Effect of Tetraakis-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

Muhammad Farid Khan, Muhammad Fayyaz Khan and Gul Majid Khan

The effects of tetraakis-μ-3,5-di-isopropyl Salicylatodiaquodicopper (II) (3,5-DIPS)$_4$ (H$_2$O)$_2$ and sodium gold (I) thiomalate (Myocrisin) Au(I) (S-CHCH$_2$(CO$_2$)$_2$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 Cu (II) (3,5-DIPS)$_2$ (H$_2$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

Faculty of Pharmacy, Gomal University, Dera Ismail Khan, NWFP, Pakistan
Khan et al.: Effect of 3, 5 DIPS of the metabolism of thiols

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, antioxidant and participate 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, tissue protection against oxidative damage, recovery and repair against oxidizing environment (oxygen, ozone etc) (Chassaud, 1973). Alteration and/or deficiency of thiols leads to life threatening acidosis, defective function of vital organs, herniation 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 leucopenia, cardiomegaly due to radiotherapy, acute and chronic encephalopathy, 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 (Elchol, 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 tetraakis-3, 5-di-isopropyl salicylato diaquocopper (II) Cu(II), (3,5-DIPS), (H2O)2 (Fig. 1) have varied pharmacological activities including: antitumor, antitumorogenic, antimitogen, anticonvulsant, anti-inflammatory, antidiabetic, radiation protection and recovery, and recovery of the CNS syndrome (Sorenson, 1989; Sorenson et al., 1993; Baquial and Sorenson, 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 rheumatic arthritis (Sigler et al., 1972).

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

Fig. 2: Structure of di-sodium gold (I) thiosalate 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 Cu(II), (3,5-DIPS), (H2O)2 and sodium gold (I) thiosalate 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 Cu(II), (3,5-DIPS), (H2O)2 have been described by Khan et al. (1997, 1997 a).

Different concentrations of Cu(II), (3,5-DIPS), (H2O)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 Cu(II), (3,5-DIPS), (H2O)2, in 0.5ml of plasma fraction of blood was maintained at 5.0, 20 and 10 μmol⁻¹. Control of plasma fraction of blood was also carried out by adding 0.5ml of ethanol-water(C6H12OH-H2O) mixtures 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 thiosalate (Myocorin) (50mg) was administered weekly by intra-muscular injection following an initial 10 mg test injection to 30 RA patients (median 18 years, range 74-40 years, median duration of disease 4 years, range 32-1 years). Treatment was stopped if a potentially serious adverse effects such as rash, mouth ulcers, leukopenia, proteinuria, thrombocytopenia, lack of effect etc.

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

<table>
<thead>
<tr>
<th>Treatment with myocorin (No. withdrawn)</th>
<th>No. of patients</th>
<th>Reasons for withdrawing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocorin (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Rash</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mouth Ulcer</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Leukopenia</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Nitric Reaction</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Proteinuria</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Lack of Effect</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Other</td>
<td></td>
</tr>
</tbody>
</table>

Khan et al.: Effect of 3, 5 DIPS of the metabolism of thiol

GL 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, 1959).

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, 1976). Albumin decreases in the acute stages of RA and increases during periods of disease remission (Hopes et al., 1954). Plasma thiol levels are reduced in patients with rheumatoid arthritis. Mycorisin produced improvement in this biochemical parameter and this was improved during therapy. The graphical representation for mycorisin, which showed statistical improvement in this biochemical parameter is given in Fig. 3. The major reason for patient's 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(I)Cl, (3,5-DIPS) (H₂O), an increase in the plasma thiol levels was observed, which was time-dependent (Fig. 4). This may be due to slow dissolution of Cu(I)Cl, (3,5-DIPS) (H₂O), 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 thione 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 mycorisin and D-penicillamine produce improvement in RA in man during six weeks to three months therapy. Most of gold (Au) from gold complex (Mycorisin) is tightly bound to the plasma or serum proteins. Gold also has 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 mycorisin 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 in complex with thiol groups of the thiol-containing proteins and thus established thiol equilibrium in vivo.

Thiol-containing drugs D-penicillamine (dithiothreitol) and Mycorisin produce several similar effects thus suggests the evidence on the involvement of gold with thiol (Jaillium et al., 1979). It seems to appear that most of the in vivo chemistry is concerned with the reaction of gold species with thiol. 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 molecule. 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 the area of research will be of considerable importance.
Khan et al.: Effect of 3, 5 DIPS of the metabolism of thiols

Copper(II) of 3,5-diisopropyl Salicylic acid, Ca(II)(3,5-DIPS), has attracted the attention of biomedical scientists because of its varied pharmacological properties (Sorensen, 1999). Human serum or plasma albumin, HSA or PSA, is an abundant blood serum of protein plasma. This protein is known to enter into the bonding interactions with drugs and metal ions 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. Cu(II)(3,5-DIPS), is extremely lipophilic, water-insoluble complex. The forms of this complex undergoes tissue distribution in biological systems remain unknown and the relevant questions of stability and fate of the administered Cu(II)(3,5-DIPS) in vivo remain unanswered. In our previous findings Khan et al. (1997, 1997a), suggested that a ternary complex of Cu(II)(3,5-DIPS), and HSA may account for distribution and enables Cu(II)(3,5-DIPS), to be distributed to all tissues via blood (Bustard and Sorensen, 1997). This appears to suggest that various forms of Cu-DIPS complex such as Cu(3,5-DIPS), Cu(II)(3,5-DIPS), and/or Cu(II)(3,5-DIPS), 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 Cu(II)(3,5-DIPS), to plasma fraction of blood raised the concentration or level of thiol confirms the suggestion that Cu(II)(3,5-DIPS), 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 (cysteiny). These functional groups are good potential co-ordinating and bonding sites for copper, 3,5-DIPS and Cu(II)(3,5-DIPS), 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 albumins through interaction with histidines 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., 1999).

The uptake of copper or copper complexes from the blood by the liver via a plasma-membrane-receptor-mediated process that recognizes albumin-bonded copper or copper complexes offers a protective mechanism against copper depletion in extra hepatic and/or extra cellular tissue (Farid et al., 1999). 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 1B of the periodic table of elements. They are also called the *coinsage metals* as they have been used for ornamental and coinage purposes. Their salts and 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 to use the proper prevention and the treatment of human diseases.

Acknowledgments

The work was partially supported by the University Grants Commission through the Gomal University, Dera Ismail Khan, Pakistan.

References


MS received 17th July, 2001; Accepted 7th August, 2001.