Oxidative and Antioxidant Changes during Formation of Unstable Atherosclerotic Plaque

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Oxidative and antioxidant parameters (content of LPO products and oxidized proteins, initial level of paraoxonase, content of α-tocopherol, retinol, and β-carotene) were studied at different stages of atherosclerotic foci development in coronary arteries: intact intima, lipid spot, stable young plaque, unstable plaque, stable plaque with fibrosis/calcinosis, and in various types of unstable plaques. The most typical sign of unstable plaques is high level of LPO products and low retinol content.

Key Words: *atherosclerotic foci in coronary arteries; unstable plaque types; lipid peroxidation; oxidized proteins; antioxidants*

Thrombus formation on the endothelial surface of atherosclerotic focus leading to occlusion of the artery, ischemia, and necrosis, is the pathogenetic basis of myocardial infarction. The triggering mechanism of thrombus formation is disintegration of the endothelium at the site of cap ulceration/destruction in vulnerable unstable atherosclerotic plaque [5,6]. Stable plaque is characterized by a thick cap, homogeneous lipid core, and absence of inflammatory changes, while unstable plaque has a thin cap (<65 μ) or a thinned site of the cap with destruction of the endothelium, inflammatory infiltration (>25 cells in a 0.3-mm long field) and loose lipid core with necrotic foci [6,14].

Inflammation plays an important role in plaque destabilization; significant infiltration of unstable plaques with macrophages and T lymphocytes was demonstrated [4,5]. High levels of inflammatory cytokines and matrix metalloproteases in atherosclerotic plaques and blood in acute coronary syndrome were demonstrated [5,9]. The role of oxidative stress factors and oxidized lipids in this process is less studied. It has been shown that macrophages, T lymphocytes, and smooth-muscle cells produce active oxygen metabolites causing, together with matrix metalloproteases, smooth-muscle cell necrosis/apoptosis, leading to thinning of the fibrous cap and ulceration of the plaque [8,11].

We studied oxidative and antioxidant changes over the course of atherosclerotic focus development to the stage of an unstable plaque and in unstable plaques of different types.

MATERIALS AND METHODS

Specimens of the coronary artery intima/media were collected during endarterectomy, carried out by intraoperative indications during bypass surgery in 23 male patients aged 40-65 years with coronary atherosclerosis and stable effort angina (functional classes II-III). The intima/media specimens were divided into fragments for histological and biochemical studies.

Histological analysis of the samples was carried out after staining with hematoxylin and eosin and after van Gieson. Intact intimal tissue was detected in 7, lipid spot/strip in 8, stable young athero-

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TABLE 1. Oxidative/Antioxidant Changes during Formation of Atherosclerotic Foci (*M*±*m*)

Parameter	Intact intimal tissue	Lipid spot/strip	Stable young plaque	Stable plaque with fibrosis/ calcinosis	Unstable plaque
LPO products, nmol MDA/mg protein	0.6±0.3	0.5±0.1	0.9±0.3	1.0±0.2	2.0±0.3^{+}
Oxidized proteins, units/mg protein	16.1±1.8	47.8±3.3^{++}	180.4±30.1**	209.5±41.7**	120.6±18.1**
Initial PO activity, μg/mg protein	21.3±1.4	22.1±2.2	103.2±27.6**	68.0±12.1**	72.9±20.7**
α-Tocopherol, μg/mg protein	330.9±36.4	357.0±35.2	383.0±31.4	387.0±32.6	346.9±31.0
Retinol, μg/mg protein	9.5±0.7	11.3±1.9	6.4±1.0*	6.1±1.0*	4.3±0.5^{+}
β-Carotene, unit/mg protein	92.6±16.1	98.1±16.3	108.3±21.9	102.1±20.4	96.4±17.7

Note. *p<0.05, **p<0.01 compared to intact tissue and lipid spot/streak; ^{+}p<0.05, ^{++}p<0.01 compared to all tissue types.

sclerotic plaque in 14, stable plaque with fibrosis/ calcinosis in 26, unstable plaque with liability to ulceration or rupture in 20 of 75 specimens. The types of unstable plaques were identified: fibroatheroma with a thin fibrous cap [14] (lipid type [3]) in 5, plaque with inflammation, erosion [3,14] in 7, and plaque with calcified core [14] (degenerative necrotic type [3]) in 8 samples.

For biochemical studies, samples frozen in liquid nitrogen were homogenized in PBS. Protein content in the homogenates was measured by the method of Lowry, the content of LPO products (MDA) was evaluated fluorometrically [10] on a Versafluor spectrofluorometer (Bio-Rad), α-tocopherol and retinol were measured by the fluorometric and β-carotene by spectrophotometric methods [13]. Solutions of 1,1,3,3-tetramethoxypropanol, α-tocopherol, retinol, and β-carotene (Sigma) served as the reference solutions. Oxidative modification of proteins in homogenates was evaluated spectrophotometrically after reaction with 2,4-dinitrophenylhydrasine [1]. The initial level of paraoxonase (PO) in homogenates was evaluated spectrophotometrically in Tris-HCl buffer containing paraoxone (Sigma) [7].

The results were processed statistically using analysis of correlations and one-way ANOVA with Dannet's test for multiple comparisons.

RESULTS

Since oxidative stress plays an important role in atherogenesis [2,12], we evaluated oxidative changes in lipids and proteins at different stages of atherosclerotic focus development (Table 1). The level of LPO products in unstable plaques was higher than in intact intima, lipid spots, young and stable plaques by 3.3, 4.0, 2.2, and 2.0 times (p<0.05), respectively. No regularity of this kind was detected for oxidized proteins. The content of carbonyl groups in proteins (markers of their oxidative modification [1]) was maximum in stable young and fibrous plaques and surpassed the corresponding parameter in lipid spots by 3.8 and 4.4 times, respectively (p<0.01).

In addition to oxidative parameters, we studied some characteristics of antioxidant potential in atherosclerotic foci reflecting the degree of protection of the vascular wall from oxidation [2,8]. The initial level of PO indicates activity of this antioxidant enzyme *in vivo* at the moment of collection of

TABLE 2. Oxidative/Antioxidant Changes in Unstable Plaques of Different Types (*M*±*m*)

Parameter	Fibroatheromas	Plaques with inflammation, erosion	Plaques with calcinosis/ fibrosis
LPO products, nmol MDA/mg protein	1.0±0.1^{*+}	1.8±0.4	2.9±0.7
Oxidized proteins, units/mg protein	145.3±28.5*	143.6±27.1*	85.1±17.3
PO activity, μg/mg protein	79.2±22.1	88.8±26.5	44.4±20.4
α-Tocopherol, μg/mg protein	170.0±41.4**$^{++}$	410.0±61.5	448.0±65.9
Retinol, μg/mg protein	2.7±0.3^{*+}	4.7±1.2	5.3±1.3
β-Carotene, unit/mg protein	43.2±15.8**$^{++}$	105.6±20.9	138.6±26.2

Note. *p<0.05, **p<0.01 compared to plaques with calcinosis/necrosis; ^{+}p<0.05, ^{++}p<0.01 compared to plaques with inflammation, erosion.

biomaterial [7,12]. The initial activity of PO in stable and unstable plaques was higher than in lipid spots by 4.7 and 3.3 times, respectively ($p<0.01$), which indicates high requirement for PO for neutralization of active oxidative processes. No differences in α-tocopherol and β-carotene levels at different stages of atherosclerotic focus development were detected. However, the level of retinol gradually decreased from the stage of lipid spot to unstable plaque, where its content was by 1.4 times lower ($p<0.05$) than in stable plaque. Hence, the most characteristic feature of unstable plaques is high level of LPO products, which agrees with published data [6,8], and low level of retinol.

The detected correlations between LPO activity and levels of α-tocopherol, retinol, and β-carotene (Pearson's coefficients -0.316, -0.364, and -0.405; Kendall's coefficients -0.276, -0.232, and -0.256, respectively, $p<0.01$) and between oxidative modification of proteins and the initial level of PO activity (Pearson's and Kendall's coefficients 0.275 and 0.228, $p<0.01$) correspond to changes in oxidative/antioxidant activity at the stage of stable plaque, when protein oxidation processes are intensive, while LPO processes have not yet reached peak activity, presumably because of pronounced antioxidant function of PO.

Study of oxidative changes in unstable plaques of different types showed higher levels of LPO products in inflammatory and degenerative plaques in comparison with lipid plaques (by 1.8 and 2.9 times; respectively, $p<0.05$; Table 2). Oxidative modification of proteins was similarly elevated (1.7 times; $p<0.05$) in lipid and inflammatory plaques in comparison with degenerative plaques. The content of lipid-soluble antioxidants in lipid plaques was minimum, in contrast to other types. In fibroatheromas, the levels of α-tocopherol and β-carotene were lower by 2.4 times ($p<0.01$) and of retinol by 1.7 times ($p<0.05$) than in inflammatory plaques and by 2.6, 3.2 ($p<0.01$), and 2.0 times ($p<0.05$) in comparison with degenerative plaques, respectively. However, despite more than 2-fold increase in the content of lipid-soluble antioxidants in inflammatory and degenerative plaques in comparison with fibroatheromas, it was insufficient for inhibiting LPO processes, which were highly active in plaques of these types.

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