EFFECT OF ALUMINIUM METAL ON GLUTATHIONE (GSH) LEVEL IN PLASMA AND CYTOSOLIC FRACTION OF HUMAN BLOOD

HAROON KHAN, M.FARID KHAN, SYED UMER JAN* AND NASEEM ULLAH

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University, D.I. Khan, Pakistan
**Department of Pharmacy, University of Baluchistan, Quetta*

ABSTRACT

Aluminium is being used in the medicines in the form of antacids. The Aluminium metal can be leached from our utensils and can harm the body for its side effects, if become available to the systemic circulation. So it is important to check the effect of Aluminum on the Glutathione *in vivo* condition. Ellman method was used to determine the effect of Aluminum on GSH level in whole blood spectrophotometerically. 5,5-Dithiobis, 2-Nitrobenzoic Acid, Glutathione, Aluminium sulphate, phosphate buffer, HCl (Hydrochloric acid) and other laboratory instruments were used to conduct the research work. Time dependent effect of Aluminum on Glutathione level in whole blood was also checked and decrease was observed. This study also shows the effect of Aluminum as helping agent for the Glutathione to enhance the antioxidant system of the body or a cause for depletion of reduced Glutathione.

Keyword: Aluminium (Al), Glutathione (GSH), Whole blood, Ellman's method, 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB).

INTRODUCTION

Glutathione (g-glutamylcysteinylglycine, GSH) containing (SH) group and act as enzyme cofactor, antioxidant and antitoxin. GSH is also present in microorganism, plant and in animal. GSH also having water solubility so it is present in cell cytosolic and aqueous portion of living system (Kosower *et al*., 1978, Meister, 1976, Lomaestro *et al*., 1995). GSH is present in two forms; Reduced GSH and Oxidized GSH (Lewin.1976). The high reduction-oxidation potential makes the GSH to act as cofactor for enzymatic reactions and antioxidant (Kehrer *et al*., 1994). The free radical scavenging effect shows the reducing power of GSH. Glutathione consist of three animo acids, glutamic acid, cysteine and glycine. Glutathione is present in reduced form inside cells. GSH in it's oxidizes state rarely not more then 10% of total amount of GSH in healthy cell (Kosower *et al*., 1978). GSH presence inside the cell shows its heath and provides resistance to toxic challenges. Depletion of GSH in cell is the suicide of cell and this phenomenon is called apoptosis (Duke *et al*., 1995; Nobel *et al*., 1996). Aluminum has many uses such as antacids, astringent, buffered aspirin and antiperspirants (Sidney, 2007). Increased use of aluminum containing antacids and antiperspirant cause's toxicity. Aluminium attracts electron and Glutathione donates electron that is why aluminum has affinity for GSH present in blood. This attraction is mainly present between Aluminium metal glutathione (Quig, 1998). This attraction leads to reduction of blood GSH level, but GSH level is restore from cysteine via γ-glutamyl cycle but if GSH is not supplied properly then continues metal exposer (Stohs *et al*., 1993; Quig, 1998; Hultberg *et al*., 2001) leads to harmful effect to the body defense system The present study is designed to check the time dependent effect of Aluminum on the GSH level in whole blood.

MATERIALS AND METHODS

Sodium Hydroxide (Fluka), Aluminum Sulphate(Fluka), Sodium chloride(Fluka) and potassium Dihydrogen phosphate (Merck) HCL 35% (Kolchlight), Disdium (Riedel Dehean AG Sleez Hannover), Distilled water (Doubled distilled), Chloroform (Merck), Ethanol (Merck), Water for Injection (Elixir Laboratories), UV-1601 spectrophotometer (Shimadzu), pH Meter Model NOV-210 (Nova Scientifc Company Ltd. Korea), Oven Memmert Model U-30,854 Schwabach (Germany), Magntic stirrer, hot Plate 400 (England), Micropippetes 200µl, 500µl, 1000µl Socorex swiss (Finland), Sortorius balance, Centrifuge (H-200, Kokusan Ensink company Japan), Eppendolf's tubes (Plastic, 10l), Siliconized Glass test tubes, sterile Pyrogen Free Disposable Syringes (B.D), fresh Human volunteer blood (Three Healthy Volunteers of 20-25 years of age) and Disposable rubber gloves, were used in research work.

General procedures

Blood Collection; 5ml of blood was taken from vein of three volunteers by syringe dipped with 0.5M Di-sodium edetate solution. Then softly mixed the blood in the syringe. Isolation of plasma; 1.8ml of blood was transferred to Eppendolf's test tube (2.0ml).Centrifuged softly for 10,000rpm for 2 min, red blood cell precipitated down. Then 0.5ml of supernatant was taken and mix

Corresponding author: e-mail: suj55@yahoo.com

softly with 50(l of 5mM Di-sodium Edetate and place in refrigerator till use.

Isolation of cytosolic fraction

0.5ml red cell fraction was taken, which was left after isolation of plasma and washed 2-3 times with 1ml of 0.9% NaCl stock solution (Mixed and centrifuge softly for 5minutes and discard supernatant). Then 0.5ml of washed red cell fraction was taken and added 0.5ml of 5mM Di-sodium Edetate solution. Mixed it softly and stored in refrigerator for 1hr. Then 0.6ml of cold chloroform: Ethanol (3:5) mixture was added and mixed thoroughly to precipitate hemoglobin. Mixed it softly and 0.1ml of water was added. Then centrifuged it hard for 10minutes at 10000-12000 rpm. Then supernatant (pale yellow)-(Lysate or cytosolic fraction) was separated and kept it in refrigerator till used. Preparation of isotonic solution of 0.4mM 50ml solution of Aluminum Sulphate; First of all 50ml of 0.4mM Aluminum sulphate (M.W 342.14) solution was prepared in 50 ml of water for injection. Then make it isotonic with sodium chloride.

METHODS

Standard curve for glutathione

Different concentrations have been made from 1mM solutions of GSH and 0.2ml of GSH solution, 2.3 ml of phosphate buffer PH 7.6 and 0.5 DTNB were mixed together by constant shaking and incubated for 10 minutes. The absorbance was taken at 412 nm. The DNTB blank solution was prepared by mixing 0.25ml (500µl) of 1mM DTNB stock solution and 2.3ml (2300 µl) of 7.6 pH phosphate buffer solution and the absorbance of this blank solution of DTNB was observed at 412nm The subtraction of DTNB blank solution absorbance from the absorbance of GSH plus DTNB mixture gives the real absorbance. The standard curve obtained as show in fig. 1.

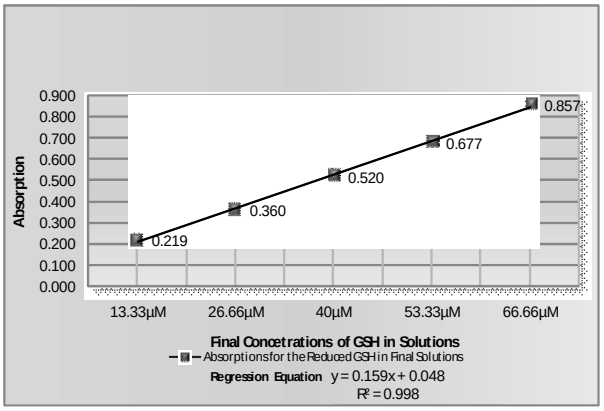

Fig. 1: Standard Curve for GSH + DTNB Mixture taken at 412nm on UV spectrophotometer.

Time dependent effect of Aluminum Sulphate effect on GSH chemical status present in plasma after adding to the whole

The effect of Aluminum sulphate, added to the whole blood on the chemical status of GSH in plasma was studied in term of concentration of GSH in mixture by well known Ellman method. Mixture of Aluminum and whole blood was prepared by taking equal volumes of stock solution of Aluminium sulphate and whole blood and placed in refrigerator till used. 2ml of Aluminium and blood mixture was collected, for extraction of plasma by standard procedure of isolation of plasma from blood. The remained cells fraction of blood latter on was being used for 0 minute reading in determination of the effect of Aluminuim sulphate on GSH concentration in cytosolic fraction with time. 0.2ml (200µl) of collected plasma was added to 2.3ml (2300 µl) of phosphate buffer of pH 7.6, followed by addition of 0.5ml (500 µl) of 1mM of DTBN stock solution in test tube. The mixture was shaken thoroughly and incubated for 5minutes. After 5 minute

Table 1: Effect of Aluminium Sulphate on the chemical status of Glutathione (GSH) in plasma with time

UV Spectrophotometer absorption readings of different solutions for GSH at 412nm wavelength									
S. No.	Time Interval	Three readings on different volunteers blood mixtures with Aluminium			Average of 3 Readings	DTNB Blank (ABS)	Real absorbance* (ABS)	GSH Blank (ABS)	Real Absorbance for GSH Blank
		1st	2nd	3rd					
1	0 min	0.309	0.296	0.316	0.307	0.060	0.247	0.456	0.396
2	30 min	0.299	0.286	0.205	0.263	0.053	0.210	0.450	0.397
3	60 min	0.289	0.179	0.247	0.238	0.058	0.180	0.460	0.402
4	90 min	0.276	0.263	0.184	0.241	0.056	0.185	0.453	0.397
5	120 min	0.266	0.154	0.223	0.214	0.050	0.164	0.440	0.390
6	150 min	0.249	0.236	0.160	0.215	0.055	0.160	0.449	0.394

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

the absorbance were taken at fixed wavelength of 412nm. In (a same manner, absorption readings of plasma GSH control solution at 0 minute were noted. For this purpose mixture of 0.9%NaCl isotonic solution and whole blood was prepared. After isolation of plasma from the control mixture, 0.2ml (200µl) of plasma was added to 2.3ml (2300 µl) of phosphate buffer of pH7.6, followed by addition of 0.5ml (500 µl) of 1mM of DTNB stock solution in test tube. The mixture was shaken thoroughly and incubated for 5 minutes. Absorbances were taken at fixed wavelength of 412nm. Absorbances were noted for this control solution for 0, 30, 60, 90, 120, 150 minutes after repeating the same steps for isolation of plasma from stock solution of 0.9% NaCl and blood 1:1 mixture. The absorption of DTNB blank solution, prepared by adding 0.25ml (2500 µl) of 1mM DTNB stock solution in 2.3ml (2300µl) of phosphate buffer of pH7.6, was also obtained at fixed wavelength of 412nm. By adopting the same steps mentioned above after every 30 minutes of interval of time Absorbances at 412nm on UV spectrophotometer were noted. The concentration of GSH in plasma was determined from standard curve of GSH.

Effect of Aluminum Sulphate on chemical status of GSH present in Cytosolic fraction after adding to the whole blood

The effect of Aluminum sulphate, added to whole blood on chemical status of GSH in cytosolic fraction was studied in terms of determination concentration of GSH in mixtures by a well known Ellman method. The remained cells fraction of blood was collected and processed for extraction of cytosolic fraction, after the isolation of plasma at each time interval for determination of effect of Aluminium sulphate on GSH in cytosolic fraction. 0.2ml (200µl) of collected cytosolic fraction was added to 2.3 ml (2300 µl) of phosphate buffer of 7.6 pH, followed by addition of 0.5ml (500 µl) of 1mM DTBN stock solution in test tube. The mixture was mixed by thoroughly shaking and incubated for 5 minutes. The absorbances were taken at fixed wavelength of 412nm. In same manner, absorption readings of cytosolic fraction GSH control solution at 0 minute were noted. For this purpose mixture of 0.9%NaCl isotonic solution and whole blood was prepared. After isolation of cytosolic fraction from the control mixture, 0.2ml (200µl) of cytosolic fraction was added to 2.3ml (2300 µl) of phosphate buffer of 7.6 pH, followed by addition of 0.5ml (500 µl) of 1mM DTNB stock solution in test solution in test tube. The mixture was shaken thoroughly and incubated for 5 minutes. Absorbances were taken after 5 minutes at fixed wavelength of 412nm. The absorbances are noted for this control solution for 0, 30, 60, 90, 120, 150 minutes after repeating the same steps for isolation of cytosolic fraction from stock solution of 0.9% NaCl and blood 1:1 Mixture. The absorption of DTNB blank solution, prepared by adding 0.25ml (500 µl) 0f 1mM DTNB stock solution in 2.3 (2300 µl) of phosphate buffer at fixed wavelength of 412nm. By adopting the same steps mentioned above after every 30 minutes of interval of time, absorbances at 412nm on UV spectrophotometer were noted. The concentrations of Glutathione (GSH) in cytosolic fraction were determined the GSH standard curve, regression equation (Equation 2).

RESULTS

Effect of Aluminium metal on the GSH concentration in whole blood studied in term of determination of GSH concentration. in plasma and cytosolic fraction after mixing the whole blood with 0.4mM concentration of Aluminium sulphate. As the time passed from 0 minute interval to 150 minutes, after mixing the whole blood with a specific concentration of Aluminium, it was seen that the normal level of GSH in plasma was reduced with time where as the blank mixtures of plasma showed the little variation of thiol status with each interval of time.

Table 2: Effect of Aluminium Sulphate on the chemical status of Glutathione (GSH) in Cytosolic Fraction (C.F) with time

UV Spectrophotometer absorption readings of different solutions for GSH at 412nm Wavelength									
S. No.	Time Interval	Three readings on different volunteers blood mixtures with Aluminium			Average of 3 Readings	DTNB Blank (ABS)	Real absorbance* (ABS)	GSH Blank (ABS)	Real Absorbance for GSH Blank
		1st	2nd	3rd					
1	0 min	0.290	0.278	0.293	0.287	0.047	0.240	0.456	0.409
2	30 min	0.250	0.240	0.254	0.248	0.053	0.195	0.450	0.397
3	60 min	0.230	0.218	0.233	0.227	0.047	0.180	0.460	0.413
4	90 min	0.225	0.215	0.229	0.223	0.058	0.165	0.453	0.395
5	120 min	0.215	0.203	0.218	0.212	0.056	0.156	0.440	0.384
6	150 min	0.190	0.185	0.197	0.191	0.060	0.131	0.449	0.389

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

Similarly as the time passed from 0 minute to 150 minutes, after mixing the whole blood with a specific concentration of Aluminium, it was seen that the normal concentration of Glutathione in cytosolic fraction was decreased with time whereas the blank mixtures of cytosolic fraction showed the little variation of thiol status with each interval of time.

STATISTICAL ANALYSIS

Statistical analysis applied to check the effect the effect of Aluminum on GSH status in plasma and cytosolic fraction and found 95% confidence interval level. The paired comparison t-test was used for comparison GSH level present in plasma blank & Aluminum affected plasma

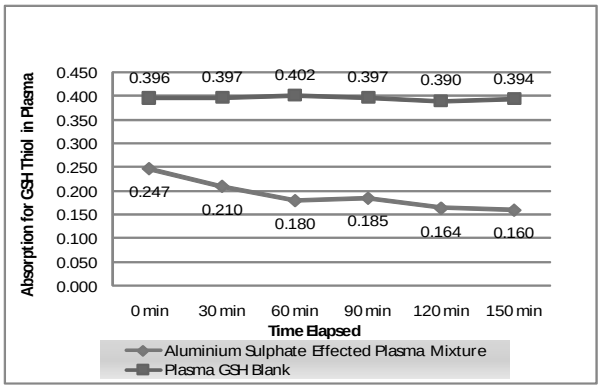

Fig. 2: Time dependent curves for $Al_2(So_4)_3$ effected plasma thiol level & control levels of plasma thiol levels.

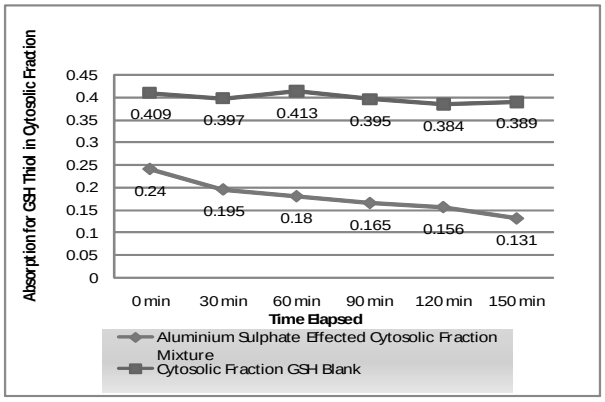

Fig. 3: Time Dependent curves for $Al_2(SO_4)_3$ affected cytosolic fraction thiol level & control levels of cytosolic fraction thiol.

Table 3: Calculation for GSH concentrations in plasma

S. No.	Real absorbance (ABS) of Plasma GSH depleted by Aluminium Sulphate	Concentration of GSH (μM) remained in Plasma of whole blood after depleted by Aluminium Sulphate	Real absorbance (ABS) of Plasma blank solution for GSH	Concentration of GSH (μM) remained in Plasma of whole blood after treated as blank
1	0.247	1.245	0.396	2.180
2	0.210	1.013	0.397	2.186
3	0.180	0.825	0.402	2.218
4	0.185	0.856	0.397	2.186
5	0.164	0.725	0.390	2.142
6	0.160	0.699	0.394	2.168

Table 4: Calculation for GSH concentrations in Cytosolic faction

S. No.	Real absorbance (ABS) of cytosolic fraction of GSH depleted by Aluminium Sulphate	Concentration of GSH (μM) remained in Cytosolic Fraction of Whole Blood after depleted by Aluminium Sulphate	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	0.240	1.201	0.409	2.262
2	0.195	0.919	0.397	2.186
3	0.180	0.825	0.413	2.287
4	0.165	0.731	0.395	2.174
5	0.156	0.674	0.384	2.105
6	0.131	0.518	0.389	2.136

Table 5: Statistical analysis of effect of Aluminium on GSH chemical status in plasma of whole blood

1- Paired Samples Statistics		Mean	N	Std. Deviation	Std. Error Mean
Pair	Aluminium + Plasma	0.89383	6	0.205014	0.083696
	Blank Plasma	2.18	6	0.024916	0.010172

2. Paired Samples Correlations		N	Correlation
Pair	Aluminium + Plasma	6	0.259
	Blank Plasma		

3- Paired Samples Test		Paired Differences					t	df	t-Critical (1-Tail)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	Aluminium + Plasma	-1.286	0.200	0.082	-1.496	-1.076	-15.752	5	2.02
	Blank Plasma								

Table 6: Statistical analysis of effect of Aluminium on GSH chemical status in Cytosolic Fraction (C.F) of whole blood

1- Paired Samples Statistics		Mean	N	Std. Deviation	Std. Error Mean
Pair	Aluminium +C.F	0.81133	6	0.234511	0.095739
	Blank C.F	2.19167	6	0.070696	0.028861

2- Paired Samples Correlations		N	Correlation
Pair	Aluminium +C.F	6	0.697
	Blank C.F		

3- Paired Samples Test		Paired Differences					t	df	t-Critical (1-Tail)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	Aluminium +C.F	-1.380	0.192	0.078	-1.581	-1.178	-17.6	5	2.02
	Blank C.F								

GSH showed that there is effect of Aluminum on GSH level present in Aluminum treated plasma of whole blood as compared to blank plasma treatment.

DISCUSSION

This scientific data about the interaction and effect of Aluminum on the chemical modulation of GSH in whole blood will enable us to understand further the role of Aluminum and GSH and strengthen our knowledge about there therapeutic uses in many disease. The Aluminum induced the depletion of GSH has interesting physiological as well as pharmacological use like detoxification by participation in redox system. Activation of SH- coenzyme, Co-enzyme action and conjugation. Thus it was of interest to further study the interaction of these metals in vivo to establish further the scientific data. In case of whole blood the results obtained from the plasma and cytosolic fraction of whole blood for the effect of Aluminium on whole blood was positively correlated between duration of Aluminum presence in whole blood and (the depletion of reduced (GSH. The results obtained from the cytosolic fraction part of whole blood for the Aluminum effect on whole blood was promising and showed that the Aluminium can cross the semi permeable membrane of the RBCs, though not to much extent but can induce a change in the chemical status of GSH and brought reduction in level of reduced GSH. The effect of Aluminum for its pharmacological actions is using worldwide for the antacids and water treatment as alum but this study provides a caution in the use of Aluminium as it depletes the reduced Glutathione which is our main antioxidant material found in the body and due to increased use of Aluminium without measuring its effect on reduced GSH, it can enhance other complications related to presence of

free radicals in the body. This study also provides the verification of studies conducted for the passage of metals across RBCs membrane**.**

REFERENCES

Duke RC, Ojcius DM and Young JDE. (1996). Cell suicide in health and disease. *Scientific American* (Dec), 79-87.

Ellman GL (1959). Determination of sulfhydryl group. *Arch. Biochem. Biophys.,* **82**: 70-74.

Hultberg B, Andersson A and Isaksson A (2001). Interaction of metals and thiols in cell damage and glutathione distribution: Potentiation of mercury toxicity by dithiothreitol. *Tox.,* **156**: 93-100.

Kehrer JP and Lund LG (1994). Cellular reducing equivalents and oxidative stress. *Free Rad. Biol. Med.,* **17**: 65-75.

Kidd PM (1991). Natural antioxidants Ñ first line of defense. *In*: Kidd PM, Huber W. Living with the AIDS Virus: A Strategy for Long-Term Survival. Albany, California: PMK Biomedical-Nutritional Consulting, pp.115-142.

Kosower NS and Kosower EM (1978). The Glutathione status of cells. *Intl. Rev. Cytology,* **54**: 109-156.

Lewin S (1976).Vitamin C: Its Molecular Biology and Medical Potential. Academic Press, New York, pp.42-59.

Lomaestro BM and Malone M (1995). Glutathione in health and disease: Pharmacotherapeutic issues. *Annals Pharmacother*, **29**: 1263-1273.

Meister A (1976). Glutathione metabolism and transport. *In*: Nygaard OF, Simic MG (ed.). Radioprotectors and Anticarcinogens. Academic Press, New York.

Nobel I, Slater AFG and Stefan C (1995). Signalling mechanisms and oxidative stress in apoptosis. *Toxicol. Letts.,* **82/83**: 149-153.

Quig D (1998). Cysteine metabolism and metal toxicity. *Alter.Med. Rev*. **3**: 262-270.

Sidney Draggan (Topic Editor) (2007). Health effects of Aluminum. *In*: Encyclopedia of Earth. Cutler J. Cleveland (Eds.), Environmental Information Coalition, (Washington, D.C.)

Stohs SJ and Bagchi D.(1993). Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.*, **18**: 321-336.