Trimetazidine as Indirect Antioxidant

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One-month therapy with trimetazidine sharply decreased the content of free radical oxidation products, lipid peroxides and malonic dialdehyde, in atherogenic low-density lipoproteins in patients with coronary heart disease. Activity of glutathione peroxidase utilizing lipid peroxides in the plasma markedly increased during trimetazidine therapy. The data suggest that trimetazidine not directly interacting with free radicals attenuates the adverse effects of intensive free radical oxidation in coronary heart disease. This effect is mediated via activation of antioxidant enzymes, which diminishes negative consequences of ischemia.

Key Words: *trimetazidine; lipid peroxides; glutathione peroxidase; free radical oxidation; antioxidants*

New approaches to the therapy of coronary heart disease (CHD) attenuating metabolic disturbances in ischemic cardiomyocytes are now extensively elaborated. Trimetazidine (1-[2,3,4-trimethoxyphenyl)methyl] piperazine, Preduktal, Servier) produces cytoprotective, antiischemic, and antihypoxic effects on the myocardium and stimulates ATP synthesis by changing energy metabolism from fatty acid to glucose oxidation [1]. Trimetazidine normalizes ion balance, attenuates intracellular acidosis, inhibits platelet aggregation by decreasing the content of thromboxane A_2, and suppresses accumulation and activation of neutrophils in the ischemic zone [1,14]. It was shown that trimetazidine has antioxidant properties and affects utilization of oxygen radicals [1,8,10,14]. Trimetazidine decreases the content of lipid peroxidation products during intensification of reactive oxygen species formation under conditions of ischemia-reperfusion [10]. Moreover, trimetazidine increases the resistance of cells and tissues to prooxidants [8]. At the same time, our *in vitro* experiments showed that trimetazidine does not modulate utilization of oxygen radicals and has no intrinsic antioxidant properties [5]. However, the possibility that trimetazidine indirectly affects free radical oxidation can not be excluded. Our studies showed that probucol (hypocholesterolemic drug) used for the therapy of CHD activates superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), the key antioxidant enzymes utilizing reactive oxygen species and lipid peroxides [6]. Here we studied the effects of trimetazidine on SOD and GSH-Px activities in the plasma and erythrocytes and the contents of lipid peroxides and malonic dialdehyde (MDA) in atherogenic low-density lipoproteins (LDL, most readily oxidized plasma lipoproteins) from patients with CHD.

MATERIALS AND METHODS

Eleven men (48±2 years) with CHD were examined. The diagnosis of class I and II stable effort angina was made in 8 patients; medical history of 7 patients included myocardial infarction. After a control period (1 week), the patients were withdrawn from antianginal preparations, but received monotherapy with 20 mg trimetazidine (3 times a day for 3 months).

Venous blood was taken monthly from fasting individuals before and during the therapy and placed into tubes with 1 mg/ml EDTA. The plasma was centrifuged 2 times in a NaBr density gradient at 42,000 rpm and 4°C for 2 h in a Beckman L-8 ultracentrifuge equipped with a 50Ti angle rotor [5,13]. LDL were dialyzed at 4°C for 16 h. Protein content was measured by the method of Lowry. LDL were diluted with a solution containing 0.154 M NaCl and 50 mM phos-

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phate buffer (pH 7.4) to a concentration of 50 μg protein/ml. LDL oxidation was induced with 30 μM $CuSO_4$ at 37°C. The content of lipid hydroperoxides was measured on a Hitachi 220A spectrophotometer at 233 nm [5]. The content of LDL lipid hydroperoxides was estimated by the reaction with xylenol orange after lipid peroxide reduction with triphenylphosphine on a Hitachi 557 spectrophotometer [11]; MDA content was evaluated by the reaction with thiobarbituric acid [2]. To estimate the activity of antioxidant enzymes in erythrocytes comprising not less than 95% enzyme activity, whole blood (0.1 ml) was mixed 1:9 with 5 mM hypotonic K,Na-phosphate buffer (pH 7.4). After hemolysis, the mixture was frozen and stored at -20°C for no more than 1 month [6]. Cold chloroform-ethanol mixture (3:5, 0.5 ml) was added to 1 ml sample on an ice bath, shaken, and precipitated by gem centrifugation [6]. SOD activity was estimated by inhibition of NBT reduction with superoxide anion radical generated in the xanthine-xanthine oxidase system at 25°C and measured on a Hitachi 557 spectrophotometer at 560 nm [11]. The amount of SOD causing a 50% inhibition of the reaction was taken as a unit of enzyme activity. GSH-Px activity in blood plasma and lysates was determined at 25°C as described elsewhere [12] with modifications [3]. We measured the kinetics of oxidized glutathione formation estimated from NADPH oxidation in coupled glutathione reductase reaction using tert-butyl hydroperoxide as the substrate on an FP-901 chemical analyzer (Labsystems Oy) at 340 nm and kinetic regimen. The amount of GSH-Px oxidizing 1 μmol reduced glutathione over 1 min was taken as a unit of enzyme activity [3]. The content of hemoglobin was measured using kits (Biolar) on a FP-901 chemical analyzer (Labsystems Oy) at 540 nm.

RESULTS

Three-month therapy with trimetazidine decreased the incidence of anginal attacks from 4.6±1.0 to 3.8±0.4 per week, reduced the demands for short-acting nitrates from 3.9±1.1 to 3.3±0.3 nitroglycerine tablets/week, and improved the tolerance to physical exercises (the time of exercise and performed work increased by 182±59 sec and 3.8±0.6 metabolic units, respectively; 601±54 sec and 10.9±0.9 metabolic units in the control, respectively).

One-month therapy with trimetazidine sharply decreased the contents of lipid peroxides and MDA in plasma LDL from patients with CHD (Table 1). The amount of LDL lipid peroxides and MDA decreased by 46-48 and 44-64%, respectively, 1-3 months after the start of therapy. However, there were no significant changes in the kinetics of Cu^{2+}-induced free radical oxidation of LDL in the plasma of patients treated with trimetazidine for 3 months. Before the start of therapy, the lag phase of *in vitro* LDL oxidation was 63.0±5.9 min and V_{max}=1.60±0.14 nmol lipid peroxides/min. After 3-month therapy with trimetazidine, these parameters were 55.0±3.4 min and 1.50±0.29 nmol lipid peroxides/min, respectively. The data suggest that parameters of LDL oxidation *in vitro* do not reflect the state of plasma antioxidant systems and are less informative, than the content of *in vivo* accumulated lipid peroxides, probably, due to the absence of endogenous plasma factors (*e.g.*, antioxidant enzymes) affecting LDL lipid peroxidation in the model system.

Trimetazidine had no effect on SOD and GSH-Px activities in erythrocytes, but markedly changed GSH-Px activity in the plasma (Table 1). GSH-Px activity in the plasma 1 month after the start of treatment

TABLE 1. Activities of Antioxidant Enzymes in Erythrocytes and Blood Plasma and Content of Free Radical Lipid Oxidation Products in LDL from CHD Patients Treated with Trimetazidine (*M*±*m*)

Parameter	Initial level	Therapy, months		
		1	2	3
Glutathione peroxidase activity				
in erythrocytes, U/g hemoglobin	6.00±0.59	4.20±0.36	4.90±0.43	5.40±0.85
in blood plasma, U/ml	0.220±0.014	0.480±0.022*	0.480±0.038*	0.560±0.043*
% of control	100±6	218±10*	218±17*	255±22*
SOD activity in erythrocytes, U/g hemoglobin	738±63	779±56	770±81	647±70
Lipid peroxide content,				
μmol/ml plasma	1.77±0.25	0.97±0.31*	0.92±0.29*	0.93±0.27*
% of control	100±14	55±18*	52±16*	53±15*
MDA content, μmol/ml plasma	10.1±1.5	4.8±0.7*	5.7±0.6*	3.7±0.9*
% of control	100±15	47±7*	56±6*	36±9*

Note. *$p<0.05$ compared to the initial level.

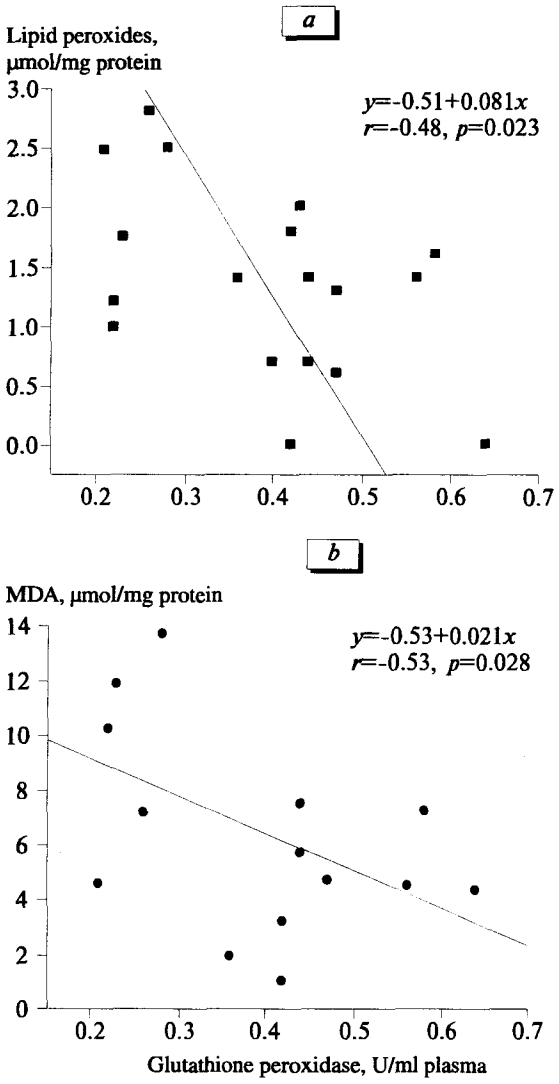

Fig. 1. Correlation between the content of primary (*a*) and secondary (*b*) products of free radical oxidation of blood plasma LDL lipids and glutathione peroxidase activity in the plasma of CHD patients treated with trimetazidine.

2-fold surpassed the initial level and remained high to the end of observations (Table 1). Unlike cytosolic GSH-Px [9], plasma GSH-Px [15] can reduce oxidized phospholipid acyls without their enzymatic hydrolysis [4]. Free radical oxidation of LDL phospholipids during atherogenesis results in their oxidative modification and rapid uptake by monocytes and macrophages, which cause preatherosclerotic damages to the vascular wall (lipoidosis) [4]. During trimetazidine therapy, plasma GSH-Px activity was inversely related to the contents of lipid peroxides (Fig. 1, *a*) and MDA in LDL (Fig. 1, *b*). It can be hypothesized that plasma GSH-Px is one of the most potent natural antioxidants protecting LDL from oxidative atherogenic modification. The decrease in LDL lipid peroxide content with increasing GSH-Px activity during trimetazidine therapy (Table 1) and the interrelation between the content of oxidation products and activity of enzymes responsible for their *in vivo* utilization and formation (Fig. 1) confirmed our assumption. Hence, trimetazidine does not directly interacts with free radicals, but attenuates adverse effects of free radical oxidation intensified during CHD. This action is mediated by activation of natural protective systems (antioxidant enzymes). Since free radical reactions play an important role in ischemic myocardial damages [4], these antioxidant effects of trimetazidine *in vivo* compensate the negative consequences of ischemia.

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REFERENCES

1. O. M. Eliseev, *Ter. Arkh.*, No. 8, 57-63 (1996).
2. V. Z. Lankin and S. M. Gurevich, *Biological Antioxidants* [in Russian], Moscow (1975), pp. 146-150.
3. V. Z. Lankin and S. M. Gurevich, *Dokl. Akad. Nauk SSSR*, **226**, No. 3, 392-395 (1976).
4. V. Z. Lankin, A. K. Tikhaze, and Yu. N. Belenkov, *Kardiologiya*, No. 6, 58-71 (2000).
5. A. K. Tikhaze, V. Z. Lankin, S. V. Kolycheva, *et al.*, *Byull. Eksp. Biol. Med.*, **126**, No. 11, 551-554 (1998).
6. A. K. Tikhaze, V. Z. Lankin, V. P. Mikhin, *et al.*, *Ter. Arkh.*, No. 9, 35-41 (1997).
7. C. Beauchamp and J. Fridovich, *Anal. Biochem.*, **44**, 276-278 (1971).
8. E. Fantini, L. Demaison, E. Sentex, *et al.*, *J. Mol. Cell. Cardiol.*, **26**, 949-958 (1994).
9. L. Flohe, *Free Radic. Biol.*, **5**, 223-254 (1982).
10. C. Harpey, P. Clauser, C. Labrid, *et al.*, *Cardiovasc. Drug. Res.*, **6**, 292-312 (1989).
11. J. Nourooz-Zadeh, J. Tajaddini-Sarmadi, and S. P. Wolf, *Anal. Biochem.*, **220**, 403-409 (1994).
12. D. E. Paglia and W. N. Valentine, *J. Lab. Clin. Med.*, **70**, 158-169 (1967).
13. V. V. Tertov, V. V. Kaplun, S. N. Dvoryantsev, and A. N. Orekhov, *Biochem. Biophys. Res. Commun.*, **214**, 608-613 (1995).
14. F. M. Williams, K. Tanda, M. Kus, and T. J. William, *J. Mol. Cell. Cardiol.*, **24**, Suppl. 1, 90 (1992).
15. Y. Yamamoto, Y. Nagata, E. Niki, *et al.*, *Biochem. Biophys. Res. Commun.*, **193**, 133-138 (1993).