PROTECTIVE ROLE OF GLUTATHIONE IN TUNGSTEN-INDUCED TOXICITY IN BLOOD COMPONENTS: A PHARMACOLOGICAL AND TOXICOLOGICAL PERSPECTIVE

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ABSTRACT
Sodium tungstate (Na₂WO₄), a pharmacological agent lacks information on its molecular mechanism of action. Therefore, it was of interest to examine the effect of Sodium tungstate (ST) on the metabolic status of glutathione (GSH) in plasma and cytosolic fraction of blood of healthy human volunteer. In the current study, the effect of Sodium Tungstate on the chemical and metabolic status of Glutathione in human venous blood components was carried out spectrophotometrically. Results showed that concentration of GSH lowered upon addition of ST to venous blood of volunteers. The lower content of GSH was found to be ST concentration and time dependent. The lower GSH content in venous blood components i.e. plasma and cytosolic fraction upon addition of ST may be either due to formation of ST-SG complex or conversion of reduced GSH to GSSG.

KEY WORDS: Reduced Glutathione (GSH), Sodium Tungsten (ST), 5, 5-Dithiobis, 2-nitro benzoic acid (DTNB), venous blood and Ellman’s method.

INTRODUCTION
Glutathione (GSH) is an endogenously synthesized tripeptide thiol, which plays an important role in intra and extracellular antioxidants defense. Glutathione is oxidized to glutathione disulfide (GSSG) upon exposure to oxidants and thus cause damage to cells of the body. Glutathione is exclusively found in its reduced form (GSH), and the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) within the cells is often used scientifically as a measure of cellular toxicity. Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases. Thus, glutathione has vital role in maintenance of body defense system in fighting against diseases along with metal and drug poisoning. Considerable data on the chemistry and pharmacological properties of Sodium tungstate is available. Interest in tungsten compounds including Sodium tungstate has been increased due to its varied pharmacological properties which include; anti-diabetic, anti-obesity, protective role in Liver necrosis and hepatic failure. It has also been reported that tungstate causes activation of insulin secretion and regeneration of β-cells population Sodium tungstate has a low toxicity profile but cases of intoxication have been reported. Since both GSH and ST have several important pharmacological properties, Therefore, it was of interest to evaluate the interaction of ST with GSH in vitro as a model of in vivo reaction to establish further scientific data which can strengthen our knowledge about the pharmacological and toxicological profile of tungsten, and the role of GSH in the improvement of the current use of tungstate in medicine along with control on growing problem of its resistance. The interaction of ST with GSH in blood components and the proposed formation of W-SG complex may contribute towards the pharmacological perspectives of ST.

MATERIALS AND METHODS
Chemicals, Reagents and Equipments
L. Glutathione (GSH) (Fluka), DTNB (Sigma), Sodium Hydroxide (Fluka AG), Sodium chloride (Merck), Disodium Edetate (Riedel Dehean AG Sleze Hannover),

Potassium Dihydrogen Phosphate (Merck), HCl 35% (Kolchlight), Sodium Tungstate (Across, Belgium), Distilled Water (Double Refined), Chloroform (Merck), Ethanol (Merck), Spectrophotometer: UV. Visible, 1601 (Schimadzu, Japan), PH Meter: Model NOV-210 (Nova Scientific Company Ltd. Korea), Analytical Balance AX 200 (Schimadzu, Japan) Centrifuge H-200 (Kokusankensin Company Japan), Eppendoff’s tubes (Plastic, 101) Oven: Memmert Model U-30,854 (Schwa Bach, Germany), Magnetic Stirrer. Purchased from local market

Methods

Preparation of Solutions

0.9% NaCl solution was prepared by dissolving 9-mg of NaCl in 100ml of water. 50-ml solution of Sodium Tungstate (1mM) was prepared by dissolving 18.96 mg of ST in distilled water. Glutathione standard solution (1mM) was prepared by dissolving 30.74mg of GSH in 100ml of 0.1 N HCl. Di, thiobis, dinitrobenzoic acid (DTNB) (1mM) was prepared by dissolving 39.6mg of DTNB in 100ml of buffer solution. Phosphate buffer (0.2M) having pH 7.6 was prepared by mixing 42.2ml of NaOH (0.2M) and 50ml Mono-basic potassium phosphate solution (0.2M) and making the volume up to 200ml with distilled water.

Preparation and isolation of Blood Components

Isolation of Plasma

12 ml fresh venous blood treated with 0.5mM Na-EDTA (500µl) to prevent clotting was collected from healthy human volunteers, 1ml venous blood was taken and mixed with 1ml of each concentration of ST (200-1000 µM) solution and incubated for 10 minutes. Final concentration of ST in each tube was 100-500 µM. Each of this 2ml sample containing blood and ST solution in 1:1 ratio was then centrifuged on centrifuge at 10000rpm for five minutes. The supernatant fluid 0.8ml (plasma) was removed with Pasteur pipette, transferred to sample tubes and kept on ice till use. The packed cells were washed twice with isotonic saline (NaCl) solution and the blood cells were lysed at 4°C with an equal volume (1:1) of distilled water for 1hour. After one hour lysis at 4°C, 0.8ml of cold mixture of chloroform- ethanol (3:5 V/V) at 0°C was added to 2ml of lysed cells to precipitate the hemoglobin, followed by addition of 0.3ml of distilled water. The resulting mixture was centrifuged as before and the pale yellow clear supernatant (cytosolic fraction) was removed by Pasteur pipette and transferred to sample tubes and stored on ice till use. Control containing 1ml of venous blood and 1ml of 0.9% NaCl solution was processed and centrifuged for the collection of Cytosolic fraction as described before.

Determination of Biological inorganic parameters

1. Plasma glutathione (Extracellular)
2. Lysate Glutathione (Intracellular)

All glutathione estimation were carried out following the modified standard Ellman’s method as given below. 2.3ml buffer was added to 0.2ml of the sample (plasma or cytosolic fraction of blood) followed by the addition of 0.5ml of DTNB. This mixture was transferred to a spectrophotometer cell. The reference cell contained buffer solution. DTNB blank consisting of 2.5ml buffer, 0.5ml DTNB was measured against a reference cell containing 3ml buffer.

Standard Curve

Standard Curve was prepared using 13.33-66.66µM GSH following the Ellman’s method as prescribed above as shown in figure 1.

RESULTS

ST Effect on GSH content of Extracellular plasma fraction of blood

1ml solution of different concentrations (200-1000µM) of ST was added to human venous blood in 5 separate tubes. Final concentration of ST in each tube was 100-500 µM. Plasma fraction in each tube of the blood was isolated after 10 minutes of incubation period and transferred to separate tubes.

Reduced Glutathione (GSH) content was measured in each tube by method as described. The GSH content of plasma fraction of blood decreased with increasing concentration of ST addition as shown in figure 2. A significant change in the content of plasma GSH from control was observed at p<0.05. Plasma GSH content at time intervals (0-90 min) was also measured when the lowest and the highest dilutions of ST having final concentrations of 100 and 500 µM respectively were added. The GSH content of extracellular plasma decreased with time (Figure 3). These results show that decrease in plasma GSH content were both ST concentration and time dependent.
ST effect on GSH content of Intracellular Cytosolic fraction of blood

1ml of ST (200-1000 µM), solution was added to 1ml venous blood. Final concentration of ST in each tube was 100-500 µM. Upon addition of ST to venous blood, measurement of intracellular Cytosolic GSH content showed gradual decrease in GSH content as shown in figure 4. Intracellular Cytosolic GSH content was also measured at 0-90 Minutes after the addition of ST to venous blood. A significant change in the content of cytosolic fraction GSH from control was observed at p<0.05. Results show the time dependent decrease in GSH content as shown in figure 5.

DISCUSSION

We have studied the effect of Sodium Tungstate (ST) on GSH level in plasma and Cytosolic fraction of human venous blood spectrophotometrically. The concentrations of ST used in the present study ranged from 100uM - 500uM ST. The effect of Tungstate on the chemical and metabolic status of GSH was studied in terms of determination of concentration of GSH at λ max 412nm. This λ max (412nm) is being used for the determination of GSH concentration in samples according to a well known Elman’s method⁹. Thus the interaction of this metal with glutathione in vitro as a model of in vivo reaction will establish further scientific data and will strengthen our knowledge about the toxicological profile of tungsten and the role of GSH in the protection of our body from its harmful effects. According to our findings the tungsten in the form of sodium tungstate induced the depletion of GSH in a dose dependent manner. The results also show positive correlation between the exposure of glutathione to the above given concentrations of ST and the depletion of GSH as the time passed from 0 to 90 minutes.

The fact that Sodium tungstate (ST) has considerable pharmacological effects prompted us to examine the interaction of ST with glutathione (GSH), the most important intra and extracellular antioxidant in plasma and cytosolic fraction of healthy human volunteers. Furthermore little information is available on ST mechanism of action at the molecular level. Our experimental work is the first to investigate the effect of ST on the metabolic modulation of GSH in blood components and to propose the molecular mechanism of action of ST. Our research hypothesis was that ST directly or indirectly or through enzymatic or non-enzymatic pathway causes modulation in the status of GSH either to glutathione disulfide (GSSG) or formation of tungstate-glutathione (W-SG) complex.

Our results support the hypothesis that incubation and addition of ST to venous blood, separation of plasma and cytosolic fraction and determination of GSH decreased the concentration of GSH in these components, one of the characteristics of ST as an oxidant. Furthermore the concentration effect of ST was not similar but rather different in plasma and cytosolic fraction of blood. The decrease in GSH concentration was more pronounced in plasma, probably due to non-lipid solubility of ST. Therefore ST has affected decrease of GSH more in plasma than cytosolic fraction.

The exact mechanism of action of ST on GSH metabolic status in this study is not known. However the proposed hypothetical mechanism of action of ST on GSH metabolic status is the formation of W-SG complex. This hypothetical mechanism of action and formation of W-SG complex is in agreement with our research work, where antibacterial activity of ST, GSH and ST and GSH mixture were examined.

The results indicate that antibacterial activity of ST was very low (almost Zero), GSSG, GSH were high and ST and GSH mixture was lower than GSH and higher than ST (not shown).

These results indicating that antibacterial activity of GSH and GSSG were the same and GST and ST mixture was very low than GSH and GSSG and ST had almost Zero leads to the conclusion that ST had not caused the oxidation of GSH to GSSG but consistent with the reaction taking place at the active site of GSH and that is S-H group and form W-SG complex.

Such reaction through a mechanism involving the coordination of W in ST with the S-H group of GSH with the formation of W-SG complex is as under,

N₄W₂O₇ → 2Na⁺ + WO₄²⁻

WO₄²⁻ + 2GSH → W-SG + H₂O

In conclusion, we have examined interaction of ST with GSH spectrophotometrically and hypothesized the formation of W-SG complex, which might have clinical implication.

REFERENCES

2. Schafer FQ, buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione/glutathione complex. Free Medical Biol Med 2001; 30(11): 1191-1212
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Figure 1. Standard Curve of Glutathione

Figure 2. Effect of ST Concentration on Extracellular plasma GSH Content

Results are the mean ±SE of 3 experiments of plasma GSH

Figure 3. Effect of ST Concentration on the extracellular plasma GSH content with time incubation period (0-90 min)

Results are the mean ±SE of 3 experiments of plasma GSH
Figure No.4 Effect of ST Intracellular Cytosolic Fraction GSH Content

- Control (1ml 0.9% NaCl/1ml of blood)
- ST (100-500 µM)

Results are the mean ±SE of 3 experiments of Cytosolic Fraction GSH

Figure 5. Effect of ST on intracellular Cytosolic Fraction GSH content with time incubation period (0-90 Min)

- Control (1ml 0.9% NaCl/1ml of blood)
- ST (100 µM)
- ST (500 µM)

Results are the mean ±SE of 3 experiments of Cytosolic Fraction GSH

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