MORPHOLOGICAL EFFECT OF COMBINATION OF FENOFIBRATE AND SAXAGLIPTIN ON KIDNEY OF DIABETIC RATS

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INTRODUCTION

Diabetes mellitus, a metabolic syndrome is known to trigger various complications including retinopathy, neuropathy, nephropathy etc. Diabetic nephropathy is the leading cause of end stage renal disease and becoming a serious social and health problem worldwide. In clinical practice, functional parameters such as albuminuria, serum creatinine and blood urea nitrogen are commonly used marker of diabetic nephropathy. Although numerous experimental effort have been made to develop ground-breaking therapeutic concepts in preventing the development and progression of diabetic nephropathy. But in recent reports most attention has focused on functional markers and has drawn attention from structural markers. Interestingly, structural changes may already be present once micro-albuminuria or other markers occurs. It’s worth mentioning that diabetic nephropathy is characterized by a series of ultra structural changes in all renal compartments and the hallmarks of diabetic nephropathy include glomerular hyper-filtration, thickening of glomerular basement membrane, expansion of extracellular matrix in mesangial areas which ultimately results in functional consequences, including progressive albuminuria, and reduction in glomerular filtration rate [1-3]. Thereby, the correlation between structural and functional consequences coerce for more insightful on structural marker for evaluating any pharmacological agent for its renoprotective potential. Thereby, the present study has been designed to investigate the effect of combination of PPAR agonist (fenofibrate) and DPP-4 inhibitor (saxagliptin) on detailed pathological changes for their direct renoprotective effect in the kidney of diabetic rats.

METHODS

After 8 weeks of streptozotocin administration the kidneys were excised and sections of 3-5 μm in thickness were made and stained with H&E and PAS using standard histologic procedures to assess mesangial cell expansion; tubular injury and glomerular basement membrane thickening using light microscopy. In addition, kidney/body weight ratio was assessed.

RESULTS

The increased kidney/body weight ratio, glomerular basement membrane thickening, tubular injury, mesangial matrix expansion, interstitial inflammation and tubular atrophy were observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Concurrent administration of fenofibrate (30 mg/kg p.o., 7 weeks) and saxagliptin (3 mg/kg p.o., 7 weeks) markedly prevent the interstitial inflammation and tubular atrophy in the diabetic kidney as compared to treatments with lisinopril (1 mg/kg, p.o., 7 weeks) in diabetic rats.

CONCLUSION

The present study provides the evidence for direct nephroprotective effect of combination of fenofibrate and saxagliptin. However, long-term clinical studies demonstrating their rationale in preventing diabetic nephropathy are mandatory.

KEYWORDS: Diabetes, DPP-4 inhibitor, Glomerular basement membrane, mesangial expansion, PPAR-α agonist.
MATERIAL AND METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched young wistar rats weighing about 200-240 g were employed in the present study. Rats were fed on standard chow diet and water ad libitum. They were acclimatized in institutional animal house and were exposed to normal cycles of day and night.

Drugs and chemicals

Streptozotocin was obtained from Sigma-Aldrich Ltd., St. Louis, USA. 1,1,3,3-tetra methoxy propane and carboxymethyl cellulose were purchased from R. K. Enterprises, Meerut, India. Fenofibrate was obtained from Ranbaxy Laboratory Ltd., Gurgaon, India. Saxagliptin was obtained from Bristol-Myers Squibb, Mumbai, India. Lisinopril was obtained from Dr. Reddy’s Laboratory Ltd., Hyderabad, India. All other chemicals used in the present study were of analytical grade.

Induction and assessment of diabetes

The experimental diabetes mellitus was induced in rats by single injection of streptozotocin (STZ) (55 mg/kg, i.p.) dissolved in freshly prepared ice cold citrate buffer of pH 4.5. The blood sugar level was monitored once daily for first week after administration of STZ. Then, at the end of the experimental protocol (8 weeks after administration of STZ), the blood samples were collected and serum was separated.

The serum samples were frozen until analysing the biochemical parameters. The serum glucose concentration was estimated by glucose oxidase peroxidase (GOD-POD) method [13] using the commercially available kit (Transasia Bio-Medical Ltd., Solan, India).

Histopathological study

The early diabetic changes in glomeruli were assessed histologically as previously described [14, 15]. The kidney was excised and immediately immersed in 10% formalin. The sections from kidney were dehydrated in graded concentrations of alcohol, immersed in xylene and then embedded in paraffin.

From the paraffin blocks, sections of 3-5 μm thickness were made and stained with hematoxylin-eosin and periodic acid-Schiff (PAS) using standard histologic procedures to assess the pathological changes occur in kidney using light microscopy.

Experimental protocol

Eight groups were employed in the present study and each group comprised 6 rats. The fenofibrate and saxagliptin were suspended in 0.5% w/v of carboxy methyl cellulose (CMC). Group I (Normal Control) rats were maintained on standard food and water and no treatment was given. Group II (Diabetic Control) rats were administered STZ (55 mg/kg, i.p., once) dissolved in citrate buffer (pH 4.5). Group III (Fenofibrate per se), the normal rats were administered fenofibrate (30 mg/kg, p.o.) suspended in 0.5% w/v of CMC for 7 weeks. Group IV (Saxagliptin per se), the normal rats were administered saxagliptin (3 mg/kg, p.o.) suspended in 0.5% w/v of CMC for 7 weeks. Group V (Fenofibrate Treated), the diabetic rats, after 1 week of STZ administration, were treated with low dose of fenofibrate (30 mg/kg, p.o.) for 7 weeks. Group VI (Saxagliptin Treated), the diabetic rats, after 1 week of STZ administration, were treated with saxagliptin (3 mg/kg, p.o.) for 7 weeks. Group VII (Fenofibrate + Saxagliptin Treated), the diabetic rats, after 1 week of STZ administration, were treated with the combination of low dose of fenofibrate (30 mg/kg, p.o.) and saxagliptin (3 mg/kg, p.o.) for 7 weeks. Group VIII (Lisinopril Treated), the diabetic rats after 1 week of STZ administration, were treated with lisinopril (1 mg/kg, p.o.) for 7 weeks.

Statistical analysis

All values were expressed as mean ± SD. The data obtained from various groups were statistically analysed using one way ANOVA, followed by Tukey’s multiple comparison test. The p value of less than 0.05 was considered to be statistically significant and the p values were of two tailed.

RESULTS

Administration of fenofibrate at lower dose (30 mg/kg p.o., 7 weeks) or saxagliptin (3 mg/kg p.o., 7 weeks) did not produce any significant per se effect on various parameters assessed in normal rats. Administration of streptozotocin (STZ) (55 mg/kg, i.p., once) produced hyperglycemia after 72 h (serum glucose >180mg/dL). After 7 days of STZ administration, the rats showed blood glucose level of greater than 270 mg/dL were selected and were named as diabetic rats. Fenofibrate (30 mg/kg, p.o., 7 weeks), saxagliptin (3 mg/kg, p.o., 7 weeks) and lisinopril (1 mg/kg, p.o., 7 weeks) were administered to diabetic rats after 7 days of single injection of STZ and their treatments were continued for 7 weeks. All the parameters were assessed at the end of 7 weeks in normal and diabetic rats with or without drug treatments. Less than 12% of mortality rate was observed in diabetic rats with or without drug treatments.

Effect of pharmacological interventions on serum glucose

The marked increase in serum concentration of glucose was noted in diabetic rats as compared to normal rats. Treatment with fenofibrate (30 mg/kg, p.o., 7 weeks) did not affect the serum glucose concentration in diabetic rats. However, treatment with saxagliptin (3 mg/kg, p.o., 7 weeks) incompletely reduced the elevated glucose level in diabetic rats. In addition, the effect of saxagliptin in incompletely reduction of elevated serum glucose level in diabetic rats was not altered by its combination with fenofibrate (30 mg/kg, p.o., 7 weeks). The lisinopril (1 mg/kg, p.o., 7 weeks) treatment slightly lowered the glucose level, but the results were not statistically significant (Figure 1).

Effect of pharmacological interventions on bodyweight, kidney weight and kidney/body weight ratio

Diabetic rats after 7 weeks (8 weeks after STZ administration) showed decrease in body weight as compared to normal rats. In addition, the kidney weight of diabetic rats was noted to be increased as compared to normal rats. Treatment with either fenofibrate (30 mg/kg, p.o., 7 weeks) or saxagliptin (3 mg/kg, p.o., 7 weeks) partially prevented the diabetes-induced decrease in body weight and increase in kidney weight. The concurrent administration of fenofibrate (30 mg/kg, p.o., 7 weeks) and saxagliptin (3 mg/kg, p.o., 7 weeks) markedly attenuated the diabetes-induced decrease in body weight and increase in kidney weight as compared to treatments with either drug alone or lisinopril (1 mg/kg, p.o., 7 weeks) in diabetic rats (Table 1). Increased kidney/body weight ratio in the diabetic rats as compared to the normal rats was found to be reversed by the concurrent administration of fenofibrate (30 mg/kg, p.o., 7 weeks) and saxagliptin (3 mg/kg, p.o., 7 weeks) as compared to treatments with either drug alone in diabetic rats (Figure 2).
Table 1: Effect of fenofibrate (feno), saxagliptin (saxa) and lisinopril on body weight and kidney weight

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Feno per se</th>
<th>Saxa per se</th>
<th>Feno Treated Diabetic Group</th>
<th>Saxa Treated Diabetic Group</th>
<th>Feno + Saxa Treated Diabetic Group</th>
<th>Lisino Treated Diabetic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm)</td>
<td>231.66 ± 8.16</td>
<td>182.5 ± 10.36a</td>
<td>226.66 ± 9.35</td>
<td>275 ± 7.35</td>
<td>199.16 ± 9.70a</td>
<td>205.83 ± 5.24b</td>
<td>217.5 ± 6.89b</td>
<td>207.5 ± 5.09b</td>
</tr>
<tr>
<td>Kidney weight (gm)</td>
<td>0.922 ± 0.08</td>
<td>1.342 ± 0.068*</td>
<td>0.904 ± 0.068</td>
<td>0.903 ± 0.051</td>
<td>1.300 ± 0.051b</td>
<td>1.272 ± 0.059</td>
<td>1.128 ± 0.081b</td>
<td>1.246 ± 0.082</td>
</tr>
</tbody>
</table>

All values are represented as mean ± SD. a = p < 0.05 versus normal control; b = p < 0.05 versus diabetic control.

The glomerular basement membrane thickening and tubular injury was observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Treatment with concurrent administration of fenofibrate (30 mg/kg p.o., 7 weeks) and saxagliptin (3 mg/kg p.o., 7 weeks) markedly protected the diabetic kidney by halting the glomerular basement membrane thickening and tubular injury as compared to treatments with lisinopril (1 mg/kg, p.o., 7 weeks) in diabetic rats (Figure 3).

Effect of pharmacological interventions on mesangial matrix expansion

The mesangial matrix expansion was observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Treatment with concurrent administration of fenofibrate (30 mg/kg p.o., 7 weeks) and saxagliptin (3 mg/kg p.o., 7 weeks) markedly prevent the mesangial matrix expansion in the diabetic kidney as compared to treatments with lisinopril (1 mg/kg, p.o., 7 weeks) in diabetic rats (Figure 4).

Effect of pharmacological interventions on glomerular basement membrane and tubular injury

Fig. 2: Effect of combination of fenofibrate and saxagliptin on kidney/body weight ratio. a = p < 0.05 versus normal control; b = p < 0.05 versus diabetic control; c = p < 0.05 versus fenofibrate treated diabetic group; d = p < 0.05 versus saxagliptin treated diabetic group.

Fig. 3: Effect of combination of fenofibrate and saxagliptin on glomerular basement membrane and tubular injury.

Fig. 4: Effect of combination of fenofibrate and saxagliptin on mesangial matrix expansion.
Effect of pharmacological interventions on interstitial inflammation and tubular atrophy

The interstitial inflammation and tubular atrophy was observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Concurrent administration of fenofibrate (30 mg/kg p.o., 7 weeks) and saxagliptin (3 mg/kg p.o., 7 weeks) markedly prevent the interstitial inflammation and tubular atrophy in the diabetic kidney as compared to treatments with lisinopril (1 mg/kg, p.o., 7 weeks) in diabetic rats (Figure 5).

DISCUSSION

In the present study, we corroborate our previous report that combination of fenofibrate and saxagliptin affords renoprotective potential by preventing the development and progression of diabetic nephropathy. Present data expands our previous reports by illustrating effect of combination of fenofibrate and saxagliptin on detailed morphological study in the kidney of diabetic rats. The major structural markers of diabetic nephropathy, namely renal enlargement; mesangial cell expansion; tubular injury and gomerular basement membrane (GBM) thickening were noted in the kidney of diabetic rats (Figure 5).

REFERENCES


