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Genetic effects of hyperbaric oxygen therapy

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Summary

Patients with several diseases have been examined for detection of chromosome aberrations in peripheral blood cells after 10 sessions of hyperbaric oxygen (HBO) at 0.15–0.20 MPa for 40 min. The present study reveals that HBO increases the level of chromosome aberrations, and that individual reactions to HBO differ. Pure erythrocytes treated with high-pressure oxygen (HBO) at 0.7 MPa for 1 h are clastogenic for intact syngeneic lymphocytes.

The effect of HBO (0.3 MPa, 5 sessions of 1 h daily) on induction of chromosome aberrations in somatic cells and germinal tissues of rat males has been studied. Induction of aberrations in bone marrow cells after HBO was seen for 3 months. In lymphocytes of patients, it was seen for 9 months. Chromosome rearrangements at the first meiotic division were detected only 90 days after exposure.

HBO affects neither the functional nor the morphological condition of gonads and does not induce dominant lethals. It is proposed that a high quantity of chromosome breaks in cells of somatic tissues is an adaptive reaction of organisms to HBO.

Oxidative stress appears to be a factor inducing instability of the genome (Ames, 1983). Wide use of hyperbaric oxygen (HBO) for the treatment of different diseases leads to the unavoidable lengthy exposure of a significant number of people to high-tension pure oxygen. The mutagenic effect of increased oxygen tension has been shown in various organisms: microorganisms, plants, mammalia (Guskov et al., 1985, 1987; Moutschen-Dahmen et al., 1959). Our present observations evaluate the genetic consequences of HBO in peripheral blood cells of humans and in bone marrow and germinal cells of rats.

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Material and methods

Blood of patients with different diseases (in particular chronic ischemic disease, ulcerative disease of the stomach, bronchial asthma) was used as the material for our study. Drugs the patients took included broncholytics (amino phylline, theofedrine, tavegil and calcium chlorate), β-blockers (propranolol), coronarolytics (dipyridamole, phenozepam, nitrates, erinit, nitroglycerine) and in case of ventricular ulcer vicalinum or almagel, before and after HBO treatment. All patients were

hospitalized for a complex course of therapy, and underwent HBO – 0.15–0.20 MPa, 10 periods of exposure, about 40 min each daily – in the barocamera 'Oka-MT'. Time for compression and decompression was 15 min. Blood for analysis was taken just before the first period of exposure and just after the last one, as well as 9 months after the end of the HBO course. In some cases whole blood, drawn before the beginning of HBO and after phytohemagglutinin (PHA) stimulation, was put into the barocamera with 0.7 MPa tension supplied for 1 h.

Blood cells were cultured in glass penicillin bottles, each containing 0.5 ml of whole blood, 4.5 ml of medium 199, 0.5 ml of bovine serum, 100 units of penicillin, 5 units of heparin, 0.1 ml PHA (Difco). The cultures were fixed 72 and 96 h after PHA inoculation. Preparations with metaphase plates were stained according to Romanovsky (pH 6.8).

To study red blood cell clastogenic activity, peripheral blood cells of a healthy volunteer (25 years old) were separated on a phycol–verografine gradient (gradient density 1.08), and then were thrice washed in Eagle solution. Erythrocytes were exposed to high-pressure oxygen (0.7 MPa for 1 h) and then added to the cultures in different concentrations.

The mutagenic effect of HBO was followed on inbred rats (60 males and 120 females). The initial weight of the rats under study was 160–180 g. Male animals were exposed to HBO (0.3 MPa, 1 h daily, for 5 days). Immediately after the exposure they were mated with virgin female rats. Mating was followed daily for a fortnight (2 ♀ : 1 ♂) to determine any mutagenic effects on mature spermatozoa (the first week of mating). After 3 months, the mating experiment was repeated in order to identify any possible effects of HBO on the stem cells. The increase in embryo mortality determined by autopsy, done on the last day of embryogenesis, was used as a measure of mutagenic effect. Bone marrow and testes of the exposed animals were used for cytogenetic analysis. After acetoalcohol fixation and acetoorcein staining of the material we determined chromosome aberrations in cells in anaphase and early telophase. All results were analyzed statistically by Fisher–Student *t*-test treatment.

Results and discussion

The analysis of chromosome aberrations in peripheral blood cells taken from patients before the beginning of the HBO sessions showed that the drug therapy did not give the obvious rise in the level of spontaneous mutagenesis (Table 1). A considerable increase in the number of chromosome aberrations was registered in peripheral blood cells of patients after HBO compared with the level before treatment. All main types of chromosome aberrations are presented. Moreover, some cells with breaks of centromeres and endoreduplications were registered. Many cells had more than one aberration (Fig. 1).

Blood samples of 10 patients were treated with HBO in vitro to compare individual reactions of the genome of somatic cells to HBO. Table 2 demonstrates the correlation between the level of chromosome aberrations induced by the treatment in vitro and the level of chromosome aberrations in patients after sessions of HBO. According to the data in vitro we can distinguish 2 groups of reaction of the genome to HBO: up to 25% and greater than 25% aberrations.

Nine months after the HBO treatment, patients' blood was cytogenetically analyzed again. The data of this analysis of patients of the second group (greater than 25% aberrations) showed an increased level of chromosome aberrations in comparison with the first group. This indicates that blood treatment in vitro can reveal the sensitivity of the genome in patients before HBO treatment.

The organism as a whole 'turns over' the endogenous effects of pure oxygen, increasing in its turn the clastogenic effect of the latter (as can be seen from Table 2). Treating intact whole blood with oxygen only once in vitro under increased tension (0.7 MPa) for 1 h induces about the same number of chromosome aberrations as are found after the end of in vivo HBO therapy under the same conditions.

As can be seen from the literature, oxygen itself is not characterized by high mutagenic effects. Its genetic effects are mediated by intermediators of a free radical nature (Vodolazkin et al., 1988; Sawada et al., 1987). However, it may be possible to maintain free radical reactions for long periods

TABLE 1

CHROMOSOME ABERRATIONS IN LYMPHOCYTES OF PATIENTS BEFORE AND AFTER A COURSE OF HBO (0.15–0.2 MPa, 40 min, 10 days)

Number, Age (years), Sex		Number of cells scored	Cells with aberrations	% Metaphases with aberrations	Type of aberration [a]								
					ctg	ctb	acb	dc	tr	in	cte	bc	pp
1, 27, F	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	80	16	20.0+3.6	3	5	6	4	2	0	0	0	0
2, 35, F	before	54	3	5.5+2.3	0	4	3	1	1	0	0	0	0
	after	70	10	14.3+4.2	4	0	6	6	0	0	0	0	1
3, 21, F	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	60	9	15.0+4.2	4	5	3	3	0	0	0	0	0
4, 61, F	before	50	1	2.0+1.8	0	1	0	0	0	0	0	0	0
	after	70	14	19.4+4.5	5	6	4	5	0	0	0	0	1
5, 53, F	before	50	6	3.0+1.6	0	3	2	1	0	0	0	0	0
	after	70	17	24.7+5.1	20	16	2	2	0	0	0	0	0
6, 35, M	before	100	5	5.0+2.4	0	2	2	2	0	0	0	0	0
	after	50	11	22.0+3.5	6	3	1	3	3	0	0	0	0
7, 40, M	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	53	11	20.9+6.2	9	8	3	1	0	0	0	0	4
8, 49, M	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	46	14	30.4+6.7	6	8	8	6	2	0	0	2	0
9, 43, M	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	90	15	16.6+3.9	2	7	8	4	0	0	0	0	2
10, 63, M	before	70	4	5.7+2.8	0	3	2	0	0	0	0	0	0
	after	52	16	30.9+6.4	2	8	5	4	2	0	0	0	0
11, 36, M	before	55	4	7.3+4.0	0	3	1	1	0	0	0	0	0
	after	100	78	78.0+7.3	10	32	20	16	13	0	0	0	2
12, 25, M	before	40	1	2.5+0.7	0	1	0	0	0	0	0	0	0
	after	81	12	14.8+3.7	15	8	1	3	1	0	0	4	0
13, 25, M	before	78	5	6.5+2.8	1	2	2	1	0	0	0	0	0
	after	120	33	27.6+4.1	8	7	9	14	0	3	0	0	0
14, 20, M	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	70	29	41.4+5.9	2	18	7	12	0	0	0	0	1
15, 18, M	before	44	2	4.5+3.3	0	0	1	1	0	0	0	0	0
	after	46	12	26.3+6.5	2	3	4	4	0	0	1	1	0
16, 34, M	before	77	4	5.1+2.6	0	2	1	1	0	0	0	0	0
	after	138	14	10.0+2.5	2	4	6	5	1	0	0	0	0
17, 45, M	before	35	1	3.0+2.9	0	1	0	0	0	0	0	0	0
	after	40	20	50.0+7.9	2	10	4	5	2	0	0	3	0
18, 24, M	before	102	7	6.9+2.5	0	3	1	2	0	0	1	0	0
	after	87	20	23.0+4.5	6	5	2	5	3	0	2	0	0
19, 30, M	before	85	4	4.3+2.2	0	1	1	2	0	0	0	0	0
	after	70	14	20.0+2.9	8	3	4	6	0	0	0	4	0
20, 49, M	before	50	0	0	0	0	0	0	0	0	0	0	0
	after	88	15	17.0+4.0	3	8	2	5	0	0	1	0	2

[a] ctg, chromatid and iso-chromatid gaps; ctb, chromatid breaks; acb, acentric breaks; dc, dicentrics; tr, translocations; in, inversions; cte, chromatid exchanges; bc, breaks of centromers; pp, polyploid.

of time in multicellular systems at the expense of macromolecules containing metals with alternating valency. As has been shown earlier, some proteins, ferritin in particular, have clastogenic activity (Whiting et al., 1981).

HBO induces red cell hemolysis with an ex-

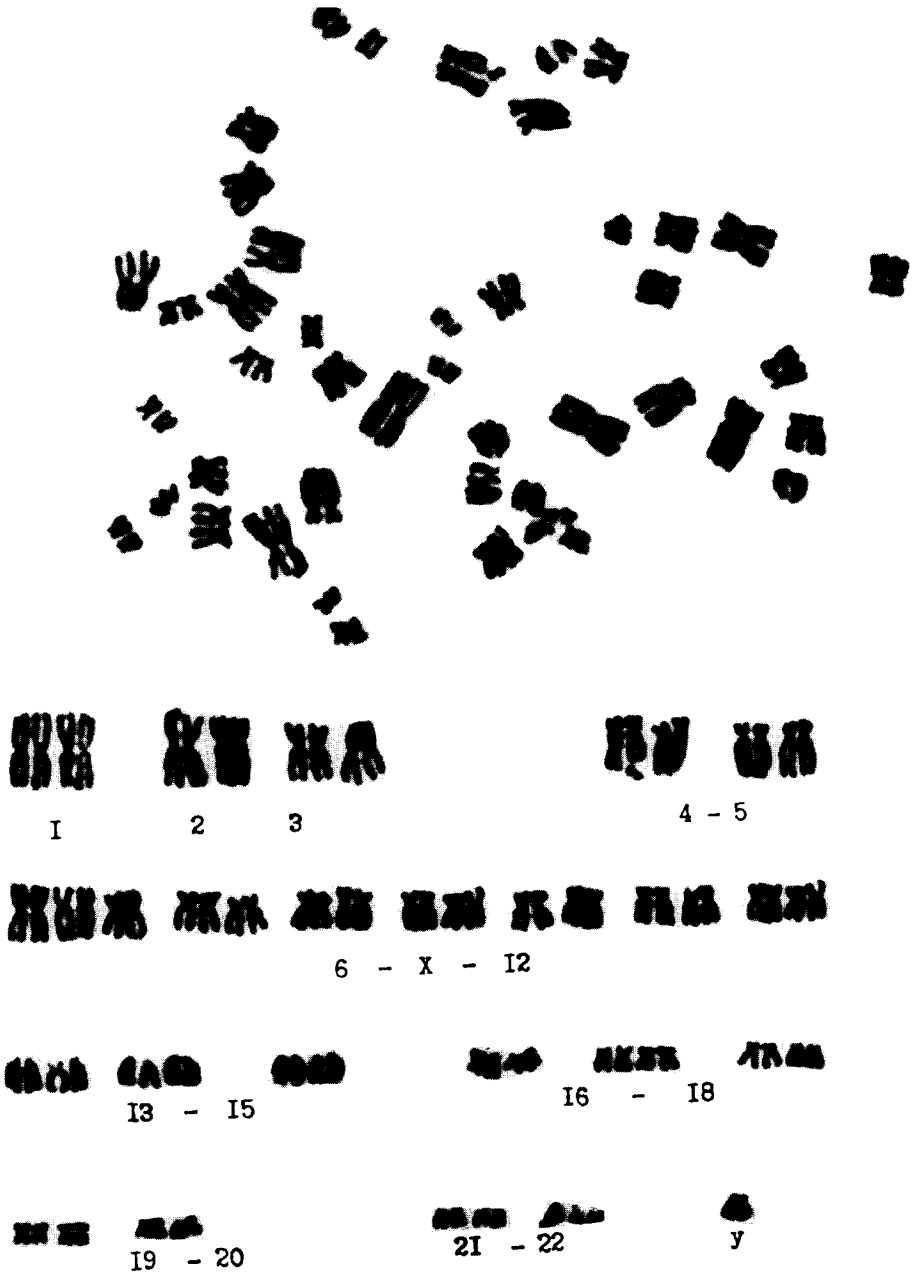

Fig. 1. Endomitosis of human peripheral blood lymphocytes after HBO.

tracellular hemoglobin increase in plasma, as is evidenced by clinical and experimental data on animals and people (Lukash and Vnukov, 1985). Pure erythrocytes treated with oxygen under high tension can, after mixture with syngeneic lymphocytes, induce genome instability (Table 3). It is seen that there is a clastogenic effect, but it depends strongly upon the volume of red cells added to the culture. Increases in erythrocyte count do not induce linear increases in chromosome

TABLE 2

INDUCTION OF CHROMOSOMAL ABERRATIONS BY HBO IN VIVO (0.15 MPa, 40 min, 10 days) AND IN VITRO (0.7 MPa, 1 h)

Patient	Before HBO			After HBO in vitro			After HBO in vivo			9 months after HBO in vivo		
	Number of cells scored	With aberrations	% Metaphases with aberrations	Number of cells scored	With aberrations	% Metaphases with aberrations	Number of cells scored	With aberrations	% Metaphases with aberrations	Number of cells scored	With aberrations	% Metaphases with aberrations
1	85	4	4.3 + 2.2	100	25	25.0 + 4.2	70	14	20.3 + 2.9	177	35	20.0 + 3.0
2	55	4	7.3 + 4.0	42	21	50.0 + 7.7	100	78	78.0 + 7.3	80	16	20.1 + 4.5
3	78	5	6.5 + 2.8	108	3	30.0 + 4.4	81	12	27.6 + 4.1	106	22	21.2 + 4.2
4	50	0	0	76	2	36.0 + 5.5	70	29	41.4 + 5.9	47	8	17.3 + 5.5
5	35	1	3.0 + 2.9	80	24	30.1 + 5.1	40	20	50.0 + 7.9	114	27	24.2 + 4.0
6	102	7	6.9 + 2.5	115	18	16,0 + 3.4	87	20	23.0 + 4.5	102	3	2.7 + 1.6
7	50	0	0	97	10	11.0 + 2.8	88	15	17.0 + 4.0	106	6	6.0 + 2.3
8	40	1	2.5 + 0.7	72	6	11.6 + 2.7	81	12	14.8 + 3.7	98	8	8.4 + 2.8
9	44	2	4.5 + 3.3	76	14	18.7 + 4.5	46	12	26.3 + 6.5	103	6	5.8 + 2.3
10	77	4	5.1 + 2.6	94	16	17.0 + 3.9	138	14	10.0 + 2.5	104	9	9.0 + 2.8

TABLE 3

CLASTOGENIC EFFECTS OF ERYTHROCYTES TREATED WITH HIGH-PRESSURE OXYGEN (0.7 MPa, 1 h) ON HUMAN LYMPHOCYTES

Treatment [a]	Number of cells scored	Number of metaphases with aberrations (%)	Type of aberration							
			ctg	ctb	cte	de	tr	in	acb	pp
25 μg/ml E + L untr.	100	6.0 + 2.9	3	4	0	1	1	0	1	0
50 μg/ml E + L untr.	100	22.0 + 4.7	12	12	0	8	0	1	8	0
100 μg/ml E + L untr.	100	17.0 + 3.7	4	1	2	10	2	0	6	4
250 μg/ml E + L untr.	85	32.9 + 5.1	11	17	0	4	2	0	6	0
500 μg/ml E + L untr.	82	24.2 + 5.0	10	10	1	8	1	1	3	1
Blood tr.	100	26.0 + 4.7	7	9	0	8	0	0	5	0
L. tr.	32	11.0 + 3.1	2	3	0	1	0	0	1	0
Control	100	4.0 + 1.9	1	0	1	2	0	0	0	0

[a] E, erythrocytes; L, lymphocytes; tr., treated with HBO; untr., untreated.

aberrations. This may be accounted for by the individual stability of red cell membranes of any given donor to the injurious factor.

Experiments on animal models have been done to consider other results of HBO. Levels of chromosome aberrations in bone marrow and germinal tissue are presented in Table 4. Increased levels of chromosome aberrations in bone marrow cells were preserved for 18 days. Some increase in chromosome aberrations in secondary spermatocytes was registered 90 days after HBO.

Cell analysis of germinative tissue did not show essential changes in relative mass of testes, osmotic resistance, concentrations, duration of life, of spermatozoids and did not show any pathological forms of spermatozoids.

A comprehensive consideration of dominant lethality (Table 5) has revealed no significant dif-

TABLE 4

INDUCTION OF CHROMOSOMAL ABERRATIONS IN BONE MARROW AND PRIMARY AND SECONDARY SPERMATOCYTES OF RATS AFTER HBO (5 days, 0.3 MPa, 1 h daily)

Cells	Days after exposure	Number of animals	Anaphase				
			Number of cells scored	Cells with aberrations	% Cells with aberrations	Chromatid and acentric breaks	Chromatid and chromosome exchanges
Bone marrow	Control	7	2500	59	2.3 + 0.3	12	58
	18	10	4800	416	8.5 + 0.4	68	492
	90	7	2500	88	3.5 + 0.3	16	124
Spermatocytes							
primary	Control	5	3680	65	1.3 + 0.1	40	40
secondary			1320	30	0.6 + 0.1	20	12
primary	18	5	2599	69	1.9 + 0.2	44	26
secondary			881	14	0.4 + 0.1	18	2
primary	90	5	2260	85	2.8 + 0.3	65	42
secondary			718	11	0.4 + 0.1	10	3

TABLE 5

DOMINANT LETHALITY IN A PROGENY OF MALE RATS EXPOSED TO HBO (0.3 MPa, 1 h daily, 5 sessions)

Breeding after exposure (days)	Potential fertility	Real fertility	Implantations	Resorptions	Fetal lethality		
					Total	Before implantation	After implantation
7							
Control	10.8+0.3	9.9+0.3	10.4+0.3	0.4+0.1	7.9+1.6	5.1+1.2	3.4+1.2
Experiment	11.7+0.5	8.6+0.5	9.9+0.7	1.3+0.4	18.9+4.7	7.2+4.0	11.9+2.1
14							
Control	10.5+0.3	9.2+0.2	9.4+0.2	0.6+0.2	11.6+1.8	6.5+1.4	5.4+1.3
Experiment	10.3+0.2	9.1+0.5	8.8+0.2	0.8+0.3	12.0+4.0	4.7+1.6	8.6+3.6
90							
Control	12.1+0.2	11.3+0.6	11.8+0.6	0.4+0.2	6.2+2.8	3.6+1.9	4.1+2.1
Experiment	12.6+0.4	10.4+0.4	11.4+0.4	1.0+0.2	17.4+3.6	9.0+2.4	10.4+2.7

ferences between the results for the exposed group and the controls. This fact may reflect the efficiency of meiotic division in eliminating chromosomal aberrations.

Although our data are preliminary, not yet conclusive and need further study, they have allowed us to come to some conclusions.

Oxygen has been shown to be a weak mutagen and recombinogen for microorganisms (Guskov et al., 1986) and does not induce recessive sex-linked lethals in *Drosophila melanogaster*. Somatic mammalian tissues, blood and bone marrow in particular, are characterized by large numbers of cells, and some part of those cells can be used to extend the endogenous antioxidant pool. Red cells with old and defective cell walls after contact with oxygen may undergo destruction, there appears to be activation of plasma ferro-containing proteins, starting a chain of free radical reactions, and inducing genome instability in peripheral blood lymphocytes. The destruction of cell biopolymers augments the level of low-molecular-weight blood antioxidants, such as urea and uric acid, that effectively suppress free radical reactions. Unlike the cells of somatic tissue the cells of germinative tissue are protected by the blood/testis barrier which prevents the metalloproteins from penetrating into seminiferous tubules (Mancini, 1964). From such a point of view, increases in chromosome aberrations in somatic cells should be considered as an intermediate stage between oxidative stress and adaptation.

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