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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Lead: Detection, absorption, oxidative effect, and antioxidant treatment in human organism

by

Oksana Zagorodna

Biosciences Program
The University of Iowa
Iowa City, IA 52242-1181

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Abbreviations

AAS	Atomic absorption spectrometry
ALAD	δ-aminolevulinic acid dehydratase
DCT1	Divalent cation transporter
DMT1	Divalent metal transporter (synonym of DCT1)
G6PD	Glucose-6-phosphate dehydrogenase
GI tract	Gastrointestinal tract
NAC	*N*-acetylcysteine
RBC	Red blood cells
ROS	Reactive oxygen species
SOD	Superoxide dismutase

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Abstract

Lead is one of the most studied toxins in the environment that has been widely used by humans due to its malleability, resistance to corrosion, and low melting point. The main sources of lead are: industry, gasoline (although recently reduced), and lead-containing paint. In the human organism, lead is not distributed homogeneously but rather into several compartments, such as soft tissues (blood, liver, kidney, etc.) and mineralising systems (bones and teeth). Determining lead levels in blood and kidney cortex is performed through electrothermal and Zeeman-effect flameless atomic absorption spectrometry methods. The absorption of lead can occur in three different ways: through skin, through inhalation, or by ingestion. Once in the organism, lead can cause oxidative stress and lipid peroxidation. Chelators and antioxidants tend to decrease lead levels in the organism, which contributes to treating lead poisoning.

Introduction

The word “lead” is of an Anglo-Saxon heritage and stands for the element, initially known by the Latin word *plumbum* (Pb). The Latin word serves as the root of plumbism, which means “lead poisoning” [1]. During the Roman Empire, lead was used to sweeten wine [2]. Also, the pots and containers for boiling grape juice and storing beverages were lead-lined. Perhaps they contributed to the fall of the Roman Empire [3]. It is quite possible that due to a heavy consumption of wine by the British upper class, plumbism was the reason of a drastic decline among aristocracy in the 18th and 19th centuries [4].

Lead is one of the most studied toxins in the environment. It is soft, silvery-grey in color, and very malleable. Because of its ability to resist corrosion, mix well with other metals, and its low melting point, lead has been widely used by humans for several centuries. A wide range of applications for lead continues to rise, which leads to contamination of the environment. Lead is toxic to living organisms, although knowing about lead toxicity has not eradicated its use from daily life [2; 3]. This paper will review main sources of lead exposure, methods of lead detection, and its mechanism of absorption and transport in a human organism. Oxidative effects of lead along with antioxidative treatment of lead poisoning will be addressed.

Sources of lead exposure

Lead is widely used in metal-related industry. Due to its low degradability, lead accumulates in the environment and causes pollution. New technologies, mechanical vehicles, and various industrial activities contribute systematically to augmenting lead levels in the atmosphere [5]. Lead toxicity is of a particular concern with children, because they take in more lead (with soil and dust) and absorb it, from the gut, in far greater quantities than adults.

Leaded gasoline used to be the main source of lead contamination in the environment worldwide [6], and it is still the main source of lead contamination in the developing countries.

When lead burns in the vehicle, it causes the emission of organolead vapor, which is extremely harmful to organisms [7].

Another source of lead exposure is lead-containing paint. It was widely used in the US until late 1970's. Therefore, old (pre-1970s) paints should be assumed to contain lead unless tests prove otherwise. Contaminated homes and yards are the major source of acute (short-term high-dose) lead poisoning. Replacing the paint in old houses causes even greater problems because lead stays in the house dust [8]. Other sources of lead exposure include water pipes, canned foods, leaded glass, lead-glazed pots, jewellery, cosmetics, soil, farm equipment, *etc*. [Reviewed in 8].

Methods of lead detection in organisms

In the human organism, lead is not distributed homogeneously but rather into several compartments, such as soft tissues (blood, liver, kidney, etc.) and mineralising systems (bones and teeth). In soft tissues, the half-life of lead is lower compared to mineralising systems. Thus the effect of lead on the organism as well as its excretion from the systems occurs faster in soft tissues. Bones can be adversely affected by lead, but also serve as an harbour of this compound, which poses a risk of endogenous source of the metal to the organism. The highest amount of lead, once in the body, is in blood[1]. There have been various methods designed to detect lead *in vivo*. Especially valuable is measuring metal levels in blood and soft tissues since they can be easily harmed by toxic compounds that contain lead [9, 10].

Determining lead in human whole blood. The presence of lead in blood is considered to be a reliable index of recent metal uptake. Therefore determining lead in blood can be used in the diagnosis of incipient poisoning. Among the techniques used for such measurements, the most sensitive and selective for lead is electrothermal atomic absorption spectrometry (AAS). In this

[1] Centers for Disease Control. Preventing lead poisoning in young children: a statement by the Centers for Disease Control. Atlanta. GA: US Dept of Health and Human Services. http://www.yale.edu/ynhti/curriculum/units/1993/5/93.05.06.x.html. Visited on 03/08/2005.

method, trace metal contamination is minimized (compared to flame AAS) by avoiding preliminary chemical extractions and additions of reagents. However, the matrix interferences make the process inaccurate. And it is difficult to establish the precise charring temperature when using simple diluents, such as water. In one of the research papers aimed to improve the accuracy of the technique, the electrothermal AAS approach was extended to the graphite-furnace AAS using diammonium hydrogen phosphate–Triton medium. This combination allowed for stabilizing the solution that contained lead samples, which increased precision of lead measurements [9].

Measuring lead in human kidney cortex. The interest in studying human kidney cortex has been prompted by its accumulation of various heavy metals one of which is lead. To measure lead in kidney cortex, Pleban *et al*. used Zeeman-effect flameless AAS method. In comparison with other techniques, such as neutron activation analysis and x-ray fluorescence, Zeeman-effect flameless AAS allows for good sensitivity, operational simplicity, and relatively inexpensive instrumentation. All these features are similar to earlier described electrothermal AAS. However, similarly to measuring blood lead, chemical and spectral backgrounds in kidney cortex interfere with determining the analyte metal. Therefore, the interferences need to be removed before measuring lead. Zeeman-effect allows for an analyte-shifted AAS. It corrects the interference by electronically correcting for the background absorption. The method allows for accurate results during atomization for broad-band molecular absorption, light scattering, and wavelength-dependent radiation [10].

Mechanisms of lead absorption

The absorption of lead can occur in three different ways: through *skin*, through *inhalation* (the most common exposure route in adults), or by *ingestion* (the most common exposure route in children).

Skin absorption. It was thought that lead can be absorbed through the skin only when it is in an organic form, such as tetraethyl lead (**Fig. 1**) or lead naphthane.

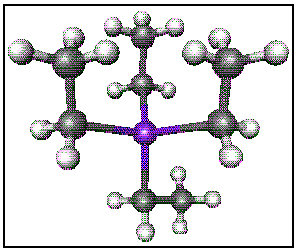

Fig. 1. The crystal structure of tetraethyl lead[2].

Lilley *et al*. found that lead metal or lead nitrate solution, when placed on the skin, can be quickly absorbed and transported in the body [11]. The lead that was absorbed could be found in sweat and saliva, although was not detected in blood and urine. To prove that lead was transported through the body, Lilley *et al*. applied a high lead nitrate amount to the left arm of the volunteer. As a result, lead concentration was increased in the sweat sample on the right arm, which served as an evidence of lead being transported in the body. Another conclusion made from the study was that the rate of lead absorption through the skin increases with increased sweating of the skin. No increase in blood lead must have meant that lead does not get to the erythrocytes. Instead, it gets transported through the plasma and concentrates in the extracellular fluid pool, sweat and saliva. That is why workers who are occupationally exposed to lead have high levels of lead in sweat and saliva, but not in blood [11]. The only way lead can increase blood levels through skin is when being a part of organic molecules [12].

Absorption through Inhalation. The most common route of lead absorption in adults is through inhaling it into the lungs. However, lead particles do not tend to stay in the inhalation system. Instead, they travel up the mucociliary escalator and are swallowed into the gastrointestinal tract [13].

Absorption by ingestion. In addition to being swallowed from the lungs, lead absorption occurs through the diet. The mechanism of lead absorption is similar to the absorption of such metals as iron [14]. The absorption of iron takes place in the upper intestine and is divided in three steps: 1)

[2] Hosting website: http://ifa.ukf.net/lead.htm. Visited on 03/08/2005

the transport of the metal at the border membrane occurs with participation of a membrane transporter; 2) transfer of the metal to the basolateral side; 3) metal is transferred basolaterally to the plasma. It has been shown that the main lead absorption in GI tract occurs in the upper intestine with participation of the mediated carrier [15], the divalent metal transporter (DMT1), also known as divalent cation transporter (DCT1). DMT1 was originally detected as the transporter of nonheme iron at the intestinal surface. Bannon *et al*. used a *Saccharomyces cerevisiae* strain that was defective for carrying iron to determine whether lead can be transported by DMT1. The results showed that both iron and lead can be transported by DMT1 in yeast, and the experiment was confirmed in a human cell line. The study also revealed that iron inhibits lead transport (**Fig. 2**) [16].

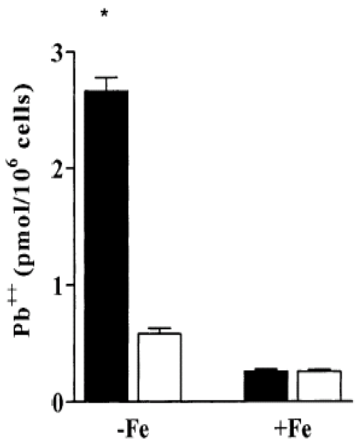

Fig. 2. Effect of iron on lead transport by DMT1 in yeast. Cells transfected with pSM703-DMT1 were incubated with 10 μM Pb-citrated in Mes buffer (pH 5.5, black pillar) or pH 7.4 in the presence of 250 μM iron (+Fe) or in ints absence (-Fe) for 30 min. Data points are means +/ - SD of triplicates. Asterisk indicates $p< 0.05$ compared to controls using Student's *t* test for unpaired samples.

Oxidative effect of lead and lead toxicity

Lead can induce a wide range of physiological, biochemical, and behavioural dysfunctions [17]. It can cause oxidative stress and lipid peroxidation in the liver, kidney, brain, and read blood cells [17, 18]. One of the studies investigated the effect of lead exposure in young (1.5 months) and adult (10 months) male Fisher 344 rats. Lead was administered to the rats through drinking water for five weeks. As a result, lead levels in red blood cells (RBC) were profoundly increased [17]. The increase of lead in RBC causes hemolysis that is associated with peroxidation of RBC membranes. Thus, it is unlikely that lead initiates peroxidation of membrane lipids directly. In one of the studies

by Ribarov *et al*. it was found that lead can enhance the autoxidation of hemoglobin in an *in vitro* liposome model [19]. The effect of autoxidation was inhibited by superoxide dismutase (SOD) and catalase, which suggests the involvement of superoxide and hydrogen peroxide. Thus the speculation was that lead may increase accumulation of reactive oxygen species (ROS) through interaction with oxyhemoglobin [19]. It was also reported that in lead-exposed animals the concentrations of antioxidant molecules, such as glutathione (GSH), were lower than normal and levels of glutathione disulfide (GSSG) were higher [17]. This can be explained by the characteristic pattern of lead to inhibit compounds having functional SH groups. Through the same inhibitory mechanism of binding to SH groups, lead can inhibit such important enzymes as δ-aminolevulinic acid dehydratase (ALAD) and glucose-6-phosphate dehydrogenase (G6PD). The decreased levels of these enzymes eventually lead to increased levels of ROS [mentioned in 18].

Antioxidants in treating lead poisoning

The main therapeutic approach to eliminating consequences of lead poisoning is to excrete lead from the organism by chelating agents (shown to reduce lead levels in blood). However, because of the rebound effect[3] of chelators, chelation therapy cannot be started if the subject was located near lead in the periodic table. There is an alternative pathway of treatment – using antioxidants. Gurer *et al*. investigated how an antioxidant (*N*-acetylcysteine) and a chelator (succimer) could treat the symptoms of lead poisoning in rats [20]. In the study, 344 rats were given lead acetate in their drinking water for five weeks. The next step was to remove lead-contaminated water and replace it with either NAC or succimer. At the end of the experiment, RBC levels were measured. The group of rats that was treated with lead had induced oxidative stress, evidenced by lipid peroxidation through the increase of malondialdehyde content and G6PD, as well as decrease in

[3] *Rebound effect* – a reversal of response upon withdrawal of the stimulus. http://cancerweb.ncl.ac.uk. Visited on 03/08/2005.

GSH. When NAC was given as a treatment, blood lead levels decreased from 35 μg/dL to 25 μg/dL. At the same time, the group treated with succimer chelator lowered blood lead levels to 2.5 μg/dL. From the results, it can be concluded that lead clearance from the blood stream is significantly enhanced by succimer and only slightly enhanced by NAC treatment. Such data are consistent with the hypothesis that the primary mode of action for succimer is through chelation and clearance of lead; and the primary mode of action for NAC is through enhancing thiol antioxidant capacity [20]. It would be interesting to see whether the effect of decreasing lead levels in blood could be amplified when both the chelator and antioxidants are used together.

Conclusion

Despite its toxicity, lead has been widely used by humans for many centuries. As a result of its use, lead poisoning has been an old but persistent problem. The accumulation of knowledge about main sources of lead along with its detection, pathways of absorption, and oxidative effects contribute to treating and preventing lead intoxication cases.

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