Oxidative Stress in Atherosclerosis and Diabetes

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We measured the content of lipid peroxides in plasma LDL from patients with chronic CHD not accompanied by hypercholesterolemia; CHD and hypercholesterolemia; type 2 diabetes mellitus and decompensation of carbohydrate metabolism; and CHD, circulatory insufficiency, and type 2 diabetes mellitus (without hypercholesterolemia). The content of lipid peroxides in LDL isolated from blood plasma by differential ultracentrifugation in a density gradient was estimated by a highly specific method with modifications (reagent Fe^{2+} xylene orange and triphenylphosphine as a reducing agent for organic peroxides). The content of lipid peroxides in LDL from patients was much higher than in controls (patients without coronary heart disease and diabetes). Hypercholesterolemia and diabetes can be considered as factors promoting LDL oxidation *in vivo*. Our results suggest that stimulation of lipid peroxidation in low-density lipoproteins during hypercholesterolemia and diabetes is associated with strong autooxidation of cholesterol and glucose during oxidative and carbonyl (aldehyde) stress, respectively. These data illustrate a possible mechanism of the progression of atherosclerosis in patients with diabetes mellitus.

Key Words: *type 2 diabetes mellitus; circulatory insufficiency; low-density lipoproteins; free radical oxidation; autooxidation of cholesterol and glucose*

High cholesterol concentration in blood plasma (particularly, in circulating atherogenic LDL) is a risk factor for the development and progression of atherosclerosis [2-4]. Atherosclerosis is accompanied by activation of free radical oxidation of unsaturated acyl glycerides in LDL (oxidative stress) [1,3,4,12], which leads to the formation of a considerable amount of carbonyl compounds and structural modification of LDL [1,2,14]. They are recognized by scavenge receptors on macrophages in the interendothelial space of the vascular wall and internalized by these cells [1,2,14]. Lipid-overloaded macrophages are transformed into foam cells. Clusters of these cells form lipoidosis zones in the aorta and coronary arteries (lipid spots). These changes are considered as primary atherosclerotic injury [2-4]. Secondary products of lipid peroxidation (*e.g.*, malonic dialdehyde, 4-hydroxynonenal, and other α,β-unsaturated aldehydes) form intermolecular bonds with terminal amino groups in LDL apoproteins and serve as the major LDL-modifying agents [1,11, 14]. Other low-molecular-weight aldehydes, including glyoxal, methylglyoxal, and 3-deoxyglucosone, are produced during glucose autooxidation in patients with diabetes mellitus and hyperglycemia [6,8,10]. It was hypothesized that these carbonyl compounds cause atherogenic modification of LDL [10]. These data explain rapid progression of atherosclerosis in patients with decompensated diabetes mellitus [9].

Here we studied the severity of oxidative stress (degree of LDL oxidation *in vivo*) in patients with chronic coronary heart disease (CHD) and/or type 2 diabetes mellitus.

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MATERIALS AND METHODS

We examined 38 patients (30 men and 8 women, average age 46±3 years) with the diagnosis of CHD. Group 3 patients (n=29) had severe hypercholesterolemia (HCH); heterozygous familial HCH was verified in 15 patients of this group. Cholesterol concentration in group 2 patients (n=9) approached the normal. Under normal conditions, total plasma cholesterol concentration is 5.2 mmol/liter. Control group 1 included 7 men (45±5 years) without CHD and HCH. During examination the patients received standard antianginal drugs (β-adrenoceptor antagonists, calcium antagonists, and antiaggregants). The scheme of treatment did not include preparations decreasing cholesterol concentration (*e.g.*, inhibitors of hydroxymethyl-glutaryl coenzyme A reductase, statins). We also examined 30 patients (15 men and 15 women, 57±9 years) with type 2 diabetes mellitus. At the start of therapy these patients were characterized by decompensation of carbohydrate metabolism (HbA_{1c}=8.10±0.03%, group 5). They perorally received sugar-decreasing drugs for 2 months (sulfonyl urea preparations alone or in combination with metformin). This treatment considerably decreased the concentration of glycated hemoglobin (HbA_{1c}=7.10±0.18%, group 4). Group 6 included 21 patients (17 men and 4 women, 63±2 years) with chronic CHD, functional class II circulatory insufficiency (NYHA classification, ejection fraction ≤45%), and type 2 diabetes mellitus (HbA_{1c}=7.20±0.34%). CHD was not accompanied by HCH, and these patients did not receive statins.

Venous blood was taken from fasting patients using 1 mg/ml ethylenediaminetetraacetic acid as an anticoagulant and antiaggregant. LDL were isolated by preparative ultracentrifugation in a NaBr density gradient using a Beckman L-8 device (fixed-angle rotor 50Ti) [15]. After dialysis the content of LDL lipid peroxides was measured by a highly specific method with modifications. Fe^{2+} xylene orange was used before and after organic peroxide reduction with triphenylphosphine [13]. Protein content in LDL was estimated by the method of Lowry. The concentration of total cholesterol was determined enzymatically on an Airone-200 chemical analyzer using Biocon test kits.

TABLE 1. Content of Lipid Peroxides in LDL from Patients with CHD and Type 2 Diabetes Mellitus

Group	Number of patients	Content of LDL lipid peroxides, nmol/mg protein	Total plasma cholesterol concentration, mmol/liter
Group 1 (control, patients without signs of CHD and diabetes)	7	8.0±3.2	4.70±0.45
Group 2 (CHD without HCH)	9	32.0±8.1	4.80±0.31
		p_{1-2}<0.002	p_{1-2}>0.05
Group 3 (CHD and HCH)	29	65.0±11.9	8.6±0.5
		p_{1-3}<0.001	p_{1-3}<0.001
		p_{2-3}<0.05	p_{2-3}<0.001
Group 4 (type 2 diabetes mellitus, HbA_{1c}=7.10±0.18%)	30	138.0±16.6	6.10±0.36
		p_{1-4}<0.001	p_{1-4}<0.05
		p_{2-4}<0.001	p_{2-4}<0.001
		p_{3-4}<0.001	p_{3-4}<0.001
Group 5 (type 2 diabetes mellitus, HbA_{1c}=8.10±0.03%)	30	199.0±19.5	6.50±0.42
		p_{1-5}<0.001	p_{1-5}<0.001
		p_{2-5}<0.001	p_{2-5}<0.001
		p_{3-5}<0.001	p_{3-5}<0.001
		p_{4-5}<0.05	p_{4-5}>0.05
Group 6 (CHD, CI II, and diabetes mellitus; HbA_{1c}=7.20±0.34%)	21	285±54	4.9±0.2
		p_{1-6}<0.001	p_{1-6}>0.05
		p_{2-6}<0.001	p_{2-6}>0.05
		p_{3-6}<0.001	p_{3-6}<0.001
		p_{4-6}<0.002	p_{4-6}<0.001
		p_{5-6}>0.05	p_{5-6}<0.001

Note. CI II, functional class II circulatory insufficiency; p, significance of differences (Student's t test).

RESULTS

Published data show that HCH serves as a risk factor for oxidative stress during atherosclerosis [3,12]. However, our study showed that the content of LDL lipid peroxides in group 2 patients with CHD and normal level of plasma cholesterol 4-fold exceeds that in patients with CHD not accompanied by HCH (control group 1, Table 1). It should be emphasized that the degree of LDL oxidation in group 3 patients with CHD and severe HCH *in vivo* more than 8-fold surpassed the control. These data support the results of our previous studies. We showed that plasma LDL from patients with CHD and familial HCH *in vitro* undergo rapid free radical oxidation [5]. Therefore, HCH is a risk factor for the development of oxidative stress during atherosclerosis. Under conditions of HCH free radical oxidation of LDL involves not only unsaturated glyceride acyls, but also cholesterol molecules [12]. Our previous experiments showed that products of cholesterol autooxidation (oxysterins) exhibit atherogenic activity [12]. The content of LDL lipid peroxides in patients with type 2 diabetes mellitus (groups 4 and 5) reached maximum and was higher than in controls and patients with CHD and HCH (by 17-25 and 2-3 times, respectively). Progression of carbohydrate metabolic dysfunction was accompanied by an increase in the degree of LDL oxidation (Table 1, Fig. 1). The presence of glucose *in vitro* stimulates free radical oxidation of LDL [7]. High degree of LDL oxidation in patients with diabetes mellitus was probably associated with activation of glucose autooxidation during decompensation of carbohydrate metabolism and development of carbonyl (aldehyde) stress [6,8]. These changes should promote a sharp increase in oxidative modification of LDL and progression of atherosclerosis. Heart failure also contributes to intensification of free radical oxidation of LDL *in vivo* (Table 1, Fig. 1). The degree of LDL oxidation in patients with CHD, circulatory insufficiency, and diabetes mellitus not accompanied by HCH (group 6) was much higher than in patients with diabetes mellitus, disturbances in carbohydrate metabolism, and HCH (group 4). The mechanism for initiation and progression of oxidative stress during heart failure is poorly understood [1]. It is clear that this phenomenon affects the course and prognosis of the disease. A rational scheme of therapeutic treatment should be developed in further studies.

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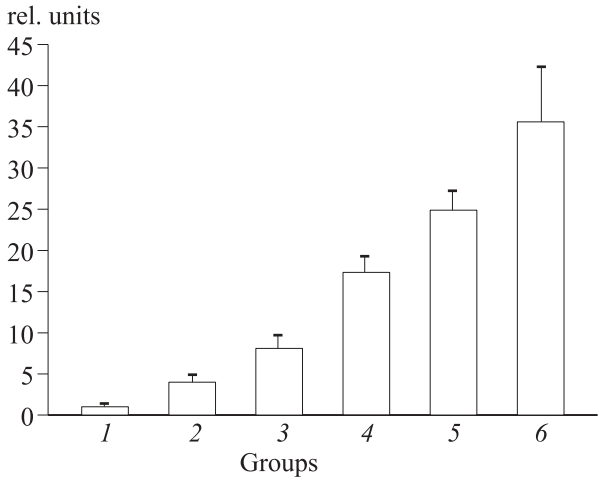

Fig. 1. Relative content of LDL lipid peroxides in patients with CHD and type 2 diabetes mellitus. Level in the control group was taken as 1 unit.

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REFERENCES

1. N. K. Zenkov, V. Z. Lankin, and E. B. Men'shchikova, *Oxidative Stress. Biochemical and Pathophysiological Aspects* [in Russian], Moscow (2001).
2. A. N. Klimov and N. G. Nikul'cheva, *Normal and Abnormal Metabolism of Lipids and Lipoproteins* [in Russian], St. Petersburg (1999).
3. V. Z. Lankin, A. K. Tikhaze, and Yu. N. Belenkov, *Kardiologiya*, **40**, No. 7, 48-61 (2000).
4. V. Z. Lankin, A. K. Tikhaze, and Yu. N. Belenkov, *Ibid*., **44**, No. 2, 72-81 (2004).
5. V. Z. Lankin, A. K. Tikhaze, G. G. Konovalova, *et al*., *Byull. Eksp. Biol. Med*., **136**, No. 7, 49-52 (2003).
6. J. W. Baynes and S. R. Thorpe, *Diabetes*, **48**, 1-9 (1999).
7. R. Bucala, Z. Makita, T. Koschinsky, *et al*., *Proc. Natl. Acad. Sci. USA*. **90**, 6434-6438 (1993).
8. C. A. L. S. Colaco and B. J. Roser, *BioEssays*, **16**, 145-147 (1994).
9. W. B. Kannel and D. L. McGee, *Diabetes*, **241**, 2035-2038 (1979).
10. H. M. Knott, E. B. Brown, M. J. Davies, and R. T. Dean, *Eur. J. Biochem.*, **270**, 3572-3582 (2003).
11. V. Lankin, *Free Radicals, Nitric Oxide, and Inflammation: Molecular, Biochemical, and Clinical Aspects*, Amsterdam (2003), Vol. 344, pp. 8-23.
12. V. Lankin and A. Tikhaze, *Ibid*., pp. 218-231.
13. J. Nourooz-Zadeh, J. Tajaddini-Sarmadi, and S. R. Wolff, *Anal. Biochem.*, **220**, 403-409 (1994).
14. D. Steinberg and A. Lewis, *Circulation*, **95**, 1062-1071 (1997).
15. V. V. Tertov, V. V. Kaplun, S. N. Dvoryantsev, *et al*., *Biochem. Biophys. Res. Commun*., **214**, 608-613 (1995).