

# Effects of reduced glutathione on nitric oxide level, total antioxidant and oxidant capacity and adenosine deaminase activity

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**Abstract.** – The aim of this study was to determine the effect of exogenous GSH, an important antioxidant containing thiol group, on total antioxidant capacity (TAC) and total oxidant capacity (TOC), adenosine deaminase (ADA), a significant indicator of cellular immunity, and nitric oxide (NO) concentrations in rabbits.

Sixteen healthy New Zealand rabbits were divided into 2 groups as control and GSH-treated group. Animals in control group received single intraperitoneal (ip) administration of 0.9% saline. Rabbits in GSH-treated group received reduced L-glutathione (10 mg/kg) (ip). Blood samples were taken from the marginal ear vein 0, 3, 6 and 12 hours after injection. Plasma TAC and NO levels were not statistically different between control and GSH group at 0 and 12 hours. Plasma TAC level was found to be significantly higher ( $p<0.05$ ) while NO level was found to be significantly lower ( $p<0.05$ ) in GSH treated group when compared to control group at 3 and 6 hours. Plasma TOC and ADA activity were not statistically different between control and GSH group during experiment.

In conclusion, exogenous GSH resulted in an alteration of TAC and NO but not TOC and ADA, so exogenous GSH may be a valuable enhancer of the antioxidant system.

## Key Words:

Total antioxidant capacity, Total oxidant capacity, Nitric oxide, Adenosine deaminase, Reduced glutathione, Rabbit.

## Introduction

Reduced glutathione (GSH) is synthesized by two sequential adenosine triphosphate (ATP)-dependent reactions. In the first reaction,  $\gamma$ -glutamylcysteine synthetase (EC 6.3.2.2,  $\gamma$ -GCS) catalyzes

the formation of  $\gamma$ -glutamylcysteine from l-glutamate and l-cysteine. In the second step, catalyzed by GSH synthetase (EC 6.3.2.3, GS), glycine is added to the C-terminal of  $\gamma$ -glutamylcysteine to form GSH<sup>1,2</sup>. The key function of GSH molecule is the maintenance of protein structure and function, the regulation of protein synthesis and degradation, the maintenance of immune function, protection against oxidative damage, and detoxification of reactive chemicals<sup>3,4</sup>. GSH molecule also plays role in immune function<sup>3</sup>.

Adenosine deaminase (ADA) is an essential enzyme of purine metabolism. ADA catalyzes the irreversible hydrolytic deamination of adenosine and 2-deoxyadenosine to inosine and 2-deoxyinosine, respectively<sup>5,6</sup>. ADA is necessary for lymphocyte proliferation and differentiation. Therefore, it is considered as an important immunoenzyme marker of cell mediated immunity<sup>7,8</sup>.

Nitric oxide (NO) is an endogenous reactive free radical, produced from the amino acid L-arginine by the enzymatic action of nitric oxide synthase (NOS)<sup>9</sup>. Although various NOS isoforms have been described, only three forms, inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS), have been described in detail<sup>10,11</sup>. NO is stabilized by carrier molecule like reduced thiol species that preserves its biological activity. Low molecular weight thiols such as cysteine, GSH, and penicillamine are prime candidates for such carrier molecules, and they can form S-nitrosothiols on reaction with oxides of nitrogen<sup>12</sup>. This molecule readily reacts in the presence of NO to yield biologically active S-nitrosothiols that is more stable and potent than NO itself. S-nitrosothiols are unstable in aqueous solution. For example, S-nitrosoglutathione (GS-NO) undergoes decomposition over hours, whereas S-

nitrosocysteine has a half-life of less than 2 min<sup>13,14</sup>. GS-NO is a bioactive intermediary that may regulate cellular functions and inhibits the sarcoplasmic reticulum bound creatine kinase<sup>3</sup>. Generally S-nitrosoglutathione (GS-NO) has been found endogenously in neutrophils and human airways at  $\mu\text{M}$  concentrations<sup>15</sup>. S-nitrosothiols may play the same role in the mechanism of action of Endothelium-Derived Relaxing Factor (EDRF) as NO<sup>3</sup>. Some Authors have assumed that the biological effects of these compounds are due to the spontaneous release of NO. However, this hypothesis is not fully supported yet<sup>16-18</sup>.

This study therefore aimed at determining the effect of exogenous GSH, an important antioxidant containing thiol group, on total antioxidant capacity, total oxidant capacity, and adenosine deaminase a significant indicator of cellular immunity and NO concentrations in rabbits.

## Materials and Methods

Sixteen New Zealand rabbits (Laboratory Animal Unit of the University of Kafkas, Kars, Turkey) of both sexes, aged between 7 and 9 months were used. The mean body weight was  $2.39 \pm 0.42$  kg. They were kept in cages at room temperature (22-25°C) with a 12:12h light:dark cycle and fed a special pelleted rabbit diet (Bayramoglu Yem AS, Erzurum, Turkey) *ad libitum*. Animals were divided into 2 groups as control and GSH-treated group. Each group consisted of 8 animals and treated as follows: animals in control group received a single intraperitoneal (ip) administration of 0.9% saline (Baxter, Mediflex, Eczacibasi, Istanbul, Turkey). Rabbits in GSH group were treated with a single ip administration of L-glutathione reduced (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) dissolved in 0.9% saline at a dose of 10 mg/kg body weight. Blood samples were taken from the marginal ear vein at 0, 3, 6 and 12 hours after injection into heparin treated tubes. Plasma was collected by centrifugation at 3000 rpm for 10 min and stored at -50°C until analyses.

## Biochemical Analysis

### **Determination of Total Antioxidant Capacity**

Total antioxidant capacities were determined colorimetrically (PowerWave XS, BioTek, Instru-

ments, Winooski, VT, USA) using commercial kit (Rel Asssay, Gaziantep, Turkey) in plasma samples. Antioxidants in the sample reduce dark blue-green colored 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample<sup>19</sup>.

### **Determination of Total Oxidant Capacity**

Plasma total oxidant capacities were determined via spectrophotometer (PowerWave XS, BioTek, Instruments, Winooski, VT, USA) with commercial kit (Rel Asssay, Gaziantep, Turkey). Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are present in reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity is related to the total oxidant molecules present in the sample at 530 nm. Trolox and hydrogen peroxide standards were used for total antioxidant and total oxidant capacities<sup>19</sup>.

### **Determination of Nitric Oxide Levels in Plasma**

Nitric oxide concentrations were determined using a spectrophotometer (PowerWave XS, BioTek, Instruments, Winooski, VT, USA) in plasma samples. Plasma samples were deproteinized with 10% zinc sulphate. Total NO (nitrate and nitrite) concentrations were determined colorimetrically by the acidic Griess reaction<sup>20</sup>.

### **Determination of ADA.**

Adenosine deaminase activity (ADA) in plasma was determined at 37°C according to the method of Giusti and Galanti<sup>21</sup> based on the Bertholet reaction, formation of coloured indophenol complex from ammonia liberated from adenosine, and quantified colorimetrically with spectrophotometer (UV-1201, Shimadzu, Japan). One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay condition. Results were expressed as international unit of enzyme activity.

### **Statistical Analysis**

The data for biochemical parameters were analyzed by ANOVA followed by post hoc Tukey test using SPSS Windows 10.0. All data were presented as mean  $\pm$  SE. Values were considered statistically significant if *P* value was less than 0.05.

## Results

Plasma TAC, TOC, NO levels and ADA activity at 0, 3, 6 and 12 hours following the treatments are presented in Table I. Plasma TOC and ADA activities were not statistically different between control and GSH group during the experiment.

Plasma TAC and NO levels were also not statistically different between control and GSH group at 0 and 12 hours. Plasma TAC level was found to be significantly higher at 3 ( $1.16 \pm 0.08$  vs  $0.64 \pm 0.10$ ;  $p < 0.05$ ) and 6 hours ( $0.93 \pm 0.07$  vs  $0.71 \pm 0.08$   $\mu\text{mol Trolox Equiv./L}$ ;  $p < 0.05$ ) while NO level was found to be significantly lower in GSH treated group when compared to control group at 3 ( $13.72 \pm 1.26$  vs  $18.61 \pm 0.85$ ;  $p < 0.05$ ) and 6 hours ( $12.28 \pm 0.68$  vs  $19.29 \pm 1.38$   $\mu\text{mol}$ ;  $p < 0.05$ ).

## Discussion

Our data showed that rabbits who have received reduced glutathione had higher TAC at 3 and 6 h of injection as compared to controls.

GSH, a thiol antioxidant, is known to act as protector in various cytotoxic conditions<sup>22-24</sup>. In addition, GSH is found within the cell at millimolar concentrations that serves several essential functions including protein and DNA synthesis and amino acid transport<sup>25-27</sup>. In condition of oxidative stress, the GSH/GSSG ratio from 100 to 10 or even 1 have been described and found to correlate with the amount of protein mixed disulfide formation. The ratio of the reduced pool to glutathione disulfide (GSSG) is critical to cellular redox balance<sup>28</sup>.

Studies have reported reduced GSH pool during cytotoxicity and oxidative stress situations<sup>22-27</sup>. In a research involving isolated rat liver exogenous GSH was shown to protect liver damage induced cyanide or hypoxia and reoxygenation. Furthermore, several Authors have shown that addition of exogenous GSH into perfusion medium resulted in an increase in intracellular level in both damaged and healthy liver by entering either directly or indirectly into liver cells<sup>29</sup>. When GSH, an endogen antioxidant, was given exogenously, intracellular GSH level might determined an increase in TAC level. In the present study, we assayed oxidative status of the reduced glutathione given rabbits by using TOC and TAC as indicator

**Table I.** Plasma total antioxidant capacity (TAC), total oxidant capacity (TOC), nitric oxide (NO) and adenosine deaminase (ADA) activity of healthy rabbits in the treatment and control group (n=8).

Parameters	Time	Groups	
		Control	Reduced glutathione
TAC (mmol Trolox Equiv./L)	0	$0.60 \pm 0.10^a$	$0.60 \pm 0.05^a$
	3	$0.64 \pm 0.10^a$	$1.16 \pm 0.08^b$
	6	$0.71 \pm 0.08^a$	$0.93 \pm 0.07^b$
	12	$0.73 \pm 0.09^a$	$0.83 \pm 0.09^a$
TOC ( $\mu\text{molH}_2\text{O}_2$ Equiv./L)	0	$0.58 \pm 0.04^a$	$0.62 \pm 0.09^a$
	3	$0.72 \pm 0.05^a$	$0.57 \pm 0.05^a$
	6	$0.57 \pm 0.05^a$	$0.62 \pm 0.06^a$
	12	$0.56 \pm 0.09^a$	$0.41 \pm 0.06^a$
NO ( $\mu\text{mol/L}$ )	0	$19.48 \pm 1.13^a$	$20.41 \pm 1.18^a$
	3	$18.61 \pm 0.85^a$	$13.72 \pm 1.26^b$
	6	$19.29 \pm 1.38^a$	$12.28 \pm 0.68^b$
	12	$20.50 \pm 0.91^a$	$19.16 \pm 1.50^a$
ADA (U/L)	0	$6.68 \pm 0.81^a$	$6.16 \pm 0.87^a$
	3	$5.44 \pm 0.44^a$	$5.38 \pm 0.40^a$
	6	$5.45 \pm 0.60^a$	$5.18 \pm 0.31^a$
	12	$6.20 \pm 0.60^a$	$5.45 \pm 0.30^a$

Values with different superscripts in the same row are significantly different at  $p < 0.05$ .

of oxidative stress, reflecting the redox balance between oxidation and antioxidation. It is well known that oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity, and various oxidants and antioxidants have additive effects on oxidative status. Although the concentration of plasma level of oxidants and antioxidants can be measured individually, it may not accurately reflect the oxidative status<sup>30</sup>. Exogenous GSH increased TAC but did not alter TOC level in this study. This may be attributed to rabbits used that were healthy and there was no condition that incurred enough stress to cause alteration in oxidant/antioxidant system. Therefore, the plasma TOC levels did not change.

Nitric oxide is a biologically active molecule with different effects on many physiological and pathological processes<sup>31</sup>. In a study by Sing et al.<sup>13</sup> NO was shown to be released from nitrosoglutathione molecule or GSH molecule forming compound with electrophilic molecules as NO. Nitrosoglutathione (GS-NO) molecule, formed by a reaction between GSH and NO, plays role in many physiologic and pathologic process through a mechanism similar to that of NO via EDRF<sup>3,32</sup>. NO level was found decreased at 3 and 6 hours of GSH application in this study. Decreased NO level may be related to formation of GS-NO. In fact, after administration of GSH, NO was bound to GSH to form GS-NO and this resulted in reduced plasma NO level. Moreover, GSH injection resulted in an alleviation of adverse effect of NO, also a free radical<sup>32,33</sup>. This latter result may also explain why GSH injection resulted in increased plasma TAC level.

ADA, an important enzyme in the purine metabolism, serves in the catalytic deamination of adenosine to inosine and ammonia. It is required for the lymphocyte proliferation and differentiation<sup>6,21,34,35</sup>. Elevation in the ADA activity is attributed to the stimulated cell mediated immune response and could also be used as a marker of activated neutrophil functions during the oxidative stress<sup>21,36</sup>. ADA results decreased in severe combined immunodeficiency disease characterized by incompetence of both humoral and cellular immunity<sup>34</sup>. GSH also plays an important role in immune system along with its antioxidant effect. Although ADA activity changes in diseases where immune system is activated or inhibited<sup>6,7</sup>, it does not change in healthy subjects<sup>37</sup>, likewise to rabbits of the present study, where ADA activity did not alter after exogenous GSH applica-

tion. Therefore, exogenous GSH may not affect immune system of healthy rabbits.

In conclusion, exogenous GSH resulted in alteration of plasma TAC but not plasma TOC and plasma ADA, and reduced plasma NO may be due to reaction between NO and GSH.

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