Original Article

Toxic Effects of Lithium

Detrimental Effect of Lithium Carbonate on Cerebellar Purkinje Neurons in Albino Rats

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ABSTRACT

Objective: To observe the damaging effect of Lithium carbonate on Purkinje neuron in the light of available literature so this study was under taken to see the toxic effects of Lithium carbonate on permanent Purkinje neuron.

Study Design: Experimental study

Place and Duration of study: This study was carried out at Animal House, BMSI, JPMC, Karachi from April 2012 to June 2012.

Materials and Methods: Thirty male albino rats of 190-240 grams were selected and divided into two major groups (control) A and B (Lithium Carbonate-treated) comprising of 15 animals each. According to the time period of the study which was 2 weeks, 6 weeks and 12 weeks. Group A served as control group which was given normal diet and B was given Li2CO3 in powder form mixed in flour. Lithium carbonate was given at a dose of 20mg/kg/day for two, six and twelve weeks. At the end of 2^{nd}, 6^{th} and 12^{th} weeks the cerebellum was removed and fixed in 10% formal saline. After processing in paraffin five micron thick section were prepared four Purkinje cell count.

Results: Group A revealed normal Purkinje cell count but the mean values of the Purkinje cell count in group B was highly significantly decreased p-value<0.001 as compared to group A.

Conclusion: Our study concludes that acute and chronic ingestion of lithium carbonate at therapeutic level is detrimental to the survival of Purkinje cerebellar neuron and patients who are prescribed lithium carbonate should be monitored carefully.

Key Words: cerebellum, Purkinje, neuron, Lithium carbonate.

Citation of article: Kohari T, Najmi N. Detrimental Effect of Lithium Carbonate on Cerebellar Purkinje Neurons in Albino Rats. Med Forum 2016;27(2):41-44.

INTRODUCTION

The word Lithium comes from Greek word "Lithos"[1], which means stone, with symbol "Li" [2]. Lithium is the gold standard, mood stabilizer [3]. Its salt is useful in treatment of bipolar [4] depressions. Lithium carbonate is often referred to as an anti-maniac drug but in many parts of the world, it is considered as the stabilizer of mental status because of its role in preventing mood swings with bipolar affective disorder.[5] It is used for other disorders, such as cycloid psychosis, major depression, cluster headache [6], and schizophrenia.[7] The Food and Drug Administration of USA approved lithium as preventive or prophylactic treatment for depressive illness.[8] A cohort study conducted in United Kingdom for the usage of lithium as a mood stabilizer determined that increment in prescribing lithium for women was 33% and 24.1% for men in the year 1995 to 2009. [9]

It has multiple effects on biological processes. The enzyme inositol monophosphatases is a potential target for lithium's damaging effect. It was found that GSK-3 inhibition increased translocation of nuclear factor of activated T-cells, ¾ (NFAT C3/4) transcription factors to the nucleus leading to increased Fas ligand (FASL) levels and Fas activation which causes cell death (apoptosis), and it is worth noting that levels of lithium-induced apoptosis was highest in cerebellum.[10] Lithium effects have been investigated in detail in the brain, intestine, liver and thyroid functions.

De Cerqueira[11] et al. had reported cerebellar degeneration secondary to Lithium carbonate ingestion. The neuropathological manifestations reported were loss of Purkinje and granule cell leading to cerebellar atrophy. [12] Central neuronal damage by lithium is due to increase in glutamate secretion which causes increase sequestration of calcium ions in the mitochondrial cisternae resulting in disruption and distortion of mitochondria in the neurons, these changes are accompanied by nuclear clumping. As neuronal cell degeneration causes release of reactive oxidant species.[13] The lipid peroxidation of the cerebellar cortex leads to decrease in concentration of the antioxidant enzymes, resulting in DNA fragmentation and cell death of cerebellar neurons with a decrease in the gray matter of cerebellar cortex.[14, 15]

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Many studies have been done on cerebellar degeneration by various agents and radiation, but my study was undertaken to evaluate the cytotoxic effect of Lithium carbonate on Purkinje neurons in the Purkinje layer of cerebellar cortex in albino rats.

MATERIALS AND METHODS

This study was conducted in the Animal House affiliated with Basic Medical sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi. For this study 30 male albino rats of 190-240 grams of weights were used. They were kept under observation for 7 day prior to commencement of study. The animals were randomized into two experimental groups comprising of 15 animals each according to the time period of the study that is 2 weeks, 6 weeks and 12 weeks respectively. Group A served as control and group B received Lithium carbonate Adamjee Pharmaceuticals at a dose of 20mg/kg/day[16,17] in powder form mixed in flour pellets for 2 weeks, 6 weeks and 12weeks. The standard laboratory Chow and tap water were available ad libitum at the end of each time period the animals were sacrificed under ether anesthesia. The cerebellums were removed by parietal bone approach. The tissue was fixed and processed for haemotoxylin and eosin staining. Morphomeetric examination was performed under light microscope and the results were recorded and tabulated.

Data collected were analyzed using students t-test. Results were expressed mean ± P-<0.001 and P-<0.05 was considered statistically significant. All the calculations were done by utilizing computer software SPSS 16 through Microsoft excel in windows.

RESULTS

The mean Purkinje cell count was highly significantly (P<0.001) decreased as compared to control group A table 1 and the Purkinje cell count of group B was highly significantly decreased at 6th wks than 2nd weeks and highly significantly decreased at 12th week than 2nd week.

Table No.I: Mean* Purkinje cell count (mm) in different group of albino rats.

	N	2nd week			6th week			12th week		
		Mean	S.D	SEM	Mean	S.D	SEM	Mean	S.D	SEM
A	15	24.2	4.44	1.98	24.9	2.30	1.03	26.9	1.90	0.85
B	15	16.0	1.00	0.45	11.4	0.55	0.24	8.2	0.84	0.37

Mean*±SEM

Statistical analysis of Mean* weekly Purkinje cell count in different group of albino rats

P-value		
2wk Vs 6wk	2wk Vs 12wk	6wk Vs 12wk
0.551	0.392	0.331
0.002	0.001	0.001

Significant** Highly Significant***

Stats analysis of major group comparison of P-value

P-value	B Vs A	0.001	0.001	0.001

Significant** Highly Significant***

DISCUSSION

Since 1949 Lithium carbonate has been in use for Bipolar disorder. Its therapeutic effects are undeniable but its adverse effects cause neurological manifestations[18]. The cerebellum is an important component of the hind brain, involved in the control of locomotion and balance. The cerebellar cortex contains eight types of neurons: Purkinje, granule, stellate, basket, Golgi, Lugaro, unipolar brush cells and candelabrum cells. Purkinje cells provide the sole output of the cerebellar cortex and are the pivotal element around which the cerebellar circuit is organized.[19]

Lithium salt therapy entails neurologic damage in persisting form. Due to lithium injection cerebellar features tend to be the most prominent .[20] The most frequent clinical feature is a permanent cerebellar syndrome and it was reported that lithium disrupt calcium hemostasis in Purkinje cell. Cerebellar syndrome has also been described after short term lithium medication. Neuropathological studies have demonstrated neuronal loss and spongiosis in the cerebellum.[21] Persisting neurologic damage follows lithium salt therapy. There are usually signs of damages at multiple sites, but cerebellar features tend to be most prominent.[22] Lithium carbonate causes neuronal Purkinje cell death due to lipid peroxidadtion which causes an increase imbalance in antioxidant enzymes which are superoxide dismutase (SOD), Catalase (CAT) and glutathione synthetase (GST), there by leading to excessive generation of free radicals hence resulting in enhanced oxidative stress.[23]

Our study showed a marked decrease in the Purkinje cell density in the Li2CO3 treated group B animals as compared to control group A. This is an agreement with Kaidanovich et al., whose work showed that lithium

ingestion causes a decrease number of the Purkinje cell in the Purkinje cell layer and this may be due to the reason that lithium causes inhibition of Glycogen Synthase Kinase-3. GSK-3 is an evolutionary conserved ubiquitous serine / thionine protein kinase. It is abundant in the neurons and neuroglia. GSK-3 acts downstream to suppress the activity of several prominent pathways such as Wnt signaling pathway, phasphoinositol 3 protein kinases and neurotropic pathway. GSK-3 is implicated in fundamental brain functions cytoskeletal stabilization axonal growth cone collapse, cell adhesions, synaptic plasticity and memory formation.

The decrease in Purkinje cell count in albino rats due to Lithium administration was also studied by Vijaymohan et al (2010). They have in their researches emphasized the fact that decreased in the concentration of the antioxidant enzymes, causes release of ROS.[24] This results in the decrease ATP production in the mitochondria and Purkinje neuron. This whole process causes excessive secretion of calcium ion resulting in fragmentation and death of Purkinje neurons.

The observations of decrease Purkinje cell density was also reported by Grignon[25] et al (1996). Cerebellar syndrome has also been described after short term as well as chronic lithium as is seen in our study.

CONCLUSION

Our study concludes that acute and chronic ingestion of lithium carbonate at therapeutic level is detrimental to the survival of Purkinje cerebellar neuron and patients who are prescribed lithium carbonate should be monitored carefully.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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