Effect of atorvastatin on the apoptosis of human umbilical vein endothelial cells and its drug mechanism

Yanmei Li1, Hong Liu2, Yanfang Wu1 and Mingzhe Zhu2*
1Vascular surgery of Yantaishan Hospital, Yantai, China
2Department of Pediatrics, Yantai Affiliated Hospital of Binzhou Medical University, Yantai, China

Abstract: Recent studies have shown that statins can inhibit the apoptosis of vascular endothelial cells. Many pharmacological actions of statins have nothing to do with their cholesterol lowering effects. These effects are called non lipid lowering cardiovascular protective effects. In this study, by establishing a high glucose induced vascular endothelial cell apoptosis model, we explored the mechanism of the non lipid - related cardiovascular protective effect of atorvastatin. The results showed that atorvastatin could protect human umbilical vein endothelial cells from damage induced by new high glucose and inhibit apoptosis. High concentration atorvastatin can up regulate the expression of Bcl-2 and down regulate the expression of Bax protein (P<0.05). This suggests that statins may play a role in cardiovascular protection by inhibiting endothelial cell apoptosis. This result is confirmed by experiments, which can provide clues for clinical treatment, and combine drug therapy and lifestyle intervention to reduce blood sugar.

Keywords: Apoptosis, atorvastatin, high glucose induction, protein expression, glucose concentration.

INTRODUCTION

Diabetic angiopathy is a major cause of disability or death in diabetic patients. Dysfunction of vascular endothelial cells is closely related to diabetic vascular complications (Balmadrid et al., 2015). Numerous studies have shown that high glucose environment can induce apoptosis of endothelial cells, thereby promoting the development of atherosclerosis (Cahill et al., 2015). Statins are the most effective drugs for the treatment of hypercholesterolemia. Long-term use of statins can significantly reduce mortality and cardiovascular events in patients with hyperlipidemia and coronary heart disease, such as the incidence of myocardial infarction, sudden death, and unstable angina (Dai et al., 2010). Many pharmacological actions of statins have nothing to do with their cholesterol lowering effects (Hou et al., 2015). These effects are called non lipid lowering cardiovascular protective effects (Dindo et al., 2004). Recent studies have shown that statins can inhibit the apoptosis of vascular endothelial cells (Ghoneum et al., 2015). This provides a new research direction for statins in non lipid lowering related cardiovascular protection. To this end, the experiment is to establish the basis of high glucose induced vascular endothelial cell apoptosis model and to explore the mechanism of atorvastatin, a newly discovered non lipid-lowering cardiovascular protective effect.

MATERIALS AND METHODS

Flow cytometry (from American BD company), polyacrylamide gel electrophoresis vertical electrophoresis slot and transfer electrophoresis slot; Rabbit anti human Bcl-2 monoclonal antibody and Rabbit anti human Bax monoclonal antibody, Rabbit anti human beta -actin monoclonal antibody; DMEM cell culture medium; atorvastatin. This experiment was approved by ethics committee of Yantaishan Hospital, ethical approval number as 2015YTSHMD2.

Apoptosis refers to the autonomous and orderly death of cells controlled by genes in order to maintain stable internal environment (Jean et al., 2017). Apoptosis is different from cell necrosis. Apoptosis is not a passive process, but an active process (Inzucchi et al., 2015). It involves the activation, expression and regulation of a series of genes. It is not a phenomenon of autologous damage under pathological conditions, but a kind of death for better adaptation to the survival environment (Chen et al., 2009). Apoptosis is a natural mechanism regulating organisms within the environment stable indispensable (Larsen et al., 2013; Kargulewicz et al., 2016). Once the mechanism is regulated, it will lead to disease if it is out of control. Recent studies have shown that statins can inhibit the apoptosis of vascular endothelial cells (Chen et al., 2015). This provides a new research direction for statins in non lipid lowering related cardiovascular protection. To this end, this study is based on the establishment of a high glucose induced vascular endothelial cell apoptosis model and to explore the mechanism of atorvastatin, a newly discovered non lipid-lowering cardiovascular protective effect.
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CO₂ incubator. After the cell grew to the confluence, it was passed through the passage.

The experiment was divided into 5 groups as: control group (10% fetal bovine serum + glucose DMEM 5.6 mmol/L); medium sugar group (10% fetal bovine serum + glucose concentration of 17.6 mmol/L DMEM culture medium); high glucose group (10% fetal bovine serum + glucose concentration of 33.3 mmol/L DMEM culture medium); low concentration atorvastatin group (10% fetal bovine serum +33.3 mmol/L glucose and 0.2 mol/L atorvastatin DMEM medium); medium concentration atorvastatin group (10% fetal bovine serum +33.3 mmol/L glucose and 2 mol/L atorvastatin DMEM medium); and high concentration of atorvastatin group (10% fetal bovine serum +33.3 mmol/L glucose and 20 mol/L atorvastatin DMEM medium).

Observation of cell apoptosis by brominated pyrididine fluorescence staining
The disinfected cover glass was placed in the 6 Hole culture plate, and the cells were grouped into 1 x 108 /L cells with different concentration of glucose and atorvastatin DMEM culture. After 24 h culture in 37°C and 5% CO₂ culture box, the cover glass was removed, and PBS liquid bleaching cells were 3 times, 3 min each time. After staining with acridine orange and bromopyridine, they were observed by fluorescence microscope.

Determination of the proliferation rate of human umbilical vein endothelial cells
5*10⁸ /L cell suspension was made from the logarithmic growth period cells, inoculated in 96 hole culture plate, and inoculated into 96 hole culture plates with 100 micron holes per hole. Each group had three compound holes, zero holes and 100 µL culture liquid, and continued to cultivate as 12 h, 24 h, 48 h, before the end 4h was sucked to the supernatant, plus 0.5g/ L four methyl azazolazolium Blue 100 mu L. Remove all the supernatant, then add 200 µL two methyl submaple to each pore, shake it well, make the crystal fully dissolved, and measure the absorbance of each hole by enzyme-linked immunosorbent assay. The above experiments were repeated three times.

Determination of early apoptosis rate by flow cytometry
According to cell group, adjust the cell concentration by 5 *10⁸ / inoculated in 6-well plates, each with three. After 24h culture, the cells were collected and PBS solution was rinsed for 3 times. The cells were suspended in a pre cooled 500µL buffer solution, 5µL AnnexinV-FITC and 5 µLPI were added to the cell. The number of early apoptotic cells was detected by flow cytometry in 1h, at room temperature, and the results were analyzed with CELL Quest software. The above experiments were repeated three times.

STATISTICAL ANALYSIS
The experimental data were all expressed by mean ± standard deviation (±s). Using SPSS17.0 software processing and analysis, the comparison of the average number of multiple groups of data using the single factor analysis of variance (One-Way ANOVA) method, 22 data comparison between the LSD-t method analysis.

RESULTS

Morphological observation of apoptotic cells
Under fluorescence microscope, most of the chromatin in the control group was green and normal, and the color was lighter. The sugar group showed more nuclear chromatin bright green and showed early apoptotic cells pyknosis or bead like. In the high glucose group, there were lots of early apoptotic cells and late apoptotic cells with orange color. The apoptotic cells of the Atorvastatin group with different concentrations were significantly reduced. The late apoptotic cells in the low concentration atorvastatin group and the medium concentration atorvastatin group were significantly lower than those in the high glucose group, and only a few apoptotic cells were found in the high concentration atorvastatin group. As shown in fig. 1, A as control group, B as middle sugar group, C as high sugar group, D as low concentration atorvastatin group, E as medium concentration atorvastatin group, F as high concentration atorvastatin group.

Effect of atorvastatin on the proliferation of human umbilical vein endothelial cells induced by high glucose
When HUVEC was cultured for 12 h under different concentrations of glucose, there was no significant difference in the proliferation rate of the three groups, and there was no statistical difference between the medium sugar group and the control group after 24 h, while the proliferation rate of the high sugar group was lower than that of the control group (P<0.05). After 48 h culture, the middle glucose group was lower than that of the control group (P<0.05), while the cell proliferation rate of the high glucose group was significantly lower than that of the control group (P<0.01). Different concentrations of atorvastatin can significantly increase cell proliferation. The proliferation rate of HUVEC in the medium concentration atorvastatin group increased significantly after 48 h culture (P<0.05). The proliferation rate of HUVEC in 12h, 24h and 48h was significantly higher than that in the high glucose group and the low concentration atorvastatin group (P<0.05; table 1) after the treatment of atorvastatin with 20 mol/L.

Determination of apoptosis rate by flow cytometry
After 24 h treatment with different concentrations of glucose, the early apoptosis rate of endothelial cells increased with the increase of glucose concentration. The
apoptotic rate in the glucose group and the high glucose group were statistically different from those in the control group (P<0.01). Atorvastatin could significantly inhibit the apoptosis of endothelial cells induced by high glucose. The difference between the concentration of atorvastatin group and the high glucose group was statistically significant (P<0.05). The early apoptosis rate of the high concentration atorvastatin group was significantly lower than that in the high glucose group (P<0.01) (table 2).

**DISCUSSION**

Atherosclerosis is characterized by thickening of the wall of the arteries, reducing the elasticity of the vessels and reducing the lumen. The vascular involvement usually starts from the intima, and then there are a variety of complications (Liu et al., 2013). Then the middle layer of the artery is degenerated, and the arteriosclerosis thrombosis is seen in the lesion (Zhu et al., 2015). Now more scholars believe that chronic vascular inflammation is the process of generation and progression of AS disease (Muraki et al., 1985; Lu, 2014). Oxidative stress is an important mechanism of its injury. At the same time, the typical manifestation of DM patients is increased blood glucose. The increased glucose environment can enhance oxidative stress and further aggravate endothelial cell injury. The effect of AS on coronary artery system can cause coronary atherosclerotic heart disease (Okuyama et al., 2015). If the patient has high blood sugar and poor control, it will cause more vascular disease, increase the patient's difficulty and social medical burden. Therefore, it is of profound and important significance to understand the mechanism of endothelial cell injury, the mechanism of drug action, the target of the action and the promoting effect on the formation of atherosclerosis, and it is of...
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Table 1: Effect of atorvastatin on the proliferation of human umbilical vein endothelial cells induced by high glucose

<table>
<thead>
<tr>
<th>Group</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>0.75±0.13</td>
<td>1.05±0.18</td>
<td>1.42±0.24</td>
</tr>
<tr>
<td>Middle Sugar Group</td>
<td>0.83±0.07</td>
<td>0.94±0.12</td>
<td>1.28±0.25</td>
</tr>
<tr>
<td>High Glucose Group</td>
<td>0.91±0.08</td>
<td>1.09±0.17</td>
<td>1.15±0.22</td>
</tr>
<tr>
<td>Low Concentration Atorvastatin Group</td>
<td>0.85±0.12</td>
<td>0.95±0.20</td>
<td>1.08±0.18</td>
</tr>
<tr>
<td>Medium Concentration Atorvastatin Group</td>
<td>0.92±0.13</td>
<td>1.06±0.18</td>
<td>1.20±0.15</td>
</tr>
<tr>
<td>High Concentration Atorvastatin Group</td>
<td>0.87±0.17</td>
<td>0.96±0.12</td>
<td>1.13±0.17</td>
</tr>
</tbody>
</table>

Table 2: Changes in the early apoptosis rate of endothelial cells after 24 h

<table>
<thead>
<tr>
<th>Group</th>
<th>Apoptosis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>3.45±0.27</td>
</tr>
<tr>
<td>Middle Sugar Group</td>
<td>6.72±0.38</td>
</tr>
<tr>
<td>High Glucose Group</td>
<td>8.41±0.55</td>
</tr>
<tr>
<td>Low Concentration Atorvastatin Group</td>
<td>7.28±0.34</td>
</tr>
<tr>
<td>Medium Concentration Atorvastatin Group</td>
<td>6.52±0.27</td>
</tr>
<tr>
<td>High Concentration Atorvastatin Group</td>
<td>6.23±0.32</td>
</tr>
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</table>

Table 3: Effect of atorvastatin on the expression of Bcl-2/Bax protein in endothelial cells induced by high glucose

<table>
<thead>
<tr>
<th>Group</th>
<th>Bax/β-actin</th>
<th>Bcl-2 /β -actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>0.423±0.071</td>
<td>0.946±0.132</td>
</tr>
<tr>
<td>Middle Sugar Group</td>
<td>0.485±0.056</td>
<td>0.917±0.125</td>
</tr>
<tr>
<td>High Glucose Group</td>
<td>0.531±0.051</td>
<td>0.724±0.105</td>
</tr>
<tr>
<td>Low Concentration Atorvastatin Group</td>
<td>0.608±0.064</td>
<td>0.851±0.062</td>
</tr>
<tr>
<td>Medium Concentration Atorvastatin Group</td>
<td>0.552±0.053</td>
<td>0.872±0.073</td>
</tr>
<tr>
<td>High Concentration Atorvastatin Group</td>
<td>0.519±0.046</td>
<td>0.961±0.095</td>
</tr>
</tbody>
</table>

great significance to the prevention and treatment of this kind of disease (Rosenthal et al., 2015). In this study, the flow cytometry was used to detect the human umbilical vein endothelial cells with different concentrations of glucose after 24 h. The early apoptosis rate of the cells in the control group, the middle sugar group and the high sugar group were 3.45±0.27%, 6.72±0.38% and 8.41±0.55% respectively, and the difference between the two groups was statistically significant (p<0.01). Through the detection of apoptosis related protein Bcl-2 and Bax, it was found that the expression of Bcl-2 protein gradually weakened and the expression of Bax protein gradually increased with the increase of glucose concentration, and the expression of Bcl-2 protein and Bax protein in the high glucose group had a significant difference compared with the control group (P<0.05) after the glucose concentration increased (Souich et al., 2013). These studies suggest that atherosclerosis may be associated with increased apoptosis of vascular endothelial cells in diabetic patients.

Statins are the most effective drugs for the treatment of hypercholesterolemia, but a large number of studies have shown that many pharmacological actions of statins have nothing to do with the cholesterol lowering effect (Szewczyk et al., 2015). These effects are also called non-lipid lowering related cardiovascular protective effects, mainly including anti oxidation and improvement of vascular endothelial cell function. And anti-inflammatory effect, inhibiting neurohormonal system activity, improves the compliance of arteries (Shim et al., 2010). The present study showed that atorvastatin could inhibit high glucose induced endothelial cell apoptosis (P<0.05), and 20 u mol/L atorvastatin could up regulate the expression of Bcl-2 protein and down regulate the expression of Bax protein. This suggests that statins may play a role in non lipid-lowering cardiovascular protection by inhibiting endothelial cell apoptosis (Yoshio et al., 2013). The mechanism may be associated with statins that inhibit the induction of high glucose on the expression of Bcl-2 and Bax proteins.

The increase of vascular endothelial cell apoptosis will lead to the increase of vascular endothelial permeability and the imbalance of vascular regulation (Schneider et al., 2011). The destruction of vascular endothelial integrity will undoubtedly contribute to the migration and deposition of lipid to the intima of blood vessels, and then the migration of monocytes and smooth muscle cells to the intima to phagocytic lipid, thus promoting the development of atherosclerosis (Tang et al., 2014). Hyperglycemia may induce the apoptosis of vascular
endothelial cells by down regulation of the expression of Bcl-2 protein and up regulation of Bax protein expression, which provides a new way of thinking for the prevention and treatment of vascular complications in diabetes (Xuan, 2015). Atorvastatin may regulate the expression of Bcl-2 /Bax protein and inhibit the apoptosis of endothelial cells induced by high glucose, thereby playing a role in cardiovascular protection of non lipid lowering. This provides a new theoretical basis for clinical application of statins in the treatment of diabetic patients. However, in vitro experiments can not fully simulate the internal environment of patients with type 2 diabetes, hormone level, blood lipid level and individual factors will affect the inhibitory effect of statins on the apoptosis of endothelial cells induced by hyperglycemia. Therefore, further deep study on endothelial cell apoptosis will be of great clinical significance.

CONCLUSION

In conclusion, the experimental study showed that high glucose had obvious damage to endothelial cells and high glucose was more effective than persistent high glucose, and atorvastatin had a significant protective effect on the injury of human umbilical vein endothelial cells induced by new high glucose and inhibited apoptosis. Poor control of blood sugar, excessive frequent fluctuations in the corresponding target organs cause more serious damage, at the same time there will be more dangerous chronic complications, resulting in increased morbidity of diabetes fatal complications, the worse the prognosis. It is proved that this result can provide a clue for clinical treatment, and the combination of drug therapy and lifestyle intervention to reduce blood sugar and reduce sugar as far as possible, do not simply pursue the purpose of hypoglycemic and cause excessive blood glucose fluctuations.

REFERENCES


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