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

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

The molecular oxygen is not cytotoxic for most of the living organisms. On the contrary, free radicals produced in incomplete oxygen reduction process are very detrimental.

The small amounts (approximately 2-5%) can undergo an incomplete reduction. During this reaction there can be generated free radicals as the ultimate products. (10). In the physiological situation these products are not dangerous for the cell. Free radicals produced in biological systems are bound with the active centre of the enzymes. This relationship assures the steady state of balance between the production and the decomposition of the cell. In the case of the disorders of cellular respiration being the result of the excess of oxygen, the process of the formation of free radicals can increase many times.

Free radicals may cause cell damage, if production of these compounds goes beyond the limit of the protective possibilites of enzymatic and nonenzymatic scavengers within the cell (4, 6). Free radicals play a decisive part in the pathophysiology of different diseases, like: autoimmune disease, radiation sickness, ischaemic disease andreperfusion and also degenerative disease (senescence and atheromatosis), as well as diabetes mellitus and acute pancreatitis (2, 11, 15).

When it was shown for the first time that activation of oxygen-derived free radicals occurred in tissues where the inflammatory process was noticed it be-

came clear that active neutrophilic granulocytes which play a part in inflammation, were the source of considerable amounts of oxygen free radicals (2). As there are preclinical studies on the influence of normobaric hyperoxide process on pancreas it needs to be tested clinically and should be criticized in the designing trials.

This work was undertaken to determine the influence of normobaric hyper-oxide process on peroxidation occurring in the rat's pancreas.

The author is interested in getting the answers to the following key questions:

1) What is the role of antioxidant enzymes in the inhibiting process of free radicals in the pancreas? 2) Moreover, what is the degree of dependence between the time of hyperoxide process and the activity of antioxidant enzymes? 3) What is the level of the products of lipid peroxidation and what is the degree of the progression of peroxide process?

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 the metal cages. The diet was standard. Rats were divided randomly into four experimental groups. Each group included 11 animals. The animals which were included into the groups labelled as group I, group II and group III lived in the germetic metal chamber. The oxygen passed as constant-flow ventilation at the rate of 2 dm^3 per minute.

Oxygen concentration inside the chambers was approximately 92%. Carbon dioxide concentration was determined using Capnograf. This concentration was less tha 0.1%.

The pressure within the chamber was analogous to the barometric pressure. Inside the chamber as well as in the environment the average temperature was 23-25°C. The time of exposure to the influence of chemically pure oxygen was the base of the classification of the rats into four experimental groups labelled as follows: group I, group II and group III. The exposure time was 12, 24, and 48 hr, respectively. The control group (Group K) included 11 rats. The animals lived in the typical environmental conditions and breathed atmospheric air.

The 10% homogenate in the phosphotic buffer at pH 7.4 was obtained using the pancreatic specimens. This homogenate was rotated for 5 min. In this homogenate the activity of the examined enzymes was measured spectrophotometrically. Lipid peroxidation activity was determined using the Specord UV VIS produced by the Cord Zeiss-Jena Company.

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

RESULTS

The results of this study were listed in two tables. Table 1 shows the activity of antioxidant enzymes like: catalase – CAT; peroxidase – PX; glutathione peroxidase – GPX; glutathione reductase – RG; Cu, Zn-superoxide dismutase; Cu, Zn-SOD; Mn – superoxide dismutase – Mn – SOD.

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)	1010 ± 144	1080 ± 164	1105 ± 192	1180 ± 205 c
Peroxidase (PX)	9.30 ± 1.45	9.92 ± 1.69	10.47 ± 1.84	11.32 ± 2.10 c
Glutathione peroxidase (GPx)	3.85 ± 0.56	4.72 ±0.81 a	5.27 ± 0.96 b	6.00 ± 1.07 c,e
Glutathione reductase (GR)	2.81 ± 0.53	3.10 ± 0.60	3.81 ± 0.71 b,d	4.36 ± 0.80 c,e
Cu, Zn-superoxide dismutase (Cu,Zn-SOD)	0.232 ± 0.040	0.282 ± 0.051a	0.364 ± 0.064 b,d	0.438 ± 0.073 c,e,f
Mn-superoxide	0.052 ± 0.011	0.076 ± 0.016 a	0.105 ± 0.020 b,d	0.162 ± 0.029 c,e,f

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 – $p<0.05$ – gropu III: group II

Simultaneously, the results obtained after 12 hr and after 24 hr, were compared with the results in the control group. Group II was compared with group I, group III with group I and also, group III with group II, respectively.

The obtained results were statistically analysed. Table 2 shows the contents of lipid peroxidation products like malonyl dialdehyde (MDA); CD. – conjugated diens and HPETE – lipid hydroperoxide in the parenchyma of pancreas. The results of this study which were obtained after 12 hr (group I), after 24 hr (group II) and after 48 hr, (group III) of peroxide process were compared with group II.

The statistic analysis of these results was made.

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)	4.4 ± 0.6	5.6 ± 0.8 a	6.1 ± 1.1 b	6.6 ± 1.0 c,e
Linked diene (LD)	0.360 ± 0.070	0.450 ± 0.085 a	0.520 ± 0.100 b	0.590 ± 0.115 c,e
Lipids hydroperoxide (ROOH)	0.010 ± 0.002	0.010 ± 0.002	0.030 ± 0.007 b,d	0.040 ± 0.010 c,e,f

Concentration of malonaldehyde – nM/mg protein
Concentration of particle with linked diene – O.D. 233 nm/g wet tissue
Concentration of lipids hydroperoxide – O.D. 353 nm/g wet 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 – $p<0.05$ – group III: group II

DISCUSSION

According to the current state of knowledge free radicals play a decisive part in the pathological processes related to the entire organism. The hyperoxidation is responsible for pathomechanism of these processes (1, 8, 12). When the levels of enzymatic and nonenzymatic protective systems increase above the limit organism damage may be observed (14).

The important role of oxygen radicals in the pathogenesis of acute pancreatitis is well documented. The first indirect observation also suggests that in human acute pancreatitis oxygen free radicals are generated and add to the damages seen (11, 15).

The protective effect of the free radical exogenous scavengers like: superoxide dismutase – SOD; catalase – CAT; Ebselen's substance; glutathione peroxidase and allopurinol – the inhibitor of xanthine oxidase was studied on the different experimental modes of acute pancreatitis (11, 15).

In humans as well as in animals dismutase is generely measured in islet Betta cells. Dismutase inhibits insulin release by the activation of oxygen free radicals (3, 7). Furthermore, oxygen free radicals are believed to be at least partly responsible for a change of structure of nucleic acids. On the other hand, oxygen radicals can lead to deficiency of insulin because these substances are able to inactivate proinsulin synthesis and also, to damage insulin's structure (13, 16).

The aim of the present study was to evaluate the activity of antioxidant enzymes which are enzymatic oxygen free radcal scavengers. During the study the author investigated the level of antioxidant enzymes in the rat's pancreas after normobaric hyperoxide process. The rats underwent hyperoxide process over 12 hr. And next the increase of the activity of peroxidase and of glutathione peroxidase-GPX and of glutathione reductase in the parenchyma of pancreas was observed. After 12 hr of hyperoxide process the activity of these enzymes increased from 7% to 23% compared to the control group. In the other groups (I, II) after 24 and after 48 hr of oxygen infusion the author observed the steady and unsignificant increase of enzymes activity.

The results indicate increased oxygen radical production in pancreatic tissues of rats in group I – from 13% to 37% and in group III from 22% to 56% (Table 1).

The most important finding in the study was the significant increase of peroxide dismutase (GPX). SOD activity in pancreatic tissues was also determined during the experiment. SOD is the main source of oxygen free radical scavengers (5).

In this experimental study the level of Cu, Zn – SOD activity and also of Mn – SOD activity in the parenchyma of pancreas was analysed separately. The data indicated the increased production of these isoenzymes in parenchyma of pancreas in each experimental group.

The results indicate a significant increase of antioxidant enzymes activity in the parenchyma of the rat's pancreas. Furthermore, it may be an organism answer to the increasing production of free radicals. The obtained date suggest that normobaric hyperoxide process may be responsible for lipid peroxidation in pancreatic tissues. Therefore the levels of lipid peroxidation by measuring malondialdehyde (MDA) were investigated.

In all the experimental groups which underwent the hyperoxide process the level of malondialdehyde increased.

The aim of the present study was also to establish the level of conjugated diens and lipid hydroperoxide in the tissue homogenate.

This compounds, as well as MDA are products of lipid peroxidation. The most important finding of the study after 12 hr, was the markedly increased amounts of the substances linked with conjugated diens. In the parenchyma of the rat's pancreas SD level was significantly elevated. After 24 hr, of hyperoxide process, lipid hydroperoxide activity has increased and after 48 hr this activity has increased to 300%.

Vitamin E (Tocopherol) concentration in intracellular organelles may be responsible for amounts of cell structures within which lipid peroxidation occurs. Vitamin E is particularly active free radical scavenger. There is a reverse proportional dependence between lipid peroxidation process and the level of vitamin E (9).

Lipid peroxidation activity may lead to disintegration of cells in the experimental rats. The changes of the properties of memebrane phospholipids could contribute to destruction of both cellular and subcellular membranes. The increased permeability is due, at least in part, to the action of phospholipids on the membrane. Moreover, the destruction of enzymatic systems together with disorder of transmembrane electrolytic gradient may be observed. In the critical cases the cellular damages may occur.

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

1. Normobatric hyperoxide process leads to a marked increase in antioxidant enzymes activity in the rat's pancreas. There is the dependence between the enzymatic activity and the time of the exposure.

2. Normobaric hyperoxide process causes the increase in lipid peroxidation in the rat's pancreas and this process is also related to the time of hyperoxide process.

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STRESZCZENIE

Współcześnie prowadzone badania eksperymentalne i kliniczne wskazują na istotną rolę wolnych rodników powstających pod wpływem hyperoksji w patogenezie chorób. Spośród wielu czynników biorących udział w patogenezie i rozwoju ostrego zapalenia trzustki oraz cukrzycy insulinozależnej coraz większą rolę przypisuje się wolnym rodnikom. Problem ten jednak dotyczy związków chemicznych, które poprzez generowanie wolnych rodników prowadzą do powstania zmian patologicznych w obrębie trzustki.

W prowadzonych przeze mnie badaniach podjąłem próbę określenia zmian biochemicznych zachodzących w trzustce szczurów poddanych działaniu hyperoksji. Ekspozycja na normobaryczną hyperoksję była jednym czynnikiem mogącym działać toksycznie na badane zwierzęta. Materiał doświadczalny stanowiła grupa 44 szczurów szczepu Wistar, które podzielono na cztery grupy, po jedenaście osobników w każdej. Grupy które oznaczono jako I, II i III, poddane były 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 III. Jedenaście pozostałych szczurów stanowiło grupę kontrolną.

Aktywność enzymów utleniających w homogenacie trzustki oznaczono metodami spektrofotometrycznymi. Badane były następujące enzymy: katalaza (CAT), peroksydaza (Px), peroksydaza glutationu (GPx), reduktaza glutationu (RG), dysmutaza ponadtlenowa Mn-SOD i dysmutaza ponadtlenowa Cu, Zn-SOD.

Analogicznymi metodami określono poziomy zawartości produktów peroksydacji lipidów, to jest aldehydu malonowego (MDA), sprzężonych dienów (SD) oraz hydronadtlenków lipidów (ROOH).

We wszystkich grupach badanych zwierząt stwierdzono zmiany biochemiczne (w większości były one istotne statystycznie), polegające na zwiększeniu aktywności badanych enzymów antyoksydacyjnych oraz na zwiększeniu zawartości produktów peroksydacji lipidów. Jedynie w przypadku hydronadtlenków lipidów po 12 godz. trwania hyperoksji nie zaobserwowano wzrostu ich zawartości w miąższu trzustki.