Original Article
Probucol decreases 8-iso-PGF2α and MMP-9 in patients with unstable angina after percutaneous coronary intervention

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Abstract: Oxidative stress and lipid peroxidation play important roles in the development of unstable angina (UA). Probucol reduces inflammation and oxidative stress. The aim of this study was to evaluate the effects of probucol on levels of 8-iso-prostaglandin F2alpha (8-iso-PGF2α) and matrix metalloproteinase (MMP)-9 in patients with UA that underwent percutaneous coronary intervention (PCI). This was a single-center prospective cohort study of consecutive adult patients with UA that underwent PCI between March 2016 and July 2016 at the Yidu Central Hospital of Weifang (China). Control patients received conventional medication (aspirin, clopidogrel, rosuvastatin, and isosorbide mononitrate). In addition to conventional therapy, patients in the probucol group took oral probucol (250 mg bid) for 3 months. Levels of 8-iso-PGF2α and MMP-9 were measured the day after PCI and after 3 months. Major adverse cardiac events (MACE) were evaluated during follow-ups (mean of 14 months). Eighty-three patients were enrolled: 40 controls and 43 treated with probucol. Levels of MMP-9 of control and probucol groups were decreased by 14.0% and 47.9%, respectively, at 3 months after PCI (P=0.003). Levels of 8-iso-PGF2α of control and probucol groups were decreased by 13.1% and 49.8%, respectively, at 3 months after PCI (P=0.001). One patient in the control group underwent a coronary angiography for angina pectoris recurrence. There was no acute myocardial infarction or death events in either group during follow-ups. Probucol with rosuvastatin may significantly decrease 8-iso-PGF2α and MMP-9 in patients with UA after PCI.

Keywords: Probucol, rosuvastatin, 8-iso-PGF2α, matrix metalloproteinase 9, percutaneous coronary intervention, unstable angina

Introduction
Unstable angina (UA) is one of the common types of coronary heart disease, with a propensity to progress to acute myocardial infarction or sudden cardiac death [1-4]. Atherosclerosis is a complex process involving oxidative stress, lipid peroxidation, and inflammation [5-8]. Iso prostanes are derivatives of arachidonic acid oxidized by reactive oxygen species (ROS). 8-iso-PGF2α is a stable isoprostane and reliable marker of oxidative stress in vivo [9]. In addition, 8-iso-PGF2α has been shown to be a sensitive and independent risk marker of coronary heart disease [10]. Matrix metalloproteinase (MMP)-9 is involved in the degradation of the fibrous cap of atherosclerotic lesions. MMP-9 levels have been directly associated with plaque scores and instability [11]. Monocyte chemoattractant protein (MCP)-1 has been associated with intima-media thickness (IMT), but not with plaque scores or stability. MMP-9 and MCP-1 may be useful biomarkers in distinguishing stable and unstable plaques and predicting future cardiovascular events [12].

Percutaneous coronary intervention (PCI) is recommended for class I patients with UA with severe stenosis [3]. However, PCI itself elicits a systemic inflammatory response that can be measured in the plasma by inflammatory markers C-reactive protein (CRP), interleukin (IL)-6, and serum amyloid A [13, 14]. Release of inflammatory factors after PCI can lead to vulnerable plaque instability and plaque rupture, resulting in adverse cardiovascular events caused by restenosis, stent thrombosis, and endothelial dysfunction [15].
Probucol is a synthetic antioxidant, first used as a lipid-lowering drug because it can significantly reduce total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) [16-19]. Probucol has also been used to treat atherosclerosis [20]. Probucol alleviates atherosclerosis by improving HDL function by accelerating the process of reverse cholesterol transport and increasing HDL-associated paraoxonase activity, thereby improving anti-inflammatory and anti-oxidant function [19, 21]. Probucol can decrease plaque areas through decreasing inflammation and oxidative stress [5, 6]. Meng et al. [22] reported that probucol significantly inhibits the generation of ox-LDL and MMP-9. PAS (probucol, aspirin, and atorvastatin) therapy reduces plaque thickness and decreases the rate of adverse events in patients with atherosclerosis [22]. Probucol has also been used in patients that underwent PCI to reduce oxidative stress, incidence of restenosis, and major coronary events after PCI [17, 18, 23, 24].

Data are lacking concerning the effects of probucol on markers of oxidative stress (8-iso-PGF2α) and plaque stability (MMP-9) in patients with UA after PCI. Therefore, the aim of this study was to measure MMP-9 and 8-iso-PGF2α levels in patients with UA that underwent PCI and were treated with probucol and rosuvastatin for 3 months. Patients of the control group were treated with conventional drugs (aspirin, clopidogrel, rosvastatin, and isosorbide).

Materials and methods

Study design and patients

This was a single-center prospective cohort study of consecutive adult patients with UA that underwent PCI, between March 2016 and July 2016, at the Department of Cardiology of Yidu Central Hospital of Weifang (China). The study was approved by the Human Research Ethics Review Committee. Informed consent was obtained just after the successful procedure, upon successful revascularization without intraoperative death.

Two experienced cardiologists, blinded to clinical and treatment characteristics of the patients, assessed the extent of coronary artery disease and morphology of all coronary artery stenoses. A significant lesion was defined as ≥75% stenosis of the arterial luminal diameter on coronary angiography, according to the ACC/AHA lesion classification scheme (A, B1, B2, or C) [25].

Procedural success was defined as a minimum diameter stenosis of <10% and final thrombolysis in myocardial infarction (TIMI) flow grade 3, without occlusion of a significant side branch, flow-limiting dissection, distal embolization, or angiographic thrombus.

Patient exclusion criteria were: 1) >80 years of age; 2) Pregnant women; 3) Liver or kidney dysfunction; 4) Acute myocardial infarction; 5) Body temperature ≥38.0°C before or during procedure; 6) Inflammatory diseases (infections or autoimmune diseases); 7) Malignancies; 8) Recent major surgery; 9) History of PCI or coronary artery bypass grafting (CABG); or 10) History of statin or antioxidant medication in the previous month.

Treatment

Patients in the control group were treated with oral conventional drugs (lifelong aspirin, 100 mg qd; clopidogrel, 75 mg qd for at least one year; lifelong rosuvastatin, 10 mg qn; and isosorbide mononitrate, 20 mg bid at the time of hospitalization). In addition to conventional therapy, patients in the probucol group took oral probucol (Jingfukang Pharmaceutical Group Co., Ltd., Chengde, China; 250 mg bid) beginning the next day after PCI, for 3 months. Patients were required to avoid other antioxidants during this period.

Blood sampling and enzyme immunoassay

Fasting venous blood samples were collected the next day and 3 months after PCI. Blood samples were centrifuged at 3000 rpm for 15
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Statistical analysis

Continuous data are presented as mean ± SD (normal distribution) or median (range) (non-normal distribution). Categorical data are presented as counts and proportions (percentages). The normality of distribution of continuous variables was tested using Kolmogorov-Smirnov test. Differences in means were assessed using independent t-test (inter-group analysis) or paired-samples t-test (intra-group analysis). Categorical data were analyzed using Chi-square test. Significance is set at two-sided P<0.05 in all tests. Analyses were carried out using SPSS 18 for Windows (IBM, Armonk, NY, USA) by a data analyst blinded to grouping.

Results

Characteristics of patients

Ninety-five patients were screened for eligibility and 83 patients were enrolled: 40 in the control group and 43 in probucol group. Baseline clinical characteristics of enrolled patients are listed in Table 1. The age range of the control group was 35–79 years, with males accounted for 65.0%. The age range of the probucol group was 43–78 years, with males accounted for 67.4%. There were no significant differences between the two groups in age, sex, smoking, and main risk factors of coronary heart disease (diabetes, hypertension, and hyperlipidemia) (all P>0.05).

Procedural features of the patients are listed in Table 2. All patients received at least one drug-eluting stent. There were no significant differences between the two groups (all P>0.05).

MMP-9 and 8-iso-PGF2α levels

MMP-9 and 8-iso-PGF2α levels are shown in Figure 1. Levels of MMP-9 of control and probucol groups were decreased by 14.0% (from 416±256 to 358±265 µg/L) and 47.9% (from 405±219 to 211±145 µg/L), respectively, at 3 months.
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Figure 1. MMP-9 (A) and 8-iso-PGF2α (B) levels of control and probucol groups. *P<0.05; **P<0.01; ***P<0.001 by the t-tests. Error bars indicate 95% CI.

Blood lipids

Blood lipids are presented in Table 3. There were significant decreases in TC (P<0.001) and LDL-C (P<0.001) 3 months after PCI in the two groups. HDL-C was decreased in the probucol group after 3 months of therapy (P<0.001).

Adverse effects

Three patients in the probucol group felt gastrointestinal discomfort for 3-5 days, but without influencing treatment. No other adverse effects, such as headache, dizziness, paresthesia, tinnitus, insomnia, skin rash, and skin itching, were observed. No electrocardiogram Q-T prolongation, ventricular tachycardia, thrombocytopenia, or abnormal liver or kidney function were found in the two groups.

Major adverse cardiac events during follow-up

No patients were lost to follow-up. One patient in the control group underwent a coronary angiography for angina pectoris recurrence. Additionally, 90% restenosis was determined and another drug-eluting stent was placed four months after the first operation. There were no acute myocardial infarction and death events in either groups during follow-up (P=0.963).

Discussion

Oxidative stress and lipid peroxidation play important roles in the development of UA [5-8]. Probucol reduces inflammation and oxidative stress [16-19]. This study evaluated the effects of probucol on levels of 8-iso-PGF2α and MMP-9 in patients with UA after PCI. Results showed that, compared to the control group, 8-iso-PGF2α and MMP-9 levels of the probucol group were decreased more significantly (47.9% vs. 14.0% and 49.8% vs. 13.1%) after 3 months of treatment. There were significant decreases of TC and LDL-C after 3 months of therapy in the two groups. HDL-C levels were decreased in the probucol group after 3 months. There were no differences in occurrence of MACE in the two groups during follow-up.

The pathogenesis of UA is complex [5-8], generally considered to involve ruptures, erosion, or hemorrhaging of vulnerable plaque, thrombosis, coronary stenosis, spasms, and embolisms, eventually leading to acute or subacute decreases of myocardial oxygen supply. During PCI treatment, a large number of inflammatory cytokines are released from the coronary atherosclerotic plaque due to the direct mechanical effects applied on the atherosclerotic plaque by balloon inflation and stent deployment [13, 14]. The important roles of inflammation in in-stent restenosis are well understood [15]. From animal models and clinical studies, it is known that endothelial injuries, platelet and leukocyte interactions, and subcellular chemoattractant and inflammatory mediators are pivotal in the development of inflammatory responses following stent implantation [26].
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Table 3. Blood lipids analysis (before PCI and 3 months after PCI)

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood lipids</th>
<th>Before PCI Median (range)</th>
<th>3 months after PCI Median (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=40)</td>
<td>TG (mmol/L)</td>
<td>1.30 (0.64-3.99)</td>
<td>1.38 (0.65-3.79)</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>TC (mmol/L)</td>
<td>4.11 (2.33-8.32)</td>
<td>3.01 (2.31-4.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HDL-C (mmol/L)</td>
<td>1.15 (0.61-1.58)</td>
<td>1.06 (0.57-1.82)</td>
<td>0.440</td>
</tr>
<tr>
<td></td>
<td>LDL-C (mmol/L)</td>
<td>2.03 (1.14-4.27)</td>
<td>1.52 (0.72-3.14)</td>
<td>0.001</td>
</tr>
<tr>
<td>Probucol (n=43)</td>
<td>TG (mmol/L)</td>
<td>1.27 (0.40-3.64)</td>
<td>0.85 (0.38-3.15)</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>TC (mmol/L)</td>
<td>4.18 (2.71-6.68)</td>
<td>2.53 (1.63-4.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HDL-C (mmol/L)</td>
<td>1.20 (0.85-1.55)</td>
<td>0.97 (0.46-1.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>LDL-C (mmol/L)</td>
<td>2.16 (1.25-4.02)</td>
<td>1.30 (0.76-3.57)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Inflammation is essential for initiation and progression of vascular remodeling, entailing degradation and reorganization of the extra-cellular matrix (ECM) scaffold of the vessel wall, leading to the development of atherosclerotic lesions [5-8]. MMPs are zinc-dependent endopeptidases found in most living organisms, acting mainly by degrading ECM components [27]. MMP-9 is secreted by monocytes, macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts [27]. MMP-9 is involved in ECM degradation, thereby thinning the plaque fibrous cap [28]. Fiotti et al. [29] showed that MMP-9 expression was significantly higher in patients with acute coronary syndrome, compared with patients with stable angina, after PCI treatment, while high expression of MMP-9 suggests plaque instability and higher risk for adverse cardiovascular events. Therefore, reducing MMP-9 expression could lead to more stable plaque, potentially reducing the risk of major adverse cardiac events after PCI. In the present study, results showed that the decrease of MMP-9 levels was more important after probucol combined with rosuvastatin compared to rosuvastatin alone. These results are supported by a study in cultured cells and apoE knockout mice [6].

Oxidation of lipids, proteins, and nucleic acids is involved in the pathogenesis of many diseases, including atherosclerosis [5-8]. Since circulating 8-iso-PGF2α levels predominantly reflect the production of 8-iso-PGF2α rather than its metabolism and excretion, it can be used as a reliable indicator of oxidative stress in vivo [9], supporting its use as a risk marker for CVD [30]. 8-iso-PGF2α levels are significantly higher in patients with UA, compared with matched patients with stable angina and control subjects [31]. Results of the present study showed that the decrease of 8-iso-PGF2α levels was more important after probucol combined with rosuvastatin, compared to rosuvastatin alone.

A previous animal experiment [32] showed that probucol could inhibit macrophage infiltration and expression of MMP-9 in plaque, thereby reducing coronary artery stenosis and increasing plaque stability. Li et al. [33] suggested that probucol may attenuate the enlargement of atherosclerotic vessel walls and may be associated with a negative remodeling pattern, without affecting lumen size. This effect of probucol may involve inhibition of ECM degradation and prevention of apoptosis in atherosclerotic plaques. A randomized controlled trial showed that probucol can significantly decrease ox-LDL-C levels and inhibit expression of MMP-9 in patients with coronary heart disease [22]. A meta-analysis showed that probucol reduced restenosis after PCI [18], supporting the concept of decreased inflammation, oxidative stress, and plaque remodeling.

Reduction of LDL-C levels has been associated with slowed atherosclerosis progression and decreased restenosis after stenting [34]. The present study showed that, in both groups, TC and LDL-C levels were significantly decreased after 3 months of therapy compared with baseline. The decrease of TC and LDL-C in the probucol group was more obvious than in the control group. Results suggested that probucol combined with rosuvastatin could more significantly reduce levels of TC and LDL-C, compared with rosuvastatin alone, probably leading to better long-term outcomes. Longer follow-ups will be necessary to examine this point thoroughly.

Kasai et al. [17] collected data from 1,694 consecutive patients that underwent complete revascularization (PCI and/or bypass surgery). Mortality was compared between patients that received probucol and those that did not. Probucol was associated with significantly reduced risk of all-cause mortality. A meta-
analysis by Liu et al. [18] showed that probucol is more than a lipid-lowering drug, also effective in reducing the risk of restenosis and incidence of MACE after PCI. In the present study, no differences in MACE were observed between the two groups. This could be due to the limitations of the study, including the small sample size and short follow-up. In addition, patients with MI and those with a prior history of PCI or CABG were excluded, reducing the overall risk of MACE in enrolled patients. Furthermore, follow-up angiographies were not performed for some patients, at their discretion. Additional studies are necessary to improve the generalizability of present results.

Probucol with rosuvastatin may significantly decrease 8-iso-PGF2α and MMP-9 in patients with UA after PCI.

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Disclosure of conflict of interest

None.

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