EFFECT OF LITHIUM METAL ON THE CHEMICAL STATUS OF GLUTATHIONE (GSH) PRESENT IN WHOLE BLOOD (ESPECIALLY IN PLASMA AND CYTOSOLIC FRACTION IN HUMAN BLOOD)

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ABSTRACT

Lithium remains a mainstay in the acute and prophylactic treatment of bipolar affective disorder. It is used in the augmentation of antidepressant treatment and, less frequently, in the augmentation of antipsychotic treatment of schizophrenia. It is reported to have specific anti-suicidal effects. Thus the effect of Lithium was interesting to study on the glutathione (GSH) level *in vivo* conditions. Ellman's method has been used to see the effect of lithium on glutathione (GSH) level in whole blood. The time dependent effect of Lithium on the chemical status of glutathione (GSH) was determined in the whole blood (Plasma and cytosolic fraction) of human. The concentration of Glutathione was drastically decreased. The decrease in the glutathione level was concentration and time of interaction dependent, probably due to oxidation of glutathione (GSH) to corresponding disulphide (GSSG). In this paper the effect of Lithium on the Thiol/GSH level was discussed *in vitro,* which in principal may present a model of *in vivo* reaction.

Keywords: Lithium (Li), Reduced Glutathione (GSH), Oxidize Glutathione (GSSG), 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB).

INTRODUCTION

Glutathione (g-glutamylcysteinylglycine, GSH) is a sulfhydryl (SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is found in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system (Kosower *et al*., 1978; Meister, 1976; Kidd, 1991; Lomaestro *et al*., 1995).Glutathione exists in two forms: The antioxidant "reduced glutathione" tripeptide is conventionally called glutathione and abbreviated GSH; the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress.

GSH has potent electron-donating capacity, as indicated by the high negative redox potential of the GSH/GSSH "redox couple" (E'0 =-0.33v) (Lewin, 1976). Its high redox potential renders GSH both a potent antioxidant and a convenient cofactor for enzymatic reactions that require readily available electron pairs (Kehrer *et al*., 1994). The reducing power of GSH is a measure of its free radical scavenging, electron-donating, and sulfhydryl-donating capacity. The reduced glutathione molecule consists of three amino acids - Glutamic acid, Cysteine, and Glycine - covalently joined end-to-end. The sulfhydryl (-SH) group, which gives the molecule its electron-donating character, comes from the cysteine residue. Glutathione is present inside cells mainly in its reduced (electron-rich, antioxidant) GSH form. In the healthy cell GSSG, the oxidized (electron-poor) form, rarely exceeds 10 percent of total cell glutathione (Kosower *et al*., 1978). Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Duke *et al*., 1996; Nobel *et al*., 1995).

Lithium in pharmacology refers to the lithium ion, Li^+, used as a drug (Hecht *et al*., 2000). Lithium is administered in a number of chemical salts of lithium, which are used primarily in the treatment of bipolar disorder as mood stabilizing drugs. In bipolar disorder they have a role in the treatment of depression and mania acutely and in the long term. As a mood stabilizer, lithium is probably more effective in preventing mania than depression, and may reduce the risk of suicide. In depression alone (unipolar disorder) lithium can be used to augment other antidepressants. Lithium carbonate (Li_2CO_3), sold as is the most commonly prescribed, whilst the citrate salt lithium citrate ($Li_3C_6H_5O_7$), the sulfate salt lithium sulfate (Li_2SO_4), aspartate and the orotate salt lithium orotate are alternatives (Nih.gov, 2007). Lithium has affinity for the glutathione present in aqueous phases of blood. This affinity is mainly formed between metal and sulfhydryl groups of glutathione (Quig, 1998).This affinity can cause a depletion of the reduced form glutathione in the blood, but with the depletion of the

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glutathione (GSH), GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle but if GSH is usually not effectively supplied, however, if GSH depletion continues because of chronic metal exposure (Stohs *et al*., 1993; Quig, 1998; Hultberg *et al*., 2001) then the pharmacological benefits of the metal being used for the help of body defenses can be harmful in nature to the body defense system. The following study makes a design to see the effects of Lithium metal, in respect of time, on the chemical status of glutathione in whole blood especially in plasma and cytosolic fraction in human blood.

MATERIALS AND METHODS

Materials

Sodium hydroxide, L.glutathione (GSH) was purchased from Fluka, 5, 5-dithiobis,2-nitrobenzoic acid (DTNB) from Sigma, Potassium dihydrogen phosphate and Disodium edetate was supplied by Reidel-ed-Haen, HCl 35% from (Kolchlight), lithium carbonate was obtained from BDH, Sodium Chloride, Chloroform and Ethanol from Merck, Water for Injection from (Elixir Laboratories), All other chemicals used were of analytical reagent grade with no further purification

1- Standard Curve for Glutathione

Different concentrations have been made from 1mM solutions of glutathione (GSH) and 0.2ml (200µl) of them was added to 2.3ml (2300µl) of phosphate buffer pH 7.6, followed by the addition of 0.5ml (500µl) of 1mM 5, 5-Dithiobis, and 2-Nitrobenzoic Acid (DTNB) stock solution. The mixtures were shaken thoroughly and incubated for 5 minutes. Absorbances were taken after 5 minutes at fixed wavelength of 412nm. The absorption of 5,5-dithiobis,2-nitrobenzoic acid (DTNB) blank solution, prepared by adding 0.5ml(500µl) of 1mM 5, 5-dithiobis, 2-nitrobenzoic acid (DTNB) stock solution in 2.3ml (2300µl) of phosphate buffer pH 7.6, was also obtained at fixed wavelength of 412nm. A real absorbance was obtained by subtracting the absorbances for 5,5-dithiobis,2-nitrobenzoic Acid (DTNB) blank solution from the absorbances for glutathione (GSH) plus 5,5-dithiobis,2-nitrobenzoic acid (DTNB) mixtures with different concentrations of glutathione (GSH). Standard curve was obtained as shown in fig. 1.

By this upper mentioned method we found the concentration of GSH in different solutions by observing the absorbances of GSH solutions at 412nm.

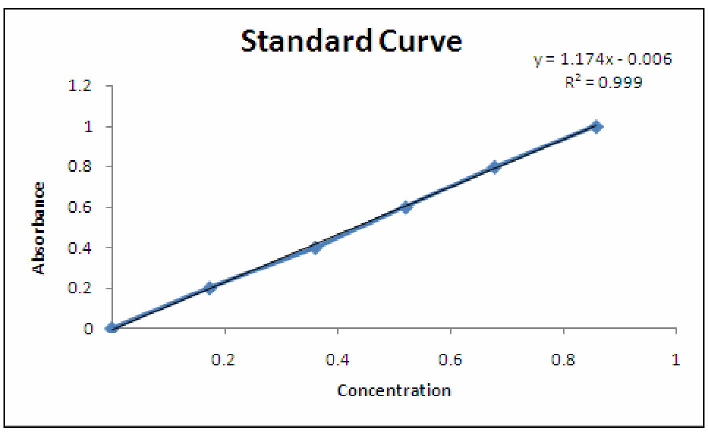

Fig. 1: Standard Curve for GSH + DTNB Mixture taken at 412nm on UV spectrophotometer.

Table 1: Effect of Lithium Carbonate on the Chemical Status of Glutathione (GSH) in Plasma with time.

UV Spectrophotometer absorbance readings of different solutions for GSH at 412nm Wavelength						
S. No.	Time Interval	Absorbances of Plasma + Lithium Carbonate	DTNB Blank (ABS)	Real absorbance* (ABS)	GSH Blank (ABS)	Real Absorbance for GSH Blank
1	0 min	0	0	0	0	0
2	30 min	0.341	0.044	0.297	0.456	0.412
3	60 min	0.330	0.049	0.281	0.450	0.401
4	90 min	0.317	0.043	0.274	0.460	0.417
5	120 min	0.311	0.048	0.263	0.453	0.405
6	150 min	0.308	0.049	0.259	0.440	0.391
7	180 min	0.294	0.050	0.244	0.449	0.399

* Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution

5 ml of human venous blood was taken and treated with heparin to prevent clotting was collected. The effect of Lithium (concentration, 2mM, added to the whole blood) on the chemical status of glutathione (GSH) in plasma and cytosolic fraction was studied in terms of determination of concentration of GSH in the mixtures by the well known Ellman's method (Ellman, 1959). Mixture of Lithium (concentration, 2mM) and whole blood was prepared by taking equal volumes of stock solution of Lithium (5ml) and whole blood (5ml) and keeping in refrigerator till used. The blood was then centrifuge on H-200 centrifuge at 10,000 rpm for two minutes. After centrifugation two layers of blood were formed. The upper layer was plasma and was removed with a Pasteur pipette and the lower layer which was precipitated down was cytosolic fraction and placed in refrigerator till use.

Determination of GSH in plasma

The assay of glutathione (GSH) with DTNB was performed followed by a standard Ellman's method (Ellman, 1959) for plasma of blood. And 2.3ml of potassium phosphate (0.2M, PH 7.6) buffer was taken in the cell and added 0.2ml aqueous solution (plasma of blood) and to this solution 0.5ml (0.001M) of 5, 5-dithiobis, 2-nitrobenzoic acid (DTNB) in a buffer was added. An absorbance of reaction product was observed after 5 minutes at 412 nm using UV/Visible double bean spectrophotometer (Shimadzu 1601, Japan) and GSH level was determined from standard curve of reduced GSH obtained with 0.2, 0.4, 0.6, 0.8 and 1mM GSH concentration.

Determination of GSH in cytosolic fraction

After isolation of plasma, 0.5ml of the red blood cells fraction was taken and washed 2 to 3 times with 1ml of isotonic saline (0.9% NaCl) solution and lysed with an equal volume (1:1) of distill water for one hour at 4^oC. And 0.6 ml of cold chloroform and ethanol (3:5 v/v) mixture at 0^oC was added to 2ml of lysed cells. Hemoglobin was precipitated followed by the addition of 0.3ml of distill water. Clear supernatant (pale yellow) cytosolic fraction of red blood cell was removed by pasteur pipette, after centrifugation and was analyzed for glutathione (GSH) level. For cytosolic fraction of blood.2.3ml of potassium phosphate (0.2M, PH 7.6) buffer was taken in the cell and added 0.2ml aqueous solution (plasma of blood) and then to this solution 0.5ml (0.001M) 5, 5-dithiobis, 2-nitrobenzoic acid (DTNB) in a buffer was added. An absorbance of reaction product was observed after 5 minutes at 412 nm using UV/Visible double bean spectrophotometer (Shimadzu 160, Japan) and GSH level was determined from standard curve of

Table 2: Effect of Lithium Carbonate on the Chemical Status of Glutathione (GSH) in Cytosolic Fraction (C.F) with time.

UV Spectrophotometer absorbance readings of different solutions for GSH at 412nm Wavelength						
S. No.	Time Interval	Absorbances of Plasma + Lithium Carbonate	DTNB Blank (ABS)	Real absorbance* (ABS)	GSH Blank (ABS)	Real Absorbance for GSH Blank
1	0 min	0	0	0	0	0
	30 min	0.184	0.040	0.144	0.200	0.160
2	60 min	0.160	0.060	0.100	0.198	0.138
3	90 min	0.134	0.050	0.084	0.196	0.146
4	120min	0.115	0.058	0.057	0.201	0.143
5	150min	0.109	0.056	0.053	0.203	0.147
6	180 min	0.098	0.060	0.038	0.197	0.137

*Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution

Table 3: Calculation for Concentration of GSH in Plasma after reaction with Lithium carbonate by Ellman's Method.

S. No.	Real Absorbance (ABS) of Plasma GSH depleted by Lithium Carbonate.	Concentration of GSH (μM) Remained in Plasma of Whole Blood after depleted by Lithium Carbonate	Real Absorbance (ABS) of Plasma blank solution for GSH	Concentration of GSH (μM) Remained in Plasma of Whole Blood after treated as blank
1	0.297	1.559	0.412	2.280
2	0.281	1.459	0.401	2.211
3	0.274	1.415	0.417	2.312
4	0.263	1.346	0.405	2.237
5	0.259	1.321	0.391	2.149
6	0.244	1.226	0.399	2.199

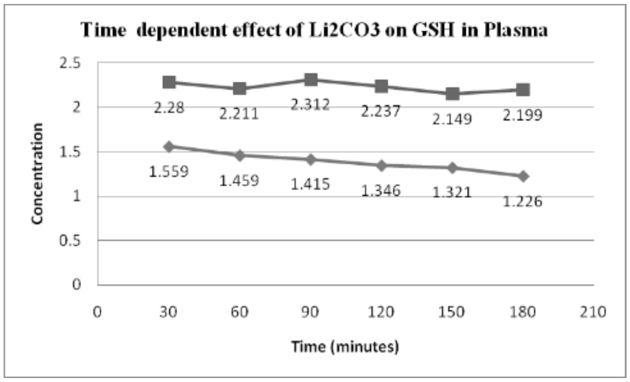

Fig. 2: The effect of lithium metal (2mM) on GSH level.GSH level was determined before (■) and after adding lithium metal (■). Upon addition of lithium metal in the plasma, decrease in GSH level in plasma was observed and found to be time dependent.

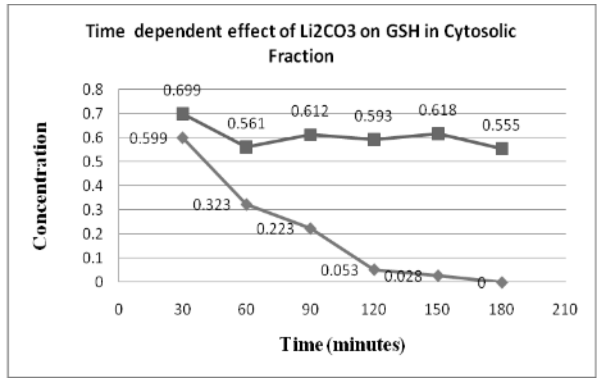

Fig. 3: The effect of lithium metal (2mM) on GSH level.GSH level was determined before (■) and after adding lithium metal (■). Upon addition of lithium metal in the cytosolic fraction of blood, decrease in GSH level in cytosolic fraction of blood was observed and found to be time dependent.

Table 4: Calculation for Concentration of GSH in cytosolic fraction after reaction with Lithium carbonate by Ellman's Method.

S. No.	Real Absorbance (ABS) of Cytosolic Fraction GSH depleted by Lithium Carbonate	Concentration of GSH (μM) Remained in Cytosolic Fraction of Whole Blood after depleted by Lithium Carbonate	Real Absorbance (ABS) of Cytosolic Fraction blank solution for GSH	Concentration of GSH (μM) Remained in Cytosolic Fraction of Whole Blood after treated as blank
1	0.144	0.599	0.160	0.699
2	0.100	0.323	0.138	0.561
3	0.084	0.223	0.146	0.612
4	0.057	0.053	0.143	0.593
5	0.053	0.028	0.147	0.618
6	0.038	0.000	0.137	0.555

reduced GSH obtained with 0.2, 0.4, 0.6, 0.8 and 1mM GSH concentration.

RESULTS

Effect of Lithium metal on the chemical status of glutathione present in the whole blood was studied in terms of concentration of glutathione (GSH) in plasma and cytosolic fraction by mixing the whole blood with 2mM concentration of Lithium metal. The decrease in normal concentration of GSH was observed with the time (30-180 minutes) in whole blood containing Lithium metal, where as the blank mixtures of plasma showed negligible variation of thiol status with the passage of time.

The decrease in GSH level in the plasma was less as compared with cytosolic fraction. The fig. 2 indicates decrease level of GSH in whole blood having lithium. The reason for decrease in concentration is that as with the passage of time concentration of lithium increases in RBCs which causes decrease in GSH level. This result confirms the finding that decrease in GSH level occurs as lithium metal crosses the cell membranes of RBCs and accumulates inside the RBCs (Khan and Khan, 2001).

STATISTICAL ANALYSIS

The paired comparison t-test of blank plasma & Lithium metal affected plasma gives the decision that there is an effect of Lithium metal on the chemical status of GSH in plasma as compared to blank.

Similarly the paired comparison t-test of blank cytosolic fraction & Lithium metal affected cytosolic fraction indicates that there is an effect of Lithium metal on the chemical status of GSH in cytosolic fraction as compared to blank cytosolic fraction.

Table 5: Statistical analysis of effect of lithium on the chemical status of GSH in plasma

1- Paired Samples Statistics					
		Mean	N	Std. Deviation (SD)	Std. Error Mean (SEM)
Pair	Lithium + Plasma	1.38767	6	0.116196	0.047437
	BLANK Plasma	2.23133	6	0.058558	0.023906

2- Paired Samples Correlations			
		N	Correlation
Pair	Lithium + Plasma / *BLANK Plasma*	6	0.575

3- Paired Samples Test									
		Paired Differences					t	df	t-Critical (1-Tail)
		Mean	Std. Deviation (SD)	Std. Error Mean (SEM)	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	Lithium + Plasma / *BLANK Plasma*	-0.844	0.095	0.039	-0.944	-0.744	-21.662	5	2.02

Table 6: Statistical Analysis of Effect of Lithium on GSH chemical Status in Cytosolic Fraction (C.F) of Whole Blood

1- Paired Samples Statistics					
		Mean	N	Std. Deviation (SD)	Std. Error Mean (SEM)
Pair	Lithium +C.F	0.19333	6	0.243533	0.099422
	BLANK C.F	0.60633	6	0.052198	0.02131

2- Paired Samples Correlations			
		N	Correlation
Pair	Lithium +C.F / *BLANK C.F*	6	0.71

3- Paired Samples Test									
		Paired Differences					t	df	t-Critical (1-Tail)
		Mean	Std. Deviation (SD)	Std. Error Mean (SEM)	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	Lithium +C.F / *BLANK C.F*	-0.413	0.209	0.086	-0.633	-0.193	-4.82	5	2.02

DISCUSSION

There is increasing interest in glutathione GSH due to its varied physiological and pharmacological properties including detoxification through participation in the redox system, activation of SH-Enzymes, co-enzymatic action and conjugation. Lithium toxicity may cause acute renal failure, hypotension, hypertension, Electrocardiogram changes, Impair consciousness, seizures and coma may progress to death (Lang & Davis, 2002). This toxicity may be reduced due the interaction between metal (Lithium) and GSH. Thus it was of interest to study the interaction of this metal *in vivo* to establish further scientific data. This scientific data about the interaction and the effect of Lithium metal on the chemical modulation of GSH in whole blood will enable us to understand further the role of Lithium metal and GSH and strengthen our knowledge about their therapeutic uses in many diseases. The Lithium metal induced the depletion of GSH in case of whole blood. The results obtained from the plasma and cytosolic fraction part of whole blood for the effect of Lithium metal on whole blood has a positive correlation between the time duration of Lithium metal presence in whole blood and the depletion of GSH. The results were also promising and showed that the Lithium metal can cross the semi permeable membrane of the

(RBCs) red blood cells (Khan and Khan, 2001), although not upto too much extent but can induce a change in the chemical status of GSH and caused a decrease in the concentration of reduced GSH. The effect of Lithium metal for its toxicological actions is recognized worldwide, the depletion of GSH by this metal proved a conjugation reaction between this metal and GSH. So the use of GSH from external source, in the case of toxicity to Lithium metal, can greatly decrease the Lithium ions in the body both in plasma as well as in cytosolic fraction of human blood. This study also provides the verification of studies conducted for the passage of metals across red blood cells membrane. This shows that 2mM is our inhibitory concentrations (IC_{50}), below which lithium will show no reaction with GSH and above 2mM lithium completely depleted GSH level both in plasma as well as in cytosolic fraction.

CONCLUSION

Lithium metal caused a decrease in the concentration of GSH. Effect of Lithium metal on the chemical status of glutathione was studied for the time dependency and noted that the concentration of GSH gradually decreased as the time passes from 30 minute (first reading) to 180 minutes in plasma and cytosolic fraction.

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