

Effect of Tetrakis- μ -3,5-di-isopropylsalicylatodiaquodicopper (II) and Sodium Gold (I) Thiomalate (Myocrisin) on the Metabolism of Plasma Thiol in the Rheumatoid Arthritis Patients and Volunteer Human Blood

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The effects of tetrakis- μ -3,5-di-isopropyl Salicylatodiaquodicopper (II) $(3,5\text{-DIPS})_4(\text{H}_2\text{O})_2$ and sodium gold (I) thiomalate (Myocrisin) $\text{Au(I)}(\text{S-CHCH}_2(\text{CO}_2)_2\text{Na})_2$ on the metabolism of plasma thiol in volunteer human blood and/or rheumatoid arthritis (RA) patients phase-I were compared and examined. Human blood and/or plasma was incubated with $\text{Cu(II)}(3,5\text{-DIPS})_4(\text{H}_2\text{O})_2$. Plasma thiol was measured by spectrophotometer using Ellman's reagent for derivatization. Myocrisin was administered to RA patients in phase-I and blood was collected for plasma thiol determination using Ellman's method. Both copper and gold complexes and/or drugs increased the concentrations of plasma thiol as compared to control group and/or in RA patients. Both copper and gold were found to have the potential to enhance the release of thiol from plasma by affecting the exposure of hidden thiol, contained in plasma proteins and enzymes. These results suggested that these complexes and/or drugs perhaps caused the leakage of the hidden plasma thiols present in proteins and enzymes preferably on albumin, a major thiol containing protein in plasma. This alteration in plasma thiol concentration in blood and/or plasma by copper and gold complex and/or drug may count towards their some pharmacological effects.

Key words: Copper (II), gold(I), blood, plasma thiol, metabolism, rheumatoid patients

Introduction

The interest in investigating the thiol and sulphhydryl (-SH) containing compounds including glutathione (GSH) has been increased tremendously during recent years mainly because of its involvement in organic, medicinal and radiation chemistry, cell physiology, molecular biology, genetics, cancer therapy, pharmacology, toxicology, enzymology and agriculture (Arias, 1976). SH groups are chemically the most active groups found in cells and are involved in many biological processes *in vivo* (Friedman, 1973). SH groups or SH groups containing compounds are known to act as reducing agent, nucleophile, anti-oxidant and participates in detoxification of xenobiotics, toxic chemicals and/or drugs and metabolism of cellular components, required for synthesis of some prostaglandins and involved in cell cycle regulation, tissues protection against oxidative damage, recovery and repair against oxidizing environment (oxygen, ozone etc) (Chasseaud, 1973). Alteration and/or deficiency of thiols leads to life threatening acidosis, defective function of vital organs, hemolysis and increased incidence of cancer (Arias, 1976).

A thiol containing product GSH is clinically used in drug, metal poisoning and toxicity, hepatic diseases, to prevent adverse reactions to anti-cancer drugs, prevention and treatment of leukopenia, carcinoma due to radiotherapy, acute and chronic eczema, corneal diseases, radiation sickness, pigmentation after inflammation, toxemia of late pregnancy and inflammation of oral mucosa due to radiation (Japan Pharm. Research, 1993).

Copper complexes which represent a group of compounds, have been used in the treatment of variety of diseases including tuberculosis, rheumatoid arthritis and degenerative diseases (Eichol, 1934 and Sorenson *et al.*, 1977). Copper complexes and/or salts were also used for the treatment of wound healing and eye infection. Copper(II) complexes including tetrakis- μ -3, 5-di-isopropylsalicylate diaquodiacopper (II) $\text{Cu(II)}_2 (3,5\text{-DIPS})_4 (\text{H}_2\text{O})_2$ (Fig. 1) have varied pharmacological activities including; anticancer, anticarcinogenic, antimutagenic, anticonvulsant, antiinflammatory, antidiabetic, radiation protection and recovery, and recovery of the CNS syndrome (Sorenson, 1989; Sorenson *et al.*, 1993; Baquial and Sarenson, 1995). Gold complexes which also represent a group of compounds, have been used in the treatment of tuberculosis since long (Koch, 1927). Gold complexes and/or drugs are now standard prescriptions for the treatment of rheumatoid arthritis (Sigler *et al.*, 1972).

Sodium gold (I) thiomalate (Myocrisin) (Fig. 2) is conventional gold (I) drug which is usually administered by intra-muscular

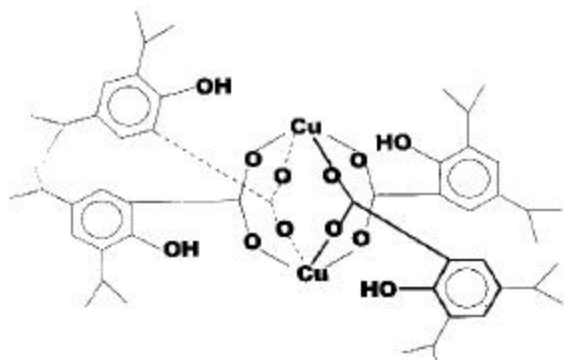


Fig. 1: Structure of Tetrakis- μ -3,5-di-isopropyl salicylate diaquodiacopper (II)

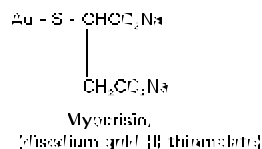


Fig. 2: Structure of di-sodium gold (I) thiomalate

injection, is being used in the treatment of R A. Serum thiol concentrations are significantly lowered in R A, emphasizing the importance of thiol changes *in vivo*. Medical studies are of little help so far to the chemists to suggest a possible mode of action of gold and therefore, there still exists some confusion on the role of gold in the treatment of R A.

The objective of this study was to compare the effects of $\text{Cu(II)}_2 (3,5\text{-DIPS})_4 (\text{H}_2\text{O})_2$ and sodium gold (I) thiomalate on the metabolism of serum or plasma thiols of human blood in two different models. A possible mode of action and/or mechanism of action of both copper and gold complexes and/or drug has been proposed.

Materials and Methods

Materials with specification, grades and methods of preparation of solutions, standard curve for GSH and synthesis of $\text{Cu(II)}_2 (3,5\text{-DIPS})_4 (\text{H}_2\text{O})_2$ have been described by Khan *et al.* (1997, 1997 a).

Different concentrations of $\text{Cu(II)}_2 (3,5\text{-DIPS})_4 (\text{H}_2\text{O})_2$ was added to 0.5ml of the plasma fraction of blood of human volunteers (students of the Faculty of Pharmacy) with their history of previous diseases, if any, including the usage of medicines. The final concentration of $\text{Cu(II)}_2 (3,5\text{-DIPS})_4 (\text{H}_2\text{O})_2$ in 0.5ml of plasma fraction of blood was maintained at 50, 20 and $10\mu\text{mol}^{-1}$. Control of plasma fraction of blood was also carried out by adding 0.5ml of ethanol-water ($\text{C}_2\text{H}_5\text{OH}-\text{H}_2\text{O}$) mixture to 0.5ml of plasma. The concentration of GSH and/or plasma thiol in the plasma of blood was determined from the standard curve of GSH at intervals by Ellman's method (Sorenson, 1989).

Gold (I) Sodium thiomalate (Myocrisin) (50mg) was administered weekly by intra-muscular injection following an initial 10 mg test injection to 30 RA patients (median 58 years, range 74-40 years, median duration of disease 8 years, range 32-1 years). Treatment was stopped if a potentially serious adverse effects such as rash, mouth ulcers, leucopenia, proteinuria, thrombocytopenia, lack of effect etc. Table 1.

Samples of 5ml human venous blood of RA patients treated with heparin to prevent clotting was supplied by the center for Rheumatic Diseases, Baird Street, Glasgow, U. K. The blood was centrifuged on a Mintral

Table 1: Patients withdrawing from the trial due to lack of effect or deterioration (weeks 0-48)

Treatment with myocrisin (No. withdrawn)	No. of patients	Reasons for withdrawing
Myocrisin (18)	6	Rash
	3	Mouth Ulcer
	2	Thrombocytopenia
	1	Leucopenia
	1	Nitrid Reaction
	1	Proteinuria
	3	Lack of Effect
	1	Other

6L centrifuged at 4000 p.m for 10 minutes. The plasma was removed with a pasteur pipette for the determination of plasma thiol concentration by Ellman's method (Sorenson, 1989).

Results

Plasma thiol levels arise from the thiol group on albumin. Alterations in albumin thiol content contribute significantly to the lowered thiol level found in rheumatoid plasma (Thomas and Evans, 1975). Albumin decreases in the active stages of RA and increases during periods of disease remission (Ropes, *et al.*, 1954). Plasma thiol levels are reduced in patient with rheumatoid arthritis. Myocrisin produced improvement in this

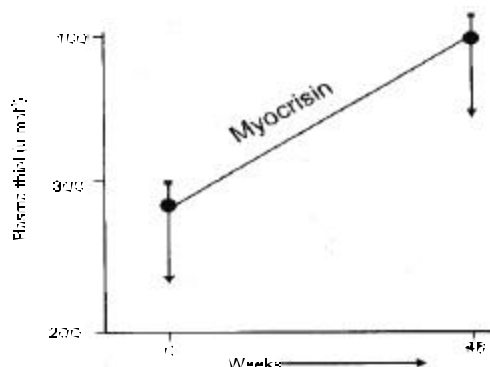


Fig. 3: Change in the median values at the inorganic biochemical parameters, after 48 weeks treatment.

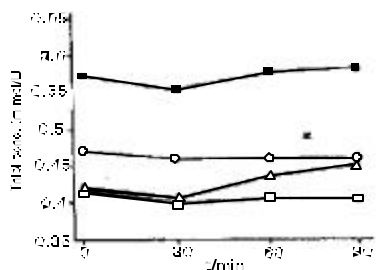


Fig. 4a: Time dependent effect of different concentrations of 3,5-DIPS on plasma thiol levels at 0, 30 and 60 minutes after the addition of plasma
 Plasma +
 ■ 500 uM (3,5-DIPS)
 ○ 250 uM (3,5-DIPS)
 △ 100 uM (3,5-DIPS)
 □ control (Plasma + 1 ml $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ molar: 13:97)

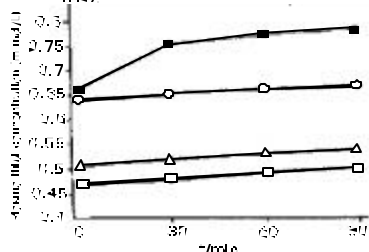


Fig. 4b: Time dependent effect of different concentrations of Cu(II) (3,5-DIPS) on plasma thiol levels at 0, 30 and 60 minutes after the addition of plasma
 Plasma +
 ■ 50 uM Cu(II) (3,5-DIPS) (H2O)
 ○ 25 uM Cu(II) (3,5-DIPS) (H2O)
 △ 10 uM Cu(II) (3,5-DIPS) (H2O)
 □ control (Plasma + 1 ml $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ molar: 13:97)

biochemical parameter and this was improved during therapy. The graphical representation for myocrisin, which showed statistical improvement in this biochemical parameter is given in Fig. 3. The major reason for patients drop-out during this period of trial was due to lack of effect or to deterioration (Table 1).

During the addition of different concentration of Cu(II) (3,5-DIPS) $_4$ (H_2O) $_2$, an increase in the plasma thiol levels was observed, which was time-dependant (Fig. 4). This may be due to slow dissolution of Cu(II) (3,5-DIPS) $_4$ (H_2O) $_2$ in a serum fraction of blood, where human serum albumin (HSA) is abundant. Blood plasma or serum thiol containing protein is known to enter into bonding interactions with drugs and metalloelements following absorption and to assist in transporting them to all tissues, resulting in the leakage of thiols from the albumin.

Discussion

Gold therapy has played an important role in the treatment of rheumatoid arthritis for many years. The most effective and least toxic dosage schedule for administration of gold has not yet been established. Drugs such as myocrisin and D-penicillamine produce improvement in RA in man during six weeks to three months therapy. Most of gold (Au) from gold complex (Myocrisin) is tightly bound to the plasma or serum proteins. Gold has also been found to accumulate more in inflamed than in normal joints and in synovial tissue (Japan Pharmaceutical Reference, 1993). Attempt have been made on the mechanism of action of myocrisin but little chemical progress has been made. Free thiol concentrations in serum or plasma are significantly important because thiol concentrations are reduced or lowered in RA.

An increase in thiol level during gold therapy was observed. This could involve formation of a gold thiol complex and then the release of thiol by exchange ligand interaction present in a large number of biological systems. The other possible mode of action of gold-drug is the binding with thiol containing protein albumin and it is known that gold binds to albumin. Gold may react in such a way as to cause the leakage of thiol, hidden in the loops and crevices of albumin, thus resulting to an increase in the plasma thiol concentration. Another possibility of an increase its concentration may relate to the remission of the disease and release of thiomalate, resulting in an increase in plasma thiol concentration.

It is also suggested that gold (Au) compounds might have been bound/complexed with thiol groups of the thiol-containing proteins and thus established thiol equilibrium *in vivo*.

Thiol-containing drugs D-penicillamine (dimethylcystein) and Myocrisin produce several similar effects thus suggests the evidence on the involvement of gold with thiol (Jellum *et al.*, 1979). It seems to appear that most of the *in vivo* chemistry is concerned with the reaction of gold species with thiols. The mammalian systems is such a complex one as that may produce Au (0), Au (I) and Au (III) during the metabolic process and that both monomeric and polymeric gold species are to be expected. Blood is rich in thiol containing low molecular weight and high molecular weight, biologically active molecules. Gold is circulated in blood mainly by plasma or serum proteins (Kamal *et al.*, 1977). Gold is divided among these serum proteins, with the largest amount being carried on albumin (Kamal *et al.*, 1977) which is the largest thiol containing protein. Gold is deposited in many tissues and Au (0), Au (I) Au (III) are the likely storage forms and are perhaps the active moieties. Although at present gold treatment is a rather toxic form of therapy and it would be clearly helpful if the toxicity is reduced for a better understanding of the likely reaction of gold complexes in mammalian systems and this area of research will be of considerable importance.

Copper(II) of 3,5-di-isopropyl Salicylic acid, $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ has attracted the attention of biomedical scientists because of its varied pharmacological properties (Sorenson, 1989). Human serum or plasma albumin, HSA or PSA, is an abundant blood serum of plasma protein. This protein is known to enter into the bonding interactions with drugs and metallo elements following absorption and tissue distribution. Serum or plasma albumin contains large number of functional groups, which offer many potential bonding sites for copper, 3,5-DIPS and copper-3,5-DIPS compounds. $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ is extremely lipophilic, water-insoluble complex. The forms of this complex undergoing tissue distribution in biological systems remain unknown and the relevant questions of stability and fate of the administered $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ *in vivo* remain unanswered. In our previous findings Khan *et al.* (1997, 1997a), suggested that a ternary complex of $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ and HSA may account for distribution and enables $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ to be distributed to all tissues via blood (Shuff *et al.*, 1992). This seemed reasonable to suggest that various forms of Cu-DIPS complex such as Cu (3,5-DIPS), $\text{Cu(II)} (3,5\text{-DIPS})_2$, and/or $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ are transported as albumin-bonded Cu-DIPS-HSA or HPA ternary complexes. A small fraction of this ternary complex may ultimately reach the site of pharmacological action thus accounts for its effects.

During the addition of $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ to plasma fraction of blood raised the concentration or level of thiols confirms the suggestion that $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ is absorbed or taken up by albumin resulting in decomposition or dissolution and both Cu(II) and 3,5-DIPS appear to be bonded at different coordinating sites, possibly at aromatic amino acids including histidine. In addition to these functional groups, HSA contains 1 thiol, and 17 disulfides (cystinyl). These functional groups are good potential co-ordinating and bonding sites for copper, 3,5-DIPS and $\text{Cu(II)}_2 (3,5\text{-DIPS})_4$ compounds (Khan *et al.*, 1997).

The suggestion that the primary copper bonding sites on HSA or HPA involves ligand atoms from HSA or HPA, far from thiol groups may cause the loops or cervices widen up, resulting in exposure of thiol groups, readily available for Ellman's reagent, obtained higher concentration or level of thiol in the serum or plasma. The observation that most of the drugs bond to HSA and the pharmacological efficacy is dependent on drug-albumin bonding interactions, enables it to be distributed to all tissues (Rowland and Tozer, 1992). It is also known that absorbed copper or copper complexes are initially bonded to albumin through interaction with histidine and other potential bonding sites on albumin. It is generally suggested that albumin is the main transport protein for copper from intestine to the liver, which is a key player in the maintenance of copper homeostasis (Farid *et al.*, 1998).

The up-take of copper or copper complexes from the blood by the liver via a plasma-membrane-recaptor-mediated process that recognizes albumin-bonded copper or copper complexes offers a protective mechanism against copper depletion in extra hepatic and/or extracellular tissue (Farid *et al.*, 1998). This type of mobilization of copper or copper complexes in biological systems is a physiological approach and may account for its pharmacological effects.

Both Au and Cu belong to group 1 B of the periodic table of elements. They are also called the "coinage metals" as they have been used for ornamental and coinage purposes. Their salts and/or complexes have been used for the treatment of human diseases. Both are absorbed and carried out in large amounts by serum or plasma proteins, especially albumin and

further distribution and utilization by tissues. Both have raised the concentration of thiols in two different models of investigation.

There is need for further study and further understanding of the role of copper and gold complexes in biological systems in the belief that detailed knowledge will help biomedical scientists the best use for the prevention and the treatment of human diseases.

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