

THE EFFECT OF LEAD ON RED BLOOD CELL SODIUM-LITHIUM COUNTER TRANSPORT AND INDUCTION OF HYPERTENSION

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Abstract: *The relationship between lead and rabbit erythrocyte lithium efflux (as a sodium lithium countertransport activity) has been investigated in vivo and in vitro. Male rabbits (1350 ± 50g) were used for the experiments. In vivo studies were performed by intraperitoneally injection of lead acetate [(CH₃COO) 2Pb.3H₂O] every other day for 2 weeks (13mg/kg body weight as an acute dose) and for 7 weeks (6.5mg/kg as chronic dose) and then lithium efflux was evaluated. It was shown that lead increased maximal efflux rate, lithium efflux, Vmax/Km, Vmax and decreased Km 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 to 150mM) and different lead concentrations (2.5 to 100µM) on lithium efflux were studied in vitro which indicated a positive relationship between the activation of erythrocyte lithium efflux and incubation time, sodium and lead concentrations (p<0.05). These observations suggest that abnormalities in lithium efflux may be a causal factor in the pathogenesis of lead-induced hypertension.*

Key words: Sodium-Lithium, Counter transport, Lead

INTRODUCTION

There are many reports indicating the changes in the biochemical characteristics of sodium lithium countertransport activity (SLC) in relation to hypertension [1]. Further, some trace elements which are related to hypertension could affect SLC activity [2]. Due to this relationship between SLC activity and hypertension and the involvement of trace metals it is plausible to investigate the effect of lead on the activity of SLC (as a lithium efflux). Lead is one of the first metals to be mined and smelted. Its uses can be documented for over 8,000 years [3]. It has been used worldwide since ancient time for its malleability, resistance to corrosion, and low melting point [4] although it appeared soon to be a very dangerous toxic agent for all living organism [5]. In fact, lead exposure was the third most common hazardous occupational (after radiation and organic solvents) noted in developed countries. Lead is not a normal constituent of body and causes serious damage in all human tissues [6].

Lead is toxic to many organ systems of human body, such as the central and peripheral nervous system, the red blood cells, the kidney, the cardiovascular system, and the male and female reproductive organs. The absorption and transport of lead by crops are of great concern, especially its accumulation in the edible part [4]. It is logical, therefore, to study the toxic effect of this toxic metal on animal metabolic systems. Na⁺/Li⁺ countertransport (Na⁺/Li⁺ CT) across the red blood cell membrane was first described by the Tosteson's group in 1975 [1] and showed to be a heteroexchanger that operates in either direction across the cell membrane. SLC in erythrocytes represents one of the membrane sodium transport systems and binds either Li⁺ or Na⁺ on one side of the membrane and it exchanges the transported species for either Li⁺ or Na⁺ on the opposite in a stoichiometric ratio of one to one [7,8]. The activity of SLC was farther shown to be ouabain and amiloride insensitive but can be inhibited by phloretin. It does not require the presence of cellular ATP for its activity [1]. The maximum rate of transport

shows inherited differences [9], but it acts in a manner that follows michaelis-menten kinetics [10].

In 1980, Canessa et al. [11] reported that an association exists between elevated activity of erythrocyte SLC, assayed under carefully defined conditions and essential hypertension. There is a positive relation between SLC activity and fasting blood insulin levels as well as hypertension and hyperlipidemia. This could well explain why raised SLC activity has been associated with a family history of both hypertension, hyperlipidemia and related cardiovascular disease [12,13]. The same correlation was also noted with resting diastolic blood pressure and maximal systolic blood pressure at peak exercise [14]. Based upon these observation, this study was designed to the investigate the effect of lead on lithium efflux both *in vivo* and *in vitro*. The investigation is important in understanding the mechanism by which lead induces hypertension.

MATERIALS AND METHODS

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 with coinciding with day light h) and had free access to food and water. For *in vivo* acute dose, animals (1350 ± 50g) were divided in four groups (5 animals in each group).

To the first group (experimental), lead (as lead acetate) was administered intraperitoneally (13 mg/kg every other day for 2 weeks). Control (2nd group) received deionized water at the same time. For chronic dose the 3rd group (experimental) was administered 6.5mg/kg every other day for 7 weeks. Control (4th 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 lithium efflux. 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 150mM of sodium chloride before measuring lithium efflux. 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 to 150 mM. To study the effect of lead

on lithium efflux cells were incubated in the presence of different lead concentrations (2.5 up to 100µM).

The erythrocyte lithium efflux was determined according to the method of Canessa et al. with minor modification [15-19]. Briefly after collecting 10ml of the blood into a tube containing heparin (125 IU), it was centrifuged at 2000g for 10 min at 4 °C and the packed erythrocytes were repeatedly washed with ice-cold choline chloride solution containing (139 mmol/l choline chloride, 1mmol/l MgCl₂, 10 mmol/l glucose, 10 mmol/l TRIS-Mops [PH 7.4] with the final osmolality of 290 ± 2 mosmol / kg). Aliquots of packed erythrocyte (2ml) were resuspended in 8ml of lithium loading buffer solution (140 mmol/l lithium chloride, 10 mmol/l lithium carbonate. 10 mmol/l glucose, 10mmol/l TRIS-Mops at pH=7.5 and osmolality 290 ± 2 mosmol/kg). Loading solution was pre-gassed with 95% oxygen and 5% carbon dioxide until the lithium carbonate was completely dissolved, generally 1.5 hours at 37°C. The loaded cells were then washed five times with ice-cold choline chloride solution to remove extracellular lithium. After the final washing, the packed cell volume of the erythrocyte was measured using a microhematocrite technique. Aliquots (0.2-0.25 ml) of packed cells were incubated in 4ml of choline ouabain medium (as above but containing 0.1mmol/l ouabain and 150mmol/l choline chloride) with the range of sodium concentrations as 0 to 150 mmol/l prepared by replacing the choline with sodium-ouabain medium (150mmol/l NaCl, 1mmol/l MgCl₂, 10mmol/l glucose, and 10mmol/l TRIS-Mops [pH=7.4]; osmolality 290± 2 mosmol/kg, in the presence of 0.1mmol/l ouabain).

After centrifugation of the mixtures at 2000g for 3 min., the lithium concentration in the supernatant was determined by atomic absorption spectrophotometry (Philips, model PU 9100). The kinetic parameters Km and Vmax of SLC determined essentially 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 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, as shown in tables 1 and 2,

Table 1: The lithium efflux at different incubation time. Figures are mean \pm SD of five experiments. As shown the lithium efflux was significantly higher (* = $P < 0.05$) in the presence of Na^+ . Lithium efflux (mmol Li^+ /L RBC)

Incubation time (min)	150mM sodium chloride medium	150 mM choline chloride medium
30	4.501 \pm 0.467*	1.209 \pm 0.158
60	7.456 \pm 0.492*	1.53 \pm 0.242 *
90	9.482 \pm 0.51*	1.884 \pm 0.239 *

Table 2: The effects of Na^+ concentrations on lithium efflux. Results are mean \pm SD of 5 experiments. Starred values are significantly different from control. Control medium contained 150 mM choline chlorides with no sodium ions, which were replaced by sodium up to 150 mM, see text for more details.

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

Table 3: The effect of different concentration of lead on lithium efflux in 150mM sodium chloride medium. Values are mean \pm SD of 5 experiments. Stars indicate that values are significantly different ($P < 0.05$).

Lead concentration (μM)	Lithium efflux (mmol Li^+ / L RBC/h)
0	7.43 \pm 0.185
2.5	7.83 \pm 0.263 *
5	8.03 \pm 0.152 *
12.5	8.096 \pm 0.262*
25	8.185 \pm 0.262*
50	8.23 \pm 0.311*
100	8.274 \pm 0.37*

Table 4: Lithium efflux in red blood cells prepared from animals pretreated with lead acetate (13mg/kg) in comparison with control group. Values are mean \pm SD of 5 experiments. Stars indicate that values are significantly different ($P < 0.05$). Lithium efflux (mmol Li^+ / L RBC /h)

Na^+ concentration (mM)	Treated group	Control group
0	2.637 \pm 0.245	2.595 \pm 0.144
10	4.505 \pm 0.271*	3.813 \pm 0.374
20	5.039 \pm 0.211*	4.71 \pm 0.227
40	6.22 \pm 0.438*	5.543 \pm 0.345
60	7.107 \pm 0.306*	6.44 \pm 0.144
80	7.908 \pm 0.502*	6.632 \pm 0.447
100	8.042 \pm 0.559*	6.952 \pm 0.371
120	8.175 \pm 0.354*	7.145 \pm 0.408
140	8.308 \pm 0.324*	7.337 \pm 0.41
150	8.375 \pm 0.279*	7.349 \pm 0.399

indicated that the lithium efflux is time and dose dependent. Further, the lithium efflux is increased with time and is totally Na^+ dependent (Table 2). Table 2 reveals the effect of different concentrations of Na^+

Table 5: Changes in the kinetic parameters of SLC induced by lead in acute dose. Treated cells were obtained from animals injected with lead acetate (13mg/kg) for 2 weeks, which compared with control group.

Parameter	Lead Injection group	Control group
V_{max} (mmol Li^+ /L RBC/h)	8.65	7.7
K_m (mmol Na^+ /L)	10.68	11.16
V_{max} / K_m	0.81	0.69
Maximum efflux rate (mmol Li^+ /L RBC/h)	5.738	4.754

Table 6: Lithium efflux in red blood cells prepared from animals pretreated with lead acetate (6.5mg/kg) in comparison with control group. Values are mean \pm SD of 5 experiments. Stars indicate that values are significantly different ($P < 0.05$). Lithium efflux (mmol Li^+ / L RBC /h)

Na^+ concentration (mM)	Treated group	Control group
0	1.471 \pm 0.079	1.461 \pm 0.078
10	3.627 \pm 0.36*	2.603 \pm 0.329
20	4.23 \pm 0.219*	3.175 \pm 0.329
40	4.979 \pm 0.257*	3.831 \pm 0.544
60	5.41 \pm 0.471*	4.402 \pm 0.474
80	6.416 \pm 0.258*	4.545 \pm 0.452
100	6.732 \pm 0.455*	4.974 \pm 0.226
120	6.991 \pm 0.214*	5.059 \pm 0.079
140	7.106 \pm 0.331*	5.202 \pm 0.163
150	7.22 \pm 0.331*	5.259 \pm 0.143

Table 7: Changes in the kinetic parameters of lithium efflux by lead in chronic dose. Treated cells were obtained from animals injected with lead acetate (6.5mg/kg) for 7 weeks, which compared with control group.

Parameter	Lead Injection group	Control group
V_{max} (mmol Li^+ /L RBC/h)	7.3	5.4
K_m (mmol Na^+ /L)	11.59	12.14
V_{max} / K_m	0.63	0.445
Maximum efflux rate (mmol Li^+ /L RBC/h)	5.75	3.798

on lithium efflux. Increase in Na^+ concentrations of the medium lead to significant increase in Li^+ efflux as compared to the control. Further study demonstrated that the addition of lead to the sodium chloride medium (150mM) increased Li^+ efflux significantly when compared with the control (Table 3).

In vivo acute dose results are shown in table 4. Red blood cells were prepared from animals pretreated with lead acetate incubated in the presence of difference concentration of sodium ions. In all cases the lithium efflux was significantly higher in treated cells (Table 4). Using Eadie-Hofstee method, it appeared that lead increased lithium efflux by increasing the V_{max} of the transporter and decreasing the K_m value (Table 5).

In vivo chronic dose results of red blood cells prepared from animals pretreated with lead acetate incubated in the presence of difference concentration of sodium ions are shown in table 6 and the results are self explanatory. Application of Eadie-Hofstee method showed that lead increased the lithium efflux by increasing the V_{max} of the transporter and decreasing the K_m value (Table 7). Almost all results are statistically significant.

DISCUSSION

Despite some controversy about the biological activity of lead ions, there is increasing evidence linking this toxic metal to hypertension [20]. However the mechanism by which lead might cause hypertension is not clearly defined. There are also some reports indicating a relationship between SLC activity and hypertension. Results of this study show that lead increased the activity of countertransport in RBC membrane.

The effects of lead on some membrane enzyme systems have been documented. For example it has been shown that lead inhibits the activity of red blood cell Atlases and vascular smooth muscle Na^+/K^+ ATPases [21,22]. The effect of lead on red blood cell proton transport systems explains the pathophysiology of lead induced hypertension. 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. Therefore, it is proposed as a good marker for genetically induced hypertension [21]. The Na^+/H^+ antiporter requires Na^+ and H^+ , where the external cation binding sites also accept lithium. That is why the Na^+/H^+ antiporter can be measured indirectly via sodium-lithium countertransport [23].

Our findings revealed that lead stimulated the activity of SLC and this may be a good indication of lead involvement in the induction of hypertension. This result is in accordance with the earlier reports that lead stimulated SLC *in vitro* [21]. Lead is known to induce a broad range of physiological, biochemical and behavioral dysfunction and many studies have explored the mechanism and symptoms of this toxic metal through the years but recent studies have focused on lead as a potential agent for the production of reactive oxygen species (ROS) leading to the elevation of oxidative stress.

ROS include superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH^{\cdot}) 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 [24-26]. Recent studies suggest that ROS are centrally involved in the pathophysiology of hypertension in laboratory animals and human beings. Thus it is probable that ROS interact in some way with chemical groups in the molecule leading to activity change [27]. 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. Thiol groups are reported to be important for SLC activity and Thomas et al. [28] demonstrated that the kinetics of SLC 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 [15]. Therefore, lead by increasing the production of ROS, which may react with the reduced thiol groups, or inhibiting the generation of reducing equivalents [29] probably can affect lithium efflux.

Results also show that there is a positive relationship between lithium efflux and sodium and lead 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 (30-32). The lithium efflux and maximal efflux rate was increased in cells under lead 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 lead induced hypertension [28]. 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 the two-studied groups.

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