Relationship between Free-Radical Lipid Oxidation and Efficiency of Coronary Angioplasty in Coronary Patients

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Low-dose (250 mg daily) oral probucol produces a significant antioxidant effect in coronary patients: increases activity of glutathione peroxidase (enzyme utilizing lipoperoxides) and reduces the content of free-radical oxidation products in the blood. Probucol therapy for 7 days before and for 6 months after coronary angioplasty significantly reduces the severity of coronary artery stenosis.

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

Coronary heart disease is the leading cause of morbidity and mortality in countries with well-developed economy. Transluminal coronary angioplasty (TCA) is the main method for the treatment of CHD, but the development of repeated arterial stenosis (restenosis) is the main problem of delayed period after endovascular intervention. Free-radical oxidation plays an important role in the mechanisms of thrombogenesis and inflammation [1,6] accompanying the early stages of restenosis [7]. Accumulation of lipoperoxides in the vascular wall is paralleled by inhibition of biosynthesis of prostacyclin (natural antithrombogenic factor), while expression of lipoxygenase promotes the synthesis of leukotrienes (inflammation mediators) [1]. In addition, antioxidants suppress local inflammatory reactions by inhibiting production of active oxygen species by macrophages [1-3]. Hence, the development of thrombosis and inflammation preceding the formation of neointima in restenosis can be effectively suppressed by adding antioxidants to the therapy of patients before and after TCA. We previously showed [6] that probucol in a daily dose of 250 mg exhibited antioxidant effects and did not produce side effects typical of the daily dose of 1000 mg. The antioxidant effect was determined not only by direct binding of lipid radicals, but also by expression of glutathione peroxidase (antioxidant enzyme) in tissues.

Here we evaluated the possibility of using probucol in a daily dose of 250 mg for suppressing oxidative stress and reducing the incidence and severity of restenosis after TCA.

MATERIALS AND METHODS

The study was carried out in male patients aged 32-65 years with CHD and stable angina and hemodynamically significant coronary artery stenosis. The patients were randomly divided into 2 groups; 38 patients (control) received antianginal therapy, 43 received additionally probucol (Alcolex, ICN)

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in a daily dose of 250 mg. The patients received probucol for 7-10 days before TCA and continued oral treatment for 6 months, after which repeated coronarography (CAG) was carried out. Transluminal coronary angioplasy was carried out by the standard method: balloon angioplasty in 15% patients and balloon angioplasty with stenting in 85%. Matrix stents (without coating) of analogous design were used. The study was carried out on a Goroscop 33 (Siemens), angiograms were processed using Hicor computer system (Siemens).

LPL were isolated by differential centrifugation of the plasma in NaBr density gradient on a Beckman L-8 refrigerator centrifuge [12]. After dialysis, LDL were diluted to a concentration of 50 μg protein/ml and incubated in a medium with 0.154 M NaCl and 50 mM phosphate buffer (pH 7.4) at 37°C with 3×10^{-5} M $CuSO_4$ and lipoperoxide accumulation was measured at λ=233 on a Hitachi 220A spectrophotometer after certain intervals [4]. The duration of the lag phase of LDL oxidation was evaluated by kinetic curves, hydroperoxide content in LDL was evaluated by the reaction with Fe^{2+} xylene orange before and after their reduction with triphenylphosphine on a Hitachi-557 spectrophotometer at λ=560 nm [9]. The content of MDA was evaluated by the reaction with TBA at λ=532 nm on a Hitachi-557 spectrophotometer [5]. Activity of erythrocyte Se-containing glutathione peroxidase was evaluated in a conjugated glutathione reductase system by the rate of NADPH oxidation at λ=340 nm with tert-butyl hydroperoxide as the substrate [5] on a Labsystems Oy FP-901 chemical analyzer in the kinetic mode. The content of total cholesterol (CH), triglycerides, and HDL CH was measured by the enzymatic method on a Chiron Diagnostics biochemical analyzer using Bio Systems kits. The content of CH in LDL was calculated from these values.

RESULTS

Previous data [10,11,13,14] indicate preventive effect of probucol in daily doses of 500-1000 mg reducing the incidence and severity of restenosis after TCA, but high doses exhibited side effects (decrease in HDL level, prolongation of corrected *Q-Tc* ECG interval, *etc.*). However, parameters of oxidative stress were not evaluated in these studies, which made impossible interpretations of the mechanism of probucol effect. We studied the effect of probucol in a daily dose of 250 mg on plasma content of total CH, LDL CH, HDL CH, and triglycerides and on the length of the *Q-Tc* interval on

TABLE 1. Parameters of Lipid Metabolism and Length of *Q-Tc* Interval in Coronary Patients before and after TCA (*M*±*m*)

Parameter	Control		Probucol, 250 mg/day	
	before TCA	6 months after TCA	before TCA	6 months after TCA
Total CH, mmol/liter	6.39±0.79	6.30±0.64	6.33±0.66	6.25±0.57
LDL CH, mmol/liter	4.30±0.62	4.35±0.60	4.36±0.50	4.31±0.55
HDL CH, mmol/liter	1.08±0.28	1.05±0.24	1.04±0.29	1.03±0.27
Triglycerides, mmol/liter	2.10±0.46	1.99±0.38	1.99±0.54	1.97±0.44
Q-Tc, sec	0.38±0.02	0.38±0.01	0.38±0.02	0.39±0.01

TABLE 2. Effects of Probucol (250 mg/day) on Oxidative Stress Values in Stenosed Vessels in Coronary Patients 6 Months after TCA (*M*±*m*)

Parameter	Control (*n*=38)		Probucol (*n*=43)	
	before TCA	6 months after TCA	before TCA	6 months after TCA
Hydroperoxides, nmol/mg protein	34.06±5.89	53.04±9.61	42.23±9.71	19.77±4.94*+
LDL MDA, nmol/mg protein	5.85±0.63	5.37±0.41	6.83±0.57	3.55±0.42*+
Glutathione peroxidase, units/mg hemoglobin	3.90±0.31	3.49±0.27	3.14±0.17	5.17±0.27*+
Lag phase, min	15.20±2.70	15.48±2.46	11.72±1.68	85.90±7.00

Note. *$p<0.001$ compared to values before TCA; +$p<0.01$ compared to the control.

TABLE 3. Probucol Effects (250 mg/day) on Angiographic Characteristics of Stenosed Vessels in Coronary Patients 6 Months after TCA (*M*±*m*)

Parameter	Control (*n*=38)			Probucol (*n*=43)		
	before TCA	after TCA	6 months after TCA	before TCA	after TCA	6 months after TCA
Minimum diameter of artery, mm	0.86±0.29	2.67±0.35	1.87±0.80	0.89±0.27	2.63±0.34	2.22±0.87*
Degree of arterial stenosis, %	69.54±7.25	6.71±5.66	32.58±29.83	68.41±7.87	5.81±5.50	18.57±20.09*

Note. *$p<0.01$ compared to the control.

ECG. In accordance with our previous findings [6], probucol in a dose of 250 mg/day (6-month course) did not modify the parameters of lipid metabolism, including HDL CH, and the length of *Q-Tc* interval (Table 1). Our data indicate that 6-month therapy with probucol significantly decreased the content of lipoperoxides in LDL and MDA in plasma LDL (by 2.1 and 1.9 times, respectively) and significantly (by 7.3 times) prolonged LDL oxidation lag phase *in vitro* (Table 2). In parallel, 6-month probucol therapy significantly (by 1.7 times) increased glutathione peroxidase activity in erythrocytes (Table 2). These results confirm previous data indicating the antioxidant effect of low-dose probucol in coronary patients [2,6].

Angiographic characteristics of stenosed arteries during TCA and 6 months after it (control CAG) are presented in Table 3. The minimum diameter of stenosed arterial segments and degree of stenosis before and directly after TCA were virtually the same in patients receiving probucol and controls (Table 3). Control CAG showed that the minimum diameter of the artery in patients treated with probucol was 2.22±0.67 mm *vs.* 1.87±0.80 mm in the control, the degree of artery stenosis being lower in the study group in comparison with the control. A significant positive correlation between MDA content in LDL and degree of coronary artery stenosis ($r=0.54$; $p<0.001$) and a significant negative correlation between MDA content in LDL and minimum diameter of coronary arteries ($r=-0.49$; $p<0.01$) were detected in patients treated with probucol for 6 months after TCA. In controls, the content of lipoperoxides in LDL and degree of coronary artery stenosis were also in positive correlation 6 months after TCA ($r=0.44$; $p<0.01$), while lipoperoxide content in LDL and minimum coronary artery diameter were in negative correlation ($r=-0.38$; $p<0.001$). Hence, probucol in a low daily dose (250 mg) significantly suppressed vascular restenosis, inhibited free-radical lipid peroxidation, and accelerated enzymatic utilization of lipoperoxides in the blood. These results validate the use of low-dose probucol as an economic and safe means improving the efficiency of endovascular interventions.

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