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 intraperitoneally injection of aluminium chloride (ALCL₃, 6H₂O) 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, $V_{	ext{max}}/K_m$, $V_{	ext{max}}$ 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 up to 150 mM) and different aluminium concentrations (2.5 up to 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-Ania and Fernandez-Marin, 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 its 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 Km and Vmax of SLC were determined according to the Eadie-Hofstee method.

The flux rate was plotted against flux/[Na⁺], and the maximum reaction velocity was determined from the intercept on y-axis and Km 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 in Table 1 and 2.

<table>
<thead>
<tr>
<th>Table 1: The activity of SLC at different incubation time</th>
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<tr>
<td>SLC (nmol Li⁺/ L RBC h⁻¹)</td>
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<td>Incubation time (min)</td>
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Figures are mean±SD of five experiments. As shown the activity of SLC was significantly higher (* = p<0.05) in the presence of Na⁺.

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<th>Table 2: The effects of different concentrations of Na⁺ on SLC activity</th>
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<td>Na⁺ concentrations (mM)</td>
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Results are mean±SD of 5 experiments. * values are significantly different from control (p<0.05).

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<th>Table 3: The effect of different concentrations of Aluminum on SLC activity in the medium containing 150 mM sodium chloride</th>
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<td>Aluminum (Al) concentration (μM)</td>
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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.
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 Km 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 countercurrent transport 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 AI increased the activity of countercurrent transport 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 AI 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 AI in biological systems, either in vitro or in vivo, is the destruction of membrane polyunsaturated fatty acid depending on oxygen free radical (Mossor-Pietraszwka, 2001).

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

ROS include superoxide radical \( \left( \text{O}_2^- \right) \), hydroxyl radical \( \text{OH}^- \) and hydrogen peroxide \( \text{H}_2\text{O}_2 \) that are produced 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, 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 (Vareesanghip 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 $V_{max}$ of the system is increased concomitantly with the decrease in $K_m$ of the transporter, thus the ratio of $V_{max}/K_m$ gave a much clearer distinction between two studied groups.

REFERENCES


