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METABOLISM AND MECHANISMS OF CYTOTOXIC ACTION OF THE LEAD IN MAMMALS

(review)

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Abstract

The real ecological situation in the Russian Federation is characterized by environmental pollution with lead compounds (V.V. Snakin, 1998). The mode of action, intake, distribution in animal body and excretion of this toxic heavy metal are substantial to establish its permissible limits and biological effects. These data are constantly replenished and require updating to reflect changes in climatic and environmental conditions, anthropogenic impacts, and geographic differences. Absorption of lead in the gastrointestinal tract (GIT) of mammals depends on the permeability of the membrane of intestinal epithelial cells and is influenced by physicochemical properties of a compound (concentration, particle size, mineralogical composition, solubility in the liquid environment of GIT, ionic potential, atomic mass), physiological feaatures of an organism (metabolism, body weight, age, gender, pregnancy, lactation), the diet composition and levels of protein, cellulose, calcium, zinc, iron, manganese, and vitamin D (J.A. Jamieson et al., 2006, D.J. MacLachlan et al., 2016; O.A. Levander, 1979; C.J.C. Phillips et al., 2011). These factors characterize the parameters of uncertainty, which are partially excluded in determining the content of lead in the peripheral blood of mammals. In peripheral blood, lead is transported by red blood cells and accumulates mainly in the liver, kidneys and bones. In fact, the toxic effect of lead on mammals depends on its accumulation in organs and tissues. Lead is excreted from mammals with faeces and urine, as well as through wool, milk, sweat glands and fetus. The half-life of the metal from the soft tissues and peripheral blood is 24-40 days. The toxic effect of lead on the organs and tissues is due to a decrease in the cell number of (E.B. Mirzoev et al., 2015). Reducing of viable cell number to a certain critical level leads to functional violations and toxic effects. Activation of free radical lipid peroxidation (LPO) and violation of Ca^{2+} homeostasis are the main mechanisms of cytotoxic action of Pb^{2+} ions (G. Flora et al., 2012; A. Roy et al., 2016; E.A. Veal et al., 2007; A.W. Harman et al., 1995). Mechanisms of regulation of cellular metabolism include, on the one hand, changes in the intensity of the process of free radical LPO, and on the other hand, modifications of the lipid composition of membranes (E.B. Burlakova, 2007). Activation of free radical LPO by lead is due not only to the generation of reactive oxygen species, but also to a decrease in the activity of antioxidant enzymes, superoxide dismutase and catalase. Changes in the composition of biological membranes affect the activity of membrane-bound proteins, i.e. enzymes, channel-forming proteins, receptors, which affects Ca^{2+} homeostasis and cell functioning a whole (R. Jahn et al., 2003, A.H. Kahn-Kirby et al., 2004). Mitochondria which provide cells with energy play a role in the cytotoxic action of Pb^{2+} ions (M. Bragadin et al., 2007). The big data analysis on Pb pollution will determine the strategy for further study of lead action, as well as the methods to solve the problem.

Keywords: lead, cytotoxic effect, calcium, blood, organ, feed, absorption, lipid peroxidation

Technical progress is accompanied by the rise of man-made pollution by heavy metals, particularly lead and its compounds. Lead is a global pollutant and classic toxicant. Annually this element is released into the environment in Russia, 0.6-1.4 thousand tons with industrial waste, 0.05 thousand tons with waste water, and 4 thousand tons from motor transport [1]. In a number of regions, its con-

tent in the air, soil, and water exceeds the maximum acceptable concentrations that enhance the likelihood of getting the metal to animal and human body [2].

Studying of Pb effect is necessary for reasoning of the acceptance limits of its effect on mammals and for assessment of the biological effects underpinned by specific aspects of metabolism of this toxic heavy metal in body. Such data is constantly refilled and requires updating upon integration, accounting for changing of climate and ecologic conditions, man-made effects, and wider supervision geography.

Purpose of present review is to analyze data on patterns of Pb intake, distribution, and excretion, as well as on mechanisms of its cytotoxic action.

Lead mainly gets into the body of mammals with feed and water. Feed undergoes grinding and enzyme destruction in gastrointestinal tract, as a result of which metal is transformed into the state accessible for digestion. Lead absorption in gastrointestinal tract is controlled by nervous and endocrine systems and occurs by passive diffusion, active transport, pinocytosis and endocytosis. Absorption process depends on permeability of membrane of intestinal epithelial cells; its intensity is affected by physico-chemical properties of the lead compounds (size of particles, mineralogical composition, compound solubility in liquid medium of gastrointestinal tract) [3-5], and physiological properties (sex, age, weight, metabolism, pregnancy, and lactation) [6-8], as well as type of diet and content of calcium, lead, iron, manganese, and vitamin D [3, 9].

One of the main factors determining the lead resorption into the blood is ionic potential and atomic mass, increase of which causes decrease of the absorption percentage. For the lead such coefficient varies from 0.05 to 0.2 [10, 11]. Lead bioavailability is influenced by its chemical form: it is higher in lead acetate than in oxide and sulfide [12]. Having penetrated into the liquid medium of mammal's gastrointestinal tract, many toxic substances very soluble in potable water form insoluble hydroxides. At the same time, slightly soluble substances are very soluble in alkali medium of gastrointestinal tract and are absorbed into the blood stream through the intestinal epithelium tissue [13]. Experiments with rats had shown that increase of the dosage of lead acetate from 1 to 100 mg/kg results in decrease of its resorption in the gastrointestinal tract from 42 to 2 % [14]. Evidently, absorption intensity in mammal's gastrointestinal tract depends on quantity of supplied metal and has non-linear nature. Possibly, it is associated with the saturation process of active transport of Pb^{2+} ions in the intestinal epithelium tissue.

With aging, metal resorption in animal gastrointestinal tract is reduced due to sealing of the intestinal cell membranes and decrease of pore diameter in them. In young species such process is more active since growing body needs mineral substances and has increased permeability of cell wall membranes [11]. Upon injection of ^{203}Pb in stomach of rats aged 1, 3, 6, 16, and 54 weeks, specific activity of radionuclide in 3-7 days comprised accordingly 82-57 %; 2.3 %; 0.4-1.1 %; 0.6 %; 0.3-0.5 % of the total activity [15]. Possibly, found differences are due to permeability of the cell wall membranes and are associated with the state of regulatory and protective mechanisms of homeostasis (sympathoadrenal system, central and peripheral nervous system, metabolic detoxication systems) in animals of different age.

Metal absorption process in mammal's gastrointestinal tract depends of their body weight and intensity of metabolism. Heat loss in small animals grows faster due to increase of the body surface per weight unit. Thus, such value comprises 0.15 m^2/kg in rats and 0.072 m^2/kg in rabbits. Differences in metabolism activity are linked with differences in resorption of metals in gastrointestinal tract of males and females: absorption intensity is higher in male rats than in

female rats [16]. Absorption of lead compounds in pregnant and milking species is stronger [17, 18]. Possibly, the reason is in total activity of physiological processes since not only absorption of Pb^{2+} ions, but also Cu^{2+}, Zn^{2+}, Fe^{2+}, Ca^{2+}, Cd^{2+} ions is increased during such period [19]. Moreover, synthesis of metallothioneins (MT) participating in transport of elements from gastrointestinal tract to blood during pregnancy and lactation increases [20].

Diets with low and high content of protein increase lead absorption in gastrointestinal tract in rats as compared to its optimal content. Thus, lead accumulation in kidney, liver, and heart tissues in rats getting diet from 3 % of protein had accordingly increased by 52, 32, and 27 %. Similar situation was observed upon increase of the protein in diet up to 30 %: lead content in the studied organs exceeded the control samples by 36, 29, and 24 %. At the same time, when protein content in the diet (15 %) was optimal, accumulation of the lead in kidneys, liver, and heart had increased by 24, 14, and 13 %, respectively [21]. Increase of percentage of raw fiber in the livestock diet decreases metal content in milk [22]: evidently, lead in feed rich in fiber is weakly leached since great share of the metal transferred to gastrointestinal tract is not reabsorbed and is immediately released from the body.

Information about the effect of vitamin D on lead absorption in gastrointestinal tract is contradictory. It is found that upon increase of the physiological norm of vitamin D in feed (over 500 IU/kg of feed) content of the lead in blood and tissues of chicks decreased. Conversely, such indicator grows when content of vitamin D (up to 500 IU/kg of feed) is low [23]. At the same time, accumulation of the lead in chest muscles grows in broiler chicks with increase of the dosage of vitamin D in diet from 3000 to 5000 IU/kg [24]. Possibly, not only content of vitamin D, but also content of Ca^{2+} influence the lead absorption intensity from the feed, since resorption of Pb^{2+} in gastrointestinal tract is intensified by deficit of Ca^{2+} in the diet. High correlation between the content of Ca-binding protein (Ca-BP) in the intestinal mucosa and the lead intoxication was found. It is assumed that Ca-BP promotes not only absorption of Ca^{2+}, but also Pb^{2+} from the gastrointestinal tract [23]. It should be noted that Ca-BP is activated with participation of vitamin D. At excessive content in the diet, bivalent cations Ca^{2+}, Zn^{2+}, Fe^{2+} suppress Pb^{2+} absorption due to change of the ability of the later to attach to membrane. At the same time, deficit of Fe^{2+} causes intense absorption of Pb^{2+} in gastrointestinal tracts, which may be due to functioning of carrier proteins in charge for Fe^{2+} transport.

Generally speaking, 3-10 % of supplied metal is absorbed from the mammal's gastrointestinal tract (except the neonatal period) [25, 26]. Preliminary starvation increases lead absorption [27]. It was found that 3 % of metal injected in the stomach is absorbed in the small intestine after feeding, with 60 % on an empty stomach [28]. It was established, as demonstrated on the models of inverted pockets of intestinal sections in rats, that the highest quantity of the lead is absorbed in small intestines (702.6±4.16 μmol/g of wet weight), and slightly lower in duodenum and ileum (646.7±28.2 and 520.8±21.3 μmol/g of raw weight) [29]. Flowing off the small intestines, blood flows in the portal vein and afterwards in liver. Lead in mainly accumulated in liver cells (hepatocytes) in microsomal and mitochondrial fractions. It is established that single intraintestinal injection of the lead acetate (62.5 mg/kg) is characterized by metal accumulation in hepatocyte mitochondria in male rats on day 1. In furtherance, on day 5 and day 10, the amount of Pb^{2+} decreases with simultaneous increase in the lysosomal ultrastructures, especially lysosomes and residual bodies, which implies compensatory intensification of detoxication processes in cells [30]. Under the effect of intracellular enzymes, lead forms complex compounds

in hepatocytes with bile acids, with which lead is excreted to the small intestine. Part of metal is moved from the stomach with fecal masses, whereas the other part is reabsorbed (enterohepatic circulation process).

Total lead pool in the body may be divided into slowly and rapidly exchanging parts. Slowly exchanging part is located in bone tissue, whereas metal content increases during the entire life and comprises 80-90 %. Part of metal that is faster involved in metabolic processes is located in soft tissues, mainly in kidneys (8.29 %) and liver (2.20 %), as well as in the peripheral blood (1.00 %) [3, 25, 26]. It should be noted that erythrocytes in the peripheral blood contain 99 % of lead [31]. In cells, lead is mainly accumulated in cytoplasm, with δ-amino levuline acid dehydratase (ALAD) as the main binding protein (ALAD binds 35-84 % of the total quantity of the metal). Besides, there are two more proteins of 10 and 45 kDa [32]. Most part of the lead in blood is bound with albumin and γ-globulin. The balance forms a complex with low-molecule combinations containing sulfhydryl groups (MT, cysteine, transferrin). It should be noted that quantity of Pb^{2+} ions (i.e. unbound) in blood is insignificant [25, 26, 33].

During pregnancy, lead penetrates through placenta into foetus by simple diffusion. Concentration of the metal in the foetus blood comprises 85-90 % of its content of the mother's blood. By the end of antenatal period, lead is found in foetus organs and tissues with predominant localization in bones [25, 26, 34].

Therefore, toxic effect of the lead on mammals is determined by its accumulation in organs and tissues. Herewith, content of the lead in peripheral blood could be more reliable indicator allowing us to exclude in part the uncertainty of effect of physico-chemical properties of compound, physiological state of animal, and type of diet.

Lead is excreted with fecal masses and urine, as well as through hair, milk, sweat glands, and foetus. Main quantity of the metal entered to gastrointestinal tract is not retained in the body and is extracted with fecal masses. At average, 30 mkg is extracted through kidneys due to glomerular filtration, with tubular excretion at high lead concentrations. Semi-extraction of the metal from soft tissues and blood takes 24-40 days. Lead mobilizes from the depot for a number of reasons (lactation, calcium deficit), which increases its concentration in the blood and causes toxic effects [25, 26, 35].

Toxic effect of Pb on organs and tissues in mammals is characterized by decrease of the number of viable cells [36, 37]. Decrease of such number to the critical value results in destruction of physiological functions of the organ. Activation of free-radical lipid peroxidation (LPO) and Ca^{2+} homeostasis disorder are considered the main mechanisms of cytotoxic effect of Pb^{2+} ions [38-41].

Free-radical LPO occurs in all types of membranes and plays an important role in regulation of the normal cellular metabolism. Free radicals required for many biological processes are acting as regulatory molecules in biochemical reactions involved in signal transduction ways [42, 43]. In this system an important place is occupied by signal molecules of reactive oxygen species (ROS): superoxide anion-radical ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), nitrogen oxide (NO^{-}) and hydrogen peroxide (H_2O_2). In particular, H_2O_2 generates hydroxyl radicals in the presence of Cu^{2+} and Fe^{2+} ions by Haber-Weiss (F. Haber, J. Weiss) or Fenton (H.J.H. Fenton) reactions. Such reasons proceed both in cytoplasm and also site-specifically [44].

Cell metabolism regulation mechanisms involve change of intensity of free-radical LPO and modification of membrane lipid content. Activation of LPO is characterized by accelerated extraction of easily oxidized lipids and enrichment of membranes by oxidation-resistant fractions [45]. Change in the

composition of biological membranes impacts the activity of membrane-binding proteins (enzymes, channel-forming proteins, receptors) that influences cell functioning in general [46, 47].

ROSs participate in a cascade of biochemical reactions which result in genome activation and adaptive synthesis of proteins ensuring compensatory metabolic changes [48]. Under the effect of lead, ROS is formed due to oxidation of ALAD, membrane lipids activation of nicotine amide-adenine dinucleotide phosphate oxidase NAD(P)H and inhibition of antioxidant protection enzymes. Lead is accumulated in erythrocytes in the peripheral blood and is bound with δ-ALAD, which has four lead-binding sites. Intracellular lead inhibits enzymes participating in haem synthesis, including δ-ALA-synthase, δ-ALAD and ferrochelatase [49]. Low concentration of metal (5-7 μg/dl) in the peripheral blood reduces activity of δ-ALAD, which results in increase of the concentration of δ-ALA in blood and urine [50, 51]. Oxidized δ-ALA generates ROS by reduction of ferrocytochrome activity and transfer of electron from oxyHb and metHb to other iron complexes [52, 53]. Increase of the lead concentration (8 μg/dl) in the peripheral blood of mammals is characterized by more intensive free-radical LPO and higher blood concentration of malondialdehyde (MDA) [54]. ROS generation occurs due to increase in activity of membrane-binding NAD(P)H-oxidase, which forms $O_2^{-\bullet}$ from the molecular oxygen [55]. Intensification of free-radical LPO affected by the lead is due not only to ROS generation, but also by reduction of activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Their inhibition is associated with high lead affinity with thiol groups and its ability to replace essential elements in proteins, i.e. Cu^{2+}, Zn^{2+}, and Mg^{2+} ions in catalytic SOD centers and Fe^{2+} ions in CAT.

Ca^{2+} homeostasis in cells also refers to universal cytotoxicity mechanisms [56]. Concentration of ionized Ca^{2+} in cytoplasm is maintained at 10^{-7} mol/l by various mechanisms (Ca^{2+}- pump and Ca^{2+}-specific channels), which are localized both in cell membrane, and in membranes of intracellular organelles [57]. When activator interacts with a cell, concentration of ionized Ca^{2+} in cytoplasm is shortly increased by times that ensure formation of Ca^{2+}-calmodulin complex inducing relevant metabolic reactions. In particular, protein kinase C is activated and increases phosphorilation of cell proteins. Membrane proteins of Ca^{2+}-Na^{+}-exchange system and Ca^{2+}-Mg^{2+}-ATPase which perform transferring Ca^{2+} ions against the concentration gradient are activated simultaneously with formation of Ca^{2+}-calmodulin complex [58]. Cell membrane is a dynamically changing structure, and its damages may be induced by structural changes in a membrane as a whole or in the micro environment of Ca^{2+}-pump and Ca^{2+}-selective channels.

Incubation of human erythrocytes for 1 hour at 5 μmol/l lead in medium led to an increase in ionized Ca^{2+} and pro-coagulation cell activity [59]. Increase of the Ca^{2+} concentration was also observed in splenocytes of rats after 10 min incubation in the medium with 1 μmol/l lead [60]. Increase of intracellular concentration of Ca^{2+} is possibly due to, in particular, activity of membrane proteins (Ca^{2+}-Na^{+}-exchange system and Ca^{2+}-Mg^{2+}-ATPase). In fact, inhibition of activity of Na^{+}-K^{+}-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase was observed in liver and kidney cells in rats getting lead with potable water (750 mg/l) during 77 days. Herewith, concentration of this metal in the peripheral blood was 55.6±6.3 μg/dl [61]. Similar results were obtained with erythrocytes of rats which during 35 days drank 0.2 % lead salt solution instead of potable water, at that blood concentration of Pb^{2+} was 97.56±11.8 μg/dl [62]. Reduction of the protein activity was observed at intensification of free-radical LPO.

Pb^{2+} ions at $1\text{-}5\times10^{3}$ μmol/l block transport of Ca^{2+} ions in human erythrocytes by inhibition of activity of Ca^{2+}-Mg^{2+}-ATPase [63]. Similar results

were obtained at 0.1 to 100 μmol/l concentration of Pb^{2+} in the medium [64]. It is assumed that Pb^{2+} directly affects the sulfhydryl groups of ATPase if Pb^{2+} concentration is more than 1 μmol/l, and calmodulin (at less than 1 μmol/l). Pb^{2+} ions may impact transport of Ca^{2+} ions through plasmatic cell membranes, directly affecting the potential sensitive channels. Possibly, lead blocks regions of Ca^{2+} binding at external cell surface or disturbs Ca^{2+}- dependent dephosphorylation of channels [65]. Besides, Pb^{2+} ions render modifying effect on Ca^{2+}-dependent potassium channels. Pb^{2+} ions activate the channels if Pb^{2+} concentration is less than 10 μmol/l, and inhibit these channels if concentration is higher [66].

Assessment of protein kinase C activity at incubation of rat brain cells with Pb^{2+} ions had revealed its increase, provided lack of Ca^{2+} ions in the medium [67]. It was established that protein kinase C activation coefficient with Pb^{2+} is 4800 times lower than with Ca^{2+} (5.5×10^{-5} and 25 μmol/l), but maximum values of enzyme activity are registered with Ca^{2+} ions. This is due to the fact that protein kinase C has several Ca^{2+} ion binding sites, the first of which more effectively binds Pb^{2+} ions [68].

Pb^{2+} ions influence several cell functions of calmodulin, including activation of calmodulin-dependent phosphodiesterase, by inclusion in Ca^{2+}-binding regions. Affinity of Pb^{2+} ions to Ca^{2+}-binding calmodulin sites is comparable with such in Ca^{2+} ions [69], however demonstrates lower values [70].

Mitochondria supplying energy to cells play certain role in Pb^{2+} cytotoxicity. It should be noted that low concentration of ROS in cells is maintained due to oxidative phosphorylation in mitochondria. Toxic effect of Pb^{2+} ions on cells of renal tubule and epithelial cells is accompanied by changes in their form, structure, and size of mitochondria, which may be due to predominant metal accumulation in mitochondrial fraction [71, 72]. Besides, disturbance of transmembrane transport of ions causing changes of Ca^{2+} homeostasis was noted. Herewith, Pb^{2+} ions inhibit flow of Ca^{2+} ions in mitochondria at simultaneous stimulation of its release from organelles [73, 74]. Reduction of the membrane potential and swelling of mitochondria were noted under the effect of lead, resulting in opening of pores in the internal membrane [75]. It is assumed that Pb^{2+} ions are directly bound with Ca^{2+}-sites in mitochondria pores. It should be noted that opening of pores occurs due to increase of the concentration of $O_2^{-\bullet}$ or its products, whereas closing of pores is caused by reduction of its concentration. Longstanding opening of pores results in apoptosis and cell death [76, 77].

Thus, accumulated data shed the light on specific aspects of metabolism and mechanisms of cytotoxic effect of the lead on mammals and allows us to highlight a number of external and internal factors affecting such processes (physico-chemical properties of lead compounds, physiological features of organism, levels of protein, fiber, vitamin D, as well as micro and macro elements in the diet). The highest metal accumulation occurs in bones, kidneys, and liver, and in several cases the lead is mobilized from depot. Activation of free-radical lipid peroxidation and disturbance of Ca^{2+} homeostasis are main mechanisms of cytotoxic action of Pb^{2+} ions. Free-radical lipid peroxidation becomes more intensive under the effect of the lead due to generation of active oxygen species and reduction of superoxide dismutase and catalase activity. Biological membranes and mitochondria are involved in manifestation of Pb^{2+} cytotoxic effect . Strategy of control over this toxicant in the environment should be based on patterns of Pb entry, distribution, and excretion from mammal's body.

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