) MDA (nmol/mg protein), B, GSH “nmol/mg protein”, C, SOD (U/mg protein), D, CATA (U/mg protein) in both normal and diabetic rats exposed to renal I/R for 24 hours. Values are mean ± S.D. (n= 8), analyzed by one-way ANOVA followed by Bonferroni multiple comparisons test. * P < 0.05; ** P < 0.001. *Compared with normal control group, †Compared with IR group, ‡Compared with DM+IR group, †† compared with pioglitazone group.](image)
with a predisposition to a spectrum of reperfusion-associated pathologies, collectively called reperfusion injury. Since type 2 diabetes, causes organ dysfunction and increases the sensitivity of organs to damages, the present study was undertaken to test the hypothesis that the hyperglycemia of diabetes renders the kidney more susceptible to ischemic injury. Hence, the protective effect of telmisartan on renal I/R injury in diabetic rats and, therefore, on renal marker and oxidative stress of kidney tissues has been examined in comparison with the protective effect afforded by pioglitazone.

The present study showed a significant increase of the blood glucose in diabetic rat, without a further increase of glycemia induced by renal I/R injury. These results agree with those reported in other reports. Diabetic rats showed a renal dysfunction in the form of a significant increase in the serum creatinine and urea levels associated with a reduction in urine volume and hypertension when compared with normoglycemic ones. This finding is considered as an indicator of deteriorated renal function. In addition, our data confirmed the findings of Yousef et al and Gabr et al, who have found that the

Figure 5. **A**, A photomicrograph renal sections stained with H & E showed no histopathological changes in kidney of sham operated group. **B**, Kidney sections of diabetic group showed mild vacuolization and congestion with development of mild necrosis in tubular cells. **C**, Kidney sections of I/R group showed moderate tubular cell vacuolization and necrosis. **D**, Diabetic I/R group showed immunocompetent cell aggregation with severe congestion, vacuolization and necrosis. Treatment with pioglitazone was nearly similar to sham group except that there was a light edema of tubular cells. **E**, **F**, Telmisartan showed mild congestion. (H & E 400×).
combination of renal ischemia with diabetes raised the renal dysfunction more than did diabetes alone suggesting a significant impairment, thus, of the glomerular function. These results emphasize the hypothesis of Melin et al\textsuperscript{31} who stated that, the combination of both diabetes and renal ischemia plays a major role in the development of diabetic nephropathy.

An important question in this work is how type 2 diabetes may cause the increased sensitivity to renal I/R, as observed experimentally. One possible explanation was the increased sensitivity to I/R due to hyperglycemia per se. Shortage of insulin could also be involved. Moreover, hyperglycemia induced a formation of advanced glycosylated end (AGE) products and an increased oxidative stress. Hemodynamic alterations could also be involved\textsuperscript{1}. This hypothesis has been confirmed in our study as diabetic non-ischemic animals demonstrated an increase in the renal MDA and attenuated antioxidant enzymes pool. Lipid peroxidation and antioxidant enzymes are important indexes of oxidant injury as they were associated with an impaired kidney function, leading to a marked increase in serum creatinine, urea, and uric acid level\textsuperscript{15,32}.

In addition, both normal and diabetic rats exposed to renal I/R exhibited an increase of oxidative stress products including tissue MDA. The depletion of the antioxidant enzymes pool was demonstrated by the declined activity in kidney tissues of superoxide dismutase, catalase and reduced glutathione. This notion was confirmed by Jitendra et al\textsuperscript{1} who emphasized that the oxidative stress is implicated both in the complications of T2 diabetes and renal ischemia/renal I/R and that the combined oxidative stress products including tissue MDA. The decreased activity in kidney tissues of superoxide dismutase, catalase and reduced glutathione. This notion was confirmed by Jitendra et al\textsuperscript{1} who emphasized that the oxidative stress is implicated both in the complications of T2 diabetes and renal ischemia/renal I/R and that the combined oxidative stress products including tissue MDA.

These findings are in agreement with several investigations that demonstrated significant rise in serum TNF-\(\alpha\) level in renal I/R injury\textsuperscript{35}, as well as in diabetic rats\textsuperscript{25,29}. The enhanced TNF-\(\alpha\) production in ischemic diabetic rats may result from the hyperglycemia\textsuperscript{46}, and the increased generation of ROS\textsuperscript{37}. Hyperglycemia induced oxidative stress and products of lipid peroxidation, likewise serves as activators of transcription factors, leading to induction of gene expression of pro-inflammatory cytokines and release of many inflammatory cytokines, as TNF-\(\alpha\) and IL-\(6\)\textsuperscript{38}. TNF-\(\alpha\) may produce a renal injury inducing apoptosis, necrotic cell death, alterations of intraglomerular blood flow and glomerular filtration rate as a result of the hemodynamic imbalance between the vasoconstrictive and vasodilatory mediators\textsuperscript{25}. Impairment of endothelial permeability, as well as, alterations of the barrier function of the glomerular capillary wall lead to the enhanced albumin permeability\textsuperscript{39}. At the molecular level TNF-\(\alpha\) augments the release of many inflammatory factors from renal mesangial cells\textsuperscript{40}. These findings agree with Araki at al\textsuperscript{41} and Kher at al\textsuperscript{42} who referred that the oxidative stress and the inflammatory response might play a pathophysiological role in renal I/R injury in T2 diabetes.

Synthetic PPAR agonists increased insulin sensitivity, modify lipid profiles, and decrease blood pressure\textsuperscript{42}. Furthermore, they reduced the oxidative stress\textsuperscript{43} and the biomarkers of the inflammation very determinant factors in the course of renal disease\textsuperscript{44}. In agreement with above reported, the results of our investigation demonstrated that treatment with both pioglitazone and telmisartan have the same attenuating effect on I/R-induced renal damage in diabetic rats. They prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe attenuation of antioxidant enzymes activity in rats exposed to the renal I/R. In addition, synthetic PPAR agonists significantly ameliorated the reperfusion-induced elevation of TNF-\(\alpha\) level in both normal and diabetic rats. We have found that both pioglitazone and telmisartan improved all the functional renal parameters as BUN, creatinine and the urinary volume with significant amelioration in the level of blood pressure. Also the histology has evidenced in telmisartan and pioglitazone treated rats a protection against renal I/R in diabetes. Our findings are in consistent with Liliane and Walter\textsuperscript{45} who emphasized that, the PPAR\(\gamma\) agonists have many protective effects in other experimental
conditions than that I/R alone. These include diabetic nephropathy, hypertensive nephropathy, experimental glomerulonephritis, and cyclosporine-induced renal injury. In fact, PPAR agonists can modulate crucial cellular events such as growth factor release, cytokine production, cell proliferation and migration, extracellular matrix remodeling, and cell cycle progression. PPAR agonists also have potent antioxidant effects. Moreover, PPARγ exerts its anti-inflammatory effect by negatively regulating the expression of pro-inflammatory genes induced during macrophage differentiation and activation. Therefore, the protection afforded by PPARγ stimulation reflects both improved glucose metabolism and insulin resistance as well as the anti-inflammatory, anti-oxidant, anti-fibrotic, and anti-apoptotic effects.

In conclusion, type 2 diabetes provoked an exaggerated renal I/R injury in STZ treated rats. Telmisartan treatment is equipotential as pioglitazone in attenuating acute I/R-induced renal injury in diabetic rats modifying the oxidative stress and the inflammation processes.

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References


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