EXPERIMENTAL BIOLOGY

Effect of Long-Term Exposure to Ozone on Functional Activity of Human Phagocytes

N. F. Abdrashitova, Yu. V. Balyakin, and Yu. A. Romanov

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Functional activity (biocidal properties) of whole blood phagocytes from humans exposed to ozone was studied by measuring spontaneous and zymosan-induced luminol-dependent chemiluminescence. Generation of reactive oxygen species and biocidal properties of phagocytes depended on the time of ozone exposure and development of bronchopulmonary diseases. The data suggest that generation of reactive oxygen species must be assayed for elaboration of individual patient management programs.

Key Words: *ozone; phagocytes; chemiluminescence*

Ozone is one the most hazardous toxicants [1], which occurs in the Earth's troposphere and is formed during some industrial processes. Since this gas is extremely toxic for humans, studies of its adverse effects on the body (in particular, on the respiratory system) are of considerable importance. The properties of ozone as a strong oxidant and inductor of free radical processes underlie the effects of long-term (for many years) exposure to this pollutant. Impaired regulation of free radical oxidation is a universal nonspecific mechanism playing a role in the pathogenesis of lung inflammations [3]. Generation of reactive oxygen species (ROS) by phagocytes plays the major role in the course and outcome of these diseases.

Here we studied functional activity of human phagocytes after long-term exposure to ozone.

MATERIALS AND METHODS

We examined 158 humans employed in industrial production of plasticizers and exposed to ozone. Chronic nonobstructive bronchitis (CNB) and chronic obstructive bronchitis (COB) were found in 61 and 53 employers, respectively (N. V. Putov classification); 44 individuals were considered to be apparently healthy. Employers with and without bronchopulmonary diseases, which were not in contact with ozone, served as the control (n=7-9). Ozone concentration did not exceed the maximum permissible dose for workrooms (0.1 mg/m^3). ROS generation by phagocytes was evaluated by recording luminol-dependent chemiluminescence (LCL) of the whole blood (0.1 ml) containing 20 U/ml heparin, luminol in a final concentration of 10^{-5} M, and Hanks solution (to a final volume of 1 ml) on a KhL-003 chemiluminometer [3]. Functional reserves of phagocytes were studied in the presence of 2 mg/ml luminol by analyzing changes in zymosan-induced CL compared to spontaneous CL (without stimulator).

RESULTS

Comparison of LCL parameters showed that ROS generation by phagocytes was different in humans exposed to ozone (Table 1). Even in apparently healthy individuals, the intensity of spontaneous LCL 2.4-fold surpassed that in humans not exposed to ozone. The chemiluminescent response of phagocytes to zymosan decreased with increasing the duration of employment.

Russian State Medical University, Moscow

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After 2- and 5-year ozone exposure, zymosan-induced LCL 7.3- and 3.1-fold surpassed spontaneous LCL, respectively. It was reported that spontaneous LCL of isolated blood granulocytes and whole blood samples increases by 3-10 times during acute pneumonia [2]. The intensity of respiratory burst in polymorphonuclear leukocytes caused by zymosan also increases [7]. At the same time, under conditions of hyperoxia the intensity of zymosan-induced respiratory burst in blood granulocytes evaluated by LCL remains unchanged [8], but spontaneous luminescence of polymorphonuclear leukocytes decreases [3].

In individuals with CNB employed for 3 years, ROS generation was intensified to a greater extent even without stimulation. Therefore, functional reserves (or biocidal properties) of phagocytes [5,6] decreased: their zymosan-induced activation was attenuated (by no more than 1.5-2.6 times). High activity of phagocytes plays the major role in oxidative damages during bronchopulmonary diseases [10-12]. Intense generation of ROS without stimulation attests to a strain of metabolic processes in cells [4]. These changes reflect neutrophil readiness to contact pathogens. However, *in vitro* enhanced spontaneous LCL reflects the ability of neutrophils to generate considerable amounts of free radicals even in the absence of pathogens, which probably underlies their damaging effects on the lung [4].

ROS generation markedly decreased after 5-year exposure to ozone and reached a minimum after 8 years. In these individuals (occupational exposure for 6 years or longer), zymosan did not induce ROS generation by blood cells. The development of COB was accompanied by a considerable decrease in biocidal properties of phagocytes, which manifested not only in the reduction of spontaneous LCL, but also in the absence of stimulation with zymosan. Previous studies showed that oxidative stress induced by hyperoxia or various chemical compounds (paraquat, t-butyl hydroperoxide, and vanadium oxides) leads to adaptation, reduced metabolic activity of phagocytes, and decreased ROS generation in response to stimulation [11].

Published data indicate that the degree and directionality of regulatory effects of drugs depend on func-

TABLE 1. Chemiluminescence (arb. units) of Blood Lymphocytes from Individuals Exposed to Ozone ($M \pm m$)

Group, age (years)		Employment, years (n)	LCL intensity	
			spontaneous	zymosan-induced
Apparently healthy				
	36-40	Control (9)	51.0±5.2	458.0±25.2
	26-30	2 (19)	102.0±8.4*	741.0±56.1*
	31-35	3 (11)	84.0±4.9*	465.0±36.9
	36-40	4 (10)	96.0±9.7*	360.0±37.5
		5 (4)	120.0±10.1*	373.0±35.6
CNB	36-40	Control (7)	132.0±6.4	639.0±33.7
	31-35	3 (6)	102.0±8.5*	272.0±16.8*
	36-40	4 (11)	120.0±9.7	276.0±18.1*
		5 (10)	198.0±17.3*	305.0±18.2*
		6 (6)	55.0±12.1*	57.0±10.9*
	41-45	7 (7)	33.0±3.4*	30.0±2.9*
		8 (5)	18.0±1.9*	17.0±1.8*
	46-50	9 (12)	17.0±1.9*	17.0±2.0*
		10 years or more (7)	20.0±2.4*	18.0±1.8*
COB	36-40	Control (9)	95.0±12.6	141.0±11.3
		5 (6)	41.0±5.8*	40.0±6.7*
		6 (7)	33.0±4.1*	30.0±1.9*
	41-45	7 (11)	21.0±1.4*	20.0±2.2*
		8 (7)	17.0±0.9*	17.0±0.9*
	46-50	9 (10)	9.0±1.1*	9.0±1.1*
		10 years or more (12)	10.0±0.1*	10.0±0.1*

Note. *$p<0.05$ compared to the control.

tional activity of phagocytes [9]. Various levels of ROS generation in humans exposed to ozone determine the necessity of elaborating individual therapeutic approaches.

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