Effect of beraprost sodium on renal function and p38MAPK signaling pathway in rats with diabetic nephropathy

Liangxiao Xie¹, Jiajia Dong¹, Jinzhi Wu¹, Changshun Wei¹, Kezhen Xu², Pengbin Lai¹*, Xiaoyun Zha¹

¹Department of Endocrinology and Metabolism, ²Department of Pharmacy, Zhangzhou Affiliated Hospital of Fujian Medical University, Zhangzhou 363000, Fujian Province, China

*For correspondence: Email: ptrx35@163.com; Tel: +86 05962081196

Original Research Article

Abstract

Purpose: To investigate the effect of beraprost sodium (BPS) on renal function and P38MAPK pathway in diabetic nephropathy (DN) rats.

Methods: Sprague Dawley (SD) rats (n = 30) were randomly divided into three groups, viz, normal control (NC), diabetic nephropathy (DN) and beraprost sodium (BPS). Creatinine (Cr), blood urea nitrogen (BUN), albumin (AU), and fasting blood glucose (FBG), were determined by Hitachi 7020 automatic biochemical analyzer, while low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and total cholesterol (TC) were measured by Olympus 400 automatic biochemical analyzer. Western blot analysis was performed to examine protein expression. Interleukin-6 (IL-6), hs-CRP, and TNF-α levels were evaluated using enzyme linked immunosorbent assay (ELISA).

Results: After 8 weeks of treatment, renal function indices (urine output, KW/BW, UAlb/24 h, Cr and BUN), blood lipid indices (FBG, LDL-C, TG and TC) and inflammatory factors levels (IL-6, hs-CRP and TNF-α) in DN group were higher than NC group (p < 0.05). In BPS group, renal function and blood lipid indices and inflammatory factor levels decreased when compared to DN group (p < 0.05). Furthermore, BPS inhibited the protein expression of p-P38MAPK, TGF-β1 and COX-2.

Conclusion: Beraprost sodium improves renal function in DN rats by inhibiting P38MAPK signaling pathway.

Keywords: Renal function, Diabetic nephropathy, Inflammatory factors, Beraprost sodium, P38MAPK signaling pathway

INTRODUCTION

Diabetic nephropathy (DN) is a severe microvascular complication of diabetes, which refers to chronic kidney disease caused by diabetes. Diabetic nephropathy (DN) can lead to proteinuria, edema and hypertension [1]. The causes of DN include changes in renal hemodynamics, oxidative stress, metabolic status, genetic factors, and immune inflammatory factors [2]. Diabetic nephropathy (DN) occurs mostly in diabetic patients, smokers and
hyperlipidemia. The treatment of DN includes early intervention of various risk factors and renal replacement therapy for end-stage renal disease. Diabetic nephropathy (DN) requires long-term continuous treatment. Prognosis of DN patients is poor [3]. Therefore, it is very important to find drugs with good curative effect for patients with DN. The mitogen-activated protein kinase (MAPK) family can regulate important physiological activities such as gene expression, cell proliferation and apoptosis [4]. The protein P38MAPK is a vital member of the MAPK family, which can influence the inflammatory process. It has been proposed that P38MAPK pathway may be an important pathogenesis of DN [5]. At present, many studies have found that activation of P38MAPK can promote the production and activation of inflammatory factors [6]. Abnormal production of chemokines and inflammatory factors increases the phosphorylation level of P38MAPK, thereby further activating the P38MAPK pathway [7]. The activation of P38MAPK pathway can promote mesangial cell proliferation and renal tubular fibrosis. Fibrosis causes the accumulation of renal inflammatory cells and aggravates the inflammatory response [8]. Increased inflammation leads to kidney damage.

Beraprost sodium (BPS) is a stable, orally active prostaglandin I2 analog with vasodilator and antiplatelet properties. It can increase renal blood flow, dilates renal blood vessels, and prevent glomerular thrombosis [9]. Beraprost sodium (BPS) also inhibits diabetes development and its complications (nephropathy) in obese Zucker rats [10]. These studies indicate that BPS may be very effective in preventing and treating the microvascular complications of diabetes. Nonetheless, the specific effect of BPS in DN is not yet known.

Therefore, the effects of BPS on the renal function and P38MAPK signaling pathway in DN rats were investigated in this study. In addition, the influence of BPS on blood lipids and inflammatory factors was investigated.

EXPERIMENTAL

Animals

The study was approved by the Animal Ethics Committee of Yantai Langdi Biotechnology Co. Ltd (approval no. 2019-66) and met the criteria of laboratory animal care [17]. Thirty specified pathogens free (SPF) healthy male Sprague Dawley (SD) rats were kept in a suitable environment (12-hour light, 55 ± 2 % humidity, 21 ± 2 °C). All rats (180 ± 20 g) had free access to drinking water and feed for 7 days. Thereafter, they were randomly divided into NC, DN and BPS treatment groups (n = 10). The insulin resistance model and BPS treatment were prepared based on previous literature [11]. Finally, all rats were sacrificed by tail vein injection of sodium pentobarbital (50 mg/kg) after 8 weeks of treatment and kidney specimens were collected.

Determination of renal function index

After 8 weeks of treatment, 24 h urine of rats in each group was collected. Urine microalbumin (UAlb)/24 h was measured by Coomassie brilliant blue method. The rats were weighed before sacrificing. The left kidney was removed quickly, and the renal cortex was stored in liquid nitrogen. Hitachi 7020 automatic biochemical analyzer was used to measure the fasting blood glucose (FBG), Cr and BUN levels.

Blood sample collection and measurement of blood index

All rats were anesthetized by intraperitoneal injection of 10 % chloral hydrate (4 mg/kg). Blood was taken from abdominal aorta. The blood sample was anticoagulated with EDTA. Blood index (TG, TC, LDL-C and HDL-C) were measured according to previous study [12].

Assessment of inflammatory factors

The levels of IL-6, TNF-α and hs-CRP were measured by ELISA. The experimental steps were performed according to the kit instructions (Mibio, Shanghai, China).

Western blot analysis

The experimental steps were carried out according to a previous study [13]. All antibodies were purchased from Abcam (Shanghai, China).

Statistical analysis

Data are analyzed using SPSS 22.0 software and shown as \( \bar{x} \pm SD \). The comparison was performed using One-way ANOVA. Significant difference was indicated at \( p < 0.05 \).

RESULTS

Renal function and urine output indices

Compared to NC group and BPS group, the weight of rats in DN group decreased (\( p < 0.05 \); Table 1). However, the weight has no significant difference between NC and BPS group (\( p > 0.05 \),
Xie et al

Table 1). Compared to NC group, urine output, kidney weight to body weight (KW/BW), BUN, Cr, and UAlb/24 h levels in DN group were elevated (p < 0.05, Table 1). Compared to DN group, Cr, UAlb/24 h, KW/BW and urine output levels in BPS group were reduced significantly (p < 0.05, Table 1). Compared to DN group, BUN level did not change significantly (p > 0.05; Table 1). These findings demonstrate that BPS nitrated kidney damage in DN rats and improved renal function.

Blood lipid and blood glucose profile

Compared to NC group, FBG, LDL-C, TC, and TG levels in DN group were increased, while HDL-C level was decreased (p < 0.05, Table 2). Compared to DN group, FBG, TC and in BPS group were reduced (p < 0.05; Table 2). Compared to NC group, FBG, TG and TC levels in BPS group were still high (p < 0.05; Table 2). The results show that BPS reduced blood glucose and blood lipids in DN rats.

Inflammatory factors

Compared to NC group, TNF-α, IL-6 and hs-CRP levels in DN group were increased (p < 0.05; Table 3). However, compared to DN group, the three inflammatory factors were obviously decreased in BPS group (p < 0.05, Table 3). Compared to NC group, TNF-α, IL-6 and hs-CRP levels in BPS group were still high (p < 0.05; Table 3). These findings demonstrate that BPS may reduce inflammation through the reduction of inflammatory factors levels.

Effect of BPS on p38MAPK signaling pathway

Compared to NC group, TGF-β1, COX-2 and p-P38MAPK protein expressions in DN group were elevated (p < 0.05, Figure 1 and Table 4). Compared to DN group, TGF-β1, COX-2 and p-P38MAPK protein expressions in BPS group were decreased (p < 0.05, Figure 1 and Table 4). However, p-P38MAPK, COX-2 and TGF-β1 expressions in BPS group were still higher than NC group (p < 0.05; Figure 1 and Table 4). These results indicate that BPS may reduce the renal damage caused by DN by affecting the expression of p38MAPK signaling pathway.

Figure 1: Effect of BPS on p38MAPK signaling pathway. The protein expressions of p-p38 MAPK, t-p38MAPK, TGF-β1 and COX-2 was compared between DN group and NC group or BPS group. *p < 0.05 (NC vs DN), #p < 0.05 (DN vs BPS)

Table 1: Comparison of urine output and renal function indices (mean ± SD)

<table>
<thead>
<tr>
<th>Index</th>
<th>NC group</th>
<th>DN group</th>
<th>BPS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>454.25 ± 31.23</td>
<td>351.16 ± 36.05*</td>
<td>424.64 ± 40.58*</td>
</tr>
<tr>
<td>KW/BW (g/mg)</td>
<td>3.26 ± 0.21</td>
<td>5.96 ± 0.35*</td>
<td>5.01 ± 0.89*</td>
</tr>
<tr>
<td>Urine output (ml)</td>
<td>32.68 ± 10.97</td>
<td>72.01 ± 14.42*</td>
<td>48.04 ± 15.54*</td>
</tr>
<tr>
<td>UAlb/24 h (mg)</td>
<td>4.57 ± 1.23</td>
<td>16.26 ± 1.52*</td>
<td>13.60 ± 1.02*</td>
</tr>
<tr>
<td>Cr (μmol/L)</td>
<td>48.05 ± 13.84</td>
<td>88.33 ± 18.56*</td>
<td>64.33 ± 14.38*</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>12.56 ± 2.04</td>
<td>26.54 ± 3.08*</td>
<td>24.14 ± 2.68*</td>
</tr>
</tbody>
</table>

Compared with the NC group, *p < 0.05; compared with the DN group, #p < 0.05

Table 2: Levels of blood glucose and blood lipids (mean ± SD)

<table>
<thead>
<tr>
<th>Index</th>
<th>NC group</th>
<th>DN group</th>
<th>BPS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mM)</td>
<td>6.03 ± 0.47</td>
<td>22.58 ± 1.62*</td>
<td>16.87 ± 1.02*</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.41 ± 0.47</td>
<td>2.36 ± 0.85*</td>
<td>2.05 ± 0.67*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>2.38 ± 0.34</td>
<td>3.52 ± 0.51*</td>
<td>3.11 ± 0.54*</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.25 ± 0.25</td>
<td>0.89 ± 0.21*</td>
<td>1.09 ± 0.19*</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.49 ± 0.11</td>
<td>1.18 ± 0.34*</td>
<td>0.72 ± 0.23*</td>
</tr>
</tbody>
</table>

Compared with the NC group, *p < 0.05; compared with the DN group, #p < 0.05

Table 3: Comparison of inflammatory factors levels (mean ± SD)

<table>
<thead>
<tr>
<th>Index</th>
<th>NC group</th>
<th>DN group</th>
<th>BPS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ng/L)</td>
<td>23.05 ± 1.26</td>
<td>58.03 ± 4.15*</td>
<td>41.69 ± 5.16*</td>
</tr>
<tr>
<td>hs-CRP (μg/L)</td>
<td>1.01 ± 0.20</td>
<td>2.68 ± 0.12*</td>
<td>1.92 ± 0.11*</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>39.85 ± 3.67</td>
<td>88.41 ± 5.95*</td>
<td>65.23 ± 4.25*</td>
</tr>
</tbody>
</table>

Compared with the NC group, *p < 0.05; compared with the DN group, #p < 0.05

Trop J Pharm Res, May 2022; 21(5): 941
Table 4: Comparison of p-p38 MAPK, t-p38MAPK, TGF-β1 and COX-2 expression (mean ± SD)

<table>
<thead>
<tr>
<th>Index</th>
<th>NC group</th>
<th>DN group</th>
<th>BPS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-P38 MAPK/β-actin</td>
<td>0.86 ± 0.03</td>
<td>1.30 ± 0.12*</td>
<td>1.11 ± 0.06*</td>
</tr>
<tr>
<td>t-P38 MAPK/β-actin</td>
<td>1.12 ± 0.02</td>
<td>1.11 ± 0.01</td>
<td>1.10 ± 0.01</td>
</tr>
<tr>
<td>TGF-β1/β-actin</td>
<td>1.11 ± 0.02</td>
<td>1.28 ± 0.11*</td>
<td>1.16 ± 0.09*</td>
</tr>
<tr>
<td>COX-2/β-actin</td>
<td>0.71 ± 0.04</td>
<td>1.09 ± 0.01*</td>
<td>0.89 ± 0.03*</td>
</tr>
</tbody>
</table>

Compared with the NC group, *p < 0.05. Compared with the DN group, #p < 0.05

DISCUSSION

The pathogenesis of DN is complicated. Long-term hyperglycemia promotes microvascular complications, accelerate pathological changes in the kidneys, and leads to the release of inflammatory factors, changes in blood rheology, and disorders of glucose and lipid metabolism. It has been shown that persistent high blood sugar in patients with DN can easily cause changes in renal hemorheology, further aggravating kidney disease [14]. This study found that the blood glucose and blood lipid levels in DN group were evaluated compared to NC group. However, blood glucose and blood lipids levels after interventional treatment with BPS were reduced compared to DN group. These results indicate that BPS improved blood rheology in DN rats and protect the kidneys.

Inflammation is involved in the evolution of DN. Tumor necrosis factor-α (TNF-α) can strengthen the inflammatory response and aggravates the damage of blood vessels [15]. It has been demonstrated that hs-CRP promotes the formation of atherosclerotic plaques [16]. Interleukin-6 (IL-6) acts as an anti-inflammatory myosin and pro-inflammatory cytokine [17]. The hs-CRP, IL-6 and TNF-α levels in DN group were found to be increased. After BPS treatment, hs-CRP, IL-6 and TNF-α levels were decreased. The findings indicate that BPS can treat inflammation in DN rats.

At present, P38MAPK pathway has been found to participate in the pathogenesis of DN. As we all know, p-P38MAPK represents the activity of the P38MAPK signaling pathway [18]. The p-P38MAPK protein expression was found to be increased in DN group compared to NC group, indicating that P38MAPK signaling pathway was activated in the renal cortex of diabetic rats. However, p-P38MAPK protein expression in BPS group was reduced compared to DN group. In the DN group, it was also found that P38MAPK pathway was activated, renal function decreased, and related inflammatory factor levels were increased. These findings imply that BPS may impede the production of inflammatory factors by inactivating P38MAPK signaling pathway, thereby protecting kidney.

CONCLUSION

These results indicate that BPS improves renal function and reduces blood glucose, blood lipids and inflammatory factors levels in DN rats. Moreover, BPS reduced the renal damage caused by DN by repressing p38MAPK signaling pathway. These findings are helpful for us to understand the mechanism of BPS in DN.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Liangxiao Xie and Pengbin Lai conceived and designed the study, and drafted the manuscript. Liangxiao Xie, Jiajia Dong, Jinzhi Wu, Changshun Wei, Kezhen Xu and Xiaoyun Zha collected, analyzed and interpreted the experimental data. Jiajia Dong and Pengbin Lai revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/subscribe), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES


