

Higher specific activities of alkaline phosphatase and β -galactosidase for the cellular membrane integrity in young and old aged lithium treated rats

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Abstract

Background: Differentiate the specific activity of Alkaline phosphatase and β -galactosidase in LiCl₂ treated young and old rat's brain and liver to their control.

Material and Methods: Totally 4 groups male wistar rats (each n=5) are taken to assess the level of Alkaline phosphatase and β -galactosidase for 10 and 20 days LiCl₂ treated young and old rat's brain and liver as compared to control.

Results: LiCl₂ increases protein content essential for old brain cell activation. Among all four group rats Alkaline phosphatase and β -galactosidase showed increased specific activity for 10 and 20 days LiCl₂ treated young and old rat's brain and liver as compared to their control. Infact Alkaline phosphatase showed significantly higher activity for 10 and 20 days LiCl₂ treated young brain (16.77 ± 0.309 and 27.74 ± 0.237) than their control 3.80 ± 0.230 and old rat's brain (14.28 ± 0.215 and 16.30 ± 0.24) than their control 8.65 ± 0.304 nm/min/mg of protein, similarly higher activity obtained for young liver (19.51 ± 0.487 and 21.28 ± 0.317) than their control 3.67 ± 0.402 and finally for the old liver (15.02 ± 0.355 and 18.45 ± 0.439) as compared to their control 3.26 ± 0.304 nm/min/mg of protein respectively.

Conclusion: These results confirm both membrane bound enzymes showed higher specific activity for LiCl₂ treatment in brain and liver cells. So lithium has positive effect on young and old brain cells for β -galactosidase and has more positive effect on Alkaline phosphatase for rat liver and brain for membrane integrity of cells.

Keywords: Total protein, Alkaline phosphatase, β -galactosidase, Specific activity, Lithium Chloride

Introduction

Hydrolytic enzymes are responsible for hydrolysis reaction splits different groups of biomolecules such as proteins, lipids, nucleic acids, carbohydrates and fat molecules into their simplest units esters, peptides and glycosides.

One of the main eukaryotic hydrolase and membrane bound enzyme Acid β -D-galactosidase (EC 3.2.1.23) cleaves β -linked terminal galactosyl residues from a wide range of naturally occurring substrates such as gangliosides, glycosaminoglycans, glycoproteins and glycolipids.⁽¹⁾

β -galactosidase is present in all hepatocytes of the liver and also localized in the lysosome.⁽²⁾ Various laboratories have used the SA- β -galactosidase assay on a variety of cells and tissues to demonstrate the onset of replicative senescence biomarker in culture^(3,4,5,6) and in vivo.^(7,8)

The deficiency of β -galactosidase leads to various disorders such as gangliosidosis, Gargoylism, I-cell disease, gangliosidosis, Krabbe Leukodystrophy and Morquio B disease. These diseases are due the accumulation of complete biomolecules of β -galactosidase substrates. The major β -galactosidase substrates of brain are glycolipids such as G_{M1}-ganglioside, lactosylceramide and galactosylceramide whose metabolism has been altered due to β -galactosidase deficiency in G_{M1}-gangliosidosis and Krabbe Leukodystrophy of man and animals.^(9,10,11,12) This enzymatic defect causes GM1 and its asialo

derivative (GA1) to accumulate primarily in the brain, leading to progressive neurodegeneration and brain dysfunction in humans.⁽¹³⁾

Another cell membrane bound enzyme alkaline phosphatase (ALP) (orthophosphoric-monoester phosphohydrolase, alkaline optimum), (EC 3.1.3.1) hydrolyze a wide variety of monophosphate esters at high pH optima and are widely distributed in nature.⁽¹⁴⁾ There are different classes named after the tissues in which they are predominantly expressed include the placental-like ALPs, intestinal (fetal and adult) ALPs, liver, bone, kidney ALPs and in brain as Tissue-nonspecific ALP (TNAP).

The mammalian isoforms share an alkaline pH optimum and are anchored to the membrane via a glycosylphosphatidylinositol anchor.⁽¹⁵⁾ In the adult mammalian CNS, TNAP represents the only isoform of ALPs and is associated with the blood vessel endothelium and with neuropil including synaptic contacts.⁽¹⁶⁾ During growth and development, brain alkaline phosphatase activity decreases in the mammals studied and the amount of enzyme activity change is tissue- and species-dependent. The brain alkaline phosphatase of mammals play a role in central nervous system for metabolism of such substances.^(17,18) In the Brain, ALP activity is markedly high in endothelial cells of BBB -type vessels and negative or low in those of non-BBB type blood vessels^(19,20) The ALP levels parallel the maturation of BBB in the newly formed capillary endothelial cells during normal development

of Brain⁽²¹⁾ and during repair processes after brain injury and inflammation.⁽²²⁾ Thus ALP has been accepted as a marker enzyme for the BBB.^(23,24, 25)

There are some modulators which are responsible for activation of hydrolases, IL-6 secreted by activated astrocytes may induce ALP activity in the endothelial cells under certain pathological conditions such as infarct, injury, inflammation and terminal differentiation of the BBB endothelial cells.⁽²⁶⁾

The enzymes activity monitored which can be enhanced with modulators which are proteins, hormones and drugs such as lithium chloride (LiCl₂) used to treat mood disorders and a major drug in psychiatry being used in the treatment and prophylaxis of bipolar affective disorder.^(27,28) The neuroprotective effects of lithium chloride partly depend on the inhibition of tau phosphorylation during transient brain ischemia⁽²⁹⁾ and inhibits a major tau kinase GSK-3 β ⁽³⁰⁾ being explored as a therapeutic drug to reduce tau hyperphosphorylation and pathology in Alzheimer's disease.^(31,32)

Therefore current study was attempted to show elevated levels of two enzymes β -galactosidase and alkaline phosphatase activity treated with LiCl₂ for the benefit of brain and liver cell function for degradation of complex substrates and for integrity of cell membrane. Thus determination of specific activity for two hydrolytic enzymes in brain and liver of rat model (young and old aged) treated with lithium chloride showed elevated activity when compared to their control.

Materials and Methods

Procuring of the rat models: Male Wistar Young rats (150-220 grams) and old aged rats (300-350 grams) were purchased from Vekateshwara agency, Bangalore and were housed five per cage, had free access to water and food (Libitum) and were exposed to a 12-h light/dark cycle. After a two week accommodation period the rat models were used for experiments. (Registration number- 1368/ac/10CPSEA) (IAEC approval Number of our project: MLACW/Ethics/BC-SP/12)

Lithium chloride (LiCl₂) dosage: Dosage of the drug was calculated by converting adult human therapeutic dose (600-2400 mg/day) to animal dose. The average dose of lithium chloride was prepared to 37mg/kg of rats weight per day. To study the effects of the drug, five young and five old rats were treated with modulators orally daily for 10 days and 20 days intervals.

Collection and Processing of Rat tissue specimens: The Brain and Liver tissue samples were collected by sacrificing the rat. The model was first anesthetized using Xylazine and Ketamine in the ratio 1:3.

Preparation of tissue extraction from specimens for biochemical studies: Brain and Liver tissue Specimens were grounded using pestle and mortar followed by glass homogenization with tris buffer saline (TBS pH=7.4 containing 1% (v/v) triton-X 100) and protease inhibitors (10 μ l 0.5 mM phenyl methyl sulphonyl fluoride in ethanol) to tissue to buffer ratio of 1:10 w/v (wet weight) at 5^o-10^oC to avoid protein denaturation. Homogenates were centrifuged at 10,000 rpm for 15 minutes at 4^oC. The supernatants were collected and stored at 4^oC until subjected for biochemical analysis.

Estimation of Protein by Lowry's method: Bovine Serum Albumin as standard protein (1 mg/ml).⁽³³⁾

Estimation of Hydrolytic Enzymes: Activities of the hydrolytic enzymes in supernatant of tissue extracts were assayed by spectrophotometer.

β -Galactosidase Assay:⁽³⁴⁾ β -Galactosidase activity was determined by release of O-nitrophenol from a 7 mM O nitrophenyl- β -Dgalactopyranoside (ONPG in phosphate buffer pH 7.0 at 37^oC) as substrate solution. Then the reaction was initiated by the addition of 1 ml of enzyme sample solution and incubated at 37^oC for 30 minutes. The reaction was stopped using 1ml of 2M sodium carbonate solution. Absorbance was noted at wavelength of 420 nm.

Alkaline phosphatase Assay:^(35, 36) Alkaline phosphatase activity was determined by 5 mM PNPP glycine buffer (pH 10) as substrate solution. Then the reaction was initiated by the addition of 0.5 ml of enzyme solution and incubated for 15 minutes. The reaction was stopped using 1ml of 0.1M sodium hydroxide solution. Absorbance was noted at wavelength of 450 nm.

SDS PAGE (SDS Page Gel Electrophoresis):⁽³⁷⁾ The tissue supernatant samples of 20 μ l are electrophoresed in a polyacrylamide gel (30%) having 10% sodium dodecyl sulfate (SDS) at a constant voltage of 50 Volts for 15mins, followed by 100 Volts for 2 hrs (approximately). The run was stopped when the marker dye reached 1-2 cm above the lower edge of the plate and turn off the current. The gel was stained with coomassive blue for proteins to visualize the band pattern and destained with 5% glacial acetic acid. Analyze each band with standard protein ladder and molecular weights were recorded.

Biostatistical Analysis: The mean and standard deviation was calculated for both enzymes β -galactosidase and Alkaline phosphatase with respect to young, old aged and LiCl₂ treated samples of rats by using 't' test calculator (Quick Calcs, Graphpad.com). The p-value and standard error was calculated from mean value and standard deviation of both enzymes.

Results

The specific activities for β -galactosidase and Alkaline phosphatase were determined for Brain and liver tissue samples of young, old aged, 10 days and 20 days LiCl_2 treated male wistar rats. Specific activity was calculated by total activity to total protein expressed in nm/min/mg of protein.

Estimation of Total Protein content in Rat brain and Liver: The total protein content for rat brain and liver extract in Young and old aged control, lithium treated young and old aged rats were estimated (Table 1 and 2).

Total protein content was higher in young control rats as compared to old rat's brain; however Lithium (LiCl_2) treated young rats showed lower protein content than their control. The young rats LiCl_2 treated for 10 and 20 days showed lower protein of 51.47 ± 0.54 and 35.23 ± 0.35 mg/ml respectively than their control 85.47 ± 0.41 mg/ml. Whereas 10 and 20 days LiCl_2 treated old aged rats showed higher protein content of 56.54 ± 0.46 and 50.48 ± 0.65 mg/ml respectively than their control 49.41 ± 0.43 mg/ml. Lithium showed positive effect for increase in protein content for 10 days LiCl_2 treated old aged rats than young rats (Table 1). This confirms that LiCl_2 treated for certain period time (10 days) showed increased protein has significant role for old rat brain cell activation. The two tailed P value is less than 0.0001. By conventional criteria, this difference is considered to be extremely statistically significant.

Table 1: Total protein content of male rat brain extract

Rat Brain	Young Rat Brain protein (mg/ml) std dev (n=5)	P value	Std error	Old Rat Brain protein (mg/ml) std dev (n=5)	P value	Std error
Control	85.47 ± 0.4			49.41 ± 0.43		
10 days LiCl_2 treatment	51.47 ± 0.54	0.0001	0.31	56.54 ± 0.46	0.0001	0.282
20 days LiCl_2 treatment	35.23 ± 0.35	0.0001	0.238	50.48 ± 0.65	0.0153	0.349

Total protein in liver was significantly higher than brain protein content. Total protein content is lower in young control rats liver as compared to old control rats and Lithium (LiCl_2) treated young rats showed lower protein content than their control (Table 2).

Table 2: Total protein content in male rat liver extract

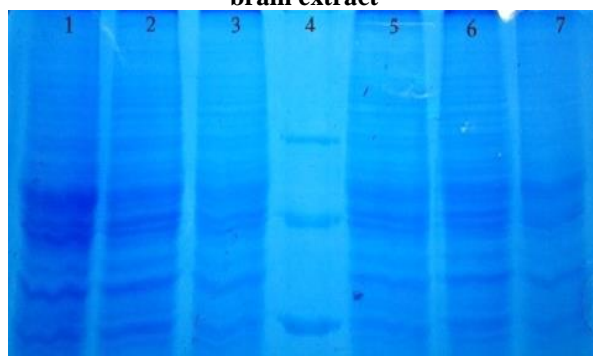
Rat Liver	Young Rat Liver (mg/ml) std dev (n=5)	P value	Std error	Old Rat Liver (mg/ml) std dev (n=5)	P value	Std error
Control	686.36 ± 5.74			827.52 ± 6.12		
10 days LiCl_2 treatment	572.47 ± 4.52	0.0001	3.267	884.64 ± 4.89	0.0001	3.503
20 days LiCl_2 treatment	526.06 ± 5.01	0.0001	3.407	856.67 ± 5.4	0.0001	3.650

The young rats LiCl_2 treated for 10 and 20 days showed lower protein of 572.47 ± 4.52 and 526.06 ± 5.01 mg/ml respectively than their control 686.36 ± 5.74 mg/ml. Whereas 10 and 20 days LiCl_2 treated old aged rats showed higher protein content of 884.64 ± 4.89 and 856.67 ± 5.4 mg/ml respectively than their control 827.52 ± 6.12 mg/ml. Lithium showed positive effect for increase in protein content for 10 days LiCl_2 treated old aged rats as compared to young rats (Fig 1) and confirms that LiCl_2 showed increased protein for significant role in old rat liver cell function.

SDS PAGE band pattern in brain and liver extracts of young, old and LiCl_2 treated rats:

The protein band pattern of SDS PAGE for different age group of rat brain showed almost similar to spectrophotometric results and few different proteins were also visible. However the intensity varies from control to lithium treated rat brain and liver (Fig. 1 and 2). Young rat brain and liver protein band intensity was less than its control where as old rat brain and liver showed high intensity band pattern for 10 days lithium treated rats but slightly intensity decreased in 20 days lithium treated rats therefore lithium has mild effect on old brain cell function.

SDS PAGE band pattern in both young and old brain extract



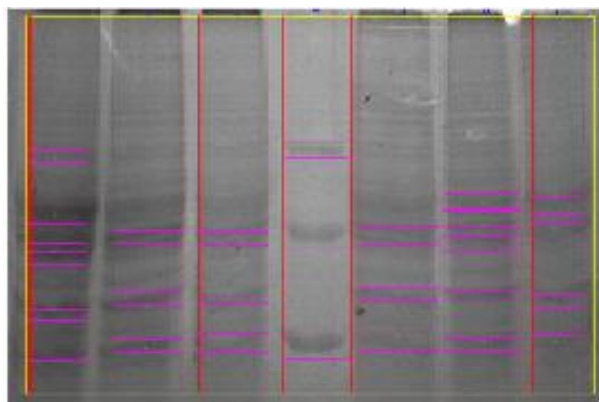


Fig. 1: Protein band patterns for both young and old brain extract

Lane 1 - Young control, Lane 2 - Young 10 days treatment, Lane 3 - Young 20 days treatment, Lane 4 - Standard marker (upper band—43000, Middle--29000, Lower--14300 Da) Lane 5 - Old 20 days treatment, Lane 6 - Old 10 days treatment, Lane 7 - Old control

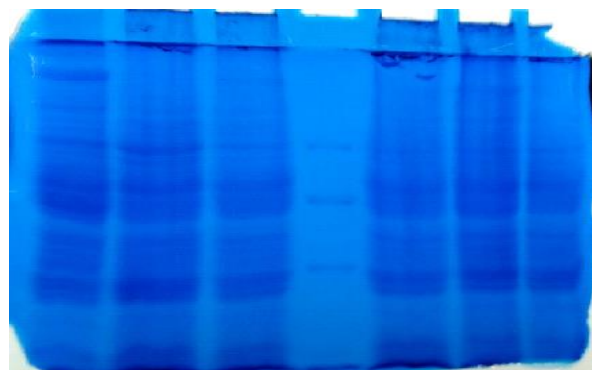


Fig. 2: Protein band patterns for both young and old liver extract extract

Lane 1 - Young control, Lane 2 - Young 10 days treatment, Lane 3 - Young 20 days treatment, Lane 4 - Standard marker (Upper band—43000, Middle--29000, Lower--14300 Da), Lane 5 - Old 20 days treatment, Lane 6 - Old 10 days treatment, Lane 7 - Old control

Estimation of Specific activity β -galactosidase in rat extracts brain Liver: Specific activity of β -galactosidase was calculated for both control and LiCl_2 treated rats of brain extract showed different activities in normal young and old rats. Further activity of this enzyme also different in 10 and 20 days LiCl_2 treated young and old aged rats (Table 3).

Table 3: β -galactosidase activity of male rat brain extracts

Rat Brain	Young rat Brain Specific Activity (nm/min/mg) Std dev (n=5)	P value	Std Error	Old rat Brain Specific Activity (nm/min/mg) Std dev (n=5)	P value	Std Error
Control	1.16 \pm 0.064			1.45 \pm 0.098		
10 days LiCl_2 treatment	3.74 \pm 0.097	0.0001	0.052	1.84 \pm 0.127	0.0006	0.072
20 days LiCl_2 treatment	6.53 \pm 0.126	0.0001	0.063	3.52 \pm 0.118	0.0001	0.069

β -Galactosidase showed higher significant specific activity of 6.53 ± 0.126 and 3.74 ± 0.097 nmoles/min/mg respectively for 20 and 10 days LiCl_2 treated young rats as compared to their control 1.16 ± 0.064 nmoles/min/mg of protein. Whereas old aged LiCl_2 treated rats showed higher β -galactosidase activity of 3.52 ± 0.118 and 1.84 ± 0.127 respectively for 20 and 10 days as compared to control 1.45 ± 0.098 nmoles/min/mg of protein but not showed significant elevated activity as compared to young rat brain samples. Two-tailed P value is less than 0.0001 except for 10 days LiCl_2 treated old brain 0.0006.

In case of rat liver, β -galactosidase showed higher specific activity of 2.26 ± 0.045 for 20 days LiCl_2 treated young rats, however 10 days LiCl_2 treated rats showed 1.72 ± 0.046 nmoles/min/mg of protein but not showed significant difference as compared to their control 1.66 ± 0.031 nmoles/min/mg of protein (Table 4). Whereas old aged 20 and 10 days LiCl_2 treated rats showed higher β -galactosidase activity of 2.70 ± 0.051 and 1.75 ± 0.037 respectively as compared to their control 1.05 ± 0.048 nmoles/min/mg of protein. It confirms that lithium has positive effect on old liver cells for elevated β -galactosidase activity especially for 20 days LiCl_2 treated old aged rats and young rat liver respectively. Two-tailed P value is less than 0.0001 except for 10 days LiCl_2 treated young brain 0.0419.

Table 4: β -galactosidase activity of male rat liver extracts

Rat Liver	Young rat liver Specific Activity (nm/min/mg) Std dev (n=5)	P value	Std Error	Old rat liver Specific Activity (nm/min/mg) Std dev (n=5)	P value	Std Error
Control	1.66 \pm 0.031			1.05 \pm 0.048		
10 days LiCl ₂ treatment	1.72 \pm 0.046	0.0419	0.025	1.75 \pm 0.037	0.0001	0.025
20 days LiCl ₂ treatment	2.26 \pm 0.045	0.0001	0.024	2.70 \pm 0.051	0.0001	0.031

Estimation of Alkaline phosphatase Specific activity in Rat Brain and liver extracts: Alkaline phosphatase showed higher significant specific activity of 27.74 \pm 0.237 and 16.77 \pm 0.309 nmoles/min/mg respectively for 20 and 10 days LiCl₂ treated young rats as compared to their control 3.80 \pm 0.230 nmoles/min/mg of protein (Table V). Whereas old aged 20 and 10 days LiCl₂ treated rats showed higher Alkaline phosphatase activity of 16.30 \pm 0.240 and 14.28 \pm 0.215 respectively as compared to their control 8.65 \pm 0.304 nmoles/min/mg of protein but not significant elevated activity as compared to control young rat brain enzyme activity lower than old rat brain cells. Two-tailed P value is less than 0.0001. By conventional criteria, this difference is considered to be extremely statistically significant.

Table 5: Alkaline phosphatase activity of male rat brain extracts

Rat Brain	Young rat brain Specific Activity (nanomoles/min/mg) Std dev (n=5)	P value	Std Error	Old rat brain Specific Activity (nanomoles/min/mg) Std dev (n=5)	P value	Std Error
Control	3.80 \pm 0.230			8.65 \pm 0.304		
10 days LiCl ₂ treatment	16.77 \pm 0.309	0.0001	0.172	14.28 \pm 0.215	0.0001	0.167
20 days LiCl ₂ treatment	27.74 \pm 0.237	0.0001	0.148	16.30 \pm 0.240	0.0001	0.173

In case of rat liver, Alkaline phosphatase showed higher significant specific activity of 21.28 \pm 0.317 and 19.51 \pm 0.487 nmoles/min/mg respectively for 20 and 10 days LiCl₂ treated young rats as compared to their control 3.67 \pm 0.402 nmoles/min/mg of protein (Table 6). Whereas old aged 20 and 10 days LiCl₂ treated rats showed higher significant ALP activity of 18.45 \pm 0.439 and 15.02 \pm 0.355 respectively as compared to their control 3.26 \pm 0.304 nmoles/min/mg of protein. Two-tailed P value is less than 0.0001.

Table 6: Alkaline phosphatase activity of liver extracts

Rat Liver	Young rat liver Specific Activity Std dev (nm/min/mg) (n=5)	P value	Std error	Old rat liver Specific Activity Std dev (nm/min/mg) (n=5)	P value	Std error
Control	3.67 \pm 0.402			3.26 \pm 0.304		
10 days LiCl ₂ treatment	19.51 \pm 0.487	0.0001	0.282	15.02 \pm 0.355	0.0001	0.209
20 days LiCl ₂ treatment	21.28 \pm 0.317	0.0001	0.229	18.45 \pm 0.439	0.0001	0.239

Lithium has shown elevated activity for β -galactosidase and Alkaline phosphatase in brain and liver of both aged rat samples as compared to respective controls. This confirms that lithium has positive effect for both brain and liver cellular enzyme activities that leads to regulation in metabolism and integrity of cells.

finding total activity (Fig. 4 and 5) and total protein (Fig. 1, 2 and 3).

Discussion

Alkaline phosphatase and β -galactosidase Specific activity was determined for all male rat groups by

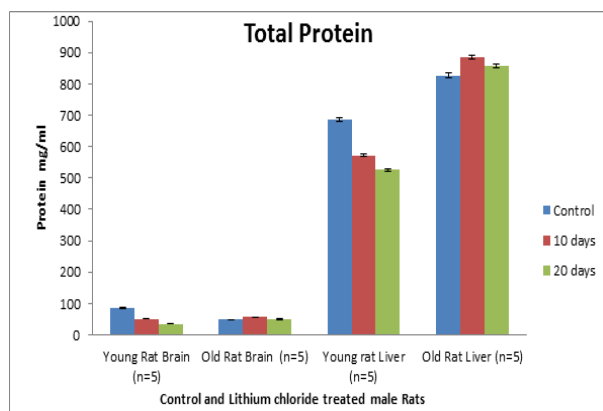


Fig. 3: Total protein content in Control and Lithium chloride treated male Rats

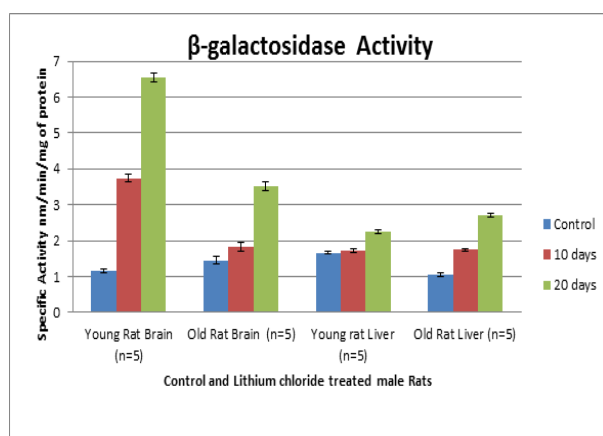


Fig. 4: β -galactosidase Specific Activity in Control and Lithium chloride treated male Rats

In brain total protein content was higher in young rats than old rats. However LiCl_2 treated young rats showed lower protein than their control. Whereas protein was slightly more in 10 days LiCl_2 treated rats than 20 days LiCl_2 treated old aged rats and control. This confirms that Brain protein showed positive effect for lithium treatment for short period of time. Even the similar results were obtained for the liver samples also. Hence Lithium play an important role for liver cell activation. The protein band pattern of electrophoresis also showed similar results to Spectrophotometric results. High intensity band pattern was noticed for 10 days LiCl_2 treated old aged rat brain and liver than other groups. Therefore lithium may be one of the best conjugative or as a drug play significant role for old rat brain and liver cell function in aged related diseases.

Specific activity for β -galactosidase and Alkaline phosphatase: Specific Activity of β -galactosidase for young male rat brain showed significantly higher for 10 and 20 days LiCl_2 treatment than their control and old rat brain cells. However old rat brain cells also showed higher activity for LiCl_2 treated rats than their control. These results are also similar to young and old rat liver which showed higher β -galactosidase activity than their

control but not significant as compared to young brain cells (Fig. 4).

β -galactosidase is not only marker for cell senescence only but also reveals that it is also for marker for growth of the cells which showed higher enzyme activity when isolated experimentally. It showed higher activity in many brain tumors especially for many meningiomas which has many no of cells in specified area than normal brain and in early passages of glioma derived cell lines as compared to parent tumors.^(38,39) This suggests that β -galactosidase localized in cell and membrane bound ie, where ever there is an increase in the number of cell growth the enzyme level was increased. Hence our current result suggests that lithium is involved in the higher β -galactosidase activity that refers to the growth of cells which applies to young brain cells of rats.

In supporting to these result earlier reports suggests Immunohistochemical analysis was undertaken 1 day after the last injection, and three-dimensional stereological cell counting revealed that lithium produced a significant 25% increase in the BrdU labeled cells in the dentate gyrus. Lithium and antidepressants may exert some of their beneficial effects by regulating hippocampal neurogenesis and may thus also have utility in the treatment of other neuropsychiatric disorders.⁽⁴⁰⁾

Therefore lithium has positive effect for β -galactosidase activity on young brain cells where the cell numbers are more with intact membrane cells. β -Galactosidase enzyme fragment complementation for the measurement of Wnt/ β -catenin signaling, important regulator of cell polarity, proliferation and stem cell maintenance during development and adulthood.⁽⁴¹⁾

From this study, lithium not only elevated the level of β -galactosidase activity for increase in the growth of brain and liver cells but also involved in defence action since it is also lysosomal localized enzyme for hydrolysis reaction, degradation of complex lipid molecules, removal of toxins and cell debris. Hence it is involved in regulation of cell function and possible for molecular therapy of brain pathology in lysosomal and neurodegenerative disorders.

Therefore it is necessary to obtain higher level of β -galactosidase even for monitoring tau self association in the treatment of neurodegeneration. Control of hyperphosphorylation of tau proteins with an increased level of Alkaline phosphatase which removes phosphate groups that prevents the neurofibrillary tangles.

The current study also deals with Alkaline phosphatase (ALP) which showed higher specific activity more significantly for normal old aged rats brain than young rats brain. However young rats treated with LiCl_2 showed significant elevated ALP activity than old rat brain treated with LiCl_2 and to their control. This confirms that lithium has more effect on young brain cells and higher membrane bound enzyme activity suggests that increase in cell numbers for young rats

(Fig. 5). While in liver ALP specific activity was significantly higher for young and old rats treated with LiCl_2 than their control. This confirms that lithium has constant effect on ALP for young and old rat liver.

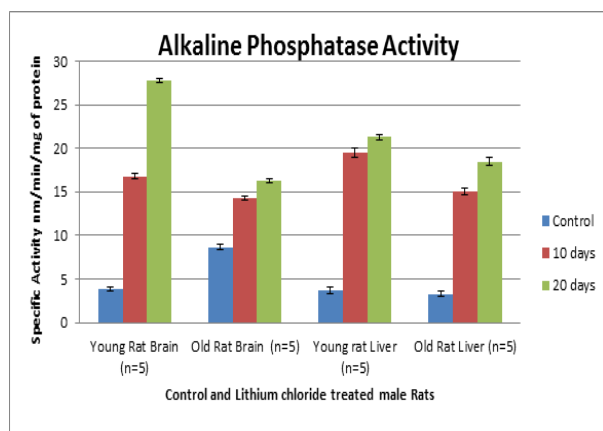


Fig. 5: Alkaline Phosphatase Specific Activity in Control and Lithium chloride treated male Rats

Alkaline phosphatase changes and play a role during brain growth and development particularly whenever the rat models treated with modulators or drugs for both young and old rats which showed elevated ALP activity due to changes in the relative cell numbers.

The quantity of enzyme expressed in a tissue may be related to its relative functional importance and as ages ALP activity decreases. Therefore lithium has positive effect on ALP activity more in young brain cells where the cells are more in number and intact membrane cells as compared to liver and old brain. However the mechanism of Lithium related to both enzyme activity yet to be proved.

Thus these enzyme activities involved in cellular activation with modulator lithium which can elicit brain cell function, liver cell function, neuroprotective, neurogenesis. Therefore lithium can be used as drug modulator by standardizing with enzyme assays to show higher activity in old cells as compared to young cells for the treatment of liver disorders, age related diseases and neurodegenerative diseases.

So the current studies which showed the increased levels of β -galactosidase with LiCl_2 may help in the degradative function of brain cells. However to find out involvement of neurons and astrocytes for degradative function by β -galactosidase and alkaline phosphatase with LiCl_2 would require the enzyme assay studies with cell culture of astrocytes and neurons separately and with coculture studies.

Therefore this may contribute a valuable therapeutic agent to the field of pharmaceuticals, which helps in regulation of enzymes believed to play an important role in regulation brain cell function, neurodegenerative diseases and liver disorders.

Conclusion

The protein content was more in normal young brain than old rat brain. Interestingly the protein was increased for LiCl_2 treated old brain than their control and young brain confirms LiCl_2 increases protein essential for old brain cell activation. β -galactosidase showed higher specific activity for young brain cells than old brain and liver, while Alkaline phosphatase showed significantly higher specific activity for 10 and 20 days LiCl_2 treated young and old rat's brain than liver and control. Hence lithium has more positive effect on Alkaline phosphatase than β -galactosidase in young brain than old brain and liver. Therefore Lithium can be a therapeutic drug to enhance the cell growth, membrane cell integrity, degradation of complex molecules in neurodegenerative diseases and helps in various brain cell function.

Acknowledgement

We express our sincere thanks to Dr. Channarayappa, Professor and Head, Department of Biotechnology, MSRIT for his encouragement. Authors would like to acknowledge Prof. Nagaveni, Head of the Department and MLACW for giving us an opportunity to carryout project work in their institute. We sincerely thank Mr. Lokesh K.N., Asst Professor, Department of Biotechnology, MSRIT for helping in calculations for this project.

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