Original Article

Protective Effects of Chronic Methylcobalamin Administration against Lithium Carbonate Induced Purkinje Cell Toxicity in Albino Rats

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ABSTRACT

Objective: The effect of Lithium on central nervous system is well known but due to paucity of literature on scavenging effect of Lithium, this study was undertaken to see Lithium toxicity on cerebellar Purkinje neurons.

Study Design: Experimental study.

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

Materials and Methods: Fifteen male albino rats of 195-245 grams were selected and divided into three major groups A, B, and C. Each major group consisted of 5 animals. Time period of this study was12 weeks. Group A served as control group which was given normal healthy lab diet and B was the Lithium Carbonate-treated group. Group C received Lithium carbonate in powder form and injection Methylcobalamin (B12) intraperitoneally. Lithium carbonate was given at a dose of 20mg/kg/day to group B and C for 12 weeks, and Methylcobalamin was injected at a dose of 200µg/kg/day/bwt. Purkinje cell count was performed with a counting reticule under light microscope.

Results: The present study concluded that Lithium carbonate when administered the 12 weeks cause the significant decrease of Purkinje cell count and Methylcobalamin restored the cerebellar cell count.

Conclusion: In the light of this study it was concluded that that Lithium carbonate causes significant permanent loss of permanent Purkinje cell neuron but Methylcobalamin provided neuroprotective effect and restored the cell count.

Key Words: Methylcobalamin, Lithium Carbonate, Toxicity

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INTRODUCTION

Lithium the bridge element[1] is an alkali metal found in group 1A, of the periodic table. The true use of Lithium carbonate as a drug was by Johan's Cade in 1949.[2] Now for the past five decades it is used for Bipolar disorder, it is also used to treat schizophrenia, cycloid psychosis[3] and major depression. In 1970 the Food and Drug Administration (FDA), United States of America[4] approved the Lithium as a treatment for mania. In a study it was observed that lithium carbonate inhibits glycogen synthase activity causing apoptosis. Glycogen Synthase enzyme is an evolutionary conserved ubiquitous serine / threonine protein kinase. It is abundant in the neurons and neuroglia. GSK-3 suppresses the activity of several prominent pathways such as Wnt signaling pathway, phosphoinositol 3 protein kinases and neurotropic pathway. GSK-3 are implicated in fundamental brain functions cytoskeletal stabilization axonal growth cone collapse, cell adhesions, synaptic plasticity and memory formation. Dysregulation of GSK-3 is implicated in schizophrenia, Alzheimer's, attention deficit hyperactivity disorder BPD and lithium inhibits GSK-3.[5,6] The highest concentration of inhibition of GSK-3 were found in cerebellum. The cerebellum is an important factor in the brain motor system. Cerebellar cortex contains 8 types of neurons and among them the purkinje cells provide the sole output of the cerebellar cortex. Immunohistological examination of GSK-3 mutant mice revealed significant degrees number of purkinje cells.[7]

It is due to such observations great concern has been documented to discourage long term use of Lithium until it is indicated. Methylcobalamin is a vitamin B-12 analogue and is necessary for the maintenance[8] of central nervous system.

B-12 analogue was discovered by British Chemist Baron Alexander[9] and later it was discovered biologically active alkyl cobalamin in 1962 by Guest. It occurs as dark red crystals or crystalline powder. Vitamin B12 has been used in the therapy of trigeminal neuralgia, MS, neuropathies, psychiatric disorders, poor growth or nutrition.[10] B12 participates in one carbon atom unit metabolism and hence is essential for methylation of DNA and proteins because B12 is

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required for homocysteine metabolism by serving as a co-factor for methionine synthase, homocysteine, and non-conversion of methionine results in oxidative stress thereby by increasing the oxidative in the endothelial cells, so B12 is necessary to eliminate the oxidative stress.[11]

It is essential for cell growth and replication. It promotes neurite growth neuronal[12] survival and these effects are mediated by methylation cycle. Mecobalamin is used in spinal cord and peripheral nerve injuries in which it restores DNA synthesis by DNA methylation.[13]

MATERIALS AND METHODS

This study was conducted in the department of anatomy and practical work of research was conducted at Basic Medical sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi. For this study 15 male Albino rats of 195-245 grams of weights were selected for study. They were kept under observation for 7 days prior to commencement of study. The animals were randomized into four experimental groups each comprising of 5 rats. Group was labeled that A, B, and C, the treatment period was 12 weeks. Group A served as control and group B received Lithium Carbonate, taken from Adamjee Pharmaceuticals, at a dose of 20 mg/kg/day in powder form mixed in flour pellets and group C received Lithium carbonate injection Methylcobalamin 200mcg/kg/day IP.[14,15] The standard laboratory Chow was available and libitum. The albino rats were decapitated. The brain was taken out and the cerebellum was separated from the rest of the brain. The cerebellum was preserved in formal saline for 24 hours the tissue was fixed and than four micrometers thick sections of the tissue were prepared. The Purkinje cell count was done by counting reticule was done for all the three groups at 12 weeks. Data collected was analyzed using students t-test. Results were expressed as mean, SEM. P<0.001 was considered statistically highly significant. All calculation was done by utilizing computer software SPSS 16.

RESULTS

The Purkinje cell count of group B animals at 12 weeks was significantly decreased P<0.001 with control group A.

Table No.I: Mean* Purkinje cell count of Albino rats in control group, Lithium treated group B and Lithium and Methylcobalamin group C at 12 weeks.

Group	12th Week		
	Mean	S.D	SEM
Control (A)	69.5	0.40	0.18
Lithium (B)	34.9	0.23	0.10
Lithium B-12 (C)	66.3	0.24	0.11

The above result shows a highly significant P-value <0.001 decreased Purkinje cell count in Lithium treated group B and a restoration of Purkinje cell count in group C which is Lithium and Methylcobalamin treated group.

Statistical Analysis of Purkinje cell count between Groups

Group	P-value
B vs. A	< 0.001***
C vs. A	<0.001***
C vs. B	<0.001***

***highly significant P-value <0.001

DISCUSSION

Lithium is a 27th most abundant ubiquitous element present in the earth crust to the extent of about 0.006%.[16] Ingestion of Lithium in the body causes several damaging effect on the body organs like brain, heart and kidney nervous system is the primary target organ of Lithium toxicity nearly 50% of all neuron of the brain allocated in this region so it may be vulnerable to injury.[17] Lithium administration caused Glycogen synthase kinase (GSK) inhibition, which increased translation of nuclear factor of activated T-cells C-3/4 (NFAT C-3/4) transcription factors to the nucleus leading to increased Fas ligand. Fas ligand cases apoptosis by binding to surface receptor as a consequence there is an activation of caspases-3 which causes cellular degradation.[18] It is of utmost important to note the levels of Lithium induced apoptosis, were highest in rat cerebellum. It is suggested that neuronal cell death due to lithium is due to the reason that it causes decrease action potential peak amplitude[19] and the amplitude of after potential following a single action potential and decreases action potential and re-polarization phase. Although depolarization results from increased Na^+ influx the effect of lithium on action potential re-polarization and after potential suggests that lithium acts to decrease outward potassium current.

Given that mammalian CNS, has limited regenerative capacity, it is of utmost importance,[20] to limit the damage than novel therapeutic strategies. Evidence suggests that caspases-3 is the key enzyme in neuronal apoptosis which is inhibited by methylcobalamin decreasing cell damage. Methylcobalamin promotes DNA repair and growth of cell.

In a study conducted by Zhang,[21] it was reported that Methylcobalamin at such an important factor restoring the normal function of DNA because it causes methylation of DNA and proteins.

It restores delayed synaptic transmission and diminished neurotransmitters[22] to normal and promotes myelination due to synthesize of lecithin, it promotes axonal transport and regeneration. Mecobalamin restores end plate potential induction in rats, by increasing nerve fiber excitability. It causes mitosis of schawn cells and incorporation of amino acids in to protein fraction of crushed sciatic nerve in rats.[23]

It was observed in our study that the Purkinje cell count was decreased in group B as compared to group C and Methylcobalamin restored the Purkinje cell count.

CONCLUSION

Our present study suggests that chronic administration of Methylcobalamin protects the Purkinje cell neuron and restores there number. So B-12 analogue ameliorates the toxic effect of Lithium carbonate.

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

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