CISPLATIN CHEMOTHERAPY EFFECTS ON TRACE ELEMENT EXCRETION AND SERUM CONCENTRATION

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

Despite medical advances in the last several years, the incidence of cancer continues to rise. Chemotherapy has long been used as a treatment for cancer. Cisplatin (cis-diamminechloroplatinum II), an inorganic platinum compound has been used for over twenty years as a chemotherapeutic treatment for several types of solid tumors. It is used primarily in the treatment of lung, ovarian, and head and neck carcinomas (1).

Regardless of the benefits of chemotherapy, there are often many side effects. Cisplatin is known to cause significant impairment in renal function resulting in the loss of some trace minerals (1). Magnesium depletion secondary to cisplatin use has been documented for many years (2). Research on cisplatin has indicated that it also may cause increased renal excretion of zinc and copper (3). An increase in serum iron and ferritin levels in patients receiving cisplatin has been noted in a few studies (4). The reason for increased iron mobilization is unclear.

Selenium has been reported to decrease cisplatin's nephrotoxicity (5). No studies were found regarding selenium status during treatment with cisplatin. Little research has been done on manganese status in the cancer patient. One study indicated a very slight elevation of serum manganese concentration in a group of cancer patients versus a control group (6). Generally, there appears to be no significant change in serum manganese levels of the cancer patient.

Trace element levels appear to vary greatly from one individual to another depending on health status and type of disease (7). The lack of knowledge regarding trace element loss and supplementation in cisplatin patients supports the need for further research

in this area. The present study examined the effect of cisplatin chemotherapy on trace element serum levels and urinary excretion.

The purpose of the study is to determine the effect of cisplatin chemotherapy on magnesium, zinc, copper, iron, selenium and manganese status in cancer patients.

The objectives of this study are as follows:

1. To determine trace element (magnesium, zinc, copper, iron, selenium, and manganese) concentrations in the serum of cisplatin patients at selected intervals in their therapy cycle.
2. To determine trace element (magnesium, zinc, iron, and manganese) urinary excretion of cisplatin patients at selected intervals in their therapy cycle.
3. To determine the creatinine clearance in cancer patients receiving cisplatin.

Hypotheses

The following hypotheses are postulated for this study:

HO1: There will be no significant effect of cisplatin chemotherapy on serum magnesium, zinc, copper, iron, selenium, and manganese concentrations.

HO2: There will be no significant effect of cisplatin chemotherapy on urinary excretion of magnesium, zinc,iron, and manganese.

HO3: There will be no significant effect of cisplatin chemotherapy on creatinine clearance.

Definition of terms

cisplatin -a water soluble inorganic platinum compound used as a chemotherapeutic drug for treatment of solid tumors.

nephrotoxic - causing destruction to kidney cells.

hypomagnesemia - reduced magnesium concentration in the blood.

creatinine clearance - a measure of the kidney's ability to excrete nitrogenous waste products.

glomerular filtration - the rate at which the kidney is able to filtrate water, small molecules and protein out of the blood as it flows through the glomerulus; an important indicator of renal function.

erythropoietin -hormone that stimulates the formation of red blood cells.

plasma - fluid portion of the blood in which microscopically visible formed elements (red blood cells, white blood cells and platelets are suspended).

serum - clear liquid that separates from blood when allowed to clot completely; blood plasma from which fibrinogen has been removed.

This thesis was written in format style according to Cancer journal specifications.

CHAPTER II

REVIEW OF THE LITERATURE

Cisplatin

Cisplatin (cis-diamminedichloroplatinum II) is an inorganic platinum compound that has been used for many years in cancer chemotherapy. Cisplatin is effective when used alone or in combination with other chemotherapeutic drugs for treatment of several types of solid tumors. Cisplatin may be a curative treatment for early diagnosed testicular and ovarian cancers. It is also used for palliative treatment in cancers of the head and neck, bladder, prostate, lung, cervix and some pediatric cancers (1).

Cisplatin is a water soluble complex that contains a central platinum atom surrounded by two chloride atoms and two ammonia moieties. Rosenberg et al (8) were the first to show that cisplatin possessed antibiotic activity. The growth of Escherichia coli was inhibited by transforming the bacteria into long filamentous structures. Subsequent studies by Rosenberg et al (9) demonstrated the antitumor activity of cisplatin in animals.

Cisplatin's mechanism of action apparently inhibits DNA synthesis resulting in inhibition of tumor growth. Antitumor activity is much greater when the chloride and ammonia moieties are in the cis position permitting rapid and bifunctional DNA binding. Studies using normal and cancerous tissue both in vitro (10) and in vivo (11) have documented the mechanism of action for cisplatin. Exposure of these tissues to equivalent therapeutic doses of cisplatin indicated a definite inhibition of new DNA synthesis. Little or no concommitant effect on synthesis of RNA or proteins was found. Following these and other studies, it became apparent that the primary target molecule of cisplatin in the cell is DNA. Antitumor action of cisplatin apparently occurs as a result of a reaction with the

purine and/or pyrimidine bases of DNA (12). The base sites, however, have not been clearly distinguished. There are several ways in which cisplatin could bind to the DNA bases. It may bind to a single nucleophilic site (monofunctional binding) or it may bind to two sites within a single DNA base or between two bases (bifunctional binding). Cisplatin could cause intrastrand cross-linking where two bases are bound or cross linked by a single platinum containing molecule on one DNA strand or it may bind to opposite DNA strands known as interstrand crosslinking. Cisplatin could also form a crosslink between DNA and other molecules such as proteins (DNA-protein crosslink) (13). Recent evidence suggests that the intrastrand crosslinking may be most important in cisplatin's cytotoxic action (14).

In the most common mode of therapy, cisplatin is administered intravenously every three to four weeks. Usual dosages range from 50-120 mg/m^2 of body surface area. Cisplatin is administered in a single dose or in divided doses over several days.

Cisplatin is diluted with physiologic saline immediately before infusion because it is an unstable compound and may produce greater toxicity if diluted in solutions that do not contain chloride ions (15). The chloride concentration is lower inside the cell. Chloride groups are replaced by water molecules. This exchange creates a positively charged compound which then may bind in more than one way as described above (14,16).

Once cisplatin is administered intravenously, it is rapidly distributed to nearly all organs, but begins to settle disproportionately in selected organs, particularly the liver and kidney. Within ten minutes after administration to dogs, the amount of renal platinum was three times that of plasma: 6.6 μg/g vs 2.6 μg/g respectively. Platinum was no longer detectable 12 days later in the plasma but was still found in the kidney at a concentration of approximately 0.6 μg/g (17). A study by Kaye and colleagues using a 14 C-labeled cisplatin analog showed that the drug appeared to be most concentrated in the outer medulla and inner cortex of the rat kidney (18). Choie and associates quantified the platinum in rat kidneys 24 hours after a single intraperitoneal dose of 6 mg/kg body weight. The

renalcorticomedullary junction showed the greatest platinum concentration followed by the renal cortex. Platinum was significantly higher in the cortex or corticomedullar junction than in the remaining renal tissue (19).

Cisplatin apparently exists in three major compartments after intravenous infusion. The three compartments include free or unbound, protein bound and erythrocyte bound (20). Belt et al indicated a decrease in mean plasma platinum levels following administration of cisplatin when given in combination with mannitol by rapid 15 minute infusion. The initial value was 2700 ng/ml 15 minutes after infusion and fell to 200 ng/ml 2 hours post infusion (21). Zumkley and collegues disputed this finding by indicating an increased concentration of platinum in the plasma by one hour after administration (less than 0.0025 μmoles/L before therapy in 9 patients to 7.14 μmoles/L at one hour after therapy in 7 patients). Twenty-four hours after cisplatin administration, platinum plasma concentration was 9.08 ± 3.1 μmoles/L in 9 patients. Cisplatin plasma concentration then dropped to 6.97 ± 1.27 μmoles/L 48 hours after therapy (3). Reasons why the plasma level continued to rise until at least 24 hrs were not stated by the authors.

Urinary excretion of cisplatin in man and animals has been studied (22, 23, 24) within 4-6 hours after cisplatin is administered, 27 to 70% is excreted in the urine. Excretion depends on the time frame of administration (21). The mean fraction of the total dose excreted is significantly greater (approximately 75%) after a 6 hour infusion versus 15 minute infusions (approximately 40%). This study also indicated that the use of mannitol with cisplatin administration reduced the amount of cisplatin excreted in the urine in 24 hours (21). Following the rapid 15 minute infusions, the mean percent of cisplatin excreted was 40%. Excretion was reduced to 18% in patients who were administered mannitol with their cisplatin dose (21). After a 6 hour infusion of cisplatin without mannitol, 75% of the dose was excreted within 24 hours. Patients who received mannitol concomitantly in a 6 hour infusion cisplatin excreted only 22% in 24 hours (21). However, Cvitkovic et al found total urinary recovery of cisplatin only slightly lower in

mannitol treated dogs versus prehydrated animals (22). Belt and coworkers suggested that differences could possibly be due to alterations in the binding behavior and reactivity of various platinum species derived from cisplatin (15).

Zumkley et al studied 15 patients who received cisplatin in an 8 hour infusion which revealed increased urine platinum excretion. A 24 hour urine was analyzed the day before, the day of therapy and two days after cisplatin administration. Initial concentration in 8 patients was less than 0.025 mg/24 hr before treatment. This value rose to 34.65 ± 12.3 mg/24 hr the first day of treatment but dropped to 8.66 ± 2.4 mg/24 hr the second day after treatment (3).

Despite cisplatin's therapeutic effects, there are significant toxic side effects associated with its use. These include impairment of renal function, hematologic depression, high frequency hearing loss, anorexia, peripheral neuropathy, nausea and vomiting. Nephrotoxicity of cisplatin has been documented pathologically and clinically but the exact mechanism for the nephrotoxicity is unclear. Pathological evidence of nephrotoxicity using renal tissue biopsies indicated that cisplatin may cause renal lesions occurring in both the distal and proximal convoluted tubules and collecting ducts (25, 26). Dentino et al (26) indicated that therapy with cisplatin resulted in a regular and persistent decrease in the glomerular filtration rate. Renal function was decreased approximately 40% after only two courses of therapy. Apparently most patients who suffer renal impairment never regain their pretreatment renal function (26).

The severity of renal impairment appears to be directly related over time to the total dosage of cisplatin. Nephrotoxicity may increase with the combined use of cisplatin and other nephrotoxic agents such as aminoglycosides (21) and with antihypertensive agents (27). Electrolyte disturbances and trace mineral loss, including hypomagnesemia, occurred in a significant number of cisplatin patients apparently as a result of renal tubular dysfunction (25, 26).

Years of study have indicated that the incidence of nephrotoxicity may be greatly

reduced by administering cisplatin with mannitol, saline or other diuretic agents. The dose and length of cisplatin treatments can be varied to decrease nephrotoxicity (28). Theoretically, the toxicity of cisplatin is reduced if the drug is given as a divided dose and as a slow infusion (29). Pretreatment hydration appears to be the most important factor in reducing nephrotoxicity (30). However even with aggressive hydration a reduction in kidney function usually occurs. Aside from the nephrotoxicity, the major dose limiting toxicities are anorexia and peripheral neuropathy (31).

Magnesium

Magnesium's importance in nutrition was first documented by noting a deficiency in rats and dogs in the 1930s. In human nutrition, the importance of magnesium was noted in 1934 in a few individuals with various disease processes. Healthy individuals rarely experience a magnesium deficiency but those who suffer from clinical disorders may become magnesium depleted (32).

The majority of the body's magnesium is found in the bone which contains approximately 834-1200 mmol (approximately 20-28 grams) in a normal adult weighing 70 kgs. Approximately 27% is in the muscle, 6-7% in other cells and 1% in extracellular fluid. An estimated 7-10 mmol are found in the liver, heart and other soft tissues. Magnesium is also found in the erythrocyte with normal magnesium content of about 2.2-3.1 mmol/L. Magnesium content of the red cell depends on the age of the cell (magnesium content drops with age) and on the analytical method used to measure its content. If atomic absorption spectrophotometry is used, the usual range for adults, children and neonates is about 0.70 - 0.75 mmol/L. Magnesium in the erythrocyte and plasma is either complexed with ions such as citrate or phosphate, protein bound or free (32). Plasma percentages of magnesium are approximately 55% free, 13% complexed and 32% bound. Magnesium is an important part of bone crystal and various cell membranes. Intracellular magnesium is approximately 6-10 mmol/kg wet weight except for the erythrocyte which has less (32).

Magnesium is a necessary ion in oxidative phosphorylation and essential to the metabolism of ATP. Its primary function is to provide the structural stability of ATP. Magnesium serves as a ligand for the ATP phosphate groups and is a part of some transport systems (32, 33). It also takes part in processes such as glucose utilization, contractibility of smooth and cardiac muscle, and synthesis of fat, muscle, protein and nucleic acids. Magnesium is needed to facilitate over 300 different enzyme systems (32, 33).

The mechanism of magnesium absorption is controversial but there is agreement that absorption efficiency is poor. The small intestine is the site where magnesium is primarily absorbed, with more absorption in the jejenum than in the ileum (32, 34). Magnesium absorption may involve two systems. One is a carrier-mediated system; the other is simple diffusion (32). Studies have indicated a very wide range of absorption of magnesium from food sources. Free living adults consuming self selected diets had an average magnesium absorption of approximately 21% in males and 27% in females (35). Other metabolic studies of magnesium intake in the usual diet showed average absorption to be $\geq$ 50-70% (32).

Once magnesium is absorbed, it may be retained for bone and tissue growth or used as turnover replacement. Magnesium is stored in at least three different pools in the body. One pool of extracellular magnesium provides quick turnover; another pool is mainly intracellular magnesium and has slower turnover. One pool is skeletal magnesium which has a very slow turnover rate (33). Magnesium that is not retained by the body is lost through the kidneys. A large amount (approximately 80%) of serum magnesium is ultrafiltrable and the proximal convoluted tubule resorbs 20-30%. The thick ascending limb of the loop of Henle is the primary resorption site (50-65%). What is not needed is excreted in the urine. Control of excretion of magnesium takes place between the thin descending limb and early distal tubule (34). The concentration of magnesium in the tubular lumen and in the plasma determines the amount of renal resorption in this area.

The distal convoluted tubule and collecting tubules and ducts reabsorb very little (32).

Physiologic and metabolic conditions may affect how much magnesium is reabsorbed in the nephron. A very important relationship exists between magnesium levels and calcium. Magnesium deficiency decreases parathyroid hormone secretion which is crucial in calcium homeostasis (36). It is possible that changes in serum magnesium may influence how the body handles calcium and thus affect cell function (36, 37). As magnesium deficiency continues, parathyroid hormone output will decline until signs of magnesium depletion are evident with symptoms such as hypocalcemia, hypocalcuria, hypokalemia, sodium retention and others (38).

Disease processes that decrease intestinal absorption or impair renal function may result in symptomatic magnesium deficiency (32). These would include malabsorption syndromes, gastrointestinal and endocrine disorders, alcoholism, diabetes, protein energy malnutrition, hypermetabolic states such as burns or trauma, prolonged low magnesium diet ingestion and renal tubular dysfunction (32). Magnesium intake may or may not have a relationship with development of heart disease, cardiac arrhythmia and hypertension (39).

Certain drugs may influence magnesium absorption and utilization. Renal loss of magnesium may be significant with the use of loop diuretics (furosemide and ethacrinic acid) and nephrotoxic drugs such as cisplatin (32).

Hypomagnesemia which occurs as a result of cisplatin chemotherapy has been documented by numerous studies over the past several years (2, 3, 40, 41). The basis for this hypomagnesemia is the significant impairment in proximal tubular resorption and distal tubular function caused by cisplatin chemotherapy (26, 27). Depletion varies with each individual dose and with the number of doses given. In some patients, it appears that the kidney may remain impaired for a number of days to months after therapy is completed (2, 3).

The severity of hypomagnesemia and its relationship to cumulative cisplatin doses were first demonstrated by Lam et al (40). Patients were evaluated after each dose of a four

cycle cisplatin chemotherapy regimen. Cisplatin dose and the relationship to low magnesium were examined using linear regression analysis. Cumulative cisplatin dose (in mg/m^2) was used as an independent variable. Out of 28 patients, 10 had a low baseline serum magnesium. Mean serum magnesium was 1.8 ± 0.1 mg/dL before chemotherapy; serum magnesium was 1.3 ± 0.1 mg/dL after one cycle in 26 patients. After the fourth cycle (in eight patients), this value dropped to 0.9 ± 0.1 mg/dL. Fourteen patients were followed for an additional three to five month period after their last cycle. Ten continued to have low serum magnesium during this followup period (2).

Plasma magnesium values may begin to fall as soon as three weeks after initiation of chemotherapy (14, 41). Buckley and colleagues (2) treated 66 patients with 6 cycles of monthly doses of cisplatin of 50 mg/m^2 in combination with other chemotherapeutic drugs. Hypomagnesemia developed in 41% of these patients after a single course of therapy. This increased to 100% after the patients had received six cycles of therapy. Serum magnesium averaged 1.76 ± 0.27 mg/dL in 27 patients after one cycle of therapy and continued to drop after each cycle to a final value of 1.20 ± 0.14 mg/dL, in 12 patients after six cycles of chemotherapy. The hypomagnesemia continued for an average of 4.5 months in 104 (79%) of 136 determinations following cessation of chemotherapy. The mean serum magnesium was 1.49 mg/dL during this post therapy time period (normal was 2.4 ± 0.3 mg/dL for this study). Average survival time was six months following cessation of chemotherapy.

Since serum magnesium levels may not denote the actual cell content of magnesium (42), a more accurate evaluation may be achieved by the study of magnesium concentration of red blood cells during cisplatin therapy. Plasma magnesium (PMg) and erythrocyte magnesium concentrations (EMg) were examined in twenty-two patients. These patients received cisplatin ranging from 50 mg/m^2 to 100mg/m^2. Samples were taken before cisplatin therapy and at one, two, four and seven days after therapy (41). PMg decreased from a basal value of 1.99 ± 0.18 mEq/L before therapy to 1.73 ± 0.25 mEq/L on the

seventh day after therapy. EMg levels decreased after cisplatin administration from 4.11 ± 0.70 mEq/L before therapy to 3.55 ± 0.62 mEq/L on the fourth day after therapy. On the seventh day after therapy, the EMg returned to pretreatment levels of 4.14 ± 0.70 mEq/L.

Urinary magnesium was measured in 14 of the 22 patients. Before cisplatin infusion urinary magnesium was 6.51 ± 3.14 mEq/24 hr; it rose to 10.1 ± 3.45 mEq/24 hr ($p<0.001$ in comparison with day 0) one day after chemotherapy was given. Urinary magnesium returned to a pretreatment level of 6.97 ± 2.71 mEq/24 hr on the second day after therapy and rose to 7.91 ± 3.93 mEq/24 hr seven days after cisplatin was given (41).

Ten of these patients had PMg and EMg serial followups through six courses (6 months) of chemotherapy. Pretreatment EMg for these patients was 4.22 ± 0.34 mEq/L. The EMg values were relatively unchanged after the first course of therapy at 4.34 ± 0.43 mEq/L and after the second course at 4.20 ± 0.40 mEq/L. Decreases were noted after the third course of therapy with a value of 3.76 ± 0.42 mEq/L ($p<0.05$) and following the sixth cycle at 3.43 ± 0.61 mEq/L ($p<0.001$ vs time 0). The mean decrease in EMg from pretreatment levels to final levels was 18.7% ($p<0.001$) (41).

During this six month period, PMg decreased after the first cycle from a pretreatment mean value of 1.98 ± 0.19 mEq/L to 1.89 ± 0.24 mEq/L. There was a statistical insignificant difference from the pretreatment levels to after the second course of therapy with a value of 1.84 ± 0.17 mEq/L ($p<0.05$), becoming more evident after the third course at 1.71 ± 0.19 mEq/L ($p<0.001$ vs time 0). The value dropped after the sixth course of therapy to 1.55 ± 0.2 mEq/L ($p<0.001$ vs the third course). The mean decrease after the sixth course of therapy was 21.7% when compared to pretreatment levels (41).

A marked decrease of plasma and red blood cell magnesium concentration levels concurrent with the increase in plasma concentration levels of cisplatin has been observed (3). Fifteen patients suffering from various malignant tumors were examined. All received cisplatin (120 mg/m^2) in an infusion lasting 8 hours. Magnesium concentrations in plasma and red blood cells were evaluated before cisplatin therapy and at 1, 24, and 48 hours

following cisplatin administration. Average plasma magnesium concentration before therapy was 1.64 mmoles/L in 15 patients. Plasma magnesium levels was decreased to 1.45 ± 0.18 mmoles/L 48 hours after therapy in 13 patients. Red blood cell magnesium concentration before therapy was 4.88 ± 0.60 mmoles/L and was 4.73 ± 0.61 mmoles/L in 10 patients 48 hr after therapy was given (3). Urinary excretion of magnesium was measured in the same study using 24 hour urine collection before, on the day of therapy and two and three days after administration of cisplatin. Urinary magnesium concentration before treatment was 81.6 ± 15.0 mg/24 hr in 15 patients. On the day of therapy this value was to 168.9 ± 136.1 mg/24 hr and on the third day after treatment 50.7 ± 30.6 mg/24 hr in 13 patients (3).

Results from these studies may suggest that cisplatin may affect magnesium metabolism at the cellular level. Because cisplatin therapy causes injury to the transport systems in which magnesium plays a stabilizing role, this injury may in turn cause an early shift of magnesium from cells into the bloodstream. Magnesium may then be taken up from the plasma during the repair process in order to recover the normal cellular content (41).

The incidence and severity of nephrotoxicity reactions and hypomagnesemia were reduced when patients receive intravenous mannitol and 0.45% saline during and after cisplatin administration (43). Hypomagnesemia may also be decreased when doses are more widely spaced and with the avoidance of rapid infusion rates (2).

Magnesium supplementation is often given before and during cisplatin infusion. Few studies have been done to determine the benefit of oral supplementation. Because the red blood cell concentration of magnesium did not decrease until after the third cycle of chemotherapy in the study by Sartori and coworkers, it is presumed that actual depletion of magnesium may not occur until later in the treatment cycle. It is possible that oral magnesium supplements should therefore be given later in the treatment course and between cycles of chemotherapy for maximum benefit and to avoid the risk of

hypermagnesemia (41). Further studies are needed to define optimal protocols for oral magnesium supplementation during cisplatin chemotherapy.

Zinc

The discovery of the nutrient role for zinc stems from research indicating its importance in plant life almost one hundred years ago (44. 45). The essentiality of zinc for animals was first reported in 1934 (46). Human zinc deficiency was first documented clearly in the early 1960s (46). This discovery was based on the finding that growth depression and delayed sexual development occurred in young Iranian and Egyptian men who had consumed vegetable protein based diets which were low in zinc (44, 48). Symptoms resolved after they were given zinc supplements (47).

Zinc is present in all cells but some tissues have a higher concentration than others (43). The human body zinc content is approximately 1.5 - 3 grams (44, 47). Most zinc is found in muscle, bone, liver and skin. Bone, prostate, and the choroid of the eye have the highest concentration of zinc (44). A substantial portion (60%) of total body zinc is found in skeletal muscle because it makes up the greatest portion of total body mass. Skeletal muscle and bone account for approximately 90% of all zinc found in the human body (45, 47).

Dietary intake of zinc may be reflected in the concentration of zinc in some tissues such as bone, testes, hair, blood, intestine and liver. Other tissues such as brain, lung, heart, erythrocytes and muscle contain stable levels of zinc and are not readily affected by dietary intake (44, 47).

Red blood cells contain 75-80% of the blood zinc stores. Plasma contains 12-22% and the remaining zinc is found in leukocytes and platelets (44, 45). Even though less than 0.5% of total body zinc is found in blood, plasma zinc is commonly used to estimate whole body zinc status (45).

Zinc is not accumulated and stored in the body. However, even though the large

amounts of zinc found the bone and muscle are not easily assessible, there are small pools of zinc in the bone, plasma, and liver that are able to exchange zinc rapidly when needed (49, 50).

Zinc plays a major role in many structural, catalytic, and regulatory functions of human metabolism (44, 47). Zinc is crucial in the functioning and structure of more than 60 enzymes (44). Zinc also plays a major role in the processes of cell replication and protein and nucleic acid metabolism (44).

The method by which zinc is transported across the intestinal mucosa is unclear. Both a carrier-mediated process and simple diffusion may be involved (44, 49). Circulating zinc in the blood is bound to albumin, transferrin, alpha- 2- macroglobulin and amino acids (49). The liver, which is the main organ involved in zinc metabolism, exchanges 30-40% of zinc in the portal blood (44).

The actual mechanism of absorption of zinc is unclear. It is absorbed throughout the small intestine but it is not clear which segment has the highest absorption capacity (49). Zinc absorption efficiency increases during dietary deficiency and decreases when intake is high (44, 47, 49).

Gastrointestinal absorption and gastrointestinal excretion of zinc are the primary ways that homeostasis is maintained. Healthy individuals excrete little zinc in the urine (49). Most zinc is lost through the feces and is a reflection of how much zinc is unabsorbed from the diet as well as endogenous zinc (44, 49). Trauma, severe burns, major surgery or a "stress" that is severe enough to cause muscle catabolism may increase urinary zinc loss (49).

Even though overt zinc deficiency is relatively rare (44), a deficiency would have an effect on numerous organ systems within the body; rapidly dividing tissues would be most susceptible to zinc deficiency (49). Enzymes dependent on zinc also are involved in collagen synthesis for wound healing. In addition, zinc also affects some immunological proteins such as thymulin (49).

Zinc interacts with a number of different hormones. In experimental animals and humans, a deficiency of zinc has been linked with impaired glucose tolerance (44, 49) suggesting involvement with insulin. Zinc also interacts with other hormones including growth hormone, gonadotropins, sex hormones, thyroid hormones and corticosteroids. These hormones may also alter zinc metabolism (44, 49).

There are a variety of physical and environmental factors which influence zinc status. Disease can affect zinc intake, absorption and excretion. Diseases involving mucosal damage and malabsorption syndromes may impair zinc absorption. Some diseases such as diabetes, sickle cell anemia, muscular dystrophy and chronic infections may result in increased zinc excretion. Anorexia causes increased urinary zinc loss secondary to increased muscle catabolism. Skin conditions such as dandruff and psoriasis may cause zinc loss. Malignant cells demand zinc for growth in many types of cancers; this increases the body's need for zinc (49). Hypermetabolic situations such as surgery or severe burns will alter zinc metabolism by increasing blood zinc levels and subsequently increasing urine losses. Prolonged hospitalization, use of some medications, gastrointestinal dysfunction, renal disease and excessive alcohol use can lead to zinc depletion and increase zinc needs (49). Symptoms of zinc deficiency may differ due to its various roles in metabolism. Symptoms may be nonspecific and include loss of appetite, dermatitis, reduced taste acuity and decreased immune function (49).

A minimal amount of research has been done on cisplatin's effect on zinc status. The actual renal processing of zinc is not well understood and zinc status rarely is measured in routine clinical lab assessments (49). However, some evidence indicates that the use of cisplatin as a chemotherapeutic drug may cause zinc depletion. A study using animals reported that cisplatin does lower hepatic zinc concentrations (52). In a short term study, Zumkley et al (3) found increased urinary zinc excretion following cisplatin therapy in a small number of human participants. This study analyzed renal loss of zinc the day before, the day of treatment and two and three days after treatment. Due to the large fluid supply of

forced diuresis, all patients had increased urine volumes. Zinc loss was elevated one day after treatment (in five patients) from 0.412 ± 0.278 mg/24 hr before chemotherapy treatment to 1.27 ± 1.05 mg/24 hr one day after treatment. Followup study on the second day indicated urinary zinc was 0.634 ± 0.296 mg/24 hr in five patients but decreased to 0.380 ± 0.258 mg/24 hr on the third day in 3 patients (3).

Plasma concentrations of zinc were also measured in the Zumkley study (3). Samples were taken before therapy and at selected hourly intervals following therapy. Plasma concentration of zinc before therapy was 10.75 ± 2.92 μmol/L in 9 patients. Levels were 11.11 ± 0.88 μmol/L at 1 hour following therapy. Following therapy 24 hrs later, the concentration was 12.68 ± 3.8 μmol/L and 13.35 ± 5.4 μmol/L 48 hours after the dose was given.

Zinc concentration in the red blood cells in this study was measured on a number of patients before therapy was begun and again at one, twenty-four and forty-eight hours after administration of cisplatin. Initial zinc concentration in the red blood cells of nine patients was 10.75 ± 2.92 μmoles/L. This value increased to 13.34 ± 5.4 μmoles/L 48 hours after treatment. Five patients were used to determine the final value (3).

Zumkley et al suggested three possible reasons for these alterations: the influence of the forced increased fluid load (that is commonly prescribed with cisplatin therapy), the negative effect of cisplatin on renal ability to preserve trace elements and the general role of renal dysfunction (3).

Sweeney et al (51) studied cisplatin therapy and and its role in zinc depletion. Using patients with biopsy confirmed squamous cell carcinoma, a twenty-four urine collection was completed and fasting blood samples were drawn for analysis. All patients in this study received cisplatin at a dose of 100 mg/m^2 over a 2 hour period or in a dose of 20 mg/m^2 daily for 5 days as a continuous infusion. Five patients receiving this therapy were assessed for the effect of day to day and diurnal variation on plasma zinc samples drawn at 7 a.m., 3 p.m., 6 p.m., and 10 p.m. Diurnal variation in plasma zinc was evident

in that the higher levels were fasting concentrations in the morning. These ranged from approximately 0.85 to 1.10 µg/mL at 7 a.m. fasting to 0.51 to 0.90 µg/mL at 3 p.m.; 0.6 to 0.9 µg/mL at 6 p.m. with a final reading of 0.61 to 0.90 µg/mL at 10 p.m. Urinary zinc variation between individuals was greater than variation within the individual.

Day to day variations in urinary excretion of zinc were studied on urine collected on two or three occasions over a one week period following cisplatin therapy. Fifteen patients were studied. Values rose from 600 µg/24 hr before cisplatin to approximately 900 µg/24 hr following cisplatin therapy (51).

The effect of cisplatin on plasma zinc was also assessed on these fifteen patients. Mean plasma zinc was 0.85 µg/mL before therapy and dropped to 0.70 µg/mL 5 days after chemotherapy. Statistical analysis using a paired t test indicated that the decrease in plasma zinc was significant ($p< 0.05$) (51). The increase in the urinary zinc did not reach significance due to small sample size and a large standard deviation (51). However, this study established a definite trend toward hypozincemia and hyperzincuria (51). The results did not indicate how cisplatin may have increased zinc excretion in the urine. Urine volume and zinc excretion were not related. The researchers hypothesized that cisplatin increased the excretion of amino acids such as histidine and cysteine (40) which has been shown to increase urinary zinc excretion (53).

Zinc loss may be important clinically in the cancer patient for many reasons. Some believe that zinc depletion may deprive the tumor of essential nutrients resulting in a benefit to the host (54). On the other hand, zinc depletion could weaken the immune system and encourage tumor growth (55). Hypogeusia is a side effect experienced by many cancer patients given cisplatin. Hypogeusia is sometimes a symptom of zinc deficiency (56).

Because past studies indicate that cisplatin may result in zinc deficiency, this possibility should be taken into consideration for patients receiving cisplatin therapy. Further studies need to be done to determine whether or not zinc supplements would be beneficial in the care of the cisplatin patient.

Copper

Copper was first recognized as necessary to cure iron deficiency anemia in rats fed a milk diet in 1900 (57). Work that clearly established the importance of copper for the formation of hemoglobin was reviewed in 1979 (58). The activities of certain metalloenzymes are dependent on copper and copper deprivation results in decreased activities of these enzymes (59). The study of Menke's disease, a genetic disease that occurs from a defect in copper metabolism and absorption, has clearly demonstrated the importance of copper in human nutrition (60).

The adult human body contains approximately 80 mg of copper with the highest tissue concentration found in the liver which contains approximately 15%. Approximately 10% is found in the brain with muscle content at 40% of the total amount (57, 58). Copper is stored in the liver and spleen. Most of the copper in the liver cells is found in the cytosol (57).

Ceruloplasmin supplies copper to other tissues to aid in the formation of cuproenzymes such as cytochrome c oxidase, superoxide dismutase and lysyl oxidase (61). The mechanism by which ceruloplasmin copper is made available to the cell is unclear. It may enter the cell intact or the ceruloplasmin copper may be transferred to an intracellular protein after it has been reduced to Cu^{+} (59).

Copper is absorbed in all areas of the gastrointestinal tract including the stomach and large intestines. The duodenum appears to be a major absorptive site. Absorption is regulated at the intestinal mucosa level (60). Newly absorbed copper is transported from the small intestine and is loosely bound to albumin and some free amino acids. Most of this copper is incorporated into ceruloplasmin after it is taken up by the liver. The ceruloplasmin is released into the blood where it makes up approximately 90% of the plasma copper pool (57).

Copper bioavailability may be affected by dietary factors. Of most importance is the adverse effect of zinc on the availability of copper. High amounts of dietary zinc may

intensify the symptoms of decreased copper status (61, 62). Generally, copper deficiency in humans is rare but has been noted in individuals taking amounts ranging from 50 mg to 150 mg of zinc per day (63). High dietary ascorbic acid also reduces the bioavailability of copper by affecting not only intestinal absorption but also its utilization (64). Most copper is excreted through the gastrointestinal tract. Only a small amount (less than 3%) is found in urine. Bile is the major contributor of endogenous fecal copper (58).

Copper deficiency may result in reduced activity of several cuproenzymes. Vascular disease, rupture of major vessels, and osteoporosis may develop as a result of decreased lysyl oxidase activity (65). Without adequate copper, the crosslinking in collagen and elastin is reduced which can result in spontaneous rupture of major blood vessels. Melanin formation is compromised when tyrosinase activity is decreased; copper deficiency also decreases cytochrome c oxidase activity which is needed for electron transport (65).

Dietary copper is important in the function of superoxide dismutase (SOD), an important enzyme in free radical defense. Copper deficiency may lead to reduced SOD activity in liver, lungs and erythrocytes (66). Adequate amounts of copper also may help prevent anemia. Presumably this anemia may be due to decreased SOD activity which causes increased free radical accumulation resulting in changes in the plasma membrane (67). This process ultimately may shorten the survival time of red blood cells (59).

Adequacy of copper affects iron metabolism. Studies in mice have indicated that the amount of dietary iron present and the sex of the animal directly affect the hematologic response to copper deficiency (68). The specific role for copper in anemia prevention and iron mobilization is unclear (59). Copper deficiency also may cause hypertrophy of the heart. Rats that were copper deficient showed cardiac enlargement as well as ventricular aneurysms. Copper deficient hearts contained less norepinephrines which may relate to cardiac malfunction. Electrocardiograms done on rats showed the occurrence of bundle-branch block and nonspecific alterations of patterns sometimes seen with metabolic

imbalance (61). Humans consuming low amounts of dietary copper also have exhibited abnormal electrocardiographs (69).

Other effects of copper deficiency include its relationship to brain catecholamine pool size (70) and the possibility that copper deficiency causes an increase in lipid peroxidation of cell membranes (57). In copper-deficient mice (depending on the degree of deficiency), a decrease in natural killer cell cytotoxicity and helper T cells was found. Immune system competence was restored with copper therapy (71).

Studies have indicated that cisplatin therapy may cause an increased renal excretion of copper and may result in copper depletion. Zumkley et al (3) studied copper concentration of plasma and 24 hour urine collections before and during cisplatin therapy. Copper status using 24 hour urine excretion as a measure was examined on the day of therapy and two and three days after cisplatin was given. Renal excretion of copper the first day after therapy was 0.026 ± 0.015 mg/hr before treatment in eight patients and 0.119 ± 0.041 mg/hr after the first day of treatment . Urinary copper on subsequent days decreased from 0.060 ± 0.018 mg/hr after the second day of treatment in eight patients to 0.029 ± 0.021 mg/hr after the third day of treatment measured in three of this group of patients (3).

Little change was noted in plasma copper concentrations before therapy, one hour, 24 hours, or 48 hours after cisplatin administration. Initial values were 21.0 ± 5.32 µmoles/L before therapy was begun and 21.87 ± 3.24 µmoles/L forty-eight hours after therapy. The reason for the alteration in trace mineral metabolism during cisplatin therapy in this study may be related to the specific effect cisplatin has on the renal excretion of trace minerals (72).

Another series of studies using samples of rat kidney tissue suggested that loss of kidney copper was due to impaired resorption secondary to cisplatin administration (73). DeWoskin and Riviere evaluated kidney tissue biopsies and indicated that although cisplatin therapy did not produce an immediate drop in kidney copper, by the fourth day after

cisplatin treatment, there was a 71% reduction in kidney copper (73). Further study is needed to define toxicity and kidney copper loss resulting from cisplatin chemotherapy.

Iron

The study of iron nutrition began as early as the 18th century (74). Use of iron supplements began in 1832 and iron deficiency in infants was first noted in 1892. The fact that iron deficiency can be prevented and that it is the most common nutritional deficiency in the United States has been clearly documented (75).

Iron is found most abundantly in the human body in hemoglobin in the erythrocyte and is bound to transferrin in the plasma. Hemoglobin transports oxygen from the lungs to tissues and makes up more than 95% of the red cell protein. More than 10% of whole blood weight is made up of hemoglobin (75).

Hemoglobin is made up of a complex of globins and heme proteins (proteins with an iron-porphyrin prosthetic group). This allows iron to stabilize in the ferrous state so that it may function as an oxygen carrier. Hemoglobin is the most abundant of the heme proteins and makes up >65% of body iron. Hemoglobin synthesis takes place in the final stages of red cell development in the bone marrow (76).

Blood levels of hemoglobin vary among individuals depending on age, sex, nutrition, pregnancy, altitude and disease. Increased levels of hemoglobin in males occur at puberty and continue to rise with age. The highest hemoglobin level for females occurs after menopause or hysterectomy when blood is no longer lost through menses (77). Men average approximately 3.8 grams of total body iron and females average 2.3 grams (78). The protein myoglobin which transports and stores oxygen for use in muscle contraction accounts for approximately 10% of total body iron (75, 78).

Storage iron in the body occurs mainly in the compounds ferritin and hemosiderin. These compounds are found in highest concentrations in the liver, spleen, and bone marrow (76, 80). Iron deficiency anemia will not develop until these iron stores are

almost totally depleted.

Iron is delivered to the tissues by plasma transferrin. Transferrin specific cell membrane receptors bind with the transferrin- iron complex and carry it into the cell where the iron is released (79). Some tissues such as placenta, liver and erythroid precursors contain large numbers of transferrin receptors so uptake of iron in these tissues is quite high (82).

Absorption takes place throughout the small intestines, and absorption is most efficient in the duodenum. This absorption also involves the cell receptors specific for transferrin. During periods of need, iron is more readily absorbed by these receptors (79). The number of receptors decrease when stores are adequate (74).

Absorption of iron is affected by iron stores in the body as well as by age, condition of the gastrointestinal tract and state of health of the individual. The amount and chemical state (heme versus nonheme) of dietary iron ingested and other dietary components also influence absorption (74). Absorption of iron from food varies from less than 1% to greater than 50%, depending on the individual's physiological need for iron, food source and composition of the diet. (78)

Iron is lost mainly in the feces from desquamated mucosal cells and from the loss of minute amounts of blood (80). Little iron is lost through the urine. Females lose more iron than males during childbearing years through menstruation.

Food contains two types of iron. Heme iron is found only in animal products; non heme iron is found in both animal and plant products. Greater than 85% of iron in the diet is non heme. Non heme iron is not absorbed as well as heme iron, and the amount of absorption depends on whole meal composition. If heme sources of iron such as meat, fish or chicken are eaten in combination with non heme iron sources, iron absorption will be approximately four times greater than if no hemo iron were consumed at that meal (81).

Iron deficiency is a nutritional problem in the United States and world-wide, possibly because the iron in the most common foods such as cereals and legumes is poorly

absorbed (82). Iron depletion usually progresses slowly and occurs in three stages. The first stage does not result in any physiological changes. It is characterized by "low iron stores" and may be measured by a decrease in serum ferritin.

Biochemical changes may indicate the second stage of depletion. In this stage, the depletion is severe enough to decrease the normal production of hemoglobin and other iron compounds. This stage can be detected by a decrease in transferrin saturation and an increase in erythrocyte protoporphyrin. In this stage, the hemoglobin level has not yet fallen to the level considered to be true anemia.

The third stage is actual iron deficiency anemia. Hemoglobin production has been suppressed to the point that the hemoglobin concentration is below the normal range of health for an individual. Iron deficiency anemia is diagnosed if it is accompanied by other lab values which are also abnormal such as a low serum ferritin (83).

Iron deficiency anemia is usually caused by decreased absorption of dietary iron, rapid growth or blood loss (85). Indications of iron deficiency anemia vary with the degree of depletion. Studies have shown that anemia may cause a decrease in work capacity (86). Some human studies have shown even mild anemia may compromise exercise performance (84). Other symptoms of iron deficiency anemia include the inability to maintain body temperature in a cold environment (85) and decreased resistence to infection (86).

Since early symptoms of iron deficiency anemia are so subtle, the deficiency is usually first suspected following a low hemoglobin or hematocrit and/or a history of low dietary iron intake. If a low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are found, then there is a strong possibility that the diagnosis is iron deficiency anemia. Additional lab tests such as erythrocyte protoporphyrin, serum ferritin and transferrin saturation may be used to confirm a diagnosis of iron deficiency anemia (83).

Changes in serum iron status following cisplatin chemotherapy have been noted in only a few studies (87). These studies have indicated that cisplatin does affect iron

metabolism. Grau et al (4) were the first to note an increase in plasma iron levels following cisplatin chemotherapy. Twenty patients with stage III lung cancer receiving doses of 120 mg /m^2 of cisplatin were studied. Plasma iron levels were obtained before treatment was begun and again after two courses of cisplatin given at 3 week intervals. Plasma iron concentrations were found to be elevated in fourteen out of twenty patients. The mean initial value was 66.50 ± 29.74 µg/dL. This rose to a value of 127.55 ± 71.47 µg/dL which was a significant increase ($p<0.001$). Plasma iron levels in these patients returned to baseline several months after cessation of cisplatin therapy. Authors of this study concluded that hydration measures and other concomitant drugs given with this therapy were not relevant in this outcome (4).

The effect of cisplatin chemotherapy on iron and unsaturated iron binding capacity (uIBC) was studied in children (87). Records of nine patients from 1984 to 1986 were reviewed. Cisplatin was given at a dose of 90 mg/m^2. These patients received 3 to 9 doses of chemotherapy at 3-4 week intervals. The average serum iron level before therapy was 75.7 ± 30.6 µg/ml. This level rose to 162.0 ± 65.3 µg/ml one day after the first course of cisplatin. The uIBC initially was 181.9 ± 33.0 µg/ml and then dropped to 86.4 ± 44.6 µg/ml after one cisplatin treatment.

Iron and uIBC levels were reviewed before and after the subsequent three doses of cisplatin. A significant decrease in uIBC and an increase in serum iron were noted within twenty-four hours after each cisplatin administration. A cumulative effect was seen in the elevation of iron (150 µg/ml before the fourth treatment of cisplatin to approximately 210 µg/ml after the fourth treatment of cisplatin). There was a decrease in the uIBC (approximately 210 µg/ml before the fourth treatment to approximately 65 µg/ml) after the fourth cisplatin treatment. This clearly indicated cisplatin's effect on iron metabolism. Two to four months after therapy was completed, iron and uIBC returned to baseline levels (87). Since over 90% of cisplatin is bound to proteins in the plasma (88), Kletter et al theorized that the serum iron increase may be the result of the competitive displacement of

iron from plasma proteins which increases the serum level and thus decreases the "free" binding sites (87).

The relationship between cisplatin and impaired erythropoiesis in patients receiving cisplatin was studied (89). Patients were divided into three groups. One group (Group A) of fourteen patients received a total of 30 courses of very high dose chemotherapy 40 mg/m^2 per day for five days in 3% hypertonic saline together with a 120 hour continuous hydration). Group B included 5 patients each of whom received 2 to 4 courses of cisplatin, 20 mg/m^2 per day for 5 days with one hour hydration in combination with other chemotherapeutic drugs. Group C consisted of twelve patients receiving 1 to 3 courses of cisplatin, 100 mg/m^2 on day 1 with 4 hour hydration followed by 5-fluorouracil 1gm/m^2/day as a 120 hour continuous infusion.

Serum iron, ferritin, and hemoglobin concentrations were evaluated before and four and six days after each course of cisplatin. In group A, serum iron increased significantly from pretreatment levels of 68.3 ± 31.8 µg/dl to 185.3 ± 67.8 by day 4 ($p<0.01$) after cisplatin administration. On day 6 levels were 191.6 ± 91.5 µg/dl ($p<0.001$). Ferritin levels in Group A rose from a pretreatment value of 166.5 ± 251.1 µg/dl to 335.8 ± 303.1 µg/dl on day 4 ($p<0.01$) and to 520.7 ± 392.7 µg/dl ($p<0.001$) on day 6.

Group B also showed an increase in serum iron from a pretreatment value of 99.2 $\pm$ 23.9 to 184.8 ± 69.8 µg/dl on day 4 ($p<0.001$) and 161.6 ± 65.4 µg/dl ($p<0.01$) on day 6. Serum ferritin concentration also rose significantly from 158.2 ± 160.3 µg/dl to $301.1 \pm$ 185.4 µg/dl ($p<0.05$) on day 4 and 408.6 ± 379.0 µg/dl ($p<0.05$) on day 6. In group C, serum iron rose from 102.9 ± 37.3 µg/dl to 211.9 ± 66.1 µg/dl ($p<0.001$) on day 4 and to 165.7 ± 64.4 µg/dl ($p<0.001$) on day 6. Ferritin levels rose from 312.0 ± 340.0 µg/dl to 471.2 ± 360.2 µg/dl ($p<0.05$) on day 4 to 491.1 ± 334.0 µg/dl ($p<0.01$) on day 6.

A decrease in the hemoglobin value was also noted in all three groups after only the first course of cisplatin. Group A indicated a 15% hemoglobin fall after the first course of chemotherapy. The lowest mean value was 10.5 ± 2.2 g/dl observed 17.6 ± 3.1 days after

beginning therapy. Hemoglobin concentrations in Group B fell 9% after the first course of therapy with a low mean value of 12.4 ± 1.5 g/dl, 18.2 ± 3.8 days from the beginning of the first course of therapy. An 11% decrease in hemoglobin was noted in group C after the first course of therapy 16.8 ± 4.9 days after starting the first course of chemotherapy. In 27.6% of all courses in group C, a fall in the hemoglobin of greater than 2 g/dl occurred.

Pollera et al concluded that iron status depends on chemotherapeutic dose but is not related to different schedules. The severity of anemia was determined by the cisplatin dose given as well as the hemoglobin pretreatment levels (89).

A previous study by Pollera et al also indicated significant changes in serum iron and ferritin levels following one treatment of cisplatin (92). Eighteen patients receiving 40 mg/m^2 per day for 5 days every 4-5 weeks were studied. Blood samples were drawn for analysis at three different times: the morning before the first drug administration, on the 7th day and 4 weeks from the start of therapy. Serum iron concentration rose progressively during the first four days of cisplatin therapy and reached a peak on day 5 of therapy. In many patients, the serum iron concentration doubled after only 24 hours. These concentrations then decreased to pretreatment levels during the second week after initiation of therapy. Serum ferritin levels began to rise on the second or third day of therapy and reached the highest point on the 7th or 8th day. The levels then returned to baseline after a two week time period. Two way analysis of variance on the first ten days after initiation of therapy indicated these changes were significant ($p<0.001$) for both iron and ferritin. The average hemoglobin concentration was increased on day 7 after treatment was finished and also returned to baseline within the second week after the start of therapy. The significant increase of serum iron and its rapid fall after discontinuation of therapy would indicate a direct effect of cisplatin on iron (90). Because the same patterns of changes in iron and ferritin were noted in all groups, the cisplatin therapy was considered to be directly responsible (89, 90).

There are several theories on the mechanism of cisplatin-induced iron mobilization.

Increased iron mobilization would increase serum iron and affect ferritin values. Concepts such as an erythropoietin deficiency syndrome secondary to nephrotoxicity of cisplatin (91), possible inhibition of erythropoesis by cisplatin (92) and the competitive displacement of iron from proteins in the plasma (87) have been suggested. Further studies are needed to clarify the mechanism by which cisplatin affects iron status.

Selenium

A requirement for selenium in human nutrition was not observed until 1979 when an association between selenium and a cardiomyopathic disease in young children was established in China (93). A simultaneous deficiency of selenium and vitamin E causes many symptoms which can be prevented or cured by supplementation with either nutrient (93). Selenium deficiency in living animals with adequate vitamin E intake has been observed only under experimental conditions. An absolute selenium deficiency was seen in rats which were fed a low selenium diet adequate in vitamin E. This resulted in poor growth, cataracts, and reproductive failure (94). Chicks fed diets severely deficient in selenium suffered from nutritional pancreatic atrophy (95).

Selenium is found in the red blood cell in two different compartments or forms. In glutathione peroxidase and selenoprotein P (a selenium transport protein in some animals), it is found as selenocysteine. The other form is selenomethionine which is derived from the diet and is not synthesized by the body. Selenomethionine is not regulated by the selenium status of the animal; it is considered to be an "unregulated" storage compartment. It may be incorporated into proteins in the place of the amino acid methionine (96). The selenomethionine pool provides selenium to the individual should the dietary supply become depleted (97).

The selenocysteine compartment is regulated and is responsible for the biological activity of selenium. Selenocysteine is synthesized and inserted into proteins by a specific mechanism which is still under investigation. Excess selenium is excreted if it is not

needed for selenocysteine formation. There may be other forms of tissue selenium that have not yet been identified (96).

Selenium functions as an antioxidant. Hydrogen peroxide and other free radicals are removed by glutathione peroxidase, a selenoenzyme present in cells and in plasma (98). Fatty acid hydroperoxides present in phospholipids are metabolized by the selenium containing phospholipid hydroperoxide glutathione peroxidase (99). Selenium may also have additional antioxidant effects (100).

Absorption of organic and inorganic selenium appears to be greater than 50%. Selenomethionine is processed metabolically the same as methionine (96). It is inserted into proteins in place of methionine and becomes a part of the tissue selenium. The selenium in selenomethionine is not released until the selenomethionine is broken down freeing selenium for use for specific activities (96). Methionine intake determines the rate of selenomethionine catabolism (101). A low methionine intake may result in a selenium deficiency which would cause decreased glutathione peroxidase activity. Because dietary selenomethionine is sequestered in protein, decreased glutathione peroxidase activity may occur despite adequate selenium intake. High methionine intake leads to catabolism of methionine and selenomethione resulting in release of selenium and repletion of the selenium deficiency (102).

Selenocysteine is formed when selenium is incorporated into serine in place of oxygen. It is then combined with a selenoprotein such as glutathione peroxidase (103). The enzyme selenocysteine B-lyase is presumed responsible for the catabolism of selenocysteine as well as the release of selenium from dietary selenocysteine (104). Selenium is excreted through the urine (96).

Selenium influences many biochemical activities. The activities of drug metabolizing enzymes may be affected by a selenium deficiency (104). Selenium deficiency studies in the rat indicate that selenium deficiency may lead to increased hepatic glutathione synthesis, increased hepatic heme turnover and changes in the utilization of

glucose (105).

Several diseases have been linked to selenium deficiency. Keshan disease, a cardiomyopathy affecting women and children in China, is controlled by selenium supplementation (106). Low selenium status may also be associated with a type of osteoarthritis, Kashin-Beck disease.

Selenium concentration in fluids and tissues may be measured in a number of ways including fluorometry, neutron activation analysis, atomic absorption and mass spectrometry (96). Measurement of glutathione peroxidase activity (107) and selenoprotein P (110) concentration may be used to estimate the biologically active selenium.

The metabolism of selenium must be understood in calculation of selenium concentration. Because selenomethione is substituted for methionine in some proteins, intake of this form of selenium will result in a higher tissue selenium concentration than intake in the selenocysteine form (96).

Intake of selenium in the selenocysteine form will affect only the selenocysteine compartment. Since this compartment is regulated, it may be used to determine selenium deficiency. Once the selenium requirement is met, it may not be used to determine selenium status (96).

The relationship between selenium and cancer has been under scrutiny for many years. Interest increased when selenium was found to be necessary for the formation of the antioxidant enzyme, glutathione peroxidase (98). Most patients in case controlled studies with cancer had lower blood concentrations of selenium than the controls (109). This could be misleading because cancer may decrease selenium status in a number of ways. The cancer may sequester the selenium (110), cause reduced dietary intake and reduce or alter nutrient absorption. As the disease advanced, the patients' blood selenium concentrations continued to drop (111) and the more advanced the disease, the lower the selenium concentration (112).

Willett et al found that average prediagnostic serum selenium values among cancer

patients were significantly lower than matched controls (113). Other studies have been less conclusive with both positive and negative results (109). There are many reasons for the conflict in the various studies which include geographical factors (soil concentration differences), dietary factors, and the relationship of selenium status with the concentraton of other nutrients such as vitamin E. Larger studies with adequate variations in selenium concentration and a sufficient number of specific cancers are needed to document further the role of selenium and its effect on human cancer (109).

No studies were found on selenium excretion in relation to cisplatin chemotherapy. However, selenium has been reported to decrease the nephrotoxicity of cisplatin when given before the treatment cycle (5, 114).

The exact mechanism of action of selenium on cisplatin is not clear. An interaction between selenium and the cisplatin molecule which prevents reabsorption by renal cells but does not impede antitumor activity has been hypothesized. This interaction may possibly increase the benefits of cisplatin and reduce toxicity (114). Further study is needed to determine the validity of such theories.

Manganese

A deficiency of manganese in animal nutrition was first recognized in mice and rats in 1931 (115). Manganese's role and possible deficiency in human nutrition was established more recently (116).

Distribution of tissue concentrations of manganese throughout the human body varies little from one individual to another (116). Manganese concentration is the highest in the mitochondria; manganese also is found in pigmented areas such as the retina and melanin granules. Tissues containing the highest concentrations of manganese are bone, liver, pancreas, and kidney. Total body manganese is highest in the bone (up to 25%), due to its mass. Dietary intake may influence bone manganese concentration.

Manganese functions as a component of metalloenzymes such as arginase,

manganese superoxide dismutase and pyruvate carboxylase (116). Arginase is responsible for urea formation. Rat studies have indicated that a manganese deficiency influences the activity of arginase (116). Pyruvate carboxylase catalyzes the initial steps of carbohydrate synthesis from pyruvate. Manganese deficiency lowers the activity of this enzyme but not enough to actually inhibit gluconeogenesis (117).

Manganese superoxide dismutase activity which catalyzes 0_2^- to H_20_2 and O_2 is also reduced in the tissues of manganese deficient rats (118). Manganese acts as an enzyme activator in various hydrolases, kinases, decarboxylases and transferases (119). Two enzymes which are specificially activated by manganese are the glycosyltransferases and phosphoenolpyruvate carboxykinase (117). Several deficiency induced pathologies and low activities of these enzymes were reported in manganese deficient animals (117).

Intestinal transport of manganese is not clearly understood. Perfusion studies have indicated that intestinal absorption of manganese is possibly a rapid saturable process which involves a high-affinity, low-capacity active-transport system (120). Manganese enters the portal blood from the gastrointestinal tract and may associate with alpha$_2$-macroglobulin or remain free. It then travels to the liver where it is almost totally removed. A small amount may enter the systemic circulation where it is oxidized to Mn^{3+} and bound to transferrin (119).

Once manganese enters the liver, it is transported to five metabolic pools. One pool represents the manganese which is taken up by lysosomes which then may be transferred to the bile canaliculus. Biliary excretion may regulate manganese in this area; 50% of intravenously injected manganese may be recovered in the feces within 24 hours. Mitochondria have a capability for manganese uptake, and this is associated with the second pool of manganese. The nuclear portion of the cell also contains a pool of manganese; the role of manganese in this area has not been defined. A fourth pool of manganese is included in the synthesis of newly formed proteins (118). Manganese is transported to and absorbed by extrahepatic tissues by a mechanism that has not been

identified. It is known that transferrin is the major manganese binding protein but it is not known to what extent transferrin assists this uptake (121). The fifth pool of manganese is free Mn^{2+}. Fluctuations in the free manganese pool may regulate cellular metabolic control similar to that of free Ca^{2+} and Mg^{2+} (118, 119).

Absorption of manganese is believed to occur throughout the small intestine. Manganese absorption is relatively low and is not thought to be under homeostatic control (119). Absorption of manganese in adults was reported to be from 2% to 15% using manganese labeled test meals. Balance studies reported absorption to be greater than 25% (122, 123).

Information regarding the effect of dietary components on manganese absorption is limited. Animal studies have indicated a need to increase manganese when consuming high amounts of calcium, phosphorus, fiber and phytate. This may be due to formation of insoluble manganese complexes in the intestinal tract which reduces the amount of manganese available for absorption . Iron also plays a role in manganese absorption. Experimental animal and human studies indicated that manganese absorption increased in the presence of iron deficiency; high dietary iron intake has been shown to cause a manganese deficiency in some species. The mechanism responsible for this phenomena is not clear (116).

Manganese deficiency has been observed in several animal species. These symptoms include impaired growth and reproductive performance, skeletal abnormalities, ataxia and complications in lipid and carbohydrate metabolism . Bone defects in the form of shortened and thickened limbs, curvature of the spine and swollen and enlarged joints due to manganese deficiency have been observed in most species (116). The probable cause for these abnormalities in animals is the reduction of the activity of glycosyltransferases secondary to manganese deficiency.

Another area where manganese deficiency has a profound effect is in pregnant animals. These offspring have irreversible ataxia with lack of equilibrium and head

retraction. Manganese deficient rats and guinea pigs also have been noted to have defects in carbohydrate matabolism with abnormal glucose tolerance (119).

Manganese deficiency also affects pancreatic function. Manganese deficient rats may exhibit depressed pancreatic insulin synthesis and increased intracellular insulin degradation (124). This effect of manganese deficiency on insulin production may be secondary to the destruction of pancreatic beta cells (119).

Manganese deficiency affects pancreatic exocrine function. Manganese-deficient rats showed an increase in pancreatic amylase content presumably due to a shift in amylase synthesis or degradation (125).

Manganese deficiency may result in abnormal lipid metabolism (116). Manganese deficient animals were found to have high liver fat concentration. Manganese is believed to play a role in steroid biosynthesis, and hypocholesterolemia has been observed in deficient animals. Abnormal lipid metabolism was shown in deficient rats which were found to have lower high-density-lipoprotein concentrations than controls (126). These rats also exhibited a shift to smaller plasma HDL particles, lower HDL apolipoproteins (apo E) concentrations and higher apolipoproteins C concentrations (126).

Research has indicated that a deficiency in manganese can lead to a number of biochemical and structural defects in experimental animals. Manganese deficiency in humans was first observed in 1972 (118). Friedman et al (127) studied a group of seven young men fed manganese-deficient diets (0.11 mg Mn/d) for 39 days. During this time, all subjects exhibited negative manganese balance and five of the seven subjects developed a fleeting dermatitis that disappeared once manganese status was restored. Serum calcium, phosphorus and alkaline phosphatase concentrations increased consistently during the depletion period which concurred with the suggestion previously made by Strause and Saltman (128) that manganese deficiency may affect bone remodeling. Although manganese deficiency has not been observed in humans consuming regular diets, there are some diseases which have been linked to manganese irregularities. Some individuals with

epilepsy have been found to have low blood manganese concentrations and children with maple syrup urine disease and phenylketonuria have been reported to have low tissue manganese concentrations (129). Furthermore, manganese deficiency may be a possible factor in the development of joint disease, hip abnormalities, congenital malformations and osteoporosis (129). Despite these findings, evidence of manganese deficiency in the general population is rare. Manganese toxicity in humans is a serious health hazard which may result in severe neurologic and psychologic disorders (130). As a result, there is little support for manganese supplementation of the typical diet.

Research regarding manganese levels in cancer patients has been scarce and generally contradictory (131). Capel and associates studied trace elements including serum manganese in patients with various tumors while they were receiving radiotherapy (7). These patients' sera was monitored at four week intervals over a 5 month period and compared with control patients from a healthy population. Blood samples were obtained after an overnight fast. Results indicated no significant differences in the serum manganese levels of the controls and the cancer patients (7). Previous studies had indicated higher levels of manganese in breast tumors than in normal breast tissue (131). However, this study and another report (6) concluded that such tissue increases were not enough to influence serum levels.

Sullivan et al investigated the role of trace elements in various diseases and concluded that evaluation of status becomes difficult because there are nutritional as well as metabolic interactions among the different trace elements (6). Patients were studied only after acute phases of disease or shock. Blood was drawn from the patient and control group after an overnight fast. Serum manganese was studied using neutron activation analysis. Serum from the group with malignancies was elevated slightly (0.011 ± 0.011 ug/ml) compared to the control group (0.006 ± 0.004 µg/ml). The serum manganese was more significantly elevated in those patients with arteriosclerotic congestive heart failure, infection and psychoses when compared to the control group.

It appears that levels of particular essential minerals are variable depending upon the the individual's health status as well as the type of cancer or disease. Routine measurement of trace mineral levels should be done in each individual patient so that proper supplementation can be administered where necessary (7).

CHAPTER III

METHODS

Study Population

This study was performed on a total of fourteen patients receiving cisplatin chemotherapy for various types of malignant tumors. A description of the patient population is given in table I. Other chemotherapeutic drugs listed (Table I) are commonly given in conjunction with cisplatin chemotherapy for additional antineoplastic therapy. They are not known to cause nephrotoxic damage to the renal tubule. The first patient entered into this study on September 22, 1993. The last patient entered on October 10, 1995. The amount of time that each patient remained on the study was dependent on the number of chemotherapeutic cycles received. This varied from a minimum of 2 months to a maximum of 6 months. This study was approved by the Institutional Review Board for Protection of Human Subjects at Oklahoma State University, Stillwater, Oklahoma and the Institutional Review Board and the Cancer Committee at St. John Medical Center, Tulsa, Oklahoma. The patients were referred to the study by two oncologists. Details of the study were explained to the patient and a patient information sheet was provided. A consent form was signed and a copy was given to the patient.

Experimental Design

Twenty-four hour urine samples were collected and blood (for serum sampling) was drawn prior to the initial chemotherapy, following the second cycle of chemotherapy, and after all chemotherapy was completed. The time period that elapsed after completion of chemotherapy before the last sample was drawn varied with some patients (Table I).

Table I: Patient Demographics

Patient Number	Age	Sex	Tumor	Body surface area/m^2	Total cisplatin dose given/ mg	Additional chemotherapeutic drugs given	Serum/urine interval post treatment
1	51	Male	Melanoma	1.81	405	carmustine	1 month
2	54	Male	Lung	1.84	600	mutamycin/vinblastine	5 months
3	65	Female	Ovarian	1.78	399	Taxol	1 month
4	65	Male	Lung	1.85	540	etoposide/vincristine	1 month
5	45	Female	Cervical	1.81	540		3 months
6	62	Female	Ovarian	1.75	780	Taxol	4 months
7	73	Male	Lung	2.0	470	etoposide/vincristine	4 months
8*	52	Female	Lung	1.65	640	etoposide	31/2 months serum 5 months urine
9**	68	Male	Lung	1.99	600	etoposide/vincristine	1 month
10	79	Female	Ovarian	1.68	751	Taxol	1 month
11	52	Male	Lung	1.77	600	etoposide	2 months
14	58	Male	Lung	2.15	200	etoposide	6 weeks
15	61	Female	Lung	1.5	450	etoposide	2 months
16	61	Male	Lung	1.75	360	etoposide	4 months

*Samples collected before 4th dose of therapy
** Samples collected before 2nd dose of therapy

Samples were drawn at the same time that routine lab work was being done for their attending physician.

Experimental procedure

Cisplatin dosage for each patient was calculated according to body surface area and protocol recommendations. The patients were also given intravenous infusion of saline and mannitol with antiemetics prior to therapy.

Instructions were given to the patient by the lab technologist or a registered nurse for the twenty four hour urine collection. The urine was collected in standard 24 hour urine collection containers which were stored in plastic bags to limit trace mineral contamination. The patient was instructed not to touch the top of the container or the urine funnel and to keep the container and funnel covered at all times. The first void was to be discarded; thereafter for the next 24 hours, all urine was to be collected in the urine container. After the container was returned to the laboratory, an aliquot of urine from the 24 hour collection was pipetted into a trace mineral free sample tube. The total 24 hour urine volume was recorded. All urine samples were labelled and frozen immediately after collection.

Blood samples were drawn using 21G x 1 1/2" trace mineral free leur cone needles into Sarstedt vacutainers containing gel and clot activator. The samples were centrifuged at 1500 to 2000 revolutions per minute for ten minutes. Serum was removed with plastic pipettes and placed in trace mineral free tubes, labelled and frozen.

Serum samples were prepared for trace element concentration using a combination of wet and dry ashing methods. Serum from each collection was pipetted into acid washed borosilicate glass tubes. To these samples a half volume of concentrated nitric acid was added and they were placed in an isotemp heating block at 85 degrees for 2 hours. Hydrogen peroxide was added every two hours to each sample until they were completely ashed and white in color (132). After ashing and evaporating to dryness, samples were diluted with 0.5% nitric acid (double distilled, G. Fredrick Smith, Columbus OH). Time

and amount of additions to each set of samples were recorded (132).

Serum samples analyzed for magnesium content were diluted with 0.5% nitric acid containing 0.1% lanthanum as chloride. Samples were then analyzed for serum concentrations of magnesium, copper, zinc, iron and urinary concentrations of magnesium and zinc by a Perkin-Elmer Model 5100PC atomic absorption spectrophotometer using an air acetylene flame at the most sensitive wave length for each element (133). Serum concentrations of manganese and selenium and urinary concentrations of manganese and iron were determined using a HGA 600 graphite furnace and Zeeman background correction(134). The same set of ashed samples were used for all serum analysis; urine samples were not ashed prior to analysis.

Serum albumin, serum creatinine and urinary creatinine were analyzed using standard methods on a Roche Fara clinical analyzer. Creatinine clearance was calculated using 24 hour urinary creatinine formula: Milliliters of plasma cleared per minute per standard surface area $= \frac{U_{cr} \times V}{P_{cr}} \times \frac{1.73}{A}$, where U_{cr} = concentration of creatinine in urine; P_{cr} = concentration of creatinine in plasma in the same units as for urine; V=volume of urine flow in ml per minute; A=body surface area in square meters (135).

Statistical Methods

Data including means and standard errors of the mean were processed stasticially using the PC SAS software. Changes in values over time were analyzed using a paired "t" test. Effects of higher dose versus lower doses were compared using the generalized linear model (GLM) (136).

CHAPTER IV

CISPLATIN CHEMOTHERAPY EFFECTS ON TRACE ELEMENT EXCRETION AND SERUM CONCENTRATION

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Introduction

Cisplatin (cis-diamminechloroplatinum II) is an inorganic platinum compound that has been used for over twenty years as a chemotherapeutic agent for several types of solid tumors. It is used primarily in the treatment of lung, ovarian and head and neck carcinomas (1). Severe nephrotoxicity is a major side effect of cisplatin use. This toxicity causes tubular dysfunction which results in hypomagnesemia and hypokalemia (2). A cumulative toxicity occurs to the glomeruli of the kidney leading to decreased glomerular filtration rate (2).

The effect of kidney damage from cisplatin on trace element levels in serum and excretion in urine is unclear. Renal excretion of iron and manganese with cisplatin treatment has not been investigated. Sartori et al reported increased renal excretion of magnesium (3). Sweeney et al reported increased urinary excretion of zinc in the urine following cisplatin. The authors stated this increase did not reach significance due to small sample size (4).

Serum levels of magnesium decrease with cisplatin use (3), whereas iron and zinc levels have shown an increasing trend (4,5). Serum levels of copper have shown little change with cisplatin chemotherapy (6).

This study was designed to examine the serum concentrations and total urine excretion of selected trace elements in patients receiving cisplatin chemotherapy after two

cycles and after the completion of therapy.

Methods

This study was performed on a total of fourteen patients receiving cisplatin chemotherapy for various types of malignant tumors. Each patients' cisplatin dosage was calculated according to body surface area and protocol. Patients were given intravenous infusion of saline and mannitol with anti emetics prior to therapy. A consent form was signed by each patient. This study was approved by the Institutional Review Board for Human Subjects Research at Oklahoma State University, Stillwater, Oklahoma and the Institutional Review Board and Cancer Committee at St. John Medical Center, Tulsa, Oklahoma. A description of the patient population is given in table I (see Chapter 3).

Blood (for serum sampling) and twenty-four hour urine collections were collected from each patient prior to initiation of chemotherapy; prior to their third cycle and at completion of chemotherapy. The time period that elapsed after completion of chemotherapy before the last sample was drawn varied with some patients (Table I).

All blood and urine samples were collected in trace mineral free plasticware and processed in acid washed glass. Serum samples were analyzed for magnesium, copper, zinc, iron and urine samples for magnesium and zinc using a Perkin-Elmer Model 5100 PC atomic absorption spectrophotometer using an air acetylene flame. Serum concentrations of manganese and selenium and urinary concentrations of manganese and iron were analyzed by the graphite furnace with Zeeman background correction. Serum albumin, serum creatinine and urine creatinine were analyzed using standard methods on a Roche Fara clinical analyzer.

Data were processed statistically using the PC SAS software. Changes in values over time were analyzed using a paired "t" test. Effects of higher dose cisplatin versus lower dose were compared by using analysis of variance using the generalized linear model (GLM) procedure.

Results

Table I lists demographic data of the patient population including the total cisplatin dose delivered to each patient and time interval that elapsed between the completion of therapy and collection of blood and urine samples. Tables II and III summarize the serum albumin, creatinine clearance and the mean serum concentration and urinary excretion of the trace elements analyzed. These levels were measured prior to starting cisplatin therapy; before the third cycle; and at various times after the completion of all therapy.

Serum albumin showed little change throughout the entire course of cisplatin chemotherapy regardless of a change in weight. These patients were found to have a significant weight loss from the beginning of the study to completion of chemotherapy ($p=0.0020$). (Data not shown). Serum magnesium concentrations dropped from the baseline value of 608 ± 38 µmol/L to 508 ± 37 µmol/L ($p=.002$) after two cycles of cisplatin. A tendency to decrease occurred in serum magnesium between baseline of 608 ± 38 µmol/L and the last level of 522 ± 35 µmol/L ($p<0.06$). Serum zinc concentration showed a tendency to increase from a baseline of 8.8 ± 0.5 µmol/L to 10.2 ± 0.4 µmol/L after the second cycle ($p<0.09$). There was a significant decrease following the second cycle from 10.2 ± 0.4 µmol/L to 8.8 ± 0.4 µmol/L after the final cycle ($p<.009$).

Serum copper concentrations changed only slightly and nonsignificantly from the baseline of 23.7 ± 2.1 µmol/L to 22.9 ± 1.4 µmol/L after the second cycle. After therapy was completed, serum copper was 24.4 ± 2.0 µmol/L. Serum iron concentrations were 13.4 ± 2.2 µmol/L initially and 17.8 ± 2.6 µmol/L after the second cycle. Eight out of fourteen patients had a higher serum iron concentration after the second cycle of therapy, even though the increase was not significant. Nine patients had a higher serum iron values after completion of therapy. Little change was noted in the serum selenium or manganese before and after chemotherapy with cisplatin.

Creatinine clearance showed a decreasing trend from 105.6 ± 14.0 mmol/d to 80.5 ± 7.4 mmol/d after the second cycle ($p<0.117$) to 57.7 ± 7.7 mmol/d after all therapy was completed ($p<0.006$). Initial magnesium excretion in the urine was $1.92 \pm .54$ mmol/d and

2.00 ± .39 mmol/d after the second cycle. After completion of therapy, the level was 2.23 ± .32 mmol/d. Urinary zinc excretions were 10.8 ± 1.7 mmol/d before therapy, 9.9± 2.2 mmol/d after the second cycle and 7.5 ± 2.9 mmol/d after the completion of all therapy. This decrease was not showed significant. Iron levels in the urine tended to decrease from baseline of 0.75 ± .12 μmol/d to 0.46 ± .05 μmol/d after the second cycle of therapy ($p<0.06$). At completion of therapy, urinary iron was 0.51 ± .09 μmol/d ($p=.12$). Urinary manganese was quite variable but showed no significant change throughout the entire chemotherapeutic cycle.

Patients were grouped according to dose (those who received a total of 540 mg or more of cisplatin versus those who received less then 530 mg for statistical analysis. There was a significantly greater decrease in creatinine clearance in the higher dose group ($p=.02$) than the lower dose group. After two cycles of therapy, patients exposed to greater amounts of cisplatin had slightly higher urinary magnesium concentrations compared to those who were exposed to lesser amounts ($p=.09$). No significant increase or decrease of the remaining trace elements in serum or urine was observed, based on total dosage.

Hematological parameters are outlined in Table IV. Hemoglobin values dropped significantly from 127± 4 g/L to 111 ± 5 g/L ($p=0.020$) after the second cycle. This level then declined to 100 ± 5 g/L ($p=0.0004$) after completion of therapy. Hematocrit levels decreased significantly from 0.38 ± 0.01 to 0.32 ± 0.01 ($p<0.005$) and then declined to 0.30 ± 0.01 ($p<0.0001$). Leukocytes dropped significantly from 10.6 ± 1.02x10^9/L to 7.3 ± 1.09 10x^9/L ($p<0.004$), then appeared to rise slightly at 8.7 ± 1.77x10^9/L. Platelets showed a significant decrease from 404 ± 49x10^9/L at baseline to 289 ± 50x10^9/L ($p=.0012$) after therapy was completed.

Discussion

Cisplatin induced hypomagnesemia has been previously reported (3,6,7) apparently as a result of significant impairment in proximal tubular resorption and distal tubular function (2,8). Our study showed a decrease in serum magnesium concentrations in

fourteen patients between the serums obtained before therapy and the second sample that was drawn after the second cycle of therapy. Magnesium levels then rose slightly after the completion of cisplatin. Four out of the fourteen patients' serum magnesium levels returned to or exceeded the baseline level 1 to 4 months after completion of therapy. Magnesium excretion in the urine appeared higher than baseline after the second cycle in 50% of the fourteen patients and after completion of chemotherapy in 57% (8 patients). This has been described in previous studies but followup was not as long as in our study (3,6) Since cisplatin therapy results in hypomagnesemia, many oncologists supplement their patients with magnesium during and after cisplatin chemotherapy. Patients in this study were prescribed magnesium supplements of 1,000 to 3,000 mg per day based on their serum magnesium levels. These supplements were prescribed throughout their chemotherapeutic cycle and after completion of therapy which may explain the apparent recovery of serum magnesium of patients on our study.

Previous studies have indicated that kidney impairment may last for days to months after completion of therapy (2). The significant decrease seen in the creatinine clearance after completion of therapy supports the acute and chronic renal damage. The continued urinary loss of magnesium despite improving serum levels could indicate that tubular function improves only slightly and that increased oral intake of magnesium is responsible for higher serum and urine levels.

The effect of cisplatin chemotherapy on serum zinc concentrations is unclear. Our study showed a tendency for increases in the serum zinc concentrations after the second cycle when compared to the initital value. This agrees with Zumkely et al, who found that baseline serum zinc concentrations increased 48 hours following treatment. (6).

However, our study showed serum zinc levels return to baseline after the final cycle of cisplatin. Urinary excretion of zinc in our study appeared to decrease in 50% of the patients after completion of chemotherapy. Zumkley et al found decreasing urinary zinc levels when baseline values were compared with levels taken three days after treatment with

cisplatin (6). The reason for changes in zinc and urine zinc concentrations is unclear.

Other studies have indicated that cisplatin use may result in increased excretion of the amino acids histidine and cysteine which have been shown to increase urinary zinc. It has also been suggested that cisplatin may cause increased release of intracellular zinc due to tumor lysis (4). These two studies would predict an increase rather than a decrease in urine zinc concentrations might be expected. The mechanism for decreased urinary zinc remains unexplained.

Previous studies indicated an increase in plasma iron levels following cisplatin therapy (5,9). Our study showed levels in serum iron concentrations at baseline to be 13.4 $\pm$ 2.2 µmol/L and 17.8 $\pm$ 2.6 µmol/L after the second cycle. Fifty-seven percent of the fourteen patients had an increase in levels during this time. The final value was 17.1 $\pm$ 1.7 µmol/L with 64% of the patient population's serum iron levels increased. Explanations for increased serum iron levels during cisplatin chemotherapy vary. The nephrotoxicity caused by cisplatin may induce an erythropoietin deficiency resulting in decreased bone marrow utilization of iron (10). Inhibition of erythropoiesis secondary to cisplatin therapy (11) or a competitive displacement of iron from proteins in the plasma would also explain higher iron levels (12).

Urinary iron excretion in our study appeared to decrease after the second course of chemotherapy. No previous studies were found that investigated urinary iron excretion in patients receiving cisplatin. Why urine iron excretions decreased is unclear. Creatinine clearance also decreased during this time period; perhaps less iron was filtered thus causing the subsequent tendency to increase in the serum iron concentrations and decreased urine iron.

Few data exist describing the effect of cisplatin on serum copper concentrations No significant change in serum copper concentrations during cisplatin therapy was found in a previous study (6). Our study also showed no significant change in serum copper or manganese from baseline levels throughout the entire course of therapy.

No prior studies were found on serum selenium concentrations in patients receiving cisplatin chemotherapy. However, Berry et al (13) has suggested that selenium may reduce the nephrotoxicity of cisplatin when given before the treatment cycle It is hypothesized that a complex of cisplatin and selenium prevents reabsorption of cisplatin by renal cells. This hypothesis has not been confirmed. Our study showed a very slight but insignificant downward trend in serum selenium levels from baseline through the completion of therapy. Further studies will be necessary to determine if decreasing serum selenium levels increase nephrotoxicity of cisplatin.

For further analysis, the patient population was divided into two groups: those who received 540 mg of cisplain and above those who received 530 mg of cisplatin and below. Our study showed a significant increase in creatinine clearance in the high dose group (p=.02). There was no difference in renal metabolism of the trace elements in the serum or the urine when looking at total dose exposure to cisplatin. It is unknown whether higher doses of cisplatin would effect serum or urinary concentrations of trace elements.

Hematological parameters showed significant decreases in hemoglobin, hematocrit, and leukocytes after the second cycle of therapy. Platelet count, hemoglobin and hematocrit showed significant decreases after completion of therapy. This confirms previously described bone marrow suppression from cisplatin therapy in chronically ill patients. If cisplatin causes erythropoietin deficiency as previously suggested (10), this may also contribute to decreased red blood cell concentrations in these patients.

Conclusion

We conclude that cisplatin therapy causes a significant decrease in serum magnesium concentrations after the second cycle. A decreasing trend in serum magnesium concentrations after completion of therapy was found but was not significant. Serum concentrations of zinc tended to increase slightly, then returned to baseline values after completion of chemotherapy. Serum selenium, manganese, copper and iron concentrations

showed no significant change throughout the entire course of therapy. Urinary concentration of magnesium appeared to increase after completion of therapy. Total urinary iron appeared to decrease after two cycles of therapy. Cisplatin has little effect on urinary zinc or manganese concentration. Creatinine clearance showed a significant decrease throughout the entire cisplatin treatment.

A significant decline occurred in hemoglobin and hematocrit levels following the second cycle and completion of therapy. Leukocytes declined significantly following the second cycle and platelets showed a significant drop in levels after completion of chemotherapy.

Table II - Serum concentrations of albumin and selected trace elements at indicated times in relation to cisplatin chemotherapy

	(1) Before therapy	(2) After 2nd cycle	(3) After final cycle	P Value 1 v 2	P Value 1 v 3
Albumin (g/L)	43.7 ± 2.99 (14)	51.2 ± 29 (14)	48.3 ± 2.45 (14)		
Magnesium (µmol/L)	608 ± 38 (14)	508 ± 37 (14)	522 ± 35 (14)	p=0.002	p<0.06
Zinc (µmol/L)	8.8 ± 0.5 (12)	10.2 ± 0.4 (12)	8.8 ± 0.4 (12)	p<0.09	p=0.009 (2 v 3)
Copper (µmol/L)	23.7 ± 2.1 (14)	22.9 ± 1.4 (14)	24.4 ± 2.0 (14)	P<0.70	p<0.72
Iron (µmol/L)	13.4 ± 2.2 (14)	17.8 ± 2.6 (14)	17.1 ± 1.7 (14)	p<0.23	p<0.23
Selenium (nmol/L)	1734 ± 213 (11)	1633 ± 131 (11)	1585 ± 298 (11)	p<0.58	p<0.71
Manganese (nmol/L)	176.2 ± 21.1 (12)	162.4 ± 14.2 (12)	194.6 ± 28.6 (12)	p<0.50	p<0.61

Table III: Urinary creatinine clearance and urine excretion of selected trace elements at indicated times in relation to cisplatin chemotherapy

	(1) Before therapy	(2) After second cycle	(3) After final cycle	P Value 1 v 2	P Value 1 v 3
Creatinine Clearance (mmol/d)	105.6 ± 14.0 (14)	80.5 ± 7.4 (13)	57.7 ± 7.77 (14)	p<0.117	p<0.006
Magnesium (mmol/d)	1.92 ±.54 (14)	2.00 ± .39 (13)	2.23 ± .32 (14)	p<0	p<0.60
Zinc (mmol/d)	10.8 ± 1.7 (14)	9.9 ± 2.2 (13)	7.5 ± 2.9 (14)	p<0.60	p<0.27
Iron (µmol/d)	0.75 ± 0.12 (14)	0.46 ± 0.05 (13)	0.51 ± 0.09 (14)	p<0.06	p=0.12
Manganese (nmol/d)	1.43 ± .384 (14)	1.36 ± .497 (13)	2.81 ± 1.32 (14)	p<0.73	p<0.26

Table IV - Hematological parameters at indicated times in relation to cisplatin chemotherapy

	(1) Before therapy	(2) After 2nd cycle	(3) After final cycle	P Values 1 v 2	1 v 3
Hemoglobin (g/L)	127 ± (14)	111 ± 5 (14)	100± 5 (14)	p= 0.020	p=0.0004
Hematocrit (l)	0.38 ± 0.01 (14)	0.32 ± 0.01 (15)	0.30 ± 0.01 (15)	p<0.005	p=0.0001
Leukocytes 10^9/L	10.6 ± 1.02 (14)	7.3 ± 1.09 (14)	8.7 ± 1.77 (14)	p<0.004	p<0.28
Platelets 10^9/L	404. ± 49.1 (14)	353 ± 51.6 (14)	289 ± 50.1 (13)	p<0.21	p=0.0012

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CHAPTER 5

Summary

The effect of cisplatin chemotherapy on trace elements in serum and their excretion in urine was studied in fourteen patients receiving cisplatin for various types of malignant tumors. Urine samples were collected and blood samples were drawn from each patient prior to beginning chemotherapy; after the second cycle and at various times after completion of therapy. Serum concentrations of magnesium, zinc, copper, iron, selenium and manganese were investigated. Urinary concentrations of magnesium, zinc, iron and manganese were also measured.

Conclusions

We conclude that cisplatin therapy causes a significant decrease in serum magnesium concentrations after the second cycle as well as a decreasing, but non-significant, trend in serum magnesium concentrations after completion of therapy. Serum concentrations of zinc appeared to increase slightly, then returned to baseline values after completion of chemotherapy. Selenium, manganese, copper and iron serum concentrations showed no significant change throughout the entire course of therapy. Urinary concentration of magnesium appears to increase after completion of therapy. Total urinary iron appeared to decrease after two cycles of therapy. Cisplatin has little effect on urinary zinc or manganese concentration. Creatinine clearance shows a significant decrease throughout the entire cisplatin treatment.

A significant decline occurred in hemoglobin and hematocrit levels following the second cycle and completion of therapy. Leukocytes declined significantly following the

second cycle and platelets showed a significant drop in levels after completion of chemotherapy.

Recommendations

The following recommendations for future research were developed from this study:

1) Final sample collection should be done at the same time interval for all patients, i.e, last urine collection and blood draw should be done one month after completion of chemotherapy for all participating patients. Too much time variation between last sample collection intervals makes it difficult to draw any conclusions regarding those levels.
2) Increase number of patients on the study if clinically possible. Too small patient population reduces chances for significance.
3) Increase amount of serum collected for analysis. Some serum samples were not available for analysis (selenium) due to shortage of serum.
4) Consider erythropoietin analysis to help reach conclusions.

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APPENDIX

APPENDIX A

CONSENT FORM

I, _____, hereby give consent for my participation in the project entitled, "The Effects of Cisplatin/Carboplatin Based Chemotherapy on Trace Element Excretion". The research for this project will take place at intervals during the entire length of my chemotherapy. I authorize LaFortune Cancer Center to perform the following treatment or procedures:

1. I understand that my physician requires a blood sample to analyze kidney function at routine intervals throughout my Cisplatin therapy. I agree to allow an additional 10 ccs of blood to be collected during these times to be used in the research of this study.

2. I understand that I will be asked to provide a 24 hour urine collection sample in addition to the routine blood samples at the beginning, prior to the third chemotherapy treatment, and at the end of my entire treatment cycle. This urine sample will be specifically for the research project. I also understand that I will be provided with a collection container at no charge to me or my insurance company.

3. I understand that I will be asked to fill out a Food Frequency Questionnaire prior to beginning my chemotherapy and at the end of the treatment cycle.

4. I understand that should I decide to start taking any type of vitamin supplementation during my treatment cycle, I will notify LaFortune Cancer Center the brand name and dosage amount of the supplement or supplements.

5. I understand that test results from my medical chart related to kidney function and to nutritional status will be analyzed as part of this research project.

6. I understand that all data obtained will become a part of my medical chart and therefore will be confidential information. Specimens collected will be classified according to number so that I am assured of confidentiality

7. I understand that this study will not interfere with my medical treatment in any way.

8. I understand that the purpose of this study is to investigate the effect of cisplatin/carboplatin based chemotherapy on the kidney's ability to maintain proper trace mineral status.

9. I understand that this participation is voluntary and that the information that I provide will be kept confidential. I understand that I may refuse to participate in this research and may withdraw from this study at any time for any reason without prejudice.

I may contact LaFortune Cancer Center (918) 744-3180 should I wish further information about this research. I also may contact Barbara Stoecker, Ph.D., R.D., L.D., Nutritional Sciences, 425 HES, Oklahoma State University, Stillwater, OK, phone (405) 744-5040 and/or Beth McTernan Stillwater, OK 74078, phone (405) 744-5700.

If I should wish to contact an individual at St. John Medical Center regarding my rights as an individual in this research protocol, I may contact Antonio C. de Leon, Jr., M.D., Chairman of the Institutional Review Board at (918) 744-2875.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Signed_____Date_____

I have discussed this research with the participant (and/or his or her authorized representative) using language which is understandable and appropriate. I acknowledge that the nature and purpose of this investigational research has been explained to the patient. There are no risks or possibility of complications involved in conjunction with this study. All of the above has been explained to the patient or his representative by me before the patient consented. I have explained to the patient that he/she may withdraw from this study at any time for any reason without prejudice.

Signed_____
(Project director or authorized representative)

APPENDIX B

PATIENT INFORMATION (Cisplatin)

Cisplatin is a potent anticancer drug used for your type of cancer but there are some things that we do not know about this drug. One question is what effect the drug has on the trace minerals in your body.

We would like to ask your help with a research project to learn more about the effects of cisplatin on trace minerals, (specifically iron, magnesium, manganese, selenium, copper and zinc) so that recommendations can be made about the trace mineral needs of patients receiving cisplatin.

Your physician will be ordering routine lab work from time to time throughout your therapy. We ask that you allow us to draw an additional 10ccs (which is equal to two teaspoons) of blood at three of these times to use for this project--before you begin your first cycle, before your third cycle and after the final or sixth cycle of treatment, whichever comes first. Since this extra blood will be drawn with the same needle as your routine lab work, there will be no additional needle sticks involved. If you participate in this study, you will be asked to provide us with a 24 hour urine sample prior to beginning your therapy, before your third cycle of therapy and after the sixth or final cycle of your chemotherapy. In addition, we will ask you to fill out a nutritional questionnaire before you begin your chemotherapy and after you have finished taking all of your chemotherapy treatment. This will help us determine how diet may or may not play a role in the loss of these minerals. This study would not interfere in any way with your therapy and has the approval of your physician. You are not required to participate in this project and you could discontinue the study at any time.

There would be no charge to you for these additional studies and the results of these studies will be added to your medical records. These analyses may be of help to your physician in providing your medical care. If you are interested in being a participant in this project we will provide you with a copy of the consent form, show you the amount of additional blood needed for the tests and answer any questions you may have about this research.

APPENDIX C

ST. JOHN MEDICAL CENTER
Medical Excellence · Compassionate Care

November 24, 1992

Kitty Mathis-Shildt, P.L.D.
224 Sunset Drive
Tulsa, OK 74114

Re: "The Effects of Cisplatin-Carboplatin Based Chemotherapy on Trace Element Excretion"

Dear Mrs. Shildt:

At the Research Committee of November 9th, the Cancer Committee of November 13th, as well as the Institutional Review Board of November 24th, members reviewed and approved the above referenced protocol and consent form.

You will be requested to provide update reports concerning your patient's experience on this protocol on a twice yearly basis. Update report forms will be mailed to you for completion at those times.

Thank you again for your continued assistance. If you should have any questions regarding this review, please contact Lori Hostick, C.M.S.C., Protocol Coordinator, in the Medical Staff Office at 744-2288.

Sincerely,

William Sheehan, M.D., Chairman
Research Committee

Antonio C. de Leon, Jr., M.D., Chairman
Institutional Review Board

WS/AD/lah

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
FOR HUMAN SUBJECTS RESEARCH

Proposal Title: CISPLATIN CHEMOTHERAPY EFFECTS ON TRACE ELEMENT EXCRETION

Principal Investigator: BARBARA STOECKER/KITTY MATHIS-SHILDT

Date: 8-28-92 IRB # HES-93-008

This application has been reviewed by the IRB and

Processed as: Exempt [ ] Expedite [xx] Full Board Review [ ]

Renewal or Continuation [ ]

Approval Status Recommended by Reviewer(s):

Approved [xx] Deferred for Revision [ ]

Approved with Provision [ ] Disapproved [ ]

Approval status subject to review by full Institutional Review Board at next meeting, 2nd and 4th Thursday of each month.

Comments, Modifications/Conditions for Approval or Reason for Deferral or Disapproval:

Signature: Marcia S. Tilley Date: 9-11-92
Chair of Institutional Review Board

VITA

Kitty Mathis-Shildt

Candidate for the Degree of

Master of Science

Thesis: CISPLATIN CHEMOTHERAPY EFFECTS ON TRACE ELEMENT EXCRETION AND SERUM CONCENTRATION

Major Field: Nutritional Sciences

Biographical:

Education: Graduated from Guymon High School, Guymon, Oklahoma in May 1973; received a Bachelor of Science degree in Food, Nutrition and Institutional Administration from Oklahoma State University, Stillwater, Oklahoma in May 1991. Passed the national registered dietitian examination in October, 1992. Completed the requirements for the Master of Science degree with a major in Nutritional Sciences at Oklahoma State University in May 1996.

Experience: Currently working as a self employed consultant dietitian in long term health care and sports nutrition.

Professional Memberships: American Dietetic Association; Oklahoma Dietetic Association; Tulsa District Dietetic Association. Member of American Dietetic Association's practice groups--Sports, Cardiovascular and Wellness Nutritionists and the Oncology Nutrition Practice Group.

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