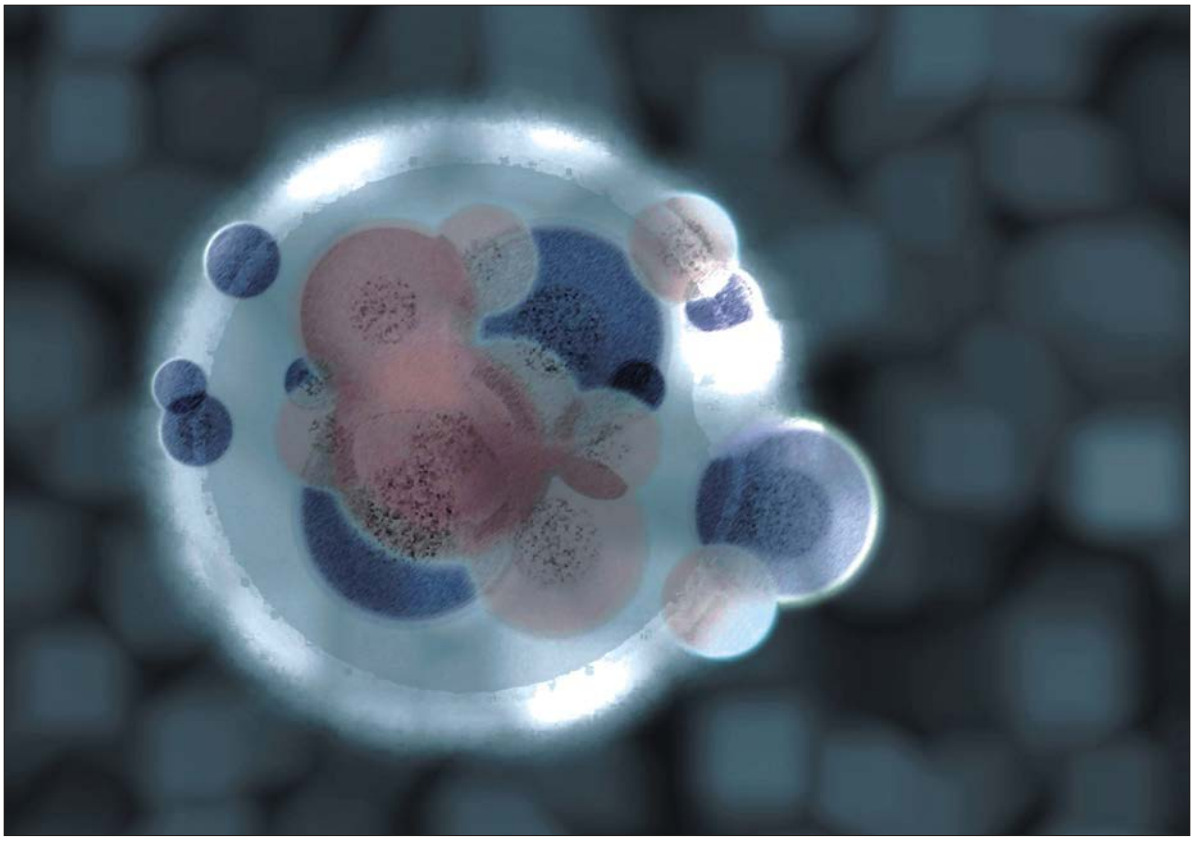

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Changes in Erythrocyte Sodium-Lithium Countertransport and Plasma Parameters Following Selenium Treatment

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Abstract: The effects of selenium as an antioxidant on erythrocyte Sodium-Lithium Countertransport (SLC) activity and plasma parameters have not already been studied in full detail. In the present study, the relationship between selenium and SLC activity, plasma parameters (lipids, lipoproteins, sodium, potassium, urea and creatinine) was investigated. Male rabbits weighed 1350±50 g were divided in to four groups (5 in each). For *in vivo* studies selenium dioxide (SeO_2) was administered intraperitoneally on alternate days for 2 weeks as acute dose (250 μg kg^{-1} body weight) and for 7 weeks as chronic dose (125 μg kg^{-1} body weight). The control groups for each doses received deionized water at the same time. The results showed that the acute dose of selenium decreased SLC activity and V_{max}/K_m, but the K_m of the transporter was increased. Also this metal decreased plasma concentrations of cholesterol, triglyceride and VLDL. All these changes may be helpful to the health. The chronic dose of the metal increased SLC activity, V_{max}/K_m and V_{max}, but it decreased K_m of the transporter. This dose of selenium increased plasma concentrations of cholesterol, triglyceride, VLDL, LDL, sodium and decreased the potassium level. *In vitro* studies showed that, the metal at low concentration (1 μM) can lower SLC activity and then it is beneficial to the health, but at high concentration (50 and 100 μM) increases the SLC activity and may cause a serious problem to the health. It is concluded that selenium at high concentration and with chronic dose increases the SLC activity and plasma lipids. But at low concentration and acute dose will have a beneficial effect to the health.

Key words: Sodium-lithium countertransport, selenium, plasma parameters

INTRODUCTION

Selenium is an essential trace metal especially as a cofactor for the critical antioxidant enzyme Glutathione Peroxidase (GPx), which inactivates hydrogen peroxide and organic hydroperoxides in the presence of reduced glutathione (Hsu and Guo, 2002; Tapiero *et al*., 2003). The major physiological role of GPx is to reduce the levels of hydrogen peroxide within the cell, thus decreasing potential free radical damage (Tapiero *et al*., 2003). Selenium is also an important constituent of antioxidant enzymes, including selenoprotein-P, gastrointestinal glutathion peroxidase, phospholipid hydroperoxide and thioredoxin reductase (Klein, 2004). The importance of selenium in the body is to prevent many disorders including atherosclerosis, specific cancers, arthritis, central nervous system pathologies, male infertility and altered immunological function and diseases related to aging (Patrick, 2004). On the other hand SLC is an ouabain-insensitive mode of sodium movement across red-cell membrane. This system promotes the exchange of sodium for sodium, lithium for lithium, or lithium for sodium (Canessa *et al*., 1980) and follows Michaelis-Menten kinetics (Thomas *et al*., 1995b; Hardman and Lant, 1996). Red Blood Cells of several mammalian species such as human, sheep, rabbit and bovine possess a countertransport system for $Li^+$and Na^+ (Duhm and Becker, 1979; Jennings *et al*., 1985). The activity of SLC is lower in blacks than whites and in white women than white men. Importantly, the activity of SLC reflects both genetic and environmental factors (Schork *et al*., 2002). During the period of 1992-1997 there have been more than 130 reports confirming the raise in SLC activity in primary hypertension, type 1 diabetes (with or without hypertension or nephropathy), hyperlipidaemia, normal pregnancy, hypertensive pregnancy, women taking steroids and in alcoholics (West *et al*., 1998). There are also reports that increased SLC activity has been shown to be associated with left ventricular hypertrophy, coronary heart disease (Gruska *et al*., 2003) and a biochemical marker or a predictor of hypertension risk in adults (Laurenzi *et al*., 1997; Vareesangthip *et al*., 2001; Mu *et al*., 2004). Therefore, the present study was designed to determine the effect of selenium on SLC activity and plasma parameters related to hypertension.

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MATERIALS AND METHODS

This study was performed in the year 2005 at the Department of Biochemistry, Isfahan University of Medical Sciences. Male New Zealand white rabbits (1350±50 g) were used for the experiments. They were purchased from Pasteur institute (Tehran-Iran), kept under standard experimental conditions (22-24°C, 40-60% relative humidity and light cycle coinciding with day light) and had free access to food and water. For *in vivo* acute dose, rabbits were divided in to two groups (5 in each). To the first group selenium (SeO_2) was administered intraperitoneally (250 μg kg^{-1}) every other day for 2 weeks. At the same time the control group was injected with deionized water. For chronic dose animals were administered with SeO_2 (125 μg kg^{-1}) on alternate days for 7 weeks. Same control group were used for the chronic doses. Before the experiment starts the rabbits were kept fasting overnight. They were anesthetized with intramuscular injection of 50 mg kg^{-1} ketamine (Lang-Lazdunski *et al*., 2000). Blood samples were collected from their hearts and poured into test tubes containing lithium heparin (125 IU/10 mL blood) (Mead *et al*., 1999; Mead *et al*., 2001). Then heparinized blood samples were centrifuged for 10 min at 2000 g and 4°C (Ragone *et al*., 1998). The packed cells washed with choline chloride and were used to measure the SLC activity. The plasma part of the sample was used to measure the concentrations of Cholesterol, Triglyceride, VLDL, LDL, HDL, Creatinine, Urea, Sodium and Potassium. For *in vitro* experiments, washed RBCs prepared from intact rabbits, were used. To evaluate the effect of selenium on SLC activity, cells were incubated with different concentrations of selenium (1 up to 100 μM). Kinetic parameters of the transporter were determined in the presence of selenium dioxide (100 μM) in the incubation media having different concentrations of sodium.

The erythrocyte SLC activity was determined according to the method of Canessa *et al*. (1980) with minor modification by Vareesangthip *et al*. (2004). The values of K_m and V_{max} of SLC were determined using Eadie-Hofstee method. The flux rate was plotted against flux/$[Na^+]_e$ and the maximum reaction velocity was determined from the intercept on y-axis and K_m from the slope. The net SLC activity then was determined by subtracting the rate of lithium efflux of erythrocytes measured in the choline medium from those measured in the sodium media. In this study the concentration of the lithium was measured by atomic absorption spectrophotometer (Philips, model PU 9100). Sodium and potassium were determined by flame photometer. Plasma parameters such as cholesterol, triglyceride, HDL, urea and creatinine measured with RI-1000 autoanalyzer using reliable kits.

The concentrations of VLDL and LDL were calculated using the following formulas:

$$\text{VLDL cholesterol (mg mL}^{-1}\text{)} = \frac{\text{Plasma triglycerides}}{5}$$

$$\text{LDL cholesterol (mg mL}^{-1}\text{)} = \text{Total cholesterol} - \text{HDL} - \frac{\text{Triglycerides}}{5}$$

Statistical analyses were performed using the SPSS statistical software program. Student's t-test was used for evaluation of the results. Data are reported as mean±SD and the significant values were considered as $p<0.05$ (Zerbini *et al*., 1995).

RESULTS

The results from *in vitro* studies performed in sodium medium (150 mM) show that selenium at low concentration can decrease SLC activity, but at high concentrations the activity gradually increases, as shown in Table 1.

Table 1: The effect of different concentrations of selenium on SLC activity in 150 mM sodium chloride medium, values are mean±SD from 5 repeated experiments

Selenium (μM)	SLC activity (mmol Li^+/L RBC/h)
0 (Control)	5.822±0.154
1	5.155±0.125*
5	5.711±0.134
10	5.844±0.152
25	5.999±0.185
50	6.244±0.169*
100	6.310±0.262*

* = Significant value $p<0.05$

Table 2: The effects of selenium (100 μM) on SLC activity in the presence of different sodium concentrations, values are mean±SD from 5 repeated experiments

Sodium medium (mM)	SLC activity (mmol Li^+/L RBC/h)	
	Treated group	Control group
0	0.00	0.00
10	1.140±0.123*	0.822±0.194
20	2.634±0.128*	2.280±0.156
40	4.280±0.188*	3.757±0.248
80	5.570±0.277*	4.972±0.290
120	6.131±0.220*	5.720±0.248
140	6.523±0.187*	6.094±0.277
150	6.543±0.189*	6.164±0.175

* = Significant value $p<0.05$

Table 3: Changes in the kinetic parameters of SLC induced by selenium (100 μM) at different sodium concentrations, values are mean±SD from 5 repeated experiments

Kinetic parameters	Treated group (Selenium)	Control group
V_{max} (mmol Li^+/L RBC/h)	8.90±0.539	8.370±0.797
K_m (mmol Na^+/L)	52.34±6.770	59.786±7.103
V_{max}/K_m	0.17±0.014*	0.140±0.010

* = Significant value $p<0.05$

Table 4: Changes in the SLC activity induced by acute and chronic dose of selenium, values are mean±SD from 5 different experiments

Sodium medium (mM)	SLC activity (mmol Li^+/L RBC/h)			
	Acute dose		Chronic dose	
	Treated group	Control group	Treated group	Control group
0	0.00	0.00	0.00	0.00
10	0.511±0.190*	1.218±0.268	1.796±0.353*	1.142±0.268
20	1.510±0.294*	2.115±0.170	2.321±0.248*	1.714±0.267
40	2.570±0.151*	2.948±0.304	3.178±0.309*	2.370±0.480
60	2.883±0.136*	3.845±0.128	3.702±0.357*	2.941±0.458
80	3.506±0.136*	4.037±0.374	3.923±0.373*	3.084±0.386
100	3.819±0.251*	4.357±0.283	4.227±0.465*	3.513±0.164
120	3.944±0.189*	4.550±0.342	4.421±0.415*	3.598±0.156
140	4.156±0.163*	4.742±0.310	4.559±0.353*	3.741±0.157
150	4.318±0.143*	4.754±0.325	4.669±0.408*	3.798±0.128

* = Significant value $p<0.05$

Table 5: Changes in the kinetic parameters of SLC induced by acute and chronic dose of selenium, values are mean±SD from 5 different experiments

Kinetic parameters	Acute dose		Chronic dose	
	Treated group	Control group	Treated group	Control group
V_{max} (mmol Li^+/L RBC/h)	5.900±0.333	6.000±0.199	5.040±0.204*	4.460±0.143
K_m (mmol Na^+/L)	62.110±5.390*	38.220±6.140	20.080±4.426*	30.760±5.840
V_{max}/K_m	0.095±0.012*	0.157±0.027	0.251±0.042*	0.145±0.021

* = Significant value $p<0.05$

Table 6: Changes in the plasma biochemical parameters induced by acute and chronic doses of selenium, values are mean±SD from 5 different experiments

Plasma parameters	Acute dose		Chronic dose	
	Treated group	Control group	Treated group	Control group
VLDL-C (mg dL^{-1})	12.20±1.240*	15.80±0.910	20.00±0.970*	18.20±1.020
LDL-C (mg dL^{-1})	76.12±3.410	80.20±5.300	98.40±12.29*	80.60±7.930
HDL-C (mg dL^{-1})	48.40±4.390	49.00±4.180	48.60±3.200	42.60±4.930
Cholesterol (mg dL^{-1})	134.00±7.110*	145.00±5.570	167.00±12.29*	140.60±12.14
Triglyceride (mg dL^{-1})	61.00±6.160*	79.00±4.530	100.00±4.850*	91.00±5.100
Urea (mg dL^{-1})	35.00±4.300	32.00±4.300	36.40±7.400	42.00±5.900
Creatinine (mg dL^{-1})	1.00±0.159	0.90±0.159	1.32±0.149	1.32±0.312
Sodium (mmol L^{-1})	138.00±4.300	139.00±5.200	160.00±9.390*	146.00±8.430
Potassium (mmol L^{-1})	4.96±0.241	4.68±0.444	3.84±0.404*	5.02±0.439

* = Significant value $p<0.05$

Using selenium (100 μM) at different concentrations of sodium indicate that SLC activity increases as compared with the controls (Table 2).

By applying Eadie-Hofstee method, it was shown that selenium could increase SLC activity by increasing the V_m/K_m value of the transporter (Table 3).

The *in vivo* studies indicate that, the acute dose of selenium decreases the SLC activity but the chronic dose increases it (Table 4).

Using Eadie-Hofstee method it was shown that the acute dose of selenium increased the K_m and decreased V_m/K_m of the transporter. But chronic dose of selenium decreased the K_m and increased V_m and V_m/K_m of the transporter (Table 5).

Acute dose of selenium decreased cholesterol, triglyceride and VLDL. But the chronic dose of this metal increased the plasma concentrations of cholesterol, triglyceride, VLDL, LDL and sodium while it decreased the potassium level (Table 6).

DISCUSSION

The results of this study show that selenium at different concentrations can have different effects on SLC activity. It is not exactly clear how selenium affect the countertransporter. But the protective effect of selenium against lipid peroxidation and a possible mechanism of action through glutathion peroxidase (GPX) or selenoprotein-P has been proposed (Thomson, 2004). Selenium can act either directly on protein sulfhydryl groups or indirectly via the action of proteins regulating the cellular redox state (Schweizer *et al*., 2004) thus reducing Reactive Oxygen Species (ROS) (Rayman, 2000) and could prevent damages to the unsaturated fatty acid of subcellular membranes caused by free radicals (Sieja and Talerczyk, 2004). Therefore, it is probable that selenium at low concentration decreases the production of ROS, which can react with the reduced thiol groups, or increase the generation of reducing equivalents

(Schork *et al*., 2002; Vaziri and Sica, 2004). Thiol groups are reported to be important for SLC activity. According to this report the kinetics of SLC are controlled by at least 2 types of thiol containing proteins. Type 1 controls the K_m for external sodium and type 2 controls the maximum velocity (V_{max}) of the transporter (Vareesangthip *et al*., 2004). Thus these key thiol groups could affect the physical properties and the function of integral transport proteins. Recent studies suggest that there is a cluster of thiol proteins that could modulate the behavior of the kinetics of SLC and the type 1 thiol protein is a member of this complex. It seems likely that this complex interacts with both the membrane cytoskeletal components and lipid bilayer and alterations of this protein complex might lead to the abnormalities in kinetics of SLC (Vareesangthip *et al*., 2000, 2004). Among all the cation transporter systems, sodium-lithium countertransport is most consistently found elevated in patients with essential hypertension, as well as in their normotensive first-degree relatives (Batuman *et al*., 1989). Thus any agent that changes the activity of SLC might be involved in the induction of hypertension (Ani *et al*., 2006, 2007). In our study high concentrations of selenium significantly increased SLC activity and V_{max}/K_m ratio of the transporter *in vitro*. This finding is in good agreement with the reports that in hypertension the ratio of V_{max}/K_m of SLC is increased and this may help to explain the mechanisms underlying selenium induced hypertension (Thomas *et al*., 1995a). *In vivo* experiments using acute dose of selenium showed a significant decrease in SLC activity in all sodium containing incubation media. This seems to be due to the decrease in V_{max}/K_m and increase in K_m of the transporter. The acute dose of selenium also decreased the plasma levels of VLDL, cholesterol and triglyceride significantly. This may be due to the role of the enzyme Phospholipid hydroperoxide glutathione peroxidase which functions to reduce fatty acid hydroperoxides and hydroperoxides of cholesterol and cholesterol ester in membrane and LDL. It also prevents LDL oxidation, which is important in the prevention of cardiovascular disease (Holben and Smith, 1999). Following chronic treatment with selenium, a significant increase in SLC activity was found. This was associated with increase in V_{max}, V_{max}/K_m ratio and decrease in K_m. This is similar to the study showing that in essential hypertension, the K_m of SLC is lower while V_{max}/K_m is higher (Rutherford *et al*., 1997; Mead *et al*., 1999). Also chronic dose of selenium increased plasma levels of VLDL, LDL, cholesterol, triglyceride and sodium, but decreased the potassium. Sodium and Potassium changes can be explained by the reports indicating that there is an increase in plasma aldosterone in rats treated with selenium (Nishiyama *et al*., 1987). Studies also showed that the ratio of Na^+ /K^+ was higher in hypertension than the control (Liu *et al*., 2004).

So, chronic exposure to selenium may be toxic because it can change the activity of SLC and plasma parameters and may increase the blood pressure. We conclude that selenium at higher concentration and chronic dose increases the SLC activity and also plasma levels of lipids. But at low concentration and acute dose by reducing the activity may have a beneficial effect on human health. The limitation of this study is the lack of enough investigations regarding the effects of selenium on SLC activity, which could conforms our findings.

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