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铜及其配合物在生物体系中的作用

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自人类有历史记载以来,铜及其配合物即用作药物。铜是人体必需的金属元素。人体的正常代谢过程需要它们,但无法体内合成,因此,需要每天从饮食摄取和吸收。生物药学家和研究者以极大的努力彻底地了解铜及其配合物在生物体系中的作用,希望获得更多地信息以利于人类疾病的预防和治疗。本综述评述了铜在组织中的分配和代谢、铜依赖酶、在病态时铜的非正常代谢、铜配合物的药疗活性和抑制氧自由基的产生。

关键词: 铜 配合物 生物体系 药疗活性 人类疾病的预防和治疗

ROLE OF COPPER AND ITS COMPLEXES IN BIOLOGICAL SYSTEMS

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Copper and its complexes have been used for their medical effect since the beginning of the recorded history. It has been recognized as an essential metalloelement. Human body requires it for normal metabolic processes but cannot be synthesized de novo, therefore daily dietary intake and absorption are required. Biomedical scientists and researchers have made tremendous efforts to completely understand the role of copper and its complexes in biological systems, which give much information relevant to the prevention and/or treatment of human diseases. This review examines and critically evaluates these research investigations with particular reference to the tissue distribution and metabolism of copper, copper dependent enzymes, altered metabolism of copper in disease states, pharmacological activities and the role of copper complexes to prevent the production and/or accumulation of oxygen derived free radicals.

Keywords: copper complex biological system pharmacological activity prevention and/or treatment of human disease

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0 Introduction

Great interest in Essential Metalloelements such as copper among academics and biomedical scientists is based in part on the conclusion that copper is required by all living systems for normal metabolic processes. It is difficult to open chemical, pharmacological, toxicological, and biological journals without seeing papers on the role of: "Copper and its Complexes in Biological Systems". The role of copper is further emphasized as functioning in many biological systems as a co-factor for some thirty enzymes involved in a wide range of metabolic functions of all cells.

The chemical nature of copper in biological systems in which it exerts its biological activity and pharmacological effects is quite variable. Copper is generally present in body tissues in two oxidation states (cupric: Cu(Ⅱ), cuprous: (Cu(Ⅰ)). Its distribution is dependent on organs region, subcellular organal and varies according to age, species, environmental, and genetic factors.

Concentrations of ionic forms of copper in tissues and in plasma are very small (10^{-18}mol · L^{-1}) since copper has a high affinity for organic ligands found in biological systems and rapidly undergoes bonding inter actions to form complexes or chelates. These complexes are primarily copperdependent enzymes, proteins such as metallothioneine, small molecular mass amino acid, carboxylic acid, phosphates, amine, diamine, thiols and small molecular mass peptides[1].

Although copper is required by all cells in all tissues, it can not be synthesized in vivo. The average adult body has 80 to 100 mg of copper[2]. The estimated safe and adequate daily intake of copper is 2 to 3 mg per day which is required to sustain a whole body requirement of 80 to 100 mg[2], which should be obtained from various dietary food stuffs (Table 1).

Table 1 Estimated Copper Concentration of Various Food Stuffs in μg per 100 grams of the Edible Portion or as Indicated[6]

Wheat		Heart	420
Hard flour first grade	150	Liver	5,300
Soft flour first grade	85	Kidney	280
Rice (brown)	250	Stomach and intestine	85
Cornflakes	65	Dairy Cow (meat)	75
Sweet Potatoes	130	Turnip	
Honey	40	Leaves raw	90
Milk Chocolate	450	Boiled	65
Peanuts (roasted)	690	Root raw	35
Kidney beans		Boiled	44
(Whole dry)	750	Pumpkin	55—100
(Boiled)	320	Cucumber	
Peas (whole dry)	490	Fruit raw	55
(Boiled)	210	Sweet corn (immature)	130
Soybeans Sprouts (Raw)	150	Tomatoes (fruit)	47
Fish (varies among various kinds)	30 to 1000	Carrot	
Calf		Root raw	55
Tongue	100	Boiled	60

Garilic (bulb)	110	Spices	
Spinach		Garlic powder	550
Leaves		Pepper Black	1,200
Raw	180	Ginger	570
Boiled	100	Grapes	
Apricots		Raw fruit	60
Raw fruit	60	Watermelon	
Strawberries		Raw fruit	38
Raw fruit	35	Melons	
Duck (meat)	400	Raw fruit	40
Chicken		Peaches	
Broiler (Thigh)	60	Raw fruit	43
Gizzard		Apple	
Thigh	40	Raw fruit	50
Intestines	65	Bananas	
Breast	34	Raw fruit	120
Chicken egg		Pineapple	
Egg Yolk fresh	80	Raw fruit	70
Egg white fresh	29	Coffee	
Milk	7—19	Instant	38
Skim milk powder domestic	95	Kitchen Salt	6
Human milk	30	Retined Salt	3
Tea (Green tea)		Tomato sauce	160
Maccha		Curry powder	800
Finely ground	640	White pepper	1000
Common Salt	18	Red-pepper powder	1,200
Tomato Ketchup	200		

@Standard tables of food composition in Japan, Minerals (magnesium, zinc and copper), Resources Councils, Science and Technology Agency, Japan, 1991

1 Tissues Distribution of Copper

Amounts of copper found in body tissues and fluids required for normal metabolic function and metabolism correlate with the metabolic rate of these tissues. Liver contains the highest copper content 680 μg per gram of tissue ash[3] since its serves as a major copper storage organ. Brain is the next richest in copper content[4] and contains about 370 μg per gram of tissue ash. The importance of copper is further implicated by the observation that the heart is also rich in copper 350 μg per gram of ash[3]. Gall bladder and bile also contain a large amount of copper, 750 μg and 547 μg per gram of tissue ash respectively, which may be due to their role in excretion and mobile copper storage[3] and the provision of antioxidant activity following secretion into the intestinal chyme. Kidney have the next highest copper content, 270 μg/g of tissue ash, which may be due to its copper conservatory role[3].

Blood is the unique source of copper that copper concentration in blood (total) is about 1. 01 μg/ml. Erythrocytes copper content is about 0. 98 μg/ml, plasma 1. 12 μg/ml and serum 1. 19

μg/ml[3].

Remaining tissues have lesser and variable amounts of copper[3], depending on the need and relatively lower metabolic activity but it is as important for normal metabolism in these tissues as it is in all other tissues. All of the copper in these tissues and fluids is complexed with large molecular weight proteins, peptides, and amino acids, which account for absorption, tissues distribution, tissue utilization, and biologically active forms of copper in vivo. These complexes of copper in tissues and in biological fluids are much more stable forms of copper than ionically bonded forms. It is important to recognize that the use of copper complex or chelate in biological systems is more relevant (appropriate) than the use of an ionic form of this essential metalloelement.

2 Copper-Dependent Enzymes

The role and essentiality of copper in biological tissues and fluids can be judged from the fact that there are about thirty enzymes[2], which are copper-dependent, performing multifarious functions in mammalian tissues and fluids. Some of copper-dependent enzymes are[3], cytochrome-c oxidase required by all cells to produce energy, extracellular and cytosolic copper-dependent and zinc modulated superoxide dismutases (Cu_2Zn_2SOD) required to prevent cellular destruction associated with the accumulation of superoxide (O_2^-) and other oxyradicals (HOO, HO, $^1O_2$) derived from it; dopamine-β-monooxygenase responsible for the synthesis of neurotransmitters norepinephrine and epinephrine from dopamine; neurocuprein also needed for the synthesis of neurotransmitters; lysyloxidase, required for cross-linking of collagen and elastin in maintenance and repair of all connective tissues; ceruloplasmin, suggested to be multifunctional having a copper transport function, serum anti-oxidant activity, mobilization of stored iron [Fe(Ⅱ)] for hemoglobin synthesis; and blood clotting factors and Ⅷ, amine oxidases for metabolism of primary amine; α-amidating mono-oxygenases responsible for the synthesis of large group of neuroendocrine hormones including gastrin, choleocystokinin, α-melanocyte, stimulating hormone, calcitonin, vassopressin, secretin and some enkephalins[5-6].

During the intervening years, interest in isolation and characterization of Cu-dependent enzymes has grown markedly and furthered research to unlock doors to a complete understanding of the metabolism of copper and the role the Cu-dependent enzymes play in tissues maintenance and function. Other possible Cu-dependent enzymes include, guanylate cyclase required for the synthesis of c-GMP, adenylate cyclase for the synthesis of c-AMP, lipolytic protein responsible for lipolysis, ACE1 and CuP_2 responsible for metallothioneine gene transcription regulatory protein[7-8].

3 Metabolism of Copper: Ingestion, absorption, utilization and excretion.

Copper is widely distributed in food stuffs (Table 1). The estimated safe and adequate daily intake of copper in complexed forms is 2 to 3 mg per day. Complexes of Cu found in various food stuffs

and beverages may give rise to other complexes through ligand exchange in the digest yielding binary or ternary complexes[6] formed with amino acids, fatty acids, amines, diamines, and albumin following absorption at pH values found in the stomach[9].

After absorption, these copper complexes undergo systemic circulation to all tissues for further utilization by cells. It is known that a portion of absorbed copper or copper complexes from the stomach or upper small intestine are initially bonded to serum albumin, possibly through an interaction with histidine and other bonding sites on albumin. It is generally assumed that albumin is the principal portal transport protein for copper from intestine to the liver. The liver plays a key role in the normal maintenance of copper homeostasis. It is known that the liver takes up copper rapidly and actively following administration via both intravenous and oral routes[10]. Serum albumin bonded copper or complexes are promptly removed from the blood by the liver via a plasma-membrane-receptor-mediated process that recognized albumin-bound copper or copper complexes[11]. Albumin and amino acid bonded forms of copper are transport forms of copper in the portal circulation taken up by hepatocytes. The active up-take of copper or copper complexes by the liver provides a potential protective mechanism against copper depletion in extrahepatic tissues.

Hepatic copper is then either secreted into the bile as small atomic mass complexes or incorporated into ceruloplasmin and returned to the circulation for the synthesis of copper-dependent enzymes and copper-dependent proteins or stored in the liver as copper-thioneine. This low-molecular-mass bonding protein has been suggested to play a role in the temporary storage of copper. To meet the normal metabolic need of tissues, copper-thioneine stored copper is released from the liver as complexed forms, ceruloplasmin, copper-amino acid complexes, and also albumin-copper complex.

This homeostatic release of copper or copper complexes from the liver meets normal copper-dependent physiological requirements of all tissues which also include de novo synthesis of other copper-dependent enzymes and proteins in all extrahepatic tissues. It is now clear and understood that the recommended daily allowance for copper 2 to 3 mg, is essential based upon its recognized need for de novo syntheses in activating of copper-dependent enzymes and proteins.

4 Altered Metabolism of Copper in Disease States

A variety of human pathologic disorders and/or various disease states are associated with larger than normal quantities of plasma copper complexes and higher than normal content in the disease affected tissues as a result of higher than normal demand for copper to support Cu-dependent metabolism required to overcome these disease states. These include; inflammation, ulcer, diabetes, ischemia, reperfusion, injury, radiation protection and recovery, infection, fever, epilepsy, cancer, carcinogenesis and pain[3]. In Wilson's disease[12], copper demand is increased in affected tissues including liver, brain, and other tissues as a result of imparied utilization which persists, and leads to tissue as a result of imparied utilization which persists, and leads to tissue damage. In Menke's disease[12], copper concentrations are low in all tissues. Low brain copper concentration is associated with enhanced lipid peroxidation accompanied by low activity of cytochrome *c* oxidase, dopamine β-monooxygenase, and su-

peroxide dismutase activities. Low activity of superoxide dismutase is accompanied by the accumulation of superoxide and finally to toxic hydroxyl and hydroperoxyl radicals as well as H_2O_2 which leads to severe damage of brain tissues. These deficits clearly demonstrate the importance of copper for human brain development, normal metabolism and protection against toxic reactive oxygen species. In rheumatoid arthritis, plasma copper level is enhanced and red cell copper level is low with increase disease severity[13]. Low red cell copper level is associated with low activity of super oxide dismutase (Cu_2Zn_2SOD), while high level of plasma copper is accompanied by increased ceruloplasmin concentration.

5 Pharmacological Activities of Copper Complexes

Copper complexes represent a group of compounds which have been used since the beginning of recorded history. There is a rich history of the use of copper complexes in the treatment of variety of diseases including tuberculosis[14], rheumatoid and degenerative diseases[15]. The therapeutic potential of copper for the treatment of rheumatoid diseases was first recognized by a German Physcian, Werner Hangarter in 1939 when he learned that Finnish copper miners were unaffected by rheumatism as long as they worked in the copper mining industry[16]. Copper complexes and/or salts were used long before 1939 for the treatment of wound healing and eye infection. The therapeutic effect of copper in the more recent treatment of rheumatic diseases was striking since rheumatism was a wide spread disease in Finland and workers in other towns and industries had more rheumatoid disease than copper miners. Werner Hangarter attributed his observations to the therapeutic effect of copper. Prior to his observations copper complexes had been used for the successful treatment of tuberculosis[16]. One of these copper complexes was Cupralene (sodium 3-(allylcuprothkouredo) 1-benzoate), used to treat both tuberculosis[17] and rheumatoid arthritis[18]. It was also reported as interesting that girls who suffered from anemia and started to work in a copper mine were soon relieved from their anemia[15]. This is consistant with the more recent observation that there is a copper-dependent iron-mobilization process required for hemoglobin synthesis[19]. With the advancement of therapy through research, another copper complex, Dicuprene (cupric bis [8-hydroxyquinoline di(diethylammonium sulfonate)], was also found to be effective in the treatment of rheumatoid arthritis and other degenerative diseases[20].

Both of these copper complexes (Cupralene and Dicuprene) were found effective and even in some instances superior to gold therapy of rheumatoid and other degenerative diseases. However nothing more was seen in scientific journals about these and other copper complex drugs after 1955. This may be due to little or no recognized requirement for copper in any biological system and the lack of multiple sources of such information as there is today[6]. Furthermore, the discovery of hydrocortisone, which was thought to be a "cure" for all rheumatic diseases, diverted the attention of rheumatologists, researchers, and physicians interested in the treatment of rheumatic diseases.

The German physcian Werner Hangarter had been using intravenous salicylic acid in his clinical department for the treatment of rheumatic fever, rheumatoid arthritis, and degenerative diseases. Based on reports[18,21] and his own experience with copper as well as intravenous salycylic acid,

Hangarter in 1950 started his research with a new copper complex under the trade name Permalon, a copper chloride and sodium salycylate mixture manufactured by the Albert Chemical Company.

Permalon, administered by intravenous injection, produced remission of fever, alleviation of pain, increased mobility, inhibition of exudation of joint effussions, decreased swelling, reddening, and erythrocytesedimentation rate.

It is interesting to point out that Hangarter had recognized the importance of copper in Permalon and concluded that Permalon's effect was not due to salicylic acid alone but due to the presence of a copper-salicylate complex or complexes.

Hangarter used Permalon by intravenous infusion at much higher doses from 1954 to 1971 and achieved equally good results. However, the use of Permalon was discontinued after 1971 due to the retirement of Hangarter and the Albert Chemical Company discontinued its manufacture.

Todate more than one hundred copper complexes of antiinflammatory drugs have been tested in various animal models of inflammation[6] and all were found to be more active as antiinflammatory agents than their parent compounds.

There are reports that reactive oxygen species (ROS) are involved[22] in at least 50 diseases including rheumatoid arthritis, cancer, atherosclerosis, myocardial infarction, and inflammatory bowl disease.

In living organisms superoxide (O_2^-) ,hydroxyl ($\cdot OH$) and peroxyl (H_2O_2) are important oxygen derived free radicals. The production of O_2^- and H_2O_2 are increased in mammalian cells by the activation of white blood cells and cause injury to body tissues. Sorenson[6] and others have suggested that O_2^- disproportionation account for the antiinflammatory activity of copper complexes. There are reports that many copper complexes including Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ are more effective than Bovine RBC Cu_2Zn_2 SOD in Fetal Calf Serum[24] and ternary Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ albumin complexes[25] do disproportionate O_2^-. That is why in rheumatoid arthritis changes in the immune system cause an influx of white blood cells into the joints, leading to an aggravation of tissue damage.

All cells of the human body have an efficient defence systems including superoxide dismutase (Cu_2Zn_2 SOD) which catalyses the dismutation[25] of O_2^- to yield H_2O_2 and O_2. H_2O_2 is removed by enzymes catalase and glutathione peroxidase.

It is interesting to point out that polymorphonuclear leukocytes (PMNLS) have reduced O_2 uptake when exposed to Cu(Ⅱ) (salicylate)$_2$, Cu(Ⅱ)$_2$(3,5-DIP)$_4$[26], or Cu(Ⅱ)$_2$(indomethacinate)$_4$[27]. This clearly demonstrates that copper complexes may become useful in the treatment of rheumatic and other degenerative diseases.

A popular theory is that the generation of O_2^- in bursts by PMNs and the lack of normal concentration of Cu-dependent SODs leads to the formation of much more reactive oxygen-derived species such as singlet oxygen ($^1O_2$), hydroxyl radical ($\cdot OH$) and hydroperoxyl radical ($\cdot OOH$) as well as hydrogen peroxide (H_2O_2)[28]. Copper complexes with SOD-mimetic activity also facilitate the de novo synthesis of copper-dependent enzymes required for normal oxygen utilization and tissues repair processes[6].

Copper complexes pose an important function in preventing radical mediated chain reaction in act-

ing physiologically as free radical scavengers and repair of damaged tissues.

6 Oxygen Derived Free Redicals Production and Copper Complexes

The adult body is composed of 70% of water while the infant body is composed of 90% of water[29]. Upon exposure to ionizing radiation including X-rays, Gamma-rays, ionizing radiolysis of water[29] takes place with the production of reactive species; such as aquated electrons, hydrogen atoms, hydroxyl radicals and protonated water;

$$H\text{-}O\text{-}H \xrightarrow{h\nu} e^{\cdot}_{(aq)} + {\cdot}H + {\cdot}OH + H_3O^+$$

Aquated electrons and hydrogen atoms are very reactive and involved in the univalent reduction of dioxygen to produce O_2^- and with further protonation yields hydroperoxyl radical.

$$O\text{-}O + 1e^{\cdot}_{(aq)} \longrightarrow O_2^-$$

$$O\text{-}O + H^{\cdot} \longrightarrow H\text{-}O\text{-}O^{\cdot}$$

H_2O_2 and OH are produced by further reduction of O_2^-,

$$O_2^- \xrightarrow{1e^{\cdot}_{(aq)}+2H^+} H_2O_2 \xrightarrow{1e^{\cdot}_{(aq)}} {\cdot}OH + HO^-$$

$$\downarrow 1e^{\cdot}_{(aq)}+H^+$$

$$H_2O$$

The half lives of HO, OOH, H and aquated electrons are very short ($\sim 10^{-3}$ s) and consequently react very fast with cell components including proteins, DNA, enzymes, and low molecular mass biomolecules. These reactions cause loss of functions, integrity, structural changes, oxidative degradation, lipid peroxidation of polyunsaturated falty acids in biological membranes and oxidation of DNA which may have more severe biological consequences. DNA damage may occur by several mechanisms, one of which is porbably attack by · OH radical, produces extensive modification of all four DNA bases, guanine, cytosine, adenine, and thymine[22]. Sorenson[30] has suggested that copper complexes may become useful in the treatment of radiation sickness and facilitate radiation recovery. Loss or low activity of copper-dependent enzymes such as SODs may account for lethality of ionizing radiation, as SODs play a vital role by catalysing the removal of O_2^- and restoration of SOD activity prevents toxico pathological consequences of O_2^- accumulation and facilitates tissue repair[31]. Copper complexes including Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ are oxygen-derived free radical scavengers[29]. The Cu_2Zn_2-SOD-mimetic activity of Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ and the prevention of O_2^- accumulation would further prevent the formation of $^1O_2$, HOO and OH. The prevention of the accumulation and formation of the above radicals and consequently the protection and repair of tissues by copper complexes may explain radiation protection and radiation recovery activities of copper complexes[29].

Copper complexes including Cu(Ⅱ)$_2$(salicylate)$_4$ and Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ having SOD-mimetic activity lead to conclusions that copper complexes might have anti-cancer activity[6]. These complexes decreased tumor growth, metastasis and increased survival of tumor-bearing mice. A mechanism of action of copper complexes as anticancer has been suggested[32] to involve glutathione oxidation and accumulation of H_2O_2. Low levels of glutathione peroxidase (GSH-PX), depleted GSH, and increased

production of H_2O_2 in neoplactic cells as compared to normal human lymphocytes, may be involved in the mechanism of action of copper complexes in eliminating neoplastic cells and not normal human lymphocytes. However the re-activation of Cu-dependent enzymes and proteins is very likely the more relevent mechanism of action accounting for anticancer activities of copper complexes.

Copper complexes including Cu(Ⅱ)$_2$(salicylate)$_4$ and Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ have also been found to have anti-diabetic activity in the streptozotocin-induced diabetic rat. These copper complexes in this model of diabetes improved glucose utilization and decreased urinary glucose excretion[6,33].

Copper complexes such as Cu(Ⅱ)$_2$(salicylate)$_4$ and Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ have anti-carcinogenic activity[34] which has been attributed to their SOD-mimetic activity. Increased production of O_2^- by stimulated PMNS and inhibition of P- 450 may account for their anticarcinogenicity since Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ and Cu(Ⅱ)$_2$(salicylate)$_4$ can undergo treanslation across cell membranes due to their lipophilic character while Cu_2Zn_2SOD cannot.

Seizures in animals and humans may be due to copper deficient diets and/or altered copper metabolism leading to inadequate brain copper content[6]. The accumulation of O_2^- and a low level of Cu_2Zn_2SOD is suggested to account for seizures due to brain tumor, trauma, and infection associated with inflammation[6]. The SOD-mimetic activity of copper complexes may account for their anti-convulsant activities due to disproportionation of O_2^- and facilitation of de novo synthesis of Cu_2Zn_2SOD and other copper-dependent enzymes.

The administration of copper complexes by various routes to biological systems is an important area of research for further understanding of the role of copper and copper complexes in biological systems.

The addition of copper to hepatoma cell cultures was shown to be chelated quickly with cellular GSH, forming a Cu-SG complex[35].

Recently Khan et al, reported that Cu(Ⅱ)$_2$(3,5-DIPS)$_4$(Fig. 1) was taken up by hepatocytes and chelated by cellular GSH, forming a Cu-SG complex[36]. It was further proposed that copper from the Cu-SG complex was transferred to copper proteins/enzymes including SODs, ceruloplasmin (CP), lysloxidase (LO) and also metalothioneine (MT) (Fig. 2). Khan et al have also suggested and

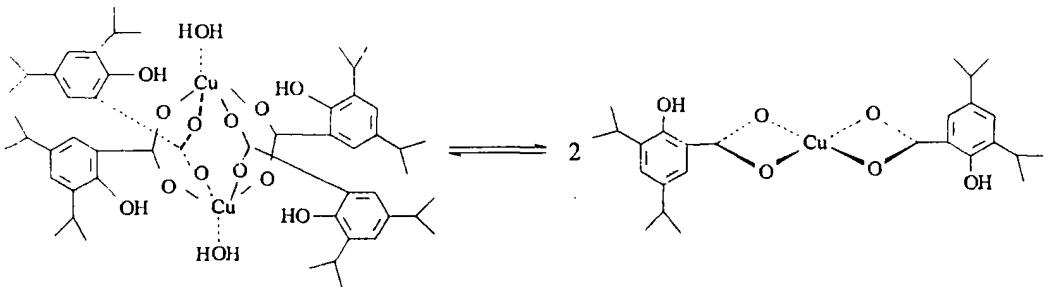

Fig. 1 Structure of tetrakis-μ-3,5-diisopropylsalicylatediaquodicopper (Ⅱ) (Cu(Ⅱ)$_2$(3,5-DIPS)$_4$(H_2O)$_2$, left) and its conversion to mononuclear complex (Cu(Ⅱ)(3,5-DIPS)$_2$(H_2O)$_2$, right)in aqueous solution

proposed that Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ is adsorbed on to the surface of cells of leuckocytes and penetrates slowly through cell membrane and bonds to GSH to yield Cu-SG complex. Copper from the Cu-GS complex is finally transferred to copper dependent enzymes (unpublished data).

Copper complexes, ligand exchange, formation of copper-ligand complexes, absorption, utilization, distribution and de novo synthesis of copper dependent enzymes/proteins, mobilization of copper to various tissues, normal physiological requirement of tissues/fluids, and tissue repair processes further support the use of copper and copper complexes to prevent and treat human diseases.

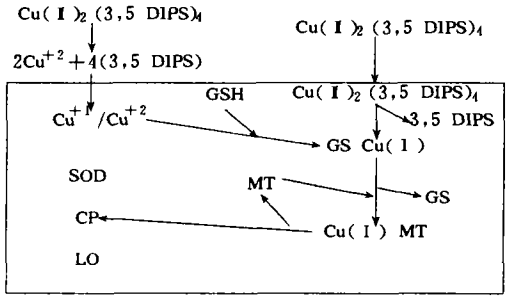

Fig. 2 This hypothetical figure underlines the physiological significance of GSH in the cell suspension in the metabolism and detoxification of Cu(Ⅱ)$_2$(3,5-DIPS)$_4$. The copper oxidation state in Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ transported into the cell is not known, therefore Cu(Ⅱ) and Cu(Ⅰ) are presented. This figure hypothesizes a mechanism for Cu(Ⅱ)$_2$(3,5-DIPS)$_4$ metabolism, copper chelation with GSH, transfer of copper to MT and copper-dependent enzymes such as SOD, CP, and LO.

It is apparent that there is need for much further research for complete understanding of the role of copper and its complexes in biological systems in the belief that further study will eventually bring detailed insight into reactions involving copper and copper complexes. This detailed knowledge will surely give us the best foundation for the prevention and possibly the treatment of human diseases. In the meantime, it is clear that tremendous efforts have gone into experiments design to obtained scientific data pertaining to the role of copper and copper complexes in biological systems. These data have given us much information relevant to the prevention and/or treatment of human diseases.

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