Experimental study on Tangshenjiangzhuo granules treatment of rats with early diabetic nephropathy

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\textbf{ABSTRACT}

\textbf{Objective:} To study the effect of Tangshenjiangzhuo granules on serum cytokines as well as the oxidative stress and endoplasmic reticulum stress in kidney tissue of diabetic rats.

\textbf{Methods:} SPF male Wistar rats were selected as experimental animals and randomly divided into control group, model group and intervention group. Model group received intragastric administration of saline after diabetes model was established, and the intervention group received intragastric administration of Tangshenjiangzhuo granules after diabetes model was established. 8 weeks after treatment, serum was collected to detect the levels of pro-inflammatory cytokines and adipocytokines, and kidney tissue was collected to detect the levels of oxidative stress molecules and endoplasmic reticulum stress molecules.

\textbf{Results:} Serum interleukin-6 (IL-6), macrophage migration inhibitory factor (MIF), regulated upon activation normal T cell expressed secreted (RANTES), Chemerin and Nesfatin levels of model group were significantly higher than those of control group (\(P<0.05\)), and serum IL-6, MIF, RANTES, Chemerin and Nesfatin levels of intervention group were significantly lower than those of model group (\(P<0.05\)); reactive oxygen species (ROS), malondialdehyde (MDA), advanced oxidation protein products (AOPP), 8-hydroxy-2-deoxyguanosine (8-OHdG), glucose-regulated protein (GRP78), CCAAT/enhancer-binding protein homologous protein (CHOP) and ATF4 protein levels in kidney tissue of model group were significantly higher than those of control group (\(P<0.05\)), and ROS, MDA, AOPP, 8-OHdG, GRP78, CHOP and ATF4 levels in kidney tissue of intervention group were significantly lower than model group (\(P<0.05\)).

\textbf{Conclusions:} Tangshenjiangzhuo granules can adjust the secretion of pro-inflammatory factors and adipocytokines, and inhibit oxidative stress and endoplasmic reticulum stress in kidney tissue of diabetic rat models.

\section{1. Introduction}

Type 2 diabetes mellitus is the endocrine system disease with highest incidence in our country, and a variety of complications will occur in the development of disease. Diabetic nephropathy is one of the most common microvascular complications in patients with type 2 diabetes, it is also a common clinical cause of end-stage renal disease, there are no effective drugs for clinical treatment of diabetic nephropathy at present, strictly controlling blood glucose, improving microcirculation and other western medicine treatment means can delay the process of renal damage to a certain extent, but there are still some patients who fail to obtain exact curative effect, and the renal damage continues to progress\textsuperscript{[1,2]}. In recent years, Chinese patent drugs have displayed positive value in the treatment of chronic diseases, and also received more and more attention from clinical scholars. In the following study, based on professor Tong Xiao-lin’s experience in treatment of diabetic nephropathy, the main ingredients of component Tangshenjiangzhuo granules include astragalus, wine-treated rhubarb, salvia miltiorrhiza, leech, and so on. The existing modern pharmacological studies have confirmed that the above drugs have the exact cytoprotection, anti-inflammatory and antioxidant effect. In order to define the value of Tangshenjiangzhuo granules for treatment of early diabetic nephropathy, diabetic rats were selected as the research objects,
and the effect of Tangshenjiangzhuo granules on the microstructure damage in the kidney tissue was analyzed.

2. Materials and methods

2.1. Experimental materials

Experimental animals were 36 SPF male Wistar rats with body mass 200–250 g, and they were provided by the laboratory animal center of North China University of Science and Technology; high sugar and high fat diet was bought from Beijing Keaoxieli feed, streptozotocin was purchased from the Sigma Company, glucometer and test paper were purchased from LifeScan Inc Company, and the enzyme-linked immunosorbent assay kits were purchased from Shanghai Westang Biotechnology Company.

2.2. Animal grouping and model establishment methods

2.2.1. Components of Tangshenjiangzhuo granules

Astragalus 15 g, wine-treated rhubarb 10 g, salvia miltiorrhiza 15 g, herba artemisiae 10 g, red peony root 10 g, rhizoma anemarrhenae 15 g, rhizoma codonopsis 10 g, rhizoma zingiberis 10 g and leech powder 3 g. Decoction-free granules were provided by Tianjin Chase Sun Tcmages Pharmaceutical Co., LTD., and the batch number was 14021821.

2.2.2. Animal modeling

High sugar and high fat feed with streptozotocin (STZ) injection was used to reproduce diabetes model, cause hyperglycemia and lead to the occurrence of microalbuminuria.

2.2.3. Animal model reproduction and evaluation methods

High fat feed formula: 2.5% cholesterol, 2.5% lard, 20% sugar, 1% cholate, 5% yolk powder and 61.5% basal feed. After a week of adaptive feeding, the normal group received ordinary feed; model group received high fat and high sugar feed. One week later, model group received intraperitoneal injection of 42 mg/kg STZ solution in one time to cause diabetes model.

2.2.4. Diabetes model evaluation standard

72 h after STZ injection, fasting plasma glucose \( \geq 16.7 \) mmol/L indicated successful establishment of diabetes model. Urine microalbumin was measured again after 4 weeks, 24 h urine protein quantitation \( \geq 30 \) mg indicated successful establishment of DN rat model.

2.2.5. Compound Chinese medicine intervention methods

According to the equivalent dosage conversion between human and rats, traditional Chinese medicine group were dosed according to 6.3 times of the normal adult dose (g/kg), received peroral intragastric administration of the corresponding drugs respectively for intervention, and were dosed for continuous 8 weeks; model group and control group were only fed with normal saline, once a day.

2.3. Serum sample collection and index detection methods

After drug intervention for 8 weeks, the rats received intraperitoneal injection of chloral hydrate, blood was collected from the heart after anesthesia and centrifuged to get serum, and then enzyme-linked immunosorbent assay kits were used to determine interleukin-6 (IL-6) and macrophage migration inhibitory factor (MIF), regulated upon activation normal T cell expressed secreted (RANTES), Chemerin and Nesfatin content.

2.4. Kidney tissue collection and index detection methods

After the blood was collected from the heart, the rats were anatomized, kidney tissue was obtained, frozen quickly in liquid nitrogen, added in protein lysis buffer, fully homogenized and centrifuged to get supernatant, and radioimmunoprecipitation kits were used to detect the levels of reactive oxygen species (ROS), malondialdehyde (MDA), advanced oxidation protein products (AOPP), 8-hydroxy-2-deoxyguanosine (8-OHdG), glucose-regulated protein (GRP78), CCAAT/enhancer-binding protein homologous protein (CHOP) and ATF4 protein.

2.5. Statistical analysis

SPSS20.0 software was used to input and analyze data, measurement data among three groups was by variance analysis and \( P<0.05 \) indicated statistical significance in differences.

3. Results

3.1. Serum cytokine levels

Analysis of serum cytokines IL-6, MIF, RANTES, Chemerin and Nesfatin among three groups of rats is as follows: serum IL-6, MIF, RANTES, Chemerin and Nesfatin levels of model group were significantly higher than those of control group (\( P<0.05 \)), and serum IL-6, MIF, RANTES, Chemerin and Nesfatin levels of intervention group were significantly lower than those of model group (\( P<0.05 \)) (Table 1).

3.2. Oxidative stress and endoplasmic reticulum stress molecule levels in kidney tissue

Analysis of oxidative stress molecules ROS, MDA, AOPP and 8-OHdG as well as endoplasmic reticulum stress molecules GRP78, CHOP and ATF4 in kidney tissue among three groups of rats is shown in Table 2. ROS, MDA, AOPP and 8-OHdG levels in kidney tissue of model group were significantly higher than those of control group (\( P<0.05 \)), and ROS, MDA, AOPP and 8-OHdG levels in kidney tissue of intervention group were significantly lower than model group (\( P<0.05 \)). GRP78, CHOP and ATF4 levels in kidney
Inflammation
These inflammatory cells invade local areas, which aggravates the inflammatory state in diabetic model rats, and Chemerin and Nesfatin are two kinds of adipocytokines participating in the development of the duration of diabetes, and they can not only recruit inflammatory cells and promote the cascade release of inflammatory factors, but can also affect peripheral tissue sensitivity to insulin and increases insulin resistance[8,9]. Chemerin and Nesfatin are two kinds of adipocytokines participating in the development of the duration of diabetes, and they can not only recruit inflammatory cells and promote the cascade release of inflammatory factors, but can also affect peripheral tissue sensitivity to insulin and increases insulin resistance[8,9].

4. Discussion

Diabetic nephropathy is the most common microvascular complication in patients with type 2 diabetes mellitus, and the inflammation, oxidative stress and endoplasmic reticulum stress caused by long-term high blood glucose are closely related to the glomerular filtration function and basement membrane barrier function damage[3,4]. In the study, homemade Tangshenjiangzhuo granules were used to treat diabetic model rats, hoping to exert the cytoprotection, anti-inflammatory and antioxidant effect of rhubarb, herba artemisiae, rhizoma coptidis, red peony root and other ingredients in Tangshenjiangzhuo granules. In order to define the effect of Tangshenjiangzhuo granules therapy on the inflammation in diabetic model rats, the pro-inflammatory factors IL-6, MIF and RANTES levels in serum were analyzed at first in the study. IL-6 is a pro-inflammatory factor with endogenous chemotaxis, and it has promoting effect the recruitment of inflammatory cells and the release of inflammatory media[5,6]; MIF and RANTES can activate mononuclear macrophages and lymphocytes and make these inflammatory cells invade local areas, which aggravates inflammation[7]. Analysis of these pro-inflammatory factors showed that serum IL-6, MIF and RANTES levels of model group were significantly higher than those of control group (P<0.05), and Chemerin and Nesfatin are two kinds of adipocytokines participating in the development of the duration of diabetes, and they can not only recruit inflammatory cells and promote the cascade release of inflammatory factors, but can also affect peripheral tissue sensitivity to insulin and increases insulin resistance[8,9].

Table 1
Serum cytokine levels of three groups of rats (x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Proinflammatory factors (pg/mL)</th>
<th>Adipocytokines (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IL-6</td>
<td>MIF</td>
</tr>
<tr>
<td>Control group</td>
<td>9</td>
<td>6.49±0.93</td>
<td>5.26±0.67</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>18.31±2.47</td>
<td>12.10±1.75</td>
</tr>
<tr>
<td>Intervention group</td>
<td>8</td>
<td>12.32±1.53</td>
<td>7.65±0.93</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with model group, P<0.05.

Table 2
Oxidative stress molecule levels in kidney tissue of three groups of rats (x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Oxidative stress molecule levels</th>
<th>Endoplasmic reticulum stress molecule levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ROS (nmol/L)</td>
<td>MDA (nmol/L)</td>
</tr>
<tr>
<td>Control group</td>
<td>9</td>
<td>5.69±0.77</td>
<td>3.39±0.51</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>13.16±1.87</td>
<td>11.27±1.57</td>
</tr>
<tr>
<td>Intervention group</td>
<td>8</td>
<td>8.94±0.93</td>
<td>5.28±0.78</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with model group, P<0.05.

tissue of model group were significantly higher than those of control group (P<0.05), and GRP78, CHOP and ATF4 levels in kidney tissue of intervention group were significantly lower than model group (P<0.05).

Tangshenjiangzhuo granules treatment can reduce inflammation and suppress the secretion of pro-inflammatory factors.

Inflammatory state may appear in early duration of diabetes, and the massively produced pro-inflammatory factors can not only cause inflammatory damage in local kidney, but can also increase systemic insulin resistance degree and accelerate the progress of the duration of diabetes. In addition to being associated with the proinflammatory factors, the inflammatory state in diabetic patients is also related to a variety of adipocytokines. Adipocytokines are the new active molecules discovered in recent years, and they are synthesized by fat tissue and secreted into the blood circulation[8,9]. The inflammation, oxidative stress and endoplasmic reticulum stress, causing the glomerular filtration function and basement membrane barrier function damage[13,14]. MDA, AOPP and 8-OHdG are the oxidation products of polyunsaturated fatty acids, and they are elevated in diabetic nephropathy[8,9].

Persistent inflammation in diabetic patients will be increasing along with the development of the course of the disease, and inflammation is closely related to the development of diabetic nephropathy[12]. Oxidative stress is mediated by ROS, and it leads to the tissue structure and function damage through causing the oxidation reaction of a variety of ingredients in glomerular cells and basement membrane[13,14]. Oxidative stress molecule levels Endoplasmic reticulum stress molecule levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ROS (nmol/L)</th>
<th>MDA (nmol/L)</th>
<th>AOPP (ng/mL)</th>
<th>8-OHdG (ng/mL)</th>
<th>GRP78 (ng/mL)</th>
<th>CHOP (ng/mL)</th>
<th>ATF4 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9</td>
<td>5.69±0.77</td>
<td>3.39±0.51</td>
<td>13.29±1.83</td>
<td>8.54±0.92</td>
<td>15.62±1.89</td>
<td>10.26±1.46</td>
<td>6.48±0.82</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>13.16±1.87</td>
<td>11.27±1.57</td>
<td>26.86±3.52</td>
<td>22.16±3.05</td>
<td>38.60±5.21</td>
<td>24.68±3.15</td>
<td>16.41±1.92</td>
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<tr>
<td>Intervention group</td>
<td>8</td>
<td>8.94±0.93</td>
<td>5.28±0.78</td>
<td>18.79±2.26</td>
<td>14.68±1.88</td>
<td>22.13±2.68</td>
<td>16.65±2.07</td>
<td>9.38±1.03</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with model group, P<0.05.
products of lipid, protein and nucleic acid in cells respectively[15,16], and analysis of the levels of the oxidation products in the kidney showed that ROS, MDA, AOPP and 8-OHdG levels in the kidney tissue of model group were significantly higher than those of control group (P<0.05), and ROS, MDA, AOPP and 8-OHdG levels in kidney tissue of intervention group were significantly lower than those of model group (P<0.05). This indicates that there is significant oxidative stress in kidney tissue of diabetes model rats, and Tangshenjiangzhuo granules can inhibit oxidative stress damage of kidney tissue. In the process of endoplasmic reticulum stress, GRP78 and ATF4 interact with each other, they are activated and then activate the expression of CHOP, and the excessively accumulated CHOP in cells can cause the endoplasmic reticulum stress and apoptosis of cells[17,18]. In the study, analysis of the levels of the endoplasmic reticulum stress molecules in kidney showed that GRP78, CHOP and ATF4 levels in kidney tissue of model group were significantly higher than those of control group (P<0.05), and GRP78, CHOP and ATF4 levels in kidney tissue of intervention group were significantly lower than those of model group (P<0.05). This means that there is significant endoplasmic reticulum stress in kidney tissue of diabetes model rats, and Tangshenjiangzhuo granules treatment can inhibit the endoplasmic reticulum stress in kidney tissue.

Based on above discussion, it is concluded as follows: Tangshenjiangzhuo granules can adjust the secretion of pro-inflammatory factors and adipocytokines, and inhibit oxidative stress and endoplasmic reticulum stress in kidney tissue of diabetic rats, and they have positive value for treatment of early diabetic nephropathy.

References


