EFFECT OF HYPERBARIC OXYGENATION ON ACTIVATION OF IMMUNOCOMPETENT CELLS AND ON LIPID PEROXIDATION DURING IMMUNIZATION WITH HETEROLOGOUS RED BLOOD CELLS

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Mechanisms of activation of immunocompetent cells are linked with changes in metabolism in the lipid bilayer of membranes, their fluidity, and the mobility of the biomolecules [5]. One of the biological phenomena of hyperbaric oxygenation (HBO) is the "membrane effect," reflecting the intensity of lipid peroxidation (LPO), changes in the lipid composition of membranes, their functional state, and "flowability" [6]. Repeated HBO increases the reactivity of the immunoglobulin receptors of lymphocytes in intact animals [3]. Inhibition of synthesis of humoral antibodies under the influence of HBO has been reported [1]. In that connection, dependence of the immune response on activation of LPO under conditions of HBO seems probable.

The aim of this investigation was an experimental verification of the hypothesis that the intensity of immune processes correlates with that of lipid peroxidation under the influence of HBO.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 170-180 g, in four series: I) intact animals (control), II) immunized, III) intact + HBO, IV) immunized animals + HBO. The rats were immunized with sheep's red blood cells (SRBC) in a single dose of $2 \cdot 10^8$ cells, intraperitoneally. Immediately after immunization a session of HBO was given, under pressure of 3 atm (300 kPa) of medical oxygen, lasting 60 min. The serum, blood plasma, and a suspension of homogenized spleen cells were investigated from each rat, immediately after immunization and on the 3rd, 5th, 8th, 10th, and 14th days thereafter. The immune response was assessed as the number of antigen-binding rosette-forming cells (RFC) in the spleen of intact animals (E-RFC) [11], and the number of antibody-forming cells (AFC) in the spleen [13]. The intensity of LPO was determined as malonic dialdehyde (MDA) in the spleen [8] and blood plasma [9], and as lipid hydroperoxides (LHP) in the plasma [2]. Antiperoxide defense of the cell was judged by the superoxide dismutase (SOD) level in the spleen; SOD activity was determined by the reaction of oxidation of xanthine by xanthine oxidase [10] and was calculated per milligram protein [14]. The results were subjected to statistical analysis. The effect of immunization and/or HBO and the strength of effect of the factors was investigated by dispersion analysis [7], using single-factor and two-factor combinations. Dependence of the immune parameters and results of LPO was determined by means of a coefficient of correlation; correlation between two parameters, determined by the effect of a third, was estimated by a partial coefficient of correlation.

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TABLE 1. Effect of Immunization with SRBC or of HBO on Immune Parameters and LPO during Test Period (14 days) and with a Single Exposure to the Factor (single-factor combination, n = 64)

Parameter	Acting factor					
	immunization with SRBC (n = 33)			HBO (n = 31)		
	str. of effect of factor		day of recording statistically signifi-	str. of effect of factor		d. rec. stat. signif. change in parameter
	$\eta^2 x \pm m$	%		$\eta^2 x \pm m$	%	
E-RFC in spleen	—	—	—	0,37±0,13**	37 2—17	5, 8, 10, 14-th absent
iRFC in spleen	0,37±0,12*	37 6—68	1, 3, 5, 8, 10-th absent	—	—	—
AFC in spleen	0,74±0,13***	74 61—87	3, 5, 8, 10, 14-th absent	—	—	—
MAD in spleen	0,40±0,12*	40 10—70	8, 10, 14-th absent	0,64±0,08***	64 42—86	1, 3, 8-th 10-th
SOD in spleen	0,19±0,15	19	3, 5, 10-th absent	0,10±0,19	10	1, 6, 10-th absent
LHP in plasma	0,20±0,15	20	1, 3, 5, 10-th absent	0,58±0,10**	58 31—85	1, 3-й absent
MDA in serum	0,49±0,09**	49	3, 14-th 25—73,5	0,29±0,14 1-th	29	3, 5, 8, 10, 14-th 1-й

Legend. Compared with intact animals; here and in Table 2: *p < 0.05; **p < 0.01; ***p < 0.001. Fraction: numerator indicates days on which an increase in the parameter was recorded, denominator — days in which a decrease was recorded.

TABLE 2. Interaction between Immunization with SRBC and HBO and Their Effect on Immune Parameters and LPO in the Case of a Single Combined Action (two-factor combination, n = 104)

Parameter	Strength of effect of factors and their interaction immediately after exposure			Day on which statist. signif. effect of factors and their interaction on parameter was recorded	Change in parameter during interaction of factors[1]	
	factor	$\eta^2_x \pm m$	%		immediately after exposure (M_{1-2})	day on which change was recorded
iRFC in spleen	A	0,38±0,09***	38,4	1, 3, 5, 8, 10-th	11,82	
	B	0,05+0,02	4,9	8-th	6,58	absent
	AB	0,15±0,02	15,0	1, 3, 5, 8, 10, 14-th		1, 3, 5, 8, 10, 14-th
	A+B	0,58+0,02***	58,3	1, 3, 5, 8, 10, 14-th	%	
AFC in spleen	A	0,35+0,10***	35,5	3, 5, 8, 14-th	(after 3 days)	
	B	0,12+0,10*	12,5	3-th	0,82	absent
	AB	0,13+0,02**	12,6	3-th	0,21	
After 3 days	A+B	0,61+0,02**	60,6	3, 5, 8, 10, 14-th	(log AFC/10^6)	3, 5, 8, 10, 14-th
MDA in spleen	A	0,10+0,13*	9,6	1, 14-th	3,13	
	B	0,36±0,09***	36,3	1, 3, 10-th	8,24	1, 3, 5, 14-th
	AB	0,09+0,02	9,3	10-й		8, 10-th
	A+B	0,55+0,02***	55,1	1, 10-th	(μmole/g)	
SOD in spleen	A	0,10+0,13	10,1	3, 5-th	0,83	1,14-th
	B	0,17+0,12*	16,9	1-st	0,93	3, 5, 8, 10-th
	AB	0,02±0,03	2,2	5-th		
	A+B	0,29+0,03	29,2	3, 5, 10-th	(unit/mg protein)	
LHP in plasma	A	0,10+0,13	9,6	3, 10-th	1,41	1-st
	B	0,24+0,11*	23,7	1, 5-th	1,97	
	AB	0,004+0,03	0,4	1, 5-th		3, 5, 8, 10, 14-th
	A+B	0,33+0,03*	32,7	1, 3, 5, 10-th	(relative units)	
MDA in serum	A	0,11+0,13**	10,7	1, 3, 10-th	17,33	
	B	0,18+0,12***	18,2	1-st	18,93	1-st
	AB	0,55+0,01***	54,8	1, 3, 5-th	18,93	3, 5, 8, 10, 14-th
	A+B	0,84+0,01***	83,7	1, 3, 5, 14-th	(μmole/g)	

Legend. [1]Compared with unoxygenated immune animals. A) Immunization with SRBC, B) treatment with HBO, AB) interaction between immunization and HBO, A + B) combined action of immunization and HBO.

EXPERIMENTAL RESULTS

In the course of development of the immune reaction (Table 1) an increase was observed in the population of immunocompetent cells (iRFC, AFC), coupled with intensification of lipid metabolism along the LPO pathway (an increase in MDA and LHP levels). Positive correlation was found between RFC – SOD (r_{12} = +0.59) and RFC – SOD – MDA ($r_{12.3}$ = +0.72) in the spleen.

After exposure of the intact animals to HBO (Table 1) levels of LHP, MDA, and SOD activity in the spleen rose ($p < 0.05$). An increase was observed in the number of E-RFC in the spleen ($p < 0.05$), which correlated positively with SOD (E-RFC – SOD r_{12} = +0.66; E-RFC – SOD – MDA serum $r_{12.3}$ = +0.83). The increase in antioxidant activity was accompanied by nonspecific stimulation of E-RFC in the intact animal.

Under the combined influence of immunization and HBO marked activation of LPO and inhibition of the immune process were observed (Table 2). Antiperoxide defense, despite some activation of SOD, was ineffective ($p > 0.05$). The immunoreactivity of the lymphoid cells (iRFC) correlated with the intensity of lipid metabolism (LPO) and of antiperoxide defense (iRFC – SOD – LHP $r_{12.3}$ = –0.97). In the metabolic system (receptors – lipids – enzymes) determining the functional state of the cells, the second component (lipids) significantly affects conformational changes in the membrane receptors, their expression, and transformation of the antigenic stimulus into the immunogenic impulse [4, 12]. The results are evidence that stimulation of immunoreactivity is connected with a definite level of intensity of LPO and of antioxidative defense (see the data for series II), whereas activation of LPO against the background of antioxidant insufficiency (see the data for series IV) has an inhibitory effect against immunocompetent cells.

Thus, activation of LPO and of the antioxidant system (SOD) creates favorable conditions for formation of the immune response, whereas intensification of LPO against a background of insufficiency of antiperoxide defense, found under the combined influence of the antigen and HBO, inhibits development of the immune process. This is evidently one of the mechanisms of hyperoxic immunodepression when HBO is used in the initial period of development of the immune response.

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