Effect of 3, 5-Diisopropylsalicylic Acid and Its Copper Complex on Glutathione Chemical Modulation and Metabolism in the Cytosolic Fraction of Human Blood

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Abstract The effect of 3, 5-diisopropylsalicylic acid (3, 5-DIPS) and its copper complex tetrakis-μ-3, 5-diisopropylsalicylatodiaquodicopper(II) $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$ on glutathione (GSH) in the cytosolic fraction of human blood has been investigated using spectrophotometric method. In the presence of both of these compounds the GSH levels of the cytosolic fraction were enhanced in a dose dependent manner. In this regard $Cu(II)_2(3,5\text{-DIPS})4(H_2O)_2$ was found to be more effective than 3, 5-DIPS.

Key words Glutathione; 3, 5-DIPS; $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$; Cytosolic fraction of human blood; Chemical modulation and metabolism

1 Introduction

There has been a growing interest in recent years in glutathione (GSH) and copper complexes because of their physiological, pharmacological, toxicological, and biochemical properties. The GSH biomolecule plays an important role in metabolic pathways involved in detoxification and excretion of various xenobiotics[1]. The well known physiological functions of GSH include maintenance of membrane integrity and cytoskeletal organization, involvement in protein and DNA synthesis, modification of protein conformation and enzyme activity, and promotion of neurotransmitter release[2]. In addition, GSH plays an important role in various detoxification reactions, including elimination of oxygen free radical, the detoxification of aldehydes and α-ketaldehydes, inactivation of electrophilic drugs and carcinogen metabolites by formation of GSH-S-conjugates and acting as free radical scavenger[3].

Concentration of GSH in various tissues, depleted and/or increased GSH levels in mutagenesis, carcinogenesis, and rheumatoid arthritis are currently very active areas of research[4]. Decreased tissue concentration of GSH has led to increased hemolysis, brain dysfunction (lack of protective role to be played by GSH in neurotransmission), as well as peripheral neuropathy, myopathy, aminoaciduria and increased incidence of cancer[5]. Recently, cancer researchers have shown an enormous interest in quantitating GSH levels in erythrocytes from cancer patients and their clinical response to the anti-cancer drugs[6]. Very recently, Demopoulos has demonstrated that up to 4000 mg/d in fractionated doses were tolerated by patients with HIV/AIDS disorders with potential benefits, as

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these patients were GSH depleted[7].

The present authors previously studied the effect of 3, 5-diisopropylsalicylic acid and its copper complex on GSH status in aqueous solutions and in the serum fraction of human blood[8]. The objective of the present study was to investigate the effect of 3, 5- diisopropylsalicylic acid and its metalloelement complex on the modulation and metabolism of GSH in the cytosolic (lysate) fraction of human blood.

2 Materials and Methods

2.1 *Materials*

Ethylenediamine tetra acetate sodium (EDTA), Chloroform, Ethanol (E. Merk, Germany), L-glutathione (GSH), 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) (Sigma Chem. Co.), Cupric chloride dihyrate ($Cu(II)Cl_2 \cdot 2H_2O$) (Beijing Chem. Works, China), Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$) (Riedle- De-Hean AG. Sleeze, Hannover) and all other reagents were of analytical grade and used without further purification.

2.2 *Methods*

Samples of 2 ml of human venous blood were taken, and dipped in to 0.5 mol/L EDTA-2Na solution to prevent clotting. The blood was centrifuged for 2 min at 10000~ 12000 r/min. The packed red cells were washed twice with 1 ml of isotonic saline solution and centrifuged at 2000~ 4000 r/min for 30 s. The supernatant was discarded each time. 0.5 ml of red cell fraction was taken and lysed at 0~ 4℃ with equal volume of 5 mmol/L EDTA solution for 1 h. 0.6 ml of ethanol-chloroform mixture (5∶3 *V*/*V*) maintained at 0℃ was added to 1 ml of lysed cells to precipitate the hemoglobin followed by the addition of 1 ml of distilled water. The resulting mixture was centrifuged as before and the pale-yellow clear supernatant was collected using a micropipette. Later on the cytosolic glutathione level was determined using to the DTNB method as mentioned previously[8].

3 Results and Discussion

3.1 *Effect of 3, 5-DIPS on the cytosolic GSH levels of human blood*

The effect of different concentration of 3, 5-DIPS on cytosolic glutathione levels are shown in Fig 1. Upon the addition of different concentrations of 3, 5-DIPS an increase in the cytosolic GSH levels was observed in a dose dependent manner. This figure also shows the time dependent effect of 3, 5-DIPS on the cytosolic GSH levels of the human blood.

Glutathione is a major low molecular weight thiol in red cells lysate. In our previous work[8] we found the oxidative effect of 3, 5-DIPS on GSH in aqueous solutions resulting in lower GSH levels as compared to the control. In the present study higher GSH levels can be observed after incubating the cytosolic fraction of the human blood with 3, 5-DIPS. The probable explanation for this effect could be the activation of GSH dependent enzymes by 3, 5-DIPS, thereby enhancing the proportion of GSH in reduced form as compared to the control.

3.2 *Effect of $Cu(II)_2(3, 5\text{-}DIPS)_4(H_2O)_2$ on Cytosolic GSH Levels of Human Blood*

The effect of different concentrations of $Cu(II)_2(3, 5\text{-}DIPS)_4(H_2O)_2$ on the cytosolic fraction of human blood can be observed clearly from the Fig 2. This figure also shows the time dependent effect of $Cu(II)_2(3, 5\text{-}DIPS)_4(H_2O)_2$ on the cytosolic GSH levels. Interactions of the cytosolic fraction of the human blood with $Cu(II)_2(3, 5\text{-}DIPS)_4(H_2O)_2$ resulted in a higher concentrations of GSH in a dose dependent manner.

The mechanism appears to be related to selective binding of 3, 5-DIPS to macromolecules with a consequent enhancement in the release of GSH.

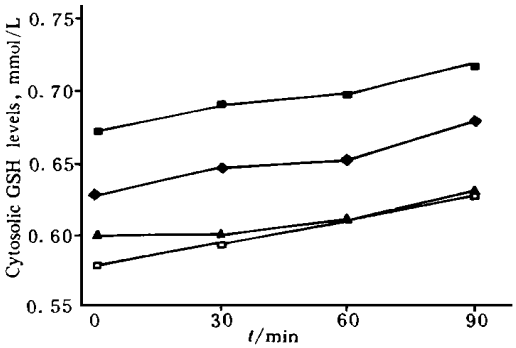

Fig 1. Time dependent effect of different Conc of 3,5-DIPS on cytosolic (lysate) GSH levels at 0, 30, 60 and 90 min
Cytosolic fraction + ■: 500 μmol/L 3,5-DIPS; ◆: 200 μmol/L 3,5-DIPS; ▲: 100 μmol/L 3,5-DIPS; □: Control 1 ml of cytosolic fraction + 1 ml of ethanol water mixture (3∶97)

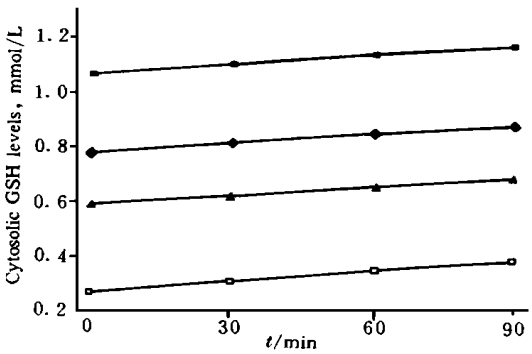

Fig 2. Time dependent effect of different Conc of $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$ on cytosolic (lysate) GSH levels at 0, 30, 60 and 90 min
Cytosolic fraction + ■: 50 μmol/L $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$; ◆: 20 μmol/L $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$; ▲: 10 μmol/L $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$; □: Control 1 ml of cytosolic fraction + 1 ml of ethanol water mixture (3∶97)

Although $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$ has shown oxidative effects on GSH in aqueous solutions[8], changes in the levels of GSH in the cytosolic fraction could be related to multiple factors, including the dissolution of the $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$ in the cytosolic fraction which presumably oxidizes and increases leakage from bound pool of GSH. Cu(II) ions oxidize and 3,5-DIPS, being greater in number than Cu(II) ions, might effectively activate bound glutathione resulting in the release of GSH in the reduced form. Furthermore $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$ was found to be more effective than 3,5-DIPS alone. This observation is in agreement with the previous reports that copper complexes of chemicals and/or drugs are more effective in treating a variety of diseases and active scavenger of free radicals than their parent chemicals and/or drugs[3,8].

The results derived from this study can be of valuable significance in the determination of appropriate directions towards the conduction of more sophisticated tests of chemicals and/or drugs to develop improved, sensitive and selective analytical methods for safety evaluation, improvement and development of new drugs and/or chemicals to ensure availability of safe and quality medicines for the desired effect in the treatment, mitigation and prevention of diseases.

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3, 5-二异丙基水杨酸及其铜络合物对人血细胞液中谷胱甘肽的化学调节与代谢作用

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摘　要　应用分光光度法研究了 3, 5-二异丙基水杨酸及其铜络合物四 μ-3, 5-二异丙基水杨酸对人血细胞液中谷胱甘肽的影响. 在这些化合物存在时,细胞液中谷胱甘肽以剂量依赖方式升高. 铜络合物较 3, 5-二异丙基水杨酸更为有效.

关键词　谷胱甘肽; 3, 5-二异丙基水杨酸;四 μ-3, 5-二异丙基水杨酸二铜二水;人血细胞液;化学调节与代谢