Full Length Research Paper

Analysis of some lipid parameters following lithium administration

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Lithium is a drug of choice for treatment of some psychological disorders including manic depression. Wistar Rats (male and female) were housed in individual improvised cages. The rats were acclimatized for two weeks after which they were randomly grouped into 3 viz control, lithium treated and lithium with saline treated group and treated for four weeks. The lithium treated group received 40 mM lithium bicarbonate per kg of feed for the first 1 week while the dosage was increased to 60mM per kg of feed for the rest of the 3 weeks. Lithium saline group received saline solution in addition to lithium. The control group was given normal feed and water liberally for the period of the experiment. At the end of the experiment the serum and the tissue cholesterol and triglycerides were quantified and the serum lithium level also determined. The results showed significant values for weight loss, serum lithium and cholesterol. The lipid parameters generally decreased. In conclusion the systemic changes in plasma lipid parameters at the cellular level induce changes in metabolism and cell membrane and this should be considered seriously in patients who are under lithium therapy for a long period because the clinically important consequence is significant.

Key words: Wistar rats, lithium, lipid parameters, cell membrane.

INTRODUCTION

Lithium is a drug of choice for treatment of some psychological disorders including manic depression (Koffman et al., 1991; Rapoport and Bosetti, 2002). The exact molecular mechanism by which lithium exerts its therapeutic and prophylactic action is not fully under-stood. It has been shown that this drug affects the metabolic cycles of inositol phosphate by inhibiting the inositol mono phosphatase (Duffy and Kane, 1996; Atack, 1996). Lithium-induced inhibition of this enzyme causes accumulation of certain inositol phosphates, primarily inositol monophosphate (Atack, 1996).

The peripheral side effects of this simple ion are very few, but some intracellular changes have already been reported such as inhibition of inositol phosphate metabolism (Lee et al., 1992; Godfrey et al., 1989), alteration in cAMP second messenger system and adenylate cyclase activity (Belmaker, 1981), inhibition of insulin release (Zawalich et al., 1989), potentiation of cerebral 5HT release (Newman and Lerer, 1988), muscarinic receptor stimulation of inositol phosphate in rat cerebral cortex slices (Batty and Stefan, 1985) and generation of lithium-sensitive gene products (Detera-Wadleigh, 2001). Lithium may also affect lipoprotein metabolism via changes in the concentration of some related hormones such as thyroid hormones (Zetin, 2004).

It has recently been reported that in type V hyper-lipoproteinemia, the activity of lithium-sodium counter transporter in cell membrane is significantly altered (Weirzbicki, 2001). Little information is, however, available on the changes in lipoprotein metabolism under lithium treatment. Due to the inter-relationship between

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Table 1. Reagent composition for cholesterol determination.

Reagent	Withal concentration
4-Aminoantipyrine	0.30
Phenol	6.0 mmol/L
Peroxidase	> 0.50 u/l
Cholesterol esterase	> 0.15u/l
Cholesterol oxidase	> 0.10u/l
PIPES Buffer	80 mmo/L pH-6.8
Cholesterol standard	5.18 mmol/L 200 mg/dl

Table 2. Procedure for cholesterol determination.

Tubes	Reagent blank (ul)	Standard (ul)	Sample (ul)
Distilled H$_2$O	10	-	-
Standard	-	10	-
Sample	-	-	10
Reagent	1000	1000	1000

some of these lithium-induced changes and the meta-bolism of lipids (Soares, 2000), this study was undertaken to investigate the changes in plasma and tissue choleste-rol and triglyceride levels following lithium administration. This is clinically very important in psychiatric patients who are under long-term treatment of this drug.

MATERIALS AND METHOD

Wistar Rat (male and female) were sourced from the animal holdings of the Department. The rats were housed in individual improvised cages containing wood shavings which were regularly changed. The rats were acclimatized for two weeks. During acclimatization, the rats were given feed and water *ad libitum.*

After the acclimatization period, the rats were randomly grouped into 3 groups viz control, lithium treated and lithium with saline treated groups. The experiment runs for four weeks. The animals were administered lithium via their meals (Dam). Lithium was mixed with their meal in the proportion calculated below:

Dam composition

0.9% saline solution
0.9 g of Nacl in 1000 m/s of distilled water
This was administered to the lithium saline treated group.

Feed preparation

40 mM lithium carbonate
73.89 g of Li2co3 in 1000 ml of distilled water = 1000 Mm
Xg of Li2Co3 in 1000 ml of distilled water = 40Mm

X g = (40 Mm x 73.89) / 1000 Mm

Xg = 2.96
X = 2.96 g
60 Mm lithium carbonates
73.89 g of Li2CO3 in 1000 ml of distilled water= 1000 Mm
X g of Li2CO3 in 1000 mls of distilled water = 1000 Mm

X = (60 Mm x 73.89) / 1000 mls

X = 4.43 g

The lithium treated group received 40 mM lithium bicarbonate per kg of feed for the first 1 week while the dosage was increased to 60 mM per kg of feed for the rest of the 3 weeks. Lithium saline group received saline solution in addition to lithium. The control group was given normal feed and water liberally for the period of the experiment.

Sample collection and chemical analysis

After 4 weeks of administration, rats were sacrificed by an overdose of Pentothal. After 4 weeks of administration, rats were sacrificed by an overdose of Pentothal.

Blood was taken from the heart and brain (prefrontal cortex) sample taken for biochemical analysis (Lipid profile).

Cholesterol determination

The method used for cholesterol determination was based on the principle of enzymatic hydrolysis and oxidation whereby the indica-tor quinoneimine is formed from the reaction of hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

N.B Samples used include serum and tissue homogenate. Table 1.

Procedure

The reagent and samples were brought to room temperature and pipetted into labeled test tubes. It was mixed in the test tubes and allowed to stand for 10minutes at room temperature 20 - 25°C. The absorbance of the sample (A sample) was read against the reagent blank within 60 min. Absorbance was read at 500 nm (Table 2).

Calculation:

(Asample \ Astandard) x Concentration of standard

Triglyceride determination

Triglyceride determination is based on the principle of enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxides, 4-Aminophenazone and 4-Chlorophenol under the catalytic influence of Peroxidase. Sample used were Serum and tissue homogenate (Table 3).

Procedure

The reagent and the sample were brought to room temperature and Pipetted into labeled test tubes.

It was mixed and incubated for 10minute at 20^C - 25^C.The absorbance was measured at 500nm of sample (Asample) and standard (Astandard) against reagent blank for 60 min (Table 4).

Table 3. Reagent composition for triglyceride determination.

Pipes buffer	40.0 mmol/L pH-7.6
4- Chlorophenol	5.5 mmol/L
Magnesium ions	0.5 mmol/L
4- aminophenazone	1.5 mmol/L
ATP	1.0 mmol/L
Lipases	$\geq$150.0 u/l
Glycerol Kinase	$\geq$0.4 u/l
Glycerol-3- Phosphate Oxidase	$\geq$1.5 u/l
Peroxidase	$\geq$0.5 u/l
Standard	2.26 mmol/L or 200 mg/Dl

Table 4. Procedure for determination of triglyceride.

	Reagent blank (ul)	Standard (ul)	Samples (ul)
Sample	-	-	10
Standard	-	10	-
Reagent	1000	1000	1000

Table 5. Lithium wavelength.

Metal	Wavelength (nM)
Lithium (Li)	670.8

Calculation:

(Asample / Astandard) X Standard Concentration (mmol/L or mg/dL)

Procedure for determination of trace metals

Sample used: Serum

Procedure

Centrifuge Blood samples at 3000 rpm for 30 min to get the supernatant

Read samples in an Atomic Absorption Spectrophotometer after standardizing the machine and checking its sensitivity (Preer et al., 1977). Read samples using respective lamps and wavelength (Table 5).

RESULTS

Mean weight changes

Figure 1 reveals significant (P < 0.03) weight decrease in the lithium treated group as compared to the control, while saline supplemented group also showed a significant (P < 0.012) weight loss which was milder when
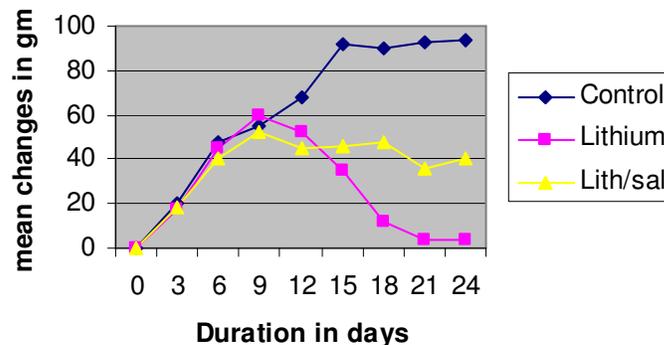
Figure 1. Mean weight changes (gm) in lithium, lithium/saline and control.

compared to the lithium group.

Determination of serum lithium level

The serum lithium level is the true measure of quantity exposed to. In this study the mean serum level increased significantly in exposed groups (Table 1).

Determination of lipids

Table 1 describes the lipid profile measured in this study. The total serum cholesterol dropped in the exposed groups, the decline was significant (P < 0.035) in the case of lithium group. The serum triglycerides decreased in the exposed groups, while the tissue level increased in the lithium group when compared to the control.

DISCUSSION

The serum levels of cholesterol and triglyceride in this study decreased, with significant value (P < 0.05) in the case of cholesterol. The triglyceride brain tissue level increased significantly while the cholesterol level de-clined. In both cases the saline seems to confer notable protection against the adverse effect of lithium administration in the saline/lithium treated groups. These observations might be as a result of changes in the activity of lipoprotein lipase, a key enzyme that plays an important role in the metabolism, transport and tissue uptake of lipid fractions.

The inhibitory effect of lithium on this enzyme is countered by the saline. This could be due the fact that once lithium is in the body and the compound (lithium carbonate) dissolves, the lithium cation competes with sodium cations, potassium cations, magnesium cations and calcium cations. Lithium cations also substitutes for sodium cations and/or potassium cations. In the case of lithium saline treated animals theexogenous administered sodium probably enhances Na-Na exchange across the

Table 1. Comparison between the lithium, saline/lithium treated and control groups.

Variable	Mean and Std error		P-value
	Exposed group	Control	
Lithium serum level in lit group	50.55 ± 1.20	18.60 ± 1.29	0.002
Lithium serum level in saline/lit gp	29.00 ± 1.29	18.60 ± 1.29	0.017
Serum chol level in lit group	28.96 ± 13.65	212.20 ± 34.97	0.035
Serum chol level in saline/lit gp	94.40 ± 16.20	212.20 ± 34.97	0.185
Brain tissue chol level in lit group	66.46 ± 34.50	76.19 ± 13.33	0.950
Brain tissue chol level in sal/lit gp	70.39 ± 9.36	76.19 ± 13.33	0.870
Serum trig level in lit group	202.95 ± 2.95	286.05 ± 42.73	0.278
Serum trig level in saline/lit gp	275.52 ± 10.67	286.05 ± 42.73	0.535
Brain tissue trig level in lit group	381.36 ± 57.14	264.97 ± 52.51	0.094
Brain tissue trig level in sal/lit gp	98.15 ± 16.48	264.97 ± 52.51	0.236

$P \leq 0.05$ (significant) Lit = lithium; Sal=saline; gp = group; chol=cholesterol; trig=triglycerides.

membrane, leading to a stable Na/K ion channels which delays or prevents the inhibitory effect of lithium on the enzyme. The exact mechanism by which lithium inhibits lipoprotein lipase activity is not known exactly, however the activity of this enzyme depends on the presence of free-SH groups (Tornqvist and Belfrage, 1976). It is probable that lithium by interacting with some essential-SH groups in the active site of the enzyme reduces enzyme activity.

Previous reports showed significantly reduced lipolysis in line with the present study. Intracellular lipolysis is achieved by the action of hormone sensitive lipase (HSL) (Mulder and Stenson, 1999) indicating that lithium may also have an inhibitory effect on HSL activity. Intracellular lipase activity is well known to be regulated through a mechanism in which adenylate cyclase system is involved. Thus, it is probable that this action of lithium on lipase activity is mediated through adenylate cyclase system. Indeed, it has already been reported that lithium could affect cell metabolism through receptor-mediated adenylate cyclase (Ebstein, 1980). The interaction of lithium with adenylate cyclase system has recently been argued by some authors (Husseini et al., 1991 and Lenox et al., 2000). The mechanism by which lithium affects lipid metabolism was not investigated in this study but could be that lithium inhibits the enzyme activity by interacting with the G-protein of the adenylate cyclase system. Thus, the intracellular level of cAMP is markedly reduced (Husseini et al., 1991). Regarding the above mechanisms and the relationship between intracellular cAMP level and lipid metabolism, it can be expected that lithium may change plasma lipid fractions. The reduction in the plasma levels of free fatty acids could be attributed to either the lowered activity of lipoprotein lipase in the presence of lithium or the inhibition of adenylate cyclase system leading to the reduction in intracellular levels of cAMP and the inactivation of HSL. Our results also showed that the serum triglycerides decreased following the administration of lithium. Triglycerides are synthe-sized from the esterification of glycerol phosphate and acyl CoA. Glycerol phosphate in cells is partly maintained by glycolytic reactions and lithium is reported to inhibit glycolytic enzymes (Mellerup and Rafaelsen, 1975) therefore, the intracellular level of glycerol phosphate is limited. Lithium, on the other hand, inhibits lipoprotein lipase and lowers the plasma levels of free fatty acids. It is probable that the limitation of triglyceride synthesis may result in lower levels of triglycerides. Our results showed that lithium increased triglycerides contents of brain. Substrates that could not reach the triglyceride synthesis pathways may contribute to phospholipids synthesis, but in this case the substrates were channeled to the synthesis of triglycerides with the concomitant reduction in the synthesis of phospholipids altering the composition of the cell membrane phospholipids and most probably affecting the BBB.

We concluded that systemic changes in plasma lipid parameters, at the cellular level, induce changes in metabolism and cell membrane and this should be considered seriously in patients who are under lithium therapy for a long period because the clinically important consequence is significant.

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