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Changes in Sodium-lithium Countertransport Activity Following Aluminium Treatment

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Abstract: The relationship between aluminium and rabbit erythrocyte sodium-lithium countertransport (SLC) activity has been investigated *in vivo* and *in vitro*. Male rabbits (1350±50 g) were used for the experiments. *In vivo* studies were performed by intrapritoneally injection of aluminium chloride ($ALCL_3.6H_2O$) every other day for 2 weeks (25 mg kg^{-1} body weight as acute dose) and for 7 weeks (12.5 mg kg^{-1} as chronic dose) and then the activity of SLC was evaluated. It was shown that aluminium increased maximal efflux rate, V_{max}/K_m, V_{max} and decreased K_m of the transporter leading to increasing of the activity when compared with control group. The effects of incubation times (30, 60 and 90 min), different sodium concentrations (0 upto 150 mM) and different aluminium concentrations (2.5 upto 200 μM) on SLC activity were studied *in vitro* which indicated a positive relationship between the activation of erythrocyte SLC and incubation time, sodium and aluminium concentrations ($p<0.05$). These observations suggest that abnormalities in SLC activity may be a causal factor in the pathogenesis of aluminium-induced hypertension.

Key words: Aluminium, sodium-lithium countertransport, hypertension, erythrocyte

INTRODUCTION

There are many reports indicating the changes in the biochemical characteristics of SLC in relation to hypertension (Van Norren *et al*., 1998). It is also well documented that some trace elements which are related to hypertension could affect SLC activity (Kedzierska *et al*., 2005).

Al is the third most abundant metallic element in the earth's crust (Moshtaghie, 1993; Flaten, 2001) and is found in many foodstuffs including corn, yellow cheese, salt, herbs, spices, tea (Yousef, 2004) and in drinking water and other beverage as well as in soil and as dust in the air (Flaten, 2001). High blood levels of Al is seen in dialysis patients (Cannata-Andia and Fernandez-Martin, 2002, Moshtaghie, 1993) which is resulted from renal function impairment. This toxic element has been suggested to be a causative factor for dialysis encephalopathy, dialysis osteodystrophy and a microcytic hypochromic anemia (Moshtaghie, 1994) and Alzheimer's disease (Moshtaghie, 1993; Flaten, 2001). In individuals without renal disease, high level of plasma Al was associated with essential hypertension (Vanholder *et al*., 2002) which justifies any study about it's mechanism of action.

Na^+/Li^+ countertransport (Na^+/Li^+ CT) across the red blood cell membrane was first described by Tosteson's group in 1975 (Van Norren *et al*., 1998) which binds either Li^+ or Na^+ on one side of the membrane and exchanges it for either Li^+ or Na^+ on the opposite direction in a stoichiometric ratio of one to one (Trelewicz *et al*., 1997; Semplicini *et al*., 2003).

The maximum rate of transport shows inherited differences, but it acts in a manner that follows Michaelis-Menten kinetics (Canessa *et al*., 1980; Hardman and Lant, 1996).

Although the physiological significance of this transporter is not fully understood but some correlations have been found between SLC activity and many metabolic disorders.

In 1980, Canessa *et al*. (1980) reported that an association exists between elevated activity of erythrocyte SLC, assayed under carefully defined conditions and essential hypertension, an observation that has been confirmed repeatedly (Weder *et al*., 2003).

The same correlation was also noted in hyperlipidemia and related cardiovascular disease (Romero *et al*., 2002; Carr *et al*., 1990) and in patients with resting diastolic blood pressure and maximal systolic blood pressure at peak exercise (Hardman *et al*., 1995).

Based upon these observation, this study was designed to the investigate the effect of aluminium on SLC activity both *in vivo* and *in vitro*.

Present results are considered to be very important in understanding the mechanism by which aluminium induces hypertension.

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MATERIALS AND METHODS

This study was performed last winter in the Department of Biochemistry, Isfahan University of Medical Sciences. Male New Zealand white Rabbits 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 h) and had free access to food and water.

For *in vivo* acute dose, animals (1350±50 g) were divided in two groups (5 animals in each group).

To the first group (experimental) Aluminium (as aluminium chloride) was administered intraperitoneally (25 mg kg^{-1} every other day for 2 weeks). Control group received deionized water at the same time.

For chronic dose the first group (experimental) was administered 12.5 mg kg^{-1} every other day for 7 weeks. Control group received deionized water at the same time.

Animals were fasted overnight before the experiment. At the time of the experiment blood samples were collected and washed RBC were prepared for determination of SLC activity.

For *in vitro* experiments, washed RBC prepared from intact animals, were used. Cells were incubated for 30, 60 and 90 min in mediums containing either of 150 mM choline chloride or 150 mM of sodium chloride before measuring SLC activity.

Cells were then incubated for 60 min in mediums containing either of choline chloride or sodium chloride at the sum concentration of 150 mM. This gives the different concentrations of Na^+ from 0.0 upto 150 mM. To study the effect of aluminium on SLC activity cells were incubated in the presence of different aluminium concentrations (2.5 upto 200 μM).

The activity of the erythrocyte SLC was determined according to the method of Canessa *et al.* (1980) with minor modification (Vareesangthip *et al.*, 2004; Senior *et al.*, 2000; Mead *et al.*, 1999; Ragone *et al.*, 1998; Stiefel *et al.*, 2001). The kinetic parameters of K_m and V_{max} of SLC were determined according to the 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 SLC maximal efflux rate was estimated from the differences between the Li^+ efflux into sodium containing and sodium free media.

Statistically, t-test was used to express the significance of the differences.

RESULTS

Preliminary experiments indicated that the activity of SLC is time and dose dependent, the results of which are shown is Table 1 and 2.

Table 1: The activity of SLC at different incubation time

	SLC (mmol Li^+/ L RBC h^{-1})	
Incubation time (min)	150 mM sodium chloride medium	150 mM choline chloride medium
30	4.501±0.467*	1.209±0.158
60	7.456±0.492*	1.53±0.242*
90	9.482±0.51*	1.884±0.239*

Figures are mean±SD of five experiments. As shown the activity of SLC was significantly higher (* = $p<0.05$) in the presence of Na^+

Table 2: The effects of different concentrations of Na^+ on SLC activity

Na^+ concentrations (mM)	SLC activity (mmol Li/L RBC h^{-1})
0	1.53±0.242
10	2.307±0.216*
20	2.813±0.268*
40	4.45±0.325*
60	5.43±0.414*
80	6.038±0.398 *
100	6.443±0.467*
120	6.95±0.52*
140	7.203±0.524*
150	7.456±0.492*

Results are mean±SD of 5 experiments. * values are significantly different from control ($p<0.05$)

Table 3: The effect of different concentrations of Aluminium on SLC activity in the medium containing 150 mM sodium chloride

Aluminium (Al) concentration (μM)	SLC activity (mmol Li^+/L RBC h^{-1})
0	7.27±0.225
2.5	7.776±0.225*
5	7.954±0.262*
10	8.004±0.242*
25	8.029±0.279*
50	8.130±0.279*
100	8.232±0.291*
200	8.308±0.268*

Values are mean±SD of 5 experiments. * indicate that values are significantly different ($p<0.05$)

As shown in Table 1 the activity of SLC (based on Li^+ efflux) is increased with time and is totally Na^+ dependent.

Results shown in Table 2 indicate the effect of different concentrations of Na^+ on SLC activity.

Increase in Na^+ concentrations of the medium lead to significant increase in Li^+ efflux compared to the control.

The effects of different concentrations of Aluminium (Al) on SLC activity are shown in Table 3. As seen in this Table 3, addition of Aluminium to the medium containing sodium chloride (150 mM) increased Li^+ efflux significantly when compared to the control.

Results obtained from *in vivo* study with acute dose are shown in Table 4. Red blood cells prepared from animals pretreated with Aluminium chloride were incubated in the presence of difference concentration of sodium ions. In all cases the SLC activity was significantly higher in treated cells, indicating that the kinetic parameters of the transporters are positively changed.

Table 4: SLC activity in red blood cells prepared from animals pretreated with aluminium chloride (25 mg kg^{-1}) in comparison with control group

	SLC activity (mmol Li^+/L RBC h^{-1})	
Na^+ concentration (mM)	Treated group	Control group
0	3.17±0.245*	2.595±0.144
10	4.351±0.323*	3.813±0.374
20	5.554±0.306*	4.71±0.227
40	6.974±0.369*	5.543±0.345
60	7.107±0.406*	6.44±0.144
80	7.287±0.287*	6.632±0.447
100	7. 42±0.389*	6.952±0.371
120	8.042±0.288*	7.145±0.408
140	8.175±0.237*	7.337±0.41
150	8.289±0.349*	7.349±0.399

Values are mean±SD of 5 experiments. * indicate that values are significantly different ($p<0.05$)

Table 5: Changes in the kinetic parameters of SLC induced by Aluminium in acute dose. Treated cells were obtained from animals injected with *Aluminium chloride* (25 mg kg^{-1}) for 2 weeks, which compared with control group

Parameter	Treated group	Control group
V_{max} (mmol Li^+/L RBC h^{-1})	8.500	7.700
K_m (mmol Na^+L^{-1})	9.884	11.160
V_{max} / K_m	0.860	0.690
Maximum efflux rate (mmol Li^+/L RBC h^{-1})	5.119	4.754

Table 6: SLC activity in red blood cells prepared from animals pretreated with chronic dose of aluminium chloride (12.5 mg kg^{-1}) for 7 weeks compared with control group

	SLC activity (mmol Li^+ /L RBC h^{-1})	
Na^+ concentration (mM)	Treated group	Control group
0	1.578±0.146	1.461±0.078
10	3.852±0.561*	2.603±0.329
20	4.20±0.515*	3.175±0.329
40	5.338±0.596*	3.831±0.544
60	6.212±0.488*	4.402±0.474
80	6.504±0.52*	4.545±0.452
100	6.678±0.692*	4.974±0.226
120	6.766±0.748*	5.059±0.079
140	7.524±0.51*	5.202±0.163
150	7.64±0.394*	5.259±0.143

Values are mean±SD of 5 experiments. * indicate that values are significantly different ($p<0.05$)

Table 7: Changes in the kinetic parameters of SLC induced by *Aluminium* in *chronic* dose. Treated cells were obtained from animals chronically injected with aluminium chloride (12.5 mg kg^{-1}) for 7 weeks and compared with control group

Parameter	Treated group	Control group
V_{max} (mmol Li^+/L RBC h^{-1})	7.500	5.400
K_m (mmol Na^+ /L^{-1})	11.190	12.140
V_{max} / K_m	0.670	0.445
Maximum efflux rate (mmol Li^+ L RBC h^{-1})	6.062	3.798

This was clearly explained using Eadie-Hofstee method, Thus Aluminium increased SLC activity by increasing the V_{max} of the transporter and decreasing the K_m value, which were shown in Table 5.

Results of *in vivo* chronic dose results are shown in Table 6. Red blood cells prepared from animals pretreated with aluminium chloride for long time, were incubated in the presence of difference concentration of sodium ions. Similar results obtained indicating the activatory effect of aluminium.

Using Eadie-Hofstee method again, it appeared that Aluminium increased SLC activity by increasing the Vmax of the transporter and decreasing the K_m value, the results of which are shown in Table 7.

DISCUSSION

The relationship between SLC activity and hypertension is well documented (Van Norren *et al*., 1998; Weder *et al*., 2003; Dunn *et al*., 2003).

Among all 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 and is therefore proposed as a good marker for genetically induced hypertension (Batuman *et al*., 1989).

The present study showed that Al increased the activity of countertransport in RBC membrane and this may be a good implication of aluminium involvement in the induction of hypertension. This is in agreement with reports that in individuals without renal disease, high level of plasma Al was associated with essential hypertension (Vanholder *et al*., 2002).

Aluminium is also suggested to potentiate the inhibition of the Na^+/K^+ ATPase activity following lipid peroxidation (Amador *et al*., 2001). This is achieved by binding to the polar regions of phospholipids or proteins on the plasma membrane leading to structural and functional alterations on membrane permeability and transport process (Martinez-Estevez *et al*., 2003).

Among the various effects induced by Al in biological systems, either *in vitro* or *in vivo*, is the destruction of membrane polyunsaturated fatty acid depending on oxygen free radical (Mossor-Pietraszewska, 2001).

Aluminium is among many agents that are able to generate Reactive Oxygen Species (ROS) (Bondy and Kirstein, 1996).

ROS include superoxide radical ($O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) that are produced as by-products during membrane linked electron transport activities as well as by a number of other metabolic pathways (Verma and Dubey, 2003).

Recent studies suggest that ROS are centrally involved in the pathophysiology of hypertension in laboratory animals and in human beings following the

interaction in some way with chemical groups of transporter molecules leading to activity change (Schork *et al*., 2002). Data obtained in cortex homogenates indicated that both glutathion (GSH) and glutathion S-transferase (GST) activity were significantly decreased with a concomitant increase in lipid peroxidation (LPO) in Al-treated rats (Mahieu *et al*., 2003).

A decrease in the activity of GST and sulfhydryl groups in plasma, liver, testes and kidney of rabbits treated with $AlCl_3$ is reported by Yousef (2004).

Thiol containing proteins generally play a major role in cellular oxidative pathway and this explains why the alteration in oxidative status may lead to atherogenesis, hypertension and other metabolic complication.

Thiol groups are also reported to be important for SLC activity and it was demonstrated that the kinetics of SLC activity are controlled by at least 2 types of thiol containing proteins. Type 1 thiol group controls the K_m for external sodium and the type 2-thiol group controls the maximum velocity (V_{max}) of this transporter (Vareesangthip *et al*., 2004).

Therefore aluminium by increasing the production of ROS, which may react with the reduced thiol groups, or by inhibiting the generation of reducing equivalents probably affect SLC activity.

Results also show that there is a positive relationship between SLC activity and sodium concentration in the incubation medium and the period of incubation.

Sodium has already been reported to be essential for the activity of many transporting systems (Prasad and Ganapathy, 2000; Takeda *et al*., 1999; Van Geest and Lolkema, 1996).

The SLC maximal efflux rate was increased in cells under aluminium treatment when compared with control and this increased activity is shown to be due to the increased V_{max} of the system and the reduction in K_m value of the transporter. 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 Aluminium induced hypertension (Thomas *et al*., 1995). In this study because Vmax of the system is increased concomitantly with the decrease in Km of the transporter, thus the ratio of V_{max}/K_m gave a much clearer distinction between two studied groups.

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