Molecular mechanisms of lead toxicity

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Abstract

Lead is a ubiquitous xenobiotic metal and because of its widespread industrial use is one of the most serious environmental pollutants. Lead is highly toxic and exposure to even low doses can produce adverse effects on all living organisms. In humans, lead exposure can affect virtually all body systems, resulting in severe health problems. On a molecular level, lead can alter or interfere with the activity of proteins and nucleic acids, ultimately resulting in changes in cell metabolism and physiology leading to pathologic states. Lead can also affect the functions of macromolecules, directly or indirectly, by inducing oxidative stress, which is regarded as a primary factor in pathophysiology of lead exposure.

Key words: lead toxicity, heavy metal, oxidative stress, RNA damage, DNA damage, genomic instability

Introduction

Lead is the most abundant heavy metal present in the environment. Both metallic lead and its inorganic and organic salts have been known and widely used since ancient times. The Romans used lead and its alloys on a large industrial scale, utilizing it in the production of water pipes, tableware, and other objects of everyday use. In addition to industrial applications, various lead compounds were used as medicines for both external and internal use (Nriagu, 1992). Because of its sweet taste, lead acetate was also used as a sugar substitute. The sweet syrup commonly used in Ancient Rome to sweeten wine was obtained by boiling wine in lead pots. The use of the "sugar of lead" as a sweetener and possibly as an antibacterial agent in wine production continued until the 19th century.

At the turn of the 20th century, increasing amount of data indicated high toxicity of lead resulting in the prohibition of use of its compounds in medicinal applications, such as internal use drugs, and as food additives. In many countries, these findings also prompted legislative regulations aimed at limiting environmental lead exposure of the general population. In the past 40 years in the United States, the initial restrictions followed by a total bans on the use of lead and its compounds in household paints, plumbing, and as a gasoline additive, have resulted in over 90% reduction of the mean blood lead levels in children. Phasing out lead from automobile gasoline reduced atmospheric lead emission levels by 98% when compared with the levels of the 1970s (Pirkle et al., 1994). Further reduction was achieved by limiting emission from metal ore processing and smelting plants. Despite these measures, lead still remains one of the most serious and health-threatening environmental pollutants. According to recent estimates, a significant fraction of U.S. children still have their blood lead levels higher than 5 μg/dl, a value set by the Centers for Disease Control and Prevention as a reference value requiring medical intervention (Brink et al., 2013).

Given its widespread occurrence and persistence in the environment, lead is considered one of the most dangerous toxins. It poses serious health threats not only to individuals directly handling it, but also to general population, particularly in the environmentally degraded regions. In numerous studies, environmental and occupational lead exposure was linked to human diseases affecting cardiovascular, immune, renal, reproductive, skeletal, and nervous systems (Silveira et al., 2014; Zhang et al., 2012; Xie et al., 2013). The primary routes through which lead enters the human organism are respiratory and gastrointestinal systems. Over one-third of the absorbed lead enters the blood and is further dispersed into other tissues (Patrick, 2006). The blood lead levels are commonly used to assess the health risk. Lead concentrations of over 0.6 μM are associated with health problems (Silbergeld, 2003), but they may be significantly

higher (up to 10 μM) in the cases of occupational exposure (Hayes, 1997). Chronic exposure to lead correlates with certain forms of cancer, and the International Agency for Research on Cancer (IARC) included lead compounds in the list of probable carcinogens (García-Lestón et al., 2010).

The effects of lead exposure are more pronounced on the central nervous system, affecting its development and functioning. Lead is recognized as a neurotoxic agent affecting cognitive and behavioral functions. Prenatal and postnatal lead exposure constitutes a key factor in the development of brain (Cecil et al., 2008). There exists a high correlation between the lead blood levels in children and their cognitive abilities and social behavior. Even low level exposure to lead may result in significantly lower performance in the IQ tests (Jusko et al., 2008; Lanphear et al., 2005), antisocial behavior, and other symptoms of attention-deficit hyperactivity disorder (ADHD) (Froehlich et al., 2009).

Molecular mechanisms of lead toxicity

It is difficult to narrow down the causes of lead toxicity on a molecular level. Lead can affect proteins interfering with their enzymatic functions or the ability to bind other cellular components. Other mechanisms involve damage of nucleic acids and induction of oxidative stress. Lead exposure affects numerous cellular processes each of which may, to some extent, contribute to the clinical symptoms of lead poisoning (Fig. 1). The contribution of each mechanism may depend on the magnitude of exposure and/or cell type.

Oxidative stress

The major toxic effect of lead observed in animal and plant cells is the increase in the levels of reactive oxygen species (ROS). In aerobic organisms, the ROS are constantly generated in cells as by-products of many biochemical processes including respiration and natural anabolic and catabolic pathways. However, under normal conditions, the concentrations of various ROS are kept at safe levels by the activity of the antioxidant enzymes and synthesis of low molecular weight antioxidant compounds. The oxidative stress is a result of the imbalance between the production and scavenging of ROS. Essentially, such an imbalance can be brought about in two ways, either by increased ROS production or by an impaired ROS detoxification/scavenging system.

Overproduction of free radicals during oxidative stress causes damage to cellular structures, tissue damage, inflammatory response, and apoptosis. ROS can target proteins, nucleic acids, and lipids, leading to their modifications, damage, and inactivation (Djordjević, 2004). Increased production of free radicals can also have long-lasting effects and some of the chronic human diseases have been linked to oxidative stress (Bocci and Valacchi, 2013). In neural cells, increased production of ROS is regarded as one of the primary factors responsible for the development of neurodegenerative disorders such as Alzheimer's and Parkinson's (Breitenbach et al., 2013).

Lead not being a redox-active element cannot directly participate in the reactions leading to the formation of ROS; however, in erythrocytes a generation of ROS has been induced by its interaction with oxyhemoglobin (Ribarov and Bochev, 1982). However, the major contribution of lead to the onset and propagation of oxidative stress is by interfering with the enzymes and other cellular components of the defense system counteracting oxidative damage (Gurer and Ercal, 2000).

Glutathione (GSH), a tripeptide of glutamate, cysteine, and histidine, is one of the most important factors protecting cell components from ROS damage. Glutathione reduces disulfide bonds in proteins to cysteines using the sulfhydryl group of cysteine as a proton donor. In the process, GSH is converted to the oxidized form, glutathione disulfide (GSSH). Glutathione reductase (GR) reduces glutathione disulfide back to glutathione using NADPH as a proton donor (Franco et al., 2007). Glutathione oxidation is one of the elements of ROS detoxification and its decreased levels, accompanied by increased levels of GSSH, are frequently observed on exposure to lead (Aoyama and Nakaki, 2013; Kasperczyk et al., 2004, 2009, 2013). Lead has a twofold effect on the levels of glutathione. First, GSH was shown to form complexes with Pb^{2+} *via* the thiol groups of cysteine (Mah and Jalilehvand, 2012), and second, lead can inhibit the activity of glutathione reductase interfering with recycling of oxidized glutathione disulfide (Sandhir and Gill, 1995).

Although glutathione and its glutathione reductase seem to be the major targets of lead, other enzymes involved in ROS detoxification are also affected by lead exposure (Kasperczyk et al., 2012), thus contributing to the increased levels of free radicals in the cell.
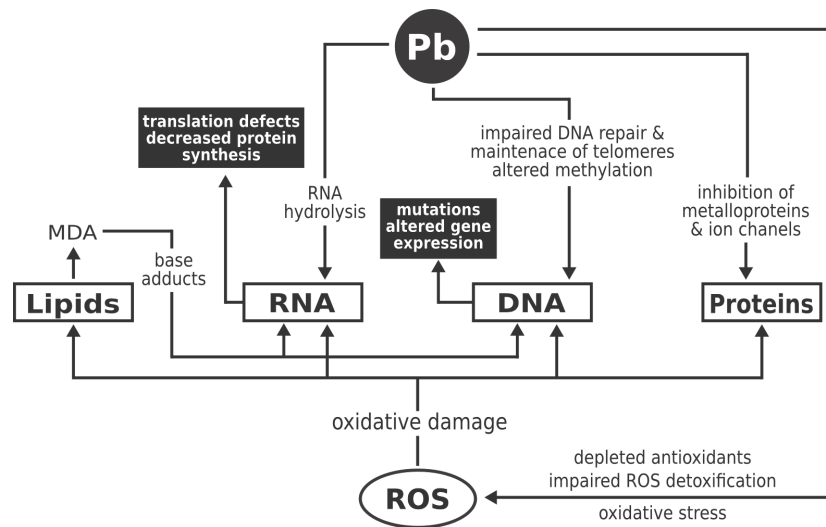
Fig. 1. Molecular targets of lead. Integrity and functions of biomolecules are affected by lead exposure directly or indirectly by promoting oxidative stress and increased levels of reactive oxygen species; see text for details

Interactions with proteins

Lead exposure was shown to affect many proteins, some of which play key roles in metabolic or regulatory pathways. Changes in the activity of enzymes or regulatory proteins can significantly contribute to its pathological state. It is assumed that Pb^{2+} can directly interfere with functions of proteins by binding thiol groups of cysteines or by ionic displacement, competing for binding with natural ligands of metal-dependent enzymes or ion transporters (Bridges and Zalups, 2005).

δ-aminolevulinic acid dehydratase

One of the enzymes that is a common target of lead toxicity in animals and plants is δ-aminolevulinic acid dehydratase (ALAD) also known as porphobilinogen synthase. ALAD catalyzes condensation of two molecules of δ-aminolevulinic acid (ALA) to form the porphobilinogen, a precursor of heme, chlorophylls, cytochromes, and cobalamins. The ALAD activity depends on the binding of a divalent metal cation Zn^{2+} in animal, fungal, and bacterial ALADs and Mg^{2+} in plant enzymes (Senior et al., 1996). In mammals, ALAD is expressed in all cell types, with the highest expression levels found in liver and erythrocytes (Warren et al., 1998). Because of its sensitivity to lead, ALAD is commonly used as a biomarker of lead exposure, and studies on the effects of lead in occupationally exposed workers demonstrated that there is a strong negative correlation between the blood lead levels and ALAD activity (García-Lestón et al., 2012).

The results of several studies suggested that the susceptibility to toxic effects of lead could depend on the genetic factors involving ALAD. In human gene encoding ALAD, multiple single nucleotide polymorphisms (SNP) have been identified. One of these SNPs involving G-to-C transversion at position 177 within the coding region results in a lysine to asparagine substitution at position 59 of the protein sequence. The combinations of polypeptides encoded by the two alleles, ALAD1 (Lys) and ALAD2 (Asn), can produce three isoenzymes-ALAD1-1, ALAD1-2, and ALAD2-2-depending on the genotype. The isoenzymes can be distinguished based on the difference in the charge resulting from the substitution of positively charged lysine with neutral asparagine, and were proposed to have different sensitivity to inhibition by lead (Wetmur et al., 1991). The results of analyses of the correlation between blood lead levels and the frequencies of ALAD alleles are contradictory (Smith et al., 1995). In one study it was shown that ALAD2 allele correlates with higher blood lead concentrations, especially at high levels of exposure (Wetmur et al., 1991). On the other hand, a study on a group of occupationally exposed workers demonstrated that blood lead levels were higher in ALAD1 homozygotes than in heterozygotes (Yang et al., 2012).

The direct consequence of decreased enzymatic activity of ALAD is increase of the concentration of its substrate – the δ-aminolevulinic acid (δ-ALA). An accumulation of δ-ALA can induce production of ROS by eno-

lization and auto-oxidation at physiological pH, producing a superoxide anion (Bechara, 1996) or *via* coupled oxidation of oxyhemoglobin (Monteiro et al., 1986). The δ-ALA accumulation can also affect the integrity of the DNA. 4,5-Dioxovaleric acid, the final product of δ-ALA oxidation, was shown to be an alkylating factor capable of modifying guanines (DiMascio et al., 2000). Because its similarity with γ-aminobutyric acid (GABA), δ-ALA can bind GABA receptors in the nervous system (Bellinger, 2011) and probably contribute to their oxidative damage (Adhikari et al., 2006) which could to some extent account for the neurotoxic effects of lead.

Calcium-binding proteins

Lead exposure was shown to affect the function of calmodulin (CaM), a second messenger protein sensitive to fluctuations in the intracellular calcium levels. A unique feature of calmodulin is its ability to interact and regulate the activity of hundreds of proteins directly linked to changes in multiple cellular processes (Hoeflich and Ikura, 2002). Calmodulin is a highly conserved protein consisting of two globular domains, each containing two helix-loop-helix EF-hand motifs involved in binding Ca^{2+} ions (Hoeflich and Ikura, 2002). The binding or dissociation of calcium ions causes conformational changes altering calmodulin's affinity for the effector proteins (Park et al., 2008). Recent studies have demonstrated the fact that though lead shows higher affinity for both N- and C-terminal domains of calmodulin than calcium ions, not all of the Pb^{2+}- and Ca^{2+}-binding sites are the same. At low concentrations, Pb^{2+} binds the calcium-binding sites, which may result in the activation of calmodulin (Ouyang and Vogel, 1998). On the other hand, at higher concentrations of lead, Pb^{2+} displaces Ca^{2+} from two of its binding sites, but also binds with the linker region, altering its structure and preventing interactions with CaM target proteins (Kirberger et al., 2013). Thus, the impact of lead on calmodulin-dependent pathways may depend on the concentration of Pb^{2+}. At low levels, lead may be responsible for hyperactivation, and at higher levels for inactivation.

Lead can also affect activities of other calcium-binding proteins. Pb^{2+} ions bind with a high-affinity calcium-binding site within osteocalcin, a protein involved in bone mineralization and resorption. In this case, the inhibitory effect of lead can interfere with bone mineral dynamics, which could explain the defects in the development of the skeletal system in children exposed to lead (Dowd et al., 1994). Lead is also a strong inhibitor of the voltage-gated Ca^{2+} and K^+ channels (Atchinson, 2003) and an activator of calcium/phospholipid-dependent protein kinase C, the proteins associated with many neuronal functions including learning and memory (Long et al., 1994).

Zinc finger proteins

Zinc finger motifs are protein domains involved in the sequence-specific recognition and thus binding of nucleic acids. The zinc finger fold is stabilized by one or two zinc ions coordinated by histidine and cysteine residues (Klug and Rhodes, 1987). Zinc finger proteins are essential components of gene regulatory networks and they participate in transcriptional activation of many essential genes involved in numerous processes (Klug, 1999; Laity et al., 2001). Because its affinity for thiol groups, Pb^{2+} can efficiently compete with Zn^{2+} for binding sites within cysteine-rich zinc fingers. The affinity of Pb^{2+} for zinc finger domains depends on the number of cysteines forming the metal-binding site. However, unlike zinc ions, Pb^{2+} are unable to stabilize the correct structure required for DNA binding (Payne et al., 1999). Substituting a native zinc ion with lead within the DNA-binding domain of a transcription factor can lead to a loss or alteration of its transcriptional activity.

The prototypic zinc finger protein is a transcription factor IIIA (TFIIIA) required for expression of 5S ribosomal RNA containing nine Cys(2)His(2) zinc fingers involved in DNA and RNA binding. *In vitro* studies demonstrated that micromolar concentrations of lead inhibited binding of TFIIIA to the promoter region of 5S rRNA gene. The inability of lead to disrupt the TFIIIA-DNA complex after it has been formed suggests that it interfered with binding of zinc ions to zinc finger domain (Hanas et al., 1999).

Transcription factor Sp1 is involved in transcription of RNA polymerase II-dependent genes from promoters rich in GC elements. The DNA-binding domain of Sp1 consists of three zinc fingers binding the GC box (Li and Davie, 2010). Mapping of the transcription binding sites in human genome revealed that Sp1 can bind at least 12,000 sites (Cawley et al., 2004) associated with promoters of genes encoding proteins involved in a wide variety of processes including growth, apoptosis, angiogenesis, immune response, neural functions, and chro-

matin remodeling (Tan and Khachigian, 2009). Many of the Sp1 target genes are important in the initiation and progression of cancerogenesis (Li and Davie, 2010). A study on DNA binding and transcriptional activity of Sp1 has revealed that lead affects the formation of DNA-Sp1 complex *in vitro* (Zawia et al., 1998; Hanas et al., 1999). Moreover, lead exposure alters expression profiles of Sp1-dependent genes involved in the development of the rat nervous system both in cell cultures and *in vivo* (Zawia et al., 1998). Similar changes in gene expression profiles in the developing rat brain were also observed for another zinc finger protein-containing transcription factor, Egr-1 (Reddy and Zawia, 2000).

These findings, although limited to a subset of developmentally regulated genes, suggest that by interfering with the activity of transcription factors, lead may affect expression of other genes, contributing to the pathophysiology of lead toxicity.

Effects of lead on DNA

Apart from inducing oxidative stress and interfering with the function of proteins, lead has a genotoxic effect affecting the integrity of a genetic material. Lead and its compounds were recognized as mutagenic factors (Roy and Rossman, 1992). These observations were further confirmed by epidemiological studies showing a correlation between elevated blood lead levels and an increased mutation rate and DNA damage (García-Lestón et al., 2012). In addition to the direct and indirect damages, lead can affect the structure of chromatin by influencing DNA methylation.

Oxidative damage

The oxidative damage of DNA was for a long time recognized as a major threat for cells, due to its far-reaching biological consequences like carcinogenesis and aging. ROS can target all components of the DNA, deoxyribose, nucleobases, and phosphodiester bonds, resulting in hydrolysis of the phosphate-sugar backbone or modification of bases (D'Errico et al., 2008). Mutations resulting from the mispairing of modified bases and breaks in the sugar-phosphate backbone can have a profound effect on the expression and functions of affected genes. By promoting an oxidative stress and an increase in ROS concentration, lead can indirectly affect oxidation of bases in genomic DNA.

The oxidation of deoxyribose at the 5′ carbon produces furfural that can react with the amino group of adenine to produce kinetin (N6-furfuryladenine) (Barciszewski et al., 1997). The presence of kinetin in the DNA as a secondary product of DNA oxidation is potentially mutagenic. During the synthesis of a complementary strand, although DNA polymerase correctly incorporates thymine at the position opposite of kinetin, replication errors may arise from nucleotide mis-incorporations occurring in a vicinity of the modified base (Wyszko et al., 2003).

Oxidation of the nucleobases in DNA can result in several products, including 8-oxo-guanine, 8-oxo-adenine, thymine glycol, and 5-hydroxyuracil (Marnett, 2000). Owing to easy detection and quantification methods, the most extensively studied oxidized base in DNA is 8-oxo-dG, and its levels are often used as a marker for DNA damage (Mangal et al., 2009). Oxidation is a result of the reaction of the ROS with the bases, but it has been suggested that lead is important catalyst in the oxidation of guanine (Banu et al., 2012). Most of the modifications of bases have a mutagenic potential. It has been shown that by the possibility of pairing with adenine, 8-oxo-dG can induce $G \rightarrow T$ and $A \rightarrow C$ transversions (Cheng et al., 1992; McCulloch et al., 2009). Studies on the effects of other oxidized DNA nucleobases have revealed that 8-oxo-adenine, thymine glycol, and 5-hydroxyuracil are also highly mutagenic (Wang et al., 1998).

Interestingly, studies on developmental lead exposure on rat brains have demonstrated that the 8-oxo-G levels in exposed animals were elevated during aging, even though lead was no longer present in the environment. This suggests that lead-induced disturbances during development can have long-term effects stimulating neurodegeneration in the aging animals (Bolin et al., 2006).

Base adducts

One of the major targets of oxygen radicals within the cell are polyunsaturated fatty acids in phospholipids. Apart from the influence on the integrity of cellular membranes, oxidation of lipids produces a range of highly reactive compounds, some of which can react with side chains of amino acids in proteins and bases in nucleic acids. They are reports that indicates peroxidation of lipids a major cause of endogenous DNA damage (Voulgaridou et al., 2011). One of the most abundant products of lipid peroxidation is malondialdehyde (MDA), which is frequently used as a marker of lead ex-

posure. Under nonstress conditions, low levels of MDA, a by-product of prostaglandin biosynthesis pathway, are detectable in the cells. Increased levels of MDA, as well as other aldehydes produced by lipid oxidation, can contribute to DNA damage, and consequent mutations, by forming exocyclic adducts with bases (Marnett, 2002; Voulgaridou et al., 2011). The deoxyguanosine adduct of MDA (M₁G-[3-(2′-deoxy-beta-D-erythropentofuranosyl)-pyrimido[1,2-alpha]-purin-10(3H)-one]) is present in the DNA from healthy human tissues, healthy at levels of 1-4 $\times 10^{-8}$ nucleotides. M_1G was shown to be mutagenic in prokaryotic and eukaryotic cells, introducing point and frameshift mutations (Marnett, 2002; Yau, 1979, Maddukuri et al., 2010). M_1G increase was also shown to induce cell cycle arrest, increased expression of p53 and p21 proteins, and inhibition of kinase activities associated with cyclins E and B (Ji et al., 1998).

Maintenance of the telomeres

One of the early outcomes observed within a few hours of lead treatment of the cells is the formation of γH2Ax foci, which is associated with the double-stranded DNA breaks (Scully and Xie, 2013). It was proposed that the DNA damage inducing the formation of γH2Ax foci resulted from a direct interaction between Pb^{2+} and chromosomal DNA (Gastaldo et al., 2007). An analysis of the lead-induced γH2Ax foci revealed that they are primarily associated with the telomeric regions (Pottier et al., 2013), suggesting the presence of uncapped or shortened telomeres recognized by the protein complexes responsible for repairing DNA damage as double-strand breaks. An increased telomere loss and doublet formation in lead-treated cells were observed due to the presence of dysfunctional telomeres, possibly resulting from defects in the replication of the lagging strand (Pottier et al., 2013). The observed telomere instability may be due to the stabilization of the G-quadruplexes by Pb^{2+} ions. G-quadruplex structures consisting of stacked G-tetrad planes connected by Hoogsteen hydrogen bonds were shown to have been associated with telomeres in a wide range of organisms including humans (Millevoi et al., 2012). The studies on thermal stability of G-quadruplexes demonstrated that Pb^{2+} ions contribute to the formation of more compact and significantly more stable structures than those depending on stabilization by monovalent sodium or potassium ions (Liu et al., 2011). Such an explanation is also consistent with

observations that telomeres instability resulting from inhibition of telomerase activity can be caused by other factors stabilizing (Zahler et al., 1991; Burger et al., 2005; Huang et al., 2008). Owing to the high stability of G-quadruplexes, replication of the telomeres requires their enzymatic unwinding, and the mutations affecting these enzymes are often associated with telomere instability (Web et al., 2013). Replacement of K^+ or Na^+ ions in the telomeric G-quadruplexes by lead might render them inaccessible to the unwinding helicases.

The correlation between lead exposure and deficiencies in the maintenance of telomeres is consistent with epidemiological studies. An analysis of the correlation between long-term occupational exposure to lead and the telomere length in peripheral white blood cells was carried out on a group of laborers employed at one of the battery manufacturing factories in China. The telomere shortening was shown to have been primarily associated with the blood lead levels and the duration of the exposure (Wu et al., 2012), indicating that a loss of telomere maintenance is one of the consequences of the chronic lead exposure. When compared with intrachromosomal regions, telomeric sequences show much higher susceptibility to damage by oxidative stress. Such instability of telomeres might also be due to lower effectiveness of DNA repair (Ayouaz et al., 2008; Kong et al., 2013).

DNA repair

The maintenance of the genome integrity being essential for the functioning and viability of the cells, there exist efficient mechanisms of DNA repair preventing propagation of damages introduced by mutagenic agents. In addition to its involvement in the processes leading to DNA damage, lead was also shown to affect the process of DNA repair.

In mammals, lead affects the DNA repair machinery by interfering with the activity of the apurinic/apyrimidinic endonuclease 1 (APE1/Ref-1) (McNeill et al., 2004). The inhibitory effect can be observed even at very low (below 1 μM) concentrations of metal ions, and in the genomic DNA of cultured Chinese hamster ovary cells, lead exposure results in an accumulation of apurinic/apyrimidinic sites (McNeill et al., 2007). APE1 is an ortholog of the bacterial Xth nuclease and is important in the DNA base excision repair (BER) at the abasic sites and single-stranded breaks produced as a result of

oxidative stress and base modifications with alkylating agents (Mol et al., 2000a; Teebor et al., 2004). APE1 nicks the DNA upstream of the abasic sites in an Mg^{2+}-dependent reaction. The resulting gap is subsequently filled in by the DNA polymerase. It has been suggested that the inhibition of nucleolytic activity of APE1 might be because of the binding of the Pb^{2+} ions with the two divalent cation-binding sites revealed in the crystal structure of the protein (Beernink et al., 2001). Subsequent studies using NMR demonstrated that the lead-dependent inhibition of the activity of APE1 involves direct interaction between Pb^{2+} and a conserved histidine responsible for the catalysis (Lowry et al., 2003).

In addition to the hydrolytic activity against apurinic/apyrimidinic sites, the APE1 was shown to possesses other nucleolytic activities of 3 DNA phosphodiesterase/phosphatase (Demple and Harrison, 1994), $3 \rightarrow 5$ DNA exonuclease, and RNase activity (Kim et al., 2014). Nucleolytic activities of APE-1 depend on the same catalytic center involving the conserved His309 residue responsible for Pb^{2+} binding (Mol et al., 2000b). Thus, under the conditions of lead overload, it is likely that all of these functions would be impaired.

The association between genotoxic effects of lead and the ability of cells to deal with DNA damages is also consistent with the observation that the extent of DNA damage does not depend exclusively on the lead levels. Differences in various indices of genotoxicity in lead-exposed individuals were shown to correlate with polymorphisms in genes involved in DNA repair, including APE1, DNA ligase 4 (LIG4), X-ray repair cross-complementing protein 4 (XRCC4), and 8-oxoguanine glycosylase (OGG1) (García-Lestón et al., 2012).

DNA methylation

The gene expression depends to a great extent on the epigenetic modifications of the chromatin. One of the key epigenetic marks is methylation of cytosines producing 5-methylcytosine (m5C) in the DNA. DNA methylation is involved in the regulation of gene expression (Blattler and Farnham, 2013; Kulis et al., 2013), in the regulation of chromatin structure, and in genomic imprinting (Weaver and Bartolomei, 2014). Correct methylation patterns are vital for the development, establishment, and maintenance of cell identity (Koh and Rao, 2013). The changes in global methylation of the genomic DNA or altered methylation patterns on the

levels of particular genes can contribute to the development of various pathologies, including cancer and neurological disorders (Brocato and Costa, 2014).

Exposure to lead as well as to other heavy metals was shown to have been associated with altered DNA methylation status (Jirtle and Skinner, 2007; Reichard et al., 2007; Sciandrello et al., 2004). It is predicted that the changes in DNA methylation may constitute a link between environmental exposure to lead (or other heavy metals) and some of the exposure-associated diseases. They could also account for the latency between lead exposure and the appearance of the syndromes of malignancies (Li et al., 2013). A direct link between lead exposure and the methylation status of DNA was shown in humans (Pilsner et al., 2009; Wright et al., 2010). An analysis of LINE-1 repetitive elements in the cultured cells and the occupationally exposed workers of the battery plant revealed that there is a significant inverse correlation between the lead exposure and the extent of DNA methylation (Li et al. 2013). The altered DNA methylation was also proposed to account for a decreased activity of ALAD. An analysis of the methylation status of the ALAD gene revealed that occupational lead exposure correlates with increased methylation of the promoter-associated CpG islands, and consequently its reduced transcriptional activity (Li et al., 2011).

Effects of lead on the functions of RNA

The influence of lead exposure on the integrity of RNA molecules remains a largely unexplored territory. One of the reasons for this is the perception of the distinct roles played by the two types of nucleic acids in the cell. Being the repository of genetic information, DNA cannot be replaced, and its damages that can ultimately result in mutations endangering correct functioning or the viability of the cell must be promptly repaired. When compared with the consequences of DNA damage, the RNA damage does not seem to have such significant impact on the cellular functions. Damaged RNA can be replaced by correct transcripts, provided that the information contained in the DNA remains intact. On the other hand, unlike DNA, RNA molecules with some bases not involved in a secondary and tertiary structure formation and more readily accessible than paired bases within the DNA double helix may be more sensitive to damage by ROS produced during oxidative stress. A detailed analysis of nucleic acids isolated from cultured cell

lines demonstrated that RNAs are in fact subject to oxidative damage to a much greater extent than DNA (Hoffer et al., 2005, 2006; Weimann et al., 2002). Although the oxidative RNA damage does not have any potentially lethal effect on the integrity of genetic information, it has been proposed that it may contribute to the pathophysiology of complex diseases including neurodegenerative disorders (Nunomura et al., 2012; Poulsen et al., 2012). Currently, there are no reports on the oxidative RNA damage in the context of lead exposure. However, given that the oxidative stress seems to be a major factor in lead toxicity, it is reasonable to believe that, just as DNA, RNA molecules are also targets of ROS-induced damage as is in the cases of other xenobiotic-inducing oxidative stress followed by RNA oxidation (Manini et al., 2009, 2010).

Similar to the case of oxidative damage of DNA, the major targets of RNA oxidation are the guanine-containing nucleotides. Since RNA polymerase can handle oxidized nucleoside triphosphates, it is possible that 8-oxo-G, as well as other oxidized bases, can be incorporated into RNA in the process of transcription. However, co-transcriptional incorporation cannot account for the amount of observed oxidized nucleotides in RNA. In the cell, there exist mechanisms for eliminating oxidized nucleoside di- and triphosphates by their enzymatic hydrolysis to monophosphates. Also, the incorporation of oxidized nucleotides into transcripts by RNA polymerase, when compared to their unoxidized counterparts, is much less efficient (Hayakawa et al., 1997; Nakabeppu et al., 1999, 2006; Taddei et al., 1997). The most obvious consequences of the presence of oxidized bases in RNA are deficiencies in the process of protein biosynthesis. Oxidized bases, having different base-pairing properties from those of their unmodified counterparts, may be a source of translational errors leading to the production of defective proteins or premature translation termination (Ishibashi et al., 2005; Tanaka et al., 2007).

Apart from the introduction of errors in proteins, mRNA oxidation may result in ribosome stalling or slowing down the translation and, ultimately, in a decreased expression of proteins, as evident from the increase in the amount of polyribosomes in the *in vitro* translation assays programmed with oxidized mRNA (Shan et al., 2007). A decreased efficiency of translation was also shown to be associated with the oxidation of nucleotides in the ribosomal RNA (Honda et al., 2005). Due to a slow turnover rate of rRNAs, such damage can have a long-lasting effect on cellular functions. Finally, there are data indicating that in extreme cases, RNA damage can lead to cell-cycle arrest and cell death in a p53-dependent or independent manner (Bellacosa and Moss, 2003). In addition to the indirect influence on the stability of RNA by promoting the production of ROS and oxidative damage, lead can also directly bind and hydrolyze RNA. Currently, the contribution of a specific targeting of RNA by lead ions to lead toxicity remains open.

An *in vivo* study aimed at determination of the impact of lead on the integrity of cellular mRNA in rabbit reticulocytes demonstrated that there was no difference in the amount of total RNA between the exposed and control animals. However, the RNA preparations from lead-treated cells were deficient in the *in vitro* translation assays and showed a significantly higher amount of free $5'$-OH groups, suggesting RNA hydrolysis. Moreover, the ratio of polyribosomes to monoribosomes in the cells from animals exposed to lead was lower than in the unexposed controls. The *in vitro* experiments using globin mRNA treated with micromolar concentrations of Pb^{2+} suggested that the loss of translational activity of mRNA was due to RNA cleavage within the $5'$-terminal region, although the detailed mechanism was not explored (Aethranis and Farkas, 1995). A specific lead-induced cleavage was also observed in 5S RNA, group I RNA intron, as well as ribosomal RNA (-[3-(2′-deoxy-beta-D-erythropentofuranosyl)pyrimido[1,2-alpha]-purin-10(3H)-one]; Winter et al., 1997). However, since the hydrolysis occurred at millimolar concentrations of Pb^{2+}, it is unlikely that it could contribute to toxic effects of lead at physiological conditions. One of the first examples of RNA hydrolysis catalyzed by lead ions was the observation that Pb^{2+} promotes cleavage of the D loop of the yeast tRNA^{Phe} at a very low Pb^{2+} concentration (Werner et al., 1976; Brown et al., 1983). The reaction was also shown to occur *in trans* using different fragments of tRNA as catalysts and substrates (Deng and Termini, 1992). Based on these observations, the SELEX technique was used to isolate an RNA motif capable of self-cleavage in the presence of lead ions (Pan and Uhlenbeck, 1992a, 1992b). The lead-dependent ribozyme, or the leadzyme motif, is an asymmetric, purine-rich internal RNA loop closed by base-paired regions. The cleavage of *cis*- and *trans*-acting leadzyme is induced by

micromolar concentrations of Pb^{2+} (Ohmichi et al., 1998; Pan et al., 1994). The formation of structures containing the leadzyme motif in cellular RNAs either *in cis* or *in trans* could result in a site-specific cleavage RNA at a sufficient concentration of Pb^{2+} ions. Such a possibility was demonstrated in tobacco plants infected with tobacco mosaic virus (TMV). The RNA oligonucleotide carrying a catalytic sequence of leadzyme and capable of forming a leadzyme motif with the target site within TMV RNA induced hydrolysis of the viral RNA and reduced its proliferation and propagation (Wyszko et al., 2006). Whether such a mechanism of lead-dependent specific RNA hydrolysis could contribute to lead toxicity in humans remains an open question.

Acknowledgments

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