CLINICAL STUDY

The redox state of glutathione in erythrocytes of individuals with Down syndrome

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Abstract

Background: **The redox state of glutathione has been used as indicator for the redox environment of the cell.**
Objectives: **To investigate relationships between the redox environments, the SOD activity, total antioxidant status and the oxidation stress markers production (MDA and lipofuscin).**
Methods: **Individuals with Down syndrome and age-matched healthy controls were enrolled into a study. Some parameters of oxidative stress in serum were determined: reduced glutathione, oxidized glutathione, redox potential of this couple (E_h), activity of superoxide dismutase in the red blood cells as well as malondialdehyde and lipofuscin.**
Results: **In the group of persons with DS statistically significant decrease in the GSH concentration was found, however, no differences in the GSSG concentration versus controls was observed. The redox potential values for couple GSH/GSSG are a statistically significantly increased in DS individuals compared to controls.**
Conclusion: **In this study we highlighted the different ways of view at the role of GSH in metabolism of persons with DS. It is useful to look at the GSH and GSSG concentrations separately as well as at redox potential value, which influence total redox state of organism *(Tab. 2, Fig. 3, Ref. 30)* Full Text (Free, PDF) *www.bmj.sk*.**
Key words: Down syndrome, redox potential, glutathione, malondialdehyde, lipofuscin.

Abbreviations: DS – Down syndrome, Ery – erythrocytes, GPx – glutathione peroxidase, GR – glutathione reductase, GSH – reduced glutathione GSSG – oxidized glutathione, Hb – hemoglobin, MDA – malondialdehyde, NADPH – nicotinamide adenindinucleotide phosphate (reduced form), P – protein, SEM – standard error mean, SOD – superoxide dismutase, TBA – thiobarbituric acid

Persons with Down syndrome are under unusual oxidative stress arising from the presence of 3 chromosome 21 (1–3). The elevation of Cu/Zn superoxide dismutase (SOD) by about 50 percent in all cells causing disturbance of antioxidant enzyme balance in persons with DS may be a key point in the pathogenesis of DS. Apparently it leads to a disturbance in the balance of reactive oxygen species (ROS) in the organism. Trisomic cells are more sensitive to oxidative stress. This sensitivity may be due to an imbalance in the hydrogen peroxide metabolism or another unknown factor.

Resistence of the cells to oxidative stress is associated with sufficient antioxidant status especially adequate intracellular level of glutathione (4, 5). GSH can act either directly as a free radical scavenger by neutralizing HO°, restores damaged molecules by hydrogen donation, reduce peroxides, and maintains protein thiols in the reduced state (6) or through GSH-dependent enzymes,

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as glutathione peroxidase (GPx), and glutathione-S-transferase (7). Oxidative stress may cause changes in the glutathione redox state of different tissues.

Glutathione is considered to be a major thiol-disulfide redox buffer of the cell (8). In average, the GSH concentration in the cytosol is 1–11 mmol/l. This is far higher than most other redox active compounds. Therefore, the redox state of this couple has been used as indicator for the redox environment of the cell (9). Although, there are several methods for estimating the intracellular redox potential, the most accessible technique is the measurement of intracellular concentration of GSH and GSSG, and the calculation of E_h using Nernst equation (10). The intracellular GSH redox homeostasis is strictly regulated to govern cell metabolism and protect cells against oxidative stress (11).

The aim of this study was to investigate extensive array of redox status indices: reduced glutathione (GSH), oxidized glutathione (GSSG), redox potential of this couple (E_h), and the most important antioxidant enzyme – superoxide dismutase in red blood cell of persons with Down syndrome (DS) as well as malondialdehyd (MDA) and lipofuscin in serum of these individuals. We were also interested in the relationships between the redox environments, the SOD activity and the oxidation stress markers production (MDA and lipofuscin).

Subjects and methods

Subjects. Sixty one individuals with Down syndrome (mean age 20.76 years, from 1.67 to 46.75 years) and 45 an age-matched healthy controls (mean age 19.57 years, from 2.67 to 47.5 years) were enrolled into a study. Down syndrome diagnosis was verified by a cytogenetic examination by Down Syndrome Department of the Slovak Medical University in Bratislava and it determined to be non-disjunction trisomy 21. Persons with DS were out-patients, who visit the Down Syndrome Department on regular basis. Parents of the individuals participating in the study provided written informed consent and ethical approval was obtained from the Ethical Commitee of Derer's University Hospital in Bratislava, Slovakia.

Individuals with DS suffering from heavy heart defects, other chronic diseases like diabetes mellitus and person with acute respiration diseases were excluded from the study.

The control group was consisted of healthy siblings of monitored individuals with DS or by healthy volunteers. In one case we examined dizygotic twins – a girl with DS and her brother with no trisomy 21.

Sample preparation. 5 ml venous blood was drawn from each individual into heparin coated tubes The serum sample was obtained by centrifugation at 1500 x g for 10 min and stored at -80 °C until the analysis.

For isolation of the erythrocytes heparinized venous blood (25 unit/mL) was washed three times with a 0.15 mol/L NaCl solution. After centrifugation (400 x g, 5 min), the erythrocytes were hemolyzed by adding triple volume of distilled water. Hemoglobin concentration in the hemolysate was measured using the Drabkin method.

Determination of reduced glutathione. Reduced glutathione was determined by a spectrophotometric method (lambda=400 nm, using the BIOXYTECH GSH-400 (USA) kit following deproteinization of blood by using a metaphosphoric acid. The concentration of GSH is expressed in µmol/g Hb.

Tab. 1. Clinical and biochemical characteristics of the subjects.

	Down syndrome Mean±SEM	Control Mean±SEM	Significance p
BMI (kg/m^2)	21.72±1.00 (n=61)	18.77±0.74 (n=45)	0.021
Cholesterol (mmol/L)	4.61±0.15 (n=61)	4.41±0.13 (n=45)	0.331
Triacylglycerols (mmol/L)	1.29±0.002 (n=61)	0.83±0.087 (n=45)	0.017

Tab. 2. The activity of SOD and some markers of oxidative stress.

	Down syndrome Mean±SEM	Control Mean±SEM	Significance p
SOD (mg/g Hb)	1.149±0.038 (n=57)	0.768±0.031 (n=43)	<0.001
GSSG (µmol/g Hb)	1.296±0.074 (n=61)	1.354±0.058 (n=45)	0.560
GSH (µmol/g Hb)	7.727±0.357 (n=61)	9.163±0.495 (n=45)	0.018
E (mV)	-0.290±0.001 (n=61)	-0.295±0.001 (n=45)	0.012
TAS	1.106±0.025 (n=53)	1.104±0.034 (n=37)	0.971
MDA (µmol/l)	1.249±0.054 (n=48)	1.149±0.078 (n=30)	0.280
Lipofuscin (µmol/l)	9.944±0.425 (n=52)	10.109±0.573 (n=33)	0.815

GSSG — oxidized glutathione, GSH – reduced glutathione, Eh – redox potential, TAS – total antioxidant status, MDA – malondialdehyde

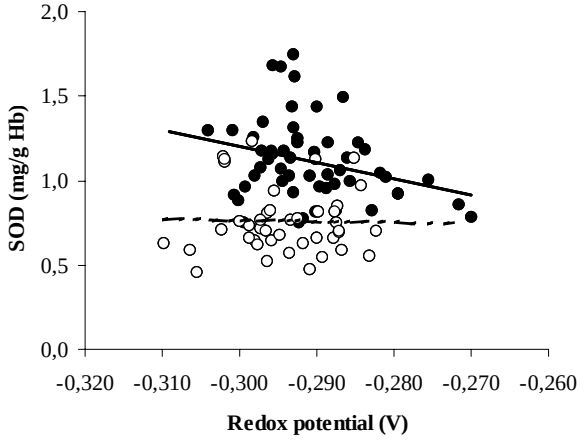

Fig. 1. Correlation between redox potential and activity of SOD in erythrocytes of persons with DS (●, solid line; r=-0.288, p=00.034, n=54) and controls (○, dotted line; r=-0.019, p=0.899, n=42)

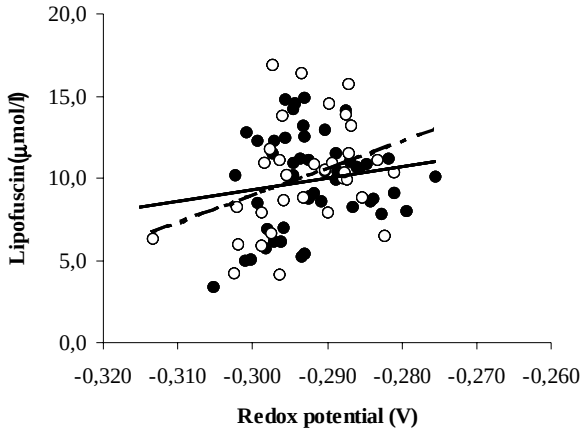

Fig. 3. Correlation between redox potential and level of lipofuscin in serum of persons with DS (●, solid line; r=0.157, p=0.277, n=50) and controls (○, dotted line; r=0.352, p=0.044, n=33).

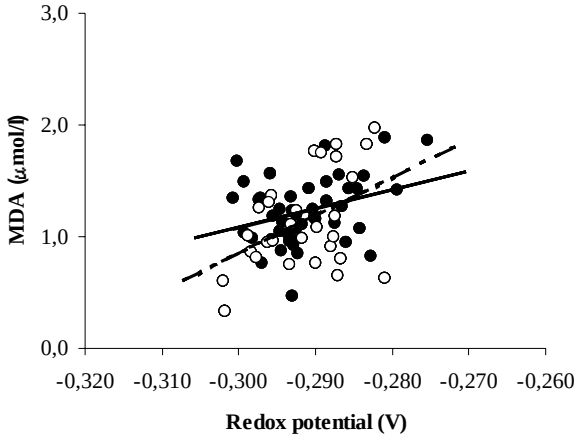

Fig. 2. Correlation between redox potential and level of MDA in serum of persons with DS (●, solid line; r=0.322, p=0.030, n=045) and controls (○, dotted line; r=0.449, p=0.014, n=29).

Determination of oxidized glutathione. Oxidized glutathione was measured according to the method of Videla and Junqueira (12), following a deproteinization of blood by using a 0.5 mol/L $HClO_4$. The determination of GSSG is based on the reduction of GSSG in presence of NADPH and glutathione reductase (GR), and on measurement of NADPH decrease at 340 nm, at 30 °C. The reaction was initiated by addition of GR. The concentration of GSSG is expressed in mol/g Hb.

Determination of superoxide dismutase. SOD activity was determined by a photochemiluminescence method using luminol. We used the commercial kit (F.A.T., Berlin) by using a bovine Cu/Zn-SOD as a standard (13). The activity of erythrocytes SOD is expressed in mg SOD/g Hb.

Determination of malondialdehyde. The production of the lipid peroxides was determined using a reaction with thiobarbituric acid (TBA). Serum TBA reaction products were measured by a HPLC method of Brown and Kelly (14) on the C18 column (Nucleosil 120-5, 250 x 4 mm, Watrex, Slovakia). The TBA-MDA products with a fluorescence detector (FP Jasco 1520, excitation at 532 nm and emission at 552 nm) were monitored. Methanol HPLC-grade in phosphate buffer was used as a mobile phase and the flow rate was 0.8 mL/min. Tetraethoxypropane was used as a standard. Lipid peroxidation was expressed in µmol/L MDA. All samples and standards were analyzed in duplicate.

Determination of lipofuscin. Lipofuscin in serum was determined using the method of Tsuchide et al (15). Fluorescence determination of lipofuscin was based on the yellow autofluorescence of lipofuscin when excited by UV light (345 nm), this was measured at 430 nm (Spectrofluorimeter AMICO-BOWMAN, USA). Lipofuscin concentration is expressed in µmol/L serum, where chinin sulphate was used as a standard.

Statistical analysis. All results are presented as mean ± SEM for given numbers of measurements. Between-group comparisons were made using the Students unpaired t-test and analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant. Correlations were expressed using the Pearson's correlation coefficient.

Results

Basic clinical parameters of individuals with Down syndrome and healthy individuals are listed in Table 1. The values of measured parameters were independent of gender.

The activity of SOD and some markers of oxidative stress in DS and control groups are given in Table 2.

In the group of persons with DS increased activity of SOD was confirmed in erythrocytes (149.6 %) when compared to controls (Tab. 2).

There is a statistically significant decrease in the GSH concentration of DS group versus controls (p=0.018), however, no differences in the GSSG concentration in DS persons versus controls was observed (p=0.560).

The redox potential values (Eh) for couple GSH/GSSG was calculated using the Nernst equation:

$E_h=E^o+(RT/2F).\ln([GSSG]/[GSH]^2)$, where E^o is standard potential (-0.24 V), R is gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature in Kelvin and F is Faraday constant (9.6485 x 10^4 C mol^{-1}) (10, 16). The redox potential accurately expresses the redox environments in the cell.

We found a statistically significant increasing E_h in DS individuals compared with controls (p=0.012).

The level of total antioxidant status (TAS) in plasma was unchanged in people with DS when compare of control (Tab. 2).

The levels of lipid peroxidation products, serum MDA and lipofuscin, are shown in Table 2. We did not observed the significant differences in MDA and lipofuscin levels of the DS and control groups.

We were interested in associations between the E_h and activity of SOD, and markers of oxidative damage to lipids – MDA and lipofuscin. We found a weak negative association between E_h and activity of SOD in individuals with DS (y=-9.58x -1.67, r=-0.288, n=54, p=0.034) (Fig. 1). In control group, no correlation was found. Correlations between Eh and MDA resp. lipofuscin are depicted in Figure 2, resp. Figure 3.

Discussion

Resistance of many cells against oxidative stress is associated with sufficient intracellular concentration of GSH. GSH can act directly as a free radical scavenger, for example, by neutralizing °OH. GSH can also donate hydrogen to oxidative damaged molecules and thereby to "cure" them, it can reduce peroxides, and keep thiol groups in reduced state. Reduced glutathione is also a cofactor for antioxidant enzyme – glutathione peroxidase, which decomposes hydrogen peroxides and other peroxides into alcohols and water when two GSH molecules are oxidized to GSSG in the process.

During the oxidative stress, GSH is depleted in various tissues, and, at the same time, the level of GSH decreased and the level of GSSG can be increased. Increased GSSG concentration in tissues and its accelerated release from cells is considered to be a signal of oxidation stress (17). In this study decreased concentration of GSH in persons with DS was observed with unchanged concentration of GSSG. The decreased GSH level may be caused by its depletion as cofactor used for the increased level of GPx, which was confirmed in individuals with DS (13, 18). Moreover, the decreased level of GSH has significant impact on the redox status in persons with DS despite unchanged GSSG level observed in the study. In the persons with DS, we observed an elevated redox potential relative to control, which was associated with a decrease of reduced glutathione level and, consequently, with a decrease GSH/GSSG ratio.

Increased expression of the SOD enzyme induces an increased GPx activity, with the SOD+GPx tandem providing frontline of the antioxidant protection. Increase in GPx activity requires sufficient levels of GSH at the unchanged activity of glutathione reductase in DS individuals (13, 18). GSSG is not regenerated to GSH sufficiently. GSH concentration, or maintaining sufficient GSH/GSSG ratio is considered a limiting factor in processes of removal of hydrogen peroxide and also other peroxides. Navarro et al (17) found and our results confirmed (13) that GPx activity in erythrocytes is much higher than GR activity, which may, at least partially, explain the shift of equilibrium towards to more positively redox status in the persons with DS. GR is not only specific for GSSG. The other disulphides exist in limited quantity in the cell and their influence on GR activity can not be excluded (19). The second possible explanation for low GSH levels in persons with DS is that GSH is being exhausted as a main low molecular weight antioxidant in cellular protection (20). The thiol group of GSH enables to reduce disulphides and other molecules. It serves as a sulfhydryl cytoplasm buffer and therefore influences and maintains overall redox potential of cells (21). By providing reductive cellular environment, glutathione directly or indirectly protects them against oxidative damage, free radical damage and other damages caused by endogenous or exogenous compounds (4). Reductive environment supports maintenance of thiols and other antioxidants, like ascorbate and alpha-tocopherol (22, 23).

The enzymes that utilize GSH as a co-substrate, can be dependent upon GSH, while processes dependent upon redox potential can be influenced by glutathione redox status (GSH/GSSG), represented also by the redox potential. At present, it is however unclear, which of these parameters, the absolute GSH level or redox potential represented mainly by GSH/GSSG ratio, is a more important risk factor of accelerated aging or as an oxidative stress marker.

GSH alone and GSH dependent enzymes, cooperate with other antioxidants and antioxidant enzymes to protect the cell against reactive oxygen metabolites. Therefore, a change in GSH concentration resp. in the redox state can subsequently affect the whole antioxidative machinery.

In this study we confirmed the statistically significant increase in SOD activity of persons with DS (Tab. 2). The shift of redox potential towards positive values leads to decrease of SOD activity (Fig. 1). CuZn-SOD can be damaged by free radicals induced by its own products H_2O_2, resulting in the inactivation and the fragmentation of the enzyme (24, 25). Under the oxidative stress conditions, when reduction capacity of cells is exhausted, thiol groups of various proteins could be oxidized resulting in modification of their structure and thereby the change in their function (26).

GSH is an important intracellular antioxidant. The decreasing of its level in erythrocytes and tissues as well as a decreasing in the GSH/GSSG ratio and increasing of redox potential could contribute to free radical diseases. The importance of GSH in protection of macromolecules against the oxidative damage is demonstrated also by our results. We found that redox potential positively correlates with serum MDA (r=0.322, n=45, p=0.03 for DS resp. r=0.449, n=29, p=0.014 for controls) and lipofuscin concentrations (r=0.157, n=50, p=0.277 for DS resp. r=0.352, n=33, p=0.044 for controls). The shift of cellular E_h to positive values showed more markedly increase of serum MDA and lipofuscin concentration in controls than in individuals with DS (Figs 2 and 3). This indicates the differences in inclination

of persons with DS and healthy subjects to oxidative damage. Gao et al (27) demonstrated the protection effects of GSH against lipofuscin production in a model system with artificially impaired GSH synthesis, supported by our observed correlation between the lipofuscin level and an increased redox potential (Fig. 3).

Changes in redox state of critical thiols of receptors, enzymes, transcription factors or transport systems are thought to function in control of fundamental processes such as gene expression, cell proliferation (28, 29) or apoptosis (30). Thus, if altered E_h could be detected in vivo in association with pathology or toxicity, this finding may provide an important link to underlying mechanism.

In this study we highlighted the different ways to look at the role of GSH in metabolism of persons with DS. On the one hand it is useful to look at the GSH and GSSG concentrations separately but on the other hand at redox potential value, which has influence on total redox state of organism.

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