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THE OXIDANT–ANTIOXIDANT EQUILIBRIUM IN THE BLOOD OF PEOPLE WITH SUDDEN SENSORINEURAL HEARING LOSS AFTER THE FIRST HYPERBARIC OXYGEN THERAPY SESSION— A PRELIMINARY STUDY

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ABSTRACT

The activity of selected antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in erythrocytes, and the concentration of thiobarbituric acid reactive substances (TBARS) in blood plasma and erythrocytes, were determined in people subjected to hyperbaric oxygen (HBO) therapy due to sudden sensorineural hearing loss (SSNHL). Venous blood samples were taken immediately before entering the hyperbaric chamber and 5 min after leaving it. In the study group, two age subgroups were distinguished: group I consisting of subjects under 35 and group II consisting of subjects over 50.
The obtained values were analysed statistically using Student's t-test. Differences were considered as statistically significant at $p < 0.05$. A statistically significant decrease in the CAT activity was shown 5 min after leaving the hyperbaric chamber in pooled subjects ($p < 0.01$) and group I ($p < 0.05$). Furthermore, a statistically significant decrease in the erythrocyte TBARS concentration was observed in group II ($p < 0.05$).
It was demonstrated that a single exposure to hyperbaric oxygen affects the oxidant–antioxidant equilibrium as evidenced by, e.g., a statistically significant decrease in the activity of catalase in erythrocytes. It is possible that the antioxidant response to HBO depends on the age of subjects.
Keywords: sudden sensorineural hearing loss (SSNHL), hyperbaric oxygen (HBO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS).

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INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) is a rapid-onset and frequent pathology. It is associated with a loss of function of one of the most important human senses. Hearing loss occurs when hearing impairment is greater than 30 dB and involves three or more frequencies. Deafness occurs in up to three days [1,4]. The incidence of this disease in Poland is approximately 5–20 cases per 100,000 people per year. The disease occurs most often between 30 and 60 years of age, with the hearing loss usually affecting one ear, rarely two [2,5,6]. The causes of sudden hearing loss usually have a vascular (50–70%), viral (28–40%) or autoimmune (approx. 15%) background [2,3].

Deafness is usually accompanied by tinnitus and an unpleasant feeling of ear blockage described by the patients as clogged ear. Moreover, frequent vertigo and balance disturbance occur [3]. SSNHL is usually treated pharmacologically with corticosteroids. During hospitalisation, the drugs are administered intravenously or intratympanically, while in an outpatient setting—mainly orally. Glucocorticosteroids are administered orally at a dose gradually tapered until discontinuation. SSNHL therapy can also include antivirals, drugs stimulating microcirculation, as well as diuretics, vitamins and diet supplements [9,12,13]. In cases of vertigo (30–40%) or tinnitus, further therapeutic procedures are required. Hyperbaric oxygen (HBO) therapy is gaining popularity in combination with pharmacotherapy. HBO therapy is the only known method that allows increasing oxygen partial concentration in the inner ear fluid.

Oxygen reaches the spiral organ in two ways: by diffusion from the stria vascularis via the cochlear duct endolymph, and by diffusion from the middle ear through the round window [11]. The presence of pure oxygen in the breathing mixture along with high pressure in the chamber (0.25 MPa) increases the pressure of oxygen in the entire body, including the middle ear. This increase is accompanied by the return of the electrophysiological function of the cochlea [12,13].

Hyperbaric oxygen therapy consists of breathing 100% oxygen in a specially adapted chamber in which the pressure is higher than the local ambient pressure. For the purposes of the therapy, a pressure of approximately 0.25 MPa is used [7]. Such pressure, along with breathing 100% oxygen through a special mask, allows oxygenation of all organs and tissues of the body, including the middle ear in order to resolve the symptoms of deafness. In the course of SSNHL treatment using HBO, a series of 15 everyday sessions in the hyperbaric chamber is used, according to the standards followed by the Mazovian Centre for Hyperbaric Therapy and Wound Treatment in Warsaw, Poland.

The increase in the concentration of oxygen in the breathing mixture may lead to an enhanced generation of reactive oxygen species (ROS) in body cells. Once the oxidant–antioxidant equilibrium in human body is disrupted, excess ROS cause damage to DNA, proteins and lipid structures of cell membranes [14,15]. ROS include, e.g., superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH)[16]. To prevent damage to cellular structures, human body has developed mechanisms capable of removing these reactive oxygen species. Among compounds endowed with antioxidative properties are non-enzymatic ROS scavengers, such as vitamins A, B, C and E, and antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [17]. A marker of oxidative stress is, e.g., the intensity of lipid peroxidation—a process consisting of repeated free radical chain reactions leading to the degradation of fatty acids forming cell membranes. Products of lipid peroxidation that are toxic to cells include thiobarbituric acid reactive substances (TBARS) [18].

MATERIAL AND METHODS

The study was conducted in a group of 32 people (mean age = 45 ± 16 years, haemoglobin—HGB = 15.0 ± 1.5 g/dL; haematocritHCT = 44.3 ± 10.4%) who were patients of the Mazovian Centre for Hyperbaric Therapy and Wound Treatment in Warsaw, Poland. The study group was divided into two subgroups: group I comprised subjects aged under 35 years (n = 11, mean age = 28.2 ± 5.8 years,HGB = 14.3 ± 2.1 g/dL;HCT = 40.9 ± 5.8%), while group II comprised subjects aged over 50 years (n = 12, mean age = 63.3 ± 8.6 years; HGB = 15.4 ± 1.10 g/dL; HCT = 43.9 ± 2.6%).

A single hyperbaric therapy session lasted 90 min. The procedure consisted of two 10-minute periods of compression and decompression at the beginning and at the end of treatment, respectively, and three 20-minute periods of breathing with 100% oxygen. The latter periods were divided by two 5-minute breaks periods during which the subjects breathed atmospheric air. The total time spent under the pressure of 0.25 MPa was 70 min, and during that time the subjects breathed hyperbaric oxygen for a total of one hour [7].

The study was approved by the Bioethics Committee of the Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland.

The subjects were treated with hyperbaric oxygen therapy in the Haux Starmed 2200 hyperbaric chamber which created the same environmental conditions for all study subjects, providing constant pressure, humidity, temperature, and allowing them to breathe pure oxygen for the same amount of time.

The material for biochemical tests was blood taken by qualified medical personnel from the basilic vein. Blood samples for the analysis were taken at two time points: before treatment in the hyperbaric chamber and approx. 5 min after treatment.

The activity of SOD, CAT and GPx was determined in erythrocytes, while the concentration of TBARS was determined in erythrocytes and blood plasma. The biochemical analyses were conducted at the Institute of Medical Biology of Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland.

The TBARS concentration was measured using the method by Buege and Aust [32] as modified by Esterbauer and Cheeseman [33]. Lipid peroxidation products were identified using thiobarbituric acid (TBA). The main lipid peroxidation product that reacts with thiobarbituric acid is malondialdehyde (MDA), therefore, for the sake of simplicity, the levels of TBARS were expressed as the concentration of MDA. The MDA concentration in erythrocytes was expressed in nmol MDA/g Hb, and that in blood plasma was expressed in nmol MDA/mL of plasma.

Determination of the SOD activity was based on the inhibition of adrenaline autoxidation to adrenochrome in alkaline conditions. To measure the SOD activity, a previously obtained haemolysate after removal of haemoglobin with a chloroform–ethanol mixture was used. Centrifugation generated two layers: upper layer containing the enzyme and lower layer containing denatured haemoglobin and chloroform [32]. The SOD activity was determined by continuous recording of the reaction using a reaction kinetics programme on a Varian spectrophotometer, and expressed in U/g Hb.

The CAT activity was determined by measuring the decrease in the absorbance of a solution of hydrogen peroxide (H_2O_2) decomposed by the enzyme. The decrease in the absorbance value is directly proportional to the reduction of the H_2O_2 concentration in the solution [34]. The CAT activity was expressed in IU/g Hb.

The GPx activity was determined at 20°C using a method based on decomposition of hydrogen peroxide by the enzyme with the concurrent oxidation of reduced glutathione [22]. The results were expressed in U/g Hb.

The study results were presented as means with standard deviation (SD) values. Statistical analysis was conducted using Student's t-test, and differences were considered as statistically significant at $p < 0.05$. The hypothesis of statistical significance of these coefficients was tested.

RESULTS

The SOD activity in the erythrocytes of pooled subjects after the hyperbaric exposure decreased in a statistically insignificant manner by approx. 2% from 778.61 ± 194.68 to 763.59 ± 152.52 U/g Hb (Tab. 1). In the erythrocytes of subjects from group I, the SOD activity after HBO therapy also showed a statistically insignificant tendency to decrease. In group I, the activity of this enzyme was 836.32± 169.33 U/g Hb before treatment, and decreased to 784.04 ± 168.40 U/g Hb after treatment. In group II, the SOD activity after HBO therapy remained roughly at the same level, with only a slight increasing tendency from 729.89 ± 119.09 to 735.30 ± 125.38 U/g Hb (Tab. 1).

The CAT activity in the erythrocytes of pooled subjects after HBO therapy decreased in a statistically significant manner by approx. 5.9% ($p < 0.01$; Tab. 1). A statistically significant ($p<0.05$) decrease in the activity of this enzyme (approx. 10.4%) was also observed in group I. In group II, a statistically insignificant tendency of the CAT activity to decrease by approx. 1.9% was seen (Tab. 1).

No statistically significant changes in the activity of GPx were noted after HBO therapy. The activity of this enzyme in the erythrocytes of pooled subjects showed a tendency to increase after treatment by approx. 18.4% from 8.8 ± 5.8 to 10.42 ± 5.24 U/g Hb. The GPx activity after the hyperbaric exposure increased by 13% in subjects from group I and by 18.5% in subjects from group II (Tab. 1).

The TBARS concentration in the erythrocytes of pooled subjects after the hyperbaric exposure had a tendency to decrease by approx. 7% from 26.31 ± 8.65 to 24.48 ± 8.53 nmol MDA/g Hb (Tab. 1). In group I, a decreasing tendency in the levels of TBARS in erythrocytes was observed. In group II, the TBARS concentration in erythrocytes decreased in a statistically significant manner after the hyperbaric exposure ($p < 0.05$). Before the exposure, the TBARS concentration in this group was 28.95 ± 6.62 nmol MDA/g Hb, and decreased by 15.6% after treatment to 24.42 ± 5.81 nmol MDA/g Hb (Tab. 1).

No statistically significant changes were noted in the TBARS concentration in the blood plasma of subjects after HBO therapy. However, a tendency of TBARS to increase in the blood plasma of pooled subjects was seen. The concentration of these lipid peroxidation products after HBO therapy decreased by 2% ($p > 0.05$) in subjects from group I and increased by 6.8% in subjects from group II ($p > 0.05$; Tab. 1).

Tab. 1

The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in the erythrocytes, and the concentration of thiobarbituric acid reactive substances (TBARS) in the blood plasma and erythrocytes of patients subjected to hyperbaric oxygen (HBO) therapy.

	Pooled subject n=32		Group I n=11		Group II n=12	
Parameter determined	Before treatment	Approx. 5 min post-treatment	Before treatment	Approx. 5 min post-treatment	Before treatment	Approx. 5 min post-treatment
SOD [U/g Hb]	778.61 ± 194.68	763.59 ± 152.52	836.32 ± 169.33	784.04 ± 168.40	729.89 ± 119.05	735.30 ± 125.38
CAT [10^4 IU/g Hb]	69.27 ± 9.45	65.60 ± 9.15**	70.20 ± 10.42	62.93 ± 9.64*	66.25 ± 10.75	65.10 ± 10.42
GPx [U/g Hb]	8.80 ±5.18	10.42 ± 5.24	9.08 ±5.60	10.34 ± 5.99	8.95 ±5.40	10.61 ± 5.18
TBARS er. [nmol MDA/gHb]	26.31 ± 8.65	24.48 ± 8.53	22.17 ± 5.93	21.90 ± 6.93	28.95 ± 6.62	24.42 ± 5.81*
TBARS os. [nmol MDA/ml]	0.47 ±0.09	0.48 ±0.09	0.51 ±0.12	0.50 ±0.13	0.44 ±0.08	0.47 ±0.05

The results are expressed as mean ± SD.
*—statistically significant difference compared to the pre-treatment activity, $p < 0.05$
**—statistically significant difference compared to the pre-treatment activity, $p < 0.01$

DISCUSSION

Breathing 100% oxygen may increase generation of reactive oxygen species in the body and affect the oxidant–antioxidant equilibrium. Respiratory chain is the main source of these toxic forms of oxygen in the cell. In the process of cellular respiration, part of oxygen naturally undergoes incomplete reduction, which leads to the formation of ROS [16,19].

Moreover, there is evidence of an effect of oral glucocorticosteroid therapy on the oxidant–antioxidant equilibrium in humans. Bednarek et al. [8] observed a decrease in the activity of CAT and SOD in people with

Graves' disease taking glucocorticosteroids. Another study demonstrated a decrease in the activity of CAT and SOD, as well as the concentration of TBARS, in the kidneys of rabbits after administration of glucocorticosteroids at a dose of 50 mg/kg b.w. following an experimentally induced ischaemia of the kidney lasting three hours [20].

In the presented study, no statistically significant changes in the SOD activity were observed. There was, however, a decreasing tendency in the activity of this enzyme seen in pooled subjects and in group I, and surprisingly an increasing tendency in the activity of the enzyme found in group II. Paprocki et al. [21] in an earlier study also showed an increasing tendency in the activity of this enzyme in the blood of volunteers treated with HBO. Freiberger et al. [22] demonstrated that an increased partial pressure of O_2 causes changes in the SOD activity. Their results suggest an increased generation of ROS, mainly superoxide anion (O_2^-)—a substrate in the dismutation reaction catalysed by SOD, in response to exposure to hyperbaric oxygen [24]. Harabin et al. [25] studied the effect of HBO on rats and guinea pigs subjected to a pressure of 2.8 ATA, as well as continuous and intermittent oxygen therapy (the latter consisting of 10-minute oxygen therapy sessions separated by 2.5-minute breaks during which the tested animals breathed air at a pressure of 2.8 ATA). The group demonstrated increased SOD activity in the lungs, and reduced CAT and GPx activity in the brains of both guinea pigs and rats subjected to continuous and intermittent oxygen therapy. They also showed that oxidative stress was less intense in animals in which the 2.5-minute breaks were introduced. In our own study, the periods of breathing 100% oxygen were separated by 5-minute breaks during which the subjects breathed atmospheric air, which could affect the obtained results.

Catalase is another enzyme of antioxidant defence in the human body, preventing the generation of hydrogen peroxide (H_2O_2) by catalysing the reaction of disproportionation into water and oxygen [34]. H_2O_2, due to its oxidizing action, is highly toxic to cells. In this study, a statistically significant decrease in the catalase activity was demonstrated in pooled subjects ($p < 0.01$) and group I ($p < 0.05$). No statistically significant changes in the activity of this enzyme were found in group II, apart from only a tendency for reduced activity. The currently presented results confirm those from a previous study by Paprocki et al. [21] in which a statistically significant decrease in the activity of CAT in subjects treated with HBO for the first time was observed. In turn, Benedetti et al. [26] showed that the activity of this enzyme increases immediately after HBO. Changes presented in this paper do not confirm that observation, with only a statistically insignificant increasing tendency in the CAT activity demonstrated in group II. It may be associated with the age of the subjects.

Liu et al. [27] suggested that the decreased catalase activity is a result of reaction between a metal and the substrate–enzyme complex, or blocked catalytic activity of catalase. Another study of the oxidant–antioxidant equilibrium in people exposed to HBO was conducted in 14 healthy non-smokers aged 25–30 years, and did not show any statistically significant changes in the CAT and SOD activity [28]. The results of that study do not confirm the results obtained in the authors' own study. It can be explained by the glucocorticosteroid pharmacotherapy that was administered to the subjects before and during the experiments.

Glutathione peroxidase catalyses the reaction between H_2O_2 and glutathione, and thereby prevents the Fenton reaction [16]. Thus, the reaction catalysed by GPx protects the body from the harmful action of ROS. Moreover, it should be remembered that GPx protects against the process of lipid peroxidation [16].

Glutathione peroxidase reduces lipid peroxides to alcohol [16,17]. In the authors' own study, no statistically significant changes in the activity of GPx in pooled subjects and in each group were observed. Similarly, an earlier study by Paprocki et al. [21] did not show any statistically significant changes in the activity of this enzyme in pooled subjects, but in turn it showed a significant increase in the activity of the enzyme in people repeatedly treated with HBO. In another study, the effect of HBO on the course of experimentally induced acute pancreatitis in laboratory rats was assessed.

Some of the rats were treated with HBO, while the control group was not treated in such a way. At the end of the experiment, the activities of SOD and GPx, and the concentration of MDA were determined in blood cell lysates. In animals treated with HBO, a statistically significant decrease in the concentration of MDA, as well as increased activities of GPx and SOD were seen [29]. Further studies in animals in which the activity of GPx and the concentration of TBARS were measured were conducted in rats after skin grafts. Forty *Sprague Dawley* rats after skin grafts were divided into two groups.

Group I (28 rats) was subjected to HBO at a pressure of 2 ATA twice per day for 28 days. Group II (21 rats) consisted of rats not subjected to such a treatment. The conducted experiment allowed observation of a statistically significant increase in the activity of oxidative stress markers in both groups, but in the group treated with HBO that increase was much larger compared to the control group without HBO. The observed phenomenon of intensification of lipid peroxidation in the grafted skin may raise concerns about the safety of HBO in people subjected to organ transplantation [30]. Another experiment showed generation of ROS and an increase in lipid peroxidation in the nervous tissue of experimental animals treated with HBO.

The examination was conducted in *Sprague Dawley* rats treated with 100% oxygen therapy for 2 hours at a pressure of 1, 1.5, 2, 2.5 and 3 ATA. At the end of the experiment, it was found that the pressure increase was accompanied by a proportional increase in the TBARS concentration and the SOD activity in the nervous tissue [23]. In another study, the concentration of MDA was assessed in male *New Zealand* rabbits experimentally subjected to complete brain ischaemia. Some of the animals after inducing ischaemia were exposed to HBO for 75 min at a pressure of 2.8 ATA, while other rabbits constituted the control group. In the brains of animals after HBO, higher values for MDA and GPx were found in comparison with the group not treated with HBO. In turn, the cortical somatosensory potentials in the rabbits treated with HBO were 50% higher than in the control group [31]. This study confirms the beneficial effect of HBO on the functioning of the brain after cerebral ischaemia.

The obtained results of the authors' own study and literature analysis suggest that the effect of HBO on oxidation–reduction processes has not been clearly explained and may depend on many factors.

CONCLUSIONS

1. A single exposure to hyperbaric oxygen affects the oxidant–antioxidant equilibrium as evidenced by, e.g., a statistically significant decrease in the activity of catalase in erythrocytes.
2. The antioxidant response to HBO may depend on the age of the subjects.

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