ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 121 SECTIO D 2004

Department of General Chemistry, Skubiszewski Medical University of Lublin

MAŁGORZATA KIEŁCZYKOWSKA, KAZIMIERZ PASTERNAK, IRENA MUSIK, JOLANTA WROŃSKA

The effect of lithium administration in a diet on the chosen parameters of the antioxidant barrier in rats

For some time the toxic effect of reactive oxygen species (ROS) has been considered to be the reason of severe organism lesions. The scientists believe that ROS can be included into pathogenesis of many of both somatic and psychical diseases (1, 8). For this reason the correct action of the antioxidant barrier – the system of enzymes and low-molecular-weight substances protecting the organism against ROS has become the problem of great importance. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) belong to the main antioxidant enzymes (8,12).

Lithium is extensively used in medicine (1, 2, 4, 5, 14). It is used in the treatment of bipolar disorder (2, 6, 14) and for the potentialization of the action of other drugs (9). Lithium is suggested to be used as the adjunctive drug in patients with Graves' hyperthyroidism subjected to radioiodine therapy (4, 5). However, its treatment can cause negative side-effects. The care of a patient with tardive dystonia is decribed (7). The authors suggest that it could be related to lithium or lithium plus carbamazepine therapy. The portrayed facts made us investigate the influence of lithium on SOD and GPx activity.

MATERIAL AND METHODS

Our study was carried out on two-months-aged, male Wistar rats (120–150 g). The animals were divided into two groups (20 animals each): control group I (K) received redistilled water, tested group II (Li) received a water solution of $Li_2SO_4 \cdot H_2O$ in the form of drinking water, at the dose of 150 mg Li $\cdot$ dm^{-3}. The animals were offered LSM food and drinking fluids *ad libitum*. A half of animals of each group were killed after three weeks and the rest after six weeks. Each time the rats were sacrificed under ketamine narcosis and blood from the heart as well as the tissues of the liver, kidney, femoral muscle, brain, spleen and heart muscle were collected. Serum was separated. 10% (w/v) tissue homogenates were prepared in 0.1 mol $\cdot$ dm^{-3} Tris-HCl buffer, pH = 7.4. Supernatants were obtained by centrifugation at 5000 x g for 30 min. In serum and supernatants GPx and SOD activities were determined using RANSEL and RANSOD kits produced by RANDOX. Protein was measured using the method of B r a d f o r d (3). The assays were carried out with the help of SPECORD M40 (Zeiss Jena) spectrophotometer. Comparisons between control and the tested groups were made using t-Student test. Values were considered significant with $p < 0.05$.

RESULTS

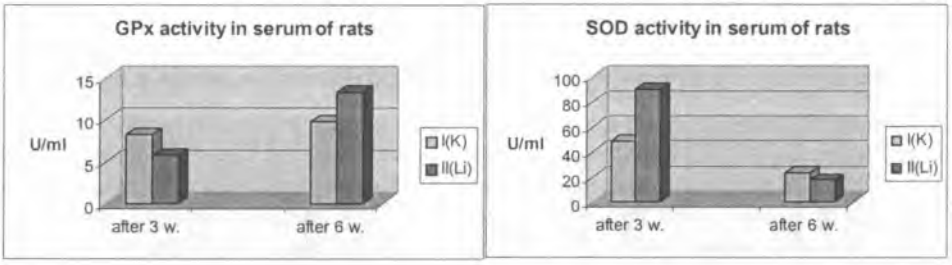
After three weeks' intoxication GPx activity in serum decreased, whereas SOD activity increased almost twice vs. control group. The next three weeks of exposure resulted in GPx increase and SOD

decrease. It should be noticed that the increment of activity of one of the measured enzymes was connected with the depletion of the other (Table 1).

Table 1. The effect of lithium administration at the dose of 150 mg • dm^{-3} on GPx and SOD activity in serum of rats

Group	GPx (U/ml)		SOD (U/ml)	
	after 3 w.	after 6 w.	after 3 w.	after 6 w.
I (K)	8.2 ± 1.0	9.8 ± 1.6	48.3 ± 8.1	23.4 ± 3.5
II (Li)	5.7 ± 0.9*	13.3 ± 1.4*	90.2 ± 14.1*	17.5 ± 2.6*

Statistical significance vs. control * $p < 0.05$

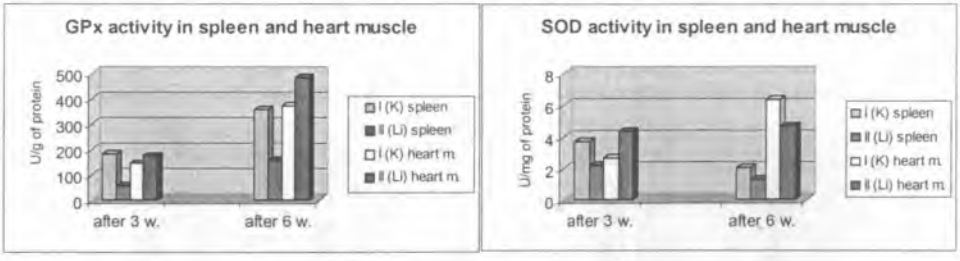


In the spleen and heart muscle enzymes' activities vs. control were changed in more univocal way. In the spleen they were diminished vs. control during all the experiment, in the heart muscle enhanced except for SOD after six weeks (Table 2).

Table 2. The effect of lithium administration at the dose of 150 mg • dm^{-3} on GPx and SOD activity in rats' tissues of spleen and heart muscle

Group	Spleen				Heart muscle			
	GPx (U/g of protein)		SOD (U/mg of protein)		GPx (U/g of protein)		SOD (U/mg of protein)	
	after 3 w.	after 6 w.	after 3 w.	after 6 w.	after 3 w.	after 6 w.	after 3 w.	after 6 w.
I (K)	183.2 ± 31.4	358.3 ± 37.0	3.7 ± 0.8	2.0 ± 0.5	145.2 ± 22.8	374.5 ± 40.5	2.7 ± 0.5	6.4 ± 0.8
II (Li)	58.5 ±14.1*	159.5 ± 15.2*	2.2 ± 0.7*	1.3 ± 0.4	175.4 ± 31.6	483.5 ± 42.4*	4.4 ± 0.8*	4.7 ± 0.7*

Statistical significance vs. control * $p < 0.05$



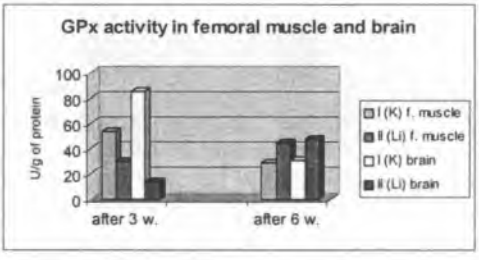
In the femoral muscle and the brain only GPx level vs. control changed significantly except for SOD in the femoral muscle after three weeks. GPx activity both in the femoral muscle and in the brain was decreased after three weeks and enhanced after six weeks. In femoral muscle similarly as in

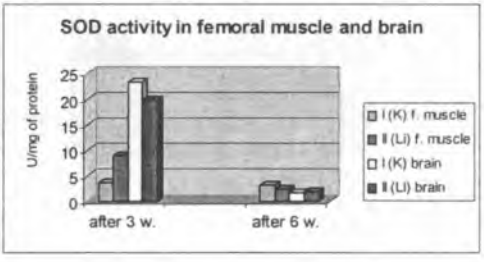
serum, during the whole experiment the increase of activity of one of the enzymes was accompanied with the diminution of the other (Table 3).

Table 3. The effect of lithium administration at the dose of 150 mg • dm^{-3} on GPx and SOD activity in rats' tissues of femoral muscle and brain

Group	Femoral muscle				Brain			
	GPx (U/g of protein)		SOD (U/mg of protein)		GPx (U/g of protein)		SOD (U/mg of protein)	
	after 3 w.	after 6 w.	after 3 w.	after 6 w.	after 3 w.	after 6 w.	after 3 w.	after 6 w.
I (K)	53.0 ± 12.6	28.6 ± 6.0	3.7 ± 1.1	3.1 ± 1.0	85.7 ± 20.2	30.8 ± 9.7	23.4 ± 3.7	1.6 ± 0.5
II (Li)	30.0 ± 11.4*	44.2 ± 7.7*	8.8 ± 2.0*	2.4 ± 0.5	14.0 ± 4.0*	47.3 ± 10.4*	19.9 ± 3.2	2.0 ± 0.5

Statistical significance vs. control * $p < 0.05$



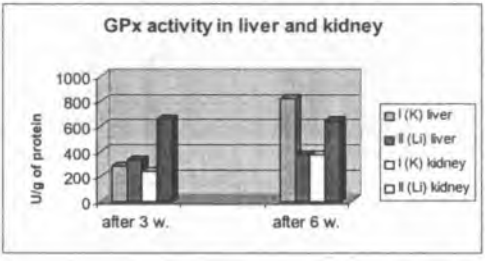


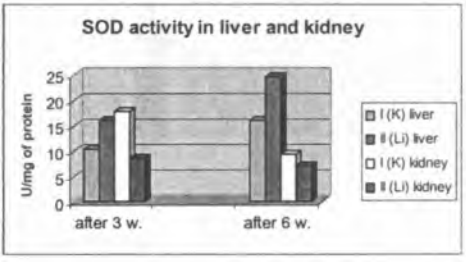
In the liver GPx vs. control decreased after six weeks, SOD was enhanced during the whole experiment. In the kidney GPx increased during the whole time of exposure, whereas in the case of SOD the depletion of its activity was observed. The increased level of one of the enzymes connected with the decreased level of the other was noticed in the liver after six weeks and in the kidney both after six and three weeks (Table 4).

Table 4. The effect of lithium administration at the dose of 150 mg • dm^{-3} on GPx and SOD activity in rats' tissues of liver and kidney

Group	Liver				Kidney			
	GPx (U/g of protein)		SOD (U/mg of protein)		GPx (U/g of protein)		SOD (U/mg of protein)	
	after 3 w.	after 6 w.	after 3 w.	after 6 w.	after 3 w.	after 6 w.	after 3 w.	after 6 w.
I (K)	284.6 ± 31.6	820.0 ± 158.1	10.4 ± 3.2	16.1 ± 4.6	239.2 ± 40.5	376.5 ± 72.1	17.9 ± 5.4	9.3 ± 1.4
II (Li)	335.0 ± 49.4	370.0 ± 77.2*	16.2 ± 3.0*	24.7 ± 5.1*	653.4 ± 98.8*	647.3 ± 71.5*	8.6 ± 3.0*	7.2 ± 1.1*

Statistical significance vs. control * $p < 0.05$





DISCUSSION

The measured enzymes' activities were changed as a consequence of lithium exposure. Results obtained by other scientists confirm ours in part. Li_2CO_3 intraperitoneal administration daily for a week resulted in no changes of SOD and GPx activities in the brain, liver and erythrocytes of rats (2). In our study brain SOD was unchanged during all the exposure, whereas liver GPx after three weeks.

Srivastava et al. (13) studied the influence of lithium therapy on GPx and SOD activities in rats with the antioxidant barrier functions disturbance caused by diabetes. In the liver Li treatment increased GPx level, whereas SOD activity remained decreased. Tandon et al. (15) investigated the effect of Li administration to rats fed diets of different protein contents. Low-protein diet decreased hepatic GPx and SOD. Lithium enhanced significantly GPx and restored SOD activity. In our investigations the GPx level in the liver was increased after three weeks but not significantly, whereas SOD level vs. control was enhanced during the whole exposure.

Full investigations on alkali metals influence both *in vivo* and *in vitro* on SOD activity in the brain were carried out by Shukla (11). One intraperitoneally administered dose did not change this enzyme activity in different brain regions. The second dose administered after 12 h caused SOD activity increase, first of all in the cerebral cortex. The studies on SOD activity changes depending on time were also carried out. After one day no significant changes in brain regions were observed, after three days the significant increment in some regions was noticed, whereas after six, nine and twelve days SOD activity was significantly enhanced in all the regions, most of all in the cerebral cortex. In our studies not significant SOD increase in the brain vs. control was obtained after six weeks' exposure.

Megrabian et al. (10) investigated SOD level in epileptic patients. Oral Li administration and vitamin E injections resulted in blood SOD increase. In our works we obtained statistically significant serum SOD increase after three weeks.

Abdalla et al. (1) studied SOD and GPx activities in erythrocytes of manic-depressive patients. Both patients treated only with lithium and those treated with lithium plus neuroleptic drugs showed increased SOD vs. control, healthy group. GPx was slightly, not significantly enhanced only in patients treated with lithium plus neuroleptics.

Song et al. (12) worked on lithium administration influence on SOD and GPx activities in neutrophils of olfactory-bulbectomised rats. LiCl administration daily for 15 days normalized SOD increased as a consequence of operation and did not affect decreased GPx in olfactory-bulbectomised rats. In sham-operated rats lithium treatment did not influence SOD and diminished GPx. The described results reveal lithium administration influence on antioxidant barrier functions. The settlement of influence of the dose and the time of exposure on organism functions will be the subject of further investigations.

CONCLUSIONS

1. The lithium oral administration influenced the antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities in serum and tissues of rats.

2. The trend of changes was depending on the time of exposure and the tissue.

3. In serum, femoral muscle and kidney the increase of activity of one of the enzymes was accompanied with the depletion of the other.

REFERENCES

1. Abdalla D. S. P. et al.: Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. Clin. Chem., 32, 805, 1986.
2. Abdalla D. S. P., Bechara E. J. H.: The effect of chlorpromazine and Li_2CO_3 on the superoxide dismutase and glutathione peroxidase activities of rat brain, liver and erythrocytes. Biochem. Mol. Biol. Int., 34, 1085, 1994.
3. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248, 1976.
4. Bogazzi F. et al.: Comparison of radioiodine with radioiodine plus lithium in the treatment of Graves' hyperthyroidism. J. Clin. Endocrinol. Metab., 84, 499, 1999.
5. Bogazzi F et al.: Treatment with lithium prevents serum thyroid hormone increase after thionamide withdrawal and radioiodine therapy in patients with Graves' disease. J. Clin. Endocrinol. Metab., 87, 4490, 2002.
6. Bosetti F. et al.: Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E_2 concentration in rat brain. Mol. Psychiatry, 7, 845, 2002.
7. Chakrabarti S., Chand P. K.: Lithium-induced tardive dystonia treated with Clozapine. Neurol. India, 50, 473, 2002.
8. Gaeta L. M. et al.: Determination of superoxide dismutase and glutathione peroxidase activities in blood of healthy pediatric subjects. Clin. Chim. Acta, 322, 117, 2002.
9. Koszewska I.: Wybrane farmakologiczne metody potencjalizacji leków przeciwdepresyjnych. Farmakoter. Psychiatr. Neurol., 1, 31, 2000.
10. Megrabian A. A. et al.: Use of lithium carbonate and vitamin E in the complex treatment of epileptics. Zh. Nevropatol. Psikhiatr. Im. S. S. Korsakova, 86, 1407, 1986.
11. Shukla G. S.: Mechanism of lithium action: *in vivo* and *in vitro* effects of alkali metals on brain superoxide dismutase. Pharmacol. Biochem. Behav., 26, 235, 1987.
12. Song C., Killeen A. A. et al.: Catalase, superoxide dismutase and glutathione peroxidase activity in neutrophils of sham-operated and olfactory-bulbectomised rats following chronic treatment with desipramine and lithium chloride. Neuropsychobiology, 30, 24, 1994.
13. Srivastava P. et al.: Insulin-like effects of lithium and vanadate on the altered antioxidant status of diabetic rats. Res. Commun. Chem. Pathol. Pharmacol., 80, 283, 1993.
14. Suwalska A., Chłopocka-Woźniak M. et al.: Długotrwała profilaktyka litem w chorobie afektywnej dwubiegunowej. Psychiatr. Pol., 36, 63, 2002.
15. Tandon A., Dhawan D. K. et al: Effect of lithium on hepatic lipid peroxidation and antioxidative enzymes under different dietary protein regimens. J. Appl. Toxicol., 18, 187, 1998.

SUMMARY

The reactive oxygen species (ROS) generated in metabolic processes can cause severe lesions. The organisms' defense constitutes the antioxidant barrier. Antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) belong to its main constituents. Lithium is widely used in medicine but its administration can cause negative side-effects. Therefore, we investigated Li oral intoxication influence on SOD and GPx activity in serum and tissues of rats in the dependence on time (three-weeks and six-weeks). In serum SOD activity vs. control increased after three weeks and decreased after six, GPx activity vs. control was changed in reverse way – diminished after three weeks and enhanced after six. In tissues the changes were diverse. The most interesting results are: the decrease of both enzymes in the spleen, no changes of SOD in the brain, the enhanced GPx and

diminished SOD in the kidney, the increased SOD in the liver vs. control during the whole experiment. In serum, femoral muscle and kidney during all the exposure the increment of one of enzymes was accompanied with the depletion of the other. The obtained outcomes allow to suggest that the lithium exposure resulted in antioxidant barrier disturbances in rats.

Wpływ podawania litu w pożywieniu na wybrane parametry statusu antyoksydacyjnego szczurów

Reaktywne formy tlenu (RFT) powstające w procesach metabolicznych mogą powodować zmiany patologiczne. Obronę organizmu przed ich działaniem stanowi bariera antyoksydacyjna. Antyoksydacyjne enzymy peroksydaza glutationowa (GPx) i dysmutaza ponadtlenkowa (SOD) należą do jej najważniejszych składników. Lit jest szeroko stosowany w medycynie, ale może powodować negatywne skutki uboczne. Dlatego podjęliśmy badania nad wpływem doustnej intoksykacji litem na aktywność GPx i SOD w surowicy i tkankach szczurów w zależności od czasu (trzy i sześć tygodni).W surowicy aktywność SOD w stosunku do grupy kontrolnej wzrosła po trzech tygodniach i zmalała po sześciu. Aktywność GPx w stosunku do grupy kontrolnej zmieniała się w sposób przeciwny – malała po trzech tygodniach i wzrastała po sześciu. W tkankach zmiany były zróżnicowane. Najbardziej interesującymi wynikami doświadczenia były: spadek aktywności obu enzymów w śledzionie, brak zmian poziomu SOD w mózgu, wzrost aktywności GPx i spadek aktywności SOD w nerce oraz podwyższenie SOD w wątrobie w stosunku do kontroli podczas całego eksperymentu. W surowicy, mięśniu uda i nerce podczas całego okresu intoksykacji wzrost aktywności jednego z enzymów wiązał się ze spadkiem aktywności drugiego. Wyniki pozwalają sugerować, że podawanie szczurom litu zaburza działanie bariery antyoksydacyjnej.