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The influence of normobaric hyperoxia on antioxidant enzyme activities and peroxidation products levels in rat brain

Wpływ hiperoksji normobarycznej na aktywność enzymów antyoksydacyjnych i poziom produktów peroksydacji w mózgu szczura

It is a well known fact that the supply of oxygen at a concentration higher or lower than those occurring under normal conditions can promote oxidative processes in cells of various tissues. The most recent experiments in hyperoxia and our previous studies have concerned lungs and indicated that hyperoxia leads to an excessive intracellular formation of reactive oxygen intermediates (ROI) as mediators of lungs injury (3, 5). There is also proposed one of possible basic mechanisms of oxidative damage in other organs including brain (2, 5).

Oxygen has been used in therapy for at least 60 years. Because oxygen concentration (50%-100%) used in some cases is potentially toxic, the patients supplemented in this way were at risk of exacerbation of previously existing lungs, brain or other organs injury (3, 9).

Free radicals may arise from a number of sources as a result of a variety of cellular mechanisms (3). The mitochondrial respiration, the xanthine oxidase pathway, the cytochrome P-450 function, the arachidonic acid pathway, invading leucocytes can all lead to the production of a variety of ROI, including superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\cdot}OH$). ROI are potentially very damaging to cells, leading to oxidation of essential cellular constituents including proteins, lipids and DNA (3). The oxidative stress in organs can be measured by malondialdehyde (MDA), conjugated dienes and hydroperoxides formation. They are important products of lipid peroxidation (2, 5).

Free radicals are dealt with a complex, interdependent system of antioxidant defences including the antioxidant enzymes (AOEs): superoxide dismutase (SOD), glutathione peroxidase (PxG), glutathione reductase (GR), catalase (CAT), and glucose-6-phosphate dehydrogenase (G6PG).

The AOEs reduce superoxide anions, hydrogen peroxide and other lipid organic peroxides. Since the AOEs are the reserve of antioxidant under genetic control, it is not susprising that they are responsible for cellular oxidative stress and can be induced under many different physiological (exercise, aging) and pathological (hypoxia, reperfusion) conditions.

The AOEs are relatively deficient in the brain (6, 8, 13). Impairment or limitation of these defences may render the brain susceptible to oxidative injury (2). The aim of this study was to evaluate AOEs activities and peroxidative processes intensity in the brains of adult rats kept under hyperoxic normobaric conditions. These parameters were assayed as a function of the duration of exposure to hyperoxia.

MATERIAL AND METHODS

The investigations were carried out on 44 male Wistar rats of 250-300 g of body weight. Before starting the experiment, animals were maintained in stainless wire cages (maximum 3 rats per cage) with free access to water and food in a natural light dark cycle. After 2 weeks of adaptation period, 33 animals were placed in thermostated (23-25 °C) hermetic chamber with glass front wall and $1m^3$ volume (100 cm × 200 cm × 50 cm). Through the chamber a stream of pure oxygen passed with 2 l/min velocity. Oxygen concentration in the chamber was monitored with a gas analyser Beckman 0260 (above 99%, 760 mmHg). Carbon dioxide concentration in outlet gas was monitored with Beckman LB-2 analyser (Beckman Instruments, Fullerton, USA). Oxygen flow was fitted in such a way that carbon dioxide concentration would not exceed 0.5%. Humidity was maintained at the level of 40-50%. After 12, 24 and 48 hours of normobaric hyperoxia, the groups of 11 animals each (B, C and D respectively) were guillotined and the brains were removed for biochemical examinations. Eleven rats were the control group (A). The brains were homogenised in 10 vol. of 10 mM Tris-HCL buffer, containing 0.145 M NaCl and 1mM EDTA and homogenate was centrifuged (5 min, 150g). Antioxidant enzyme activities were determined in brains homogenate by means of spectrophotometer, by the method of Kawaguchi (SOD), Paglia (PxG), Mizuno (RG), Bergmeyer (CAT), and De Mowa (G6PD). Peroxidation product levels were determined by means of spectrophotometer by the method described by Ledwożyw (MDA), by Buege (conjugated dienes) and by Austa (hydroperoxides).

Statistical analysis was performed by the Student's t-test for unpaired data.

RESULTS AND DISCUSSION

Table 1 shows antioxidant enzyme activities and Table 2 shows proxidations product levels in the rat brain after 12, 24 and 48 hours of hyperoxia. The rise in activities of the above-mentioned enzymes was observed as early as 12 hours of exposure to the pure oxygen and further great statistically significant increase after 24 and 48 hours as compared with control brains was found. In particular the activities of MnSOD and PxG have increased.

Table 1. Antioxidant enzyme activities (units/mg DNA) in the brain from normobaric hyperoxia-treated rat. Mean ±SD. t-Student test for unpaired data: **-p<0.01 B vs A, C vs A, D vs A

	Hyperoxia (hours)			
	O (A)	12 (B)	'24 (C)	48 (D)
Cu, Zn-superoxide dismutase	95.0±5.0	114.0±4.0	294.5±4.0**	404.7±7.0**
Mn-superoxide dismutase	7.5±0.8	18.0±1.0	47.5±4.0**	79.8±2.0**
Glutathione peroxidase	0.5±0.1	1.5±0.2**	2.8±0.1**	4.1±0.2**
Glutathione reductase	0.8±0.06	1.2±0.04**	1.9±0.02**	2.45±0.03**
Catalase	80.0±10.0	170.4±11.0**	310.4±10.0**	440.0±11.0**
Glucose-6-phosphate dehydrogenase	1.4±0.1	2.3±0.1**	3.3±0.2**	4.5±0.2**

Table 2. Malondialdehyde (nm/mg protein), conjugated dienes ($E_{233/g}$) and hydroperoxides ($E_{353/g}$) levels in the brain from normobaric hyperoxia-treated rat. Mean ±SD. t-Student test for unpaired data: **-p<0.01 B vs A, C vs A, D vs A

	Hyperoxia (hours)			
	O (A)	12 (B)	24 (C)	48 (D)
Malondialdehyde	2.1±0.2	3.7±0.2**	4.4±0.1**	4.9±0.1**
Conjugated dienes	0.45±0.05	1.55±0.04**	1.95±0.03**	2.4±0.05**
Hydroperoxides	0.01±0.002	0.05±0.002**	0.08±0.001**	0.11±0.002**

A statistically significant rise in levels of lipid peroxidation products has been observed with the advancement of hyperoxia for all of parameters but particularly for hydroperoxides. It resulted in about 400%, 700% and 1000% (for group: B, C, D respectively) increase in hydroperoxides content when compared with the control group (A). The content of conjugates dienes was about fourfold greater after 48h of hyperoxia in comparison with control. Rat brain MDA level showed a tendency to increase too.

There is some evidence that hyperoxia is involved in the development of brain damage and that oxygen at least partly acts through reactive free radicals formation (2, 5, 12). The species and tissues differences in suscep-

tibility to oxygen also exist (1, 4, 5, 8). Adult mice do not respond to an increase in AOEs activities when exposed to subtoxic (60-80%) or toxic (100%) oxygen concentration (5). The relation between hyperoxia (60% O_2 for 48h) and AOEs activity were studied in selected organ of developing chick embryos. Exposure to hyperoxia at different time points during incubation resulted in twofold to tenfold increase AOEs activity in homogenates of all organs (liver, lungs, intestine) except the brain (15). According to Frank et al. (1) adult rats are susceptible to oxygen (96-98%), through showing increase in antioxidant defence enzymes in brain. These changes are similar to the changes seen in senescent brain compared to young adult controls (10). The exposure of rats to 4 atmospheres of 100% oxygen for 90 min. is associated with increased levels of lipid peroxidation products (MDA and 4-HDA), but with decreased activities PxG and RG in lungs and brain. There is a relationship between metabolic activity and mitochondrial MnSOD levels. Tissues with high metabolic demands (and correspondingly increased ROI production) such as liver, kidney, heart and brain possess higher levels of this enzyme (8). MnSOD is also more easily induced than CuZnSOD in many oxidative stresses. Mover-Lev (11) showed that the AOEs response in the brain, liver, lungs, heart, uterus of pregnant rats to environmental oxidative stress (1 week of exposure to hyperoxia) was higher than that of nonpregnant rats. It may be a result of elevated metabolism in pregnancy. Long-term exposure to low levels of ROI causes slow damage of neurons leading to the neuronal cell death characteristic of the age-related neurodegenerative diseases (7). In these cases, like in our study, the elevations in the contents of lipid peroxidation products were correlated with an increase in AOEs levels, suggesting a compensatory response. It is possible that peroxidation of brain membrane lipids leads to damage of capillary endothelial cells and neurones (14). Many investigators have observed in new-born rats, breathing 3h with pure oxygen, the syndrome of acute toxicity to CNS with fever. Other investigations have shown the neuronal necrosis in brain (5). The results presented in this work suggest that great, dependent on time of exposure, induction of antioxidant enzymes and intensification of peroxidative processes in brain of rats exposed to hyperoxia occur. Our results support the data on ROI involvement in hyperoxic brain injury.

REFERENCES

1. Frank L. et al.: Oxygen toxicity in neonatal and adult animal of various species. J. Apply. Physiol., 45, 699, 1978.
2. Halliwell B. et al.: Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet, 23, 1396, 1984.
3. Halliwell B. et al.: Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J., 219, 1, 1984.
4. Ito T. et al.: Measurement of adenosino triphosphate in the brain of mice exposed to normobaric hyperoxygenation. Med. Sci. Res., 23/6, 373, 1995.
5. Jamieson D.: Lipid peroxidation in brain and lungs mice exposed to hyperoxia. Biochem. Pharmacol., 41, 749, 1991.
6. Kornburst P.: Relative susceptibility of microsomes from lungs, heart, liver, kidney, brain and testis to lipid peroxidation. Correlation with vitamin E content. Lipids, 15, 315, 1980.
7. Lovell M.: Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. Neurology, 45, 1594, 1995.
8. Martilla R. et al.: Oxygen toxicity protecting enzymes in the human brain. J. Neural. Trans., 74, 87, 1988.
9. Menzel M. et al.: Increased inspired oxygen concentration as a factor in improved brain tissue oxygenation and tissue lactate levels after severe human head injury. J. Neurosurgery, 91 (1), 1, Jul. 1999.
10. Mizuno Y. et al.: Regional distribution of thiobarbituric acid-reactive products, activities of enzymes regulating the metabolism of oxygen free radicals, and some of the related enzymes in adult and aged rat brains. J. Neurochem., 46, 1344, 1986.
11. Mover-Lev H. et al.: Changes in enzymatic antioxidant activity in pregnant rats exposed to hyperoxia or hypoxia. Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol., 118/3, 353, 1997.
12. Pablos M. et al.: Acutely administered melatonin reduced oxidative damage in lungs and brain induced by hyperbaric oxygen. J. Apply. Physiol., 83/2, 354, 1997.
13. Sies H.: Antioxidant activity in cells and organs. Am. Rev. Respir. Dis., 136, 478, 1987.
14. Sjoberg F. et al.: Specific blood flow reducing effects of hyperoxaemia on high flow capillaries in the pig brain. Acta Physiol. Scand., 165(1), 33, Jan. 1999.
15. Van-Golde J. et al.: Induction of antioxidant enzyme activity by hyperoxia (60% O_2) in the developing chick embryo. J. Physiol., 509/1, 289, May 1998.

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STRESZCZENIE

Badano zmiany w aktywności dysmutazy nadtlenkowej, peroksydazy glutationu, reduktazy glutationu, katalazy i dehydrogenazy glukozo-6-fosforanu, a także poziomy aldehydu malonowego, sprzężonych dienów i hydronadtlenków w homogenatach mózgów szczurów po 12, 24 i 48 godzinach hiperoksji normobarycznej. Stwierdzono znaczny wzrost aktywności badanych enzymów już po 12 godzinach ekspozycji zwierząt na czysty tlen i dalszy coraz większy wzrost w miarę wydłużania czasu ekspozycji na hiperoksję. Podobne zmiany obserwowano w stężeniach produktów peroksydacji. Wyniki świadczą o tym, że hiperoksja normobaryczna indukuje aktywność enzymów antyoksydacyjnych i wzmaga procesy peroksydacyjne w mózgu szczura.