Free-Radical Oxidation in Human Alveolar Condensates: Clinical and Diagnostic Significance

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A noninvasive method of assessing free-radical oxidation in human body is proposed. It is based on analysis of free radical formation and superoxide radical scavenging in alveolar condensate. This method provides information regarding physical health and allows a choice of measures preventing the development of free-radical pathologies.

Key Words: *free-radical oxidation; alveolar condensate; chemiluminescence; diagnosis*

The development of adequate methods for assessing free-radical oxidation in human body is hampered by a variety of factors: low concentrations and multiplicity of oxidation products, unequivocal interpretation of simple tests (for example, determination of the thiobarbituric acid-reactive products), and complexity of methods yielding unambiguous results (measurement of expired hydrocarbons). Almost all current methods for assessing free-radical oxidation and antiradical systems in human body are invasive, which hinders their wide application. Therefore, reproducible and unambiguous noninvasive diagnostic tests for quantitative evaluation of free-radical oxidation are necessary.

Generally, changes in free-radical oxidation which can be detected by the available tests characterize particular stages of a free-radical pathology that had been established by conventional diagnostic techniques. However, it is very important to identify premorbid stages of general and occupational diseases in order to take adequate preventive measures.

In an attempt to solve the above-mentioned problems we used condensate of human alveolar fluid [4]. Analysis of this condensate provides indirect information regarding the pulmonary surfactant system which plays an important role in the pathogenesis of several diseases [2].

Alveolar condensate contains transition metals in concentrations sufficient for direct determination by atomic absorption techniques [4,5] as well as monoamines, amino acids, fatty acids [3,8,11], and a number of other compounds determining the intensity of free-radical oxidation in human body [6]. The discovery of nonenzymatic superoxide-scavenging activity (SSA) in blood serum [1] implies that this activity is present other fluids of the body, including alveolar condensate.

MATERIALS AND METHODS

Alveolar condensate was collected using a modernized (separable at the joint) Polezhaev's absorber placed in an ice/NaCl mixture. Two milliliters of the condensate, which can be collected within 20-25 min of nonforced breathing, is sufficient for analysis.

Chemiluminescence (CL) has been widely applied to evaluate generation of reactive oxygen species, although there exists a variety of CL sources [12]. Analysis of the H_2O_2-induced luminol-dependent CL allows one to reveal generation of reactive oxygen species in biological material [7,10]. The chemiluminescence parameters (maximum burst and light sum) were measured for 1-2 min in a

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PKhL-01 chemiluminometer (Russia) after successive addition of 1 ml phosphate buffer (pH 7.3-7.4), 0.05 ml 0.006% aqueous solution of luminol, and 0.1 ml condensate to a thermostatically (37°C) controlled cuvette. The reaction was started by the addition of 0.2 ml freshly prepared 10% H_2O_2. Alveolar condensate was omitted from blank samples. The rate of free radical formation (RFR) was calculated as the ratio of the CL light sum in test sample to that in blank sample.

SSA was determined spectrophotometrically (at 560 nm) by measuring the extent to which tetrazolium nitroblue was reduced in the superoxide generating system phenazine methosulfate — NADH [9]. Each sample contained 1.3 ml phosphate buffer (pH 7.8), 0.5 ml 0.95 mM NADH, 0.3 ml 0.24 mM tetrazolium nitroblue, and 0.1 ml 7.5 mM phenazine methosulfate.

Results were statistically processed using Student's *t* test.

RESULTS

The study included 644 individuals: miners of various specialties (n=308), workers of metal-conversion plants (n=84), patients (n=92) with vibration-induced pathologies treated in the Neurology Department of the Clinic for Occupational Diseases (Erisman Institute of Hygiene, Moscow), and children and women (n=160) from contaminated regions. Alveolar condensate was found to exhibit both a fast burst of H_2O_2-induced CL and pronounced SSA. Chemiluminescence can be induced by transition metals (which in the presence of H_2O_2 promote generation of oxygen radicals in the Fenton and Haber—Weis reactions) [6], and oxidative substrates, such as monoamines and unsaturated fatty acids. The absence of protein in these condensates points to the nonenzymatic nature of SSA which may be due at least partially to the presence of the amino acid—metal complexes and monoamines. From the results obtained, we concluded that it is reasonable to introduce an additional prognostic indicator, namely, the RFR/SSA ratio, which permits an early detection of disturbances in the radical formation—antiradical protection system. This conclusion was verified for people of different ages living in different climatic zones.

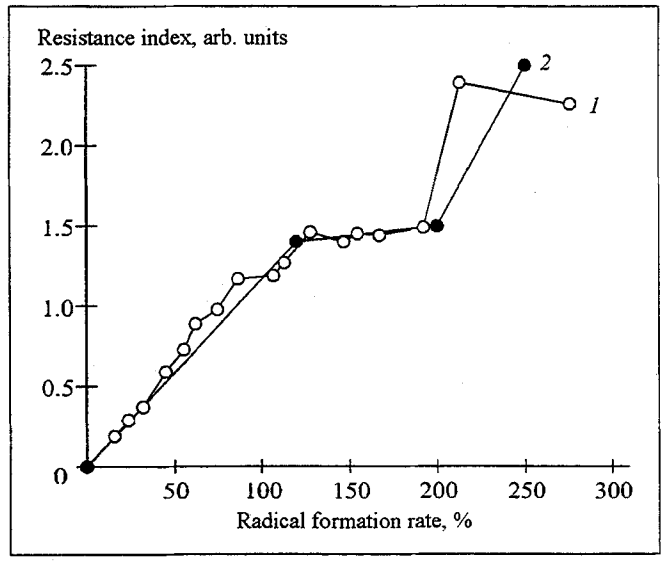

Fig. 1. Resistance index (radical formation rate/superoxide-scavenging activity) as a function of the radical formation rate. Averaged data obtained for at least 10 individuals were used to plot each point. Curve *1* was drawn through all points, curve 2 was obtained by the least squares fitting.

Table 1 shows typical data obtained in three age-matching groups of individuals who had been working for more than 5 years in the Northern region either underground in deep mines (groups 1 and 2) or overland (control group). Group 1 consisted of people working under adverse conditions involving exposure to dusts, gases, and chemicals (inhalation of dust containing nickel, cobalt, and copper, polycyclic aromatic hydrocarbons, nitric oxides, etc.). Group 2 individuals worked under conditions of increased exposure to dust. The parameters of free-radical oxidation in group 2 differed little from those in the control group, a slight increase in RFR being compensated by a similar increase in SSA, so that the RFR/SSA ratio was similar to that in the control group (Table 1). Stability

TABLE 1. Intensity of Free-Radical Oxidation in Alveolar Condensates Obtained from Miners ($M\pm m$)

Group	Chemiluminescence		SSA, arb. units	RFR/SSA
	maximum, %	light sum of RFR, %		
Control (n=22)	80.7±7.8	99.2±10.4	25.7±4.9	3.86±0.42
1st (n=19)	86.3±9.3 (106.9)	215.6±19.6* (213.3)	10.4±1.9* (40.5)	20.73±3.7* (537.1)
2nd (n=21)	86.6±11.2 (107.3)	121.3±8.6** (122.2)	30.3±3.7** (118.0)	4.0±0.6** (103.7)

Note. Percent of the control is given in parentheses. p<0.05: *compared with the control; **compared with group 1. RFR = radical formation rate; SSA = superoxide-scavenging activity.

of respiratory function, oxygen consumption, energy expenditure, basal metabolism, and heat loss from the body surface (data not shown) were demonstrated in a parallel study: these parameters did not exceed the control values. By contrast, in group 1, RFR increased more than twofold with the corresponding decrease in SSA, which resulted in a 5.4-fold rise in the RFR/SSA ratio over the control value. In group 2, the respiratory function parameters significantly dropped compared with the two other groups.

Thus, the proposed approach reveals disturbances of functional status of a particular population and allows one to recommend appropriate preventive measures.

Simultaneous measurement of CL and SSA makes it possible to evaluate both RFR and the effectiveness of antiradical protection, i.e., parameters reflecting general resistance of the organism. However, in certain circumstances (screening of large groups under field conditions) only RFR determined from the light sum of CL can be employed. This is confirmed by the RFR/SSA *vs.* RFR plots (Fig. 1).

Analysis of our results revealed a relationship between the intensity of radical formation in alveolar condensate and functional state of individuals. Four categories were defined:

1) RFR<50% (i.e., antiradical protection systems dominate over radical formation) indicates a compensated state (for example, as a result of physiological adaptation to chronically acting adverse agents);

2) RFR ranging from 50% to 150%: lower and upper borders of health;

3) RFR ranging from 150% to 200%: activation of free-radical oxidation, correction of the antioxidant status is recommended;

4) RFE>200%: breakdown of adaptive mechanisms with the development of a premorbid state, which necessitates normalization of the antioxidant status by means of antioxidant therapy and other measures.

This interpretation has been confirmed by a simultaneous determinations of the RFR and major physiological characteristics of the people included in the study.

The proposed method reveals both individual and group changes in free-radical oxidation and expands the range of modern noninvasive techniques for early diagnostics of free-radical perturbations in human body.

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