Spectrophotometric investigation of Glutathione modulation by Thallium chloride in aqueous medium

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Abstract: Thallium has been shown to significantly influence various tissues of living organisms; Exposure to Thallium can disturb mitochondrial function, degenerate neurons, and interfere with the function of critical metabolic enzymes and co-enzymes. Glutathione (GSH) an essential biomarker is considered a key factor in harnessing the thallium toxicity. In the present study the interaction of Thallium (Thallium Chloride) and glutathione was investigated spectro-photo-metrically in aqueous media. The renowned Elman's experimental protocol was followed at a wavelength of 412nm for Glutathione quantification in each sample. The pH of each sample was maintained at 7.6 using Phosphate buffer during the entire course of the experiment. A concentration as well as time dependent depletion of glutathione after exposure to various concentration of Thallium metal was observed, revealing chemical interaction between the metal and glutathione. The exact mechanism of interaction of Thallium and glutathione is still to be investigated. However, this piece of research suggests that a decrease in the concentration of Glutathione may be due to Thallium-GSH abduct or oxidize glutathione (GSSG) formation. This study was performed in-vitro as a model of in vivo.

Keywords: Elman's reagent, Glutathione, GSSG, Thallium Chloride, Phosphate Buffer, Spectrophotometer, Thallium-GSH abduct.

INTRODUCTION

Glutathione (GSH) is one of the primary non-protein tri-peptide thiol molecules found in mammalian-cells and has bio-reducing action (Lomaestro and Malone 1995). Inside the cells, Glutathione occurs in two states i.e. in reduced form (GSH) and oxidized form (GSSG) where the reduced state (GSH) is up to 95% of the cumulative (GSH + GSSG) content. However, the concentration of the Intracellular oxidized Glutathione (GSSG) may increase with oxidative-stress or neurotic conditions and reduced Glutathione consumption happens in the meantime (Cereser *et al.,* 2001). GSH and GSSG comprise of a thiol redox couple and have part, as a controlling factor, in gens regulation and intra-cellular signal transduction (Dalton *et al*., 1999). Glutathione in reduced state (GSH) has critical role in cell division, immune-reaction, signals processing control, apoptosis, and in detoxification of some xenobiotics and heavy metals (Ogawa 2005) and (Meister and Anderson 1983). Glutathione has antioxidant action and is a cofactor for enzymatic responses that need promptly accessible electron pairs. Recent studies have shown that metals such as Palladium, Vanadium and other elements (Khan *et al.,* 2010, 2011a, 2011b and 2012; Muktiar *et al.,* 2012, 2013, 2017 and 2018; Shah *et al.,* 2013; Naseem *et al*., 2015 & Hashmat *et al*., 2016) show the ability to produce reactive oxygen species, initiating lipid per oxidation. Glutathione is exceptionally receptive to physico-chemical substances due to its sulfhydryl moiety (-SH) and conjugates to different molecules including the overwhelming metal particles (Balendiran *et al*., 2004 & Vitecek *et al*., 2006). A raise in GSH/GSSG ratio is an early indication of oxidative stress or risk of serious illness (Cereser *et al.,* 2001). Metal toxicity includes geno-toxicity or carcinogenicity, neuro-toxicity (Flora and Saxena 2006) and thus the interaction of metals with GSH has attracted the attention of biomedical scientists. Thallium is a chemical element occurring between two very toxic elements: lead and mercury in the periodic table. However, the toxicity of Thallium is much greater than that of its neighbors. Thallium and its compounds contact with skin are dangerous and adequate ventilation should be provided when melting this metal. Some Thallium compounds in a very low dose in experimental animals produced mild to moderate enteritis and moderate to severe colitis. Severe degenerative changes were found in mitochondria of kidney, liver, brain and small intestine (Herman and Bensch 1967). Thallium compounds also exhibited reproductive toxicity on rates i.e. adverse effects on sperm cell maturation and motility, alterations in the epithelium of the seminiferous tubules, as well as

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ultra-structural changes in Sertoli cells (Formigli, *et al.*, 1986). Thallium compounds may also affect human. Various studies showed that its compound toxicity, may affect children more than adults (Kazantzis *et al.*, 1994). Thallium poisoning symptoms include; hallucination, lethargy, delirium, convulsions, tingling pain in the extremities and muscular weakness, followed by coma. Some neurological and mental disturbances may persist and death is caused by respiratory failure or cardiac arrest (Change *et al*., 1996). In a recently published report of a man who ingested thallium compound has the ability to interfere with chromosome distribution during cell division (Hantson *et al*., 1997). There are reports on the interaction of divalent TI^{+3} ions with thiol (SH) Group (Fabrik *et al.*, 2009). In present study the Thallium-Glutathione interaction was investigated in aqueous medium spectrophotometrically. Therefore it was of interest to see the chemical effect of this metal (Thallium Chloride) on the chemical and metabolic status of Glutathione in aqueous phase.

MATERIALS AND METHOD

Materials

L-Glutathione (GSH) (Fluka), 5,5-dithiobis,2-nitobenzoic Acid (DTNB) (Sigma), Sodium Hydroxide (NaOH) (Fluka AG), Thallium Chloride (Fluka), Potassium dihydrogen Phosphate (Merk), Distilled water (Double distilled), HCl 37% (Kolch light). UV/Visible spectrophotometer 1601(Shimadzu).pH Meter: NOV-210 (Nova scientific company Ltd. Korea), Oven: Memmert Model U-30,854 Schwabach (Germany), Magnetic stirrer (England) Hot Plate: 400 (England). Weighing balance (Sartorius) Oven: Memmert Model U-30,854 Schwabach (Germany), Micoropipettes (200, 500 and 1000) Socorex Swiss (Finland).

Preparation of stock solutions

1mM Glutathione Stock solution

30.75mg (Mol. weight 307.4g) of Glutathione was dissolved in sufficient quantity of 0.1M phosphate buffer (pH 7.6). The volume was adjusted to 100ml by adding more 0.1M phosphate buffer (pH 7.6) to make 100ml of 1mM Glutathione stock solution. This stock solution was then kept in refrigerator till use.

1mM 5-5-dithiobis, 2-nitrobenzoic acid), DTNB/ Ellman's Reagent

39.64mg of 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB) (M.W 396.35) was dissolved in sufficient quantity of 0.1M phosphate buffer pH (7.6) to make 100ml of 1mM DTNB solution. This stock solution was then kept in refrigerator till use.

0.1M phosphate buffer pH 7.6 stock solution

0.1M NaOH solution was prepared by dissolving 1g NaOH (Molecular weight of NaOH is 40g) in 250ml distilled water, then 0.1M monobasic potassium solution was prepared by dissolving 3.4g of monobasic potassium (KH_2PO_4) (Molecular weight of KH_2PO_4 is 136.09g) in 250 ml distilled water. Then 200 ml of 0.1M phosphate buffer (pH 7.6) was prepared by mixing 42.4ml of 0.1M NaOH solution with 50ml of 0.1M monobasic potassium phosphate solution and was diluted to 200ml with distilled water. The pH was then adjusted to 7.6 with 0.1M NaOH or 0.1M monobasic potassium phosphate solution.

Preparation of 2mM Thallium chloride stock solution

31.074mg (Mol. weight 310.74g) of Thallium Chloride was dissolved in sufficient quantity of distilled water to make 50ml of 2mM Thallium Chloride stock solution. This stock solution was then kept in refrigerator till use.

Determination of glutathione, by Ellman's (DTNB Modified) method

The Elman's modified method (Elmans, 1959) was followed for the determination of Glutathione in aqueous solution, after treatment with different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 2mM) of Thallium Chloride.

Experimental protocol

2ml of different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 2mM) of Thallium Chloride was mixed with 2ml of 1mM Glutathione, separately in test tubes; the mixtures were left for 10 minutes. 0.2ml of these mixtures were taken and mixed with 2.3ml of 0.1M phosphate buffer pH 7.6, followed by the addition of 0.5ml 1mM DTNB separately in various test tubes, Kept for 5 minutes. Absorbance was recorded as a function of Glutathione, through UV spectrophotometer at fixed wave length of 412 nm against reference cell. (Reference solution contained 2.8ml of 0.1Mphosphate buffer pH (7.6) and 0.2ml of 1mM Glutathione, solution). Average results were obtained by repeating the experiment three times. Control Glutathione solutions were without metal solution).

Standard curve of glutathione

1mM stock solution of Glutathione was prepared by dissolving 30.74 mg of Glutathione, in sufficient volume of 1mM Phosphate buffer pH 7.6 in 100ml volumetric flask. This stock solution was diluted to prepare a series of Glutathione solutions containing 0.2, 0.4, 0.6, 0.8 and 1.0mM Glutathione respectively. Reading samples were prepared by taking 0.2ml (200µl) from each of these diluted solutions of Glutathione and each sample was added to 2.3ml (2300µl) of phosphate buffer having pH of 7.6, followed by the addition of 0.5ml (500µl) of 1mM 5, 5-Dithiobis, 2-Nitrobenzoic acid (DTNB) solution. The mixtures were then shaken thoroughly and incubated for five minutes. Then absorbance of each sample was taken at a fixed wavelength at 412nm against reference cell. The Glutathione absorbance of each concentration was then plotted as a function of final concentration of Glutathione in mixture to produce a standard curve.

Linear regression analysis was performed using Microsoft Excel® 2010. The spectrophotometric analysis was determined by performing a linear regression analysis by using the Glutathione standard curve. The correlation coefficient (R2) with a Value of 0.999 indicates an optimum regression within the given range of concentrations that was analyzed in this study.

The data of the standard curve calculation was obtained by the following equation:

$$Y = m\,x + b$$

Where,

Y = Absorbance at 412 nm.

m = Slope of Glutathione standard curve of known concentration.

b = Intercept.

X = Concentration of Glutathione, standard curve of known concentration.

$$c = Y - b/m$$

If, Y, b and m are known, "x" the unknown concentration of Glutathione - can be determined. The above equation was used to calculate the concentration of Glutathione in aqueous solution after treatment with different concentrations of Thallium Chloride.

RESULTS

Glutathione standard cure

Glutathione standard cure was constructed as mention in (fig. 1).

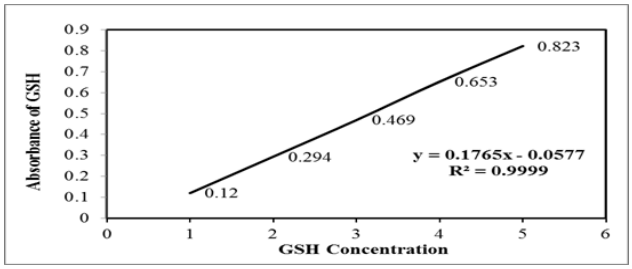


Fig. 1: Standard Curve of GSH

Effect of different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 2 mM) of Thallium Chloride on the chemical status of Glutathione (1mM)

2mL of different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 2mM) of Thallium Chloride solutions were added separately to 2mL of 1.0mM Glutathione taken in six separate test tubes and were shacked well. The concentration of Glutathione in each of the above test tube was half i.e. 0.50mM (500µM) and that of Thallium Chloride were also half i.e. 0.00005mM (0.05µM), 0.0005mM (0.5µM), 0.005mM (5µM), 0.05mM (50µM), 0.5mM (500µM) and 1mM (1000µM), respectively. Then reading samples for UV spectorophotmeter were prepared by taking 0.2mL from each one of the above test tubes followed by the addition of 2.3mL of phosphate buffer pH (7.6) and 0.5mL of 1mM DTNB solution. The final concentration of Glutathione in the test tubes remained 0.03333mM (33.33µM) and that of Thallium Chloride were 0.000003mM (0.003 µM), 0.00003mM (0.03µM), 0.0003mM (0.33 µM), 0.003mM (3.33µM), 0.0333mM (33.33µM) and 0.06666mM (66.66µM), respectively.

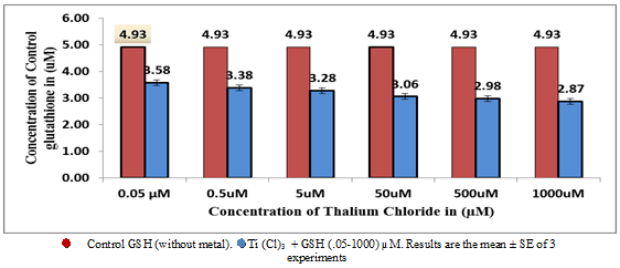


Fig. 2: Effect of Different Concentration of Ti (Cl) $_3$ on status of GSH

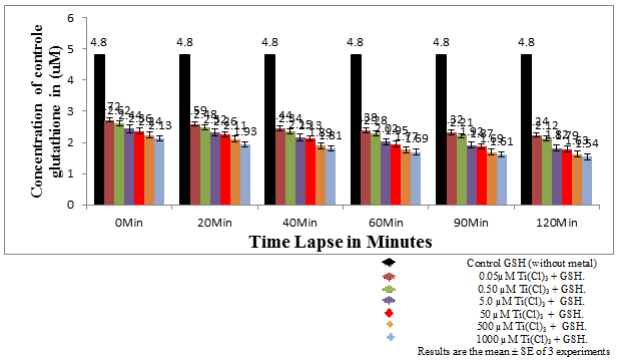


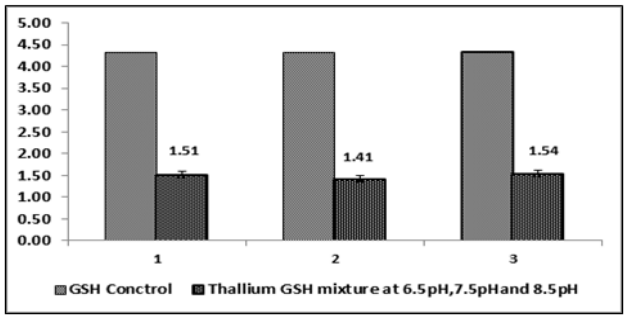
Fig. 3: Effect of Ti (Cl) $_3$ (0.05-1000µM) with time, incubation period (0-120 min).

Control solution (Glutathione blank) was prepared by mixing 2mL of 1mM Glutathione solution and 2mL of phosphate buffer having pH of 7.6. The final concentration of Glutathione in control solution (Glutathione blank) was also 0.5mM (500µM), as in the sample. The absorbance of each sample was recorded after 5 minutes at 412 nm against control samples, was then converted into concentration of Glutathione in mixtures with the help of standard curve of known concentration of Glutathione. The concentration of Glutathione was then plotted against the concentration of Thallium Chloride in mixture samples as shown in fig. 2.

Effect of different concentration (0.0001, 0.001, 0.01, 0.1, 1 and 2mM) of Thallium Chloride on the chemical status of Glutathione (1mM) with time

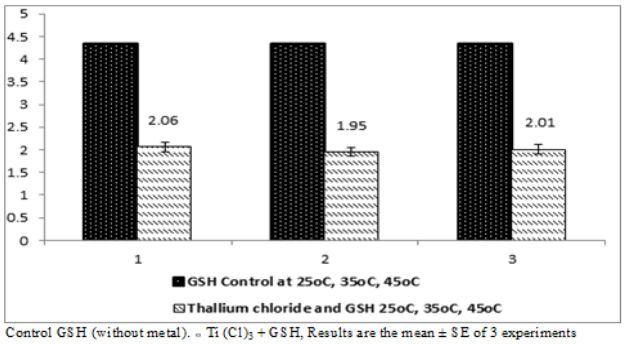
2mL of different concentrations (0.0001, 0.001, 0.01, 0.1, 1 and 2mM) of Thallium Chloride solutions were added to 2mL of 1mM Glutathione. The reading samples for UV spectrophotometer were prepared according to standard Elman's protocol. The absorbance was recorded at 0, 20, 40, 60, 90 and 120 min and was converted to concentration of Glutathione (determined from the

Glutathione standard curve) left after treatment with Thallium Chloride, were plotted against the time interval and are shown in fig. 3.



Control GSH (without metal). ■ $Ti(Cl)_3$ + GSH. Results are the mean ± SE of 3 experiments

Fig. 4: Effect of Ti $(Cl)_3$ (500 µM) at 6.5, 7.5, 8.5 pH



Control GSH (without metal). ▫ $Ti(Cl)_3$ + GSH, Results are the mean ± SE of 3 experiments

Fig. 5: Effect of Thallium Chloride (3.33 µM) on GSH (3.33µM) at 25°C, 35°C, 45°C temperatures

Effect of different pH (6.5, 7.5, 8.5) buffer on the chemical status of Glutathione solution in the absence and presence of Thallium Chloride

Three different reading samples each of (6.5, 7.5, 8.5 pH Glutathione) and Thallium Chloride were prepared by mixing 2mL of 1mM Glutathione (6.5, 7.5 and 8.5pH) were mixed with 2mL of 1mM Thallium Chloride in test tube, respectively, the pH was adjusted by adding few drops of either Conc. HCl or NaOH, shacked well and left for 5 minutes. The final concentrations of Glutathione and Thallium Chloride in reaction mixture were calculated to be 0.5mM (500) µM, each. Absorbencies were taken after 5 minutes at 412nm against control Glutathione, were then converted into concentration of Glutathione with the help of standard curve of known concentration Glutathione as shown in fig. 1. Finally, the concentrations of Glutathione (determined from the Glutathione standard curve) left after treatment with Thallium Chloride, was plotted against the final concentration of Thallium Chloride in mixture samples (fig. 4).

Effect of Thallium on the chemical status of Glutathione (0.1 mM) at different temperatures (at 25, 35 and 45 °C)

2mL of 0.1mM Thallium Chloride solution was added separately to 2mL of 0.1mM Glutathione taken in three separate test tubes, shacked well. These test tubes were kept in water bath for 10 minutes to maintain the temperature at (25, 35 and 45°C). Final concentration of Glutathione and Thallium Chloride in each of the above test tube was 0.05 (50µM), respectively. Reading samples for both control and reaction mixtures of Glutathione and Thallium Chloride were prepared according to standard Elman's protocol. The final concentration of Glutathione in both control and that of Glutathione and Thallium Chloride mixtures in the sample test tubes was 0.0033mM (3.33µM), respectively. Absorbance was taken after 5 minutes at 412 nm against reference cell. The absorbance was converted into concentration of Glutathione in mixtures by using standard curve of known concentration of Glutathione. Finally the concentrations of Glutathione (determined from the Glutathione standard curve) left after treatment with Thallium Chloride, were plotted against the final concentration of Thallium Chloride in the mixture samples (fig. 5).

DISCUSSION

From our results it was found that while adding thallium chloride to the Glutathione it depleted the Glutathione (GSH) level both concentration as well as with time elapse, as Glutathione can undergo oxidation, so Glutathione acts as an electron donor (Kidd 1997), thus may reducing thallium and oxidized by itself and thus its level may dropped. Our study is also supported by literature as reported to literature that thallium undergoes interaction in its trivalent Ti ion form of thiol (SH) Group (Galvan-Arzate *et al.*, 1994). The mechanism of reduction is still to be investigated but the level of depletion of Glutathione may be proposed by either Glutathione may undergoes oxidation, forming oxidized glutathione (GSSG) or making complex formation i.e. $(Tl(SG)_3)$. Our study further revealed that Optimum interaction between Glutathione and metal was observed at pH of 7.5 and temperature of 35°C.

CONCLUSION

It is concluded from our finding that depletion of Glutathione by Thallium, to either formation of GSSG or Tl $(SG)_3$ complex, might have clinical impact. This *in vitro* study was made as possible model for the *in vivo* studies.

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