CV 247 is under investigation and its safety and efficacy have not yet been demonstrated

COPPER GLUCONATE

Introduction

Copper (Cu) like manganese and zinc is chemically classified as a transitional element in the periodic table. Following zinc and iron, Cu is the third most abundant trace element in the body, an adult man contains between 80 and 150mg Cu, widely distributed throughout the body (Mann, 2002). Tissue concentrations range from 1.5 to 2.5 microg/g with the exception of the liver with concentrations in the range 30 to 50 microg/g dry tissue. It's essential role in animals and man was first realised in 1926 after it was found to be required for haemaglobin synthesis in rats (Mann, 2000.

Cu can form complexes in which the metal serves as a central atom and as a result its function is closely associated with its binding to biological ligands, particularly in enzyme systems. Like manganese, Cu can adopt distinct redox states allowing the metal to play a pivotal role in cell physiology as a catalytic co-factor in the redox chemistry of enzymes, mitochondrial respiration, iron absorption, free radical scavenging and elastin cross-linking. Vertebrate enzymes containing or activated by Cu are:

Lysyl oxidase, which is used in collagen synthesis
Peptidylglycine α-amidating monooxygenase used in neuropeptide synthesis
Superoxide dismutase (specifically cytosolic Cu/Zn SOD) used to detoxify superoxide.
Ceruloplasmin, another anti-oxidant enzyme.
Ferroxidase/ceruloplasmin for release of stored iron.
Cytochrome c oxidase important in oxidative phosphorylation.
Dopamine β-hydroxylase used for the neurotransmitter synthesis.
Tyrosine oxidase used in melanin synthesis.

Other Cu proteins include, metallothionein, α-fetoglobulin, and transcuprein (Mann, 2002).

In vitro studies have demonstrated that extracellular Cu modulates the secretory function of peptidergic neurons, and is present in high concentrations in nerve terminals and secretory vesicles, suggesting that Cu is important in neurosecretion (Hartter, 1988).

Copper homeostasis

If present in excess, free Cu ions can cause damage to cellular components, largely due to its ability to take part in Fenton-like reactions in which the highly reactive hydroxyl radical is formed. As a consequence mammals have evolved means of minimising levels of free Cu ions and destructive Cu complexes which involves a delicate balance between uptake and efflux determines the amount of cellular Cu. The liver has a pivotal role in the homeostasis of several essential trace metals including Cu. The detoxification of exogenous metals is attributed to its ability to extract metals from plasma, metabolise, store and redistribute them in various forms either into the bile or back into the bloodstream. Cu homeostasis, which maintains a small but constant body pool, is co-ordinated by several proteins including glutathione, metallothionein, membrane bound Cu

transporting P-type ATPases, Menkes and Wilson proteins and by cytoplasmic transport proteins called Cu chaperones that ensure delivery to specific sub-cellular compartments. These homeostatic mechanisms which include mobilisation of secretory mechanisms and excretory pathways when dietary intake is high, prevent accumulation of Cu to toxic levels. Diseases, which are caused by dietary Cu overload primarily affect the liver, and are usually based on genetic pre-disposition (Schuemann, 2002). Conversely when Cu intake is low, Cu levels in organs such as the brain and heart are efficiently conserved, though conservation in the liver is induced only after considerable Cu loss (Levenson, 1998).

Metallothionein (MT) increases the resistance of cells to exposure to high Cu levels and thus is crucial for safe intracellular Cu storage and cell survival at normal and supra-physiological exposure levels. When Cu intake is high, metallothionein increases dramatically in the liver, kidney and intestine. Endogenous Cu excretion is a major point of regulation of the body's Cu stores

Characterisation of the MT-Cu complex suggests that MT has an important role in the cellular storage and delivery of Cu ions to cuproenzymes. At sub-physiological levels (0.4 microM), the Cu content was not found, in a rat fibroblast cell line model, to depend on MT. However when the extracellular levels were increased to physiological levels (10microM) MT was required for the cells ability to accumulate Cu. The sub-cellular localisation of the accumulated Cu in the cytoplasm was MT dependant. Following supra-physiological exposure to Cu (>50microM), MT cells had a decreased capacity for Cu storage and an elevated sensitivity to a minor increment in intracellular Cu levels, suggesting that Cu toxicity is not due to the metal content, but to the interaction of the metal with cellular components. Moreover, as exposure increases further, MT cells failed to show increased levels of mRNA encoding MT, SOD and the Cu chaperone for SOD. However it is evident that increased levels of MT, probably as a consequence of a specific cellular response to harmful levels of Cu, is essential for sequestering an intracellular excess of the metal in response to supra-physiological Cu exposure (Tapia, 2004).

As a consequence, except under certain genetic conditions, Cu is likely to be benign to most mammals and not responsible for genomic instability, including fragmentation of and/or alterations to DNA, induction of mutation, or apoptosis, or other toxic events (Linder, 2001).

The Cu content of a human body may decrease with age. About 10% of the Cu pool is in the blood. The plasma content is about 15microM/L, up to 90% of which is associated with caeruloplasmin. Caeruloplasmin is one of the acute phase proteins and via its ferroxidase activity, catalyses the oxidation of ferrous iron. This latter reaction is essential for the mobilisation of iron as a complex with transferrin, and maybe one of the mechanisms by which Cu is able to regulate iron homeostasis (Mann, 2002).

Copper deficiency

Cu deficiency results in abnormal bone metabolism and skeletal abnormalities in most species. This is as a consequence of collagen matrix being incompletely formed due to reduced activity of the enzyme lysyl oxidase. The lack of the cross linking by lysyl oxidase also affects elastin which is a protein which gives the aorta its flexibility. During Cu deficiency, the aorta is weakened and aortic rupture may occur. Additional cardiac abnormalities due to Cu deficiency may include cardiac hypertrophy, altered electrocardiograms, abnormal mitochondrial structure and reduced levels of ATP and phosphocreatine.

Copper deficiency and carcinogenesis

Manifestations of Cu deficiency include decreased Cu/Zn SOD, Increased LDL cholesterol, decreased HDL cholesterol, decreased glucose clearance, decreased methionine and leucine enkephalins, and abnormal cardiac function (Sandstead 1995).
The importance of Cu can be attributed to its role as a co-factor in a number of enzymes including cytochrome oxidase and particularly SOD, that are involved in the defence against oxidative stress, and a deficiency compromises the anti-oxidant defence system of cells thus increasing their susceptibility to oxidative DNA damage (Pan, 2000). SODs are part of the body's natural defense against reactive oxygen species, the free radicals that cause the damage, by catalysing the reaction by which superoxide is removed. The hydrogen peroxide that is generated is then further metabolised by either catalase or glutathione peroxidase. Cu is an essential part of both extracellular and cytosolic Cu/Zn SOD, and in the absence of Cu, this SOD is completely inhibited. In contrast to Mn SOD, for which there is no substitute for manganese, the zinc moiety of Cu/Zn SOD may be replaced by a similar metal, even Cu itself, though activity is reduced (Stipanuk, 2000). Copper containing SOD has been found to be lower in malignant cell lines as compared to normal tissues (Marklund,1982), and reduced amounts of copper/zinc SOD and MnSOD have been implicated in multistage carcinogenesis in both rodents and man (Davis, 1999). The activity of SOD has been examined in the liver cytosol and mitochondria of copper loaded rats where it was found that SOD activity increased by more than 100% (Russanov, 1986).
The results of an in vitro study on human cultured mammary cells (MCF 12A), found that after incubation with increasing concentrations of Cu, that there was a consistent protective and/or stimulating affect at even the lowest concentration. The study demonstrated that, though Cu and other trace metals could exert a hormesis effect in cultured mammary cell, Cu might play a role in controlling cellular processes and proliferation (Schmidt, 2004).
An extreme form of Cu deficiency is seen in the Menkes' syndrome, a rare X-linked genetic defect in Cu transport and storage resulting in fatal vascular and cerebral degeneration early in life.

Immunoregulation

Copper deficiency is known to impair immune function as a consequence of both neutropenia and T lymphocytopenia resulting from reduced production. It has been shown that the addition of Cu to cultures of HL 60 cells (a promyelocytic cell line) promotes differentiation toward the granulocyte/neutrophil phenotype by enhancing the progression of the cells from promyelocytes to myelocytes. Copper deficiency also inhibits the proliferation of T cells (especially the T helper or CD4+ cells) in response to mitogens (Stipanouk, 2000).

Recommended Daily Allowance

There are many copper containing veterinary compounds available at doses considerably in excess of those proposed on a daily basis for CV 247.

The Feeding Stuffs Regulations (Statutory Instrument 2000 No 2481) allows a maximum content of copper between 15 and 175 mg/kg of feed depending on age and type of animal.

According to the National Research Council, the estimated safe and adequate daily dietary intake (ESADDI) for Cu is 1.5 to 3.0mg, but most Western diets do not furnish this amount, and indeed the average daily intake of Cu in the USA is only about 1mg. Dietary Cu intake requirements may differ between men and women.

The clinical use of Cu salts often exceeds the upper ESADDI, especially in patients with a Cu deficiency. Typical therapeutic daily doses vary between 5 and 8 mg. Copper gluconate is regarded as the safer compound (Rosado, 2003). Therapy with doses of Cu gluconate of 9mg/day for periods of 6 months or more have been reported to treat haematologic disorders associated with Cu deficiency (Bartner, 2005), and doses of 10mg Cu gluconate /day for 12 weeks have been used to treat chronic low back pain without ill effect (Pratt, 1985).

Studies from animal models and human volunteers suggest that acceptable intakes of Cu that would avoid Cu deficiency and/or toxicity may vary between 10 and 50mg/kg body weight (Aggett, 1999).

CLINICAL PHARMACOLOGY

Absorption, Distribution, Metabolism and Excretion in Man

The bioavailability of Cu from the diet is about 65-70% but this can vary greatly and is influenced by a number of factors including chemical form, interaction with other metals and dietary components. Intestinal absorption of Cu has been reported to be inhibited by zinc, and the risk of Cu deficiency is increased when the molar ratio of zinc to Cu is high (Sandstead, 1995). Diets high in Cu result in a reduced absorption, and Vitamin B6 depletion in young women has been found to inhibit Cu absorption (Turnlund, 1991). Calcium supplements improve body Cu retention, whilst ascorbic acid is known to inhibit Cu absorption (Kies, 1989), though this might be dose dependant as moderate supplemental intakes of ascorbic acid do not supress intestinal Cu absorption (Jacob, 1987).

The biological t1/2 of 67Cu from the diet is about 13-33 days (Johnson, 1992), with biliary excretion being the major route of elimination. The serum concentration ranges up to 1.5mg/L in healthy persons. Gastrointestinal symptoms can occur at whole blood concentrations around 3mg Cu/L (Barceloux, 1999).

The normal upper limit of free serum Cu (non ceruloplasmin bound) in infants is 0.3 microM/L or 1.6% of total serum copper. The normal total serum Cu for both children and adults is 21.4 microM/L and the normal upper limit for urinary Cu excretion in adults is 15 microg/dl (Eife, 1999).

Absorption

In adult humans, the net absorption of dietary Cu is about 1mg/day, though efficiency varies greatly depending on dietary intake. Dietary Cu joins some 4-5mg of endogenous Cu flowing into the gastrointestinal tract through various digestive juices. Most of this Cu returns to the circulation and to the tissues including the liver (Linder, 1998). Copper absorption has been reported to be greater in women under the age of 60 years, than men (Johnson, 1992).

Copper can be absorbed in the stomach and throughout the length of the small intestine, but predominantly in the jejunum. Copper absorption is regulated at the intestinal level. Absorption can be separated into a saturable, regulated portion and a nonregulated diffusional component. Copper absorption is inhibited by metallothionein, which is produced in response to high levels of dietary copper. Some studies have shown that high levels of zinc can also inhibit intestinal Cu absorption, possibly due to the former's stimulation of metallothionein. Conversely high levels of dietary Cu do not inhibit zinc absorption. Studies using human intestinal Caco-2 cells found that at pH 6, uptake of Cu was linear over the first 6 minutes and between 10 and 80 microM exhibited non-saturable transport kinetics. In addition Cu uptake was energy dependant and was affected by the valency state of Cu, preferring Cu 2+ over Cu 1+ (Ferruzza, 2000).
The effects of Cu ingestion on gastric and intestinal permeability have been investigated in a double blind crossover study in a group of 31 healthy subjects. Each subject ingested 200ml water and, on a separate occasion, 200ml water containing 10mg/L of Cu sulphate, followed, after 15 minutes, a solution containing 40g sucrose, 7.5g lactulose and 2g mannitol. Ingestion of the Cu solution significantly increased gastric permeability to sucrose, but did not change intestinal permeability to lactulose/mannitol. The changes to permeability had no effect on the reported gastrointestinal symptoms (mainly nausea and to a lesser extent, vomiting) which affected 22% of the subjects after ingestion of the Cu solution, mainly in the first 15 minutes. The results strongly suggest that the ingestion of Cu exerts an affect on gastric, but not intestinal mucosa, reducing the gastric mucosal barrier capacity (Gotteland, 2001).
Copper is poorly absorbed through the skin, whatever the vehicle used (Pirot, 1996).

Effects of dietary components on copper absorption

Interactions with dietary components can influence how well Cu (and manganese) are absorbed from the diet. Chemical similarities with other nutrients may result in competition for common binding sites between related minerals. Also binding of minerals to organic components of the diet in the lumen of the gastro-intestinal tract can alter absorption. One well- known example of this is the inhibitory effect of the phosphate rich plant compound, phytate on Cu absorption (and other minerals). Ascorbic acid inhibits Cu absorption, probably due to its ability to reduce Cu2+ to Cu1+, suggesting that Cu is optimally transported in its Cu2+ state (Stipanuk, 2000).

Copper has been reported to be less efficiently absorbed from a vegetarian diet then from a nonvegetarian diet (Hunt, 2001), though the 3 fold difference in the phytic acid content of the comparative diets may have been the main contributory factor.
The factors affecting absorption of Cu in vegetarian subjects have been evaluated in 6 healthy volunteers. Each received a dose of 2.7-5.2 mg Cu/day for 12 weeks. It was observed that the apparent absorption from this intake was between 10.6 and 21.7%, which was lower than that from a non-vegetarian diet. Factors enhancing absorption included dietary levels of riboflavine, cellulose, milk proteins, oxalates and zinc, whilst phosphorus, niacin, calcium and pulse protein inhibited absorption of Cu (Agte, 1994).

Whilst Cu absorption in rats is affected by the level of intake of sucrose and fructose in the diet, even the consumption of diets of which 20% of the calories is made up these sugars, had no affect on Cu absorption in man (O'Dell, 1990).

The affect of ascorbic acid (AA) on absorption of Cu has been investigated in a study in healthy young males over a 14 week period. During 4 intake periods of doses up to 605 mg/day of AA, it was found that Cu absorption, Cu retention, total serum Cu and ceruloplasmin serum levels were not significantly affected by changes in the daily intake of AA, though the oxidase activity of serum ceruloplasmin was decreased by about 20% during the high AA dose period (Jacob, 1987).

Effect of age on Cu absorption

The effect of age on Cu intake and absorption has been evaluated in 39 healthy infants during the first 3 months of life. One half of the subjects were randomly assigned to receive oral supplementation of 80mg Cu (as Cu sulphate)/Kg for 15 days. At the end of the period Cu absorption was measured by using orally administered 65Cu as a tracer, followed by faecal monitoring of excreted labelled Cu. Mean Cu absorption at 1 month of age was 83.6%, whilst at 3 months it was 77.6%. However statistical analysis showed that the interaction between age and Cu supplementation did not have a significant effect on Cu absorption in neonates and small infants (Olivares, 2002). Copper is better absorbed from breast milk than from infant formulae (Loennerdal, 1998).
Copper absorption has been compared in young and elderly men. A group of 6 healthy young men were confined to a metabolic unit for 78 days to study Cu (and iron) absorption from constant diets containing 3mg Cu/day (and 10mg iron/day). Absorption was determined twice using Cu enriched with the stable isotope 65Cu. Data was compared with data from a similar group of elderly men. It was found that Cu absorption was similar amongst the individual subjects regardless of age, and serum Cu was in the normal range for all subjects. However intersubject levels of both serum and urine Cu varied considerably (Turnlund, 1988).

Distribution

Once absorbed into the blood plasma, Cu is primarily bound to albumin and transported to the liver where it is incorporated into ceruloplasmin. The complex is then released into the circulation for delivery to the peripheral tissues. Ceruloplasmin is a large glycoprotein which is produced in the liver and whose synthesis is regulated by both Cu, and certain inflammatory mediators, such as interleukin-1 and glucocorticoids. These and other factors associated with the acute-phase response are probably responsible for the increase in serum Cu and ceruloplasmin that occur following acute inflammation or infection. Cell culture studies have demonstrated that the mechanism of Cu uptake into cells is through the binding of the ceruloplasmin-Cu complex to a cell surface receptor, following which Cu is taken up as the free metal (Percival,1990). Intracellular Cu is initially bound mainly to low molecular weight components such as glutathione and subsequently shifted to high molecular weight components such as metallothionein and SOD (Ferruzza, 2000). Adaption to high and low Cu intake depends upon the cellular control of influx and efflux of cellular Cu concentrations. The mechanisms of Cu transport involved in cellular adaption to low and high Cu exposures are different (Arredondo, 2000).

Menkes' syndrome and Wilson's disease are genetic disturbances in Cu metabolism. Both are the result of a genetic mutation in a class of genes encoding Cu transporting P-type ATPases.

Excretion

Under normal circumstances less than 3% of dietary Cu is lost through urine or through cutaneous losses. Although some Cu can be lost by direct sloughing off of intestinal cells, especially when intake is high, almost all Cu excretion is via the bile, complexed in such a way that re-aborption cannot occur. Biliary Cu excretion in man ranges from 0.5 to 1.3 mg/day.

Human pharmacology summary table

Species and age	Gender and no.	Molecule and dose	Route of admin	Period of admin	Observations	Ref
Man	31	CuSO4 2mg in sol	po	single	Ingestion of Cu increases gastric permeability of sucrose. GI symptoms affected 22% of subjects	Gott-Eland 2001
Man Veget-arian	6	2,7-5.2 mg/d in diet	po	12wks	Absorption of Mn between 10.6 and 21.7% which is lower than that from non-vegetarain diets. Riboflavine, cellulose, milk and Zn in diet enhance absorption of Mn, whilst P, niacin, Ca and pulse protein inhibit absorption	Agte 1994
Man young				14wks	During 4 pds of doses of ascorbic acid up to 605mg/d it was found that absorption, retention and total serum levels of Cu were not significantly affected.	Jacob 1987
Man infants	Up to 3m 39	CuSO4 80mg/kg	po	15d	Mean Cu absorption at 1m of age was 83.6%, but 77.6% at 3m	Oliv-Ares 2002
Man Young & old	6 of each	3mgCu/d In diet	po	78d	Cu absorption was similar regardless of age and serum Cu levels, though variable, were in the normal range for all subjects	Turn-Lund 1988

CLINICAL EXPERIENCE

Clinical use of copper gluconate

Cu gluconate has been used to treat the haematologic disorders associated with copper deficiency at a dose of 9mg/day for 6 months (Bartner, 2005), and to help modulate, as part of a micronutrient supplement, the blood anti-oxidant status in patients with major trauma (Berger, 2001). Other clinical uses of Cu gluconate as a part of a cocktail of substances, include the treatment of melanoma (De Oliveria, 1998).

Copper gluconate has been investigated as a treatment for low back pain. A double blind study was undertaken in 14 subjects randomised to receive either 10 mg Cu gluconate or placebo every day for 12 weeks. There was no difference in the clinical response. Copper levels were measured in blood urine and hair and found that there was no increase over the 12-week period. Similarly there was no significant change in haematocrit, tryglyceride, SGOT, GGT, LDH, cholesterol or alkaline phosphatase in the group receiving the Cu salt (Pratt, 1985). Copper gluconate has been claimed to have tumour-inhibiting properties and has been used in high doses for the direct treatment of cancer tumours (Nieper, 1979), though details are not available

Cu gluconate has also been used in combination with manganese gluconate. A randomised double blind, placebo controlled study was undertaken in 97 patients for the local treatment of breast fissures related to breast feeding, in which it was found that the combination after 5 days was significantly better in the treatment of fissures less than 14 days old, when compared with placebo (Dreno, 1997). The combination has also been evaluated for the treatment of superficial wounds (Mallet, 1994).

A review of the basis and clinical aspects of copper is appended as reference 311A (Harris, 2003).