Introduction

Annually, over one million people in the United States experience a sudden cardiac event as a result of acute coronary syndrome (ACS), with approximately 20 million people affected worldwide (1). Previous studies have found that dyslipidemia, enhanced oxidative stress, and inflammation are involved in the development of atherosclerosis and its related complications, such as the cardiovascular disease (CVD), although the underlying mechanisms remain unknown (2,3). Serum lipids are the most important factors contributing to the occurrence and development of ACS. Moreover, oxidative stress plays an important role in the various stages of thrombosis development (4).

Overwhelming evidence from epidemiological and clinical studies has demonstrated that low-density lipoprotein (LDL) is a key element in the development of atherosclerosis (5). Oxidized LDL (ox-LDL) can induce atherothrombosis by stimulating monocyte infiltration and smooth muscle cell migration and proliferation. Further, it contributes to atherothrombosis by inducing endothelial cell apoptosis and subsequent plaque erosion, impairing the anticoagulant balance in the endothelium,
stimulating the tissue factor production of smooth muscle cells, and triggering macrophage apoptosis (6). High-density lipoprotein (HDL) plays an anti-atherogenic role in prevention of atherosclerosis partly due to its anti-oxidative and anti-inflammatory properties by preventing LDL oxidation (3,7). HDL can also transport free cholesterol (FC) from peripheral tissues (including arterial walls) to the blood, with the help of the cell membrane protein ATP-binding Cassette transporter A1 (ABCA1) and the macrophage Scavenger Receptor BI (SR-BI); this mechanism can reverse atherogenesis and is called reverse cholesterol transport (RCT) (8).

The HDL-associated enzyme paraoxonase-1 (PON1) is largely responsible for the antioxidant action of HDL due to its ability to hydrolyze oxidized phospholipids (9). Experiments have shown that knocking out the PON1 gene has pro-inflammatory and pro-atherogenic effects by increasing the levels of ox-LDL, while the trans-gene or over-expression of PON1 is anti-atherogenic by suppressing LDL oxidation and inflammation (10). Lower plasma PON1 activity is associated with increased CVD risk, which may be affected by both genetic polymorphisms and environmental factors, such as pharmaceutical interventions (3).

Probucol is a bisphenolic compound with unique anti-atherogenic properties, including LDL-C-lowering, antioxidant, and anti-inflammatory functions. Although probucol may also significantly decrease the levels of HDL-C, it has been shown to have a strong anti-atherosclerosis effect (11). The mechanism underlying this effect is not entirely clear; however, it is hypothesized to be related to the decreased levels of ox-LDL and the increased levels of PON1 (12). Statins are widely used for lowering plasma LDL and play a pivotal role in the primary prevention of CVD mortality and major cardiac events. It has been suggested that statins may have additional anti-atherogenic effects, such as stabilization of atherosclerotic plaques and inhibition of vascular inflammation and lipid oxidation (13). One of these so-called pleiotropic effects of statins could be a reduction in oxidative stress even before the lipid-lowering effect becomes apparent. These antioxidant functions are considered to be at least partly associated with the beneficial effects that occur very early in the course of statin therapy (14).

Therapy with probucol as well as atorvastatin leads to lipid regulation with antioxidant effects (12). However, little is known regarding the combined use of these two drugs. Whether such combined therapy may act synergistically in antioxidant therapy in patients with ACS has been briefly studied (15). This study aimed to examine the therapeutic effects of the combined use of probucol and atorvastatin on atherosclerosis in ACS patients and discuss the possible therapeutic mechanisms of this combination.

Methods

Subjects

This clinical study was performed in the Cardiology Department of the Chinese PLA General Hospital in Beijing, China. Overall, 126 consecutive patients (77 men and 49 women; mean age, 61.3±8.9 years) who presented with symptoms of acute coronary disease were recruited for this study from December 2010 to July 2011. The patients were admitted based on the history, physical examination, electrocardiogram (ECG) and dynamic ECG, levels of myocardial necrosis markers (CK/CK-MB/cTNT), and coronary angiography for the diagnosis of acute ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP). Informed consent was obtained from all the patients after explaining the nature and the purpose of the study. The study was approved by the Ethical Committee of Chinese PLA General Hospital.

STEMI was diagnosed in patients with chest symptoms suspected of being caused by myocardial infarction (MI) and persisting for at least 20 min within the last 24 h before admission along with ECG findings of ST-segment elevation of ≥1 mm in two or more limb leads, two or more contiguous precordial leads, or left bundle branch block (LBBB). NSTEMI was diagnosed in patients with chest symptoms suspected of being caused by MI and persisting for at least 20 min within the last 24 h before admission; no ST-segment elevation ≥1 mm or LBBB; and elevated levels of the biochemical markers of myocardial necrosis, including cardiac troponin T ≥1.0 nm/dL or creatine phosphokinase MB (CK-MB) two times above the normal range. UA was diagnosed in patients with resting or nocturnal chest pain persisting ≥20 min along with any of the following findings: T-segment depression of ≥0.5 mm, T-wave inversion of ≥3 mm, and serum troponin T ≥1.0 nm/dL. Confirmation of significant stenosis was based on diagnostic imaging, a recent reduction in LV contractions detected by ultrasound echocardiography, or reversible drug or exercise-induced myocardial hypoperfusion on thallium perfusion scintigraphy (5).

Patients with hepatic, endocrine, or renal disorders...
(serum creatinine level >130 mmol/L); type 2 diabetes mellitus; alcoholism; drug dependence; gallstones; malignancy; pregnancy; or lactation or patients receiving anticoagulant or lipid-lowering therapy were excluded from the study.

**Serum specimen preparation**

Elbow venous blood was drawn after overnight fasting. Laboratory biochemical tests were performed using these samples. Further, serum samples were separated by low-speed centrifugation (3,000 rpm, 10 min at 4 °C) and aliquots were frozen at −80 °C under nitrogen until measurement. The serum levels of the HDL subfractions HDL$_2$ and HDL$_3$ along with ox-LDL and PON1 were detected using enzyme-linked immunosorbent assay kits.

**Patient groups**

The 126 ACS patients were divided into odd (group A) and even (group B) groups based on the order of enrollment. Group A (n=62; 37 men and 25 women), received only 20 mg of atorvastatin qd (Lipitor, 20 mg/tablet, Pfizer, Inc, Dalian, China) and were considered the control group. Group B was designated as the treatment group, and patients were given 750 mg of probucol bid (Zhile, 0.125 g/tablet, Qilu Pharmaceutical Co., Ltd, Jinan, China) as well as 20 mg of atorvastatin qd (Lipitor, 20 mg/tablet). In addition to these, both the groups were prescribed conventional nitrate ester and dual anti-platelet drugs. At the beginning of treatment and at follow-up examination at 12 weeks, 12-lead ECGs were obtained and the QT interval was measured, along with hepatic and renal function tests and serum levels of ox-LDL and PON1.

**Statistical analysis**

Normal data were presented as mean ± standard deviation (SD), and comparisons between the two groups were made using a number of independent-samples $t$-tests. The parameters before and after treatment in the same group were compared using paired $t$-tests. For the various measurement data, analysis of variance (ANOVA) was used to test for differences. If homogenous, Fisher’s least significant difference test was used for multiple comparisons between groups. Otherwise, Tamhane’s T2 test was used. For enumeration data, the chi-square test was used. Statistical analysis was performed using SPSS 15.0 for Windows (SPSS Inc., USA). P<0.05 was considered statistically significant.

**Results**

**Characteristics of the study population**

The basic characteristics of the two groups are outlined in Table 1. There were no statistically significant differences in terms of age, gender, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and serum glucose (GLU), K$^+$, and creatinine (Cr) levels between the two groups (P>0.05).

The clinical characteristics of the two groups are outlined in Table 2. There were no statistically significant differences in each Type of ACS, culprit vessel, left ventricular ejection fraction (LVEF), and the ratio of hypertension between the two groups (P>0.05).

**Serum lipid levels**

Table 3 shows the serum lipid levels in the patients before and after treatment. There were no significant differences in the serum lipid levels between the two groups before treatment (P>0.05). After treatment, in the control group, the total cholesterol (TC), triglyceride (TG), and LDL levels were noted to have decreased; the HDL level increased significantly; and the levels of HDL$_2$ and HDL$_3$, as well as the HDL$_3$/HDL$_2$ ratio did not change significantly. In the treatment (combined) group, the TC, TG, and LDL levels were significantly decreased after treatment, being significantly lower than those in the control group; further,
the levels of HDL, and the HDL/HDL ratio increased significantly compared with those in the control group (P<0.05).

Table 2 Clinical characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control group (n=62)</th>
<th>Treatment group (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of ACS (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>10 (16.1)</td>
<td>11 (17.2)</td>
</tr>
<tr>
<td>STEMI</td>
<td>12 (19.4)</td>
<td>17 (26.6)</td>
</tr>
<tr>
<td>UA</td>
<td>40 (64.5)</td>
<td>36 (56.2)</td>
</tr>
<tr>
<td>Culprit vessel (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>25 (40.3)</td>
<td>27 (42.2)</td>
</tr>
<tr>
<td>RCA</td>
<td>16 (25.8)</td>
<td>19 (29.7)</td>
</tr>
<tr>
<td>LCX</td>
<td>21 (33.9)</td>
<td>18 (28.1)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>50.7±7.3</td>
<td>51.2±7.9</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>45 (72.6)</td>
<td>47 (73.4)</td>
</tr>
</tbody>
</table>

NSTEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; UA, unstable angina pectoris; LAD, left anterior descending artery; RCA, right coronary artery; LCX, left circumflex branch; LVEF, left ventricular ejection fraction.

Table 3 Serum lipid levels (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=62)</th>
<th>Treatment group (n=64)</th>
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</thead>
<tbody>
<tr>
<td>TG (mmol/L)</td>
<td>1.94±0.54</td>
<td>1.64±0.41*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.20±1.00</td>
<td>3.85±0.91*</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.63±0.58</td>
<td>2.39±0.32*</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.09±0.19</td>
<td>1.17±0.22*</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.53±0.14</td>
<td>0.51±0.27</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.46±0.10</td>
<td>0.48±0.12</td>
</tr>
<tr>
<td>HDL/HDL ratio</td>
<td>0.89±0.15</td>
<td>0.91±0.17</td>
</tr>
</tbody>
</table>

Table 4 Serum levels of Ox-LDL and PON1

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control group (n=62)</th>
<th>Treatment group (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox-LDL (mg/L)</td>
<td>485.4±190.1</td>
<td>396.8±130.6*</td>
</tr>
<tr>
<td>PON1 (KU/L)</td>
<td>31.9±13.7</td>
<td>38.9±15.3*</td>
</tr>
</tbody>
</table>

a, post-treatment levels compared with pre-treatment levels, P<0.05; b, significant difference between the two groups, P<0.05. Ox-LDL, oxidized low-density lipoprotein cholesterol; PON1, paraoxonase-1.

Serum levels of ox-LDL and PON1

Using the t-test, we compared these two indicators of lipid and antioxidant levels before and after the treatment in each group as well as between the two groups after treatment. The Ox-LDL level decreased significantly after treatment as compared to the baseline in both the groups; further, it was significantly lower in the treatment group than in the control group (P<0.05) (Table 4). The PON1 level increased significantly after treatment as compared to the baseline in both the groups; further, it was significantly higher in the treatment group than in the control group (P<0.05).

The relationship between ox-LDL and PON1

The serum levels of ox-LDL and PON1 showed a significant negative correlation by the Pearson correlation analysis (r=−0.5) (Figures 1-4).

Follow up and side effects of the therapy

The follow up of these patients involved in the presents study lasts for 12 weeks:

Blood samples were collected at the start and the end of the research respectively; the occurrence of adverse reactions was followed up during this period. No serious effects of the therapy on hepatic or renal function and no evidence of muscular damage were observed. Reversible mild elevation of transaminase in one case in the control group and two cases in the treatment group were observed in the first 2 weeks, which returned to the initial level after treatment at the end of the follow-up. No severe
gastrointestinal reaction or prolonged QT interval in the ECG was observed in any of the patients.

**Discussion**

Atherosclerosis is an inflammatory disease that is associated with oxidative stress and thrombotic agents (16). Oxidative stress is generally considered as the dynamic balance between the production of reactive oxidant species and antioxidant capacity. It has been reported that levels of antioxidants can be affected by various lipoproteins, including LDL (5). Circulating ox-LDL is generally believed to be pro-atherogenic (6). Oxidative modification of LDL in the arterial wall is a complex process involving several biological pathways (17). Ox-LDL can promote the production of chemoattractants, adhesion molecules, and colony stimulating factors for monocytes by endothelial cells. Preliminary adhesion of circulating monocytes to the vascular endothelium and their subsequent transmigration into the arterial intima comprise the early steps of the formation of atherosclerotic lesions. In this pathway, HDL inhibits atherosclerosis by suppressing LDL oxidation and the biological effects of the mildly oxidized LDL on monocyte-endothelial cell interactions (18).

PON1 is an esterase enzyme synthesized by the liver that is associated with HDL in the blood. The antioxidant activity of HDL is largely attributed to PON1 present the HDL molecule (19). PON1 can hydrolyze aromatic carboxylic acid esters, organophosphates, and oxidized phospholipids, and the hydrolysis of oxidized phospholipids...
by PON1 can destroy the biologically active lipids in the mildly oxidized LDL (9). Since the activity of PON1 is associated with HDL, it possibly protects LDL and HDL from oxidation (18).

Probucol is a bisphenolic compound that was originally synthesized as an antioxidant for its ability to reduce cholesterol levels. However, its utilization has been limited mainly because it leads to considerable reductions in HDL levels (20). Yamashita et al. found that probucol was useful in lowering the risk of cardiovascular events without any significant adverse effects, especially in combination with statins (21). Experiments showed that the effects of multiple anti-atherogenic agents were mainly due to the inhibition of the oxidative modification of LDL. Probucol inhibits foam cell formation from THP-1 cells by suppressing lipid accumulation and by enhancing the release of cholesterol from macrophages (20). Probucol also plays a role in mediating cellular cholesterol efflux and can facilitate reverse cholesterol transport (RCT) from the arterial wall and peripheral tissues to the liver (22).

The development of 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase (HMGR) inhibitors or statins has improved the widespread use of lipid-lowering drugs. In addition to the known traditional effects such as lipid regulation, statins can also affect plaque stability, improve the balance of oxidative stress, and reduce the multiple effects of inflammatory reactions (12,23). Oxidative stress and/or weak antioxidant defense systems are considered as important factors in the multi-mechanistic pathogenesis of atherosclerosis. Based on abundant evidences, atorvastatin has been found to exert beneficial cardiovascular effects independent of its lipid-lowering ability, possibly due to its antioxidant properties (24).

The present study aimed to determine whether the treatment of ACS patients using atorvastatin with/without probucol could reduce the levels of ox-LDL and PON1 and explore whether PON1 could regulate the pathogenesis of atherosclerosis. Based on abundant mechanistic evidences, atorvastatin has been found to exert beneficial cardiovascular effects independent of its lipid-lowering ability, possibly due to its antioxidant properties (24).

The main side effect of statin therapy is liver and muscle toxicity (31,32), and the most common adverse reactions of probucol are gastrointestinal discomfort and prolonged QT intervals in the ECG (21). The present study does not find any serious effect on the hepatic or renal functions, indication of muscle damage, and severe gastrointestinal reaction. Therefore, our results suggest that probucol...
combined with atorvastatin is a safe and effective treatment for ACS patients. Lastly, the present study suggests that the addition of probucol to atorvastatin has synergistic effects in lowering cholesterol levels and improving antioxidant activity.

Acknowledgements

Funding: This work was supported by the Supporting Fund from the Ministry of Science and Technology of China (2009BAI86B04) (Li Fan).

Disclosure: The authors declare no conflict of interest.

References


