Protective effect of calcium dobesilate combined with benazepril therapy on renal injury in patients with early diabetic nephropathy and the possible molecular mechanisms

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Objective: To explore the protective effect of calcium dobesilate combined with benazepril therapy on renal injury in patients with early diabetic nephropathy and the possible molecular mechanisms.

Methods: A total of 50 patients with early diabetic nephropathy treated in our hospital between May 2012 and January 2016 were collected, and according to the random number table, the patients were divided into observation group (n=25) and control group (n=25). On the basis of conventional treatment, control group of patients received benazepril therapy, observation group of patients received calcium dobesilate combined with benazepril therapy, and the treatment lasted for 3 months. Before and after treatment, automatic biochemical analyzer was used to detect the levels of renal injury indexes in peripheral blood, RIA method was used to detect the levels of renal injury indexes in urine, ELISA method was used to detect the levels of renal fibrosis indexes and Western-blot method was used to detect the protein expression of TGF-β1/BMP-7 and Smad signaling pathway molecules in renal tissue.

Results: Before treatment, differences in renal injury index levels, renal fibrosis index levels and signaling pathway molecule protein expression were not statistically significant between two groups of patients. After treatment, BUN, SCr and β-TP levels in the peripheral blood as well as KIM-1 level in urine of observation group were lower than those of control group; renal fibrosis indexes TGF-β1, CTGF, TIMP-1, LN and HA levels in serum of observation group were lower than those of control group; TGF-β1 and Smad2/3 protein expression in renal tissue of observation group were lower than those of control group while Smad7 and BMP-7 protein expression were higher than those of control group. Conclusion: Calcium dobesilate combined with benazepril therapy can reduce the renal injury and inhibit the fibrosis process in patients with early diabetic nephropathy, and it achieves the above effect by regulating the TGF-β1/BMP-7 and Smad signaling pathway function.

1. Introduction

Diabetic nephropathy (DN) is the most common kidney disease complication of diabetes, early diabetic nephropathy (EDN) is the most commonly studied disease stage in clinical practice, there has been a certain degree of urinary protein in patients in the stage, but the condition is still reversible, and active treatment measures should be taken to avoid the occurrence of end-stage renal disease[1-2]. Benazepril belongs to angiotensin-converting enzyme inhibitor and can be used for the treatment of hypertension, congestive heart failure and other diseases, diabetic patients are mostly complicated with different degree of hypertension, the severely fluctuating blood glucose and blood pressure levels can increase the patients’ renal injury, and the vascular dilation effect of benazepril can ease the body’s renal injury to a certain extent[3]. Current study has shown that the benazepril is more effective to dilate large vessels, its improvement on microcirculation is limited, and therefore,
other microcirculation-improving drugs need to be added in the treatment of diabetic nephropathy to expand the curative effect. The major component of calcium dobesilate is calcium dobesilate, it is applicable to the treatment of all kinds of microvascular lesions and varicose vein syndrome, and some scholars have currently proposed to use the drug for adjuvant treatment of early diabetic nephropathy\[4\]. In the study, calcium dobesilate combined with benazepril was used for the treatment of patients with EDN in our hospital, and the renal injury and molecular mechanisms were elaborated, now reported as follows:

2. Information and methods

2.1 General information

A total of 50 patients with early diabetic nephropathy treated in our hospital between May 2012 and January 2016 were included, and the patients themselves signed informed consent. According to the random number table, the patients were divided into observation group (n=25) and control group (n=25). Observation group included 14 male cases and 11 female cases, they were 49-78 years old, the course of diabetes was 8-25 years and (14±2) years in average, and the course of diabetic nephropathy was 5 months-2 years and 1 year in average; control group included 13 male cases and 12 female cases, they were 50-76 years old, the course of diabetes was 9-23 years and (14±2) years in average, and the course of diabetic nephropathy was 6 months-3 years and 1 year in average. Two groups of patients were not statistically different in the distribution of gender, age, course of diabetes and course of diabetic nephropathy (P>0.05), and the research was approved by hospital ethics committee.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) clearly diagnosed with type 2 diabetes; (2) urine albumin excretory rate (UAE) 20-200 μg/min; (3) with glomerular nodule-like lesions after renal biopsy; (4) ≤80 years old. Exclusion criteria: (1) with kidney neoplasms, hydronephrosis, kidney stones and other primary kidney diseases; (2) with secondary hypertension caused by hyperaldosteronism, pheochromocytoma and so on; (3) allergic to calcium dobesilate and (or) benazepril; (4) dropping out the treatment and with incomplete clinical data.

2.3 Treatment methods

Both groups of patients received regular hypoglycemic and antihypertensive therapy, and the control group of patients received captopril treatment on the basis, which was as follows: oral administration of benazepril hydrochloride tablet (Beijing Novartis Pharmaceutical Co., LTD., approved by H20000712), 20 mg/time, 1 time/d, for continuous 3 months of treatment.

On the basis of conventional treatment, the observation group received calcium dobesilate combined with benazepril hydrochloride tablet treatment, which was as follows: oral administration of calcium dobesilate (Xi’an Lijun Pharmaceutical Co., LTD., approved by H20000712), 500 mg/time, 3 times/d; benazepril hydrochloride tablet usage and dosage were the same as those of control group, and the treatment lasted for three months.

2.4 Observation indexes

2.4.1 Renal injury indexes

Before treatment and after 3 months of treatment, 1-2 mL of fasting cubital venous blood was extracted from two groups of patients and cryopreserved in a -20 °C refrigerator, and 24 h urine of two groups of patients was collected. Automatic biochemical analyzer (Abbott Laboratories LTD., model Aeroset) was used to detect blood urea nitrogen (BUN), serum creatinine (SCr) and \(\beta\)-trace protein (\(\beta\)-TP) levels, and RIA method was used to detect kidney injury molecule-1 (KIM-1) level in urine.

2.4.2 Renal fibrosis indexes

Before treatment and after 3 months of treatment, 1-2 mL of fasting cubital venous blood was extracted from two groups of patients, let stand at room temperature for 12 h and then centrifuged at low speed to get supernatant, and the enzyme-linked immunosorbent assay (ELISA) was used to detect the serum levels of renal fibrosis indexes, including transforming growth factor \(\beta\) 1 (TGF-\(\beta\) 1), connective tissue growth factor (CTGF), tissue inhibitor of metalloproteinase-1 (TIMP-1), laminin (LN) and hyaluronic acid (HA).

2.4.3 TGF-\(\beta\) 1/BMP-7 and Smad signaling pathway

Before treatment and after 3 months of treatment, the renal biopsy was performed, and Western-blot method was used to detect the TGF-\(\beta\) 1, Smad2/3, Smad7 and BMP-7 protein expression. The electrophoresis apparatus needed in operation process was purchased from Wuhan Shijie Youhong Technology Co., LTD., model SPIFE 4000; electric transmembrane apparatus was purchased from Thmorgan Company, and the model was TB10.

2.5 Statistical methods

SPSS 15.0 software was used for statistical processing, measurement data was in terms of Mean ± SD, comparison between two groups before and after treatment was by group t test, comparison before and after treatment was by paired t test and P<0.05 meant statistical significance in differences.
3. Results

3.1 Renal injury indexes

Comparison of renal injury indexes BUN (mmol/L), SCr (μmol/L), β-TP (mg/L) and KIM-1 (pg/mL) levels between two groups of patients was as follows: before treatment, differences in BUN, SCr and β-TP levels in the peripheral blood as well as KIM-1 level in urine were not statistically significant between two groups of patients (P>0.05). After treatment, BUN, SCr and β-TP levels in the peripheral blood as well as KIM-1 level in urine of both groups were lower than those before treatment, and differences within same group were statistically significant (P<0.05). After treatment, renal fibrosis indexes TGF-β1, CTGF, TIMP-1, LN and HA levels in serum of observation group were lower than those of control group, and differences between groups were statistically significant (P<0.05), shown in Table 1.

3.2 Renal fibrosis indexes

Comparison of renal fibrosis indexes TGF-β1 (μg/L), CTGF (μg/L), TIMP-1 (μg/L), LN (μg/L) and HA (μg/L) levels in serum between two groups of patients was as follows: before treatment, differences in renal fibrosis indexes TGF-β1, CTGF, TIMP-1, LN and HA levels in serum were not statistically significant between two groups of patients (P>0.05). After treatment, renal fibrosis indexes TGF-β1, CTGF, TIMP-1, LN and HA levels in serum of observation group were lower than those before treatment, and differences within same group were statistically significant (P<0.05). After treatment, renal fibrosis indexes TGF-β1, CTGF, TIMP-1, LN and HA levels in serum of observation group were lower than those of control group, and differences between groups were statistically significant (P<0.05), shown in Table 2.

3.3 TGF-β1/BMP-7 and Smad signaling pathway

Comparison of TGF-β1/BMP-7 and Smad signaling pathway molecule protein expression in renal tissue between two groups of patients was as follows: before treatment, differences in TGF-β1, Smad2/3, Smad7 and BMP-7 protein expression in renal tissue were not statistically significant between two groups of patients (P>0.05). After treatment, TGF-β1 and Smad2/3 protein expression in renal tissue of both groups were lower than those before treatment while Smad7 and BMP-7 protein expression were higher than those before treatment, and differences within same group were statistically significant (P<0.05). After treatment, TGF-β1 and Smad2/3 protein expression in renal tissue of observation group were lower than those of control group while Smad7 and BMP-7 protein expression were higher than those of control group, and differences between groups were statistically significant (P<0.05), shown in Table 3.

Table 1.
Comparison of renal injury index levels before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>BUN (mmol/L)</th>
<th>SCr (μmol/L)</th>
<th>β-TP (mg/L)</th>
<th>KIM-1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>25</td>
<td>Before treatment</td>
<td>15.38±1.93</td>
<td>124.37±14.69</td>
<td>1.18±0.16</td>
<td>54.82±6.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>6.38±0.75</td>
<td>78.66±8.93</td>
<td>0.67±0.07</td>
<td>31.24±3.95</td>
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<tr>
<td>Control</td>
<td>25</td>
<td>Before treatment</td>
<td>15.17±1.89</td>
<td>125.63±14.25</td>
<td>1.16±0.18</td>
<td>54.19±6.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>11.56±1.89</td>
<td>92.31±10.54</td>
<td>0.92±0.09</td>
<td>40.12±5.48</td>
</tr>
</tbody>
</table>

Note: compared with same group before treatment, *P*<0.05; compared with control group after treatment, *P*<0.05.

Table 2.
Comparison of renal fibrosis index levels in serum before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TGF-β1 (μg/L)</th>
<th>CTGF (μg/L)</th>
<th>TIMP-1 (μg/L)</th>
<th>LN (μg/L)</th>
<th>HA (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>25</td>
<td>Before treatment</td>
<td>120.38±14.27</td>
<td>15.82±1.79</td>
<td>583.29±67.55</td>
<td>160.73±17.29</td>
<td>154.82±17.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>75.86±8.19</td>
<td>9.05±0.98</td>
<td>312.84±40.62</td>
<td>109.37±13.28</td>
<td>102.37±12.48</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>Before treatment</td>
<td>120.38±14.27</td>
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</tr>
</tbody>
</table>

Note: compared with same group before treatment, *P*<0.05; compared with control group after treatment, *P*<0.05.

Table 3.
Comparison of TGF-β1/BMP-7 and Smad signaling pathway molecule protein expression in renal tissue before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>TGF-β1 Smad2/3 Smad7 BMP-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>25</td>
<td>Before treatment</td>
<td>98.23±10.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>71.38±7.12</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>Before treatment</td>
<td>97.59±10.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>85.49±9.05</td>
</tr>
</tbody>
</table>

Note: compared with same group before treatment, *P*<0.05; compared with control group after treatment, *P*<0.05.
4. Discussion

Benazepril is a common drug to treat patients with EDN, and many clinical studies have confirmed that it can increase the renal blood perfusion and improve kidney function. But study also shows that EDN is progressive, and the renal function is still deteriorating after patients receive benazepril therapy alone, indicating that other drugs with different mechanisms of action are needed for such patients in order to expand the overall efficacy and optimize treatment outcome[5]. Calcium dobesilate is also known as calcium dobesilate capsule, its active ingredient is water-soluble calcium dobesilate, it has multiple effects such as improving abnormal hemodynamics, inhibiting excessive oxidative stress and relieving glucose metabolism disorders in renal tissue, and it currently regarded as one of the reliable drugs for the treatment of EDN[6]. In the study, calcium dobesilate and benazepril were used as new treatment, and the therapeutic effect of the drug compatibility for patients with EDN and the possible mechanism were elaborated.

There is already urine protein excretion rate increase in patients with EDN, generally between 20-200 μg/min, persistent urine protein can further worsen kidney damage, the typical manifestation is the increased BUN and SCr levels in the circulating blood, and it is directly related to the impaired glomerular filtration as well as the middle molecular and macromolecular substance accumulation in the body[7,8]. β-TP is a kind of low molecular weight protein, and study has found that when the kidney function declines, the β-TP level in circulating blood increases significantly, so it is considered to be the sensitive and reliable index to reflect the renal injury degree[9]. KIM-1 is the transmembrane protein in renal proximal tubular epithelial cells, its expression in kidney tissue is little under physiological conditions, KIM-1 expression is vigorous after renal injury, and it can enter into the urine through the glomerular filtration membrane[10]. In the study, the levels of above renal injury indexes in the circulating blood and urine were detected, and it was found that compared with the control group of patients, the observation group of patients were with lower BUN, SCr and β-TP levels in circulating blood and lower KIM-1 level in urine after treatment, indicating that adding calcium dobesilate therapy on the basis of benazepril treatment can further optimize the renal function and reduce renal injury in patients with EDN.

Renal fibrosis is an important pathological mechanism of EDN, which is mainly characterized by glomerular hypertrophy, basement membrane thickening and extracellular matrix increasing[11,12]. After renal fibrosis, both hemodynamics and glomerular function will change, LN, HA and other mesenchyma increase, and glomerular filtration on them declines, which all lead to the increased serum LN and HA levels in the body. TGF-β1, CTGF and TIMP-1 mediate epithelial-mesenchymal transition and extracellular matrix accumulation, the TGF-β1 is considered as the most critical factor for glomerular sclerosis and renal tubular interstitial fibrosis, and it is also one of the latest EDN treatment targets[13]. In vitro study has shown that high glucose medium can increase the TGF-β1 expression in renal proximal tubules and glomerular cells. It has been confirmed that CTGF is involved in liver fibrosis process, it up-regulates the expression of extracellular matrix (ECM) to induce fibroblast proliferation, and some scholars speculate that it may also play an important role in the process of renal fibrosis[14]. TIMP-1 and matrix metalloproteinases (MMPs) maintain the dynamic balance of ECM together, MMPs expression increases when EDN occurs, and TIMP-1 also reactively increases to contain the excessive ECM production. In the study, serum levels of above fibrosis indexes were detected, and it was found that compared with the control group of patients, the observation group of patients were with lower serum TGF-β1, CTGF, TIMP-1, LN and HA levels after treatment, indicating that adding calcium dobesilate treatment can effectively restrain the renal fibrosis process, and this is associated with its specific inhibition on protein kinase C and TGF-β1.

The molecular mechanism of calcium dobesilate treatment of EDN is still not clear at present, it has been made clear that the drug helps to inhibit renal fibrosis process, and therefore, it is speculated that it might regulate the signaling pathways associated with fibrosis[15]. GF-β1/BMP-7 and Smad signaling pathways are the major signaling pathways of renal fibrosis, and their downstream factor expression imbalance can directly result in renal fibrosis and renal injury[16]. TGF-β1 is currently the most important pro-fibrosis factor that can increase the ECM synthesis and accumulation; Smad is the intracellular kinase substrate of TGF-β1 receptor, and mediates its intracellular signal transduction. Through transmembrane TGF-β1 receptors, the activated TGF-β1 further stimulates the downstream Smad2/3, make it phosphorylated and then transmit signals to the inside of cells. TGF-β1 activation also activates the Smad7, and it competes with Smad2/3 to be combined with TGF-β receptors, and then block TGF-β1 signaling pathway. BMP-7 is the most important anti-renal fibrosis factor in the body, it influences the TGF-β1/BMP-7 and Smad signaling pathways to antagonize renal fibrosis, and it has been found in rat models with DN that BMP-7 protein expression is low in kidney tissue[17,18]. In the study, the protein expression levels of the TGF-β1/BMP-7 and Smad signaling pathway downstream molecules were detected, and it was found that compared with the control group of patients, the observation group of patients were with lower TGF-β1 and Smad2/3 protein expression as well as higher Smad7 and BMP-7 protein expression in renal tissue, confirming that the effect of calcium dobesilate on resisting renal fibrosis and relieving renal injury is achieved by regulating TGF-β1/BMP-7 and Smad signaling pathways.
To sum up, it is concluded that calcium dobesilate combined with benazepril therapy can reduce the renal injury and inhibit the fibrosis process in patients with early diabetic nephropathy, it achieves the above effect by regulating TGF-β1/BMP-7 and Smad signaling pathway function, and it’s worth popularization and application in clinical practice in the future.

References


