PRACE POGLĄDOWE
REVIEW PAPERS

Medycyna Środowiskowa - Environmental Medicine 2018, Vol. 21, No. 4, 44-52
www.medycynasrodowiskowa.pl
www.journal-em.com
DOI: 10.19243/2018406

Molecular basis of lead toxicity

Molekularne podstawy toksyczności ołowiu

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ABSTRACT

Lead represents a metal widespread in the environment, mainly due to its broad application in several branches of industry. It represents an extremely toxic an extremely toxic agent for living organisms, affecting several systems and organs, including central nervous system, hemopoetic system, circulatory and immune systems, liver and kidneys. The studies conducted in the last years enabled to evaluate numerous metabolic pathways responsible for lead toxicity. In the present study the actual data on the basic molecular pathology of lead compounds toxicity were presented: generation of oxidative stress and lesions in genetic material, modifications of neurotransmitters pathways in brain, abnormalities in immune system functioning and changes in metabolism of red cells. Summing up the above we should note that differentiation of molecular mechanisms in lead toxicity, resulting in multiple biological effects of the exposure, causes that the exposure continues to pose a significant challenge for environmental medicine and occupational medicine.

Key words: lead; toxicity; oxidative stress; neurotransmitters; immune system

STRESZCZENIE

Ołów jest metalem szeroko rozpowszechnionym w środowisku, głównie ze względu na szerokie zastosowanie w wielu gałęziach przemysłu. Ołów charakteryzuje się wysokim stopniem toksyczności dla żywych organizmów, wpływając na wiele narządów i układów narządów, w tym na ośrodkowy układ nerwowy, układ hemopoetyczny, układ krążenia, system odporności, wątrobę i nerki. Badania przeprowadzone w ostatnich latach umożliwiły ocenę licznych szlaków metabolicznych odpowiedzialnych za toksyczność ołowiu. W niniejszym opracowaniu przedstawiono aktualne dane dotyczące podstawowych mechanizmów patologii molekularnej związków ołowiu: powstawanie stresu oksydacyjnego i zmian w materiale genetycznym, modyfikacje szlaków przekaźników nerwowych w mózgu, nieprawidłowości w funkcjonowaniu układu odpornościowego oraz zmiany w metabolizmie krwinek czerwonych. Podsumowując, należy zauważyć, że zróżnicowanie mechanizmów molekularnych toksyczności ołowiu, skutkujące zróżnicowaniem biologicznych efektów narażenia, powoduje że narażenie na ołów nadal stanowi poważne wyzwanie dla medycyny środowiskowej i medycyny pracy.

Słowa kluczowe: ołów; toksyczność; stres oksydacyjny; neuroprzekaźniki; układ immunologiczny

Nadesłano: 20.06.2018
Zatwierdzono do druku: 13.10.2018

INTRODUCTION

Lead represents a metal widespread in the environment, mainly due to its broad application in several branches of industry, i.a. as an antiknock agent, element of water-supply/ sewage system, component of paints, raw material for production of lead glass, ammunition and batteries [1, 2]. It manifests numerous advantageous properties, such as plasticity, resistance to corrosion, low melting point [1]. Nevertheless, it remains to represent an extremely toxic agent for living organisms, affecting several systems and organs, including central nervous system, hemopoetic system, circulatory and immune systems, liver and kidneys. Moreover, it is an element recognized by the International Agency for Studies on Cancer as a potential carcinogen (group B2: a possible human carcinogen) [1, 3, 4]. Till now, evidence for carcinogenic activity of lead originates from animal studies [5]. Human body is penetrated by the lead with polluted water, food or air [6]. Even if several potential mechanisms explaining toxic action of lead have been suggested, none of them has been unequivocally proven.

OXYDATIVE STRESS

Numerous studies suggest that factors which probably mainly cause lead induced pathology include lead-provoked oxydative stress [7]. Proofs originating from studies conducted in recent few years indicate that lead may inhibit activity of antioxydative enzymes, such as glutathione peroxidase, catalase and superoxide dismutase (SOD) [1]. Moreover, it leads to production of reactive oxygen forms, to exhaustion of antioxydative reserve and it stimulates lipid peroxidation [1]. Xu et al. provided evidence for augmented production of reactive oxygen forms (ROS) and malonyl dialdehyde (MDA) in tissues of livers originating from mice exposed to action of lead acetate. ROS react with many macromolecules, in particular with residues of polyunsaturated fatty acids, which are particularly sensitive to oxidation [1]. Similar conclusions were reached by Zhang et al., who confirmed reduction in activity of superoxide dismutase in hepatic tissues originating from fishes of Brachydanio rerio species exposed to lead compounds [2]. Superoxide dismutase is a metalloenzyme containing a single ion of copper, a single ion of zinc and a single residue of tyrosine.It is contained in extracellular matrix and plays a significant role in protection of tissues against the damage induced by reactive forms of oxygen [2]. It was proven that lead may induce decrease in activity of dismutase by direct interaction at the molecular level, including effects of complex formation due to electrostatic forces, binding with the enzyme active center, preventing access of the substrate to tissues, change in secondary structure of SOD and release of copper and zinc ions from the active center [2]. Similarly, Hasanein et al. described an increased peroxidation of lipids in livers of animals subjected to action of lead, indicated by a significant increase in concentration of malonyl dialdehyde (MDA). Moreover, they detected a reduced concentration of SH groups, which might have resulted from the high ability of lead to bind these groups [8]. In the study administration of carnosine (beta-alanyl-L-histidine) significantly reduced content of MDA and elevated the level of SH groups [8]. Carnosine is a well known antioxidant, which blocks production of ROS and scavenges products of lipid peroxidation during reaction of free radicals. In addition carnosine upregulates expression of mRNA for hepatocyte catalase [8]. Patra et al. in their study confirmed that exposure to lead results in an increased lipid peroxidation with specific lesions in tissues of liver, kidneys and brain. Administration of alpha tocopherol and ascorbic acid reduced the level of lipid peroxidation, providing evidence for their antioxydative function [7]. Results of the study conducted by Abdallah et al. pointed to reduction of reduced coenzyme Q concentration in the group exposed to lead, as compared to the control group. Coenzyme Q acts as a transporter of electrons in mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes and in plasma membrane. The effect of lead on concentration of coenzyme Q reduced form and absence of such effect on concentration of its oxidised form may suggest that lead reduces activity of enzymes responsible for reduction of ubiquinone to ubiquinol, such as reductases of NADPH-CoQ [3]. Ubiquinol plays the role of an antioxydative compound, inhibiting lipid peroxidation and trapping free radicals [3]. El-Sokkary et al. provided evidence for an augmented lipid peroxidation and reduced activity of superoxide dismutase and a reduced glutathione concentration in tissues of liver and kidneys of rats subjected to action of lead. Moreover, they documented a decreased surface and volume of cell nucleus and a reduced ratio of nucleus to cytoplasm in the studied hepatocytes. Administration of melatonin inhibited lipid peroxidation, returned SOD activity and GSH concentration as well as the nuclear-cytoplasmic parameters. Conclusions of the study suggested that melatonin might be useful in reversing effects of the lead-induced oxydative stress [9].

DAMAGE TO DNA

The induced due to lead action oxydative stress results in peroxidation of lipids in cell membranes, of sulphhydryl groups in proteins and it damages DNA [9]. Xu et al. in their experiment documented an increased number of DNA lesions resulting from induction of oxydative stress and, in addition, an augmented expression of p53 and Bax proteins which resulted in an imbalance between Bax and Bcl2. The above allows to draw the conclusion that also induction of apoptosis represents one of lead's mode of action [1]. The damage to DNA may be induced directly by oxygen reactive forms or by action of peroxidated lipids. In this way single or double breaks in DNA thread may develop, chromosomal aberrations, modifications within purine, pyrimidine bases or deoxyribose, DNA–DNA interstrand cross-links or DNA-protein cross-links may develop and also frequency of micronuclei formation may increase. All the changes may lead to genome instability [1]. In response to DNA damage an increase takes place in concentration of p53 protein, resulting in activation of several genes, stop in cell cycle, apoptosis or DNA repair [1]. Bax contains a part binding p53 protein in its promoter. In normal conditions Bax is present in cytosol as a monomer, following activation it becomes transplaced to mitochondria and forms an integral protein of cell membrane. After inclusion to the cell membrane Bax may form channels allowing for a release from mitochondria of proteins associated with apoptosis, such as cytochrome C. In contrast to Bax, Bcl-2 represents an integral protein of mitochondrial membrane and forms heterodimers with Bax in order to prevent apoptosis. Therefore, the Bax/Bcl-2 ratio plays a significant role in determination whether the cell will or will not undergo apoptosis. In the study of Xu et al. an augmented expression of Bax was detected in presence of an unchanged expression of Bcl-2, which might lead to mitochondrial dysfunction and may introduce the cell to apoptotic pathway [1]. Using commet assay, Danadevi et al. detected a genotoxic effect of lead in 45 employees exposed to its action, as compared to the control group [10]. In turn, Donmez et al. described an increased frequency of sister chromatid exchange in lymphocytes originating from 32 men occupationally exposed to lead and zinc. The exchange of genetic material between sister chromatids (SCE) represents one of the methods in evaluation of environmental genotoxicity [11]. Several of the till now conducted studies indicate that lead may cause an increased incidence of chromosomal aberrations and may inhibit DNA repair [11]. Examining a group of 37 employees of battery-producing factory, exposed to action of lead, Fracasso et al. found an increased incidence of DNA breaks, down-regulation of protein kinase C activity and an altered cellular redox state [5]. In addition, a positive correlation was proved between lead concentration and production of oxygen reactive forms and a negative correlation with concentration of GSH [5]. Studies in vitro suggested that a proportion of effects induced by lead may be linked to its reaction with calcium ions and to activation of protein kinase C with the resulting translocation of PKC from cytosol to cell membrane. Protein kinase C plays a significant role in transmission of signals, cell growth and differentiation. It is activated not only by extracellular factors (hormones, growth factors, cytokines), but also by environmental factors, through the second messenger intermediaries. Activators of PKC include, i.a, phorbol esters, the recognized tumor promoters. They stimulate its translocation from cytosol to the cell membrane. Therefore, the fact that lead interacts with calcium ions in activation of PKC suggests that the metal may also act as a tumor promoter [5]. Mechanisms leading to procarcinogenic action of lead remain to be fully recognized [4]. Nevertheless, the in vivo and in vitro studies allowed to suggest few possible mechanisms. Lead may induce carcinogenesis through, i.a., augmenting the probability of DNA damage through the direct or indirect way [4]. The indirect mechanisms may include induction of ROS production, disturbed processes of DNA repair (base excision repair – BER and nucleotide excision repair – NER), and also by substitution of zinc and calcium in DNA-binding proteins [4, 12]. Pb may also substitute elements such as Zn, Ca, Mg, Hg, Cu, required for synthesis and repair of DNA [13]. By the direct interactions lead may alter secondary DNA structure by covalent binding with oxygen atom of nucleic acids or nitrogen atom in DNA bases [4]. As found by Zhang et al., Pb^{2+} bound DNA with four binding sites to form Pb^{2+} – DNA complex by minor groove binding effects and electrostatic forces, which resulted in destruction of the double DNA thread structure [4]. Similarly, Shaik et al. found that lead may induce breaks of a single DNA thread, probably by competing with metal binding sites [14]. In their studies, Bonacker et al. found that lead may also disturb function of microtubules [15]. Lead salts inhibited assembly of tubulin, mobility and rate of microtubule transplacement in the dose-dependent extent. The disturbances in microtubules most probably follow interaction of lead with proteins,

through the reaction with sulphhydryl groups and acceleration of nonspecific ATP hydrolysis [15]. Luo et al. proved in the study conducted on rats that lead may participate in pathogenesis of hyperactivity syndrome, affecting epigenetic mechanisms, in this case inducing changes in histone proteins [16]. In hippocamps of rats exposed to lead action, the investigators detected an increased acetylation of histones. Such a posttranslational modification of histones, the proteins around which DNA envelopes forming nucleosomes, modulates gene expression by altering structure of chromatin [16]. Sui et al. found that lead induced stress in cells of cardiofibroblasts, promoting the process of autophagy which, by inhibition of the pathway linked to mTOR kinase promotes survival of the cells in toxic environment [17]. Autophagy represents a very conservative cellular process, in which cytoplasmic material, including organelles, becomes sequestered in vesicles covered by a double cell membrane, termed autophagosomes and, subsequently it is supplied to lysosomes, to become degraded or recycled. The process remains under control of several signalling pathways, including those of 3-phosphatidyl inositol kinase (PI3K), the serine-threonine protein mTOR kinase (mammalian target of rapamycin) and mitogen-activated protein kinase (MAPK). [17]. Autophagy was observed both during normal cell growth and differentiation and in pathological situations, during hunger, bacterial infections, production of poorly folded proteins or upon presence of damaged structural cell components. In many studies an effective autophagy was found to protect from apoptosis. Even if it plays an important pro-life function and warrants cell, in certain situations it may also lead to cell death [18].

NEUROTOXICITY

Ma et al. proved in their study that significant mechanisms of lead neurotoxicity include inhibition of differentiation involving oligodendrocyte precursors, through i.a. reduced expression of oligodendrocyte transcription factor, olig2 and of CNPase proteins. Moreover, lead inhibits expression of mRNA for NCX3 sodium-calcium exchanger 3, which results in markedly elevated intracellular concentration of calcium [19]. Many investigative reports point to significant importance of calcium transport for differentiation and myelinization of oligodendrocytes. Alterations in intracellular concentration of calcium not only influence transformation of oligodendrocyte precursors into mature oligodendrocytes but they participate also in beginings of myelinization and remyelinization processes [19]. The direct inhibition of NCX3 immediately affects differentiation of oligodendrocyte precursor cells, blocking their development at the immature stage [19]. N-methyl-D-aspartate receptor (NMDAR) plays a principal role in processes of learning, memory and synaptic plasticity. Toscano et al. proved that in brains of rats subjected to action of lead alterations take place in expression of genes and proteins forming receptor subunits, specifically in an augmented proportion of NR2B subunits in receptor molecules. Such changes may lead to disturbances in signalling pathways linked to calcium and transcription factors indispensable for learning processes [20]. Another mechanism of lead neurotoxicity was suggested by Rahman et al. [21]. Excessive expression of serine-threonine phosphatases – PP1, PP2A, PP2B – is linked to inhibition in processes of learning and memory. Activation of such phosphatases may lead to alterations in cytoskeleton, which may negatively influence the processes. Rahman et al. proved that exposure to lead resulted in an augmented expression of certain serine-threonine phosphatases, which inhibits processes of memory development. In a similar manner damage to microtubules is linked to cognitive disturbances and to death of neurons [21]. A microtubular network is stabilized by proteins linked to microtubules (MAP). One of such proteins is protein tau. Phosphorylation of tau at the stechiometric ratio of 2–3 phosphatase moles per mole protein is required for initiation of formation and stabilization of mikrotubules (for promoting assembly and stability of microtubules), while hyperphosphorylation (9–10 moles phosphatase per mole tau) results in damage and disintegration of microtubules, followed by loss of memory and death of neurons. In the study of Rahman, Pb was proved to induce hyperphosphorylation of tau at positions of Ser 199/202 and Thr 231 [21]. Yun and Hoyer found that exposure to even low concentrations of lead may result in inhibition of activity involving certain enzymes engaged glycolytic required for pathway, in this way resulting in energy deficits in neurons. The investigators provided evidence for a significant inhibitory effect of lead on the complex of cerebral pyruvate dehydrogenase (PDHc-puryvate dehydrogenase complex) and hexokinase. The hexokinase is the first rate-limiting enzyme in glycolysis, while its concentration manifests good correlation with principal indices of glucose utilization in the brain. In turn, PDHc participates in oxydative decarboxylation of pyruvate to acetyl-

CoA. [22]. This, in turn, is required for synthesis of acetylocholine and for production of ATP. Acetylocholine plays role of an intermediate in processes of learning, memory and perception while ATP is required for processes such as synaptic transmission, ionic homeostasis, processing and phosphorylation of proteins, long term synaptic potentiation (LTP) [22]. The inhibitory action of lead reflects most probably its binding of enzymic SH groups. Because an abnormal metabolism of glucose provides background to neurodegenerative diseases, such as Alzheimer's disease, lead can be regarded to represent a potential risk factor or a factor accelerating development of such diseases. Feng et al. proved that lead down-regulates expression of SIRT 1, and in this way reduces phosphorylation of CREB, inducing in this way cognitive deficits. SIRT 1 represents a NAD+ dependent deacetylase and affects processes linked to genome stability and repair, transcription and metabolism. Sirtuins (or SIRs-silent information regulators) were discovered originally in yeast cells and classified as the IIIrd class of histone deacetylases (HDACs). Recently conducted studies indicate that SIRT 1 controls post-translational modification of CREB. CREB represents a nuclear transcriptional factor, the activation of which forms a significant component of intracellular signaling pathways, responsible for several biological functions, including memory [23]. In turn, Schneider et al. documented effect of lead on expression of methyltransferases (DNMT1, DNMT3a) and MeCP2 (methyl cytosine-binding protein) in the hippocamp. This indicates that lead controls gene through its effect on epigenetic mechanisms, in this case on DNA methylation [24]. Recent studies point to a significant role of DNA methylation in cerebral development and function, i.a. in control over synatic plasticity. An abnormal course of such processes is linked to several diseases characterized by cognitive disturbance: schizophrenia, Rett's syndrome, syndrome of fragile X chromosome. Deficit of DNMT1 and DNMT 3a in neurons results in a deficient plasticity of synapses and in deficits in processes of learning and memory. MeCP2 represents a DNA-binding protein, engaged in transcriptive control of many genes. Changes in expression or methylation of MeCP2 were linked to, i.a., syndrome of Rett, autism, mental retardation, ADHD [24]. Moreover, MeCP2 affects also plasticity of synapses. A degree of MeCP2 expression in cerebral cortex and hippocamp correlates with synaptogenesis in the two regions [24,25]. Lidsky and Schneider in their study pointed to at least few mechanisms of a direct neurons injury by lead: apoptosis, excitotoxicity, effect on neurotransmiter storage and release, secondary transmitters, cerebral endothelial cells, astroglia, oligodendroglia [26]. Apoptosis or a programmed cell death may be induced by several factors, including, i.a., an elevated intracellular calcium concentration. Lead disturbs calcium homeostasis, causing its marked cummulation in cells exposed to its action. Moreover, it causes release of calcium from mitochondria [26]. In nanomolar concentrations, lead substitutes for calcium in activation of, i.a., calmodulin and other secondary messengers. The activated calmodulin stimulates protein kinases, c-AMP and phosphodiesterases and it affects ion channels. It also inhibits the calcium-dependent release of acetylocholine, dopamine and of aminoacid neurotransmitters but it increases their basic release. Moreover, it affects also the structure and function of synapses and receptors for neurotransmitters. Synaptosomes of rats subjected to action of lead were found to contain less numerous synaptic vesicles, damaged mitochondria and an increased density of NMDA receptors. A destructive effect of lead was described on dopaminergic system, in the form of, i.a., necrosis and apoptosis in dopaminergic cells of mesencephalon [26]. Lead exerts its toxic effect also on cells of oligodendroglia and astroglia, i.a., through the delay in differentiation of glia progenitor cells, inducing in this way hypomyelinization and demyelinization [26]. In cases of exposure to high concentration of lead, leading to acute encephalopathy, damage of blood/brain barrier develops with the resulting cerebral edema and cerebral ischemia. Another indirect effect of lead action in the brain involved the disturbed transport of thyreotropic hormone to brain, the hormone indispensable for cerebral development and the deficiency of which causes mental retardation [26]. Glutamate represents the principal stymulatory neurotransmiter in mammalian brain while activation of its receptor, NMDAR plays a significant role in processes of memory and learning and in neurodegenerative diseases [6]. Guilarte and McGlothan proved for the first time, as mentioned above, that exposure to lead induces a significantly elevated expression of mRNA for NR1 subunit of the receptor and a decreased expression of mRNA for NR2A subunit, with an unchanged expression of NR2B subunit. This results in an increase in numer of receptor complexes with prevalence of NR2B subunit [6]. The changes may lead to a slowed down processing of signals with a decrease in NMDAR activity-dependent plasticity of cerebral synapses (in a slower processing of synaptic events with reduc-

tion of activity-dependent synaptic plasticity in the mature brain). Nihei et al. proved that such changes in subunits of NMDAR receptor impoverished in rats their spatial abilities and caused deficits in the so called long-term synaptic potentiation (LTP) [6]. In addition, receptors with prevalence of NR2B subunit were found to exhibit extra-synaptic localization. Such observation might explain inhibition of CREB phosphorylation in hippocamp cells of rats exposed to action of lead since activation of the extra-synaptic NMDAR receptor is linked to switching off the CREB phosphorylation pathway, the process activated by the synaptic NMDAR [6]. CREB is one of the key transcription factors which are important for plasticity of synapses. It is activated by phosphorylation in the polypeptide chain position Ser-133 by one of calcium-dependent signalling pathway kinases: protein kinase A (PKA), calcium/calmodulin-dependent protein kinase (CAMK I) or mitogen activated protein kinase (MAPK). In turn, the kinases undergo activation by cAMP. Active CREB, after binding to activating proteins, such as CREB binding protein (CBP) becomes linked to the cyclic AMP response element (CRE), the strictly conservative DNA sequence, located in promoter regions of several genes. CREB plays a significant role in processes of learning, memory and synaptic plasticity. It acts as a significant element in the signal propagation pathway from synapses to cell nucleus, linking NMDAR activation, calcium-dependent signaling pathways with expression of genes indispensable for synaptic plasticity. As mentioned above, in cases of exposure to lead activation of extra-synaptic NMDAR receptor leads to dephosphorylation of CREB. Disturbances in phosphorylation and in CREB-binding activity disturbs transcription of genes associated with processes of memory. CREB is required for inhibition of LTP while phosphorylation of CREB increases in ptocesses of learning and leads to induction of LTP. Exposure to lead results in a diminished activity of adenyl cyclases with competitively increased activity of cerebral phosphodiesterases. The processes result in a reduced concentration of cAMP in lead-exposed tissues, which probably reduces activity of protein kinase A. Among mitogen activated protein kinases (MAPK), ERK1 and 2 (p44 and p42) as well as p38 play a role in signalling pathways indispensable for processes of learning. Exposure to lead results in an increased phosphorylation of ERK 1 and 2, probably through the mechanism of inhibition involving NMDAR receptor. The same effect is induced by activation of extrasynaptic NMDAR. CAMK II also plays a significant role inprocesses of learning, memory and synaptic plasticity. In normal conditions CAMK II becomes activated by an increase in intracellular concentration of calcium, which allows for attachment of CAMK II to the complex of calcium/ /calmodulin. Attachment of the complex allows for autophosphorylation of threonine residue in position 286 with the resulting calcium-independent activity. In such a condition, the enzyme may remain active even in the absence of calcium. Phosphorylation of threonine residue in position 286 CAMK II represents a key phenomenon for processes linked to memory [6]. We have a tour disposal just a restricted numer of studies which focused on lead action on the CAMK II system. However, it is probable that lead disturbs normal function of the kinase affecting its kinetics and, in particular, reducing maximum rate of the reaction [6].

IMMUNOTOXICITY

Toxic action of lead on the immmune system was proven both in studies on animals and on humans exposed to its action. The changes in immune system pertained in particular lymphocytes T but also macrophages, concentrations of immunoglobulin and complement components and production of antibodies [27]. As proven by numerous studies, lead induces a shift of immune responses toward those linked to lymphocytes Th2 [27, 28]. Such changes result in inhibited delayed type hypersensitivity reactions, augmented concentrations of IgE and IL-4 (produced by lymphocytes TH 2) and a reduced production of IFN gamma (produced by lymphocytes Th 1) [27]. The above described alterations in the immune system result in elevated concentrations of cytokines produced by lymphocytes Th-2, a risk of formation of autoantibodies and development of autoaggressive diseases [27]. Results of a few investigations indicate that exposure to lead may pose a risk for bronchial overactivity, asthma and other autoimmune diseases [27]. Gao et al. in a study conducted on bone marrow (BM) – derived dendritic cells (Dcs), proved that lead-treated bone marrow produced less dendritic cells than bone marrow cells not exposed to lead. Dendritic cells developing upon exposure to lead were characterized by an augmented expression of MHC II, they effectively polarised antigen-specific lymphocytes T toward Th-2 cells (efficiently polarized antigen-specific T cells to Th-2 cells) and enhanced the allogenic T cell proliferation. Moreover, Pb-Dcs induced Th2 skewing of HI and inhibited cell-mediated immunity (CMI). Den-

dritic cells effectively presented antigen to lymphocytes T and, depending on their phenotype, could polarize their differentiation [28]. In addition, the evidence was presented that exposure to lead reduced expression of CD80 on APC (macrophages or lymphocytes B), while it exerted to influence on expression of CD86. Expression of CD80 increases production of lymphocytes Th 1 and expression of CD 86 increases production of lymhocytes Th2. In addition, dendritic cells exposed to lead induced an increased production of cytokien associated with lymphocytes Th 2: Il-6 and Il-10 and a reduced production of IFN- gamma, produced by lymphocytes Th 1. Lead probably stimulates phosphorylation of ERK/MAPK kinases in dendritic cells, which stabilises c-Fos transcription factor, which represents an inhibitor of IL-12. Thus, activation of ERK signalisation inhibits production of IL-12, but increases production of IL-10. Lead-exposed dendritic cells manifested a significantly higher IL-10/IL-12 ratio [28].

DAMAGE TO RED CELLS

Results of several studies indicate that red cells in persons exposed to action of lead manifest a reduced concentration of ATP, numerous morphological and metabolic abnormalities [29]. Normal concentration of highly energetic purine nucleotides (ATP, GTP) in erythrocytes is indispensable for preservation of their physiological functions, including their normal shape, active transport, appropriate concentration of ions on both sides of cell membrane, reduced form of iron in hemoglobin, synthesis of glutathione and pyridine coenzymes, control of glycolysis and pentoso-phosphate pathway [29]. Purine nucleotides my be synthetised de novo or reconstructed from already existing free purine bases in salvage reactions. Resynthesis of adenine nucleotides may occur in two ways. The first, adenine pathway, dependent on 5-phosphoribosyl-1-pyrophosphatase (PRPP), requires activity of adenine phosphoribosyl transferase (APRT)). The other pathway, the adenosine pathway is independent of PRPP, but requires activity of adenosine kinase (AdoK) [29]. Baranowska-Bosiacka et al. provided evidence for inhibition of phosphoribosyl transferases activity (APRT and HPRT) in erythrocytes exposed to action of lead. One of potential mechanisms in which lead inhibits activity of PRTases involves binding to sulfhydryl groups of proteins, which may result in altered molecular configuration or in development of S-S Bridges between free sulphhydryl and amino acids in active center of the enzyme. This results in decreased catalytic abilities of the enzyme or its inactivation [29]. Reports are also available that lead may competitively bind prosthetic groups of several metalloenzymes instead of Mg^{2+}. In studies of Baranowska-Bosiacka et al. an additional effect of lead involced induction of hemolysis. It might result from either inhibition of activity of enzymes such as glucose-6-phospho dehydrogenase (G6PD) and pyrimidine-5'-nucleotidase or due to a direct effect of free radicals generated by lead [29]. Baranowska- Bosiacka and Hłynczak detected in their work change in erythrocyte shape and a reduction in them of ATP, GTP, NAD i NADP concentratio in parallel to increasing lead concentration. In view of the above, one of the mechanisms which might explain disturbances in erythrocyte morphology might involve inhibition of energetic processes in the cells [30]. On many occasions lead was proven to inhibit glycolysis in red cells due to inhibition of glyceraldehyde-3 phosphate dehydrogenase, the enzyme dependent on NAD. In addition, as found by Paglia et al., lead inhibits also pyruvate kinase (PK), which reduces concentrations of ATP, PRPP, NAD and of the total amount of adenyl nucleotides. In turn, Lachant et al. described reduced activity of enzymes in the pentose-phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD), gluconate-6-phosphate dehydrogenase (6PGD), phosphofructokinase and hexokinase in lead-exposed erythrocytes [30]. Baranowska-Bosiacka i Hłynczak demonstrated also a pronounced correlation between concentration of lead and decreased concentration of NAD and NADP in erythrocytes. Lead probably inhibits activity of NAD synthetase. Many authorsb suggested that that the principal mechanism of toxic activity manifested by lead involves induction of free radicals and of oxydative stress, which may lead to oxidation of hemoglobin, peroxidation of lipids in cell membranes and to other lesions. Moreover, lead inhibits activities of enzymes such as NADPH-dependent methemoglobin reductase, glutathine reductase, G6PD, catalase, superoxide dysmutase [30]. Another possible mechanism through which lead may induce disturbances in erythrocyte morphology is an interaction with cytoskeleton proteins [30]. Belloni-Olivi et al. documented a correlation between ATP-dependent phosphorylation of spectrin and shape of erythrocytes. In the studied in vitro human erythrocytes the investigators an increased activity of kinase C and proteins dependent on kinase C. They also proved an increase in protein phosphorylation within membrane cytoskeleton.

Such results indicate that lead stimulates phosphorylation of proteins in membrane cytoskeleton through a kinase C-dependent mechanism. Since no increase in calcium or diacylglycerol concentrations were noted, lead seems to activate the enzyme through a direct interaction [30, 31].

CONCLUSION

Summing up the above we should note that differentiation of molecular mechanisms in lead toxicity, resulting in multiple biological effects of the exposure, causes that the exposure continues to pose a significant challenge for environmental medicine and occupational medicine. In opinion of the authors, intense studies should be continued to increase the awareness of unfavorable effects of lead on health condition while reduction of exposure to lead compounds should constitute one of principal long-term goals of international and local legislation.

Acknowledgements

The study was funded by local grant from the Wroclaw Medical University. The authors report no declarations of interest.

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