Up-regulating PPAR-γ expression and NO concentration, and down-regulating PAI-1 concentration in a rabbit atherosclerotic model: The possible antiatherogenic and antithrombotic effects of atorvastatin

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Abstract

We investigated the effect of atorvastatin on the plasma concentration of plasminogen activator inhibitor-1 (PAI-1) and nitric oxide (NO) in a rabbit model, and the relationship between these effects and peroxisome proliferator-activated receptor γ (PPAR-γ). In our experiments, 24 male Japanese rabbits were divided into 3 groups: the high-cholesterol diet group (the high-C group), the high-cholesterol diet plus atorvastatin group (the atorvastatin group), and the normal diet group (the control group). All rabbits were killed after a 16-week feeding. The expression of PPAR-γ and the plasma concentrations of NO and PAI-1 were evaluated by an immunohistochemical assay while the level of the plasma lipid profile was measured using a commercially available kit. The atorvastatin not only reduces the plasma levels of the total cholesterol (TC) and the low-density lipoprotein cholesterol (LDL-C), but also increases the expression of PPAR-γ and the concentration of NO in comparison to the control group [16.11±2.35% vs 7.68±1.04%; 249.30±27.90 vs 179.12±28.51 (μml/L), p<0.05 respectively]. In addition, the concentration of PAI-1 in the atorvastatin group is lower than that in the control group (0.11±0.01 vs 0.14±0.02, p<0.05). The changes of PAI-1 and NO in the atorvastatin group are in good accordance to that of PPAR-γ. Results show that atorvastatin significantly up-regulates the expression of nuclear transcription factor, namely PPAR-γ, and induces the changes of the other two factors, which might provide mechanisms for the antiatherosclerotic and antithrombotic effects of atorvastatin.

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Keywords: Atherosclerosis; PPAR-gamma; NO; PAI-1; Lipid; Atorvastatin

1. Introduction

The pathophysiology of atherosclerosis is of great interest. Although the lipid deposition is crucial to induce athero-
activator, whose activation may decrease fibrinolysis and promote progression of thrombosis [5].

Some studies have suggested a potential antiatherogenic effect of PPAR-γ activation [6,7], whereas others associated PPAR-γ with a potential vascular effect [8]. These works demonstrate that the activation of PPAR-γ could stimulate the release of the endothelial nitric oxide (NO) [9,10] and drop PAI-1 expression in human endothelial cells, [11] which might contribute to these vascular protective effects.

Atorvastatin, belonging to the statin family, is widely used for the treatment of dyslipidemia and atherosclerotic disorders. Statins with pleiotropic effects [12,13] could not only enhance the activity of endothelial NO synthase (eNOS), but also reduce plasma PAI-1 concentration in adipose tissues [14,15]. However, whether there is a relationship between atorvastatin’s effects on PAI-1/NO and activation of PPAR-γ remains poorly known. In this contribution, we will evaluate the effect of atorvastatin on the expression of PPAR-γ, PAI-1 and NO in atherosclerotic vascular tissue to explore possible antiatherosclerotic and antithrombotic mechanisms of atorvastatin.

2. Materials and methods

2.1. Materials

24 male Japanese rabbits weighed 2.0–2.5 kg (provided by a breeder at Jilin University), high-cholesterol feedstuff (component: cholesterol 1%, vitelline powder 7.5%, cattle oil 8%, normal feedstuff 83.5%) and normal feedstuff. Commercial PPAR-γ, NO and PAI-1 assay kit were used (provided by Tiancheng Co. Ltd., Shanghai). Atorvastatin was obtained from Changchun Biocommodity Institute, China.

2.2. Animal models

The 24 rabbits were equally divided into 3 groups in random as: the high-cholesterol diet group (the high-C group), the high-cholesterol diet plus the atorvastatin group (atorvastatin group), and the normal diet group (the control group).

All rabbits were housed in air-conditioned rooms equipped with laminar flow. 200 g chow was given for each rabbit in 3 times daily. Atorvastatin for group B, mixing with plain feedstuff, was administered in 2 mg per animal per day. The dose of the atorvastatin was either 1.5 mg or 2.5 mg. The dose treating was kept from two weeks to 3 months. The levels of the plasma total cholesterol (TC) and the low-density lipoprotein cholesterol (LDL-C) were significantly lower than that of the cholesterol fed group [14–16]. The feedstuff with the drug was administered daily by adding it to a small chow dispenser that made chow pellets available and ensured the ingestion of the entire dose.

2.3. Blood and tissue samples collection

At the end of 16th week, blood of the rabbits was collected via ear vein. After that, the plasma was separated with acentric device from the blood while the animals were sacrificed with heart gas embolism method. PPAR-γ expression was tested with an immunohistochemical assay, the concentrations of NO and PAI-1 with ELISA assay, and plasma lipid were determined by an automatic biochemical analysis device.

2.4. Immunohistological assessment

2.4.1. Tissue processing

The rabbits were sacrificed with heart gas embolism method. After the animal chest was opened, the aorta was peeled and washed with the normal saline solution after the fat tissue was removed. The tissue then was fixed using 10% formaldehyde, dehydrated with ethanol, and embedded in paraffin. Finally the obtained sections (5 μm in thickness) were stained with hematoxylin and eosin. The randomly selected ten visual fields were observed under an optic microscope. The detections of PPAR-γ, PAI-1 and NO were performed according to the instructions of the test reagent.

2.4.2. Detection of PPAR-γ with the immunohistochemical assay

The sections were washed 3 times with PBS after being dewaxed and hydrated, and then were added with peroxide enzyme. The treated sections were placed at ambient temperature for 10 min, and the first antibody was added. Then the sections were kept at 4 °C for 12 h. They were added with the second antibody marked with biotin after being washed with PBS. After keeping them for 10 min, the same procedure was repeated. Streptomycin anti-biotin–peroxide enzyme solution was entered into the sections at the last. Samples were exposed to the ambient temperature for 10 min before PBS washing, and then DAB chromogenesis was carried out.

2.4.3. Detection of NO with ELISA assay

A standard solution, a buffer solution and an enzyme, each in 100 μl, were infused into the plasma sample, which was retained at 37 °C for 60 min. A chromogenic solution was further added. Retaining the solution for 10 min at 25 °C, the concentration of NO was detected by absorbance at 530 nm with a 722S spectrophotometer.

2.4.4. Detection of PAI-1 with ELISA assay

100 μl standard solution and 100 μl plasma were infused into each hole of the reaction plate. After retaining for 15 min at 37 °C, the plate was washed 5 times with distilled water. Then each hole was added with 100 μl enzyme-linked solution. At 37 °C for 15 min, the plate was then washed 5 times again. 100 μl ending solution was added to each hole. PAI-1 concentrations were determined by measuring the absorbance at 492 nm by the 722S spectrophotometer.
2.5. Lipid measurements

2.5.1. Detection of plasma lipid

The serum concentrations of TC, triglyceride (TG) and LDL-C were determined using LX-20 automatic biochemical analysis device.

2.6. Statistics

A one-way ANOVA model was used to do the statistical analysis of variables that coincide with the assumptions of normality and homogeneity of variances. The F-test was performed to examine the validity of the ANOVA model. Because some variables deviate seriously from the assumption for homogeneity of variances, a significant difference was alternatively evaluated using the Kruskal–Wallis (K–W) testing method. Comparisons between any two groups were conducted by using the q-test. The SPSS 10.0 (SPSS Inc., Chicago, US) was used for all statistical tests. The significance was defined when $p<0.05$.

3. Results

3.1. Morphology

The blood vessel endothelium in the high-C group was swollen, degenerated and partly absent, and the tunica intima was much thicker than that of the normal group. The internal elastic membrane was ruptured and illegible. The tunica media thus became incrassated as smooth muscle cells proliferated, which were bigger and turbulent in arrangements, as shown in Fig. 1. In the atorvastatin group, atherosclerosis lesion was much milder than that in the high-C group. The thickness of tunica intima and ratio of tunica intima/tunica media decreased clearly as shown in Fig. 2. In the control group, the endothelium of tunica intima was normal as shown in Fig. 3.

3.2. Plasma lipid

There were significant differences in the expressions of TC and LDL both among the three groups and between any two groups ($p<0.05$). However, the differences of the expression of TG were unconspicuous among the three groups ($p>0.05$). The summarized data are given in Table 1.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>High-C group</th>
<th>Atorvastatin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>23.51±10.58a</td>
<td>14.27±3.51b</td>
<td>1.36±0.33</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.83±0.38</td>
<td>1.08±0.52</td>
<td>1.45±0.45</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>21.39±10.00a</td>
<td>14.23±4.01b</td>
<td>0.72±0.35</td>
</tr>
</tbody>
</table>

Superscript letters a and b indicate a significant difference ($p<0.05$) from atorvastatin and control groups, respectively.
Table 2
The expression of PPAR-γ positive percent, concentration of NO in blood and absorbance of PAI-1 in plasma in the three groups (x̄±s, n=8).

<table>
<thead>
<tr>
<th></th>
<th>High-C group</th>
<th>Atorvastatin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-γ (%)</td>
<td>11.23±1.21abc</td>
<td>16.11±2.25b</td>
<td>7.68±1.04</td>
</tr>
<tr>
<td>NO (μmol/L)</td>
<td>172.07±40.66a</td>
<td>249.30±27.90b</td>
<td>179.12±28.51</td>
</tr>
<tr>
<td>PAI-1 (A)</td>
<td>0.15±0.02a</td>
<td>0.11±0.01b</td>
<td>0.14±0.02</td>
</tr>
</tbody>
</table>

Superscript letters a and b indicate a significant difference (p<0.05) from atorvastatin and control groups, respectively.

3.3. PPAR-γ expression and NO/PAI-1 concentration

As shown in Table 2, the PPAR-γ expressions of the three groups (p<0.01) were evidently different. The expression in the atorvastatin group was the highest while differences between the other groups were also significant (p<0.05). The concentration of NO in the atorvastatin group was also the highest (p<0.01) while that in the rest was similar (p>0.05). The concentration of PAI-1 in the atorvastatin group was the lowest (p<0.01), while that in the rest was similar too (p>0.05).

4. Discussions

The role of stains in the reduction of serum lipids positively impacts multiple disease states, which depends on the lipid reduction [17–19]. However, other effects independent of lipid lowering have also been reported in literatures.

Since the oxidized low-density lipoprotein (ox-LDL) can up-regulate the expression of PPAR-γ [20] and is regarded as the endogenetic activator, the expression of PPAR-γ in the high-C group was significantly higher than that in the control group and was accompanied with the increase of the plasma lipid as mentioned above. The addition of atorvastatin to the atorvastatin group decreases the amount of the endogenetic activator of PPAR-γ, namely ox-LDL, depending on its classical lipid-lowering effect, while the expression of PPAR-γ in the atorvastatin group was higher than that in the high-C group. Thus, atorvastatin could up-regulate the expression of PPAR-γ. Also, the concentrations of PAI-1 and NO in the atorvastatin group evidently differed from that in the other groups, being in accordance with the change of PPAR-γ. Consequently, the effects of atorvastatin on PAI-1 and NO were induced possibly by the enhanced expression of PPAR-γ.

It has been reported that ox-LDL could also enhance PAI-1 expression [21,22]. The expression of NO could be reduced by the increased interaction of the caveolin and the endothelial nitric oxide synthase when there was hyperlipidemia [23,24]. However, although the concentrations of PAI-1 and NO of the high-C group were different from that of the control group in our study, no any statistical difference was found, possibly due to the small size of samples and the shorter time of hyperlipidemia while the most previous studies for these were achieved in vitro. As the hyperlipidemia is complex and can induce a series of physiopathologic changes, how and when plasma PAI-1 and NO concentrations change should be clarified in further studies.

As stated above, PPAR-γ is a ligand-activated nuclear transcription factor, which can be activated by fatty acids, by fatty acid-derived eicosanoids, and by several pharmacological compounds [20]. PPAR-γ is involved in lipid metabolism, glucose homeostasis, and cell proliferation, differentiation and apoptosis [25]. Considerable evidence indicates that PPAR-γ is beneficial to inflammatory diseases including atherosclerosis. Activation of this receptor may modulate the inflammatory response in the progression of atherosclerosis. Thiazolidinediones, the PPAR-γ ligand, could up-regulate NO by enhancing the expression of eNOS [7] and decrease PAI-1 expression in human endothelial cells [8]. Our results confirm that atorvastatin has the same effect as PPAR-γ ligand thiazolidinediones where the expression of PPAR-γ is enhanced. Hence, besides the lipid reduction, atorvastatin as an ectogenic substance should contribute additionally due to the activation of PPAR-γ.

In addition, atorvastatin, as an important drug for treating atherosclerosis, is always favored by physicians. Our study shows that atorvastatin could stimulate the expression of PPAR-γ, which may provide the possible antiatherosclerotic and antithrombotic mechanisms independent of the reduction of lipid of this drug. Since our study is only a preliminary work, more studies about other effects of atorvastatin, especially potential effects contributed to activation of PPAR-γ, should be carried out.

5. Conclusions

The expressions of PPAR-γ, NO and PAI-1, in a rabbit model of atherosclerosis, are studied. It is found that atorvastatin can up-regulate NO and decrease PAI-1 expressions besides the lipid fall due to possibly the enhancement of the expression of PPAR-γ while the mechanism of the effect of atorvastatin on the above factors should be further studied.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [26].

References


