Selenium Effect on Hyperoxia-Induced Glutathione Peroxidase Activity and Free Radicals Production in the Brain

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ABSTRACT

The purpose of this study was to examine the behavior of Glutathione Peroxidase (GPx) activity and Free Radicals (FR) production in the brain during graded hyperoxia exposure with and without selenium preventive intake. Forty two adult male rats were assigned to seven groups, six animals each. The first group served as control and the second, third and fourth groups were exposed to hyperoxia for 24, 48 and 72 h, respectively. The fifth, six and seventh groups were exposed to hyperoxia for 24, 48 and 72 h with selenium treatment. Following the exposure period for each group animals were sacrificed and brain tissues were homogenized for GPx and FR analysis. The results of 2-way ANOVA showed that the main effects of both selenium and hyperoxia were significant ($p < 0.05$) for GPx. However, FR production was significantly ($p < 0.05$) affected by hyperoxia only. Pair-wise means comparisons showed that the corresponding means ($\pm$) SD of GPx activity decreased significantly ($p < 0.05$), from baseline non-selenium values of 13841.72±1245.67 and 14594.89±6711.50 to 5741.72±949.31 and 4829.98±775.52 following 24 and 48 h of hyperoxia exposure respectively, then increased to 7846.19±2467.69, following 72 h of hyperoxia exposure, as compared with non-selenium mean value of 4346.38±349.14. These differences were attributed to the ability of brain tissue to use selenium to reduce the requirements for GPx during 24 and 48 h as such to spare the integrity of its antioxidant mechanism during 72 h. Based on the results of the present study selenium's supplement and diet rich selenium are recommended for TBI patients.

Keywords: Selenium, Brain, Hyperoxia, Free Radicals, Reactive Oxygen Species

1. INTRODUCTION

Hyperoxia inducts mitochondria oxidative stress in variety of body tissue, including the brain (Haffor, 2004; Bin-Jaliah *et al.*, 2009; Alattas and Haffor, 2010; Haffor and Alattas, 2010) and that result in brain tissue injury and cellular death in the central nervous system (Jankov *et al.*, 2003; Bin-Jaliah *et al.*, 2009). Clearly, Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) mediate many processes of CNS, hence make its tissue strategic environment to induce rapid rate of ROS and RNS, leading to cellular death and variety of tissue damages.

The role of glutathione in the detoxification of reactive oxygen intermediates has been well established (Jalili *et al.*,

2011). Reduced glutathione (GSH) is oxidized in the presence of glutathione peroxidase (GSH-Px) and hydrogen peroxide (H_2O_2) or lipid hydroperoxides (Shrivastava, 2011), resulting in the formation of nontoxic compounds. Two molecules of GSH are oxidized during this reaction resulting in the formation of glutathione disulfide (GSSG). The disulfide can then be reduced to GSH in the presence of the enzyme glutathione reductase (GSSG-Red) and adequate concentrations of NADPH (Jalili *et al.*, 2011). GSH-Px is a selenoenzyme (Se GSH-Px) and animals deficient in selenium have markedly decreased tissue GSH-Px activity and enhanced toxicity when exposed to normobaric hyperoxia.

Selenium, through the incorporation into selenoproteins, may provide protection from hyperoxia-

induced Reactive Oxygen Species (ROS)-mediated cell damage in different tissues (Bin-Jaliah, 2008a). An effective antioxidants system has been shown to protect both rats and mice from hyperbaric hyperoxia (Haffor and Al-Johany, 2005). However, the exact period of exposure for O2 of protection provided by exogenous glutathione administration remain unknown.

Selenium (Se) is a naturally occurring micronutrient that is essential for antioxidant defense systems (Al-Taie *et al.*, 2003; Guney *et al.*, 2005). The dietary intake of selenium has a delicate balance between the harmful effects of excessive selenium uptake leading to selenium toxicity and the damaging effects on selenoprotein function during selenium deficiency. Interestingly, the CNS appears to be resistant to fluctuations in antioxidant, hence can maintain stable levels of its antioxidant reserve. In the present study we examined the consequences of selenium on GPx and FR production in the brain during exposure to different exposure periods of 24, 48 and 72 h in order to estimate the antioxidant sustainability of CNS against oxidative stress which has not been reported previously. In this regards, the selenium role in up-regulating the brain antioxidants sustainability has not been examined prior to this study. We hypothesized that selenium intake would provided protection to rats via a free radical quenching mechanism requiring activation of the glutathione redox cycle, then selenium-deficient rats might not exhibit GSH-induced protection. To explore this hypothesis, a graded length of HP exposure to rats with water drinking and rats with selenium-supplemented match and then exposing these animals to 100% O2 for 24, 48, 72 h.

In view of the previous introduction summarized above and the preventive role of selenium to brain's oxidants products, the current study was designed to compare the effects of selenium with graded length of oxygen breathing on free radicals production and glutathione peroxidase activity in tissue known to be susceptible to oxygen toxicity and rich with nitroso-redox which generates both ROS and RNS. Some preliminary results of this study had been published previously in abstract form during the 2008 Beijing joint conference of the international physiological societies (Bin-Jaliah, 2008b).

2. MATERIALS AND METHODS

2.1. Experimental Design

Forty two adult Wister albino male rats, Rattus norvigicus, matched with age and body weight, were randomly assigned to 7 groups, 6 animals each. The first group served as Control (C) and the 2nd, 3rd and 4th groups were exposed to hyperoxia for 24 h (HP-24), 48 h (HP-48) and 72 h (HP-72) without selenium whereas the 5th, 6th and 7th groups were on selenium supplement with selenium. Following the treatment conditions for each group, animals were sacrificed and brains tissues were homogenized immediately in 0.9 saline solutions (4:1 ratio), for the determination of glutathione peroxidase activity and free radical production. All procedures were approved by the Ethical Committee of Physiology at the King Khalid University Medical School (Abha, KSA) and were performed in agreement with the Principles of Laboratory Animal Care, advocated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.

2.2. Hyperoxia Exposure

Animals of the three O_2 breathing groups were placed in a closed box that has an inlet flow which was connected to 100% O_2 tank (medical grade) on which a regulator was connected to maintain flow at 5 Liter Per Minute (LPM). The out flow of the regulator passed through a humidifier in order to saturate the inspired air with H_2O. The outlet ventilation rate of the box was adjusted at 5 LPM to ensure that the concentration of oxygen in the box remains equal to 100% O_2 and maintain normal flow and maintain normal barometric pressure at 767 mmHg. The temperature inside the box was adjusted at room temperature (22-24°C). Animals of the first O_2 breathing groups (HP-24) was exposed to 24 h whereas the second was exposed to 48 h (HP-48) and the third was exposed to 73 h (HP-72).

2.3. Tissue Samples Preparation

Following the completion of the respective treatments by each group, rats were euthanized by cervical dislocation and their brains were isolated. Seven rats per group had their brains utilized for analyses of endpoints outlined here. Tissues of the brain of a given rat was placed in 0.9% saline solutions (4:1 mL per mg wt tissue) and homogenized, using homogenizer (Ultra-Turrax System-Germany) with the sample tube held on ice. The homogenates were then centrifuged for 10 min at 3000 rpm (ZENTRIFUGEN, Model 2405, Germany). The resultant supernatant fraction from each brain homogenate was then recovered and used for Free Radical (FR) and glutathione peroxidase activity determinations.

2.4. Free Radical Determination

Free radicals production was measured, using the d-ROMs-4 test kits (Health and Diagnostic, Italy) according to the manufacturer's instructions. The test

measures the levels of Hydroperoxides (R-OOH) which are generated by peroxidation of biological compounds; lipid, amino acids, nucleic acids. This test is based on the principle of the ability of hydrogen peroxides to generate free radicals after reacting with some transitional metals (Fe^{2+}/Fe^{3+}), according to Fenton's reaction as follows:

$$H_2O_2 + Fe^{++} = *OH + OH - +Fe^{++}$$

Thus, the hydrogen peroxides of biological sample generate free radicals (alcoxy and peroxyl radicals) after exposure to a transitional metal (F^{2+}/Fe^{3+}).When a correctly buffered chromogen substance (N, N-diethyl-phenylendiamine) lead to the reduction of hydrogen peroxides which in turns colored as radical cation. Color intensity was read using spectrophotometer with peak absorbance of 505 nm. In the d-ROMs test results were expressed in CARR units (CARR U). One CARR U relates to 0.08 mg H_2O_2/100 mL.

2.5. Glutathione Peroxidase (GPx) Determination

GPx was determined using Randox protocol (Randox, UK). The principle of this method is based on the specificity of GPx to catalyze the detoxification of hydrogen peroxide according to the following reaction:

$$2GSH + H_2O_2 \xrightarrow{\text{GPx}} GSSG + 2 H_2O$$
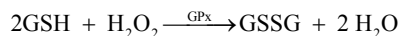
2.6. Statistical Analysis

Mean group differences for the dependent variables; Free Radicals (FR) and GPx were evaluated using two-way Analysis of Variance (ANOVA) to reveal the main effect of each group on the dependent variables. Tukey-Kramer multiple comparisons were used to compare differences between each means pairs. All statistical analysis was conducted using SPSS program.

3. RESULTS

3.1. Descriptive Results

Hyperoxia elevated the average (±SD) GPx activity in the brain tissue from the baseline control value 5467.80±2852.66 to 13841.72±1245.67 and 14594.89±6711.50 with increasing length of exposure period from 24 to 48 h, then dropped to 4346.38±349.14 U/L. The corresponding change in selenium supplemented groups were 5741.72±949.31, 4829.98±775.52, then increased to 7846.19±2467.69 U/L, respectively (**Table 1**). These results demonstrated that selenium supplements in drinking water elicited GPx activated stimulus that could be pre and or post transcriptional stage which allowed for sustain GPx activity between 24 to 48 h of hyperoxia exposure (**Fig. 1**). Similarly, hyperoxia elevated the average (±SD) FR production in the brain tissue from the baseline control value 73.33±20.19 to 132.17±21.12 with increasing length of exposure period from 24 to 48 h, then dropped to 65.33±21.12 Carr (**Table 2**). The corresponding change in selenium supplemented groups were 110.83±17.03, 121.83±7.91, then dropped to 68.33±18.25 Carr, respectively (**Table 2**). **Figure 2** displays the behavior of FR during graded exposure to hyperoxia, but the observed changes were different among selenium and non selenium treated groups following 24 h of hyperoxia exposure.
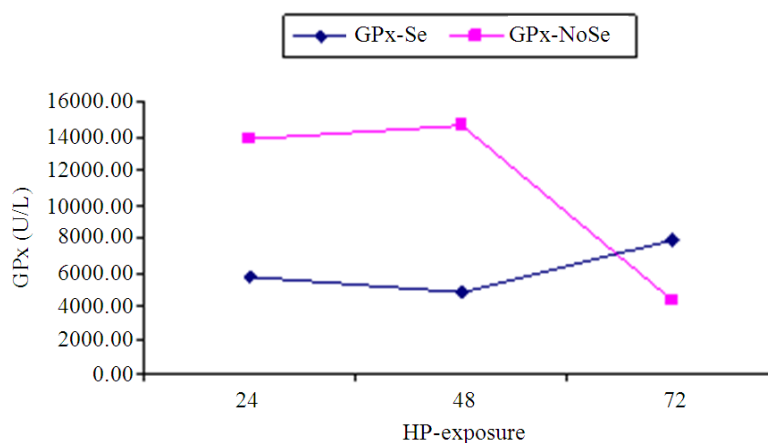
Fig. 1. Behavior of GPx activity during graded hyperoxia exposure with selenium (diamond) and without selenium (square)
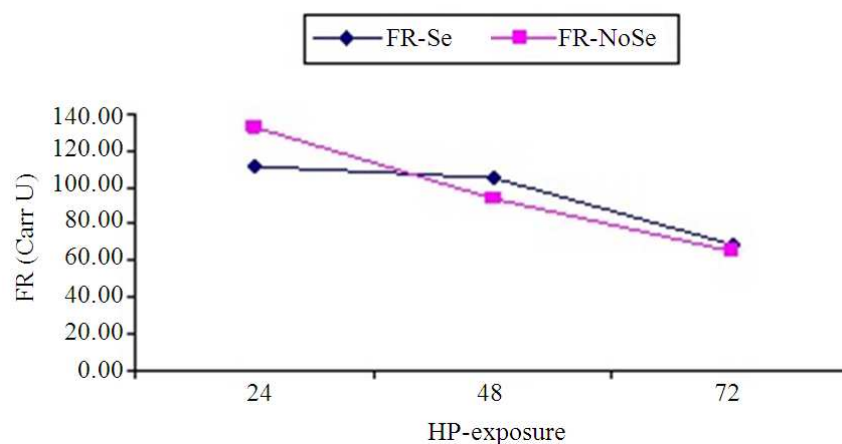
Fig. 2. Behavior of FR production during graded hyperoxia exposure with selenium (diamond) and without selenium (square)

Table 1. Descriptive statistics: Dependent variable: GPx Acivity

Group membership	Treatment condition	Mean	Std. deviation
	Control	5467.80	2852.66
No selenium	Hyperoxia-24 h	13841.72	1245.67
	Hyperoxia-48 h	14594.89	6711.50
	Hyperoxia-72 h	4346.38	349.14
	Control	5467.80	2852.65
Selenium	Hyperoxia-24 h	5741.72	949.31
	Hyperoxia-48 h	4829.98	775.52
	Hyperoxia-72 h	7846.19	2467.69

Table 2. Descriptive statistics: Dependent variable: Free radicals production

Group membership	Treatment condition	Mean	Std. Deviation
	Control	73.33	20.19
No selenium	Hyperoxia-24 h	132.17	21.77
	Hyperoxia-48 h	94.33	14.56
	Hyperoxia-72 h	65.33	21.12
	Control	73.33	20.19
Selenium	Hyperoxia-24 h	110.83	17.03
	Hyperoxia-48 h	121.83	7.910
	Hyperoxia-72 h	68.33	18.25

Table 3. Tests of between-subjects effects dependent variable: GPx activity

Source	Type III sum of squares	df	Mean Squar	F	Sig.
Corrected model	346308743.887(a)	4	86577185.972	5.186	0.002
Intercept	2895707852.823	1	2895707852.823	173.469	0.000
Group	154767073.507	1	154767073.507	9.271	0.004
Condion	191541670.379	3	63847223.460	3.825	0.016
Error	717794744.773	43	16692901.041		
Total	3959811341.482	48			
Corrected total	1064103488.659	47			

Table 4. Multiple comparisons: dependent variable: GPx activity: Tukey HSD

					95% Confidence interval	
(I) Treatment condition	(J) Treatment condition	Mean difference (I-J)	Std. error	Sig.	Lower bound	Upper bound
	Hyperoxia-24 h	-4323.9217	1667.9779	0.0600	-8781.5138	133.6705
Control	Hyperoxia-48 h	-4244.6317	1667.9779	0.0670	-8702.2238	212.9605
	Hyperoxia-72 h	-628.4867	1667.9779	0.9820	-5086.0788	3829.1055
	Control	4323.9217	1667.9779	0.0600	-133.6705	8781.5138
Hyperoxia-24 h	Hyperoxia-48 h	79.2900	1667.9779	1.0000	-4378.3021	4536.8821
	Hyperoxia-72 h	3695.4350	1667.9779	0.1350	-762.1571	8153.0271
	Control	4244.6317	1667.9779	0.0670	-212.9605	8702.2238
Hyperoxia-48 h	Hyperoxia-24 h	79.2900	1667.9779	1.0000	-4536.8821	4378.3021
	Hyperoxia-72 h	3616.1450	1667.9779	0.1490	-841.4471	8073.7371
	Control	628.4867	1667.9779	0.9820	-3829.1055	5086.0788
Hyperoxia-72 h	Hyperoxia-24 h	-3695.4350	1667.9779	0.1350	-8153.0271	762.1571
	Hyperoxia-48 h	-3616.1450	1667.9779	0.1490	-8073.7371	841.4471

3.2. Inferential Results

The results of 2-way ANOVA (**Table 3**) showed that the main effects of both selenium and hyperoxia were significant ($p<0.05$) for GPx. However, FR production was significantly ($p<0.05$) affected by hyperoxia only. Pair-wise means comparisons showed that the corresponding means ($\pm$) of GPx activity decreased significantly ($p<.05$), from baseline non-selenium values of 13841.72 ± 1245.67 and 14594.89 ± 6711.50 to 5741.72 ± 949.31 and 4829.98 ± 775.52 following 24 and 48 h of hyperoxia exposure respectively, then increased to 7846.19 ± 2467.69, following 72 h of hyperoxia exposure, as compared with non-selenium mean value of 4346.38 ± 349.14 (**Table 4**).

4. DISCUSSION

The first major findings of the present study showed that gradual lengthening of the period of exposure to oxygen breathing up to 48 h resulted in sustained rise in 2 folds for GPx activity and 1.5 folds for FR production in the brain tissue. This sustainable rise reflects brain tissue antioxidants defense system ability resistance against the continued rise of ROS. The possible mechanisms for continuous hyperoxia-induced reactive species reflect an additive effect of mitochondria oxidative stress-MOS (Haffor, 2004). Under oxidative stress mitochondria releases free radicals by-products such as hydrogen peroxide to the cytosol at higher rate than its elimination rate by cellular antioxidants (Sawyer and Colucci, 2000; Manizheh *et al.*, 2011; Amutha and Subramanian, 2012). A progressive accumulation (build up) of proton (H^+) leak from the inter-mitochondria membrane region to the matrix. It

has been shown that exposure to hyperoxia for 24 h resulted in morphologic changes in the inner mitochondrial membrane (Haffor and Al-Johany, 2005; Bin-Jaliah *et al.*, 2009) that are similar to brain inflammation (Manizheh *et al.*, 2011; Fridovich, 1998; Jankov *et al.*, 2003; Jafari *et al.*, 2004). During long duration of hyperoxia exposure such as 48 h the body's antioxidant defenses are overwhelmed by the buildup of ROS in the mitochondria, nucleus, cytosol, membranes and extracellular fluid compartments. Oxidative Mitochondrial Stress (OMS) occurs when O_2 tension is increased because the buildup of superoxide anion ($^*O_{2\text{-}}$) cannot be controlled. When $^*O_{2\text{-}}$ is increased during the exposure to hyperoxia, the electron transport chain becomes limited by the buildup of a large proton gradient in the inner mitochondrial membrane which, in turn, results in variety of mitochondrial pathological changes such as swelling, concentrated cristae, dilution of the inner and outer membrane (Haffor, 2004). Besides MOS, brain tissue produces nitro oxide which contributed relative excess shift in nitrasive-redox balance (Hare, 2003; 2004) and the subsequent high turnover rate in cellular death and brain tissue damage. These findings emphasize the rise of ROS, in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and Duchenne muscular dystrophy (Kubo *et al.*, 1994), hence there may be a preventive and treatment roles for selenium in these disorders. It was shown recently that mice fed a selenium-deficient diet exhibit severe motor dysfunction because of neuron degeneration. It appear that reduced dietary selenium can have significant effects on levels of selenoproteins involved in oxidative stress, such as glutathione peroxidases, thioredoxin reductases and methionine sulfoxide reductases (Jalili *et al.*, 2011).

The second major findings of the present study is higher GPx activity at 72 h of HP exposure, with the treatment of selenium, as compared with non-selenium suggest that the process of selenium transport needed for selenocysteine-protein incorporation is important for normal CNS function. It was reported that approximately 60% of selenium in plasma is present as selenoprotein P (Satia et al., 2005; Stranges et al., 2006). This protein differs from other selenoproteins in that it incorporates up to 10 Se atoms per molecule in the form of selenocysteine as opposed to the single selenocysteine incorporated in other selenoproteins (Miettinen et al., 1983). Selenoprotein P is abundant throughout the body suggesting that one function is to serve as a primary transporter in systemic selenium delivery (Rayman, 2000; 2005). This is especially evident in the CNS where selenoprotein P levels can be maintained independent of plasma selenium (Miettinen et al., 1983; Sajdel-Sulkowska et al., 2008). However, genetic ablation of selenoprotein P results in reduced, but not a commensurate decrease in CNS-associated selenium levels, suggesting that other selenium proteins compensate for the selenoprotein P deficiency and supporting the hypothesis that basal selenium levels are essential for the brain and have a priority for available selenium (Sajdel-Sulkowska et al., 2008; Stranges et al., 2006). Dietary selenium can exist as selenomethionine, elenocysteine, selenate or selenite (Cigarroa et al., 1993) and is incorporated as selenocysteine into a subset of specific seleniumdependent proteins selenoproteins (Berthe et al., 1986; Barilla et al., 1991; Rajpathak et al., 2005) of particular interest are the selenoproteins involved in oxidative stress, such as the glutathione peroxidase enzymes (classical GPX-1, gastrointestinal GPX-2, plasma GPX-3, phospholipids hydroperoxide GPX-4) and the Thioredoxin Reductase 1 and 2 (TR).

5. CONCLUSION

Based on the results of the present study it can be concluded that hyperoxia continous exposure inducted the buildup of ROS, a proposed new mechanism for brain injury and diseases. Brain tissue has the ability to use selenium to reduce the requirements for Gpx during 24 and 48 h as such to spare and sustain its antioxidant defense mechanism. Selenium's supplement and diet rich selenium are recommended for TBI patients.

6. ACKNOWLEDGMENT

7. DECLARATION OF INTEREST

The researcher reports no conflict of interest.

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