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Effect of vitamin B3 supplementation on glutathione redox cycle

Adem Keskin[1*]

[*1]*Aydın Adnan Menderes University, Institute of Health Sciences, Department of Biochemistry (Medicine), Aydın, Turkey*

*Corresponding author : ademkeskin78@gmail.com
Orcid No: https://orcid.org/0000-0003-1921-2583

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Abstract: In this study, the effect of vitamin B3 supplement given to rats was investigated on glutathione redox cycle by looking at glutathione peroxidase and glutathione s transferase activities. 20 Wistar albino male rats were used. Vitamin B3 supplement was given to one of the two groups that were formed. The other group was determined as the control group. 360 mg/kg/day vitamin B3 supplement was given by oral gavage method for 10 days. At the end of 10 days, intracardiac blood samples were taken. Glutathione peroxidase activity level was determined as 1033.44±198.05 U/L in the vitamin B3 supplement group and 526.00±99.54 U/L in the control group. The glutathione peroxidase activity level of the vitamin B3 supplemented group was found to be statistically significantly higher than the glutathione peroxidase activity level of the control group ($p<0.001$, $t=7.239$). This difference has huge impact size ($d=3.24$). Glutathione s transferase activity level was determined as 6.50±0.97 U/mL in the vitamin B3 supplement group and 7.50±0.67 U/mL in the control group. The glutathione s transferase activity level of the group given vitamin B3 supplementation was found to be statistically significantly lower than the glutathione s transferase activity level of the control group ($p=0.015$, $t=2.698$). This difference has a very large effect size ($d=1.20$). As a result, vitamin B3 supplementation led to an increase in glutathione peroxidase activity at a huge effect size and a decrease in glutathione s transferase activity at a very large effect size.

Keywords: Vitamin B3, Glutathione peroxidase, Glutathione s transferase, Glutathione redox cycle

1. Introduction

Glutathione is a simple sulfur compound that is non-protein and consists of three amino acids. The functions of glutathione are very diverse. In particular, redox and homeostatic buffering are in the foreground. Glutathione status is modulated by oxidants as well as nutrition and other factors. Changes in the thiol-disulfide balance can affect its structure and activity (Noctor et al. 2011).

Glutathione's functions include detoxifying electrophilic xenobiotics such as chemical carcinogens, environmental pollutants, and antitumor agents (Hayes et al. 2005). The classical activity of glutathione transferases is the conjugation of compounds with electrophilic centers to the tripeptide glutathione (Oakley 2011).

Selenium-containing glutathione peroxidase, which has an important role in redox reactions and is involved in the glutathione cycle, protects against oxidative damage, inhibits inflammation and oxidant-induced regulated cell death (Brigelius-Flohe and Flohe 2020). Oxidized glutathione is formed by glutathione peroxidase, which is involved in the redox cycle of glutathione. Glutathione reductase, which is involved in the conversion of this oxidized glutathione to reduced glutathione, is responsible for maintaining the reduced glutathione supply (Couto et al. 2016). Glutathione reductase catalyzes the reduction of glutathione disulfide, dependent on nicotinamide adenine dinucleotide phosphate, for the supply of reduced glutathione (Belorgey et al. 2013).

The functional cofactors derived from vitamin B3 are nicotinamide adenine dinucleotide (NAD^+), its phosphorylated form, nicotinamide adenine dinucleotide phosphate ($NADP^+$), and their reduced forms (NAD(P)H). These cofactors, called the NAD(P)(H) pool, are closely related in all major bioenergetic, anabolic and catabolic pathways in all life forms. This pool also contributes to post-translational protein modifications and second messenger generation (Makarov et al. 2019).

Vitamin B3 is the precursor of NADPH, a cofactor in the glutathione redox cycle, which has an important role in redox, homeostatic buffering and xenobiotic detoxification. In this study, the effect of vitamin B3 supplementation on the activities of glutathione peroxidase and glutathione transferase enzymes involved in the glutathione redox cycle was investigated.

2. Materials and Method

The study is qualitatively an experimental animal study. The ethics committee decision required for the study was obtained from the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University. In the study, 20 Wistar Albino male rats weighing between 380-465 grams were used. Two groups of ten were formed. One group was given a vitamin B3 supplement. The other group was determined as the control group.

2.1. Operations on rats

The study was planned as 10 days. During this time frame, one group was given a daily vitamin B3 supplement. Vitamin B3 was obtained from Sigma-Aldrich as a powder preparation of 100 grams. A solution was prepared from the powder preparation in order to give it to the rats by oral gavage method. Determination of solution dose; It was determined based on the study of Kwon et al. (2018). Each animal was weighed every day and 360 mg/kg/day vitamin B3 supplement was given by oral gavage method. Both groups exercised 30 minutes after vitamin B3 supplementation. The purpose of exercise was to activate the glutathione cycle more by increasing the oxidative stress level in rats. One day after the tenth day of vitamin B3 supplementation, blood samples were taken by intracardiac route.

2.2. Biochemical processes

Approximately seven ml of blood samples were taken intracardiacly and centrifuged at 3000 rpm for five minutes. Serum samples were taken. The method of Paglia and Valentina (1967) was used for glutathione peroxidase activity analysis. In this method, glutathione peroxidase activity is calculated by monitoring the decrease in the absorbance value of NADPH, which is oxidized per minute at a wavelength of 340 nm. The glutathione s transferase colorimetric analysis kit, which is the kit of the Elabscience brand, was used for glutathione s transferase activity analysis,. Biotek Epoch (Canada) device was used for these analyses.

2.3. Statistical analysis

SPSS for Windows 22.0 program was used for statistical analysis. Data obtained from the study were given as mean±standard deviation (X±SD), and P values below 0.05 were considered statistically significant. The groups were compared with an independent sample t-test. The effect sizes were calculated based on the t values obtained and the studies of Cohen (1988) and Sawilowsky (2009).

3. Results

In this study, glutathione peroxidase activity level was determined as 1033.44±198.05 U/L in the vitamin B3 supplement group and 526.00±99.54 U/L in the control group (Table 1). Glutathione peroxidase activity levels of the groups were compared with an independent sample t test. As a result of this comparison, the glutathione peroxidase activity level of the group given vitamin B3 supplementation was found to be statistically significantly higher than the glutathione peroxidase activity level of the control group ($p<0.001$ $t=7.239$). According to the effect size calculation based on Cohen (1988) and Sawilowsky (2009) studies on effect size, a significant difference with a huge effect size ($d=3.24$) was found between the groups.

In this study, glutathione s transferase activity level was determined as 6.50±0.97 U/mL in the vitamin B3 supplement group and 7.50±0.67 U/mL in the control group (Table 1). Glutathione s transferase activity levels of the groups were compared with independent sample t test. As a result of this comparison, the glutathione s transferase activity level of the vitamin B3 supplemented group was found to be statistically significantly lower than the glutathione s transferase activity level of the control group ($p=0.015$ $t=2.698$). According to the effect size calculation based on Cohen (1988) and Sawilowsky (2009) studies on effect size, a significant difference with a very large effect size ($d=1.20$) was found between the groups.

Table 1. Glutathione Peroxidase and Glutathione S Transferase enzyme activity level mean and standard deviations of the groups

Enzyme	Vitamin B3 Group	Control Group	p	d
Glutathione Peroxidase (U/L)	1033.44± 198.05	526.00± 99.54	<0.001	3.24
Glutathione S Transferase (U/mL)	6.50± 0.97	7.50± 0.67	0.015	1.20

4. Discussion

Vitamin B3 and its derivatives, nicotinamide adenine dinucleotides, play important roles in the cellular redox state, Ca^{+2} stores, DNA damage and repair, stress responses, cell cycle timing, and lipid and energy conservation and regulation. It is stated that the metabolism associated with them occupies a central place in the aging processes of mammals (Xu and Sauve 2010). One of these associated sites of metabolism is the glutathione redox cycle. Glutathione is at the center of the glutathione redox cycle, has a thiol tripeptide structure. Glutathione is found in almost every compartment of the cell, including the nucleus. Transport between different intracellular compartments is crucial for the regulation of cell proliferation (Vivancos et al. 2010).

Glutathione is involved in many other reactions such as glutathionylation of proteins, neutralization of superoxides, and detoxification of metabolites through conjugation (Bachhawat and Yadav 2018). The basic and earliest known function of glutathione is thiol-disulfide interactions, where it is converted back to reduced glutathione by NADPH-dependent glutathione reductase. The central role of glutathione in defense metabolism in animals has long been established as selenium-dependent glutathione peroxidase is a central pillar of animal antioxidant metabolism (Noctor et al. 2012).

Glutathione s transferase plays a multifunctional role in the detoxification mechanism, including the activation of

cytochrome P450 as well as the conjugation of phase two system oxidants (Jones et al. 2007).

Many studies have been conducted on the link between glutathione and vitamins. Liang et al. (1999) investigated the effect of vitamin B2 deficiency on glutathione levels in their study with rats. In this study, glutathione levels were found to be low in rats fed a diet devoid of vitamin B2 for 6 weeks. Another study with a diet deprived of vitamin B2 for 12 weeks found a decrease in glutathione levels (Huang et al. 2010). In a study conducted with rats infected with Trichinella spiralis, a decrease in glutathione peroxidase activity was found with vitamin B2 deficiency (Tumkiratiwong et al. 2003). A similar conclusion was reached in a another study on vitamin B2 and beta carotene, precursor of vitamin A (Shenhu et al. 1999). A review on vitamin B2 states that vitamin B2 has an antioxidant effect as a component of the glutathione redox cycle (Ashoori and Saedisomeolia 2014). In the study of Ponce et al. (2011), they concluded that the daily release of the glutathione redox cycle decreases in vitamin A deficiency. They stated that they found retinoid- as well as clock-responsive sites on regulatory regions of glutathione reductase and glutathione peroxidase genes. Vitamin D has also been found to increase glutathione in the brain by upregulating brain gamma-glutamyl transpeptidase, an enzyme involved in the glutathione cycle (Garcion et al. 2002). Van Haaften et al. (2003), stated that antioxidants such as vitamin E have protective effects on glutathione-dependent enzymes. In addition, it has been stated that vitamin E, together with glutathione and a heat-sensitive membrane-bound factor, can prevent the harmful effects of reactive oxygen species on polyunsaturated fatty acids in biomembranes. In the study of Waly et al. (2015), it was determined that the consumption of glutathione increased in healthy young people who were fed low in vitamin C, and as a result, it induced oxidative stress.

In our study, glutathione peroxidase activity was found to be significantly higher in the vitamin B3 given group compared to the control group. The effect size of this result has been found to be huge. This is due to NADPH, a derivative of vitamin B3. At the same time, NADPH is the cofactor of the glutathione reductase enzyme. In addition, glutathione reductase is an enzyme involved in the glutathione redox cycle. The Meyer-Ficca et al. (2016) study confirms this result. Meyer-Ficca et al. (2016) stated that there is a intimate connection between dietary vitamin B3 intake and resulting NAD concentrations. In a study conducted with rats, it was stated that retinal NAD levels decrease with aging and this causes a tendency to glaucoma. In the same study, it was stated that glaucoma did not develop with oral vitamin B3 administration (Williams et al. 2017). Lappas et al. (2011) found that nicotinamide (a vitamin B3 derivative) treatment of human placenta caused an increase in glutathione peroxidase gene expression. Overdose of acetaminophen causes severe oxidative stress. In a study with rats whose liver was damaged by acetaminophen, they determined the prophylactic and therapeutic effects of vitamin B3 (Mahmoud and Mahmoud 2016).

In our study, Glutathione s transferase activity was found to be significantly lower in the vitamin B3 given group compared to the control group. The effect size of this result has been found to be very large. This is because reduced glutathione is a substrate for both glutathione s transferase and glutathione peroxidase. Therefore, an increase in glutathione peroxidase enzyme activity at a huge effect size led to a very large effect size decrease in glutathione s transferase activity.

5. Conclusion

As a result, vitamin B3 supplementation in rats caused an increase in glutathione peroxidase activity at a huge effect size and a decrease in glutathione s transferase activity at a very large effect size. In cases where oxidative stress increases (exercise, infection, excessive drug use, etc.), glutathione levels decrease. In addition, glutathione levels decrease in old age and long-term vitamin deficiency. In these cases, vitamin B3 supplementation can be used to continue the Glutathione redox cycle in order to reduce the damage caused by oxidative stress.

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