EVALUATION OF RED BLOOD CELL SUPEROXIDE DISMUTASE, CATALASE, ZINC AND COPPER, AND PLASMA ZINC AND COPPER LEVELS IN MICE EXPOSED TO DARK INDUCED STRESS

FULYA TEKSEN, ALI BILGILI1, PELIN ARIBAL KOCATURK2, AND LEVENT ALTINTAS1

Basic Health Sciences, Biotechnology Institute and Faculty of Health Education, Ankara University, 06290, Kecioren, Ankara, Turkey
1Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, 06110, Diskapi, Ankara, Turkey
2Department of Physiopathology, Ankara University, School of Medicine, 06100, Sihhiye, Ankara, Turkey
fteksen@hotmail.com

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Abstract

The effects of dark induced stress on some antioxidant enzymes and their cofactors were evaluated. The study was performed on 280 male mice. Measurements for red blood cell copper/zinc-superoxide dismutase (Cu/Zn-SOD) and catalase activities, as well as plasma and red blood cell zinc and copper concentrations were taken. Mice exposed to dark induced stress are susceptible to antioxidant enzyme activities such as Cu/Zn-SOD, catalase (CAT), plasma and red cell zinc and copper, and were significantly different (P<0.001) compared with the values for the control group.

Key words: mice, dark induced stress, superoxide dismutase, catalase, zinc, copper.

As oxygen has invaded progressively to the anaerobic world, living organisms have invented defence systems and has thoroughly adapted to a 21% of atmospheric oxygen in the course of evolution (19). Reactive oxygen species (ROS) are produced in the human body in both healthy and diseased states. In healthy bodies, they may arise as regulatory mechanisms, such as intercellular signalling species (3) or bactericidal agents (8). The production is normally controlled by antioxidant defence mechanisms that include enzymes, mainly glutathione peroxidase, and reductase, superoxide dismutase, catalase, and glucose-6-phosphate dehydrogenase with their substrates, such as vitamins E and C, flavonoids, selenium, zinc, copper, and reduced glutathione (2). If there is a marked imbalance between the production and removal of reactive oxygen, then oxidative stress arises (8, 19).

Material and Methods

The control group was composed of 56 mice and the experimental group was composed of 224 mice. All the mice were male with their weight ranged from 30 to 35 g, and their age around 12-14 weeks. The mice from both the study and control groups were fed the same diet for 10 days before being exposed to darkness. Red blood cell (RBO) Cu/Zn-SOD, and CAT activities, also plasma RBO zinc and copper concentrations were determined on days 15, 30, 45, and 60 after exposure to darkness.

Blood samples of the control and experimental groups were collected every morning at the same time, into heparinized tubes. Plasma was kept in polypropylene tubes; and each sample was studied daily.
Cu/Zn-SOD and CAT activities were determined spectrophotometrically (1, 20). The Zn and Cu concentrations were determined by atomic absorption spectrophotometry (16, 18).

The mice were exposed to the darkness, and the blood was collected at the Pharmacology and Toxicology Laboratories, plus all the biochemical measurements were performed at the Physiopathology Department Laboratories.

For statistical analysis of data, variance analysis and Duncan tests were used where appropriate.

**Results**

All red blood cell Cu/Zn-SOD, CAT activities, plasma, red blood cell Zn, and Cu concentrations of the experimental group were significantly different (P<0.001) from the control group as shown in Table 1.

Cu/Zn-SOD enzyme activity started to increase, as the effects of darkness started to take hold and reached the highest level on the 30th d (5.275.5±272.6 U/gHb) of exposure, and then slightly decreased on the 45th (5001.2±307.5 U/gHb) and 60th d (4818.7±282.1 U/gHb). In contrast, the activity of CAT enzyme was at its lowest level on the 30th d (162.23±2.0 k/gHb) of dark exposure, and then increased on the 45th d (165.27±1.8 k/gHb), and again decreased on the 60th d (162.5±2.9 k/gHb) of darkness.

Red blood cell Zn concentration levels were higher on the 15th d (6.3±0.4 µg/mL) but then decreased to 4.6±0.3 µg/mL on the 30th d, and then again increased to (5.6±0.5 µg/dL) on the 45th d of dark exposure. Finally, the level decreased to its lowest level of (4.1±0.3 µg/mL) on the 60th d of dark exposure.

On the other hand, plasma Zn concentration in the experimental group was found to decrease on the 15th d (90.27±7.2 µg/dL), and then increased to the highest concentration (121.1±9.8 µg/dL) on the 30th d of dark induced stress. Finally, it decreased to 102.0±9.3 µg/dL and 88.8±12.0 µg/dL on the 45th and 60th d, respectively.

In case of other Cu/Zn-SOD cofactor Cu levels, as observed in Zn concentrations, the RBO concentration was also high on the 15th d (0.3±4.7 µg/dL) of dark exposure, but then decreased on the 30th (0.2±7.5 µg/dL), 45th (0.1±6.1 µg/dL), and 60th (0.1±5.1 µg/dL) d of exposure.

Plasma copper concentration was low (50.2±7.5 µg/dL) at 15th d of exposure and then started to increase on the 30th d (58.2±4.0 µg/dL) and reached its highest level on the 45th d (84.3±8.4 µg/dL). Finally, the level decreased to the lowest level of (43.5±4.2 µg/dL) on the 60th d of dark exposure.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Enzyme activity and levels of trace elements in plasma (P) and erythrocytes (E) of experimental and control groups of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>E SOD (U/gHb)</td>
<td>Control</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 15</td>
</tr>
<tr>
<td>4393.7±163.8a</td>
<td>4767.1±173.4b</td>
</tr>
<tr>
<td>E CAT (k/gHb)</td>
<td>165.9±1.2a</td>
</tr>
<tr>
<td>P Zn (µg/dL)</td>
<td>111.2±5.6a</td>
</tr>
<tr>
<td>P Cu (µg/dL)</td>
<td>66.2±8.9a</td>
</tr>
<tr>
<td>E Zn (µg/mL)</td>
<td>5.57±0.3a</td>
</tr>
<tr>
<td>E Cu (µg/mL)</td>
<td>0.2±3.5a</td>
</tr>
</tbody>
</table>

Means the same line with different superscripts differ significantly ** P<0.01, *** P<0.001.

a,b,c: Differences between the values of the same line marked with different letter are significant.
Discussion

Stress is an important factor for today’s society, especially for individuals living in very difficult conditions; and these factors can lead to many kinds of diseases acting in many different ways. On the other hand, it has been shown that the organisms in stress produce more free oxygen radicals (10). The gene encoding Cu/Zn-SOD (Sod Cc2) was induced by environmental stress factors (10) and in eliminating these radicals, which cause great damage in the tissue; SOD takes place and is the first step, as observed in our study.

In an examination of stress conditions for plant cells, it was also observed that the Cu/Zn-SOD gene quickly responded to salinity treatment in light, but not in the dark. It was suggested that phytohormone and active oxygen species (AOS’s) are associated with the regulation of SOD genes under specific environmental stresses (10), one of them being under the exposure of darkness.

It was again reported that long-action protection of DNA from radiation might be provided by antioxidants and healthy functioning of the DNA repair systems. The Cu/Zn-SOD enzyme takes place in the DNA repair system by the elimination of breakage factor from plasma of radiation exposed people (17).

It has also been well established that antioxidant enzymes are necessary for the survival of the cell even under normal conditions, and it was shown that Cu/Zn-SOD and CAT enzymes in addition with selenium-glutathione peroxidase, acted in a co-operative or synergistic way to ensure a cell’s protection (14). Similarly, this was observed in our study. In our experimental group, the Cu/Zn-SOD and CAT enzymes seemed to be acting in co-operative way. Firstly, the Cu/Zn-SOD enzyme in red blood cells responded to the stress factor, and the activity of the enzyme increased significantly, then it decreased and an increase in catalase enzyme activity was observed.

In a study on biorhythm and circadian variation in rats, it was reported that oxidative stress was higher in the early stages of darkness (12) these are similar to our results. Hence, the increase in red blood cell Cu/Zn-SOD activities on days 15 and 30, suggested the elevated dismutation of oxygen and significant increase in Cu/Zn-SOD activities was a compensatory mechanism against excess oxygen.

Catalase is another antioxidant enzyme that protects the cells in aerobic organisms, by catalysing the rapid decomposition of hydrogen peroxide (2). In a study, Jansens et al. reported that CAT activity did not change in fish, due to their metabolic activity or the depth of sea that they live in (9).

On the other hand, in patients with septic shock, they found higher levels of plasma and red blood cell SOD and CAT enzyme activities (11), and in another study, high catalase activity was observed in patients having diseases related to oxidative stress (2).

In our study, in exposure to dark induced stress, the catalase enzyme activity was at the lowest on the 15th and 30th d, but on the 45th d it reached its highest level of activity. On the 30th d, red blood cell Cu/Zn-SOD activity peaked, but catalase activity was at its lowest level, presenting a reverse correlation to the activity of Cu/Zn-SOD.

Trace elements, namely Zn and Cu, have important roles in red blood cell Cu/Zn-SOD enzyme activity, being cofactors of the enzyme. Hence, due to changes in the enzyme activities, corresponding alterations in Zn and Cu concentrations were observed, as expected, in our study. Zn and Cu concentrations in plasma and red blood cell, showed a very concordant pattern with the Cu/Zn-SOD enzyme activity. Therefore, on the 15th d of dark exposure, while the Cu/Zn-SOD levels were increasing, the concentration of Zn in the red blood cells was also high, contrastingly, for the plasma it was at the lowest level during the same time, showing the usage of Zn in the plasma firstly and red blood cells later on. Additionally, there was a direct correlation between the red blood cell Cu/Zn-SOD activity, where it peaked on the 30th d, and concentrations of Zn in red blood cells and plasma on the same day. This time, Zn concentration was at a low level in red blood cells, but higher in the plasma. A similar pattern for the Cu concentration was observed. When the first deposits in the plasma were consumed, then Cu in the red blood cells was used. On the 60th d of exposure, both the antioxidant enzymes and trace element levels decreased.

Couinaud (5) reported that Zn deficiency in the body was increased during stress. Another investigator found that cold stress induced an efflux of Zn from plasma (15).

Levy et al. (13) reported that Cu/Zn-SOD expression had a marked influence on circulating Cu homeostasis, indicating that the enzyme functions in Cu homeostasis via mechanisms distinct from its superoxide scavenging properties.

The trace element changes could be explained by the increased levels of activity for Cu/Zn-SOD and CAT enzymes against the oxidative stress radicals, and at the same time by the increased utilisation of Zn and Cu in the plasma firstly. Meanwhile, it was thought that the levels of Cu and Zn were raised in the plasma in order to compensate this situation.

Finally, it was observed that in mice exposed to dark induced stress, the antioxidant defence enzymes start to respond with Cu/Zn-SOD enzyme, and then the response continued with CAT enzyme and that during this time the cofactors of the enzymes, namely Zn with Cu, found in red blood cell along with plasma, were used accordingly.

This was concluded with the results that red blood cell Cu/Zn-SOD, CAT enzyme activities, and concentrations of the cofactors Zn and Cu were found to be significantly affected when compared to control group, and it will be of great value to plan more coherent studies on the subject in the future, in order to explain the pathophysiology of the antioxidant mechanism in more detail.
References


