PROTECTIVE EFFECT OF VITAMIN E ON THE OXIDATIVE DAMAGE CAUSED BY ACUTE SODIUM NITRITE INTOXICATION*

Onur ATAKIŞI**

Ayla ÖZCAN**

Geliş Tarihi : 01.11.2002

Summary: The purpose of this study is to determine possible changes in the antioxidant systems of rabbits caused by acute sodium nitrite intoxication and to examine the role of vitamin E as an antioxidant to prevent the damage. Twenty male rabbits were used as material. In order to determine control values blood samples were taken from 10 rabbits before the experiment started. Rabbits were divided into two groups as experiment and treatment. Sodium nitrite at the dose of 60mg/kg was given to the experiment group subcutaneously (SC), and 60mg/kg sodium nitrite (SC) followed by 100mg/kg tocopherol (IP) was administered to the treatment group. Blood samples were collected at 1,12 and 24 hours post injection. GSH, MDA, total protein and glucose levels were measured colorimetrically. It was observed that the level of GSH in the experiment group at 12 hours post the injection was lower than the control group while MDA level was higher. This differences were statistically significant (p<0.01).

Key Words: Sodium nitrite, Free radical, Nitric oxide, Vitamin E

Sodyum Nitrit ile Akut Zehirlenme Sonucunda Olusan Oksidatif Hasar Üzerine E Vitaminiinin Koruyucu Etkisi

Özet: Bu çalışmanın amacı, akut nitrit zehirlenmesi sonucunda oluşan tavanada antioksidan sistemlerde meydana gelebilecek değişiklikleri saptamak ve bu hassas önemlindedir bir antioksidan olan E vitamininin rolünü araştırmaktır. %60 olarak 20 adet erkek tavşan kullanılmış. Denemeden önce kontrol değerlerinin saptanması için 10 tavşanın kan numuneleri alınmıştır. Yırtmağın tavan denemeleri ve tedavi olmak üzere 10'arla 2 gruba ayrılmıştır. Deneme grubuna 60mg/kg dozda sodyum nitrit (SC), tedavi grubuna da 60mg/kg dozda sodyum nitrit (SC) + 100mg/kg dozda α-tokolferol (IP) uygulandı. Enjeksiyondan 1, 12 ve 24 saat sonra kan numuneleri toplanmıştır. GSH, MDA, glutok ile total protein düzeyleri kloromericik yöntemle ölçüldü.

Enjeksiyondan 12 saat sonra deneme grubundan alınan kan örneklerinde GSH düzeyi kontrol grubuna göre istatistiksel olarak önemli şekilde düşük (p<0.01), MDA düzeyi istatistiksel olarak önemli şekilde yüksek (p<0.01) olarak saptandı.

Anahtar Sözcüklər: Sodyum nitrit, serbest radikal, nitrık oksid, E vitaminı.

INTRODUCTION

There is such a perfect balance in nature and organism that all mechanisms are arranged in a perfect order. For example, oxygen necessary for life, if increases by 20% forms reactive oxygen species (ROS) which is then toxic and increases production of free radicals. In recent years, studies have shown that stress is very effective in the formation of free radical and thus brings about ageing, infertility unwanted cell death, alzheimer's disease, cancer and diabetes. Nitrite and nitrate found in well water and processed meat as protective agent cause oxidation and cancer in consumer and also release nitric oxide (NO) from their molecules which is then transferred to peroxy nitrite, a reactive molecule playing an important role in the pathology of various diseases as well. Lipid peroxidation is a chain reaction which provides a source for the free radicals that start further peroxidation and cause irreversible membrane injury.

It is reported that oxidant materials cause decarboxylation in proteins, hydrolysis in peptide bonds, damage in hem albumine. A decrease of glycolitic ATP synthesis and an increase in the use of ATP through its effect on carbohydrate metabolism. In a number of research, it was demonstrated that stress factors increase the level of malondialdehyde (MDA) which is the product of lipid peroxidation and decrease the level of reduced glutathion (GSH). Glutathion, endogen an antioxidant reacts, with free radicals and peroxides, which results in protection of cells against the oxidative damage and prevents oxidation by keeping -SH groups at reduction.

When produced to outperform the defence mechanisms, free radicals, which are formed as a result of oxidative, xenobiotic, enzymatic and nonenzymatic reactions, cause various defects in organism. They mostly effect lipid, protein, DNA, enzyme, carbohydrate and important compounds of all these as well. It is reported that exogen antioxidants such as vitamin E, C, tiols, carotinoids, protect the body against tissue and macromolecule (DNA, protein, lipoprotein) damages caused by the harmful effect of free radicals and enhance the antioxidant defence system of the cell. Experimental data shows that the
use of antioxidants which demolishes or decreases the effect of free radicals probably prolongs the life expectancy.\textsuperscript{7,17-20}

The aim of this study was to determine the changes which may occur in the antioxidant systems as a result of acute nitrite intoxication in rabbits as well as the role of vitamin E, which prevents this oxidative damage as an antioxidant.

**MATERIALS and METHODS**

In this study, 20 rabbits approximately, 8-9 months old, were used, as material. Animals were dewormed before the experiment started. The rabbits were kept in a place at room temperature (18-22°C) for adaptation. All the animals were fed by commercial rabbit feed and water was provided ad libitum. In order to obtain the control values, blood samples were taken into EDTA coated tubes through the ear vena of the rabbits which were left hungry before the experiment. After two days, rabbits were divided into two groups, formed of 10 rabbits each, as experiment and treatment groups. They were given number cards and weighted individually.

First group were injected sodium nitrite at the dose of 60mg/kg, and the second were injected the same amount of sodium nitrite and α-tocopherol (IP) at the dose of 100mg/kg. After the injection (1, 12 and 24 hours), the blood samples were taken from both groups, and GSH analysis was made by using Beutler method\textsuperscript{21} and MDA analysis by means of thiobarbituric acid method\textsuperscript{22}. Serum were held at -25°C until the analysis, to measure the total protein and glucose concentration. Those parameters were measured by means of commercial kits spectrophotometrically.

The statistical analysis of results were performed by student’s t test.

**RESULTS**

Levels of the GSH, MDA, glucose and total protein in the groups (control, experiment and treatment) were displayed in Table 1.

When compared with the control group, GSH concentration at 12 hours post injection in experimental group, was low (p<0.01), and MDA level was high (p<0.01). The differences in GSH and MDA levels, taken at 1 and 24 hours of experiment and treatment groups were statistically in significant. The changes of the total protein and glucose in the experiment and treatment groups were not statistically important.

| Table 1. Change of GSH, MDA, glucose and total protein levels. |
|------------------|------------------|------------------|------------------|
|                  | GSH (mg/dl)      | MDA (μmol/L)     | Glucose (mg/dl/L)| T. Protein (g/dL) |
|                  | $\bar{x}±S_x$    | $\bar{x}±S_x$    | $\bar{x}±S_x$    | $\bar{x}±S_x$     |
| Control          | 37.01±1.83       | 5.38±0.46        | 106.4±2.15       | 5.38±0.46         |
| Experiment       |                  |                  |                  |                  |
| 1 hour           | 36.21±2.15       | 5.78±0.22        | 108.0±2.77       | 6.0±0.09          |
| 12 hour          | 31.70±1.23       | 7.39±0.54        | 113.4±3.71       | 5.84±0.16         |
| 24 hour          | 38.21±1.68       | 5.9±0.45         | 113.0±2.48       | 5.90±0.13         |
| Treatment        |                  |                  |                  |                  |
| 1 hour           | 39.30±2.15       | 5.48±0.26        | 107.3±3.21       | 6.16±0.11         |
| 12 hour          | 39.76±2.83       | 5.90±0.28        | 111.3±3.21       | 6.01±0.17         |
| 24 hour          | 41.01±2.08       | 5.47±0.39        | 112.5±3.08       | 6.04±0.09         |

n: animal number  *p<0.01

**DISCUSSION**

Peroxynitrite is formed by the reaction between $\cdot O_2$ and NO released by nitrite and nitrate compounds taken from diet. Peroxynitrite, a very reactive molecule, is very important in the pathologizing of various diseases. ROS, occurring in the tissues, may give harm to DNA, carbohydrate and lipids. Preoxidants and free radicals are controlled by enzymatic and non enzymatic antioxidants. Glutathione is an important substrate in the functions of enzymatic antioxidants. Relation between tocopherol, abundant in biological membrane, and the most effective cleaner of hydroxyl radical, and tiols and other compounds, increase the cellular antioxidant defence.\textsuperscript{7,11,14,19}

The parameters, investigated after 1 and 24 hours of the injection of sodium nitrite, didn’t show any important changes when compared with the control groups. It has been thought that sodium nitrite does not form any oxidative effect within an hour, and that oxidative stress is restrained by antioxidants 24 hour later.

Sireli and his colleagues\textsuperscript{23} have studied the effect of acute nitrite poisoning, the formation of methemoglobin and hemolytic anemia in cavies. They reported that the amount of methemoglobin increases after an hour of the application of sodium nitrite, and a decrease in the amount hemoglobin. This was thought to be due to the oxidation of hemoglobin to methemoglobin by nitrite ions. In our study, GSH and MDA levels do not change after an hour of injection of sodium nitrite when compared with the controls. This may reflect a lack of oxidative effect of
sodium nitrite within an hour and may also be due to different species of animal used in our study34.

In this study, GSH level significantly decreased and MDA level significantly increased (p < 0.01) after 12 hours of application when compared with the control group. No important changes in GSH and MDA level were determined in the treatment group.

In rats intoxicated with carbon tetrachlor the concentration of MDA was found to be high and GSH level low4. In our study similar results were obtained. This results also show the tissue damage caused by sodium nitrite intoxication.

Rats treated with pesticide had high concentration of GSH and MDA in liver. It was thought that high GSH level in liver was due to changes in antioxidant system and high MDA level was a result of celluler damage caused by pesticide and its product, ROS. It was also noted that in animals given vitamin E, GSH and MDA levels returned to the normal range, this was attributed to the protective effect of vitamin E35. In this study, there was an increase in MDA level and a decrease in GSH. The values in the treatment group were found close to that in the control group. Our results also indicated the prevention of oxidative stress by vitamin E.

In a study carried out on the patients with hyperthyroidism the plasma MDA level was found to be high and the GSH level was low; yet it was normal in the group to which vitamin E was given. Therefore vitamin E was believed to prevent oxidative stress in hyperthyroidism36. A similar result was found in our study. The main target of ROS is the long chain unsaturated fat acids in membrane lipids, and ROS is thought to bring about the loss of the membrane structure and functions due to the lipid peroxidation.

In a study, by Park and his colleagues37, total glutathion in lungen of rats subjected to the cigarette smoke 3 times a day for 30 days was significantly low. They speculated that decreased glutathion may play a negative role in the protection of DNA and protein structure.

Ayakci et al.38 reported significant increase in the concentration of GSH and MDA in liver of rats ingested ethanol for long period. It is suggested that chronic ethanol consumption stimulates liver lipid peroxidation and as a result an increase in GSH synthesis in liver. In another study, in which rats were given ethanol39, it was reported that the increase in plasma and erythrocyte MDA levels may occur because of the stimulation of lipid peroxidation in liver. In our study, increased blood MDA levels may be the result of the stimulation of lipid peroxidation by sodium nitrite.

The effect of α- tocopherol upon oxidative damage formed with doxorubicin in human erythrocye was studied by Geetha et al.40 they found that the membrane damage decreased in the group to which α-tocopherol was given, and it was thought that this was the result of antioxidant characteristic of α-tocopherol.

In our study insignificant decrease in total protein and an increase in glucose concentration at 12 hours of experimental was determined.

Bourdon and his colleagues41 found that albumine molecule was the most effected compound by free radicals, and the low level of albumine diabetic patients was the result of oxidative stress in vascular complication.

In a study carried out upon 33 individuals to determine the effect of oral glucose tolerance test on erythrocyte GSH level, 2 hours after glucose intake, GSH level was found statistically low. Moreover, there was a reverse relationship between erythrocyte GSH, insulin and C-peptid. Furthermore, the glucose loading brings about an oxidative stress42. In our study, an insignificant increase in glucose level may be caused by the of sodium nitrite on carbohydrate metabolism in liver.

In conclusion in this study, sodium nitrite may be create oxidative damage, and vitamin E protects from this effect. It was also found that glucose and total protein levels are not good criterion to predict oxidative stress.

REFERENCES

6 Mayer B, Pfeiffer S: A new pathway of nitric oxidedicyclic