Research Article

Effects of Pioglitazone and Irbesartan on Endothelial Dysfunction on Experimentally Streptozotocin-Induced Diabetic Nephropathy in Rats

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Abstract. Background and Aim. Diabetes mellitus is associated with an induction of vascular endothelial dysfunction (VED) that leads to the pathogenesis of diabetic nephropathy (DN). Diabetic nephropathy (DN) is a main cause of end stage renal disease. This study was designed to investigate the protective effects of irbesartan (IRB) and its combination with pioglitazone (PIO) on impaired endothelial dysfunction, oxidative stress, and inflammation beyond blood glucose control in streptozotocin- (STZ-) induced diabetic nephropathy in rats. Materials and Methods. Forty-eight male albino rats were divided equally and randomly into six groups: A (control non-diabetic non-treated) and B (diabetic non-treated) injected intraperitoneally by streptozotocin (65mg/kg) and nicotinamide (110 mg/kg) to induce diabetic nephropathy; C (diabetic treated with IRB (30mg/kg/daily) orally), D (diabetic treated with PIO (30mg/kg/daily) orally), E (diabetic treated with IRB and PIO in doses (30 mg/kg/day and 30 mg/kg/day) orally), and F (diabetic treated with IRB and PIO in doses (30mg/kg/day and 15mg/kg/day, resp.) orally for 12 weeks). Fasting blood glucose, urea, creatinine, albumin in urine levels, IL-6, nitric oxide (NO), and Malondialdehyde peroxidase (MDA) were determined in serum of the different groups at the end of the experiment; renal blood flow and aortic and renal histopathological changes were also evaluated. Results. Administration of irbesartan and pioglitazone to diabetic rats caused significant decrease in levels of urea, creatinine, albumin in urine levels, IL-6, nitric oxide (NO), and Malondialdehyde peroxidase (MDA) were determined in serum of the different groups at the end of the experiment; renal blood flow and aortic and renal histopathological changes were also evaluated. Results. Administration of irbesartan and pioglitazone to diabetic rats caused significant decrease in levels of urea, creatinine, albumin, serum inflammatory mediators (IL-6 and NO), and oxidative stress markers. The effects of irbesartan and/or pioglitazone were associated with improvement of renal blood flow, and they markedly reduced vascular and renal damage induced by diabetes. Conclusion. The results of the present study support the fact that the combination between irbesartan and pioglitazone was better than monotherapy on endothelial dysfunction and diabetic nephropathy through inhibition of the pro-inflammatory mediators, NO bioavailability, and oxidative stress, which are independent of their glucose-lowering properties. And there was no significant difference between the combination of pioglitazone and irbesartan in large doses and in low doses.

Keywords: irbesartan, pioglitazone, endothelial cell (EC), type 2 diabetes, oxidative stress, diabetic nephropathy.
1. Introduction

Diabetic nephropathy has become the most frequently seen cause of the end stage renal failure. Many of the complications of diabetes are related to increased serum glucose and increased generation of reactive oxygen species, which lead to endothelial dysfunction [13]. Endothelial dysfunction and, possibly, inflammation are novel predictors of progression to diabetic nephropathy in patients with type 2 diabetes and microalbuminuria independently of traditional risk factors [39]. Many investigations showed that intensive glycemic and heart rate control prevent occurrence and progression of diabetic nephropathy [8]. However, particularly in some of type 2 diabetic patients, complications develop at the time of diagnosis, and strict glycemic control cannot be always attained. Therefore, development of treatment modalities preventing occurrence or progression of diabetic nephropathy becomes necessary.

Thiazolidinediones are insulin-sensitizing agents used in the treatment of type 2 diabetes. They demonstrate these effects by activating peroxisome proliferator, activating receptor gamma (PPARγ), which is a type of nuclear receptor and acts as a PPARγ agonist [30]. PPARγ receptors are found in vascular smooth muscle cells, macrophages vascular endothelial cells, colonic epithelial cells, and renal glomerular cells [34]. Beneficial effects of thiazolidinedione on diabetic nephropathy have been reported [23, 32].

Irbesartan is a potent and selective angiotensin II subtype 1 receptor antagonist used in patients with hypertension, including those with type 2 diabetes mellitus and nephropathy. Treatment with angiotensin type 1 receptor blockers (ARBs) improved renal dysfunction and glucose metabolism in obese diabetic animal models and humans [35]. Antidiabetic activity may be due to improved glucose metabolism via blocking of the inhibitory effect of angiotensin II (Ang II) on insulin signal transmission and a moderately potent partial peroxisome proliferator activated receptor gamma (PPARγ) agonist.

In the present study, irrespective of their glycemic control, the effects of irbesartan and pioglitazone on endothelial dysfunction and diabetic nephropathy in experimentally induced type 2 diabetes mellitus in rats were examined.

2. Materials and Methods

2.1. Experimental design

The rats were randomly divided into six groups.

Group (A): control group; this group did not receive any drug.

Group (B): diabetic non-treated rats; rats received a single dose of 65 mg/kg/I.P streptozotocin (STZ) 15 minutes after intraperitoneal administration of 110 mg/kg of nicotinamide for induction of type 2 diabetes [19]. STZ was dissolved in citrate buffer (0.1 M, pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma determined at 72 h and then on day 7 after injection. Animals with blood glucose concentration more than 200 mg dl were used for the study.

Group (C): diabetic rats were treated with irbesartan (30 mg/kg/day, orally) for 12 weeks [35].
Group (D): diabetic rats were treated with pioglitazone (30mg/kg/day, orally) for 12 weeks [23].

Group (E): diabetic rats were treated with irbesartan (30 mg/kg/daily, orally) and pioglitazone (30 mg/kg/daily, orally) for 12 weeks.

Group (F): diabetic rats were treated with irbesartan (5 mg/kg/daily, orally) and pioglitazone (15mg/kg/daily, orally) for 12 weeks.

The doses of drugs used were according to previous literatures and experiments [23, 35].

At the end of the experimental period, rats were anaesthetized by inhalation of ether, and blood samples were collected from retrobulbar sinus and processed for biochemical measurements. Then rats were sacrificed, and their kidneys and aorta were rapidly excised and put in 10% formalin for histopathological examination.

Bidirectional blood flowmeter with FFT analysis (Hadeco, Japan) was used. After setting the mode of pulsed Doppler blood flowmeter, we used ultrasonic gel on the probe top and turned the volume control to the maximum. The probe pressed softly on the measurement area at an angle of 45-60°. After hearing the optimal sounds, we waited for 5 seconds without moving the probe, then pressed the freeze key to freeze the waveform [16].

2.2. Biochemical measurements

2.2.1. Fasting blood glucose levels (FBG)

One drop of blood from the adult rats was obtained by puncture of the retrobulbar sinus; the capillary end of the glass tube was inserted into the medial canthus of the eye. The sinus was punctured, and blood entered the tube by its own pressure forming a free flow of blood. The blood was biochemically investigated for FBG according to Fossatip (1982). Diabetes was confirmed in rats by showing blood glucose levels above 200 mg/100 ml [7].

2.2.2. Estimation of renal excretion of albumin

All rats were housed individually in metabolic cages for 24 hours with free access to water and a normal chow. Albumin concentrations were measured in 24-hour urine (mg/24 hours) samples using a Minineph Microalbumin kit (The Binding Site, Birmingham, UK).

2.2.3. Measurement of serum urea and serum creatinine

At the end of the 12th week, rats were anaesthetized with ether. Venous blood samples were collected by heparinized capillary tubes from the retroorbital plexus of rats [38].

Serum levels of urea: A serum level of urea was determined using a commercially available kit according to the manufacturer’s instructions [20].

Serum creatinine: The kit used for measuring creatinine levels (Randox Laboratories, Crumlin, County Antrim, Northern Ireland) is based on the Jaffe’ reaction. In an alkaline
solution, creatinine combines with picric acid to form an orange-red complex (the creatinine-picric acid complex). The increase in absorbance using a spectrophotometer at 510 nm is proportional to creatinine concentration (mg/dl) [29].

2.2.4. Measurement of malondialdehyde

Malondialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals, is considered a reliable marker of oxidative stress and was determined by measurement of the chromogen obtained from the reaction of malondialdehyde with 2-thiobarbituric acid, according to Aruoma et al. (1989). The MDA values are expressed as umol/ml.

2.2.5. Nitric oxide

The concentration of serum nitrate (a stable end product of NO, in µmol/l) was measured by a one-step enzymatic assay using nitrate reductase (Roche Diagnostic Group, Basel, Switzerland). The concomitant reduction of nitrate to nitrite by NADPH was reflected by the oxidation of the coenzyme and the decrease in absorbance using a spectrophotometer at 340 nm [3].

2.2.6. Estimation of IL-6 by ELISA technique using (Ray Bio ® Mouse IL-6)

By following the manufacturer’s instructions according to the protocol of Howard, O’Gara (1992).

2.3. Renal blood flow (RBF) [16]

At the end of the experiment, rats were anaesthetized with ether and then fixed on operating table; the abdominal cavity was opened and the kidneys were exposed. The flow probe was placed on top of the right renal artery for the measurement of renal blood flow by the flowmeter (Hadeco ES 1000 SPM, Japan); bidirectional blood flowmeter with FFT analysis (Hadeco, Japan) was used. After setting the mode of pulsed Doppler blood flowmeter, we used ultrasonic gel on the probe top and turned the volume control to the maximum. The probe pressed softly on the measurement area at an angle of 45-60°. After hearing the optimal sounds, we waited for 5 seconds without moving the probe, then pressed the freeze key to freeze the waveform.

2.4. Histopathological parameter

The rats in all groups were sacrificed, and their kidneys were cut; each kidney was divided into two halves. The specimens were preserved in 10% formalin, dehydrated in ascending grades of ethyl alcohol (50%, 70%, 90%, and 100%), and cleared; the two halves were embedded in soft and hard paraffin, respectively. Paraffin blocks were generated, and sections (3-µm thick) were cut on a microtome and subjected to haematoxylin and eosin staining [6]. In addition, the
The thoracic aorta was cut as near the heart as possible and dissected free as far as the diaphragm. The aortic arteries were cleaned from surrounding tissues and fat, then fixed in 10% phosphate buffered formalin. Fixed specimens were prepared for paraffin sections. Aorta cross sections (4μm) were cut at the aorta and stained with hematoxylin & eosin.

3. Statistical Analysis

Data were represented as mean ± SEM. Multiple comparisons were performed using one-way ANOVA (analysis of variance) followed by Tukey’s test as a post hoc test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using GraphPad InStat version software package [14].

4. Results

4.1. Effect of streptozotocin-induced changes in rats

A single dose of streptozotocin (65mg/kg/day i.p.) and nicotinamide (110mg/kg/i.p.) significantly increased (p < 0.05) fasting blood glucose level (FBG), 24-hour urinary albumin excretion (Table 1). Also, STZ significantly increased plasma urea, creatinine concentration, lipid peroxidation (MDAp), and IL-6 in all diabetic groups compared with normal control group (Table 1). On the other hand, streptozotocin significantly decreased renal blood flow (Table 2).

4.2. Effects of irbesartan and pioglitazone administration on streptozotocin-induced changes

4.2.1. Fasting blood glucose level, serum urea, and serum creatinine level

Administration of irbesartan alone had no significant effect on blood glucose level, but significantly decreased serum urea and creatinine levels in diabetic rats. On the other hand, administration of pioglitazone (30mg/kg) alone and combination of irbesartan and pioglitazone both in large or small doses significantly decreased blood glucose, serum urea, and creatinine levels in comparison with diabetic group (Table 1). And there was no significant difference between the combined drugs in large and small doses groups.

4.2.2. Renal excretion of albumin

Administration of irbesartan alone (30mg/kg/day, orally), pioglitazone alone (30mg/kg/day), and combination of irbesartan and pioglitazone in large and small doses significantly reduced the effects of streptozotocin on renal albumin excretion compared with diabetic non-treated group (Table 1). And there was no significant difference between the combined groups.
4.2.3. Lipid peroxidation (MDAp)

Administration of irbesartan alone (30mg/kg/day), pioglitazone alone (30mg/kg/day), and combination of irbesartan and pioglitazone significantly reduced lipid peroxidation (MDAp) compared with diabetic non-treated group. And there was no significant difference between the two combined groups (Table 2).

4.2.4. Serum IL-6 and NO levels

Administration of irbesartan (30mg/kg/day), pioglitazone (30mg/kg/day), and combination of irbesartan and pioglitazone significantly reduced IL-6 and normalized nitric oxide levels compared with diabetic non-treated group. And there was no significant difference between the two combined groups (Figure 1).

4.3. Renal blood flow

Administration of irbesartan (30mg/kg/day), pioglitazone (30mg/kg/day), and combination of irbesartan and pioglitazone significantly improved the effect of streptozotocin on renal blood flow in compared with diabetic non-treated group. And there was no significant difference between the two groups (Table 2, Figure 4).

4.4. Renal histopathological structure

Histopathological examination of renal tissues of diabetic rats revealed diffuse diabetic glomerular sclerotic lesion, which is characterized by diffuse thickening of glomerular capillary wall and a generalized increase in mesangial matrix in all mesangial regions of all glomeruli (Figure 2), while histopathological examination of renal tissues in pioglitazone and/or irbesartan treated rats showed mild thickening of glomerular capillary wall and mild changes in mesangial matrix (Figure 2).

4.5. Histopathological changes of aorta

Histological examination of a cut section of the aorta of control group showed that the wall of the aorta consists of tunica intima, tunica media, and tunica adventitia. In diabetic non-treated group, there were ulcerated endothelial cells of the intima with collection of foamy histiocytes and fat globules and formation of fatty streaks with degeneration. The media and adventitia showed fibrosis and inflammatory cell infiltration (Figure 3B). Irbesartan and pioglitazone treatment markedly decreased the size of fatty streaks and foamy and inflammatory cell infiltration. Regeneration of the endothelial cells of the intima was observed (Figure 3C, D, E, F).
Table 1: Effects of irbesartan (30mg/kg), pioglitazone (30mg/kg), and combination of irbesartan and pioglitazone in high dose and low dose on fasting blood glucose level (FBG) and 24-hour urinary albumin excretion (UAE), serum urea, and creatinine (mg/dl) in diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>FBG (mg/dl)</th>
<th>Urine albumin (mg/24 h)</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>88 ± 2.9</td>
<td>3.89 ± 2.3</td>
<td>36.4 ± 2.8</td>
<td>0.9 ± 0.06</td>
</tr>
<tr>
<td>DM group</td>
<td></td>
<td>200 ± 20.1</td>
<td>14.5 ± 1.1</td>
<td>145 ± 4.6</td>
<td>3.7 ± 0.17</td>
</tr>
<tr>
<td>Irbesartan-treated</td>
<td></td>
<td>190 ± 18.4</td>
<td>8.7 ± 0.45^ab</td>
<td>48.2 ± 3.1^b</td>
<td>2.1 ± 0.13^b</td>
</tr>
<tr>
<td>Pioglitazone-treated</td>
<td></td>
<td>112 ± 5.2^c</td>
<td>7.2 ± 0.53^b</td>
<td>45.4 ± 2.9^b</td>
<td>2.3 ± 0.11^b</td>
</tr>
<tr>
<td>IRB+PIO high dose treated</td>
<td></td>
<td>105 ± 3.9</td>
<td>5.0 ± 0.4^bcd</td>
<td>42.1 ± 3.9^bcd</td>
<td>1.7 ± 0.1^bcd</td>
</tr>
<tr>
<td>IRB+PIO low dose treated</td>
<td></td>
<td>115 ± 4.1^ab</td>
<td>5.6 ± 0.45^bcd</td>
<td>39.1 ± 4.2^bcd</td>
<td>1.6 ± 0.1^bcd</td>
</tr>
</tbody>
</table>

^aSignificant difference from control group.  
^bSignificant difference from diabetic group.  
^cSignificant difference from irbesartan group.  
^dSignificant difference from pioglitazone group.

Table 2: Effects of irbesartan (IRB), pioglitazone (PIO), and their combination on renal blood flow (RBF) and lipid peroxidation (MDAp) in diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Renal blood flow (ml/min.)</th>
<th>MDAp (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>11.4 ± 0.9</td>
<td>3.2 ± 0.31</td>
</tr>
<tr>
<td>DM group</td>
<td></td>
<td>2.3 ± 0.15^c</td>
<td>7.11 ± 0.31^a</td>
</tr>
<tr>
<td>Irbesartan-treated</td>
<td></td>
<td>6.5 ± 0.39^b</td>
<td>5.13 ± 0.19^b</td>
</tr>
<tr>
<td>Pioglitazone-treated</td>
<td></td>
<td>7.1 ± 0.45^b</td>
<td>5.6 ± 0.41^b</td>
</tr>
<tr>
<td>IRB+PIO high dose treated</td>
<td></td>
<td>9.9 ± 0.40^bcd</td>
<td>4.1 ± 0.29^bcd</td>
</tr>
<tr>
<td>IRB+PIO low dose treated</td>
<td></td>
<td>10.1 ± 0.46^bcd</td>
<td>4.51 ± 0.23^bcd</td>
</tr>
</tbody>
</table>

^aSignificant difference from control group.  
^bSignificant difference from diabetic group.  
^cSignificant difference from irbesartan group.  
^dSignificant difference from pioglitazone group.

Figure 1: Effects of pioglitazone (30mg/kg/day), irbesartan (30mg/kg/day), and their combination in large and small doses on serum levels of IL-6 (ng/ml) and NO (µmol) in streptozotocin induced diabetic nephropathy in rats. a: Significant difference from control group. b: Significant difference from diabetic group. c: Significant difference from irbesartan group. d: Significant difference from pioglitazone group.
Figure 2: Effects of irbesartan and pioglitazone on histopathological changes in rats with diabetic nephropathy.
A) Cut section of normal renal tissue of control. B) Cut section of renal diabetic nephropathy revealed diffuse diabetic glomerular sclerotic lesion, which is characterized by diffuse thickening of glomerular capillary wall and a generalized increase in mesangial matrix in all mesangial regions of all glomeruli. C) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with irbesartan showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E x 40). D) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with pioglitazone showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E x 40). E) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with large doses of pioglitazone (30mg/kg/day) + irbesartan (30mg/kg/day) showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E x 40). F) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with small doses of pioglitazone (15mg/kg/day) + irbesartan (5mg/kg/day) showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E x 40).

5. Discussion

In our study, experimental induction of diabetes mellitus resulted in diabetic nephropathy, which was assessed by measuring 24hrs. Urinary albumin excretion, serum creatinine, and serum urea on the 12th week of the experiment. It was noted that there was a significant increase in urinary albumin excretion, serum creatinine, and serum urea noted in rats after STZ administration. This is in agreement with Mianzhi et al. (2012). Various factors are involved in the pathogenesis of diabetic nephropathy. It is believed that hyperglycemia induces a defect in the mitochondrial electron transport chain, resulting in increased production of reactive oxygen species and increased oxidative stress. This is a common mediator of the pathophysiological effects of hyperglycemia and subsequent diabetic nephropathy [41]. The increased oxidative stress activates glycation and formation of advanced glycation end products with the formation of cytokines and growth factors [42]. Also increased angiotensin II plays an important part in the pathogenesis of diabetic nephropathy; it causes a preferential constriction of the efferent glomerular arteriole, increases glomerular capillary permeability to protein, stimulates advanced glycation end product formation, and stimulates mesangial cell proliferation with accumulation of mesangial
matrix by inducing many pro-inflammatory and fibrogenic cytokines, chemokines, and growth factors [9].

The current study showed that endothelial dysfunction, proteinuria, inflammatory mediators, and diabetic nephropathy were significantly improved by combination therapy of large doses and small doses of irbesartan and pioglitazone in streptozotocin-nicotinamide induced type 2 diabetes in rats, independent of plasma glucose. But there were no significant differences between the combined groups. Three-month administration of large and small doses of irbesartan and pioglitazone to diabetic rats significantly ameliorated endothelial dysfunction and improved renal blood flow. Moreover, administration of irbesartan and pioglitazone to diabetic rats caused significant decrease in levels of urea, creatinine, albumin, serum inflammatory mediator (IL-6), and oxidative stress markers. These findings are congruent with those of Hartner et al. (2014), who reported that irbesartan significantly ameliorated proteinuria and improved glomerular, arteriolar, and tubulointerstitial lesions in diabetic rats. This renal protection effect of irbesartan might be related to reduction of inflammation and oxidative stress. Similarly, long term administration of irbesartan in rats was reported to diminish the elevation in urinary protein excretion, plasma creatinine, and urea nitrogen levels and reduce the extent of glomerular and tubule-interstitial lesions [43]. Minoru Satoh et al. (2008) demonstrated that blockade of angiotensin II signaling in diabetic glomeruli reduced ROS production, improved
Figure 4: Effect of irbesartan and pioglitazone on renal blood flow in diabetic rats: a) Doppler ultrasound record of renal blood flow in normal rats. b) Doppler ultrasound of renal blood flow in diabetic rats. c) Doppler ultrasound of renal blood flow in irbesartan (30mg/kg/day) treated group. d) Doppler ultrasound of renal blood flow in pioglitazone (30mg/kg/day) treated group. e) Doppler ultrasound of renal blood flow in irbesartan (30mg/kg/day) + pioglitazone (30mg/kg/day) treated group. f) Doppler ultrasound of renal blood flow in irbesartan (5mg/kg/day) + pioglitazone (15mg/kg/day) treated group.

the eNOS coupling statement, and, indeed, restored NO bioavailability in glomeruli. In clinical practice, the effects of irbesartan to inhibit the early and progression of diabetic nephropathy were reported in two prospective randomized, double-blind clinical trials, without a reduction in blood pressure [31, 44].

Moreover, in this study, administration of pioglitazone significantly decreased serum creatinine levels, microalbuminuria, and renal alternations. These findings are in line with those of previous studies, where administration of pioglitazone significantly reduced albuminuria and regressed development of histopathological lesions and vascular wall consolidation [22, 23, 32]. These beneficial effects of pioglitazone might be related to decreased levels of malondialdehyde, superoxide dismutase, interleukin-6, and tumor necrosis factor alpha [1, 23], which was confirmed in the present work.

Many studies investigated the effect of pioglitazone on histopathological changes in diabetic nephropathy. Tanimoto et al. (2004) reported that normalization of the Bowman capsular volume, decrease in the amount of eNOS (endothelial constitutive nitric oxide synthase) protein in the glomerular vascular endothelium, and improvement in glomerular hyperfiltration were detected after treatment with pioglitazone 10 mg in rats. On the other hand, Majithiya et al. (2005) demonstrated that pioglitazone increased NO expression with a decrease in renal oxidative stress.

Another important finding of the current study is that treatment with irbesartan and pioglitazone ameliorates endothelial dysfunction and enhances renal blood flow in diabetic rats. Endothelial dysfunction and inflammation are novel predictors of progression to diabetic nephropathy in patients with type 2 diabetes and microalbuminuria independently of traditional...
risk factors. Endothelial dysfunction is caused by diabetes and also accelerates the progression of diabetes. Thus, improvement of endothelial dysfunction is beneficial for reducing renal impairment in diabetic patients [45]. These findings correspond with those of Tobli et al. (2011) who found that treatment of pioglitazone at low doses is associated with improvement of tubular and endothelial integrity and angiogenesis in diabetic rats. And effects occurred by normalization of the renal levels of connective tissue growth factor and fibronectin, TNF-\(\alpha\), IL-6, and the ratio between eNOS and iNOS. Moreover, Huang et al. (2008) reported that pioglitazone significantly ameliorates endothelial dysfunction and increases blood low recovery after tissue ischemia in diabetic mice. These beneficial effects of pioglitazone due to activation of eNOS appear to be essential for pioglitazone to promote angiogenesis in ischemic tissue [18]. Also, Ceriello et al. (2005) reported that the decrease in endothelial dysfunction as assessed by flow-mediated vasodilation and the increase in IL-6 and intercellular adhesion molecule-1 were significantly attenuated by irbesartan treatment in type 2 diabetic patients. In this study, experimental induction of diabetic nephropathy resulted in significant reduction of renal blood flow. This is in line with the results of Chan et al. (2011) who mentioned that diabetic nephropathy is one of the vascular complications of diabetes mellitus and is associated with hemodynamic alterations in the diabetic kidney revealed by a reduction of renal blood flow. This result was explained by Garg & Rabelink (2011) who reported that chronic hyperglycemia resulted in alterations in size and charge-selective properties of the glomerular capillary wall, alterations in the glomerular basement membrane (GBM) composition, and alteration of podocyte biology. Moreover, chronic hyperglycemia could induce reactive oxygen species production and glycation of proteins.

In the present study, combination therapy in large and small doses of irbesartan and pioglitazone significantly improved endothelial dysfunction, renal blood flow, microalbuminuria, and diabetic nephropathy. However, there was no significant difference between combined groups. These combinations improved renal and vascular structure more significantly than either individual drug did. These findings are supported by the previous studies, where administration of pioglitazone with angiotensin II receptor blocker significantly reduced endothelial dysfunction and had cardiorenal protective effects in diabetic obese mice [11]. In addition, Morikawa et al. (2011) found that combination therapy of pioglitazone with angiotensin II receptor blocker was more effective than angiotensin II receptor blocker monotherapy to reduce proteinuria in diabetic hypertensive patients with proteinuria renal disease.

6. Conclusion

Irbesartan and pioglitazone have renoprotective effects and attenuate the endothelial dysfunction in diabetic rats. Moreover, the combination of both drugs at high and low doses significantly ameliorated streptozotocin induced endothelial dysfunction in rats and might prevent the development of diabetic nephropathy by ameliorating vascular dysfunction and anti-inflammatory and antioxidant effects. Data of the present study pointed out that the use of combination therapy of irbesartan and pioglitazone in small doses had nearly the same renoprotective effects of combination of both drugs in large doses. So, we prefer using the combination in small doses in diabetic nephropathy for better effects, decreasing the side effects of each drug alone.
Competing Interests

The authors declare no competing interests.

References


