Pomeranian J Life Sci 2023;69(2):33-39 doi: 10.21164/pomjlifesci.939

Selected biochemical mechanisms of lead neurotoxicity

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ABSTRACT

Elevated levels of lead ions (Pb^{2+}) in the bloodstream present a fatal risk to all age demographics. Furthermore, a wealth of research underscores that chronic exposure to even low, non-symptomatic doses can trigger developmental disorders in children. Various studies have illustrated the competitive nature of Pb^{2+} with divalent metals from the metabolic pool, notably calcium ions (Ca^{2+}). By exploiting transport pathways and binding sites on specific proteins, Pb^{2+} can infiltrate nearly every organ, including the brain. The N-methyl-D-aspartate receptor (NMDAR) is recognized as one of the key molecular targets for Pb^{2+}. Mitochondria are also the subject of many studies investigating the toxicity of lead. Maintaining the health of the fragile developing nervous system during prenatal and neonatal stages necessitates diligent monitoring and reassessment of what constitutes safe lead ion concentrations in the bloodstream.

Keywords: divalent metals; lead; mitochondria; neurotoxicity; neurodegenerative disorders; NMDAR; oxidative stress; synaptic conduction.

INTRODUCTION

Human use of lead has a long history, with its earliest known applications dating back to 7500 BCE. The metal's availability, together with its unique physical and chemical traits, fueled its widespread adoption worldwide. Yet, as the centuries unfolded and the uses of lead multiplied, its harmful health implications began to eclipse its utility.

The late 18th century ushered in the Industrial Revolution, a crucial juncture in lead exploitation, resulting in widespread human exposure to a plethora of lead compounds and the beginning of research into the metal's toxicological attributes. In the 20th century, lead poisoning became a well-documented phenomenon, largely traced back to the pervasive presence of lead in everyday commodities like paint, batteries, and gasoline. The absorption pathways of lead have been discovered, along with the diverse interaction of the element with the systems of the human body. Efforts have been made to reduce the environmental emissions of lead by implementing measures such as the ban on the sale of leaded gasoline in the United States in 1986 and in Europe in 2005 [1, 2].

Despite significant cutbacks in its usage, lead is still common in various sectors including industry, construction, and transportation. Especially alarming is the affinity of lead ions (Pb^{2+}) for neural structures. Though the full spectrum of lead's direct and indirect effects on neuronal development and function remains unexplained, a clear correlation has been established between exposure to lead compounds and the onset of certain neurological disorders.

Elevated levels of Pb^{2+} in the bloodstream present a fatal risk to all age demographics. Furthermore, a wealth of research underscores that chronic exposure to even low, non-symptomatic doses can trigger developmental disorders in children [3, 4]. The role of Pb^{2+} in the pathogenesis of autism, attention deficit hyperactivity disorder, and schizophrenia has been highlighted [5]. Additionally, early-life lead exposure may hasten neurodegenerative changes, potentially leading to conditions like Alzheimer's or Parkinson's disease later in life [6].

Given these potential risks, maintaining the health of the fragile developing nervous system during prenatal and neonatal stages necessitates diligent monitoring and reassessment of what constitutes safe lead ion concentrations in the bloodstream. Until 2012, the term "level of concern" was used to refer to children, indicating a minimum blood lead concentration of 10 µg/dL. This concept was replaced by the blood level reference value, which represents the average blood lead concentration in this age group. Currently, the accepted value for this reference is 3.5 µg/dL [7].

ABSORPTION AND DISTRIBUTION PATHWAYS OF LEAD IN THE BODY

The absorption of lead via inhalation primarily depends on particle size and solubility. Studies have revealed that small inorganic lead particles in aerosol form (less than 1 µm) are absorbed with striking efficiency – approx. 95% – within the bronchiolar-alveolar region [8]. Conversely, larger particles (exceeding 2.5 µm) are usually ensnared by the ciliated epithelium of the upper respiratory tract and subsequently expelled to the nasopharyngeal region and then swallowed [9]. When it comes to organic forms of lead, such as tetraethyllead and tetramethyllead, the absorption rate is estimated to range between 60–80% [10].

Lead absorption via the gastrointestinal tract remains an intricate process with many unknowns. The potential

mechanisms for both transcellular and paracellular transport through the intestinal epithelium are currently under scrutiny. It has been ascertained that inorganic lead compounds are most effectively absorbed from the duodenum after forming complexes with phosphates and bile acid residues [11]. The possibility of membrane carriers for ionized lead, akin to those for calcium and iron ions (Fe^{2+}), which facilitate active transport across mucous and serous membranes or into enterocytes, remains a viable hypothesis [12, 13].

Several factors are known to modulate the efficiency of lead absorption through the gastrointestinal tract, including age, the volume of gastrointestinal content, dietary calcium and iron content, pregnancy status, lead dose, and the bioavailability of lead compounds [14, 15, 16, 17]. It is noteworthy that, statistically, the gastrointestinal tract is the primary avenue for lead intoxication in children [18].

Transdermal absorption is seemingly of minor quantitative relevance for inorganic forms of lead. Notably, trace amounts of lead in the blood correlate with observable levels of lead compounds in the outermost layers of the stratum corneum in individuals with occupational exposure [19]. The absorption of tetraalkyl lead compounds is considerably more efficient, as shown in studies on rodent skin exposed to tetraethyllead [20].

Regardless of the route of absorption, inorganic lead infiltrates the bloodstream, where it binds to red blood cells in 99% [21, 22]. Several mechanisms for the transport of Pb^{2+} across the erythrocyte cell membrane have been put forth. The process likely involves HCO_3^--dependent anion exchangers, and the possibility of calcium channel involvement cannot be discounted [23, 24]. Within the erythrocyte, Pb^{2+} bind to protein ligands, primarily delta-aminolevulinic acid dehydratase (ALAD) [25]. It is postulated that the release of lead from erythrocytes likely involves active transport via Ca^{2+}-ATPase [26].

A significant proportion of Pb^{2+} ions – approx. 90% – gradually accumulates in bones due to their propensity to form complexes with phosphate residues, much like calcium ions (Ca^{2+}) [27]. The ensuing deposits, structurally akin to hydroxyapatites, ensure consistent availability of Pb^{2+}, even amidst declines in the element's blood concentration due to excretion. Conditions marked by predominant osteolytic processes lead to an enhanced release of lead from this bone reservoir, followed by redistribution to other organs [28, 29]. Beyond bones, lead primarily accumulates in organs including the liver, skeletal muscles, skin, adipose tissue, lungs, kidneys, aorta, and brain [27, 30]. The precise transport mechanism of lead to soft tissues remains a mystery; however, it is suggested that lead may utilize pathways originally designed for Ca^{2+} and Fe^{2+}.

TRANSPORT OF LEAD IONS TO THE CENTRAL NERVOUS SYSTEM

Entry into the intricate and delicate environment of the central nervous system is constrained by the blood–brain barrier (BBB) – a specialized formation comprising tightly conjoined endothelial cells of brain capillaries, enveloped by astrocytic processes [31]. Despite the BBB's selective ion permeability – integral to the delivery of vital macro- and micronutrients to the brain – lead, in its ionic state, can permeate it via simple diffusion or by forming inorganic compounds dependent on anion exchangers. This transportation process may involve voltage-dependent calcium channels (VDCC), calcium release-activated channels, and potentially the transport protein DMT1 [32, 33, 34]. The release of Pb^{2+} into the bloodstream implicates Ca^{2+}-ATPase, mirroring the mechanism observed in red blood cells [35].

Severe symptomatic lead poisoning has been proven to directly impair and compromise the structural integrity of the BBB, while concentrations beneath 80 μg/dL do not elicit discernible structural changes [36, 37]. A linear association has been established between the maturation of the BBB and the concentration of Pb^{2+} accumulated in representative brain regions, such as the hippocampus, underscoring the neuroprotective importance of a properly functioning, fully matured barrier [38, 39].

Performing a function analogous to the BBB, the blood–cerebrospinal fluid barrier (BCB) consists of endothelial cells of the choroid plexus vessels in direct contact with cerebrospinal fluid. The deleterious effects of lead on the BCB appear to be more severe in children than in adults. The inhibitory influence of Pb^{2+} on the expression of claudin-1, a protein integral to intercellular junctions of the endothelium, indicates that it may compromise BCB's integrity during its development [40]. Lead's impact on the functionality of the choroid plexus is further exemplified by diminished transthyretin expression, a protein crucial for the transport of thyroid hormones that are essential for brain development [41].

IMPACT OF LEAD ON GLIAL CELLS

Astrocytes serve as a bridge between endothelial cells and neurons. Research has shown that these types of glial cells have the capacity to accumulate significant concentrations of Pb^{2+}, thus restricting the spread of Pb^{2+} in the central nervous system [42, 43]. The entry of lead into astrocytes likely occurs through L-type VDCC [44]. Intracellular organization of lead deposits bypasses the mitochondrial network, preserving the energetic efficiency of astrocytes [42]. The process of Pb^{2+} accumulation is most efficient in immature astroglia, resulting in the inhibition of the expression of specific proteins necessary for the physiological progression of the cell cycle [45]. Prolonged exposure leads to altered cell morphology, improper distribution of mitochondria and Golgi apparatus, expansion of the endoplasmic reticulum, and excessive production of gliofilaments. Regions of astrocyte proliferation become apparent, particularly in the hippocampus and prefrontal cortex [46]. Potential disruptions in the interaction between Pb^{2+}-burdened astrocytes and endothelial cells can result in dysfunction of brain capillaries [42]. The limited Pb^{2+} buffering capacity in astroglial cells poses a potential threat to more vulnerable neurons.

Oligodendrocytes play a crucial role in the formation of myelin sheaths in the central nervous system. It has been demonstrated that oligodendroglia exhibits a particular vulnerability to the toxic effects of lead, especially during the early stages of cell differentiation [47]. The direct effects include the inhibition of olig2 transcription factor expression, CNPase protein levels, and the suppression of sodium-calcium exchanger 3 activity [48]. Consequently, this disrupts the regulation of intracellular calcium concentration, leading to the arrest of oligodendrocyte development at the precursor stage and a significant reduction in the efficiency of myelination and remyelination processes [49].

IMPACT OF LEAD ON NEURONS

Ionic disturbances

When discussing the direct effects of lead exposure on the central nervous system, it is crucial to consider the interactions between Pb^{2+} and the metabolic pathways of specific cations. Lead enters the interior of neurons through at least 3 membrane transport mechanisms. The first mechanism does not involve any carrier proteins, while the involvement of VDCC and NMDA-activated channels is suggested in the other mechanisms [50].

Calcium

Under physiological conditions, VDCC facilitates the transport of Ca^{2+} into cells. Maintaining a low intracellular concentration of Ca^{2+} is necessary in the resting state of presynaptic neuron membranes. Depolarization triggers the opening of VDCC, allowing calcium influx to form complexes with proteins involved in synaptic vesicle formation. Lead likely binds to the extracellular region of VDCC, blocking the channel's access to Ca^{2+} while promoting the transport of Pb^{2+} into the neuron [51]. Lead exhibits a high affinity for protein domains dedicated to Ca^{2+}, influencing pathways involved in neurotransmitter release. Calmodulin is activated through the binding of Ca^{2+} to EF hand domains. At nanomolar concentrations, lead inappropriately occupies these domains, partially stimulating calmodulin and its dependent phosphodiesterase [52]. Conversely, higher concentrations of Pb^{2+} lead to a reduction in calmodulin activity, possibly due to the availability of additional binding sites and allosteric modifications of the protein [53]. The influence of Pb^{2+} on the expression of genes related to calmodulin has been demonstrated [54]. Changes in calmodulin metabolism result in disruptions in signal transmission at the synaptic level. Another protein susceptible to the effects of Pb^{2+} is protein kinase C (PKC), with certain isoforms depending on Ca^{2+}. Protein kinase C plays various roles in cells, contributing to the initiation of signaling pathways that activate transcription factors. It has been shown that picomolar concentrations of Pb^{2+} partially activate the enzyme, while higher concentrations can have inhibitory effects on PKC [55].

Zinc

Zinc serves essential functions in the body, including the formation of complexes with proteins involved in antioxidant processes. It also complements specific Cys2-His2 domains in zinc finger proteins, which are transcription factors that bind to specific DNA sequences. It has been shown that Pb^{2+} competes with zinc ions (Zn^{2+}) for binding sites on various zinc finger proteins due to its high affinity for thiol groups [56]. Consequently, this leads to the inhibition of specific transcription factors necessary for the proper development of the central nervous system, such as TFIIIA, Sp1, and Egr-1 [57, 58, 59].

Zinc ions are also crucial for the proper functioning of ALAD in red blood cells. Lead ions show a high affinity to the sulfhydryl groups present in ALAD subunits, which originally bind zinc as a cofactor [25]. The inclusion of Pb^{2+} inhibits ALAD activity, resulting in a decrease in heme synthesis and the production of free radicals [60]. This lead-induced anemia poses an indirect threat to brain energy metabolism.

An important mechanism of lead neurotoxicity is its interaction with the N-methyl-D-aspartate receptor (NMDAR), whose activation depends, in part, on the presence of zinc. While the precise binding site of Pb^{2+} to the receptor is still uncertain, the antagonism of lead against NMDAR has been demonstrated, with 1 potential target being the allosteric binding site of Zn^{2+} [61]. Consequently, the blockade of NMDAR leads to a decline in the ability to acquire cognitive functions and shape memory through the mechanism of long-term synaptic potentiation (LTP) [62].

Changes in neurotransmission

The NMDAR belongs to the ionotropic, heteromeric receptors for glutamate [63]. It plays a significant role in glutamatergic neurotransmission, serving as a key player in shaping synaptic plasticity through the mechanism of LTP [64]. This process occurs during the development of neuronal pathways and the formation of memory traces, which is why NMDAR exhibits high expression levels in the hippocampus, cerebral cortex, and basal ganglia [65]. Selective blockade of the receptor using aminophosphonovaleric acid in rat brain cells resulted in a noticeable decline in learning and memory abilities comparable to states of hippocampal damage [66]. On the other hand, the detrimental effects of excessive NMDAR stimulation have also been demonstrated, leading to the uncontrolled influx of Ca^{2+} into neurons and neuronal apoptosis [67].

The receptor consists of the NR1 subunit, which is present in every NMDAR variant, and one of the NR2 subunits (A, B, C, or D), whose type depends on the stage of cell development and the location within the body [68]. During the prenatal and neonatal periods, the NR2B-NMDAR form predominates and gradually gets replaced and supplemented by the NR2A-NMDAR form or, in the case of the cerebellum, the NR2C-NMDAR form [38, 68]. In fully developed brains, specific regions of receptor localization, such as the forebrain and posterior horns of the spinal cord, exhibit the NR2B subunit [69, 70].

In mature glutamatergic synapses, the production and release of presynaptic vesicles containing the neurotransmitter stimulate the NR2A-NMDAR on the postsynaptic membrane. The complete activation of the receptor is a multifaceted process involving the binding of glutamate and glycine,

depolarization-induced removal of the magnesium ions (Mg^{2+}) block, and the involvement of Zn^{2+} as a cofactor. The channel associated with NMDAR mediates the flow of Ca^{2+}, sodium (Na^{+}), and potassium (K^{+}) ions [65]. Controlled elevation of intracellular Ca^{2+} concentration in the postsynaptic cell promotes cascades of events involving calcium-binding proteins such as calmodulin and PKC.

The activated NMDAR influences the phosphorylation pathway of the cAMP response element-binding protein (CREB), which is a transcription factor necessary for the synthesis of brain-derived neurotrophic factor (BDNF). Released retrogradely from the postsynaptic cell, BDNF plays a crucial role in strengthening the signal in glutamatergic synapses by activating the tropomyosin receptor kinase B in the presynaptic cell [71]. Stimulation of presynaptic terminals enhances the production of vesicles containing glutamate and mobilizes proteins from the SNARE family to participate in neurotransmitter release [72]. In this way, brief high-frequency neuronal stimulation leads to long-term enhancement of synaptic transmission effectiveness, exemplifying the essence of the LTP model. The significance of BDNF in this process is underscored by the promising results of pharmacological trials for Alzheimer's disease using agents that increase BDNF levels [73].

The NMDAR is recognized as one of the key molecular targets for Pb^{2+} [74]. Lead's affinity for the NR2A subunit and noncompetitive inhibition of the allosteric Zn^{2+} binding site are suggested mechanisms [62]. This leads to the blockade of receptor activation and the inability to open the ion channel. The impermeability of the postsynaptic membrane to Ca^{2+} inhibits pathways involved in protein phosphorylation that modulates synaptic transmission [75]. Additionally, there is an excessive expression of NR2B-NMDAR in extrasynaptic locations, indirectly inhibiting the pathway associated with CREB [76]. This results in a reduced level of BDNF protein, disruption of feedback signaling, and insufficient reinforcement of synaptic transmission for efficient memory retention.

Other direct targets for Pb^{2+} are the proteins involved in the organization of synaptic vesicles and the release of neurotransmitter into the synaptic space. Lead has been shown to have inhibitory effects on the expression of synaptophysin and synaptobrevin , proteins responsible for vesicle docking to the presynaptic membrane [77]. Dysfunction of synaptotagmin due to Pb^{2+} binding at the Ca^{2+} binding site manifests as impaired vesicle fusion and a decrease in neurotransmitter release [78].

Mitochondrial metabolism

The central nervous system exhibits particularly intense energy metabolism. Insufficient substrate supply or impairment of any step in adenosine triphosphate (ATP) synthesis poses a lethal threat to neurons, leading to cognitive impairments, loss of consciousness, or even death. Key steps of cellular aerobic metabolism take place in mitochondria, semi-autonomous organelles with a double membrane system. Differences in the permeability of mitochondrial membranes give rise to the intermembrane space and the mitochondrial matrix, which have distinct chemical and enzymatic compositions. The inner membrane houses complexes I–IV of the electron transport chain (ETC), which generate a proton gradient used by ATP synthase to carry out oxidative phosphorylation.

Currently, mitochondria are the subject of many studies investigating the toxicity of lead, which report new potential molecular targets for Pb^{2+} [79]. The rationale behind these investigations is based on the interactions of Pb^{2+} with cation pathways and the possibility of their substitution in mitochondrial protein composition. The potential competition between Pb^{2+} and ions such as: manganese (Mn^{2+}), Fe^{2+}, copper (Cu^{2+}), and Zn^{2+} poses a risk to mitochondrial homeostasis, particularly in terms of losing control over the concentration of oxidative factors [80]. For example, reactive oxygen species (ROS) are products of physiological processes in the ETC. At low concentrations, ROS can participate in intercellular signaling, and their excess is reduced by specific enzymes such as glutathione (GSH) peroxidase, catalase, and superoxide dismutase. The presence of lead inhibits antioxidant enzymes and depletes the capacity for ROS processing by GSH and natural antioxidants, leading to severe cell damage or death [81]. Particularly harmful is the peroxidation of polyunsaturated fatty acids, resulting in the production of malondialdehyde (MDA) and 4-hydroxyn--onenal, toxic aldehydes that alter the physical properties of cell membranes and form complexes with enzymatic proteins and nucleic acids [82].

One of the functions of mitochondria is initiating apoptotic pathways in response to external or internal signaling stimuli. The primary process preceding cascades of apoptotic changes is the opening of mitochondrial permeability transition pores (MPTP). The lead's role as an inducer of apoptosis seems to be related to its impact on cellular Ca^{2+} metabolism. A proposed mechanism involves Pb^{2+} interacting with endoplasmic reticulum calcium release receptors [83]. Sustained high cytoplasmic Ca^{2+} concentration stimulates calcium influx into mitochondria, leading to MPTP opening.

It has been demonstrated that Pb^{2+} can promote apoptosis in neurons not only by modifying the permeability of mitochondrial membranes but also by disrupting the expression of mitochondrial nucleic acids. This impact is manifested through an increase in the release of cytochrome c, particularly detrimental is the peroxidation of polyunsaturated fatty acids, resulting in the production of MDA and 4-hydroxynonenal, toxic aldehydes that alter the physical properties of cell membranes and form complexes with enzymatic proteins and nucleic acids [82]. Additionally, it affects the expression of caspase 3 and 9, an excessive ratio of Bax to Bcl-2, and the activation of p53 protein [84].

INDIRECT NEUROTOXIC EFFECTS

The central nervous system's maturation relies on a network of systemic metabolic pathways which can be adversely affected by exposure to lead. Specifically, the impact of Pb^{2+} triggers a reduction in the activity of an enzyme called aminolevulinic

acid (ALA) dehydratase, which in turn leads to impaired heme synthesis and the accumulation of delta-aminolevulinic acid (δ-ALA) in red blood cells [25]. This unstable compound transforms into a self-oxidizing enolic form, which generates singlet oxygen ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2) when reacting with oxyhemoglobin. Both $O^{2\bullet-}$ and H_2O_2 play a role in generating highly reactive hydroxyl radicals, potentially amplifying oxidative stress [85]. An elevated level of δ-ALA also inhibits neurotransmission involving γ-aminobutyric acid (GABA). Although the exact mechanism behind this occurrence remains unclear, it likely involves the inhibition of potassium-dependent GABA release by synaptosomes or complications with the binding of GABA by synaptic membrane receptors [86].

Furthermore, lead's influence on the body's iron metabolism is a significant concern. The availability of Fe^{2+} is crucial for numerous processes vital for the developing brain, such as oxygen binding and transport, the Krebs cycle, oxidative phosphorylation, lipid metabolism, and nucleic acid synthesis and expression [87]. Lead ions can decrease Fe^{2+} absorption in the intestines, potentially leading to severe iron deficiency, sideropenic anemia, and failure of bioenergetic processes. The reduced uptake of Fe^{2+} from the gastrointestinal tract might be due to competition for binding sites of divalent metal transporters [88]. Another detrimental effect involves the obstructed removal of Fe^{2+} from the brain because Pb^{2+} inhibits the expression of ferroportin 1, a protein responsible for iron transport from cells into the bloodstream [89].

CONCLUSION

The progress made in understanding the molecular mechanisms of Pb^{2+} sheds light on the inherent risks and utter redundancy of lead's presence within the body. Various studies have illustrated the competitive nature of Pb^{2+} with divalent metals from the metabolic pool, notably Ca^{2+}. By exploiting transport pathways and binding sites on specific proteins, Pb^{2+} can infiltrate nearly every organ, including the brain.

A significant concern stems from the metal's tendency to accumulate in bones, thereby leading to continuous exposure to lead's detrimental effects on vital tissues, irrespective of whether the exposure occurred several years prior. Whether present in high concentrations or even at subthreshold low levels, lead disrupts neuronal homeostasis. It hampers synaptic conduction mechanisms, obstructs the formation of new neuronal connections, and diminishes the efficiency of the brain's energy processes.

During its developmental phase, the central nervous system is particularly vulnerable to the adverse effects brought about by Pb^{2+}. Moreover, an asymptomatic, hidden long-term lead intoxication can potentially precipitate serious cognitive impairments and accelerate neurodegenerative processes later in life.

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