ANTI-ATHEROSCLEROTIC EFFECT OF ROSUVASTATIN BY MODULATION OF MGF-E8 AND KLOTHO EXPRESSION

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Abstract

The aim of this study was to assess the effect of rosuvastatin-mediated MGF-E8 expression on the symptoms of Coronary Heart Disease (CHD) patients with hyperlipidaemia. Two hundred CHD patients with hyperlipidaemia who were admitted to Chong Ming Branch, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, China between 2015 and 2017 were selected and divided into two groups. The subjects in the control group received Hypocof treatment, while those in the experimental group were additionally treated with rosuvastatin 10 mg/day. After 6 weeks of treatment, the differences of low density lipoprotein cholesterol (LDL-C), high sensitive C-reactive protein (hs-CRP), lipoprotein-a (LP(a)), high density lipoprotein cholesterol (HDL-C), MGF-E8, Klotho protein expression and the levels of Th1 and Th2 cell in peripheral blood were assessed. Our results showed that MGF-E8, Klotho protein and HDL-C were higher compared to the control group (p < 0.05). The levels of Th1 cells in both groups decreased, while the levels of Th2 increased. Therefore rosuvastatin can act by altering the expression levels of related proteins, such as MGF-E8 and Klotho and by modifying the levels of Th1/Th2 cells.

Rezumat

Scopul acestui studiu a fost evaluarea efectului rosuvastatinei asupra simptomelor bolii coronariene la pacienții cu dislipidemie, prin modularea expresiei MGF-E8. În studiu au fost introdusі 200 de pacienți cu boală coronariană și dislipidemie tratați în perioada 2015-2017 în spitalul Chongming Branch, Affiliated Xinhua din cadrul Universității Shanghai Jiao Tong, China, împărțiți în două grupuri, un grup de control și un grup de studiu. Cei din grupul de control au primit tratament cu Hypocof R, în timp ce pacienții din grupul de studiu au primit tratament cu rosuvastatină 10 mg/zi. După 6 săptămâni de tratament au fost evaluate lipoproteinele cu densitate scăzuta (LDL-C), proteine C reactivă înalt sensibilă (hs-CRP), lipoproteina-a (LP(a)), lipoproteinele cu densitate înaltă (HDL-C), MGF-E8, proteina Klotho precum și nivelul celulelor Th1 și Th2 din sângele periferic. Rezultatele obținute au arătat că valorile MGF-E8, a proteinei Klotho și a HDL-C au fost semnificativ mai mari comparativ cu grupul control (p < 0.05). După tratament nivelul celulelor Th1 a scăzut în ambele grupuri, în timp ce nivelul celulelor Th2 a crescut semnificativ statistic. Rosuvastatina poate acționa ca și hipolipemiant prin modularea expresiei proteinelor de tipul MGF-E8 și Klotho precum și prin modificarea nivelului celulelor Th1/Th2.

Keywords: rosuvastatin, hyperlipidaemia, MGF-E8, Klotho, Th1, Th2

Introduction

Coronary Heart Disease (CHD) is caused by myocardial ischemia, hypoxia or necrosis due to vascular stenosis or obstruction resulting from coronary artery atherosclerotic lesions, with serious risks for human life [5]. T lymphocyte can generate a large number of inflammatory cytokines to regulate atherosclerosis. T lymphocytes are divided into two cells subsets, type I T helper cells (Th1) and type II T helper cells (Th2), according to their biological roles. Th1 which can produce interferon-γ (IFN-γ) and interleukin-2 (IL-2), and can promote cellular immunologic response through strengthening the activity of cytotoxic T cells and natural killer (NK) cells. Th2 cells produce IL-4 and IL-10 and mediate humoral immunity through promoting B lymphocyte to produce antibodies. Hyperlipidaemia caused by dyslipidaemia and cholesterol accumulation is one of the complications of coronary heart disease [3, 19, 27]. Metabolic syndrome, characterized by hyperlipidaemia, obesity, type II diabetes mellitus with insulin resistance and hypertension, is associated with non-alcoholic fatty liver [8] or rare disease such as Prader Willi syndrome [4]. Insulin resistance is the link between non-alcoholic fatty liver disease and other pathological conditions including sleep apnoea syndrome and polycystic ovary syndrome, associated with modified
been approved by rosvastatin 10 mg/day for 6 weeks. The study patients aged 
40 female patients in each group. In the control group B (experimental group), with 60 male patients and 
were divided into group A (control group) and group 
c[12], angiotensin converting enzyme inhibitors and 
8.6 years old). They were under treatment with 
angiotensin and blood pressure lowering drugs or other disruptive drugs one month 
before being admitted to hospital and had no severe infection, immune system diseases, heart and lung 
dysfunction, heart rate disorders, liver, kidney, hematopoietic system diseases, mental illness, or 
allergy to statins.

Reagents and equipment. The materials included RPM1640 cell culture medium (Hyclone, USA), phosphate buffer saline (PBS) (Hyclone, USA), flow cytometry staining buffer (Tianjin Haoyang Biotechnology, China), FIX&PERM Kit (Tianjin Haoyang Biotechnology, China), centrifuge tube (Corning, USA) and flow cytometry tube (Corning, USA). The instruments included CO2 constant-temperature incubator (Thermo, USA), LD5-2A centrifugal machine (Beijing Medical Centrifuge Factory, China), inverted optical microscope (Olympus, Japan) and flow cytometry (BD FACS Aria, USA).

Biochemistry
Blood lipids. Patients were requested to abstain from high-fat food 24 hours before blood collection. Blood samples were collected from fasted patients of the two groups before treatment and 6 weeks after treatment. Afterwards, the serum biomarkers as: total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C were measured by Hitachi 7170 automatic biochemical analyser [20].

High-sensitivity C-reactive protein (hs-CRP). The serum levels hs-CRP was measured by turbidimetric method as previously described [21] using hs-CRP kit (Diazyme Laboratories, Inc., USA).

Serum lipoprotein LP (a), Klotho protein and MFG-E8. Serum levels of LP (a), Klotho protein and MFG-E8 were assessed by ELISA as previously described, using Human Lipoprotein a ELISA Kit (Novus Biologicals, Bio-Technne, China), u-Klotho ELISA kit (USCN life Science Inc., Wuhan, China), human MFG-E8 ELISA kit (Sigma-Aldrich China, Inc., China), [28].

Isolation of Peripheral Blood Mononuclear Cell (PBMC). 3 mL of blood collected on heparin sodium anticoagulant was centrifuged at 1000 t/min for 6 min. After the separation of the supernatant, 2.5 mL of lymphocyte separation medium were added, in order to separate the PBMC. After PBMC separation the samples were kept in 5% CO2 atmosphere at room temperature till further analysis.

Th1/Th2. PBMC samples after supernatant removal were treated with RPMI-1640 complete medium supplemented with 10% Foetal Bovine Serum (FBS). Cells were stimulated with a combination of 250* Monensin/BFA and incubated for 4 h at 37°C and 
5% CO2 atmosphere. The fluorescent-coupled monoclonal antibodies used for detection using the flow 
cytometry method according to manufacturer protocol
were CD4 PerCP-Cy5 and CD8APC (Tianjin Haoyang Biotechnology, China).

**Efficacy criteria for CHD.** The angina was evaluated by stress test and electrocardiogram evaluation. If the symptoms of angina completely or basically disappeared and its incidence fell by more than 80%, the effect was considered significant; if the symptoms of angina were alleviated, and the incidence reduced by above 50%, the effect was good; otherwise, the treatment was ineffective.

**Efficacy criteria for hyperlipaemia.** If TG level reduction rate was over 40% or TC level reduction rate was over 20%, the effect was considered significant; if TG level reduction rate was over 20% or TC level reduction rate was over 10%, the effect was good; otherwise, the treatment was ineffective.

**Statistical analysis.** The experimental results were analysed by SPSS 17.0. All data were expressed as mean ± standard deviation. Logistic regression [22] was used to analyse independent risk factors, T1 representing the period before treatment and that T2 after treatment. The content of protein in the two periods was recorded, and the statistical significance of the data was studied.

### Results and Discussion

#### Comparison of the treatment effects

As depicted in Figure 1, the comprehensive effective rate was by 89% higher in group B compared to group A, with statistical significance (p < 0.05), supporting what already was known, that rosuvastatin is effective in treating patients with hyperlipidaemia.

#### Blood lipids test results

The levels of the lipidic profile are presented in Figure 2.

As shown in Figure 2, data on blood lipids were not significantly different before treatment in the two groups (p > 0.05). As expected for T2, the content of TC, TG and LDL-C in blood, decreased in group A and B, and the parameters for group B decreased significantly, while HDL-C was up-regulated in both groups with best results for group B showed a higher degree of up regulation (p < 0.05).
Comparison of serum hs-CRP levels

As shown in Figure 3, hs-CRP content in T1 was not statistically significantly different between the two groups (p > 0.05). In T2, levels of hs-CRP in the control group significantly decreased from 12.35 ± 1.88 mg/L to 5.63 ± 1.01 mg/L (p < 0.05) while in group B it decreased from 12.58 ± 2.13 mg/L to 2.26 ± 0.46 mg/L (p < 0.05). The levels of hs-CRP was significantly reduced in group B compared to group A after treatment (p < 0.05).

Serum lipoprotein LP (a), Klotho protein and MFG-E8. Serum lipoprotein LP (a), Klotho protein and MFG-E8 test results are shown in Table I.

Table I

Comparison of serum lipoprotein LP (a), Klotho protein and MFG-E8 between the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>MFG-E8 (ng/mL)</td>
<td>204.90 ± 4.93</td>
<td>297.88 ± 12.03#</td>
</tr>
<tr>
<td>LP (a) (mg/L)</td>
<td>375.42 ± 38.22</td>
<td>175.65 ± 20.36*</td>
</tr>
<tr>
<td>Klotho protein (pg/mL)</td>
<td>27.43 ± 2.86</td>
<td>32.48 ± 3.78#</td>
</tr>
</tbody>
</table>

# p < 0.05 comparing T1 with T2; * p < 0.05 comparing group A with group B

As shown in Table I, the expression levels of MFG-E8 and Klotho protein in groups A and B were similar before treatment (p > 0.05). After treatment, the expression levels of MFG-E8 and Klotho protein in group B was significantly increased. The expression level of MFG-E8 increased from 194.88 ± 5.03 ng/L to 507.85 ± 24.12 ng/L, and the expression level of Klotho protein increased from 27.32 ± 2.85 pg/L to 41.63 ± 5.14 pg/L; the differences for group A showed statistical significance (p < 0.05). The expression levels of MFG-E8 and Klotho protein of both groups increased significantly after treatment, and the differences had statistical significance (p < 0.05). There was no significant difference in LP (a) level between the two groups before treatment (p > 0.05). After treatment, LP (a) level decreased from 365.23 ± 35.12 mg/L to 144.23 ± 23.42 mg/L in group B and decreased from 375.42 ± 38.22 mg/L to 175.65 ± 20.36 mg/L in group A (p < 0.05). The difference of LP (a) level was significant before and after treatment in both groups (p < 0.05).

Percentage of Th1 and Th2. It can be seen from Table II and Figures 4-6 that the percentage of Th1 and Th2 of group A and B were not significantly different before treatment (p > 0.05). After 6 weeks of treatment, the percentage of Th1 decreased in both groups, but the percentage of Th2 increased, and the percentage of Th1 and Th2 of the two groups before and after treatment were significantly different (p < 0.05). The percentage of Th1 of group B was lower than that of group A (19.07 ± 2.09% vs. 22.14 ± 1.97%), while the percentage of Th2 of group B was higher than that of group A (1.72 ± 0.13% vs. 1.44 ± 0.14%); the differences were statistically significant (p < 0.05).

Table II

The changes in percentage of Th1 and Th2 in the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Th1 cells (%)</td>
<td>27.04 ± 1.85</td>
<td>22.14 ± 1.97#</td>
</tr>
<tr>
<td>Th2 cells (%)</td>
<td>1.12 ± 0.17</td>
<td>1.44 ± 0.14*</td>
</tr>
</tbody>
</table>

# p < 0.05 T2 vs T1; * p < 0.05 group A vs B
In recent years, more and more people tend to suffer from cardiovascular diseases. Coronary atherosclerosis is one of the main causes of CHD. Hyperlipidaemia is an independent risk factor for CHD and it is caused by excessive levels of proteins such as hs-CRP and LP (a) due to the lipid rise in the plasma [10]. Lipids in human plasma are essential metabolic biomolecules and they can easily cause a variety of sudden illness [7] in a high level. CHD combined with hyperlipidaemia is one of the most common comorbidities, which poses a great threat to the human body. Hence, it is urgent to find new effective drugs. Rosuvastatin is one of the most common lipid-lowering drugs [1, 2, 16], which can effectively inhibit the formation of HMG-CoA reductase to reduce the body's cholesterol production. It is commonly used in the treatment of dyslipidaemia, although cases of severe myopathy have been reported especially in elderly patients treated with high doses of statins [14]. Moreover, rosuvastatin can inhibit inflammatory responses [17]. As a pro-inflammatory biomarker, hs-CRP can reflect the occurrence of vascular inflammation in patients [13]. Rosuvastatin can effectively inhibit the production of inflammatory factors, thereby reducing the risk of CHD in patients. The decreased expression of MFG-E8 and LP (a) can accelerate the accumulation of apoptotic membrane fragments and the induction of atherosclerosis. Therefore, the
content of MFG-E8 and LP (a) has important values for evaluating the severity of CHD patients with hyperlipidaemia [15, 25]. Klotho protein as an important anti-aging factor in human body can protect vascular endothelial cells, i.e. slow the aging and apoptosis of vascular endothelial cells, and effectively inhibit atherosclerosis.

In this study, serum lipoprotein LP (a), Klotho protein and MFG-E8 levels were analysed in order to assess their implication in rosuvastatin hypolipaemiant mechanism of action. Rosuvastatin up-regulated the levels of MFG-E8 and Klotho protein and decreased the content of LP (a) in patients, leading to reduced risks of developing CHD associated complications. It was found that the expression level of Klotho protein in group B was higher than that of group A after treatment (p < 0.05); compared to the control group, the expression level of serum Klotho protein.

In this study, serum lipoprotein LP (a), Klotho protein and MFG-E8 were regulated effectively reduce the incidence of hyperlipidaemia and the expression level of LP (a) in patients with CHD, leading to reduced risk of developing CHD associated complications. It was found that the expression level of Klotho protein in group B was higher than that of group A after treatment (p < 0.05); compared to the control group, the patients with rosuvastatin had more obvious changes, rosuvastatin modulating Th1/Th2 levels, as part of the immune system.

Conclusions

Roustatin can reduce the levels of TC, TG, LDL-C and hs-CRP by changing the expression of MFG-E8 and other proteins and regulating some components of the immune system, namely the percentage of Th1 and Th2 cells. Uprregulation of MFG-E8, Klotho protein and LP (a) levels may effectively reduce the incidence of hyperlipidemia in patients with CHD. This study provides a new scientific reference for the use of rosuvastatin in the treatment of patients with CHD.

References


