

BIOPHYSICS AND BIOCHEMISTRY

Low Daily Dose of Antioxidant Probucol Decreases Incidence and Severity of Restenosis after Transluminal Coronary Balloon Angioplasty

A. I. Kaminnyi, V. Z. Lankin, A. N. Samko, A. L. Sozykin, S. I. Provatorov, G. G. Konovalova, E. I. Perepelitsa, A. K. Tikhaze, T. Yu. Poleyaya, V. V. Kukharchuk, and Yu. N. Belenkov

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Antioxidant probucol in both high (1000 mg) and low (250 mg) daily doses effectively reduced manifestations of oxidative stress in patients with atherosclerosis (assessed by *in vivo* accumulation of lipoperoxides in atherogenic LDL). When probucol was administered in a dose of 250 mg/day for 7-10 days before transluminal balloon coronary angioplasty and then for 6 months after surgery, the incidence of restenosis decreased to 25% compared to 45% in the control (without probucol therapy). In the group of operated patients receiving probucol (250 mg/day for 6 months) the minimal artery lumen was significantly higher, and the degree of artery occlusion significantly lower than in the control group not treated with probucol.

Key Words: *transluminal balloon coronary angioplasty; restenosis; thrombosis; inflammation; antioxidants*

Transluminal balloon coronary angioplasty (TBCA) is an efficient method of myocardial revascularization during hemodynamically significant coronary artery stenosis. However, restenosis (occlusion of the vessel at the site of intervention) is the major complication of TBCA (30-50% cases) [1,4]. Restenosis after TBCA includes such important stages as thrombosis and inflammation [10]. Free radical oxidation plays an important role in both these processes [2,4]. Specifically, low activity of antioxidant enzymes in the vascular wall during atherosclerosis can provoke accumulation of lipoperoxides and inhibition of biosynthesis of prostacyclin, a natural antithrombotic factor [4], while expression of lipoxygenase in the endo-

thelium promotes synthesis of proinflammatory leukotrienes [2,8]. Antioxidants inhibit production of oxygen radicals by activated macrophages, thereby arresting the local inflammatory reaction [2-4]. Therefore, the development of thrombosis and inflammation, which precede the formation of neointima during restenosis, can be significantly prevented by antioxidants administered both before and after TBCA.

Our aim was to examine the possibility of reducing the incidence and severity of coronary artery restenosis by synthetic antioxidant probucol administered in a low daily dose (250 mg) before and after TBCA.

MATERIALS AND METHODS

In the first part of the study, 28 men aged 52 ± 1.4 years with chronic coronary heart disease (CHD) and hyper-

A. L. Myasnikov Institute of Cardiology, Russian Cardiology Research and Production Center, Russian Ministry of Health, Moscow. **Address for correspondence:** akam67@mail.ru. A. I. Kaminnyi

cholesterolemia were randomized into two equal groups and received probucol (Alcolex, ICIV Hungary-Rt) in a daily dose of 1000 mg (2×500 mg) or 250 mg (2×125 mg) for 6 months. Plasma LDL were isolated by differential centrifugation in NaBr density gradient [5] in a Beckman L-8 ultracentrifuge. Then the content of lipoperoxides in LDL was determined in Hitachi-557 spectrophotometer at $\lambda=560$ nm by the reaction with Fe^{2+} xylene orange before and after reduction of organic hydroperoxides with triphenylphosphine [11]. Activity of Se-containing glutathione peroxidase in erythrocytes was determined in the conjugated glutathione reductase system by the rate of NADPH oxidation at $\lambda=340$ nm using tert-butyl-4-hydroxyphenyl as the substrate as described elsewhere with modifications [5] using an FP-901 chemical analyzer (Labsystems Oy) in the kinetic mode.

In the second part of the study, we examined 40 men aged 55.0 ± 5.4 years with chronic CHD and angiographically documented stenosis ($\geq 70\%$) of one of the major arteries. The total cholesterol content in these patients was 6.90 ± 0.28 mmol/liter. They were randomized into two equal groups. In addition to routine antianginal therapy, group 1 patients received probucol in a daily dose of 250 mg for 7-10 days before TBCA and for 6 months after it. Group 2 patients (control) received routine therapy before and after angioplasty. TBCA was carried out by the routine method [7] with a Crosco (Siemens) apparatus. After TBCA, the residual stenosis did not exceed 25%. Six months after angioplasty, the patients were subjected to repeated coronarography [6] on the same apparatus. The presence of restenosis in the operated artery was documented, if it was constricted by at least 50%. The angiograms were quantitatively analyzed using a Hicor system (Siemens).

RESULTS

For a long period, probucol in a daily dose of 1000 mg was used as a hypolipidemic agent. However, this

synthetic phenol antioxidant was inefficient in decreasing the total and LDL cholesterol [3,5], while 2-4-fold decrease in the daily dose of probucol had no effect on lipid metabolism [2,4,5]. In our study, the high dose of probucol significantly decreased the total and LDL cholesterol, while the low dose of this drug produced no significant effect on these parameters (Table 1). After 6-month probucol therapy in both high and low doses, the concentration of lipoperoxides in plasma LDL decreased to the same extent (Table 1, differences are insignificant). Therefore, low daily dose of probucol, which is four times lower than that providing the hypolipidemic effect, is sufficient to improve the antioxidant status of the organism and to protect it from oxidative stress. In other words, probucol can be used as an efficient non-toxic antioxidant, which exerts its *in vivo* effect via direct [2,4,5] and indirect (by increasing activity of GSH-peroxidase, Table 1) mechanisms [2-4]. These features favor probucol among other antioxidant preparations used in clinical studies.

Some studies, carried out in different countries, corroborated the possibility of suppression of restenosis of the coronary arteries after balloon angioplasty by administration of antioxidants to animals [13] and humans [12,14,15]. All these studies report virtually identical (40-50%) decrease in the incidence of post-TBCA restenosis by the high doses of probucol [12, 14,15]. Our data agree with these findings (Table 2). They show a possibility to use low and safer doses of probucol to eliminate the post-TBCA restenosis. The control group of patients and the patients treated with probucol in a daily dose of 250 mg were comparable by X-ray morphological characteristics of vascular damage both before and immediately after successful TBCA (Table 2). However, the incidence of restenosis was only 25% in the experimental group vs. 45% in the control group. Repeated angiography carried out 6 months after TBCA revealed significant differences between the control and probucol-treated groups by the following parameters: minimum diameter of artery

TABLE 1. Effect of Low and High Doses of Probucol on Lipid Metabolism and Intensity of Free Radical Processes in Patients with CHD ($M \pm m$)

Index	Before treatment (n=28)	After treatment	
		high doses of probucol (n=14)	low doses of probucol (n=14)
Total cholesterol, mmol/liter	7.20 ± 0.21	$5.70 \pm 0.18^*$	6.70 ± 0.46
LDL cholesterol, mmol/liter	5.10 ± 0.26	$4.40 \pm 0.14^*$	5.30 ± 0.42
LDL lipohydroperoxides, nmol/liter	8.10 ± 0.48	$5.90 \pm 0.19^*$	$6.20 \pm 0.02^*$
Glutathione peroxidase, U/g Hb	3.70 ± 0.37	$7.20 \pm 0.52^*$	$6.70 \pm 0.58^*$

Note. $*p < 0.05$ compared to the values before treatment.

TABLE 2. Effect of Probucol Therapy (250 mg/day) on Dynamics of X-Ray Morphological Parameters of Coronary Arteries after TBCA ($M \pm m$)

Observation period	Control (n=20)		Probucol (n=20)	
	minimum diameter of artery lumen, mm	degree of artery occlusion, %	minimum diameter of artery lumen, mm	degree of artery occlusion, %
Before TBCA	0.77±0.35	74.8±13.0	0.78±0.30	72.6±12.8
After TBCA	2.75±1.06	10.3±6.9	2.28±0.72	12.5±10.6
Six months after TBCA	1.41±0.94 ⁺⁺	46.2±29.7 ⁺⁺	1.88±0.73 ^{++o}	28.9±21.2 ^{++o}

Note. $p < 0.05$ compared to ^{*}values before TBCA, ⁺values after TBCA treatment, and ^ocontrol.

lumen and degree of stenosis (Table 2). For example, minimum diameter of artery lumen in probucol-treated group (250 mg/day for 6 months) was greater by 30% and the degree of stenosis was lower by 1.6 times than the corresponding parameters in the control group (Table 2). Our study suggests that antioxidants can be used for reduction of restenosis. Probucol was also efficient in suppression of oxidative stress and inhibition of post-TBCA restenosis even in a low dose. The data obtained open new vista for wider use of antioxidants (specifically, probucol) in invasive cardiology.

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