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The influence of normobaric hyperoxide process on antioxidant enzymes activity and on lipid peroxidation processes in the rat's liver

Wpływ normobarycznej hipoksji na aktywność enzymów antyoksydacyjnych oraz na procesy peroksydacji lipidów w wątrobie szczura

Paradoxical phenomenon is that oxygen which is indispensable for the synthesis of high-energy phosphates (ATP) under specific conditions can be cytoxic to the whole organism. Under normal physiologic conditions 95% of oxygen is approximately used during cellular respiration precesses and undergoes quadrivalence reduction in the mitochondrial chain reaction of cytochromes. The reduction occurs when each oxygen molecule is united with four electrons. The remaining amounts of oxygen (about 2-5%) play a decisive part in the production of oxygen free radicals. Free radicals may activate or inhibit enzymes and also are toxic. These compounds are generated during incomplete oxygen reaction. (6).

Under normal physiologic conditions both enzymatic and nonenzymatic systems occurring within cells, may be responsible for inactivation of free radicals. Free radicals may cause cellular damage, if production of these compounds goes beyond the limit of the protective possibilites of scavenger enzymes (7).

Oxygen is cytotoxic, if oxygen concentration is higher than its amounts in the atmosphere. This phenomenon is called hyperoxide process (4). Under normal conditions the described above situations are not observed. There are a lot of clinical indications of therapeutic application of both normobaric and hyperbaric hyperoxide processes (3). The cytotoxic action of oxygen on lungs is well-documented. This is the effect of active contact surface between lungs and respiratory gases.

The author put forward a hypothesis that oxygen free radicals may be involved in pathogenesis of pulmonary damage during normobaric hyperoxide process (5, 16). Free radicals produced in biological systems may be important pathologic mediators in different tissue injuries but the precise role of these compounds remains unclear.

The work was undertaken to determine the influence of normobaric hyperoxide process on peroxidation which occurs within hepatic cells.

In the present experiment the author is interested in getting the answers to the following questions:

1) What is the role of antioxidant enzymes in the inhibiting process of free radicals in the liver? 2) What is the degree of dependence between the time of hyperoxide process and the activity of antioxidant enzymes? 3) Is there any dependence between the time of hyperoxide process and the progression degree of peroxidation?

MATERIAL AND METHODS

The studies were carried out on 44 male Wistar strain rats. The average weight of rats was 230 - 250 g. The animals lived in metal cages. The diet was standard. The rats were divided randomly into four experimental groups. Each group included 11 animals. The animals which were included into the groups labelled as follows: group I, group II, group III lived in the hermetic metal chamber. The oxygen passed as constant-oxygen flow ventilation at the rate of 2 dm^3 per min.

Oxygen concentration within the chambers was approximately 92%. This concentration was measured using the Oxytest. Carbon dioxide concentration was determined using Capnograf. This concentration was less than 0.1%. The pressure within the chamber was analogous to the barometric pressure. Inside the chamber and in the environment the average temperature was 23-25°C. The exposure time of the influence of chemically pure oxygen was the base of classification of the rats into four exprimental groups labelled as follows: group I, group II, group III. The time of exposure was 12, 24 and 48 hr, respectively. The control group (group K) included 11 rats. The animals lived in typical environmental conditions and the rats breathed using atmospheric air.

The 10% homogenate in the phosphatic buffer at 7.4 was obtained using the liver specimens. This homogenate was rotated for 5 min. In this homogenate the acivity of the examined enzymes was measured spectrophotometrically. Lipid peroxidation acivity was determined using the Specord UV VIS produced by the Carl Zeiss - Jena Company. The total SOD activity was assayed in the homogenate according to Kawaguchi et al. (10). Catalase activity was determined by the method of Cohen (2). Peroxidase activity was assayed using the Putter's and Becher's method (14). Glutathione peroxidase activity was determined in the homogenate by the method of Paglia and Valenti's. Glutathione reductase activity was assayed by Mizuno's method (12).The activity of examined antioxidant enzymes was measured in units per mg of DNA (U/mg DNA).

The amounts of lipid-peroxidation products was assayed by the method of L e d w o ż y w et al. (11). The activity of conjugated dienes and of lipid hydroperoxide was measured using the method of B u e g e ' s . The concentration of these substances was assayed in units of optical density per gram of hepatic tissue.

The unpaired Student's t-test was used for statistical analysis of biochemical results of the studies. In each experimental group both an arithmetical average and a standard deviation were estimated for biochemical data. The level of differences of the obtained results was determined between group I, group III and group II.

The statistical data were summarized in the tables and on the diagrams.

RESULTS

The results of this study were listed in two Tables. Table 1 shows the activity of antioxidant enzymes as follows: catalase-CAT; peroxidase-PX; glutathione peroxidase-GPX; glutathione reductase-RG; Cu, Zn-superoxide dismutase-Cu, Zn-SOD; Mn-superoxide dismutase Mn-SOD in the parenchyma of rat's liver. Simultaneously, the results obtained after 12 and after 24 hr were compared with the results obtained in the control group. The obtained results were statistically analysed. Table 2 shows the contents of lipid peroxidation products; malonyl dialdehyde (MDA); CD-conjugated dienes and HPETE-lipid hydroperoxide

Table 1. Enzyme activities of pancreas tissue in studied rats

	Normobaric hyperoxia [hours]			
	0 (Control group)	12 (Group I)	24 (Group II)	48 (Group III)
Catalase (CAT)	2240 ± 351	3150 ± 510 a	3556 ± 605 b	3620 ± 642 c
Peroxidase (PX)	17.22 ± 2.10	21.50 ± 3.60 a	27.22 ± 4.80 b,d	28.10 ± 5.24 c,e,f
Glutathione peroxidase (GPx)	6.69 ± 0.93	8.81 ± 1.34 a	10.30 ± 1.75 b,d	10.80 ± 1.81 c,e
Glutathione reductase (GR)	3.40 ± 0.52	4.27 ± 0.63 a	5.42 ± 0.92 b,d	5.88 ± 1.23 c,e
Cu, Zn-superoxide dismutase (Cu,Zn-SOD)	0.440 ± 0.065	0.620 ± 0.094 a	0.710 ± 0.120 b,d	0.824 ± 0.138 c,e
Mn-superoxide dismutase (Mn-SOD)	0.095 ± 0.018	0.120 ± 0.023 a	0.183 ± 0.030 b	0.210 ± 0.036 c,e

Enzyme acivities are shown in activity units [U/mg DNA]

a – $p<0.05$ – group I: control group
b – $p<0.05$ – group II: control group
c – $p<0.05$ – group III: control group
d – $p<0.05$ – group II: group I
e – $p<0.05$ – group III: group I
f – $p<0.05$ – group III: group II

Table 2. Concentration of lipid peroxidation products in pancreas tissue of studied rats

	Normobaric hyperoxia [hours]			
	0 (Control group)	12 (Group I)	24 (Group II)	48 (Group III)
Malonaldehyde (MDA)	28.4 ± 3.4	31.5 ± 5.2	38.2 ± 7.1 b,d	44.6 ± 8.8 c,e
Linked diene (LD)	0.880 ± 0.140	0.970 ± 0.160	1.040 ± 0.200	1.220 ± 0.220 c,e
Lipids hydroperoxide (ROOH)	0.030 ± 0.005	0.030 ± 0.006	0.050 ± 0.008 b,d	0.080 ± 0.011 c,e,f

Concentration of malonaldehyde – nM/mg protein.
Concentration of compounds with linked diene – O.D. 233 nm/g wet liver tissue.
Concentration of lipids hydroperoxide – O.D. 353 nm/g wet liver tissue.

a – $p<0.05$ – group I: control group
b – $p<0.05$ – group II: control group
c – $p<0.05$ – group III: control group
d – $p<0.05$ – group II: group I
e – $p<0.05$ – group III: group I
f – $p<0.05$ – gropu III: group II

in the hepar parenchyma of the examined rats. The results of this study which were obtained after 12 hr, 24 hr and 48 hr of peroxide process were compared with the results determined in the control group. The statistic analysis of these results was performed.

DISCUSSION

The effect of the cytotoxic influence of oxygen is very complicated, however it still remains unclear. According to many investigators oxygen-derived free radicals mediate an important step in the pathologies which occur as a result of hyperoxide process (5, 7).

The reactions catalysed by enzymes like: superoxide dismutase, peroxidase, glutathione peroxidase, glutathione reductase and catalase protect human organism against undesirable effects of free radicals action. Initial indirect observations also suggest that superoxide dismutase (Cu, Zn-SOD and Mn-SOD) is indispensable for living of aerobic organisms.

It is worth our attention that SOD synthesis as well as SOD activity increase during hyperoxide process. Moreover, the dismutation catalysed by SOD is the key reaction of oxygen free radical scavenders action. This reaction may occur spontaneously but SOD presence can accelerate this process about 10 times (7).

There is some evidence that great SOD activity in citosol of cells can be responsible for protection of tissue lipids against oxidative process. SOD activity plays a decisive part in the inhibition of artheromatous process evolution. Recent studies presented evidence that hypovitaminosis caused by deficit of vitamins like: vit. A, vit. C, vit. E and also selenium deficit may be responsible for the increase of organism sensitivity on hyperoxide process as well as susceptibility to other diseases (9, 15). In addition selenium is indispensable for the normal function of glutathione peroxidase. In humans the most important source of xanthie oxidase is the liver.

On the ground of experimental findings, free radicals produced in biological systems may be important pathologic mediators in liver disease. The best known generator of free radicals is xanthic oxidase. On the other hand, the liver can contain large amounts of such substances as SOD and GSH and PG and also alfa-tocopherol. It is necessary to underline that the substances like free radical scavenders are protective potential of human organism.

Well-defined controlled clinical studies underlined that oxygen-derived free radicals have been implicated as important agents in pathogenesis of liver diseases (17).

The important finding in my study was the increased activity of peroxide dismutase, glutathione peroxidase and glutathione reductase in the parenchyma of the rat's liver. It should be stressed that in all the experimental groups which underwent hyperoxide process, after 12 hr the activity of these enzymes increased from 25% to 32% compared to the controls. In the other experimental groups after 24 hr and after 48 hr of hyperoxide process there was observed further increase of the decribed above activity. The enzyme activity in group II has increased from 54% to 59% and in group III from 61% to 73% compared to the controls, respectively.

The statistic analysis of these results was very important (Table 1). In this experimental study there was analysed the level of Cu, Zn-SOD activity and also of Mn-SOD activity in the parenchyma of liver. After 12 hr of hyperoxide process the increase in Mn-SOD activity in the liver was 27% compared to the controls. After 24 hr of hyperoxide process the increase in this activity was 93%. The highest increase in Mn-SOD activity was obtained after 48 hr (exactly 121%). After 12 hr the results indicated increased Cu, Zn-SOD activity by 41% compared to the controls. After 48 hr Cu, Zn-SOD activity was higher by 87% compared to the controls.

The results indicate the increase in antixidant enzymes activity in the parenchyma of rat's liver. Furthermore, it may be an organism answer to the in-

creasing production of free radicals. The obtained data suggest that the normobaric hyperoxide process my be responsible for lipid peroxidation in liver tissues. It was observed that in all the experimental groups which underwent the hyperoxide process the level of malondialdehyde increased compared to the controls (Table 2). The studies have demonstrated that there is interdependence between the level of MDA and the antioxidant enzymes activity. The increased activity of antioxidant enzymes can leads to inhibition of lipid peroxidation processes (8).

The important finding of the study was the marked increase of amounts of lipid hydroperoxide and of conjugated dienes after 12, 24 and 48 hr of hyper-oxide process.

The results of the trials described above support the following conclusions:

- Normobaric hyperoxide process leads to a marked increase in antioxidant enzymes activity in the rat's liver. There is the dependence betweeen the enzymatic activity and the exposure time.
- Normobaric hyperoxide process casuses the increase in lipid peroxidation processes in the rat's liver. Moreover, this process is also related to the time of hyperoxide process.

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STRESZCZENIE

W prowadzonych przeze mnie badaniach podjąłem próbę określenia zmian biochemicznych zachodzących w wątrobie u szczurów poddanych działaniu hyperoksji. Ekspozycja na normobaryczną hypoksję była jedynym czynnikiem mogącym działać toksycznie na zwierzęta.

Materiał doświadczalny stanowiła grupa 44 szczurów szczepu Wistar. Były to albinotyczne samce o ciężarze ciała nie przekraczającym 250g, które podzielono na cztery grupy po jedenaście osobników w każdej. Grupy, które oznaczono jako I, II i III, poddane zostały ekspozycji na działanie czystego tlenu pod ciśnieniem jednej atmosfery absolutnej przez 12 godz. w grupie I, 24 godz. w grupie II i 48 godz. w grupie oznaczonej jako III. Jedenaście pozostałych szczurów stanowiło grupę kontrolną.

Aktywność enzymów antyutleniających w homogenatach badanych narządów oznaczono metodami spektrofotometrycznymi. Badane były następujące enzymy: katalaza (CAT), peroksydaza (Px), peroksydaza glutationu (GPx), reduktaza glutationu (RG), dysmutaza ponadtlenkowa Mn-SOD i dysmutaza ponadtlenkowa Cu, Zn-SOD.

Analogicznymi metodami określano poziomy zawartości peroksydacji lipidów w badanych narządach, to jest aldehydu malonowego (MDA), stężonych dienów (SD) oraz hydronadtlenków lipidów ROOH.

We wszystkich badanych grupach zwierząt stwierdzono zmiany biochemiczne (w większości były one istotne statystycznie), polegające na zwiększeniu aktywności enzymów antyoksydacyjnych oraz na zwiększeniu zawartości produktów peroksydacji lipidów w wątrobie.