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Original Research

The Influence of Different Doses of Lithium Administered in Drinking Water on Lipid Peroxidation and the Activity of Antioxidant Enzymes in Rats

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Abstract

Lithium compounds are widely employed in medicine. However, both its positive and negative effects have been revealed. We have tried to evaluate the influence of oral administration of different Li_2CO_3 doses for a period of 8 weeks on the elements of antioxidant status in male rats. The activity of two main antioxidant enzymes: glutathione peroxidase (GPx) and superoxide dismutase (SOD) as well as the concentration of malonyldialdehyde (MDA) – the marker of lipid peroxidation – have been studied. In blood no significant changes of both lipid peroxidation levels and enzyme activity have been found. In tissues enzymes' activities have decreased, significantly in liver (SOD) and kidney (GPx and SOD). No evidence of lithium-induced lipid peroxidation in the tissues has been observed. In the case of brain the Li protective effect against lipid peroxidation has been displayed. In conclusion, the antioxidant status has not been affected in blood and suppressed in tissues as a consequence of Li exposure. The tissue of brain has seemed to be the least attacked organ.

Keywords: lithium, lipid peroxidation, glutathione peroxidase, superoxide dismutase, male rat.

Introduction

Lithium compounds have been employed in medicine for more than 50 years [1], particularly in psychiatry. However, a great deal of new fields of its action have been discovered, which has resulted in attempts toward using Li therapy in cases of thyroid diseases [2-4], neurodegenerative illnesses [5-7] and as a topical drug in dermatology [8]. Lithium has also been found to possess an insulin-like action [9, 10]. As for its psychiatric employment, the newest works have underlined its utmost efficacy as an adjuvant in augmentation of antidepressants [11]. The marked effectiveness of this element in prevention of bipolar disorder relapse and of suicide has also been reported [12]. On the other hand, many studies regarding its numerable adverse effects have been published [13-18].

The reactive oxygen species (ROS) are particles generated in metabolic processes as a consequence of incomplete reduction of the oxygen molecule [19]. Numerous investigations have revealed that they cause damage to the organism and can be involved in pathogenesis of the most severe diseases [20-24]. One of the oxidative processes is lipid peroxidation, succeeded by the increase of malondialdehyde (MDA) concentration [22, 23]. The organisms' defence against oxidative damage is the antioxidant barrier – the system consisting of high- and low-molecule-substances possessing the ability to scavenge ROS [23, 25].

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Having regard to the wide medical application of lithium and the importance of balance between anti- and prooxidative processes, we have tried to evaluate the influence of the oral administration of different Li_2CO_3 doses on the elements of antioxidant status in male rats. Our study has included activities of the main antioxidant enzymes – glutathione peroxidase (GPx) and superoxide dismutase (SOD), as well as the concentration of MDA – the marker of lipid peroxidation.

Materials and Methods

Our study was performed on two-month-old male Wistar rats (180–220 g). The animals were divided into seven groups (six animals each): control group (C) received redistilled water, six tested groups were provided with water solutions of Li_2CO_3, as the only drinking fluids. The concentrations of the solutions were established as follows: 0.7; 1.4; 2.6; 3.6; 7.1 and 10.7 mmol $Li^+ \cdot dm^{-3}$. The animals were offered standard feed LSM and drinking fluids ad libitum. Each animal was put in a separate cage and consumption of the provided fluid as well as body weight was monitored every day.

Using the obtained data the daily lithium intake was calculated for each rat:

$$\text{daily Li intake [mg Li} \cdot \text{kg}^{-1} \text{ b.w.]} = V \cdot c \cdot m^{-1}$$

V – consumption of the provided fluid [ml]
c – concentration of the provided fluid [mg $Li \cdot ml^{-1}$]
m – body weight [kg]

After the end of the experiment the average value was estimated for each rat and the mean of the daily Li intake in each group was calculated. After eight weeks, rats were sacrificed under ketamine narcosis and blood from the heart as well as the tissues of liver, kidney and brain were collected. 10% (w/v) tissue homogenates were prepared in 0.1 $mol \cdot dm^{-3}$ Tris-HCl buffer, pH=7.4. Supernatants were obtained by centrifuging at 5000 x g for 30 min. In blood and supernatants GPx activities were determined using RANSEL kit produced by RANDOX according to Paglia and Valentine method [26] and expressed in $U \cdot ml^{-1}$ in blood and in $U \cdot g^{-1}$ of protein in the tissues. SOD activity was measured using RANSOD kit produced by RANDOX according to Arthur and Boyne method [27] and expressed in $U \cdot ml^{-1}$ in blood and in $U \cdot mg^{-1}$ of protein in the tissues. Protein was measured using the method of Bradford (28). In plasma and supernatants the MDA concentration was determined using the Ledwożyw et al. method [29] and expressed in nmol $MDA \cdot ml^{-1}$ in plasma and in nmol $MDA \cdot mg^{-1}$ of protein in the tissues. The assays were carried out with the help of a SPECORD M40 (Zeiss Jena) spectrophotometer. Comparisons between control and tested groups were made using c-Cochran-Cox test. Values were considered significant with $p < 0.05$.

The study was approved by I Local Ethical Commission of Feliks Skubiszewski Medical University of Lublin, acceptance 435/2003.

Results

The average daily consumption of the provided fluids, body weight gain and average daily Li intake are presented in Table 1. Body weight gain and the consumption of offered fluids were not found to be significantly different in the Li administered groups vs. control. The greatest body weight gain was observed in groups receiving the lower doses of lithium. In groups provided with the high doses body weight gain was comparable with control. No symptoms of lithium toxicity were observed in tested groups during exposure.

Li administration generally caused no significant changes of the antioxidant status in blood. Lipid peroxidation levels in plasma and blood GPx activity were increased in groups receiving lower doses, whereas they diminished in groups provided with higher doses. Blood

Table 1. Daily average fluids consumption, daily average Li intake and body weight gain of rats.

Li dose [mmol/l]	Daily average drinking fluids consumption [ml]	Average daily lithium intake [mg Li/kg b.w.]	Body weight gain during experiment [g]				
			4 day	8 day	21 day	36 day	44 day
C	38 ± 8	-	210 ± 15	280 ± 30	336 ± 29	370 ± 37	393 ± 39
0.7	40 ± 8	0.6 ± 0.1	200 ± 14	292 ± 31	346 ± 40	393 ± 28	412 ± 22
1.4	35 ± 3	1.1 ± 0.2	219 ± 10	295 ± 16	359 ± 17	392 ± 13	412 ± 18
2.6	43 ± 8	2.2 ± 0.6	199 ± 8	305 ± 25	362 ± 30	385 ± 35	428 ± 18
3.6	39 ± 7	3.1 ± 0.6	212 ± 11	276 ± 26	331 ± 27	367 ± 21	384 ± 24
7.1	33 ± 4	5.1 ± 1.2	209 ± 15	273 ± 15	330 ± 19	384 ± 22	399 ± 30
10.7	32± 5	6.9 ± 0.6	209 ± 13	267 ± 24	331 ± 36	369 ± 21	395 ± 23

values are mean ± SD

Table 2. GPx and SOD activity in whole blood and MDA concentration in plasma.

Li dose [mmol $Li^{+} \cdot l^{-1}$]	GPx [$U \cdot ml^{-1}$]	SOD [$U \cdot ml^{-1}$]	MDA [$nmol \cdot ml^{-1}$]
C	60 ± 9	403 ± 80	33.7 ± 8.7
0.7	74 ± 11	304 ± 69	48.8 ± 10.8
1.4	69 ± 10	410 ± 86	37.6 ± 9.6
2.6	66 ± 12	279 ± 58	38.0 ± 10.1
3.6	54 ± 8	308 ± 71	34.0 ± 8.7
7.1	53 ± 9	439 ± 70	25.1 ± 6.8
10.7	55 ± 10	395 ± 91	24.0 ± 7.0

values are mean ± SD of six rats

SOD activity was subject to fluctuations. All those changes were not statistically significant (Table 2).

In the tissues the tendency to the depletion of the antioxidant enzymes' activities was observed. With regard to SOD a statistically significant increase was obtained in all the studied tissues, although in the case of the brain it concerned only groups administered with lower doses. Concerning GPx statistically significant depletion was observed only in the tissue of kidney. The decrease of GPx activity obtained in the tissues of liver and brain was insignificant (Table 3).

MDA concentration in tissues showed the depressed course, giving the evidence of the Li protective effect. However, this influence was significant only in the case of the tissue of brain. The only exception was the significant increase of MDA concentration in the tissue of kidney in the group given the highest dose (Table 3).

Table 3. GPx and SOD activity and MDA concentration in the tissues of liver, kidney and brain.

Li dose [mmol $Li^{+} \cdot l^{-1}$]	GPx activity [$U \cdot g^{-1}$ of protein]	SOD activity [$U \cdot mg^{-1}$ of protein]	MDA [$nmol \cdot mg^{-1}$ of protein]
liver			
C	1723 ± 520	32.7 ± 5.2	7.4 ± 1.8
0.7	1325 ± 402	12.5 ± 2.8 *	7.1 ± 1.6
1.4	1391 ± 370	13.6 ± 3.6 *	4.1 ± 1.1 *
2.6	1137 ± 350	13.9 ± 3.7 *	5.1 ± 1.2
3.6	1109 ± 290	15.0 ± 3.5 *	4.8 ± 0.9
7.1	1245 ± 390	18.5 ± 5.5 *	5.1 ± 1.2
10.7	1549 ± 500	21.7 ± 5.1 *	5.3 ± 1.4
kidney			
C	1800 ± 435	23.1 ± 5.9	5.1 ± 1.5
0.7	765 ± 218 *	10.3 ± 2.8 *	5.0 ± 1.6
1.4	850 ± 254 *	12.2 ± 3.4 *	4.6 ± 1.2
2.6	616 ± 161 *	11.8 ± 3.0 *	4.5 ± 1.4
3.6	660 ± 184 *	13.1 ± 2.9 *	4.0 ± 1.4
7.1	1055 ± 251 *	10.9 ± 2.7 *	3.5 ± 1.3
10.7	828 ± 201 *	9.2 ± 2.7 *	8.8 ± 2.0 *
brain			
C	183 ± 55	17.2 ± 2.1	36.3 ± 8.7
0.7	147 ± 48	8.8 ± 1.2 *	22.0 ± 4.1 *
1.4	102 ± 39	5.8 ± 1.5 *	22.3 ± 4.7 *
2.6	124 ± 44	11.0 ± 2.4 *	26.0 ± 5.8
3.6	118 ± 38	11.3 ± 3.3 *	21.0 ± 4.9 *
7.1	151 ± 57	12.4 ± 3.3	20.8 ± 5.4 *
10.7	145 ± 49	15.2 ± 5.7	18.7 ± 5.0 *

values are mean ± SD of six rats

* statistical significance vs. control $p < 0.05$

Discussion of Results

Lithium administration has unaffected blood antioxidant status. Our outcomes are partially consistent with those obtained by other scientists. The intraperitoneal lithium carbonate treatment for a period of 7 days resulted in no changes in erythrocytic total SOD and GPx in rats. Lipid peroxidation in erythrocytes also was unchanged [30]. In turn, Li administration for a period of 4 weeks in drinking water caused no changes of GPx activity in blood and a slight, insignificant increase of blood lipid peroxidation in rats [31]. LiCl injection insignificantly enhanced SOD and markedly decreased GPx in neutrophils of sham-operated rats. In the case of olfactory-bulbectomized rats SOD was significantly decreased and GPx unchanged as a result of LiCl treatment. It gives evidence of the differences of Li influence in the physiological and pathological states [20]. In manic-depressive patients treated with Li not significantly enhanced erythrocytic SOD and unaffected GPx in comparison with normal individuals were obtained [32].

In the case of tissues the tendency to the antioxidant barrier's impairment was displayed but it should be underlined that in the brain this negative influence was the least considerable. MDA in this organ was significantly decreased, which we consider to be evidence of the protective Li action against lipid peroxidation. This fact should be noticed in relation to lithium application in psychiatry. The results of the other scientists' studies are partially consistent with our work. Li_2CO_3 intraperitoneal administration (24h) enhanced SOD activity in different regions of the brain. 12-day-treatment also increased SOD in different regions of the brain, although in the mid-brain this effect was less significant after 3 days [33]. In our investigations the brain SOD was diminished but the period of Li treatment was considerably longer. Intraperitoneal Li treatment for a period of 7 days changed neither SOD nor GPx in brain of rats [30]. Another study concerned lipid peroxidation in the case of disturbed pregnancy (chronic restrain of prenatally Li-administered female rats during pregnancy). It was found that under such conditions lipid peroxidation in newborn rats' brain was unchanged [34]. These observations seem to be of great importance in view of the negative influence of Li on the organisms' development [13, 15, 18]. It also agrees with our conclusions that Li does not induce lipid peroxidation and in some cases plays a protective role in tissues.

In relation to the tissue of liver the reported works resulted in the observations supporting ours in some part. Intraperitoneal Li treatment for a period of 7 days changed neither SOD nor GPx in rat livers [30]. Lithium carbonate provided to rats in drinking water for a period of 4 weeks markedly influenced neither GPx nor SOD in liver. Lipid peroxidation was also unaffected. [31]. In an-other study one-month-administration in diet resulted in the decrease of hepatic lipid peroxidation in rats under different dietary regimens [35]. Chinese scientists Li and Long reported that lithium exerted the divergent effect on lipid peroxidation in rat livers. Lower doses resulted in inhibition, whereas the higher concentrations showed a stimulating influence [36]. An increased GPx activity in the liver of diabetic rats was observed, whereas SOD remained unchanged as a consequence of Li treatment [37]. However, it has already been mentioned that differences of the lithium's action in physiologic and pathologic states in rats were observed. Concerning human beings, it has also been reported that the Li effect on cognitive functions differed in healthy subjects from that found in psychiatric patients [38].

It should be noticed that the enzymes' activities were more significantly affected in the groups receiving the lower doses, whereas the higher ones looked to exert a considerably slighter effect. We can present no satisfied explanation of this phenomenon. Studies concerning this problem are being continued.

Conclusions

1. Lithium administration has exerted no significant impact on blood antioxidant enzymes' activities and on the level of plasma lipid peroxidation in rats.
2. In tissues the symptoms of oxidative stress have been observed and the brain has seemed to be the least attacked target.
3. No Li-induced lipid peroxidation has been obtained in plasma, liver and kidney, whereas in the brain evidence of a protective effect has been observed.

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