EFFECT OF SILVER METAL ON THE CHEMICAL STATUS OF GLUTATHIONE (GSH) IN AQUEOUS MEDIUM

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ABSTRACT

Various kinds of Silver compounds, or devices to make solutions or colloids containing silver, are sold as remedies for a wide variety of diseases. Silver itself is not toxic but most silver salts are, and some of them may be carcinogenic. Thus it is interesting to study the effect of silver on the glutathione (GSH). The effect of silver on the chemical status of the glutathione (GSH) in aqueous medium has been studied using U.V Spectrophotometer by Ellman's method. The effect of silver on the chemical status of glutathione (GSH) was determined in aqueous medium with different concentrations of silver salt and also with the passage of time. There was found a drastic effect on decreasing the concentration of glutathione (GSH) in aqueous medium as the concentration is increased and time has passed. The decrease in the glutathione level was concentration and time dependent, probably due to oxidation of GSH to corresponding disulphide (GSSG). In this paper the effect of silver metal on thiol/GSH level was discussed *in vitro,* which in principle may present a model of *in vivo* reaction.

INTRODUCTION

Glutathione (g-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Majority of the body's GSH is made in the liver. Liver GSH synthesis is closely linked to overall protein synthesis, and also to intakes of sulfur amino acids from the diet (Tateishi *et al.,* 1981). The body's other organs seem to draw on GSH exported from the liver, by way of the circulation as well as the bile. Hormones and other vasoactive substances increase GSH efflux into the bile, and this may contribute to the hepatic GSH loss noted under conditions of stress [Deleve and Kaplowitz, 1990). About 80 percent of the GSH synthesized in the liver is exported from the hepatocytes, and most of this is utilized by the kidneys, which also carry a major toxic burden [Deleve and Kaplowitz, 1990) with some cells of the body unable to directly utilize GSH, with cysteine's availability being the main factor limiting GSH synthesis in the cells, and with dietary L-Cysteine known to be potentially toxic, N-acetyl Cysteine (NAC) takes on important significance as a dietary GSH source. NAC is a cysteine precursor; it is well absorbed by the intestine, and becomes converted to circulating cysteine by de-acetylation. It seems not to raise GSH levels if they are already within the normal range, but it can raise abnormally low GSH levels back to normal. This is the basis for its use as an antidote to acetaminophen's liver toxicity (Hoyumpa and Schenker, 1996). 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 (GSH) (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).

Silver has affinity for the glutathione (GSH) present in aqueous phases of blood. These 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 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 (Quig,D.1998; Hultberg;2001 Stohs,1993) 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 silver, in respect of time, on the chemical status of glutathione (GSH) in aqueous medium.

MATERIALS AND METHODS

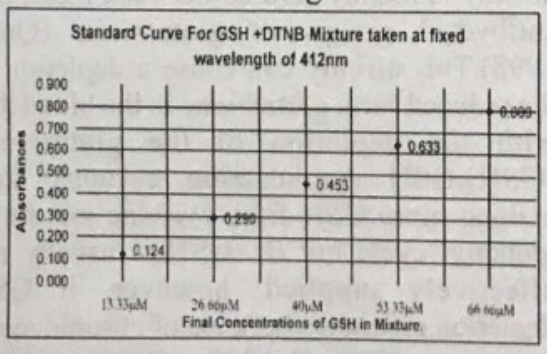
Materials

L.glutathione (GSH) was purchase from (Fluka), 5,5-Dithiobis, 2-Nitrobenzoic Acid(DTNB) was from (Sigma) chemical Co, Silver nitrate was obtained from (Across Belgium). All other reagent were of the highest purity commercially available. U.V 1601 spectrophotometer (Shimadzu). pH Meter : Model NOV-210, Nova Scientific Company Ltd. Korea, Oven: Memmert Model U-30,854 Schwabach (Germany). Magnetic Stirrer, hot plate 400(England) .Micropipettes 200 µl, 500 µl, 1000 µl were used of Socorex Swiss (Finaland), Sartorius Balance.

METHODS (Ellman, 1959)

Standard Curve for Glutathione

200µl of 0.2, 0.4, 0.6, 0.8 and 1mM solutions of Glutathione (GSH) was added to 2.3ml of phosphate buffer pH 7.6, followed by the addition of 0.5ml of 1mM 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB) stock solution. The mixtures were shaken thoroughly and incubated for 5 minutes at 30°C. Absorbances were taken after 5 minutes at fixed wavelength of 412 nm. Blank was prepared in which GSH was omitted. Standard curve was constructed by plotting the change of absorbance versus final concentration of GSH in the mixture. Straight line was drawn by using linear regression analysis. The correlation coefficient of plot was 0.9974. Standard curve was obtained as shown in figure 1.



Figure# 1- Standard Curve for Glutathione (GSH) + DTNB Mixture taken at fixed wavelength of 412nm

EFFECT OF DIFFERENT CONCENTRATIONS OF SILVER NITRATE ON THE CHEMICAL STATUS OF GLUTATHIONE (GSH) IN AQUEOUS MEDIUM

To 0.8ml of 1mM Glutathione (GSH) taken in five separate test tubes, 1ml of different concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1mM solution of silver nitrate were added separately, shaked and further diluted to 2ml with phosphate buffer (pH 7.6). The final concentration of Glutathione (GSH) in each five tubes was 0.4 mM. Five separate tubes were prepared with 0.2ml silver nitrate plus GSH mixture from each previously made five tubes diluted with 2.3ml of phosphate buffer (pH 7.6) and added 0.5ml of 1mM DTNB stock solution. The final concentration of GSH in each of these five tubes was 0.02666 mM. The effect of silver nitrate on the chemical status of GSH was studied in terms of determination of concentration of GSH in mixtures by a well known Ellman's method, as mentioned in standard curve for GSH. The results are shown in the table 1. A control for GSH was also prepared by taking 0.8ml of 1mM GSH stock solution in a test tube and diluted with 1.2ml of phosphate buffer (pH 7.6) with final concentration of 0.4 mM of GSH.

TIME DEPENDENT EFFECT OF SILVER NITRATE ON THE CHEMICAL STATUS OF GLUTATHIONE (GSH) IN AQUEOUS MEDIUM

To 0.8ml (800l) of 1mM GSH taken in a test tube 1ml of 0.1mM solution of silver nitrate was added, shaked and further diluted to 2ml with phosphate buffer (pH 7.6). The final concentration of GSH in tube was 0.4 mM and of silver nitrate was 0.05 mM. Five separate tubes were prepared with 0.2ml silver nitrate plus GSH mixture from each previously made five tubes diluted with 2.3ml of phosphate buffer (pH 7.6) and added 0.5ml of 1mM DTNB stock solution. The final concentration of GSH in each of these five tubes was 0.02666 mM. The effect of silver nitrate on the chemical status of GSH

]was studied in terms of determination of concentration of GSH in mixtures by a well known Ellman's method, as mentioned in standard curve for GSH. The absorbances were read at 0, 30, 60, 90, 120, 150 minutes

after preparing mixture of 0.8ml of GSH plus 1ml of silver and diluted to 2ml with phosphate buffer (pH 7.6). The concentrations of GSH were determined from the glutathione standard curve and are shown in the figures below.

The observation is shown in the table 2
A control for glutathione was also prepared by taking 0.8ml of 1mM GSH stock solution in a test tube and diluted with 1.2ml of phosphate buffer (pH 7.6) with final concentration of 0.4 mM of glutathione.

Table 1- Effect of different concentrations of silver nitrate on the chemical status of glutathione (GSH)

Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank solution was0.060 ABS at 412nm
Final Concentration of GSH in Mixture in final Mixture is 26.66µM

S#	Conc. Used of silver nitrate	Final conc. of silver nitrate in Mixture	1st ABS	2nd ABS	3rd ABS	Average of 3 Readings	Real absorbance*	Real Absorbance for GSH Blank
1	0.02mM	6.67µM	0.125	0.358	0.277	0.253	0.193	0.289
2	0.04mM	13.33µM	0.106	0.345	0.261	0.237	0.177	0.287
3	0.06mM	20.00µM	0.064	0.298	0.216	0.193	0.133	0.288
4	0.08mM	26.67µM	0.064	0.208	0.171	0.148	0.088	0.290
5	0.1mM	33.33µM	0.064	0.140	0.137	0.114	0.054	0.287

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

Table 2- Effect of silver nitrate on the chemical status of glutathione (GSH) with time

Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank solution was 0.060 at 412nm
Final concentration of glutathione (GSH) was 26.66µM, and of silver nitrate was 33.33µM in final mixture

S. no	Time Interval	1st ABS	2nd ABS	3rd ABS	Average of 3 Readings	Real absorbance*	GSH Blank ABS	Real Absorbance for GSH Blank
1	0 min	0.166	0.178	0.187	0.177	0.109	0.371	0.311
2	30 min	0.105	0.117	0.126	0.116	0.048	0.376	0.316
3	60 min	0.084	0.096	0.105	0.095	0.027	0.373	0.313
4	90 min	0.071	0.083	0.092	0.082	0.014	0.371	0.311
5	120 min	0.117	0.001	0.074	0.064	-0.004	0.374	0.314
6	150 min	0.147	0.038	0.108	0.098	0.030	0.370	0.310

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

RESULTS

Effect of Silver on the Chemical Status of Glutathione (GSH) In Aqueous Solution

Effect of silver metal on the chemical status of Glutathione was studied in term of determination of concentration of GSH. Silver metal caused a decrease in the concentration of GSH. Different concentrations of silver cause a gradual decrease in the concentration of GSH in aqueous medium as shown in the figure 2 and table 3.

Effect of silver on the chemical status of GSH was also studied for the time dependency and found that the concentration of GSH was gradually decreased as the time passes from (0 minute interval of time to 150 minutes) as shown in the figure 3 and table 4.

STATISTICAL ANALYSIS FOR EFFECT OF SILVER ON THE CHEMICAL STATUS OF GLUTATHIONE (GSH)

Statistical approach for the effect of silver

on the chemical status of GSH was also conducted for the concentration dependent and time dependent effects. The paired comparison T-test (Table 5) of concentration dependent effect of silver and GSH blank gave the decision that there is an effect of silver on the chemical status of GSH in aqueous solution as the concentration of silver is increased as compared to GSH blank solution treatment. Similarly the paired comparison T-test (Table 6) of time dependent effect of silver and GSH blank gave the decision that there is an effect of silver on the chemical status of GSH in aqueous solution as the passage of time is increased with a specific concentration of silver as compared to GSH blank solution treatment

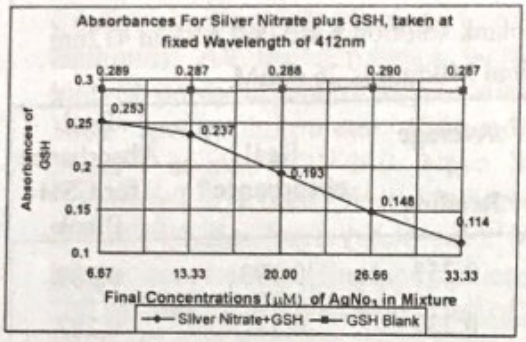


Figure 2- ***Curves for silver nitrate affected Thiol Level & Control Levels of Thiol***

Table 3- Calculation for determination concentration of GSH after reaction with silver nitrate by Ellman's Method

S/No.	Real Absorbance(ABS)	Concentration of GSH (µM) Remained
1	0.193	17.713
2	0.177	16.402
3	0.133	12.795
4	0.088	9.107
5	0.054	6.320

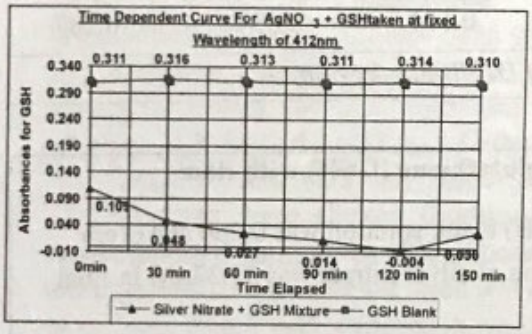


Figure-3 ***Time Dependent Curves for silver nitrate affected Thiol Level & Control Levels of Thiol.***

Table 4 Calculation for determination of concentration of GSH after reaction with silver nitrate by Ellman's Method

S/No.	Real Absorbance(ABS)	Concetration of GSH (µM) Remained
1	0.109	10.828
2	0.048	5.828
3	0.027	4.107
4	0.014	3.041
5	0.000	1.566
6	0.030	4.352

Table 5. Paired comparison t-test for concentration dependent effect of $AgNo_3$

	Silver affected mixture	GSH (blank)
Mean	0.129	0.288
Variance	0.003	0.000001
Observations	5	5
Pearson Correlation	0.029	
Hypothesized Mean Difference	0	
df	4	
t Stat	-6.080	
P(T<=t) one-tail	0.001	
t Critical one-tail	2.131	
P(T<=t) two-tail	0.0036	
t Critical two-tail	2.77	

Table 6 Paired comparison t-test for time dependent effect of $AgNo_3$

	Silver affected mixture with time	GSH (blank)
Mean	0.037	0.312
Variance	0.001	5.1E-06
Observations	6	6
Pearson Correlation	-0.190	
Hypothesized Mean Difference	0	
df	5	
t Stat	-17.00	
P(T<=t) one-tail	6.4E-06	
t Critical one-tail	2.0150	
P(T<=t) two-tail	1.2E-05	
t Critical two-tail	2.570	

DISCUSSION

There is increasing interest in 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, for instance formation of marcapturic silver plays no known natural biological role in humans, and possible health effects of silver are a subject of dispute. Silver itself is not toxic but most silver salts are, and some may be carcinogenic, thus it was of interest to study the interaction of these metals in vitro to establish further scientific data. This scientific data about the interaction and the effect of silver on the chemical modulation of GSH will enable us to understand further the role of silver and GSH and strengthen our knowledge about their therapeutic uses in many diseases and the treatment for their toxic nature to health. In the same manner the effect of silver was also studied for the concentration dependent and time dependent on the chemical status of GSH and the concentration of reduced GSH was decreased with increasing concentration of silver metal in solution and with the passage of time respectively. The following sequences of reactions are suggested to have happened in the experiment.

GSH + Silver (Ag) ----> Ag-SG

Equation# 1

The results also suggested that there was a possibility of formation of intermediate or conjugate between silver and GSH. However it was not possible to estimate or determined those conjugates under those conditions. Since both GSH and silver, are biological active compounds. It was of interest to study the possible interaction of this metal in *vitro* as a model of in *vivo* interaction

CONCLUSION

The tripeptide thiol glutathione (GSH) has facile electron-donating capacity linked to it sulfhydryl (SH) group. Glutathione is an important water phase antioxidant and essential cofactor for antioxidant enzyme. It provides protection also for the mitochondria against endogenous radicals. Its high electron donating capacity combined with its high molecular concentration endows (GSH) with great reducing power, which is used to regulate a complex thiole-exchang system. Different concentration of silver metal caused a gradual decreased in the concentration of GSH in aqueous. Effect of silver on the chemical status of glutathione was also studied for the time dependency and noted that the concentration of glutathione (GSH) gradually decreased as the time passes from (0 minute interval of time to 150 minutes) in aqueous solution.

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