CHAPTER I

INTRODUCTION

Lead (Pb) is one of the most useful metals which has been mined and worked by men for millenniums (Chisolm, 1971). The main use of lead is in the manufacture of storage batteries, the production of alkyllead petrol additives, and as alloys combined with various metals that provide certain qualities suitable for the particular use. Lead is used in bullets and shot, as its high density makes it ideal for this purpose. Lead is also used extensively in the chemical industry, and is widely used in the manufacture and handling of sulphuric acid. It is also used in the protection of pipelines, brides, and ships. Because of its excellent sound attenuation property, lead is used in construction to keep within prescribed noise and vibration levels. Lead asbestos pads are used to isolate heavy machinery. Moreover, lead combined with chromate and molybdate forms paint pigments, and its silicate is used in ceramics and in fireproofing fabrics, and the lead arsenate has been used as an insecticide (Winship,1989).

1 Lead Sources, Absorption and Excretion

Because of the inappropriate use of lead, it has resulted in outbreaks of lead poisoning in humans from time to time since antiquity (Chisolm, 1971). In the present, lead is one of the most ubiquitous toxic metal which is detectable in practically all phases of the inert environment and in all biologic systems (Goyer,1991). Information gathered over a period of years has demonstrated the sources and the quantities of lead taken into the body from the food, beverages and air (Kehoe, 1961). More recently, the contamination in food and beverages has on occasions resulted in fatal poisoning when acidic food and drinks like tomato juice, fruit juice, cola drinks, cider, or pickles, stored in improperly glazed containers have dissolved the lead in the containers. Moreover, industrial processes resulted in an increased exposure to lead, and inhalation of lead from air pollution has been increasing. Occasional cases of lead exposure have occurred from lead dust in shooting galleries, artists' paint pigments, fumes from burning painted wood, jewellers' waste, lead type, soluble lead compounds in old lead pipes, in battery factories and in air from combustion of lead-containing auto exhausts (Winship, 1989).

Lead contamination in normal conditions are estimated to have consisted of 100-300 mg of lead per kg. of food, 100 mg per liter of water, and 2.5 mg per cubic meter

of air. By contrast, large urban areas have annual lead concentrations of 1 to 3 mg per cubic meter in air with short-term mean concentrations up to 44 mg per cubic meter occuring in peak traffic periods (Naovarat Suwanabun, 1993).

The major routes of lead absorption are from the gastrointestinal tract and the respiratory system. About 5-10 % of lead ingested in food, absorbed, mainly in the small intestine; some of the absorbed lead undergoes enterohepatic recycling. Children absorb a substantially greater proportion, about 50 %, of dietary lead than do adults. Inorganic lead is not absorbed through intact skin but organic lead compounds, such as lead naphthenate and tetraethyllead may be absorbed rapidly. Fumes of organic lead compounds are highly toxic. Lead can be absorbed from all areas of the respiratory tract, including the nasal passages. It is absorbed by the lungs from dust particles (Winship, 1989). Significant amounts can also be absorbed from a bullet or shot wound, with lead shot considered particularly dangerous because of its larger surface area permitting greater absorption. There have been reports of lead poisoning occuring within a month after a bullet wound (Berman, 1966).

The most excretion of lead is mediated in part by the gastrointestinal and urinary tracts. Lead is also excreted in the sweat, hair and nails. Maternal milk contains small amounts of lead. The concentration of lead in

urine is directly proportional to that in plasma, but since most of the lead is in the erythrocytes, very little is filtered. The elimination of lead is slow. The half-life of lead in blood is variously stated to be about one month to 70 days. An average adult excretes approximately 0.3 mg lead into the feces and 0.03 mg in the urine daily. Fecal lead mostly represents lead that was ingested, but not absorbed. Urinary lead indicates the degree of lead absorption, and concentrations above 15 mg/liter in adults and 0.08 mg/liter in children are considered dangerous (Berman, 1966 and Winship,1989).

2 Clinical Manifestations of Lead Poisoning

2.1 Acute Exposure

Acute lead poisoning occurs from the accidental ingestion of acid-soluble lead compounds. The fatal dose of absorbed lead has been estimated as 500 mg. Symptoms are an intense thirst, a metallic taste, followed by nausea, vomiting, and a burning abdominal pain ; there may be diarrhoea or constipation. The vomit may have a milky appearance, due to the formation of lead chloride, and the stools may become black, due to lead sulphide. Acute central nervous symptoms, which include paraesthesia, pain, and muscle weakness, develop. An acute haemolytic crisis is seen

sometimes, resulting in anaemia and haemoglobinuria. Severe renal damage follows, with oliguria, shock, and coma ; death may occure within 1-2 days (Winship,1989)

2.2 Chronic Poisoning

Chronic poisoning is usually due to the accumulation of small quantities of lead in the body by inhalation, ingestion, or skin absorption. In general, the symptoms are mainly gastrointestinal with chronic exposure, mainly neuromuscular with subacute poisoning, and affect the central nervous system when there is more rapid, intense absorption. Detailed descriptions of the adverse effects are covered in the toxicology section (Winship, 1989). Moreover, chronic exposure leads to renal damage and can also interfere with fertility and cause females menstrual disturbances. The most severe clinical form of lead poisoning is brain damage, which produces clumsiness, subtle changes in mental attitude, sluggishness, poor memory, inability to concentrate, restlessness, and hyperirritability (Naovarat Suwanabun,1993).

3 Blood Lead Concentration and Lead Toxicity on Heme Synthesis and Neuro System

Blood lead levels also indicate the degree of lead absorption (Berman, 1966). The toxic effects of lead and the

minimum blood lead level at which the effect is likely to be observed are shown in Table 1 . These effects involve several organ systems and biochemical activities (Goyer, 1991). Moreover, blood lead levels should be interpreted as follows : (Berman, 1966).

0-210 μg/l : negative

210-600 μg/l : evidence of increased lead exposure

above 600 μg/l : lead intoxication indicated

In blood, the most obvious lead effect is the decrease in heme synthesis which leads to anemia (Chisolm, 1971). The other concession is neurological effect. Thus, it is of interest to mention the mechanism of lead on these two systems.

3.1 Effect of lead on Heme synthesis

Heme is the iron-containing constituent that combines with protein to form hemoglobin, the oxygen-carrying pigment of the red blood cells. Heme is also an essential constituent of the other respiratory pigments, the cytochromes, which play key roles in energy metabolism (Chisolm, 1971).

Disturbances in heme synthesis are demonstrated by the appearance of abnormal concentrations of heme precursors in blood, for examples : protoporphyrin IX and delta-aminolaevulinic acid (δ-ALA). Lead has been shown to inhibit heme synthesis at several sites, the enzymes concerned being

Table 1 Lowest observed effect levels for induced health effects (Goyer, 1991).

Effect	Blood Lead Concentration (µg/l) Children	Adults
HEME EFFECT		
Anemia	800-1,000	800-1,000
U-ALA	400	400
EPP	150	150
ALAD inhibition	100	<100
NEURO EFFECT		
Encephalopathy	800-1,000	1,000-1,200
I.Q. deficits	<300	-
Peripheral neuropathy	400	400
RENAL EFFECTS		
Acute nephropathy	800-1,000	-
Chronic nephropathy	-	600
Vit.D metabolism	<300	-

U-ALA = Urinarly aminolaevulinic acid

EPP = Erythrocyte protoporphyrin

ALAD = Aminolaevulinic acid dehydratase

heme synthetase , delta-aminolaevulinic acid dehydratase (δ-ALAD) , ferrochelatase , delta-amino-laevulinic acid synthetase (δ-ALAS) , uroporphyrinogen decarboxylase, and coproporphyrinogen oxidase (Winship, 1989).

Although several enzymes of heme synthesis have been reported to be affected by lead, the incorporation step has not been studied sofar. In the last step of heme synthesis, ferrochelatase catalyzes iron to combine with protoporphyrin IX (as shown in Figure 1) (Chisolm, 1971 and Orten, 1982). It is generally believed that the iron entering the blood stream from the intestine is mostly in the ferrous form. In the plasma, ferrous iron (Fe(II)) is rapidly oxidized to ferric iron (Fe(III)) by oxidase activity residing on the Ceruloplasmin (Cp), and is incorporated into the specific iron-binding protein, transferrin (Osaki, 1966). The other possibility to explain anemia caused by lead is the decrement of Ceruloplasmin's oxidase activity.

3.2 Effect of lead on Neuro system

The another major risk is toxicity to the nervous system. Lead in the central nervous system tends to concentrate in gray matter and certain nuclei. The highest concentrations are in the hippocampus, followed by cerebellum, cerebral cortex, and medulla.

Symptoms of lead encephalopathy begin with lethargy, vomiting, irritability, loss of appetite, and dizziness and

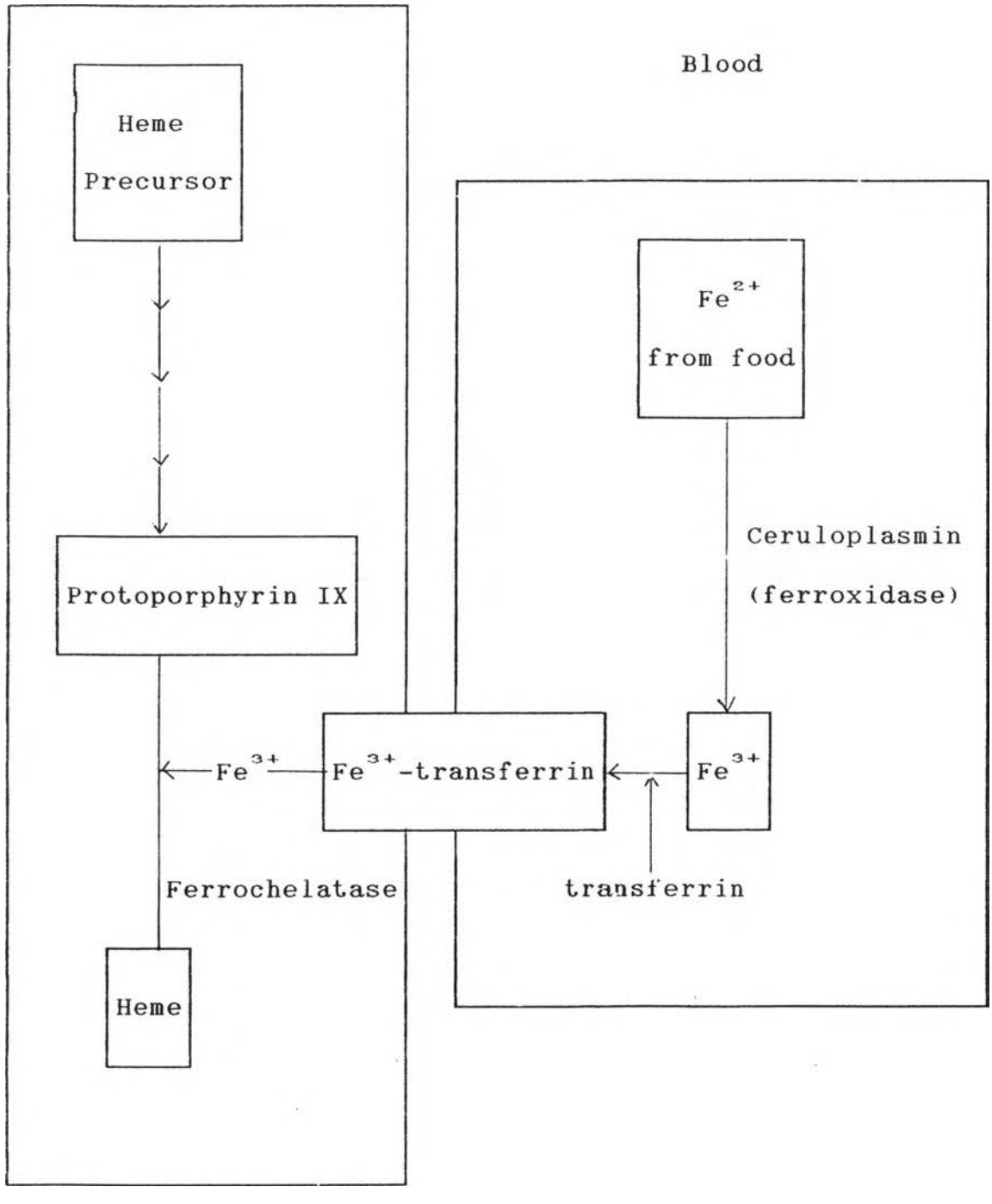

Figure 1 The relationship between ferrous iron oxidation by Cp and its transport to heme synthesis.

progress to obvious ataxia and reduced level of consciousness, which may progress to coma and death. The pathological findings at autopsy are severe edema of the brain due to extravasation of fluid from capillaries in the brain. This is accompanied by loss of neuronal and increase in glial cells. Recovery is often accompanied by sequelae including epilepsy, mental retardation, and optic neuropathy and blindness in some cases (Goyer, 1991).

The following plasma factor, Cp may explain how lead causes toxicity on different organs, resulting in different symptoms mentioned above.

4 Ceruloplasmin

Ceruloplasmin (Cp) is the blue-colored copper containing alpha-2 globulin (α_2-globulin) glycoprotein of mammalian plasma and have the polymorphic variants from the carbohydrate moiety (Gutteridge, 1981). Cp has been identified as single polypeptide chain with a molecular weight of 150,000 dalton, isoelectric point at 4.4 (Holmberg, 1948).

The obvious functions of Cp are the transport and homeostasis of copper since it binds 90 % of blood plasma copper (Oosthuizen, 1985), each molecule of Cp binds 8 copper atoms (Orten, 1982). From the physiological function of Cp as a copper-transporting protein, Cp is able to supply

copper within cells for incorporation into other copper enzymes, such as superoxide dismutase and cytochrome oxidase.

The other biological role of Cp has been suggested to be that of a "ferroxidase" which catalyzed oxidation of ferrous iron (Fe(II)) to ferric iron (Fe(III)), as shown in the following equation, and so facilitates the binding of iron to transferrin (Kaldor, 1983). It is investigated that

$$Fe^{2+} + O_2 + Cp\text{-}Cu^{2+} \rightleftharpoons Fe^{3+} + H_2O + Cp\text{-}Cu^{3+}$$

copper on Cp molecule is required for the oxidase activity of Cp (Holmberg, 1951).

Moreover, the reduction of the incorporation of copper into Cp molecule causes Wilson's disease (Hepatolenticular degeneration syndrome), which has the excessive accumulation of copper in the central nervous system, liver, cornea, kidney, and other organs, and has high of the urinary excretion of copper but low of serum copper (Kaushansky, 1987).

As the first symptom of Wilson's disease, psychiatric symptoms were as common as neurologic symptoms. The most common psychiatric symptom was depression, followed by emotional lability, personality changes, and slow mentation. The most common neurologic first symptoms were motor ones, as follows : tremor, incoordination, dystonia, rigidity, and difficulty with fine motor tasks. These were followed in

frequency by dysarthria, micrographyia, and gait difficulty. A variety of tremors were described : resting, postural or kinetic tremor, symmetric or asymmetric, and coarse or fine. Usually the tremors were described as intermittent and progressive. The early manifestations of fine motor impairment involved difficulty in buttoning, typing, playing piano, and writing. Gait difficulty was usually first evident in sports and later in walking and standing (Starosta-Rubinstein, 1987).

The symptoms (pain, stiffness, swelling) and physical finding (pain with motion and decreased range of motion) of Wilson's disease is similar to the symptoms caused by lead toxicity. This finding suggests that lead may cause reduction of Cu on Cp molecules.

Thesis Assumption

Lead reduces heme synthesis which finally leads to anemia. Other than enzyme inhibition, the impairment of Cp-dependent iron transport may also cause the reduction of heme synthesis.

Lead toxicity leads to various symptoms similar to Wilson's disease which resulted from Cp depletion.

The above findings strongly suggest that Cp is an important factor mediated to lead toxicity. Two Cp activities may relate to this effect, namely metal binding and oxidase activity.

Aim of This Thesis

Experiments will be performed on human serum and purified human Cp with the emphasis on the following studies :

1. *In vitro* experiments in lead-treated serum and in serum of lead-intoxicated patient to prove that lead affects Cp's metal binding.

2. The relationship between lead binding and oxidase inactivation on the Cp.

3. The effect of some chelators on the release of lead from Cp molecules.