The pathogenesis mechanism of diabetic cardiomyopathy and alleviation of simvastatin combined benazepril

Chao-Jie Zhou1*, Bin Hu2, Jian-Min Liu1, Han-Min Lin1

1Peng Pai Memorial Hospital, Haifeng County, Guangdong Province, China
2People’s Hospital of Guangdong Province, China

ARTICLE INFO

Objective: To explore the pathogenesis mechanism of diabetic cardiomyopathy and alleviation of simvastatin combined benazepril. Methods: SD rats were divided into 4 groups: control, diabetic group, SP00125 group and simvastatin combined benazepril group. The cardiac function and serum MDA, SOD, HLD and LDL were detected. The expression of JNK signaling pathway key proteins were analyzed by Western Blotting. Results: The content of HDL and SOD was decreased while MDA and LDL were increased in diabetic group. The expression of JNK and pJNK was also found increased in diabetic group. Simvastatin combined benazepril medication can normalize those abnormalities greatly. Conclusion: Over-activated JNK signaling pathway was involved in diabetic cardiomyopathy and simvastatin combined benazepril exert function by inhibiting JNK pathway.

1. Introduction

With the development of the mode of production and life style, the incidence of diabetes has increased with years. Diabetic cardiomyopathy is one of the most severe complications of diabetes, which will destructively affect patients’ myocardial function. As one of signal transduction pathways, C-Jun N-terminal kinase (JNK) is a major member of the Mitogen activated protein kinases (MAPKs). It can be induced and activated by multiple cytokines, stress stimulation and growth factor. It also plays a significant role in multiple physiological and pathological processes by participating in cellular growth, differentiation, proliferation and embryonic development. In following research, we explored why streptozotocin (STZ) induce diabetic cardiomyopathy and analyzed the therapeutic effects of Simvastatin combined Benazepril.

2. Materials and methods

2.1. General materials

Male Sprague-Dawley rats, weighing (220±10) g, were purchased from Zhejiang Laboratory Animal Center; and the experimental animal certification number was SCXK 20080033. Streptozotocin (STZ) was purchased from American Sigma Company; the kits of Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) from Nanjing Jiancheng Bioengineering Institute; JNK and pJNK from American Abcam Company; the kits of secondary antibody and color from Wuhan Boster Bio-engineering Limited Company; Malondialdehyde (MDA) and Superoxide Dismutase (SOD) assay kit from Nanjing Jiancheng Bioengineering Institute.

2.2. Methods

Male Sprague-Dawley rats were randomly divided into control group, diabetes STZ model group, SP60025 group and Simvastatin combined Benazepril group. All the rats were fed with water and normal food for three days. Then rats in the model group and other two drug treatment groups were intraperitoneally injected with 65 mg/kg STZ after 12-hour fasting; and the control group rates received intraperitoneal injection of citrate buffer at the same dose. After 60 d, left ventricular function of rats in each group was measured and rats were sacrificed by carotid artery letting blood subsequently.

2.3. Biochemical measurement

The assay kits of HDL, LDL, MDA and SOD were purchased from Nanjing Jiancheng Bioengineering Institute. All measure operation steps were strictly followed.
2.4. Western Blotting

All the rats’ protein of heart tissue extraction and protein quantitative method were conducted according to the manufacturers’ instructions. Equivalent PAGE was performed by electrophoresis and semi-dry method to transfer total protein membrane to cellulose acetate membrane. Cellulose acetate membrane was incubated in a 4 °C refrigerator and sealed for 1 h; and then the membrane was incubated at room temperature 20 °C with first antibody for 2 h. Washed by phosphate buffer and the membrane was incubated with second antibody for 2 h. Using image acquisition and analysis system to measure optical density after 3 times washed by sterile PBS. Quantitative analysis of the expression of protein was made.

2.5. Statistical methods

SPSS18.0 statistical software was used to input and analyze data, and the results were expressed as (mean±SD). Results with P<0.05 were considered to be statistically significant.

3. Results

3.1. Abnormal cardiac function of diabetic rats and drug intervention

Left ventricular function of diabetic rats induced by STZ was abnormal. Systolic pressure, end-diastolic pressure and ± dp/dtmax were abnormal, too. However, Simvastatin combined Benazepril can significantly improve such circumstances. Data were considered significant if P<0.01. (Table 1)

3.2. Abnormal HLD and LDL of diabetic rats as well as drug intervention

Serum HDL of diabetic rats decreased significantly and LDL increased significantly. Above anomalous features were improved after SP600125 and drug intervention. Data were considered significant if P<0.01. (Figure 1)

3.3. Increased MDA and decreased SOD as well as drug intervention

Serum oxidized stress enzyme SOD of diabetic rats decreased significantly and MDA increased significantly. Above anomalous features were improved after SP600125 and drug intervention. Data were considered significant if P<0.01 (Figure 2).

3.4. JNK pathway protein of up-regulated expression and restorative effect of Simvastatin combined Benazepril

The JNK pathway protein expression pJNK1/2 and JNK/2 of diabetic rats ischemic myocardium increased significantly. Above anomalous features were improved after SP600125 and drug intervention. Data were considered significant if P<0.01 (Figure 3).

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**Table 1.** Drug effect and injured cardiac functions of diabetic rats induced by STZ.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Diabetic</th>
<th>SP600125</th>
<th>Simvastatin combined Benazepril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular systolic pressure (kPa)</td>
<td>15.9±2.33</td>
<td>10.3±5.01**</td>
<td>13.6±2.18##</td>
<td>12.9±3.33##</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (kPa)</td>
<td>0.91±0.20</td>
<td>1.41±0.34**</td>
<td>1.24±0.39##</td>
<td>1.20±0.87##</td>
</tr>
<tr>
<td>+dp/dtmax</td>
<td>1444±301</td>
<td>912±201**</td>
<td>1235±301##</td>
<td>1002±198##</td>
</tr>
<tr>
<td>-dp/dtmax</td>
<td>822±174</td>
<td>697±200**</td>
<td>756±198##</td>
<td>745±201##</td>
</tr>
</tbody>
</table>

**p<0.01 compared to control, #P<0.05 and ##P<0.01 compared to diabetic group.
4. Discussion

Diabetes, along with hypertension and swelling, is one of major diseases that threaten people’s health and life. Its incidence and mortality rate increase steadily in recent years[7-9]. Diabetes damage people’s health mainly by means of microvascular complications and vascular complications and diabetic cardiomyopathy is one of the most severe vascular complications, which seriously threatened people’s life. Through exploring STZ induce diabetic cardiomyopathy and analyzing the therapeutic effects of Simvastatin combined Benazepril, we found that diabetic rats induced by STZ were in patients with abnormal cardiac function, their HDL and SOD decreased significantly, their LDL and MDA increased significantly, also the expression of JNK1/2 and pJNK1/2 increased significantly. However, these anomalous features in the SP600125 intervention group and the treatment group of Simvastatin combined Benazepril recovered to a great extent. It seemed that activated JNK pathway participated in the damage process of diabetic cardiomyopathy induced by STZ, and through inhibiting activated JNK pathway, Simvastatin combined Benazepril can reduce myocardial injury of diabetes.

JNK pathway widely exists in various human tissues, and participates in various physiological and pathological processes. Activated JNK pathway has been found in most of myocardial damage models. In the study of lactic acid and low dosage edaravone in reducing myocardial apoptosis and p38-JNK pathway, we found that ischemia-reperfusion would cause abnormal expression in SOD, apoptosis indexes, phosphorylated p3 and phosphorylated JNK of rat myocardium; and combined injection of lactic acid and low dosage edaravone would restore p38-JNK pathway and reduce apoptosis[10]. In the study of ERK and JNK pathway in AcSDKP inhibited fibroblasts proliferation of rat heart induced by platelet-derived growth factors, we found that AcSDKP blocked the activation of JNK pathway mediated by platelet-derived growth factors to inhibited fibroblasts proliferation[11]; and we also noticed JNK pathway’s participation in the study of sub-anesthesia doses of sevoflurane’s effects on cerebral cortex apoptosis of neonatal rat. It has been proved by previous studies that JNK inhibitor SP600125 can inhibit the activation of JNK pathway as well as reduce infarct size and improve cardiac function of rat myocardium cause by ischemia-reperfusion[14]. All the analysis showed that diabetic rats induced by STZ were in patients with abnormal cardiac function, their HDL and SOD decreased significantly, and their LDL and MDA increased significantly. At the same time, the expression of JNK pathway protein also increased significantly, and these anomalous features in the SP600125 intervention group and the treatment group of Simvastatin combined Benazepril recovered to a great extent. That is why we hold that activated JNK pathway’s participation in diabetic cardiomyopathy induced by STZ. Hyperlipidemia is one of the most serious complications caused by diabetes. We found abnormal features not only in the rats’ cardiac function, but also in their MDA and SOD level. However, such indexes recovered after the intervention of JNK pathway blocker, which indicated that activation of JNK pathway also caused abnormal blood-lipid of diabetic rats.

In a word, activated JNK pathway participated in the damage process of diabetic cardiomyopathy induced by STZ, and through inhibiting activated JNK pathway, Simvastatin combined Benazepril can reduce myocardial injury of diabetes.

References