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BIOLOGICAL EFFECTS OF LEAD AND ITS MECHANISM OF TOXICITY

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Abstract:
Lead poisoning has been recognized as a major public health risk, particularly in developing countries. Though various occupational and public health measures have been undertaken in order to control lead exposure, cases of lead poisoning are still reported. Exposure to lead produces various deleterious effects on the hematopoietic, renal, and reproductive and central nervous system, mainly through increased oxidative stress. These alterations play a prominent role in disease manifestations. Modulation of cellular thiols for protection against reactive oxygen species (ROS) has been used as a therapeutic strategy against lead poisoning. N-acetylcysteine, α-lipoic acid, vitamin E, quercetin and a few herbal extracts show prophylaxis against the majority of lead mediated injury in both *in vitro* and *in vivo* studies. This review provides a comprehensive account of recent updates describing health effects of lead exposure, relevant biomarkers and mechanisms involved in lead toxicity. It also updates the readers about recent advances in chelation therapy and newer therapeutic strategies, like nano encapsulation, to treat lead induced toxic manifestations.

Keywords: lead, nervous system, N-acetylcystein, biomakers

INTRODUCTION:

Lead (Pb) is ubiquitous and one of the earliest metals discovered by the human race. Unique properties of lead, like softness, high malleability, ductility, low melting point and resistance to corrosion, have resulted in its widespread usage in different industries like automobiles, paint, ceramics, plastics, *etc*. This in turn has led to a manifold rise in the occurrence of free lead in biological systems and the inert environment [1-3].

Lead is regarded as a potent occupational toxin and its toxicological manifestations are well known. The non-biodegradable nature of lead is the prime reason for its prolonged persistence in the environment [4-5]. Human exposure to lead occurs through various sources like leaded gasoline, industrial processes such as lead smelting and coal combustion, lead-based paints, lead containing pipes or lead-based solder in water supply systems, battery recycling, grids and bearings, *etc*. Although lead toxicity is a highly explored and comprehensively published topic, complete control and prevention over lead exposure is still far from being achieved. There is no such level of lead that appears to be necessary or beneficial to the body and no "safe" level of exposure to lead has been found. Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It is known to interfere with a number of body functions and it is primarily affecting the central nervous, hematopoietic, hepatic and renal system producing serious disorders (Kalia & Flora, 2005). Acute toxicity is related to occupational exposure and is quite uncommon. Chronic toxicity on the other hand is much more common and occurs at blood lead levels of about 40–60 ug/dL. It can be much more severe if not treated in time and is characterized by persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma [6].

Effect on nervous system:

Compared to other organ systems, the nervous system appears to be the most sensitive and chief target for lead induced toxicity [7]. Both the central nervous system and the peripheral nervous system become affected on lead exposure. The effects on the peripheral nervous system are more pronounced in adults while the central nervous system is more prominently affected in children . Encephalopathy (a progressive degeneration of certain parts of the brain) is a direct consequence of lead exposure and the major symptoms include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucinations [8-9]. More severe manifestations occur at very high exposures and include delirium, lack of coordination, convulsions, paralysis, coma and ataxia [10]. Fetuses and young children are especially vulnerable to the neurological effects of lead as the developing nervous system absorbs a higher fraction of lead. The proportion of systemically circulating lead gaining access to the brain of children is significantly higher as compared to adults [11]. Children may appear inattentive, hyperactive and irritable even at low lead exposure. Children with greater lead levels may be affected with delayed growth, decreased intelligence, short-term memory and hearing loss. At higher levels, lead can cause permanent brain damage and even death [12]. There is evidence suggesting that low level lead exposure significantly affects IQs along with behavior, concentration ability and attentiveness of the child. Repercussions of lead exposure on the peripheral nervous system have also been observed in the form of peripheral neuropathy, involving reduced motor activity due to loss of

myelin sheath which insulates the nerves, thus seriously impairing the transduction of nerve impulses, causing muscular weakness, especially of the exterior muscles, fatigue and lack of muscular co-ordinationb[13].

Effect on hematopoietic system

Lead directly affects the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anemia [15-16]. Anemia caused on account of lead poisoning can be of two types: ***hemolytic anemia***, which is associated with acute high-level lead exposure, and ***frank anemia***, which is caused only when the blood lead level is significantly elevated for prolonged periods [17].

Lead significantly affects the heme synthesis pathway in a dose dependent manner by downregulating three key enzymes involved in the synthesis of heme. δ-aminolevulinic acid dehydratase (ALAD)**,** a cytosolic enzyme that catalyzes the formation of porphobilinogen from δ-aminolevulinic acid (ALA), aminolevulinic acid synthetase (ALAS)**,** a mitochondrial enzyme that catalyzes the formation of aminolevulinic acid (ALA), and finally, the mitochondrial enzyme ferrochelatase that catalyzes the insertion of iron into protoporphyrin to form heme [18-19]. The initial and final steps of heme synthesis take place in the mitochondria, whereas the intermediate steps take place in the cytoplasm.

Lead inhibits the three aforementioned vital enzymes of this pathway but its effect on ALAD is more profound and its inhibition has been used clinically to gauge the degree of lead poisoning. Inhibition of ALAD results in the accumulation of aminolevulinic acid, detectable in the plasma and urine even at blood lead levels of less than 10 µg/dl. Although ALAD inhibition is first noted at blood lead levels of 10–20 µg/dl, heme biosynthesis does not decrease until the activity of ALAD is inhibited by 80–90%, which occurs at a much higher blood lead concentration of about 55 µg/dl . Inhibition of ferrochelatase results in increased excretion of coproporphyrin in urine and accumulation of protoporphyrin in erythrocytes (EP). Moreover, inhibition of this enzyme results in the substitution of iron by zinc in the porphyrin ring forming zinc protoporphyrin (ZPP). The concentration of ZPP thus gets increased, which can also be used as an indicator to monitor the level of lead exposure (Jangid *et al.*, 2012). Thus, the collective inhibition of these three key enzymes blocks the heme production via the heme synthesis pathway. The mechanism responsible for shortening the life cycle of erythrocytes is not well understood. One of the earliest observed hematological effects of lead revealed basophilic stipplings of red blood cells (presence of dense material in red blood cells), which is also a potential biomarker for the detection of lead poisoning. These aggregates are degradation products of ribonucleic acid [20-21].

Renal effect

Renal dysfunction occurs mostly at high levels of lead exposure (>60 µg/dL) but damage at lower levels has also been reported (~10 µg/dL) (Grant, 2008). Renal functional abnormality can be of two types: acute

nephropathy and chronic nephropathy. Acute nephropathy is characterized functionally by an impaired tubular transport mechanism and morphologically by the appearance of degenerative changes in the tubular epithelium along with the occurrence of nuclear inclusion bodies containing lead protein complexes. It does not cause protein to appear in the urine but can give rise to abnormal excretion of glucose, phosphates and amino acids, a combination referred to as ***Fanconi's syndrome***. Chronic nephropathy on the other hand, is much more severe and can lead to irreversible functional and morphological changes. It is characterized by glomerular and tubulointerstitial changes, resulting in renal breakdown, hypertension and hyperuricemia [22-24].

Cardiovascular effect

Both chronic and acute lead poisoning causes cardiac and vascular damage with potentially lethal consequences including hypertension and cardiovascular disease (Navas-Acien *et al.*, 2007). Low level lead exposure can contribute to hypertension in both animals and humans (ATSDR, 2005). Other major disorders include ischemic coronary heart disease, cerebrovascular accidents and peripheral vascular disease. Although evidence of causal relationship of lead exposure and hypertension was reported, it applies only in cases of cardiovascular outcomes of lead toxicity [25-27].

Reproductive health effects:

Lead causes a number of adverse effects on the reproductive system in both men and women. Common effects seen in men include: reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone. Women on the other hand, are more susceptible to infertility, miscarriage, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery (Flora *et al.*, 2011). Moreover, during the gestation period, direct influence of lead on the developmental stages of the fetus has also been reported [28].

Effect on bones:

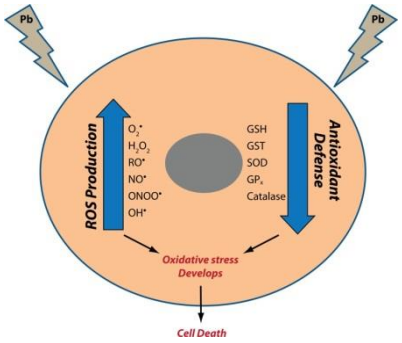
The primary site of lead storage in the human body is bones (Renner, 2010; Silbergeld *et al.,* 1993). There are two compartments in bones where lead is believed to be stored. The exchangeable pool present at the surface of bone and the non-exchangeable pool located deeper in the cortical bone. Lead can enter into plasma at ease from the exchangeable pool but can leave the non-exchangeable pool and move to the surface only when bone is actively being re-absorbed (Patrick, 2006). Stable lead isotope methodology showed that bones contibute around 40–70% of lead released into blood in adults. In adults, 85–95% of the lead is stored in bones, in contrast to 70% in children, resulting in higher concentration of lead in soft tissues in children. The storage and the mobilization of lead in bones depend on several factors, like dose/rate of lead exposure, age, pregnancy, gestation and race [29].

Mechanism on toxicity:

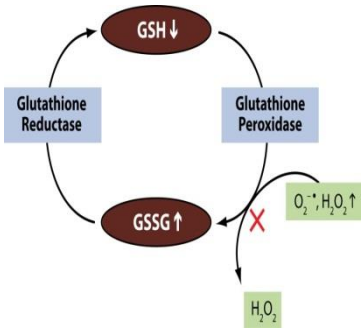
Lead is probably the most extensively studied heavy metal. Studies carried out in this field have reported the presence of various cellular, intracellular and molecular mechanisms behind the toxicological manifestations caused by lead in the body.

Oxidative stress

Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Flora, 2011). It has been reported as a major mechanism of lead induced toxicity. Under the influence of lead, onset of oxidative stress occurs on account of two different pathways operative simultaneously; first comes the generation of ROS, like hydroperoxides ($HO_2^{\bullet}$), singlet oxygen and hydrogen peroxide (H_2O_2), and second, the antioxidant reserves become depleted (Figure 1)[30].



The antioxidant defenses of the body come into play to nullify the generated ROS. The most important antioxidant found in cells is **g**lutathione (GSH)**.** It is a tripeptide having sulfhydryl groups and is found in mammalian tissues in millimolar concentrations. It is an important antioxidant for quenching free radicals (Mates, 2000). Glutathione exists in both reduced (GSH) and oxidized form (GSSG). The reduced state of glutathione donates reducing equivalents ($H^+ + e^-$) from its thiol groups present in cysteine residues to ROS and makes them stable. After donating the electron, it readily combines with another molecule of glutathione and forms glutathione disulfide (GSSG**)** in the presence of the enzyme glutathione peroxidase (GP_X**)**. GSH can be regenerated from GSSG by the enzyme **g**lutathione reductase (GR) (Figure 2). Under normal conditions, 90% of the total glutathione content exists in reduced form (GSH) and around 10% is in the oxidized form (GSSG). Under conditions of oxidative stress, the concentration of GSSG is much higher than that of GSH[30].



Lead shows electron sharing capability that results in the formation of covalent attachments. These attachments are formed between the lead moiety and the sulfhydryl groups present in antioxidant enzymes, which are the most susceptible targets for lead and which eventually get inactivated. Lead inactivates glutathione by binding to sulfhydryl groups present in it. This results in synthesis of GSH from cysteine via the γ-glutamyl cycle, which is usually not effective in replenishing the supply of GSH (Hultberg *et al.,* 2004). Similarly, lead inactivates enzymes like δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GP_X) and glutathione-S-transferase, which further depresses the glutathione levels.[31].

A few other notable antioxidant enzymes that are rendered inactive by lead include super oxide dismutase **(**SOD**)** and catalase **(**CAT). Decrease in SOD concentration reduces the disposal of superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical ($O_2^{-\bullet}$). Apart from targeting the sulfhydryl groups, lead can also replace the zinc ions that serve as important co-factors for these antioxidant enzymes and inactivates them (Flora *et al.,* 2007).

Lipid peroxidation is another biomarker of oxidative stress and is one of the most investigated consequences of ROS on lipid membranes. The generated free radical captures electrons from the lipids present inside the cell membranes and damages the cell. Apart from lipid peroxidation, lead also causes hemoglobin oxidation, which directly causes RBC hemolysis. This occurs due to inhibition of ALAD, which results in an increased concentration of substrate ALA in both blood and urine. These elevated ALA levels generate hydrogen peroxide and superoxide radical and also interact with oxyhemoglobin, resulting in the generation of hydroxyl radicals (Patrick, 2006). Progression of all the above mentioned mechanisms makes the cell extremely vulnerable to oxidative stress and may lead to cell death.

Ionic mechanism of lead toxicity

Ionic mechanism of action for lead mainly arises due to its ability to substitute other bivalent cations like Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations like Na^{+} (though bivalent cations are more readily substituted), affecting various fundamental biological processes of the body (Lidsky & Schneider, 2003). Significant effects have been found on various fundamental cellular processes like intra and intercellular signaling, cell adhesion, protein folding and maturation, apoptosis, ionic transportation, enzyme regulation, release of neurotransmitters, *etc.* (Garza *et*

al., 2006). The ionic mechanism contributes principally to neurological deficits, as lead, after replacing calcium ions, becomes competent to cross the blood-brain barrier (BBB) at an appreciable rate. After crossing the BBB, lead accumulates in astroglial cells (containing lead binding proteins). Toxic effects of lead are more pronounced in the developing nervous system comprising immature astroglial cells that lack lead binding proteins. Lead easily damages the immature astroglial cells and obstructs the formation of myelin sheath, both factors involved in the development of BBB.

Lead, even in picomolar concentration, can replace calcium, thereby affecting key neurotransmitters like protein kinase C, which regulates long term neural excitation and memory storage. It also affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine and GABA) and regulation of uptake and retention of calcium by synaptosomes. This interaction between lead and sodium seriously impairs the normal functioning of the aforementioned sodium dependent processes [32-33].

CONCLUSION:

Lead poisoning has been known to mankind since antiquity, although the situation got aggravated since the 18th century during the industrial revolution. It was the period when various important qualities of lead were discovered that made it one of the most widely used industrial metals. Lead has no known biological function in the body and once it enters the body, it is known to cause severe health effects that might be irreversible. It affects almost all the major organ systems of the body like hematopoietic, renal, nervous and cardiovascular systems. Various molecular, cellular and intracellular mechanisms have been proposed to explain the toxicological profile of lead that includes generation of oxidative stress, ionic mechanism and apoptosis. Of these oxidative stress has been found to be more pronounced and much more severe. Lead causes generation of ROS which results in critical damage to various biomolecules like DNA, enzymes, proteins and membrane based lipids, while simultaneously it impairs the antioxidant defense system. Chelation therapy has so far been used as the mainstay of the treatment that involves quenching of lead from different sites of the body and expels it through urine.

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