Interaction of palladium inorganic salt and organic complex with glutathione content of liver homogenate

Muhammad Mukhtiar[1], Syed Umer Jan[2*], IhsanUllah[3], Rahman Gul[2], Abid Hussain[1], Essa Ali[4], IzharUllah[1], Abdul Jabbar[5], Muhammad Akram[6], Sibghat Mansoor[7] and Muhammad Farid Khan[8]

[1]Department of Pharmacy, Faculty of Medical & Health Science, The University of Poonch. Rawalakot, Pakistan
[2]Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
[3]Department of Pharmacology, Poonch Medical College, Rawalakot, Pakistan
[4]Faculty of Agriculture, The University of Poonch. Rawalakot, Pakistan
[5]Faculty of Veterinary & Animal Sciences the University of Poonch Rawalakot, Pakistan
[6]Department of Eastern Medicine, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan
[7]College of Pharmacy, The University of Punjab. Lahore, Pakistan
[8]Faculty of Pharmacy, Gomal University, D.I. Khan, Pakistan

Abstract: Glutathione is an essential antioxidant of living organism that provides a primary protection against metals toxicity. A significant amount of glutathione is present in blood erythrocytes, plasma and liver hepatocytes to protect them from oxidative damage from both external and internal oxidants. Metalo-element palladium has numerous pharmacological, clinical and toxicological compensations, like palladium is used as anti-viral, anti-bacterial, neuro-protective and anti-tumor agent. However studies have also indicated some mild to serious toxic effects of palladium metallo-elements. In the presence study the interaction of palladium inorganic salt and organic complex with glutathione (GSH) content of liver homogenate was examined spectro-photometrically. 20% (w/v) liver homogenate was prepared of the collected liver of rabbit in 5% TCA (tri-chloro-acetic acid) solution and 1mm EDTA, using a potter-eveljhem homogenizer with motor driven Teflon pestle. The GSH content quantification was carried out by Elman's method. Our finding showed that there was a depletion of GSH content by both palladium inorganic salts and organic complexes, concentrations wise as well as with time elapse as level of GSH content decrease from (43.6% to 72.62%) with Palladium Nitrate and from (24.09 to 59.5%) with Bis-benzonitrile Palladium II Chloride as compared to control, and further dropped with time incubation from 0-90 minutes from (49.7 to 87.1%), with Palladium Nitrate and from (29.3% to 67.6%) respectively. The result showed that the effect of both inorganic salt of palladium was more enhanced as compare to its organic complex. It was suggested from our finding that the depletion in the glutathione content of liver homogenate may be due to oxidation of glutathione or due to glutathione metal abduct formation by both inorganic salt and organic complex of palladium. This study in situ is a model of *in vivo*.

Keywords: Glutathione (GSH), palladium, spectrophotometer, Elman's method, rabbit liver homogenate

INTRODUCTION

Transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicities associated with these metals may be due at least in part to oxidative tissue damage (Currie, 1995). Toxic metals creates high risks to juvenile, as exposures in very early age produce lifelong physical, mental and behavioral impairments, and also major chronic diseases in adults like cardiovascular, neurological, cancer and renal diseases. (WHO, 2010); (Lanphear *et al.*, 2005). 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 & 2018; Shah *et al.*, 2013 & 2013a; Khan J *et al.*, 2012; Naseem *et al*., 2015; Hashmat Ullah *et al*., 2015, 2016, 2016a), exhibit the ability to produce reactive oxygen species, resulting in lipid per oxidation. Enhanced per oxidation of lipids may result in the damage to the cells, tissues and organs. Palladium compounds suppress virus entry, cell to cell spread, capsid- assembly and trans-activation of virus genome and thus show antiviral activity (Petia *et al*., 2004) & Genoya *et al.*, 2004). A few studies have demonstrated that palladium compounds have cytotoxic activities resembling standard platinum based drugs (Gao *et al*., 2010). Some Palladium base complexes have been demonstrated better growth-inhibition results on human non-small cell lung disease *in vitro* than cis-platin (Ulukaya *et al*., 2011). Some Palladium Complexes revealed some neuro-protective activities in conditions like heart failure, ischemic attack and anesthetic or sedative mishaps (Francis *et al*., 2004). Glutathione (GSH) is present almost in all cells of the ling organism in a considerable quantity, varies in concentration depends on the ratio of metabolite and GSH interaction with in these cells and tissues (Friedman *et al.*, 1989; Hotchkiss *et al.*, 2009). The liver: which is one of the hardest working and important organs in the body, the

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

highest tissue levels of glutathione are found in the liver (Monograph GSH, 2002). Here it plays huge roles in deactivating and escorting a wide range of toxins from the body, including the breakdown products from excessive alcohol consumption (Kiefer, 2005). Of the many jobs the liver performs, detoxification is the liver's most important. The most essential element used by the liver in this process is glutathione (GSH). The liver is our ultimate filter and has two critical functions: to process nutrients and eliminate toxins from the body. It does this by cleansing the blood at about two quarts a minute. Large quantities of Glutathione are found the liver where a two-stage detoxification process takes place. Phase One begins the chemical conversion of harmful compounds that are mainly fat-soluble into intermediate forms. But Phase two is where the final transformation takes place to help convert the intermediate toxins into water-soluble substances that can be excreted through the bowel or kidneys. Only water-soluble substances can be excreted. If there is not enough Glutathione to generate the Phase two enzymes, toxins will build to dangerous levels in the liver (Koch, 2011). Glutathione is the most important physiological chelator and the glutathione in reduced form protects cells from reactive oxygen species related with heavy metals (Becker & Soliman, 2009; Kaur *et al.*, 2006; Rosenblat *et al.*, 2007); Zeevalk *et al*., 2010; Flora, 2009). GSH forms metal complexes via non-enzymatic reactions (Ballatori, 1994). GSH is one of the most versatile and pervasive metal binding ligands and plays an important role in metal transport, storage and metabolism. GSH works (a) in the mobilization and delivery of metals between ligands, (b) in the transport of metal across cell membranes, (c) as a source of cysteine for metal binding, and (d) as a reductants or cofactor in redox reactions involving metals. The sulfhydryl group of the cysteine moiety of GSH has a high affinity for metals, forming thermodynamically stable but kinetically labile mercaptides with several metals, including mercury, silver, cadmium, arsenic, lead, gold, zinc, and copper (Rannug, 1980; Van Bladeren *et al*., 1981; Rannug *et al*., 1978). In this paper we investigate the effects of heavy metals palladium inorganic Salt and organic Complex on Liver antioxidant status (the level of Glutathione GSH) and its ability to detoxifying and reducing these metals.

MATERIALS AND METHODS

Reagents

"All reagents were commercially obtained. Ellman's Reagent: 5, 5 di-thiobis 2 nitro benzoic acid i.e. DTNB), Palladium Nitrate, Bisbenzo-nitrile Palladium ii chloride, were purchased from Sigma Aldrich". Sodium di-hydrogen phosphate 95% from (Merck), HCl 35% (Kolchlight) and sodium hydroxide were purchased from (fluka), Dextrose, sodium hydroxide (NaOH), Sodium-edetate (Riedel Dehean AG Sleeze Hannover), were purchased from (Merck), Ethanol (Merck), pH Meter (NOV-210, Nova Scientific Company Ltd. Korea), UV-Visible Spectrophotometer (Shimadzue, 1601Japan, Magnetic Stirrer, hot plate 400(England), (Oven was purchased from Memmert Model U-30,854 Germany (Schwabach)), Micropipettes 200µl, 500µl, 1000µl (Socorex Swiss Finland). Centrifuge: (H-200, Kokusan Ensink company Japan). Eppendolf's tubes (Plastic, 10l), Disposable rubber gloves: Siliconized.

Preparation of stock solutions

0.1M Phosphate buffer PH 7.6 Stock Solution

200 ml of phosphate buffer pH 7.6 was prepared by mixing respective amount of Monobasic Potassium Phosphate (KH_2PO_4) and NaOH. The pH was adjusted adding few drops of strong solution NaOH /or HCl.

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

39.64mg of (DTNB) was dissolved 100ml of (0.1M phosphate buffer pH 7.6.

2mM glutathione, palladium nitrate, Bis-benzonitrile) Palladium ii chloride, stock solutions

2mM stock solutions of Glutathione, Palladium Nitrate, Bis-benzonitrile Palladium ii chloride, stock solutions were prepared by dissolving 30.75mg, 26.6 mg, 38.36mg, in 100ml of Phosphate buffer pH 7.6 respectively according to their respective molecular weight.

0.5M and 5mM disodium edetate solutions

18.861 gram of disodium Edetate was dissolved in 100ml of Phosphate Buffer ph7.6 then diluted with further phosphate buffer to make 5mM EDTA solution from this stock solution.

Normal Saline (0.9% w/v NaCl Solution (0.154 M)

100 ml of 0.9% NaCl solution was prepared by dissolving 0.90g of pharmaceutical grade sodium chloride in sufficient quantity of water for injection to make the whole volume 100 ml.

Preparation liver homogenate sample

Isolation of whole liver

The healthy animals (three rabbits) were collected from animal house of Faculty of Pharmacy Gomal University D.I. Khan KP. The Rabbit was anesthetized with diethyl ether. The fully anesthetized Rabbit was decapitated with gluiton, the abdominal cavity was opened with scissors to expose large area. The liver was carefully cut and washed with 0.9% saline to remove blood and blotted. After washing, dried and weighed the livers. Three rabbit were used to gain average weight of livers. The weight obtained was 150mg.

Isolation liver homogenate

20% (w/v) homogenate was prepared of the above collected liver in 5% TCA (tri-chloro-acetic acid) solution

and 1mm EDTA, using a Potter-eveljhem homogenizer with motor driven Teflon pestle. Centrifuged homogenate at 2,000*g for 15 minutes and collected protein free supernatant. Added 50µl HCl (0.1 N) to the supernatant to keep GSH in reduced form. Sealed it tightly and placed it on ice till further use. Toobtainclear spectra of absorbance upto 1.6 the homogenate obtained was diluted four times with phosphate buffer saline pH 7.6.

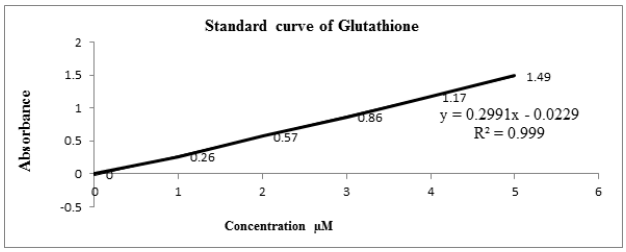


Fig. 1: Standard curve of GSH

Estimation of GSH by elman's method

Glutathione content of Liver homogenate was estimated following Elmans method 1959.

Standard calibration curve

The standard curve was constructed by taking a series of five dilution from 2mM GSH stock solution i.e. (.2mM,. 4mM.6mM,. 8mMand 1mM. Then 0.5ml of 1mM, 5-dithiobis, 2-Nitrobenzoic acid (DTNB) was taken in five different test tubes to it was added. 2ml of each of the above diluted GSH solutions the final volume was adjusted to 3ml by adding 2.3ml of Phosphate buffer PH 7.6, mixed well and incubated for five minutes, absorbance were recorded at fixed wavelength 412nm on UV-visible. Absorbance was plotted as function of the final concentration of GSH solution to obtained standard curve as shown in fig. 1). The spectro-photometric analysis was obtained by using a linear regression analysis of the absorbance versus GSH concentration plot (standard curve) on graph pad prism 5. The correlation coefficient (R2) with a Value of 0.999 indicates a good regression within the given range of concentrations, analyzed in the present study. The data of the standard curve are best described by a linear equation:

Y = m c + b

Where,

Y = Absorbance at 412 nm.

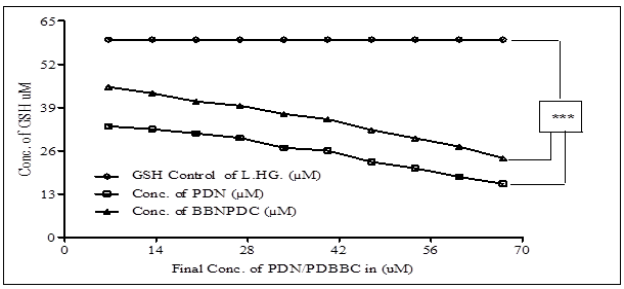
M = Slope of Glutathione standard curve of known concentration.

B = Intercept.

C = Concentration of Glutathione standard curve of known concentration.

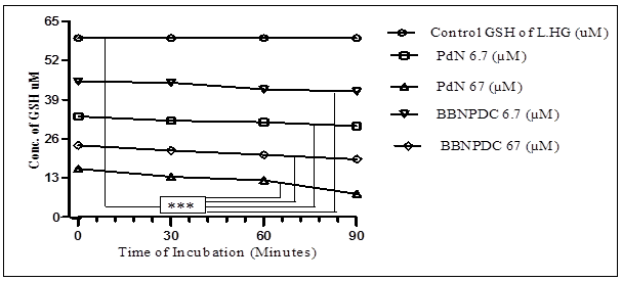
C = Y - b/m

If, Y, b and m are known, "c" unknown concentration of Glutathione can be determined. The above equation was used to calculate the concentration of GSH content of Liver homogenate after treatment with different concentrations of Palladium inorganic and organo-metallic complexes.



Results are the mean ±SE of 3 experiments of (GSH)
Values were compared statistically by using One way ANOVA followed by dunnett test. The error bars indicate the mean SE. ***P<0.001, versus control.

Fig. 2: Conc. dependent effect of (6.7 to 67µM) of PDN/BBNPDC on GSH content of liver homogenate



Results are the mean ±SE of 3 experiments of (GSH)
Values were compared statistically by using One way ANOVA followed by dunnett test. The error bars indicate the mean SE. ***P < 0.001, versus control.

Fig. 3: Time dependent effect of Lowest and highest Conc. (6.7 and 67µM) of PDN / BBNDC on GSH content of liver Homogenate.

Experimental protocol

1.10 ml of Liver Homogenate, was taken in 10 separate test tubes and was mixed with (0.2 to 2mM) of Palladium Nitrate, incubated for 10 minutes.

2.The reading samples was prepared by taking a series of ten samples cuvettes to it 0.5ml of 1mM DTNB was added from stock solution + 0.2ml of Palladium Nitrate Glutathione mixture for each one of the above dilutions,+ 2.3ml of phosphate buffer saline pH 7.6, incubated for five minutes.

3. The final concentration of Palladium Nitrate was (6.7µM 13.4µM, 20.1µM, µM, 26.8µM, 33.5µM), 40.2µM (46.9µM), 53.6µM (60.3µM) and 67.0µM respectively.

4. For control sample 0.2ml of the Glutathione content of pure plasma sample was added instead of plasma palladium mixture. The same procedure was repeated for Bis-benzonitrile palladium ii chloride sample.

5. Absorbance was then recorded on UV- visible spectrophotometer at fixed wavelength at 412nm, converted into concentration using known standard curve of Glutathione already prepared mentioned in fig. 1.

Ethical approval

This study was approved by the Departmental Ethical Committee of Pharmacy Faculty, Gomal University, D. I. Khan.

RESULTS

Results of liver homogenate GSH wit palladium compounds

Effect of Various Conc. (6.7 to 67uM) PDN/ BBNPD on Liver Homogenate GSH content, and With Time at (0 -90) Minutes),

The standard Elman's protocol was applied to liver homogenate sample according to methodology. When GSH content of liver homogenate was exposed to different concentrations (6.7µM to 67µM) of either Palladium Nitrate /or Bis-benzonitrile-palladium (II) Chloride respectively in liver homogenate after centrifugation and the results were compared with control sample GSH content of liver homogenate (without metal salt and complexes) the level of GSH content was also decreased significantly ($p<0.001$) from (43.6% to 72.62%) with Palladium Nitrate and from (24.09 to 59.5%) with Bis-benzonitrile palladium (II) chloride as compared to control as Shown in fig (2). A further decreased was observed in the level of GSH content when time of incubation was increased from 0 to 90 minutes from(49.7 to 87.1%), with Palladium Nitrate and from (29.3% to 67.6%) by addition Palladium organometalic complexes respectively after centrifugation, as compared to control as shown in fig 3.

DISCUSSION

Our study showed that there was depletion in glutathione content of liver homogenate, suggesting that palladium metal salt and complex has the deferential affinity for thiol (SH) group, from the study it's also obvious that affinity of palladium inorganic salt is higher than organic complex as the inorganic salt of palladium lower the glutathione content of liver homogenate more than organic complex. The depletion of the GSH content of Liver Homogenate may be attributed to the fact that Palladium salt/ complex being electrophilic in nature, having the property of facilitating them to react with vital cellular nucleophiles possessing SH group, GSH being a cellular non-protein sulfhydryl molecule, which on treating with palladium salt/complex is accompanied by its significant depletion in cells by reacting with SH group of glutathione. This may be resulted in formation of glutathione S-conjugates or conversion of GSH into GSSG which is the initial step in the biotransformation of electrophiles

CONCLUSION

On the basis of the results obtained we conclude that both palladium inorganic salt and organic complex interactions are involved in the induction of oxidative stress under the exposure to these substances. The disturbances in the oxidative status observed in our experimental model may indicate a risk of liver damage during exposure to Palladium inorganic salt/ organic complex via the free radical mechanisms. It appears that our metal compounds lower hepatic GSH level via extra hepatic action. Our primary experiments and the subsequent available results suggested that liver homogenate suspension may be a useful model for studding metallo-element palladium toxicity in the future. These observations also showed that metallo-element palladium may cause or induce toxicities to various organs of the body including hepato-toxicity. Also the detoxification mechanism of glutathione of heavy metals like palladium metallo-elements isan important tool that may contribute to counteract metal induced toxicity.

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