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Chemical Status of Glutathione in the Presence of a Salicylic Acid Derivative and its Copper Complex

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The objective of this investigation was to determine the chemical status of Glutathione (GSH) in the presence of a drug and its copper complex employing simple spectrophotometric analysis. A salicylic acid derivative, 3, 5-di-isopropyl salicylic acid (3,5-DIPS) and its copper complex, tetrakis-μ-3,5-di-isopropylsalicylato-diaquodicopper (II) [Cu (II)$_2$ (3,5-DIPS)$_4$.2H$_2$O] was used as a model drug and its complex, respectively. A prominent and regular decrease in the level of GSH was caused by Cu (II)$_2$ (3,5-DIPS)$_4$ 2H$_2$O as compared to the simple ligand 3, 5-DIPS. The decrease in the levels of GSH was found to be dependant upon the concentration and time of the Cu (II)$_2$ (3,5-DIPS)$_4$ 2H$_2$O. Some changes in the status of GSH might be the basis of this chemical change observed in the form of depletion.

Key words: Glutathione, chemical status, 3,5-di-isopropyl salicylic acid, tetrakis-μ-3,5-di-isopropylsalicylato-diaquodicopper (II)

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Introduction

Tetrakis-μ-3,5-di-isopropylsalicylato-diaquodicopper (II) [Cu (II)$_2$ (3,5-DIPS)$_4$. 2H$_2$O] is a binuclear copper complex (Greenway *et al.*, 1988). It has anti-inflammatory, anti-ulcer (Sorenson, 1976); analgesic (Okuyama *et al.*, 1987); antidiabetic (Gandy *et al.*, 1983); anti-convulsant (Sorenson *et al.*, 1979); anti-cancer, anti-carcinogenic, anti-mutagenic (Leuthauser *et al.*, 1981) and radioprotectant (Sorenson, 1984) activities. These pharmacological activities have been related to its disproportionation of superoxide, the facilitation of *de novo* synthesis of other copper (Cu) dependant enzymes required to overcome these disease states (Sorenson, 1984).

On the other hand interest in a xenobiotic, glutathione (GSH) is also increasing because of its varied physiological and pharmacological activities. Glutathione (L-γ-glutamylcysteinyl glycine) is the most important non-protein thiol widespread in animals, plants and micro-organisms (Kosower and kosower, 1987). SH-group of the cystein moiety is the most reactive functional group of GSH in the biological processes. It acts as a cellular reductant, catalyst in a number of reactions, reactant in various phases of metabolism, as a storage and transport form of cystein, and as a cell protector against free radicals, reactive oxygen species and toxic compounds of endogenous and exogenous origin (Wilson, 1983).

GSH and Cu (I or II) are considered to have anti-oxidant properties and they are involved in a large number of physiological and pharmacological activities (Moldeus and Jernstrom, 1973). Following the reports that copper complexes of anti-arthritic drugs are more active and less toxic anti-inflammatory agents than their parent drug (Khan *et al.*, 1992a; Khan *et al.*, 1997; 1997a), the effect of 3,5-DIPS and Cu (II) (3, 5-DIPS)$_4$.2H$_2$O on the chemical status of GSH in aqueous solutions was investigated to further understand their mechanism of action in the above mentioned diseases.

Materials and Methods

The experimental work was done at the Faculty of Pharmacy, Gomal University, D. I. Khan and at the Department of Pharmacy, Peshawar University, during 1999-2001.

Glutathione (GSH), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma Chemical Co.), 3, 5-di-isopropyl salicylic acid (3,5-DIPS) (Aldrich Chemicals) were used. Tetrakis-μ-3, 5-di-isopropylsalicylato-diaquodicopper (II) Cu (II) (3,5-DIPS)$_4$.2H$_2$O was prepared with 3, 5-DIPS and CuCl$_2$ according to the method of Sorenson, 1976a; Khan *et al.*, 1997; 1997a. UV-Dec-610, double beam spectrophotometer was used for the analysis.

One mM GSH, 10^{-2} M DTNB solutions were prepared in 0.1M phosphate buffer (pH, 7.6). One mM 3,5-DIPS and 1mM Cu (II)$_2$ (3, 5-DIPS)$_4$. 2H$_2$O solutions were prepared in water and water-ethanol (H$_2$O: C$_2$H$_5$OH); 97:3 solvent system, respectively.

To 800μL of 1mM GSH in three separate test tubes, 1000, 500 and 250μL of 1mM 3, 5-DIPS solutions were added, shook and further diluted each to 2 ml with phosphate buffer (pH, 7.6).

Similarly, 1000, 500 and 250μL of 1mM Cu (II)$_2$ (3,5-DIPS)$_4$. 2H$_2$O were added to 800 μL of 1mM GSH in three separate test tubes and final volumes of 2ml were made with phosphate buffer (pH, 7.6) in each case.

The effects of 3,5- DIPS and Cu (II)$_2$ (3, 5-DIPS)$_4$.2H$_2$O on the chemical status of GSH were investigated by determining the GSH concentration in the respective mixtures by Elman's method (Sorenson 1976a; Khan *et al.*, 1997; 1997a).

Results and Discussion

3, 5-DIPS apparently caused an insignificant decrease in the concentration of GSH at initial stages followed by a regular increase in its concentration with the increase in the concentration of 3, 5-DIPS. This increase in the concentration of GSH was also time dependent, as shown in Figs. 1 and 2. Cu (II)$_2$ (3, 5-DIPS)$_4$. 2H$_2$O caused a very prominent decrease

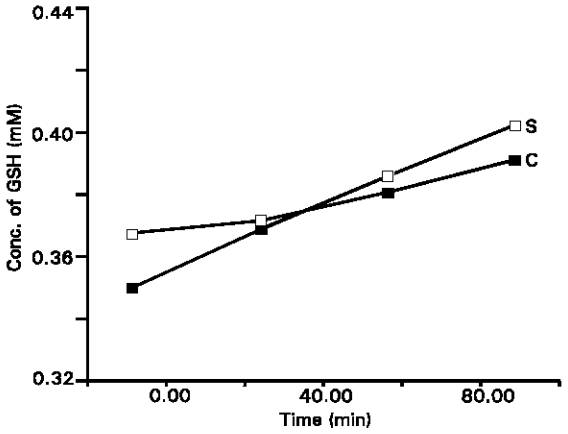

Fig. 1: Effect of 3,5-DIPS on chemical status (concentration) of GSH (400 μM) (■). The final concentration of 3,5-DIPS in the mixture containing GSH and 3,5-DIPS was 500μM (□) C, Control; S, Sample

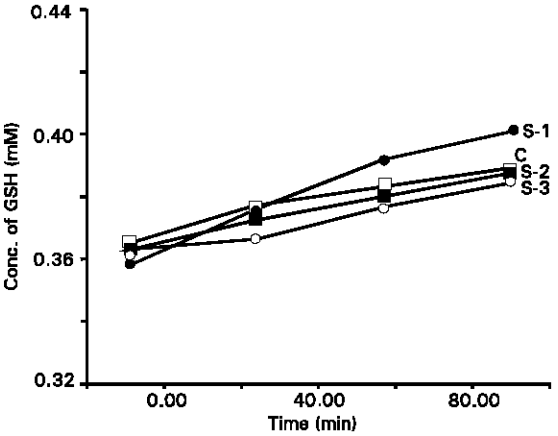

Fig. 2: Effect of different concentration of 3,5-DIPS on the chemical status Concentration of GSH 400μ M (□). The final concentration of 3,5-DIPS in the mixture containing GSH and 3,5-DIPS were 500 μM (●), 250 μM (■) and 125 μM (○) C, Control; S, Sample

in the concentration of GSH, in a concentration and time dependant manner, as shown in Figs. 3 and 4.

The effect of 3, 5-DIPS and Cu (II)$_2$ (3,5-DIPS)$_4$.2H$_2$O on the chemical status of GSH was investigated by determining the concentration of GSH at λ max 412nm (Sorenson, 1976a). During this research work it was found that Cu (II)$_2$ (3,5-DIPS)$_4$.2H$_2$O was more effective in lowering the GSH concentration than the simple ligand, 3,5-DIPS.

To summarize Cu (II)$_2$ (3,5-DIPS)$_4$.2 H$_2$O oxidized GSH and

The following sequence of reactions were suggested:

$$Cu\ (II)_2\ (3,5\text{-}DIPS)_4 \longrightarrow 2Cu\ (II)\ (3,5\text{-}DIPS)_2$$

$$2GSH + 2Cu\ (II)\ (3,5\text{-}DIPS)_2 \longrightarrow 2GS\text{-}2Cu\text{-}(I)\ (3,5\text{-}DIPS) + 2(3,5\text{-}DIPS) + 2H^+$$

$$2GS + Cu(I)\ (3,5\text{-}DIPS)_2 \longrightarrow GSSG + 2Cu\ (II)\ (3,5\text{-}DIPS)_2 + {}^1/_2\ O_2 + 2H^+ + 2H_2O$$

Thus

$$GSH + {}^1/_2\ O_2 + Cu(II)_2(3,5\text{-}DIPS)_4 \longrightarrow GSSG + H_2O$$

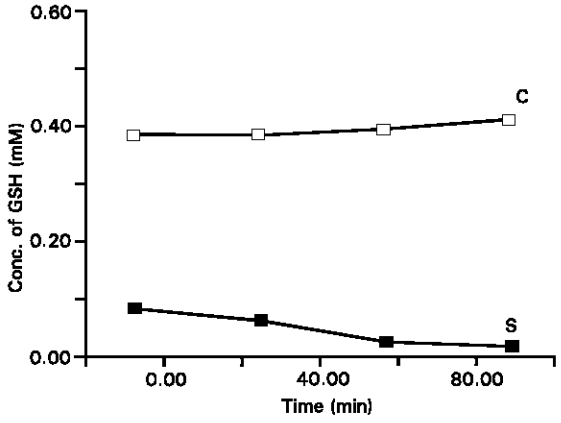

Fig. 3: Effect of $Cu(II)_2$ $(3,5\text{-}DIPS)_4$. $2H_2O$ on the chemical status, concentration of GSH 400 μM (□). The final concentration of $Cu(II)_2$ $(3,5\text{-}DIPS)_4$. H_2O in the mixture containing GSH and $Cu(II)_2$ $(3,5\text{-}DIPS)_4$. $2H_2O$ was 500 μM (■); C, Control; S, Sample

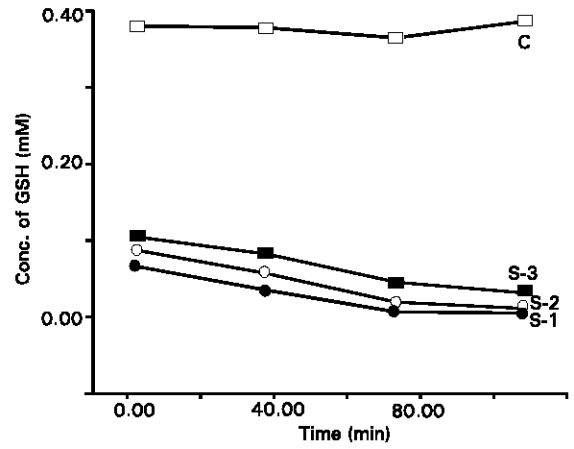

Fig. 4: Effect of $Cu(II)_2$ $(3,5\text{-}DIPS)_4$. $2H_2O$ on the chemical status, concentration of GSH 400 μM (□). The final concentration of Cu $(II)_2$ $(3,5\text{-}DIPS)_4$. $2H_2O$ in the mixture containing GSH and Cu $(II)_2$ $(3,5\text{-}DIPS)_4$. $2H_2O$ were 500 μM (●), 250 μM (○) and 125 μM (■); C, Control; S, Sample

GSSG in the presence of air oxygen (O_2). Therefore, it is to be point out that determination of GSH concentration in biological samples should be performed in the nitrogenous (N_2) atmosphere to avoid oxidation of thiols (-SH) or GSH to a greater extent, which will in turn helps us to interpreting correct results in certain clinical investigations.

The results also suggested that there was a possibility of formation of intermediate or conjugate between 3,5-DIPS, $Cu(II)_2$ $(3,5\text{-}DIPS)_4.2H_2O$ and GSH. However, it was not possible to determine or estimate these conjugates. This hypothesis is in agreement with the results obtained by Khan *et al.* (1992). The results of this research work also give clues for further investigation of the scavenging effect of mixtures containing 3,5-DIPS and/or $Cu(II)_2$ $(3,5\text{-}DIPS)_4$. $2H_2O$ and GSH on the oxygen derived free radicals in the biological samples because $Cu(II)_2$ $(3,5\text{-}DIPS)_4.2H_2O$ exhibits the property to scavenge free radicals (Khan *et al.*, 1997).

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