

Dolomatov S.I., Sataeva T.P., Zukow W. Modern aspects of regulatory, pathophysiological and toxic effects of cobalt ions during oral intake in the human body. *Health Risk Analysis*, 2019, no. 3, pp. 161–174. DOI: 10.21668/health.risk/2019.3.19.eng

MODERN ASPECTS OF REGULATORY, PATHOPHYSIOLOGICAL AND TOXIC EFFECTS OF COBALT IONS DURING ORAL INTAKE IN THE HUMAN BODY

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Abstract. Cobalt is the essential trace microelement which is an indispensable part of several enzymes and co-enzymes. Cobalt ions may enter the environment from both natural sources and human activities. This metal is very widespread in the natural environment and can be formed as an effect of anthropogenic activity. Toxic activities of cobalt and its compounds depend on the physical and chemical properties of these complexes, including their electronic structure, ion parameters (charge-size relations) and kinetics. Cobalt has both beneficial and harmful effects on human health. Cobalt is beneficial for humans because it is part of vitamin B12, which is essential to maintain human health. Exposure of humans and animals to levels of cobalt normally found in the environment is not harmful. When excessive cobalt amount enter into human body multiple and chronic harmful health effects can occur and the longer the cobalt ions are stored in the body, the more changes they cause in cells. Cobalt gets into the body in several ways: mainly by food, by the respiratory system, by the skin or as a component of various biomaterials. Despite this metal abundancy much of our knowledge of cobalt toxicity is based mainly on the animal studies. Undoubtedly, inorganic forms of cobalt are toxic, accumulate in various tissues and can evoke a chain of pathological cascade changes in the cells. Though some cobalt effects might be beneficial for medicine. Therefore, the purpose of our review is to provide the current analysis about the most significant regulatory, pathophysiological and the epigenetic effects of Co²⁺ in the human body.

Key words: cobalt, Co²⁺ salts, Co²⁺ kinetics, heavy metal, cobalt toxicology, pathophysiology, epigenetics.

1. Introduction

The environmental pollution by xenobiotics is one of global unresolved problems, the relevance of which increased even more in the 21st century. Among modern xenobiotics the leading position is occupied by salts of heavy non-ferrous metals, which are extracted in large quantities into the human habitat. These include toxic trace elements such as: lead, cadmium, cobalt, chromium, mercury, aluminum, etc. Heavy nonferrous metals enter the body not only through the gastrointestinal tract, which is most vulnerable to the action of man-made exotoxins, but also through the respiratory organs, integuments and mucous membranes, especially with a decreased barrier functions.

According to the modern data rather high bioavailability of cobalt salts and the presence of metal mineral compounds in human trophic chains can be caused by both endemic geochemical factors and the human activity itself associated with the operation of coal-fired power plants, the production of certain foods, the mining and processing of metals, medical alloys, ceramics, household waste disposal, etc. (Hokin B. et al., 2004; Gál J. et al., 2008; EFSA Panel on Additives, 2009; Tvermoes B.E. et al., 2015). There is numerous available data indicating the role of diet in the intensity of cobalt entry into the human body

(Hokin B. et al., 2004; EFSA Panel on Additives, 2009; Tvermoes B.E. et al., 2015). The results of the kinetics research of the bivalent cobalt ions (Co^{2+}) in the human body demonstrate that the maintenance of stable systemic indicators of this ion can be enhanced by the binding of the cation to plasma polyanion proteins (e.g. albumin); accumulation of significant amounts of Co^{2+} inside erythrocytes; its intense reabsorption by tubular nephron epithelium (Tvermoes B.E. et al., 2015). The predominant pathways for cobalt excretion out the body of humans and other mammals are the kidneys and the gastrointestinal tract (Gál J. et al., 2008; Tvermoes B.E. et al., 2015). The intensity of renal clearance of Co^{2+} rather accurately reflects the balance of the soluble ion fraction in the extracellular body fluid (EFSA Panel on Additives, 2009; Tvermoes B.E. et al., 2015). A modern methodology for estimating the level of cobalt consumption in human populations can be based on an analysis of the Co^{2+} dynamics in the blood and urine (Tvermoes B.E. et al., 2014; 2015; Unice K.M. et al., 2012; Wei B. et al., 2018). Along with this, in population studies it is quite interesting to compare the dynamics of cobalt content in the various human blood and hair samples (Wei B. et al., 2018). The parallel between the metabolic pathways in the human body of the Ca^{2+} and Co^{2+} cations is emphasized suggesting a preferential distribution of those cations in the intravascular fluid, in erythrocytes and in specialized cellular organelles of certain tissues with high rates of Ca^{2+} metabolism (Simonsen L.O. et al., 2012). In particular, in the rat experiments the cardiotoxic effect of bivalent cobalt salts was demonstrated mainly due to the accumulation of the Co^{2+} cation inside the cardiomyocytes (Gál J. et al., 2008). On the other hand, experimental evidence is given for the thesis stating that the intracellular metabolism of Co^{2+} may be closely linked with the metabolism of Zn^{2+} ions and the transport system of positively charged aminoacids through cationic amino acid transporter - Cat1 (Ryuko S. et al., 2012).

There are several reasons why the development of effective methods for assessing the levels of Co^{2+} intake and the study of its kinetics in the human body can have practical importance. Firstly, physiologically active Co^{2+} -containing complex compounds (cobalamins) are vital for the normal biochemistry of physiological processes. Secondly, cobalt mineral salts (Co^{2+}) are constantly present in food products, mainly of animal origin (EFSA Panel on Additives, 2009). The obtained data suggests that the permissible rate of daily intake of Co^{2+} into the human body may be limited up to 1000 μg / day (Unice K.M. et al., 2012). According to other data, the permissible daily intake dose for Co^{2+} is about 600 μg / day (EFSA Panel on Additives, 2009). The existing sanitary norms of the Republic of France stipulate the limiting values of the permissible daily consumption of Co^{2+} (depending on age), which are 1.6-8 μg / kg bw / day (Leysens L. et al., 2017). Given that normal food intake of cobalt ions is, on average, 0.012 mg day^{-1} per person, reaching higher values in some populations of India 0.23 mg day^{-1} and Japan 0.036 mg day^{-1} (Gál J. et al., 2008). Excessive physiological norms of oral consumption of Co^{2+} can cause toxic effects, activating the processes of mutagenesis, carcinogenesis and tissue necrosis through the induction of reactive oxygen species formation and suppressing the work of DNA repair systems (M. De Al., 2003; EFSA Panel on Additives, 2009; Simonsen LO et al., 2012; Paustenbach DJ et al., 2013), activation of pro-inflammatory factor products (Liang Y. et al., 2017), thyroid dysfunction (Yorita Christensen KL, 2013; Paustenbach DJ et al., 2013; Tvermoes BE et al., 2015), increased risk of cardiomyopathy (Paustenbach DJ et al., 2013), encephalopathy and congenital birth defects (EFSA Panel on Additives, 2009; Catalani S. et al., 2012; Dai Y. et al., 2013). The obtained data from population-based studies confirms that higher levels of cobalt intake into the human body may lead to a decrease in visual acuity (Mendy A. et al., 2012).

In humans as well as in other mammals the mineral salts of Co^{2+} do not participate in the processes of biosynthesis of cobalamins by the intestinal microflora (Gál J. et al., 2008; EFSA Panel on Additives, 2009). At the same time we have obtained some data reports indicating that Co^{2+} ions coming from food (not included in cobalamins) can cause regulatory effects mediated by the production of physiologically active substances that act as a kind of cation mediators in relation to various tissues and organs. Primarily, these effects are related to hypoxia-inducible factor (HIF) (Salnikow K. et al., 2000; Yuan Y. et

al., 2003; Maxwell P., Salnikow K., 2004; Gál J. et al., 2008; Jelkmann W., 2012; Muñoz-Sánchez J., Cháñez-Cárdenas ME, 2018). Meanwhile, that particular influence of cobalt on the body which is mediated through the hypoxia-inducible factor does not entirely correspond to the generally accepted views on the toxic effects of cobalt substances. Perhaps, therefore, over the past two decades, this unit of research has quite clearly transformed into an independent research direction. The presence of its own specific approaches and research methods allows it to interfere productively a range of issues concerning the pathophysiological and regulatory aspects of the Co^{2+} influence on the human body. In our opinion, this is the direction that is the most promising and relevant for both fundamental science and practical medicine.

Consequently, the purpose of our data analysis is to provide the current information about the regulatory and pathophysiological effects of Co^{2+} in the human body regarding the systemic, tissue and molecular levels after toxic food intake or during accumulation of the cobalt cation in the extracellular body fluid.

2. TOXIC EFFECTS OF COBALT

In the context of the facts under consideration, information on the pronounced direct toxic effects of cobalt can appear to be rather interesting because it allows to make a clearer distinction between the mechanisms of the regulatory and toxic effects of Co^{2+} . It should be noted that the origin of the toxic effects and the level of the toxic dose of cobalt cation largely depend on the way how it enters the body: oral, inhalation or transcutaneous. According to some authors, the threshold values of its oral intake were identified, the excess of which causes a toxic effect. In particular, for oral administration of Co^{2+} , the minimum risk threshold is associated with daily ion intake of about 0.01 mg Co / kg body weight / day (EFSA Panel on Additives, 2009). The review shows that induced Co^{2+} cardiomyopathy can be caused by food intake of an ion within 0.04 to 0.14 mg Co / kg / day. There is an opinion on the relevance of studies aimed at substantiating the threshold levels of Co^{2+} compounds in the human body (Leyssens L. et al., 2017) since the proportion of anthropogenic factor in the bioavailability of this chemical element for humans has significantly increased. It has been suggested that the threshold values of the systemic toxic effects of Co^{2+} may be due to the saturation of the intravascular deposition systems of the cation (plasma proteins and erythrocytes) and an increase in the concentration of free (ionized) cobalt in the extracellular fluids (Tvermoes BE et al., 2015; Leyssens L. et al., 2017).

Hence, not only the levels of food intake of metal ions, but the state, for example, of protein metabolism, can determine the degree of Co^{2+} toxicity. Indeed, there are direct evidences that a low protein diet can be considered as an additional risk factor for the development of the toxic effects of Co^{2+} (EFSA Panel on Additives, 2009). The results of clinical studies also confirmed the ability of plasma proteins to bind Co^{2+} ions, emphasizing the possible role of this mechanism in the neurotoxic effects of metal cations (Catalani S. et al., 2011).

The available data suggests that the direct toxic effects of Co^{2+} on humans and animals occur mainly due to the elevation of reactive oxygen species resulting in the activation of lipid peroxidation and proteins, followed by severe destructions of nucleic acids and the suppression of DNA repair systems (Jomova K., Valko M., 2011; Leyssens L. et al., 2017). It is indicated that the application of antioxidants reduces the toxic effect of cobalt (Jomova K., Valko M., 2011, Zadnipyany I.V. et al., 2017). The authors of the cited review indicate that not only salts, but also metallic cobalt possess genotoxic properties, while cobalt compounds, for example, with tungsten or tungsten carbide, can enhance their genotoxic and carcinogenic properties.

Meanwhile, there is the evidence that direct damage to mitochondria can be considered as another pathogenetic mechanism of polyorganic disorders induced by cobalt salts (Wang G. et al., 2000). According to the authors of the cited publication, the toxic effect of Co^{2+} on mitochondria contributes to the development of oxidative stress and damage to mitochondrial DNA regardless of the nuclear DNA

damage. In vitro studies on murine embryonic stem cells revealed that cobalt dichloride CoCl_2 enhances the formation of reactive oxygen species, reduces the level of antioxidant defense, promotes the release of cytochrome C from mitochondria into the cytoplasm and finally activates mitochondrial cell apoptosis (Lee J.-H. et al., 2013). The authors highlighted that toxic effects of cobalt dichloride depend on the dose and time of exposure of the cells to their growth medium containing heavy metal ions. It was reported that cobalt dichloride was able to induce the formation of a superoxide anion in the mitochondria of various types of human cells (Chamaon K. et al., 2019).

In our opinion, it should be taken into account that the toxic levels of Co^{2+} can have a specific effect on the immune system. In particular, studies on human monocytes and neutrophils in vitro have shown that Co^{2+} cations activate the Toll-like receptor-4 (TLR4) of leukocytes, stimulating cell migration, resulting in increased production of pro-inflammatory cytokines and the formation of foci of infiltrates (Lawrence H. et al., 2016). On the other hand, the induced by Co^{2+} Toll-like receptor-4 is expressed on the human microvascular endothelial cells (Anjum S.A. et al., 2016). So, the cited results go in a good agreement with the previously obtained information about Co^{2+} -dependent induction of TLR4 in murine peritoneal macrophages is accompanied by the overproduction of pro-inflammatory cytokines and tissue hormones (Shweta. Et al., 2015).

Nevertheless, the analysis of the toxic properties of a substance should be always based on the results of the study of its kinetics in the human body. On the one hand, a number of organs (myocardium, liver, kidneys) with the ability to accumulate Co^{2+} ions (Simonsen L.O. et al., 2012; Tvermoes B.E. et al., 2014) have been identified. There is indication of a change in metabolic processes in the liver and kidneys of rats exposed to prolonged exposure to cobalt dichloride at a dose of $12.5 \text{ mg cobalt kg}^{-1}$ for 7 days (Shrivastava K. et al., 2010). Pathomorphological studies have shown that cobalt dichloride leads to dose-dependent structural disorders of the liver and myocardial tissue (Liu Y. et al., 2010). In the context of the previously discussed issues, it is important to emphasize that the tissues characterized by a high intensity of Ca^{2+} metabolism can be considered as the target for the toxic effects of Co^{2+} (Simonsen L.O. et al., 2012). Indeed, the results of experimental studies suggest that salts of Co^{2+} triggered the necrotic changes in the heart and kidneys (Akinrinde A.S. et al., 2016; Oyagbemi A.A. et al., 2019). Skeletal musculature can be also considered as a Co^{2+} target organ. It has been established that Bcl-2 adenovirus E1B interacting protein-3 (BNIP3) is an important component in muscle tissue, determining Co^{2+} - induced autophagy via the HIF - BNIP3 (Chen R. et al., 2017). In connection with these facts, information about the mechanisms of Co^{2+} ion entry into the cell and into the mitochondria can be quite helpful. Divalent metal ion transporter 1 (DMT1) (Muñoz-Sánchez J., Cháñez-Cárdenas M.E., 2018) is considered to be the main channel of Co^{2+} entry (as well as divalent ions of iron and nickel). However, it is reported that the transport of the histidine amino acid through the cationic amino acid transporter - Cat1 can also be involved in the absorption of cobalt cations, whereas the Zhf1 transport protein, which is responsible for the metabolism of zinc ions, can detoxify cells from cobalt cations (Ryuko S. et al., 2012). According to the current references, the further entry of the Co^{2+} from the cytoplasm into the mitochondrial matrix is carried out according to the equivalent transport mechanism of Ca^{2+} and other bivalent metal ions - the mitochondrial permeable transitional pore mechanism mediated by the mitochondrial potential (Feng W. et al., 2016). In addition, the authors draw attention to the fact that Co^{2+} ions alike Ca^{2+} ions, can influence the system of mitochondrial translocations.

On the other hand, the results of in vitro studies on the culture of cardiomyocytes made it possible to establish that the toxic properties of cobalt dichloride occur due to a direct toxic effect on the state of redox processes in mitochondria that triggers the cell apoptosis (Niu N. et al., 2019). Perhaps a feature of the cardiotoxic action of Co^{2+} is the disruption of the fundamental processes of energy metabolism in mitochondria which are not capable to maintain the adequate contractile functions of cardiomyocytes affecting both systole and diastole (Hantson P., 2019). Arguments confirming damage to mitochondria by cobalt (Co^{2+}) ions of the tricarboxylic acid cycle (Kurhaluk N. et al., 2019; Hantson P., 2019) are also

being discussed. In vitro studies there have been established that cobalt dichloride stimulates oxidative stress and apoptosis in a dose-dependent manner (He Y. et al., 2018). The authors showed that Co^{2+} ions stimulate the expression of dynamin-related protein 1 (Drp1), which determines the pathogenetic mechanisms of cell death: a decrease in the mitochondrial membrane potential, a decrease in ATP level, the stimulation of the formation of reactive oxygen forms. The mechanisms of cell apoptosis caused by mitochondrial damage and excessive stimulation of oxidative stress are considered to be the universal pathogenetic mechanism of Co^{2+} induced damage to the central nervous system (Catalani S. et al., 2012). It is also impossible to exclude the fact that a synergistic factor determining the nature of the influence of cobalt dichloride on the fundamental metabolic processes (Krebs cycle activity, electron transport rate by cytochromes and mitochondrial DNA transcription activity) in the mitochondria can be considered as the hypoxia-inducible factor (Saxena S. et al., 2012).

Nowadays attention is drawn to the fact that most authors, discussing the toxic effect of Co^{2+} on mitochondria, point to increased production of reactive oxygen species and the induction of apoptosis, as two closely related effects. Meanwhile, in the literature there are reports that such close relationship between oxidative stress and apoptosis is more typical for the pure metallic cobalt (Liu Y. et al., 2017). At the same time, the authors of the cited publication provide the data that the path of stimulation of apoptotic signals by Co^{2+} ions may not be directly related to the activation of oxidative stress.

3. PATHOPHYSIOLOGICAL EFFECTS OF Co^{2+}

An important aspect of the problem under discussion is that cobalt mineral salts, those which are not part of cobalamins, can have their own regulatory effect on the state of metabolic processes in the human body. Cobalt salts (Co^{2+}) have been used for a long time as a pharmacological stimulators of hematopoiesis in the treatment of anemias (Ebert B., Jelkmann W., 2014; Hoffmeister T. et al., 2019). Further studies have shown that hypoxia-inducible factor is the leading mediator that determines the pathway and intensity of the effects of cobalt mineral salts on the human body (Salnikow K. et al., 2000; Maxwell P., Salnikow K., 2004). The question regarding the feasibility of pharmacological treatment of anemia with cobalt hematopoietic salts was withdrawn from wide practical application in the 70s of the 20th century. Meanwhile, the possible aspects of using cobalt mineral salts, mainly in the form of Co^{2+} still remain in the field of interests of modern practical medicine and is reflected in a number of modern publications (Tanaka T. et al., 2005; Shrivastava K. et al., 2008; Chai YC et al., 2018).

According to the modern references the hypoxia-inducible factors are considered to be the main mediators of the regulatory and pathophysiological effects of the Co^{2+} salts (Salnikow K. et al., 2000; Maxwell P., Salnikow K., 2004; Muñoz-Sánchez J., Cháñez-Cárdenas M.E., 2018). An analysis of the possible mechanisms of Co^{2+} -dependent induction of hypoxia-inducible factors allowed to express the opinion that the suppression of the metabolic clearance of HIF-1 α as a result of Co^{2+} -induced decrease in the enzymatic activity of prolyl hydroxylases is not likely to be associated with the replacement of the Fe^{2+} ion with Co^{2+} in the enzyme catalytic center (Karaczyn A. et al., 2006; Kaczmarek M. et al., 2006; Muñoz-Sánchez J., Cháñez-Cárdenas ME, 2018). According to the authors of the cited publications, the more likely mechanism of Co^{2+} -dependent stabilization of HIF-1 α is depletion in the presence of Co^{2+} ions of intracellular reserves of ascorbic acid, which is crucial for the reduction of Fe^{3+} to Fe^{2+} in the active center of prolyl hydroxylases. Some data represents the role of Co^{2+} in the metabolic clearance of HIF-1 α with the participation of von Hippel-Lindau protein (Yuan Y. et al., 2003), as well as in the stimulation of HIF-1 α translation (Muñoz-Sánchez J., Cháñez-Cárdenas M.E., 2018).

However, by analyzing the effects of the Co^{2+} salts bounded with the stabilization of HIF-1 α , a unique pattern can be revealed that is not observed during review the basic pathogenetic mechanisms of the toxic effects of this metal. Firstly, the direct toxic effects of Co^{2+} mineral salts are considered as a uniquely

negative factor. At the same time, in the case of low levels of Co^{2+} intake not over exceeding the toxic threshold values, in addition to negative body reactions, we can observe physiological effects which are potentially applicable in practical medicine. For example, in earlier studies it was suggested that cobalt dichloride could serve as a promising adaptogen in the conditions of hypobaric hypoxia (Shrivastava K. et al., 2008). The results of later studies show the promising outcomes of cobalt in vitro application in regenerative medicine in order to: form HIF-1 α -dependent Co^{2+} induce stem cells of a certain phenotype (Jeon ES et al., 2014), create conditions that are optimal for implant engraftment (Chai YC et al., 2018) and to control osteogenic differentiation (Chen Y. et al., 2019). Along with this, the results of nephroprotective properties of cobalt salts were obtained in in vivo studies (Matsumoto M. et al., 2003; Tanaka T. et al., 2005). Considering the previously identified HIF-1 α -dependent nephroprotective properties of Co^{2+} , the use of organometallic cobalt complexes have been proposed, which are able to provide strictly supervised intrarenal level of Co^{2+} production in order to inhibit interstitial fibrosis (Tan L. et al., 2019).

Secondly, the ability of Co^{2+} ions to stabilize HIF-1 α is considered to be a pleiotropic pathophysiological mechanism of cation influence associated with the production of various physiologically active molecules and affecting the mitochondrial function (Stenger C. et al., 2011; Chimeh U. et al., 2018; Muñoz-Sánchez J., Cháñez-Cárdenas M.E., 2018). In regard to this statement, the warnings that cobalt mineral salts are not the mandatory component of the human diet and their food consumption should be strictly standardized are absolutely reasonable (Jelkmann W., 2012; Simonsen L.O. et al., 2012; Ebert B., Jelkmann W., 2014). Indeed, research results show that even relatively low levels of cobalt dichloride intake can cause vivid changes in metabolic processes in the myocardium and in the renal parenchyma (Akinrinde A.S. et al., 2016; Oyagbemi A.A. et al., 2019). It was also established that cobalt dichloride even in the low doses not exceeding the toxic threshold, may trigger the tissue fibrosis and necrosis (Kong D. et al., 2015).

In earlier publications, there is evidence of demand for biomonitoring of the presence of Co^{2+} in human food, emphasizing the versatility of such pathophysiological reactions, such as inflammation, in response to the presence of a cation in the body (Simonsen L.O. et al., 2012; Czarnek K. al., 2015). Also, it is necessary to point out that the authors of the cited reviews emphasize the role of HIFs proteins in the realization of the pathophysiological effects of cobalt. It is possible that a more significant range of pathophysiological reactions to the intake of Co^{2+} can be related with the number of HIF-1 α -dependent regulatory effects of Co^{2+} (Nagasawa H., 2011; Eskandani M. et al., 2017). In particular, it is indicated that inflammation can be considered as one of the pathogenetic HIF-1 α -related effects of hypoxia (Eskandani M. et al., 2017). There are also grounds to believe that Co^{2+} ions stimulate the expression of the cyclooxygenase-2 protein gene, against the background of a decrease in the enzymatic activity of endothelial NO-synthase complexes (Liang Y. et al., 2017). In vitro studies have shown that Co^{2+} have the ability to activate in macrophages the biosynthesis of inducible NO synthase and pro-inflammatory interleukins (Kumanto M. et al., 2017). A number of publications emphasize the universal pathogenetic role of the Toll-like receptor-4 (TLR4) of leukocytes in the body's immune responses to salts of heavy metals such as cobalt and nickel (Schmidt M. et al., 2010; Samelko L. et al., 2016). It is interesting that nanoparticles of metallic cobalt can also stimulate the dose-dependent production of human monocytic IL-1 β and TNF- α (Samelko L. et al., 2016). The authors of the cited publications also provide facts confirming the role of the pro-inflammatory effect of cobalt and its compounds in the pathogenesis of the immunopathologies of muscular and bone tissues. However, there is evidence in the literature that cobalt dichloride can induce immunosuppression by suppressing the action of pro-inflammatory factors (Kwak J. et al., 2018). Although, as we have already observed, the regulatory effects of the metal may depend on the dose of exposure and some other experimental conditions.

At the same time, it has been shown that cobalt salts in the liver, in subtoxic concentrations, can stimulate the production of a transforming factor beta-pleiotropic cytokine, which determines cell cycle

restriction, stimulation of extracellular matrix protein synthesis combined with immunosuppressor effects (Kong D. et al., 2015). The authors of the cited source believe that the dynamics of TGF-beta production reflects the risk of organ fibrosis and can act as the cancerogenic factor. Attention is drawn to the fact that Co^{2+} -induced fibrosis may be systemic and get triggered by the generation of reactive oxygen species, while the another heavy metal chromium ions (Cr^{3+}) do not act this way (Xu J. et al., 2018).

Discussing HIF-1 α -dependent regulatory effects of Co^{2+} in our consideration, it is necessary to point out another important area of research due to the ability of the metal to participate in the regulation of gene expression through such mechanisms as covalent chromatin modification and synthesis of small non-coding RNA. This line of research is developing dynamically in the field of testing of new diagnostic methods, and is also valuable in the development of a fundamentally new way to treat the most lethal cancer cases (Permenter M.G. et al., 2013; Ratha S. et al., 2017; Li C.-L. et al., 2018).

4. EPIGENETIC EFFECTS OF Co^{2+}

The earliest publications on this topic contain information that Co^{2+} can stimulate the transcription of certain proteins independently of the intracellular endogenous production of reactive oxygen species (Salnikow K. et al., 2000). Further, directly induced by Co^{2+} HIF-1 α -dependent epigenetic mechanisms associated with enzyme DNA methylation and histone acetylation systems were evidenced (Maxwell P., Salnikow K., 2004). In the modern reviews the role of epigenetic mechanisms in the implementation of toxic and carcinogenic effects of heavy metals is widely emphasized (Salnikow K., Zhitkovich A., 2008; Chervona Y, Costa M., 2012; Brocato J., Costa M., 2013). The importance of the HIF-1 α -dependent epigenetic mechanisms induced by cobalt and other heavy metals is also recognized (Salnikow K. et al., 2008; Nagasawa H., 2011; Brocato J., Costa M., 2013; Eskandani M. et al., 2017). It was also shown that stimulation of cobalt dichloride with extracellular matrix deposits, as well as induction of vascular endothelial growth factor and erythropoietin are associated with HIF-1 α (Tanaka T. et al., 2005). In our opinion, the scientific novelty of the proposed approach consists in the fact that for the first time a theoretical explanation of the pathogenesis of deadly oncological diseases induced by heavy metals was explained and based on epigenetic mechanisms for controlling of gene expression. At the same time, the pathogenesis of these diseases was not considered as a result of direct DNA damage. The developed approach was based on the cancerogenic effects of heavy metals caused by a specific covalent modification of chromatin that alters gene expression (Salnikow K., Zhitkovich A., 2008; Salnikow K. et al., 2008). The sustainability of this approach was confirmed by subsequent research results (Chervona Y, Costa M., 2012; Brocato J., Costa M., 2013).

The results of *in vitro* studies on cell culture of multiple myeloma (B-cell malignancy selectively localized in the bone marrow) showed that the presence of cobalt chloride had a significant effect on the levels of HIF-1 α in cells, as well as on the state of expression of the genes identified as transcription factors, cell differentiation markers, protein kinases, cytokines and growth factors, tumor suppressors, and oncogenes (Bae S. et al., 2012). According to the authors, they managed to isolate a group of genes sensitive to Co^{2+} ions. It was also established that cobalt dichloride, through the processes of acetylation of histone proteins, regulates the expression of extracellular-superoxide dismutase (Hattori S. et al., 2016). On the other hand, valproic acid, and histone deacetylase inhibitors, has been shown to reduce the pathophysiological effects of HIF-1 α (Luo H.-M. et al., 2013; Kim Y.J. et al., 2017).

Along with this, it was shown that HIF-1 α can regulate not only the covalent modification of chromatin, but also affect the biosynthesis of small non-coding RNAs capable of determining protein biosynthesis at the level of transcription or translation (Kwak J. et al., 2018). Indeed, there is evidence in the literature that HIF may influence the metabolic systems of non-coding small RNAs (Ho J.J. et al., 2012; Ibrahim A.A. et al., 2017). At the same time, *in vitro* studies have established a link between the presence of

cobalt in the medium, HIFs with proteins, and the expression index of micro RNA cells (Silakit R. et al., 2018). There is evidence that HIF-dependent mechanisms, through the micro-RNA system, are involved in the regulation of the expression of pro-inflammatory cytokines (Kwak J. et al., 2018). The mechanisms of Co^{2+} induction of inflammation play an important role in the pathogenesis of cobalt intoxication, however, the role of epigenetic mechanisms that determine the synthesis (including micro RNA) of pro-inflammatory protein factors has not been studied deeply enough (Kumanto M. et al., 2017).

Conclusions

The relevance of studying the kinetics of heavy non-ferrous metals coming from the industrial objects is determined by significant pollution of the modern ecosystems. Ecotoxicants affect all internal organs at the molecular, cellular, tissue and systemic levels. The toxic effect of the damaging factor depends on its concentration and exposure duration, its combination with the other damaging factors, chronic human diseases and its immunological reactivity.

One of the representatives of heavy non-ferrous metals is cobalt, which simultaneously belongs to the group of microelements and therefore is vital for the functioning of living organisms, but at the same time, with its increased intake, it is toxic to the body and even destructive. There are certain concentrations in which cobalt is essential for living organisms. The main biological role of this element is its presence in the molecule of water-soluble vitamin B12 (cyanocobalamin), in which its mass fraction is 4%. However, excessive intake of cobalt in the body is geno-, enzyme- and membrane-toxic. The toxicity of high concentrations of cobalt occurs due to its hypoxic action, activation of lipid peroxidation and depletion of antioxidant systems. Cobalt, as an ion with variable valence, severely induces lipid peroxidation, promotes the formation of oxidative stress, which disrupts the function of the endothelium, damages biological macromolecules of internal organs: organs of respiration, cardiovascular system, liver and especially kidneys, where xenobiotics are neutralized.

The data from a number of studies indicate a toxic effect of cobalt chloride on the kidneys and myocardium, characterized by the development of microangiopathy, fibrotic proliferation, stimulation of apoptosis and even necrosis. The basis of the toxic effect of heavy non-ferrous metal salts, including cobalt, is the development of oxidative stress that can damage the cell and mitochondrial membranes and cause their structural and functional changes. The blocking of functionally active groups, structural proteins and functioning enzymes plays a significant role in the development of these disorders. Another mechanism of the toxic effect of heavy metals on the body is their property to replace calcium ions in specific processes.

The toxic effect of cobalt has been studied many times and has already been proven, however the mechanisms of the damaging effect of cobalt at the molecular and DNA level have not yet been fully studied. Cobalt dichloride (CoCl_2) has a similar effect to hypoxia due to expression of HIF-1 α mRNA, which indicates the dependence of the expression process of this mRNA not only according to oxygen level, but also in the presence of iron ions. Cobalt is able for firmly binding with heme than iron. It was also shown that cobalt activates HIF-1 due to depletion of the intracellular content of ascorbic acid, a co-factor for HIF-hydroxylase, which destabilizes and inactivates HIF-1 α . Hypoxia, as is known, is a typical pathological process that accompanies and determines the development of many pathological conditions. It leads to functional, and then structural changes in organs and tissues as a result of a decrease in the intracellular concentration of oxygen. This also applies to hypoxia of tumor cells (intratumoral hypoxia). Thus, many cancers include areas of hypoxia. Intratumoral hypoxia significantly worsens the prognosis of the disease, since angiogenesis in the tumor tissues is very intense. This, apparently, is one of the reasons for the rapid growth of malignant tumors and can explain cancerogenic cobalt effect. In addition, enhanced angiogenesis in the tumor contributes to the metastasis of its cells, which ultimately increases mortality among such patients. The principal mechanism of adaptation of cancer cells to hypoxia is

activation of the HIF-1 factor. The elucidation of the pathogenetic role of the HIF-1 α factor opens up new possibilities not only in the correction of hypoxia, but also in the treatment of malignant tumors.

As illustrated, the influence of cobalt ions on the body should be researched deeply, as well as interactions between cobalt and other ions and aminoacids occurring in body fluid. Perhaps these interactions can also have some specific and significant beneficial effects.

The authors declare no conflict of interest.

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