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Analysis of antioxidant enzyme activity and magnesium level in chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a respiratory pathology characterised by slow and irreversible decrease in values of one-second forced expiratory volume (FEV1). It includes both chronic bronchitis and emphysema, progressing gradually to chronic pulmonary insufficiency. In COPD there exists an imbalance between oxidant agents and antioxidant cellular defence mechanisms, the so-called oxidative stress, which leads gradually to alveolar damage in this disease (7-10,12,13,16).

Reactive oxygen species (ROS) are chemical compounds of oxygen that contain one or more unpaired electrons in their structure. They take part in oxidation and reduction, easily set up chain oxidising reactions which cause many pathological changes in cells. They are involved, for example, in peroxidation of cell membrane lipids, in protein function change or intracellular DNA damage (5,7-9). Normally, cells are equipped with many protective mechanisms against those processes. One of them are antioxidant enzymes which take part in metabolisation of free radicals. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) that we measured in our work, are one of them. A big role is also played by low-molecular-mass agents, such as vitamine C, E and glutathione which neutralise free radicals. Intracellular ion metal sequestration is very important, as well. It has been also suggested recently that magnesium be involved in antioxidant cellular defence. Its deficiency predisposes to increased production of reactive oxygen species (1,6).

In a haemostasis there is always a balance between free oxygen species and antioxidant defence systems. In pathological conditions, however, this balance is being destroyed, which leads to the oxidative stress (7,12,13,16).

The importance of oxidative stress in pathology has been proven in such diseases as adult respiratory distress syndrome (ARDS), asthma, emphysema and chronic bronchitis (10,12,13,16).

This work was aimed to examine antioxidant status of erythrocytes in patients with COPD by measuring 3 main antioxidant enzymes: superoxide dismutase (SOD, E.C. 1.15.1.1.), catalase (CAT, E.C. 1.11.1.6) and glutathione peroxide (GTX, E.C. 1.11.1.9). Simultaneously, the serum level of magnesium was examined in patients with COPD in order to assess its possible role in the disease.

MATERIAL AND METHODS

The work was done on the material obtained from outpatients of the Department of Pulmonary Diseases and Tuberculosis. The study was approved by the Ethical Committee of the Medical University of Lublin and all patients gave their formal consent. The control group consisted of 14 men and 6 women at the age of 30 to 60 years who at the time of collection of blood samples for the research, underwent routine blood control examinations. None of them had a history of a chronic disease or a current infection. The lung function tests were normal. The examined group consisted of 41 persons – 24 men and 17 women at the age of 45 to 75 years. According to the results of spirometry and gasometry the patients were divided into 2 groups: patients with mild and severe type of COPD. The patients with FEV1 >= 70 % and pO2 >= 60 mmHg were qualified to the mild type group of COPD, whereas the severe type group of COPD included patients with results of FEV1 < 70 % and pO2 < 60 mmHg.

Antioxidant enzymes were measured in erythrocytes which had been previously rinsed 3 times with the physiologic salt solution.

SOD activity was measured using the spectrophotometric method (14). In this method, the inhibition of the cytochrome c reduction rate is monitored at 550 nm at 25°C, utilising the xanthine/xanthine oxidase system as the source of O_2^-. SOD competes for superoxide and decreases the reduction rate of cytochrome c. One unit of SOD activity was defined as the amount of enzyme that inhibits by 50% the rate of cytochrome c reduction.

GPX activity was measured by following the rate of oxidation of the reduced form of glutathione, which was similar to the method previously reported (15). The formation of oxidised glutathione was monitored by a decrease in the concentration of NADPH, measured at 340 nm and 37°C, caused by the addition of glutathione reductase to the reaction mixture.

CAT activity was measured using the method reported by A e b i (2). This method uses the change in absorbance at 240 nm and 25°C of a solution of 10 mM H_2O_2 in 50 mM phosphate buffer, pH 7.0. The decrease in absorbance per unit time is a measure of the catalase activity. The plasma level of magnesium was assessed by the colorimetric method (Randox kit). Protein concentration was assessed by the Lowry method (11). All enzyme activities were reported as unit per mg of protein.

Statistical analyses and presentation of results: Data were expressed as the means ± standard deviation. Differences between groups were analysed by t-Student's test and by ANOVA. p value <0.05 was considered to be significant.

RESULTS

The increased level of SOD in the group of severe COPD was found. It was 12.4% higher than in the control group (Fig.1), which did not make the statistically important difference, however (p=0.079). The activities of CAT and GPX decreased by 16 and 22.4%, accordingly, in patients with severe type of COPD (Fig. 2,3). The decrease in CAT activity turned out to be of a statistical importance with p=0.045, whereas the activity of GPX did not show statistically important difference (p=0.08). The observed changes in antioxidant enzymes activity depended on the severity of the disease – they were bigger in the group of patients with severe COPD. Magnesium level was dependent on the severity of the disease, as well. It was much lower in the COPD group compared to that of the control group. Although in none of the COPD group the decrease was statistically important, the percentage of patients with low level of magnesium set at < 0.7 mmol/l (which is a border value of hypomagnaesemia) was 3.5 times bigger in patients with severe COPD compared to that of the control group. The numbers were 35 and 10%, accordingly.

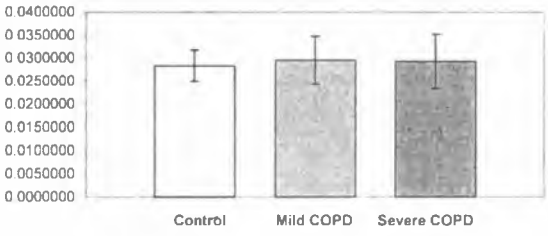

Fig. 1. SOD activity in COPD (U/mg)

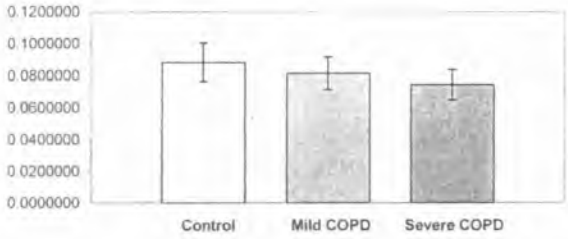

Fig. 2. CAT activity in COPD (U/mg)

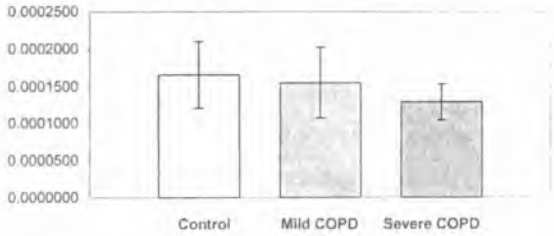

Fig. 3. GPX activity in COPD (U/mg)

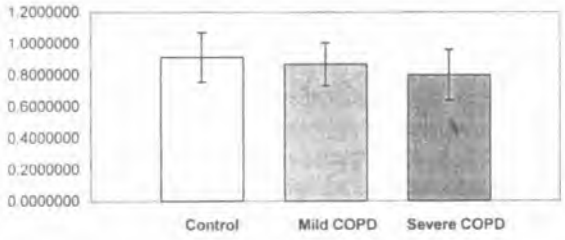

Fig. 4. Magnesium level in COPD (mmol/L)

DISCUSSION

The results of the experiment show the existence of imbalance in the antioxidant status in patients with COPD. The disease is associated with the increased production of reactive oxygen species and changes in the level of antioxidant enzymes prove the existence of cellular oxidative stress in this disease.

The existence of different tendencies in the activity of SOD, CAT and GPX is a disadvantage. Increased level of SOD activity (although not statistically important) may indicate an increase in the production of hydrogen peroxide. At the same time, the drop of CAT and GPX activities proves the inefficiency of utilisation processes of hydrogen peroxide. Its high level has been proven earlier in patients with COPD by other authors (3,4).

The released oxidants can directly damage the lung wall, making its extracellular matrix weaker, or act indirectly through inactivation of alpha1-antitripsin and other protease inhibitors. Inactivation of protease inhibitors makes it easier for the protease to destroy the lung wall, thus enhancing the possibility of emphysema and further on COPD (10).

The measured activity of antioxidant enzymes may be influenced both by genetic and environmental factors. It has been shown previously that various chemicals present in the environment can change the expression and activity of an enzyme through induction and competitive inhibition (10).

Hydrogen peroxide moves freely between subcellular structures initiating its action directly on them. The effect of its action may be, for instance, inactivation of enzymes or damage to DNA (5). Hydrogen peroxide may also generate a more toxic hydroxyl radical in reactions with transition ions (8).

The existence of the lowered level of magnesium in the examined group suggests its role in the theory of ROS caused destruction in COPD. It has been showed in the previous literature that hypomagnaesemia is a condition leading to increased production of ROS (6). This fact can be used in clinical practise by increasing the intake of magnesium in patients with COPD.

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SUMMARY

There is an increasing interest in the relationship of hypomagnesemia and oxidative stress in pulmonary diseases. It seems to be clinically relevant to assess prooxidant/antioxidant balance and its correlation with magnesium level in COPD.

In this study, there were investigated the antioxidant enzymes activity and magnesium plasma level in a group of patients with chronic obstructive pulmonary disease and in the control group.

The study group consisted of 41 patients admitted to hospital for ambulatory medical treatment in pulmonary department of the Medical University of Lublin. The control group was made up of 20 patients who were admitted for control examinations.

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activity were assessed in red blood cells using kinetics methods. Significantly decreased activity of CAT in COPD, unsignificantly decreased activity of GPX and unsignificantly increased activity of SOD were detected. Patients with COPD also showed the lowered plasma magnesium level.

The conclusion is that COPD is accompanied by a lowered magnesium level and an alteration in antioxidant status due to possible oxidative stress in this disease.

Analiza aktywności enzymów antyoksydacyjnych i poziomu magnezu we krwi osób chorych na przewlekłą obturacyjną chorobę płuc (POChP)

Przewlekłą obturacyjną chorobą płuc charakteryzuje uszkodzenie struktur pęcherzykowych dolnego układu oddechowego, spowodowane, jak się wydaje, tzw. stresem oksydacyjnym, czyli zaburzoną równowagą pomiędzy czynnikami utleniającymi a mechanizmami obrony komórkowej.

Celem pracy była ocena stanu antyoksydacyjnego i poziomu magnezu w tej chorobie. Badaną grupę stanowiło 41 pacjentów leczonych ambulatoryjnie w Klinice Chorób Płuc i Gruźlicy Akademii Medycznej w Lublinie. Grupa kontrolna obejmowała 20 zdrowych osób, u których pobrano krew przy okazji okresowych badań kontrolnych. Oceniono aktywność trzech kluczowych enzymów antyoksydacyjnych: dysmutazy ponadtlenkowej (SOD), katalazy (CAT) i peroksydazy glutationowej (GPX) w czerwonych krwinkach oraz poziom magnezu w surowicy krwi.

Wykazano statystycznie istotne zmniejszenie aktywności katalazy, zależne od stopnia ciężkości choroby. Aktywność pozostałych enzymów była również zależna od ciężkości choroby – aktywność SOD wzrastała u osób chorych, zaś aktywność GPX zmniejszała się, jednakże nie były to zmiany istotne statystycznie.

U pacjentów z POChP występowały częściej niedobory magnezu.