Oxygen-Dependent Processes in Monocytes and Metabolic Risk Factors for Atherogenesis

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Oxygen-dependent processes in peripheral blood monocytes were intensified in patients with metabolic cardiovascular syndrome. This was manifested in increased production of $O_2^{\bullet-}$ and NO. Among metabolic factors (cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triacylglycerols, glucose, *etc*.), products of glycosylation (fructosamine) and plasma triacylglycerols were most potent in modulating generation of $O_2^{\bullet-}$ and NO by monocytes.

Key Words: *monocytes; oxygen-dependent processes; fructosamine; triacylglycerols*

Activation of monocytes/macrophages and oxidative modification of low-density lipoproteins mediated by these cells are the key events of atherogenesis [1,4]. Oxidation of lipoproteins under the action of monocytes/macrophages involves superoxide radicals generated by intracellular NADPH oxidase [4,7]. At the same time, monocytes produce NO capable of initiating or inhibiting lipoprotein oxidation [1,6,9]. Prooxidant and antioxidant properties of NO depend on different factors, first of all on its concentration [1,8], which, in its turn, is determined by functional state of NO-producing cells. It can be hypothesized that functional state of monocytes depends on metabolic characteristics of the internal medium [3].

Previous experiments showed that incubation of cells in a medium with high glucose content is accompanied by intensive generation of $O_2^{\bullet-}$ and NO [2,5]. The mechanisms of this phenomenon remain unclear. Hyperglycemia and dyslipidemia are metabolic disturbances that serve as independent risk factors of atherogenesis and affect production of reactive oxygen metabolites in monocytes.

Here we studied the relationship between production of $O_2^{\bullet-}$ and NO by monocytes and severity of dyslipidemia in patients with metabolic cardiovascular syndrome (MCS).

MATERIALS AND METHODS

We examined 34 patients with MCS (non-insulin-dependent diabetes mellitus, arterial hypertension, and abdominal obesity) and 27 healthy donors. Monocytes were isolated from mononuclear cell suspension in flat-bottom flasks by the method of selective adhesion. This suspension was obtained by density gradient centrifugation (ρ=1.077 g/cm^3). NO production induced by 10 μg/ml bacterial lipopolysaccharide was studied in supernatants of a 1-day-old monocyte culture using Griess reagent. The intensity of NO production was determined by the amount of nitrite anions and stable NO metabolites. The reference sample contained monocytes cultured in a complete medium with lipopolysaccharide and NO synthase inhibitor N^{ω}-monomethyl-L-arginine (1 μmol/ml).

The intensity of $O_2^{\bullet-}$ production by monocytes was estimated by zymosan-induced luminol-dependent chemiluminescence (CL) [3]. Luminol (50 μl) was added to a cuvette containing 800 μl cell suspension and spontaneous CL and CL-response to opsonized zymosan (100 μl) were measured. The total CL yield over 16 min after stimulation with opsonized zymosan was estimated and served as an integral CL parameter.

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The contents of glucose, total cholesterol (CH), high-density lipoprotein cholesterol (HDL CH), low-density lipoprotein cholesterol (LDL CH), triacylglycerols, and fructosamine were measured using standard Biocon sets.

The significance of differences was evaluated using nonparametric Mann—Whitney test. Correlation analysis included Spearman rank correlation test (R). Multiple correlation coefficients (β) were calculated by multiple regression analysis.

RESULTS

Generation of $O_2^{\bullet-}$ by monocytes from patients with MCS was higher than in healthy donors. The CL-response of cells from patients with MCS was more pronounced than in healthy donors (Table 1). The intensity of NO production in patients with MCS was higher than in healthy donors. These data attest to activation of oxygen-dependent processes in mononuclear leukocytes in patients with MCS. The observed changes are associated with the development of metabolic disturbances during MCS. This conclusion is derived from our observations and published data on increased production of reactive oxygen metabolites by cells during hyperglycemia[6,10]. Patients with MCS had higher basal and postprandial blood glucose concentration compared to healthy donors, decreased HDL CH content, hypertriacylglycerolemia, and high level of LDL CH (Table 1). A correlation was found between the intensity of NO production by monocytes and basal glucose concentration in patients with MCS (R=0.87, p<0.05). In addition, the intensity of $O_2^{\bullet-}$ generation by monocytes from patients with MCS correlated with glucose concentration (R= 0.62, p<0.05).

Our results are consistent with published data that intravenous infusion of glucose stimulates $O_2^{\bullet-}$ generation by monocytes in obese patients [5]. Probably, hyperglycemia accompanying MCS modulates functional activity of monocytes. Multiple regression analysis revealed a relationship between plasma fructosamine concentration and production of $O_2^{\bullet-}$ and NO by monocytes from patients with MCS (β=0.7, p<0.05; and β=0.54, p<0.05, respectively). Previous studies showed that chronic hyperglycemia accompanying metabolic syndrome contributes to intensive nonenzymatic glycosylation of proteins [2]. These changes result in $O_2^{\bullet-}$ and NO overproduction by peripheral blood monocytes. A less significant relationship was revealed between plasma triacylglycerol concentration and production of $O_2^{\bullet-}$ and NO by monocytes (β=0.589, p<0.05; and β=0.44, p<0.05, respectively). It can be hypothesized that disturbances in lipid metabolism (*e.g.*, excessive accumulation of triacylglycerols in the plasma) increase the intensity of oxidative processes in peripheral blood monocytes.

TABLE 1. Content of Glucose, Total CH, LDL CH, HDL CH, Triacylglycerols, and Nitrite Anions and CL Parameter in Patients with MCS and Healthy Donors (M±m)

Index	Healthy donors (n=27)	Patients with MCS (n=34)
Glucose, mmol/liter		
basal	4.31±0.14	6.67±0.46*
postprandial	4.41±0.12	8.57±1.06*
Total CH, mmol/liter	5.78±0.14	6.17±0.46
LDL CH, mmol/liter	3.46±0.25	4.06±0.2*
HDL CH, mmol/liter	1.35±0.14	0.9±0.05*
Triacylglycerols, mmol/liter	1.41±0.12	2.6±0.33*
Integral parameter of CL, pulses per monocyte	17.84±1.8	24.76±1.21*
NO_2^-, μmol/liter	4.2±0.23	5.4±0.05*

Note. *p<0.05 compared to healthy donors.

Our results suggest that hyperglycemia, nonenzymatic glycosylation of proteins, and dyslipidemia are the major factors responsible for stimulation of oxygen-dependent processes in monocytes. These changes are accompanied by the appearance of most atherogenic oxidatively modified lipoproteins and early development and progression of angiopathies in patients with MCS.

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