Comparative Study on Renoprotective Effects of Perindopril and Telmisartan on Strepotozotocin-Induced Diabetic Nephropathy in Rats

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Abstract

Diabetic nephropathy occurs in approximately one-third of all people with diabetes and is the leading cause of renal failure in developed and developing countries. Angiotensin II plays a potent role in the initiation and progression of diabetic nephropathy by increasing glomerular pressure and glomerular selectivity as well as activation of fibrosis and cellular growth in the kidney. Thus, agents which can inhibit the formation of angiotensin-II like ACE inhibitors or agents that can block the action of angiotensin II such as AT 1 receptor blockers have potential role in prevention of diabetic nephropathy. This study was designed to evaluate and compare the renoprotective effects of (ACEI) perindopril and (ARB) telmisartan on rats with diabetic nephropathy.

Diabetic nephropathy was induced in rats by streptozotocin (STZ) 100mg/Kg/single I.P administration. Diabetic nephropathy rats were randomly divided into 3 groups. Diabetic nephropathy group, perindopril (6mg/kg/day) and telmisartan group (10mg/kg/day). Each drug was given orally for 8 weeks starting with STZ injection. Fasting blood glucose (FBG), advanced glycation end products (AGEPs), blood urea, serum creatinine, urine protein, tissue malondyaldehyde (MDA) and tissue reduced glutathione (RG) were measured by colormetric methods. Renal tissue specimens were histopathologically examined by hematoxylin & eosin staining (H & E).

Both drugs significantly reduced FBG, AGEPs, blood urea, serum creatinine, urine proteins, tissue MDA and significantly increased tissue RG. Moreover, histopathological examination of kidney tissues showed improvement of nephropathy in the form of reduction of thickening and infiltration of the infalmmatory cells with more superior effects of telmisartan over perindopril.

Key Words: Diabetic nephropathy – Strepotozotocin – Perindopril – Telmisartan – Angiotensin.

Introduction

DIABETIC nephropathy (DN) is a leading cause of end-stage renal disease. It is one of the microvascular complications of diabetes mellitus. Oxidative stress is an important leading cause for the major pathways involved in the development and progression of diabetic microvascular as well as macrovascular complications. Also there are many beneficial effects of antioxidants in prevention of renal injury owing to diabetes [1].

The cause of diabetic nephropathy is multifactorial and may include altered glucose metabolism, ischemia and superoxide induced free radical formation and increased oxidative stress [2].

It has been shown that renin angiotensin aldosterone system blockade prevents fibrosis. As aldosterone stimulates fibrosis by stimulating the production of plasminogen activator inhibitor (PAI-1) and transforming growth factor-p (TGF-J3) [3].

Telmisartan is a non-peptide angiotensin II type 1 receptor (AT1) antagonist with high lipophilicity and the longest half-life compared with other angiotensin receptor blockers (ARBs) [4].

Telmisartan has a beneficial effect in diabetic nephropathy by blocking the undesirable effects of angiotensin II which are, vaso-constriction, stimulation of aldosterone release, sympathetic activation, increase glomerular pressure, increase glomerular permeability as well as activation of fibrosis and cellular growth in the kidney [5].

In our pervious study we evaluate the role of perindopril in diabetic nephropathy [6].

The aim of this study was to evaluated and compare the effect of telmisartan and perindopril on STZ-induced diabetic nephropathy in rats."
Material and Methods

Animals:
Adult male albino rats (n=40), weighing 150-200g. They were brought from (Experimental Animal Breeding Farm, Helwan-Cairo). All animals were housed in controlled laboratory condition at 20-25°C in a 12h light/dark cycle and had free access to standered diet and water. They had been aclimatized for one week and were caged (10/cage) in fully ventilated room (at room temperature) in Pharmacology Department, Benha Faculty of Medicine from April – Sept. 2013. All experimental protocols were approved by the committee of Benha University.

Experimental protocol:
After acclimatization for 1 week, rats were randomly divided into 4 experimental groups, 10 rats each and treated for 8 weeks as follow:

Group I (Normal control group): Rats received Carboxy methyl cellulose (CMS 1%) and served as normal control group (CN).

Group II (Diabetic group): Rats received a single IP injection of streptozotocin (100mg/kg) [7,8].

Group III (Perindopril group): STZ received rats treated with perindopril (6mg/Kg daily orally for 8 weeks dissolved in CMS 1%) [8].

Group IV (Telmisartan group): STZ received rat treated with Telmisartan (10g/kg daily for 8 weeks dissolved in CMS 1%) [9].

All groups injected with a single intra-peritoneal injection of STZ (100mg/kg, i.p., provided by Sigma) prepared in 0.1 N citrate buffer at pH 4.5 were given to rats except normal control group.

At the end of the experimental period, rats were anaesthetized by inhalation of ether and blood samples were collected from rat tail and processed for biochemical investigation. Then rats were sacrificed and their kidneys were rapidly collected and divided into 2 parts. One part was put in 10% formalin for histopathological examination. The second part was kept at –80°C and used for biochemical measurements.

Parameters measured:
I- Biochemical measurements:
1- Fasting blood glucose (FBG) [10].
2- Advanced glycation end products (AGEPs) [10].
3- Blood urea (BL urea) [11].
4- Serum creatinine [12].
5- Urine proteins [10].

II- Markers for oxidative stress in kidney [13]:
A- Tissue reduced glutathione (RG).
B- Tissue malondyaldehyde (MDA).

III- Histopathological examination:
The kidney specimens were obtained, washed with ice cold saline and divided into two portions. The first one was immediately frozen at ~80°C for the different biochemical determinations, this portion latterly was minced and homogenized with Elvenhjem tissue homogenizer. The crude homogenate was centrifuged and the resultant supernatant (free of insoluble materials) was used for assay of tissue malondyaldehyde, reduced glutathione [14]. The other portion of kidney specimens fixed in formaline 10% and histopathological examination of them was done using hematoxylin and eosin (H&E) stain [18].

The degree of diabetic nephropathy was assessed using the simple scoring system of [16] was used for the evaluation of the different therapies used in the present study. In this scoring system diabetic nephropathy is classified as follows: Class I, glomerular basement membrane thickening: Isolated glomerular basement membrane thickening and only mild, nonspecific changes by light microscopy that do not meet the criteria of classes II through IV. Class II, mesangial expansion, mild (IIa) or severe (IIb): Glomeruli classified as mild or severe mesangial expansion but without nodular sclerosis (Kimmelstiel-Wilson lesions) or global glomerulosclerosis in more than 50% of glomeruli. Class III, nodular sclerosis (Kimmelstiel-Wilson lesions): At least one glomerulus with nodular increase in mesangial matrix (Kimmelstiel-Wilson) without changes described in class IV. Class IV, advanced diabetic glomerulosclerosis: more than 50% global glomerulosclerosis with other clinical or pathologic evidence that sclerosis is attributable to diabetic nephropathy.

Statistical analysis of the data [17]:
All data were expressed as mean ± S.D, data were evaluated by the one way analysis of variance. Difference between groups were compared by Student’s t-test with p<0.05 selected as the level of statistical significance.

Results
Effect of perindopril and telmisartan on FBG:
FBG level was significantly (p<0.001) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant (p<0.01) reduction in FBG level. Administration
of telmisartan significantly \((p<0.05)\) reduced FBG level compared with perindopril (Table 1 & Fig. 1).

**Effect of perindopril and telmisartan on A GEPs:**

Serum AGEPs level was significantly \((p<0.001)\) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant \((p<0.01)\) reduction in serum AGEPs level. Administration of telmisartan significantly \((p<0.05)\) reduced serum AGEPs level compared with perindopril (Table 1 & Fig. 2).

**Effect of perindopril & telmisartan on Blood urea:**

Blood urea level was significantly \((p<0.001)\) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant \((p<0.01)\) reduction in blood urea level. Telmisartan treatment significantly \((p<0.05)\) reduced blood urea level compared with perindopril (Table 1 & Fig. 3).

**Effect of perindopril and telmisartan on tissue creatinine:**

Serum creatinine level was significantly \((p<0.001)\) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant \((p<0.01)\) reduction in serum creatinine. There was insignificant \((p>0.05)\) difference between perindopril and telmisartan in reduction of serum creatinine (Table 1 & Fig. 4).

**Effect of perindopril and telmisartan on urine proteins:**

Urine protein level was significantly \((p<0.001)\) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant \((p<0.01)\) reduction in urine protein level. There was no significant \((p>0.05)\) difference between perindopril and telmisartan in reduction of urine protein (Table 1 & Fig. 5).

**Effect of perindopril & telmisartan on tissue MDA:**

Tissue MDA level was significantly \((p<0.001)\) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant \((p<0.01)\) reduction in tissue MDA level. Treatment with telmisartan significantly \((p<0.05)\) reduced tissue MDA level compared with perindopril treated rats (Table 1 & Fig. 6).

**Effect of perindopril and telmisartan on tissue RG:**

Tissue RG level was significantly \((p<0.001)\) increased in STZ diabetic nephropathy non treated rats compared with control normal rats. In perindopril and telmisartan treated rats, there was significant \((p<0.01)\) reduction in tissue RG level. Telmisartan treatment significantly \((p<0.05)\) reduced tissue RG level compared with perindopril (Table 1 & Fig. 7).

**Histopathological finding:**

Group I: Showed normal appearance of the glomeruli & tubules (Fig. 8).

Group II: Showed severe diffuse glomerulosclerosis and cellular infiltration, interstitial fibrosis and tubular dilatation (Class IV) (Fig. 9).

Group III: Showed mild improvement of glomerulosclerosis but there is tubular atrophy & dilatation (Class IIb) (Fig. 10).

Group IV: Showed improvement of both tubular dilatation and mesangial expansion and decrease of interstitial cellular infiltration (Class IIa) (Fig. 11).

**Table (1): Effects of the drugs used on different parameters in different studied groups (mean ±SD).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control (Group I)</th>
<th>DN (Group II)</th>
<th>PER (Group III)</th>
<th>TEL (Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td></td>
<td>100.5±6.5</td>
<td>302±22.1*</td>
<td>279.6±20.4**</td>
<td>230.3±15.1**a</td>
</tr>
<tr>
<td>Serum AGEs (nmol/ml)</td>
<td></td>
<td>1.78±0.08</td>
<td>6.2±0.4*</td>
<td>3.8±0.14**</td>
<td>2.9±0.1%**a</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td></td>
<td>20.5±1.8</td>
<td>56.8±2.9*</td>
<td>37.4±2.3**</td>
<td>28.2±2.2**b</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td></td>
<td>0.56±0.02</td>
<td>4.6±0.32*</td>
<td>1.96±0.09**</td>
<td>1.6±0.08**</td>
</tr>
<tr>
<td>Urine protein (mg/dl)</td>
<td></td>
<td>0.45±0.02</td>
<td>1.9±0.09*</td>
<td>1.3±0.04**</td>
<td>1.4±0.06**</td>
</tr>
<tr>
<td>Tissue MDA (nmol/g tissue)</td>
<td></td>
<td>33.6±2.1</td>
<td>89.2±4.1*</td>
<td>62.1±3.2**</td>
<td>50.5±2.9**a</td>
</tr>
<tr>
<td>Tissue RG (mg/g tissue)</td>
<td></td>
<td>0.251±0.01</td>
<td>0.043±0.002*</td>
<td>0.169±0.008**</td>
<td>0.182±0.009**b</td>
</tr>
</tbody>
</table>

N = Number of rats in each group.  
** = Significant \(p<0.001\) compared with normal rats.  
* = Significant \(p<0.01\) compared with control normal rats.  
** = Significant \(p<0.05\) compared with diabetic non-treated rats.  
a = Significant \(p<0.05\) compared with perindopril treated rats.  
b = Significant \(p<0.01\) compared with perindopril treated rats.
Fig. (1): Comparison of FBG level (mg/dl) in different studied groups.

Fig. (2): Comparison of AGEs level (n mol/ml) in different studied groups.

Fig. (3): Comparison of blood urea level (mg/dl) in different studied groups.

Fig. (4): Comparison of serum creatinine level (mg/dl) in different studied groups.

Fig. (5): Comparison of urine protein level (mg/dl) in different studied groups.

Fig. (6): Comparison of renal tissue MDA level (n mol/g tissue) in different studied groups.

Fig. (7): Comparison of renal tissue RG level (n mol/g tissue) in different studied groups.
Diabetic nephropathy (DN) is a severe, chronic microvascular complication of type 2 diabetes mellitus, and it is the main cause of endstage renal disease (ESRD) in diabetic patients [18].

In the present study, we induced diabetic nephropathy in adult male albino rats by a single (I.P.) injection of streptozotocin (STZ) (100gm/kg/I.P) and rats were examined after one month.

Single STZ successfully induced diabetic nephropathy as evidenced biochemically by induced significant increase of FBG, AGEPs, blood urea, serum creatinine, urine protein and tissue MDA with significant reduction of tissue RG and confirmed histopathologically by inflammatory cell infiltration and diffuse glomerular sclerotic lesion, these results were in agreement with [19].

In accordance with the present study [19,20], showed that serum urea & creatinine were significantly higher in diabetic rats compared to the normal control animals.

The use of STZ in rats induces diabetic nephropathy evidenced by displayed elevated levels of serum creatinine, BUN, and blood urea which were taken as direct in vivo index for nephropathy in STZ-treated rats [21].

Administration of perindpril for 8 weeks in diabetic nephropathy rats significantly attenuated both biochemical and histological evidence of diabetic nephropathy.

These results were in agreement with [8] who reported that ACE represents a significant risk factor for the progressions of DN, and concluded that perindpril treatment ameliorate STZ induced diabetic nephropathy changes in DM rats.

Concerning the results about renoprotective effects of perindopril [22], reported that perindopril suppresses the apoptosis induced by endoplasmic
reticulum stress in renal tubules in experimental diabetic rats.

Also these results were in agreement with previous study Hanan et al., [6] which compared the effects of perindopril and colonidine on STZ induced DN in rats.

Administration of telmisartan for 8 weeks in diabetic nephropathy rats significantly attenuated both biochemical and histological evidence of diabetic nephropathy.

Telmisartan-therapy in this study showed a significant decrease in level of serum AGEPs compared to diabetic rats, and this is corresponding to the study of [23] who showed that treatment with nifedipine-telmisartan decreased AGEs and increased receptor of them in hypertensive rats.

The use of telmisartan in the current study showed significant decrease of serum urea and serum creatinine levels compared to diabetic non medicated group and is in agree with [24,19].

Zhang [25], found that the administration of telmisartan to diabetic rats significantly reduced 24-h urinary albumin, serum creatinine, and BUN, and increased creatinine clearance. Moreover, telmisartan can moderate kidney hypertrophy and renal histology in diabetic rats, provides superior reductions of proteinuria compared with ramipril and is effective in reducing renal endpoints.

The result in our study is in agree with [26] who showed that telmisartan significantly reduced urinary albumin excretion and improved the blood glucose level.

In a trial to explore the mechanism of the protective effect of perindopril and telmisartan we measured renal tissue contents of lipid peroxidation product (MDA) and RG.

The current study showed a significant increase of kidney tissue malondyaldehyde (MDA) level in diabetic non medicated group compared to the control group. This result was in agreement with those of [27,28].

Latha and Pari [29] have reported an increased lipid peroxidation in liver and kidney of diabetic rats.

Previous studies done in diabetic patients and diabetic rats reported that the persistent hyperglycemia could induce reactive oxygen species (ROS) that produces a marked oxidant impact as evidenced by the increased level of lipoperoxides (LPO) of diabetic animals than control non diabetic animals [19,30].

Administration of perindopril and telmisartan significantly decreased of tissue MDA and significant increase of RG levels.

This result was in accordance with [31] who reported that perindopril attenuates renal tubulointerstitial injury by inhibiting scavenger receptor A over-expression in diabetic rats. Scavenger receptor A (SR-A) is the main receptor through which oxidized LDL and advanced glycation end products get into the cells.

This result was in accordance with [32] in which telmisartan significantly decreased kidney tissue levels of MDA and increasing level of RG of diabetic rats which was elevated.

After 8 weeks of rats treatment with telmisartan there was significant reduction of kidney tissue MDA level and significant increased in GSH content and the catalase enzyme activity [33].

Moreover, the results run in consistence with [33,34] who reported that assessed oxidative stress in both diabetes and hypertension showing higher levels of MDA and significant decrease in MDA level after only one month of therapy with either lisinopril or telmisartan.

Antihypertensive drugs (All receptor antagonist) like losartan in rat have renoprotective effect partly independent of blood pressure lowering but related to the improvement of oxidative stress, hypoxia and AGEs products formation [35].

The results of the present study may provide an evidence for the beneficial effects of perindopril and telmisartan in improving DN with more significant effect of telmisartan in reducing FBG, AGEPS, blood urea, urine proteins and tissue MDA and increasing tissue RG level. These results run in agreement with [24] who compared between the nephroprotective effect of ARB, Telmisartan and ACE 15 benazepril demonstrating that both telmisartan and benazepril attenuate the development of proteinuria, prevent kidney structural injury and elevation of blood urea. But, telmisartan reduced elevated blood urea level more effectively as compared to benazepril.

These results may be explained by three different mechanisms: Firstly angiotensin-converting enzyme is a relatively nonspecific enzyme that has substrates in addition to angiotensin I, including bradykinin and other tachykinins. In contrast ARBs may offer more complete angiotensin II inhibition
by interacting selectively with the receptor site [36]. Second the major angiotensin receptor subtypes, AT1 and AT2, usually mediate opposite actions, such as AT1-mediated vasoconstriction while AT2-mediated vasodilatation. Blockade of AT1 receptors leads to a compensatory increase in angiotensin II levels and the subsequent increased activation of AT2 receptors [37]. Third there is structural resemblance between telmisartan and pioglitazone which is a peroxisome proliferators activated receptor Y (PPARY) agonist that approved for the treatment of type II diabetes [38].

Inhibition of ACE may results in accumulation of bradykinin and other tachykinins producing bradykinin-mediated side effects, such as cough and angioedema [36].

There are not many pharmacological options to treat diabetic nephropathy, ACEIs and/or ARBs are currently the only drugs that effectively slow the progression of diabetic nephropathy [39].

Now ACE inhibitors are first choice drugs for diabetic nephropathy. These results concluded that ARB are equieffective and may be more potent than ACE inhibitor and can be used as a first line drug to retard the progression of DN and with the use of ARBs we can prevent the side effects of ACE inhibitors.

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References


