Review paper DOI: 10.5114/ceji.2015.52837

Selected aspects of the action of cobalt ions in the human body

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Abstract

Cobalt is widespread in the natural environment and can be formed as an effect of anthropogenic activity. This element is used in numerous industrial applications and nuclear power plants. Cobalt is an essential trace element for the human body and can occur in organic and inorganic forms. The organic form is a necessary component of vitamin B_{12} and plays a very important role in forming amino acids and some proteins in nerve cells, and in creating neurotransmitters that are indispensable for correct functioning of the organism. Its excess or deficiency will influence it unfavourably. Salts of cobalt have been applied in medicine in the treatment of anaemia, as well as in sport as an attractive alternative to traditional blood doping. Inorganic forms of cobalt present in ion form, are toxic to the human body, and the longer they are stored in the body, the more changes they cause in cells. Cobalt gets into the body in several ways: firstly, with food; secondly by the respiratory system; thirdly, by the skin; and finally, as a component of biomaterials. Cobalt and its alloys are fundamental components in orthopaedic implants and have been used for about 40 years. The corrosion of metal is the main problem in the construction of implants. These released metal ions may cause type IV inflammatory and hypersensitivity reactions, and alternations in bone modelling that lead to aseptic loosening and implant failure. The ions of cobalt released from the surface of the implant are absorbed by present macrophages, which are involved in many of the processes associated with phagocytose orthopaedic biomaterials particles and release pro-inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor α (TNF-α), and prostaglandin.

Key words: *cobalt, macrophages, type IV hypersensitivity reactions, biomaterials, implant.*

(Centr Eur J Immunol 2015; 40 (2): 236-242)

Introduction

Cobalt – occurrence and application

Cobalt was discovered in 1735 by Georg Brandt. It is one of the essential trace elements in the human body [1]. This element belongs to the eighth iron-group. Cobalt can occur in various degrees of oxidation forms from +1 to +5. The most popular are +2 and +3; other forms of them are rare. Ions form of cobalt, such as Co^{3+}, react with various acids and create salts; however the Co^{2+} form is less reactive [2].

Cobalt is very widespread in the natural environment and can be formed as an effect of anthropogenic activity [3]. It is present in small quantities in compounds with sulphur and arsenic. This element is used in numerous industrial applications like welding, diamond tooling, grinding, chemical catalyses, and nuclear power plants [3, 4]. In nuclear plants, it can occur in two radioactive forms of cobalt, ^{58}Co and ^{60}Co, which are formed from corrosion-erosion of alloys containing cobalt and other metals. These two forms produce high-energy γ photons, which can be used in radiotherapy to fight tumours [5, 6].

Cobalt is used in the production of alloys in plants and in ceramics, as well as chemical catalyses. Moreover, compounds of cobalt are used in industrial production of paints, e.g. Thenard's blue, and lacquers in glass and ceramics [3, 4, 7].

Organic and inorganic cobalt

This essential element occurs in inorganic and organic forms. The first form is essential and necessary for the human body but its excess or deficiency will influence it unfavourably. The organic form of cobalt is present in green parts of plants, fish, cereals, and water [8, 9].

In the human body this element is present in amounts from 1 to 2 mg: we can find it in the heart, liver, kidney, and spleen, and considerably smaller quantities in the pancreas, brain, and serum [10, 11].

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Cobalt is a necessary component of vitamin B_{12} (hydroxocobalamin) and a fundamental coenzyme of cell mitosis. Moreover, cobalt is very important for forming amino acids and some proteins to create myelin sheath in nerve cells [3, 10].

Cobalt also plays a role in creating neurotransmitters, which are indispensable for correct functioning of the organism [10]. The salts of cobalt stimulate the synthesis of erythropoietin, which is the most important function in the activation of different stages of erythropoiesis, which, in turn, is connected with the formation of erythrocytes in bone marrow [12, 13].

Deficiency of cobalt is strongly related to disturbances in vitamin B_{12} synthesis, so it might cause anaemia and hypofunction of thyroid and increase the risk of developmental abnormalities and failure in infants [10].

However, excess of this metal might increase the action of thyroid and bone marrow, which might, in turn, lead to overproduction of erythrocytes, fibrosis in lungs, and asthma [14].

For a long time the salts of cobalt have been applied in medicine in the treatment of anaemia, as well as in sport as an attractive alternative to traditional blood doping. This metal enhances synthesis of erythropoietin, which influences enlargement of erythrocyte quantity in blood, improving aerobic performance, but the mechanism of this process is not fully understood. However, it is known that hypoxia is a physiological impulse in erythropoietin production and decreases the oxygen content in circulating blood by bark of adrenal glands and liver [12].

Inorganic forms of cobalt present in ion form are toxic for the human body. When longer exposed, this element is stored in the body and causes a number of changes in cells [3, 4, 9]. Cobalt chloride and cobalt sulphide are its inorganic forms, which are widely applied in the brewing industry – they are added to beer to prevent the formation of foam. In people consuming large quantities of this drink, cardiomyopathy, a disorder of the diastole of the left ventricle, has been diagnosed. Significant accumulation of cobalt in the heart muscle in these people is formed postmortem. Some derivatives of cobalt, like ^{59}Co, are considered to be carcinogenic and are qualified as such by the International Agency for Cancer Research [3, 5, 6].

Cobalt in the human body

Cobalt daily intake in adults is 3 μg where the content of cobalt is 0.012 μg [11]. Cobalt gets into the body in a few ways. Firstly, with food; secondly by the respiratory system; thirdly, by skin; and finally, as a component of biomaterials. Biomaterials have already been in use for 2000 years, but their widespread use followed the introduction of polymethyl metacrylate (PMMA) in 1937 [15].

Intake of cobalt with food

Cobalt gets into the body with food as shown in Fig. 1, and then it is absorbed in the small intestine. Then the metal gets into the bloodstream where it is bound to proteins and transported with blood to tissues and cells [12].

The respiratory system

Substantial intake of cobalt can occur through the respiratory system, especially in people who are employed in heavy metal industry producing and using cobalt metal and cobalt compounds. The lungs absorb a considerable amount of cobalt oxide from dust, welding fumes, metallic cobalt, and other metals [12].

Particles of cobalt and other metals get through to the upper and lower respiratory system and they are displaced depending on their size. The large particles are swallowed and get to the gullet but the smaller ones get through the endothelial cells of the lungs. This situation causes many changes and a risk of lung cancer [1, 16].

Most epidemiological studies were carried out in workers employed for ten or more years in heavy metal manufacturing plants. In this case lung cancer occurs [7].

Other studies, not epidemiological, demonstrated a connection between inhalation of cobalt and lung cancer. Moreover, chronic cobalt exposure can cause rhinitis, asthma, respiratory irritation, and inflammation. The next studies on animals demonstrated that cobalt exposure can induce many respiratory lesions and alveolar and bronchiolar neoplasms [7].

Another research project demonstrated that the toxicity of Co ions during the wet grinding of alloy metals can cause a greater risk of allergic alveolitis than in people employed in dry areas with much higher levels of cobalt ions. These cobalt ions are able to induce the production of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor α (TNF-α), by alveolar macrophages as well [4].

The skin

The skin is like an external coat and it is susceptible to environmental pollution, especially in workers who are employed in heavy industry. Skin is the biggest immuno-

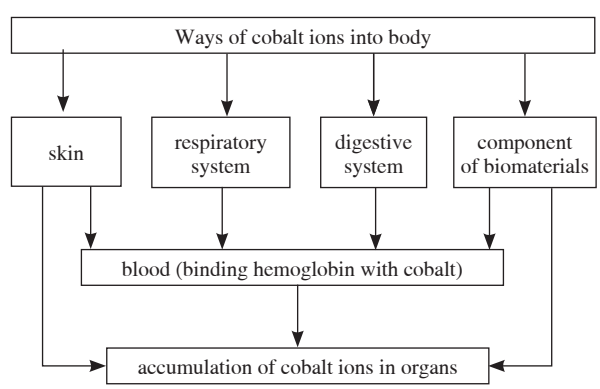

Fig. 1. Ways of cobalt ions into body

logical organ, which has a very important function in the human body. Its role is to defend the body against pathogens and harmful environmental factors and, secondly, it asserts communication with the external biotope [17].

The skin is very sensitive when exposed to chemicals and other pollution that can be found in the environment and workplace [18]. Environmental factors, especially heavy metals such as cobalt, can induce allergic contact dermatitis and may cause irritant reactions on the skin. Allergic contact dermatitis affects from 1 to 10 per cent of the total population, and metals such as cobalt, nickel, and chromium can cause contact allergy, affecting 10-15% of women and a few per cent of men [18, 19].

These diseases can occur especially in workers exposed to more specific occupational conditions, such as in industries preparing hard metals, diamond polishing, and recovering cobalt powders. Cobalt ions from different metal objects and cobalt powders in repetitive contact with the skin can be released from these objects and then they diffuse through the skin and cause allergy and irritant reaction [18, 19, 21].

Yet another study has demonstrated results of skin absorption of cobalt in industrial workers and in laboratory experiments with volunteers. These investigations were carried out in factories with high levels of pollution. No relation between cobalt ambient air and its urinary secretion was found. On the other hand, when studying heavy metal industry workers whose skin sustained contact with materials containing cobalt, high levels of this metal in serum and urine were detected [18, 21].

The next experiments were carried out in the laboratory on four people, who were asked to hold their right hands for 90 minutes in a box with freshly mixed wet powder-containing 5-15% cobalt. The level of cobalt was increased by an order of magnitude after exposure and remained unvaried for 48 to 60 hours. Another study evaluated an *in vitro* system using human abdominal skin obtained from autopsy and synthetic sweat. This research proved that cobalt passed through skin with sweat [21].

Cobalt gets to the body through the respiratory system and by gastrointestinal or skin absorption, and then it gets into the blood, where is bound to erythrocytes. Sometimes binding of metal by erythrocytes at the normal membrane potential is small but this process could be accelerated by the depolarisation of membrane or the addition of the divalent ionophore cation. This situation brings disorder to electrochemical equilibrium, which could result in extension of cobalt ions intracellularily bound [13, 22].

The digestive system

Water-soluble cobalt gets into the body through the gastrointestinal system, and it is absorbed from the small intestine to the blood. Moreover, large quantities of metallic cobalt and its oxide in dust and fumes get into the body through lungs and then they get to the lymphatic and the vascular system. Initially, the concentration of cobalt ions is high but it gradually decreases, reaching a lower level within 24 hours. It is connected with storing cobalt ions in the liver and kidneys and urinary excretion. In another experiment retention of inorganic cobalt in a normal adult human after intravenous injection was studied. The investigation showed that cobalt was reduced by 40% after 24 hours and by as much as to 70% after a week; however, after one month it decreased to about 20% and after one year to about 10%, which means that this amount of cobalt is retained [12, 21].

Cobalt is accumulated mainly in liver, kidneys, pancreas, heart, skeleton, and skeletal muscles. Moreover, the aim of the investigation was to analyse the mechanism of binding cobalt to red cells *ex vivo*. The membrane of these cells was depolarised and the results were as follows: the concentration of cobalt ions in cytosol was about 1%, which means that this metal is bound irreversibly to haemoglobin during life, and it circulates in serum penetrating all tissues and organs [13].

Implants

In America about 500,000 people and in Europe 300,000 people receive joint implants every year. This problem does not concern only old people but also younger ones [23, 24]. Cobalt and its alloys are fundamental components in orthopaedic implants and have been used for about 40 years. Cobalt and other metals, like chromium, molybdenum, and nickel, create alloys that are used as a material to construct total hip arthroplasty [25, 26].

Prostheses can be made only from metals, and they are called MoM (metal on metal), or they can contain metals and polyethylene – MoP (metal on polyethylene), or only ceramics. Biomaterials are characterised as substances different from a medicament or combination of synthetic or natural substances, and they aim to replace natural body tissues. They are also used in the construction of artificial organs and in the production of medical equipment [27].

The biokinetics model of cobalt in the human body

The model of systemic biokinetics of cobalt was developed for the International Commission on Radiological Protection (ICRP) because of concern for workers who intake radionuclides occurring in the nuclear industry and as diagnostic tools in nuclear medicine. The primary kinetics model was created by Leggett in 1992 and was bound with alkaline earth elements. The structure of the primary model was based on the framework for biokinetics of inorganic cobalt [21].

This model helps us to understand the biological behaviour of inorganic cobalt in the human body. It was based on many studies carried out earlier on humans and laboratory animals that were exposed to radioactive and also stable

cobalt under controlled conditions. The differences between inorganic and organic cobalt were established. Both forms were accumulated in a high concentration in the liver and retained in the body for a long time. This model describes the circulation of cobalt ions between blood and four systemic tissues: liver, kidneys, skeleton, and other organs. The proposed model of behaviour of inorganic cobalt is based on the earlier data by Smith *et al.* (1972) and Letourneau *et al.* (1972) after injecting $^{60}CoCl_2$ and $^{58}CoCl_2$. Measurements were presented as a fractional transfer per day from places where inorganic cobalt was bound and transferred to receptor compartments. This systemic model provides all recycling data as well as for phases of loss as urinary and faecal excretion; some of these results were based on the data from Smith *et al.* (1972). Thus, this model is mainly based on the data by Smith *et al*. and is supplemented by the data obtained from laboratory animals [21].

The biokinetics model of inorganic cobalt was created by R. W. Leggett (2008) and was based on the research of initial systemic cobalt circulation and its dislocation in compartments of the body. Among these compartments were: liver, kidneys, skeleton, other tissues, and blood. The aim of this biokinetics model was to establish the level of cobalt ions circulating in blood after intravenous injection of ^{60}Co and ^{58}Co. The distribution of cobalt was examine by autoradiography in the body and was expressed in percentages. The blood was divided into blood bound to cobalt ions, which is transferred to organs, and blood remaining in plasma, as shown in Fig. 2. Furthermore, 6% of cobalt ions are bound by blood remaining in plasma, and 94% of cobalt ions are divided among various compartments, including faecal and urinary excretion. Such organs as liver, kidneys, skeleton, and others also represented circulation of cobalt ions in blood. For example, liver received 35% of cobalt ions in blood, and then it was removed from this place in 75% of these ions into plasma. Moreover, the rest of the cobalt ions (20%) contained in bile go to the small intestine, with 5% remaining and representing the long-term retention of cobalt ions. Kidneys receive 4.5% of cobalt ions in blood and 5% of these ions remain in the kidneys. An additional 30% of cobalt ions go to the urinary bladder from blood that is transferred to organs. Moreover, 6% of ions go to the skeleton, where 3% of them are distributed on the trabecular bone surface and 3% to the cortical bone surface. In addition, 85% of cobalt ions are removed from the skeleton into plasma; 15% of these ions represent long-term retention. Moreover, other organs received 16% of cobalt ions binding in blood [21].

Application of cobalt as biomaterial

The use of biomaterials in medical practice should meet several conditions, e.g. toxicity, biological compatibility, healing of tissues, and mechanical properties [28, 29].

The time of safe use of biomaterials depends on several factors. The most important of them is biocompatibility. It is a property of biological material, which, besides its basic function in the body, does not cause worsening of the patient's condition, or any other complications. Implants with

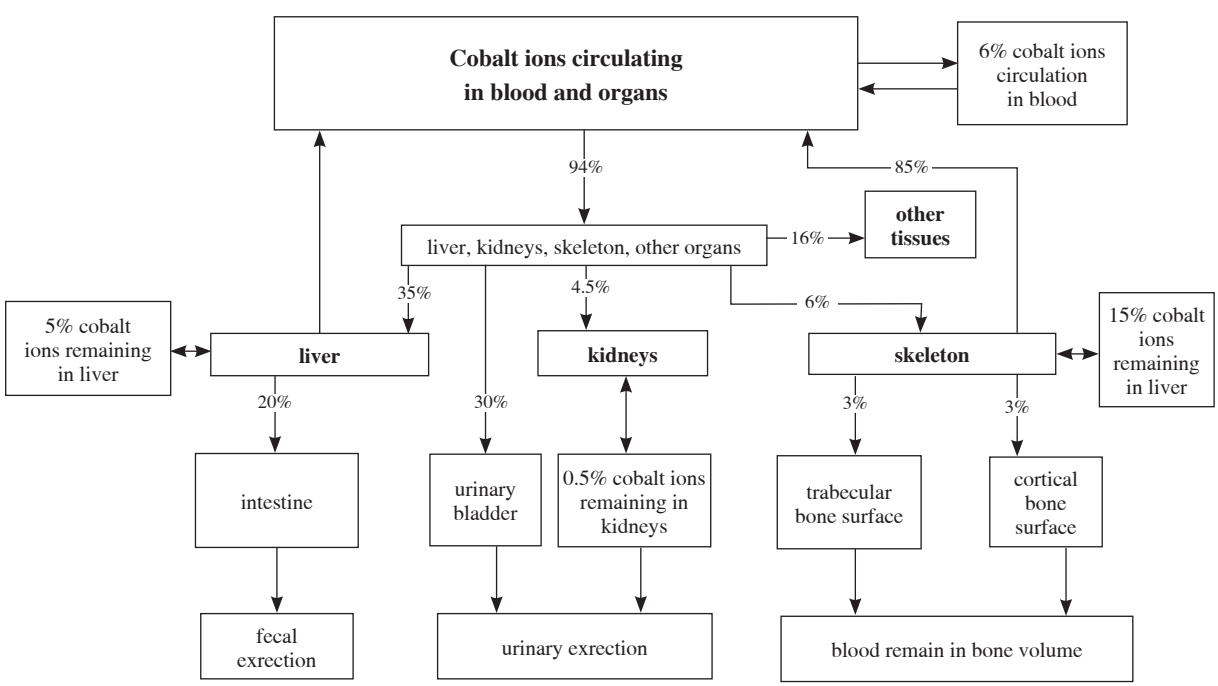

Fig. 2. Circulation of cobalt ions between blood and four systemic tissues

of good compatibility should not be toxic or immunologically active, so they should not induce chronic reactions or an inflammatory condition. In material engineering we can enhance several groups of materials, such as ceramics, polymers, carbon, and metals. All of the groups are different, and they are characterised by different properties [28].

The metal group contains steel Cr-Ni-Mo, titanium and its alloys, cobalt alloys, niobium, and precious metals. These materials are characterised by various properties such as susceptibility to stretching, brittleness, and mechanical resistance. Due to these properties they are commonly used in the production of various implants, but they are not without defects [15, 29, 30].

Biomaterials always trigger a reaction in tissues. The biomaterial used in the prosthesis plays a very important role in the success of the implant. Therefore, the use of any biomaterials in the body is determined by the material's biofunctionality and biocompatibility. The latter of these is very important but difficult to determine because of a number of interactions between body substances and biomaterials [26].

One such interaction is insufficient corrosion resistance in body fluid. The corrosion of metal is the main problem in constructing implants. Several studies have demonstrated that corrosion mechanisms can cause the release of ions of Co, Cr, and Mo into the surrounding tissue and synovial fluid [25, 31]. Moreover, these released metal ions may cause inflammatory and hypersensitivity reactions, and alternations in bone modelling that leads to aseptic loosening and implant failure [32, 33].

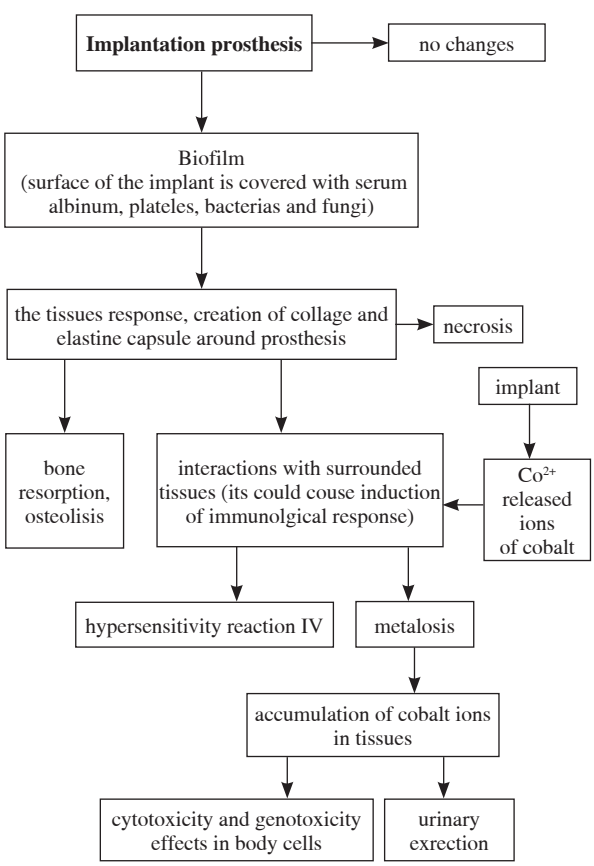

Fig. 3. Influence of cobalt ions released from surface of implant and immunological responses of body

Response of the human body to implantation

The presence of an implant, an alien body in a living organism, detected by the immunological system, triggers certain defensive mechanisms, as shown in Fig. 2.

After the implantation of a prosthesis the first defensive reaction is the creation of biofilm. It means that the surface of the implant is covered with serum albumin and platelets and is defined as an organic or non-organic deposit on the surface of the material and contains cellular elements and bacteria and fungi [15].

The bacterial forms have the ability to bind extracellular matrix albumins like collagen and fibrinogen, and cause their adhesion (such as: *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas species, Enteroccocus*). If the colonisation of bacteria is stopped by the host's cells, such as macrophages and fibroblasts, we cannot observe any changes in the body, but sometimes the defensive mechanism can be weaker than the pace of adhesion, and we can observe the growth of an increasing number of bacteria, as shown in Fig. 3. The final result of the tissue response is the creation of a collagen and elastin capsule around the prosthesis. This situation could lead to vasa's atrophy and necrosis, bone resorption, and ultimately failure of the implant [15, 25].

Moreover, this situation could be directly connected with work of implant. As mentioned earlier, the prosthesis contains various alloys of metals such as: cobalt, copper, chromium, molybdenum, and nickel, and they all release ions into the host tissues because of corrosion. The biological liquids in the body consist of many ions of chlorine, sodium, potassium, lime, phosphates, and organic components, and in addition a higher temperature of the body and changes of pH in tissues surrounding the implant cause the process of corrosion of metals [28, 29]. These ions are released over time, and their level can be highly significant because they are the source of many pathophysiological effects. Studies have demonstrated a sevenfold increase in metal ions in the synovial fluid. Another investigation has shown that Cr and Co ions increase the release of proinflammatory cytokines from macrophages and inhibition of osteoblasts, osteoclasts, and T and B cell proliferation [32]. Moreover, wear debris could be accumulated at the highest doses in the adjacent tissues and bone marrow and could circulate in the bloodstream and penetrate other organs in the body [24, 34].

The implant is an alien, foreign body, and the organism will activate some mechanisms which aim at its destruction. Macrophages are the type of leukocytes that, after reaching maturity, leave the tissue and get into the bloodstream. These cells are very important components of the immunological system and they synthesise cytokines and other factors that initiate inflammation and bone resorption. All of them cause osteolysis and aseptic loosening and failure of prostheses [26, 36].

The released ions of metals from metal on the metal surface of periprosthetics could enter the bloodstream and circulate on the body and ultimately accumulate in the heart, liver, kidney, spleen, pancreas, and lymphatic tissue, and only a part of them will be eliminated through urine. This metallic debris may have direct toxicological effects over a long time [37].

The metallic corrosion products can penetrate the cell plasma membrane, bind cellular proteins or enzymes, and modulate cytokine expression. Osteolysis, and aseptic and non-aseptic loosening are major causes of implant failure [26, 38, 39].

The reaction of the body to ions of cobalt and other metals is a type IV hypersensitivity reaction. The ions of cobalt and other metals released from the surface of the implant are absorbed by present macrophages, which are involved in many of the processes associated with phagocytose orthopaedic biomaterial particles. If these ions cannot be destroyed after phagocytosis of macrophages then the macrophages resolve and release pro-inflammatory mediators such as IL-1, IL-6, TNF-α, and prostaglandin, as shown in Fig. 4. The pro-inflammatory cytokines such as IL-1 α and β stimulate resorption of bone, and then they act synergistically to the tumour necrosis factor TNF-α. Moreover, macrophages release matrix metalloproteinases (MMPs) and chemokines [39-42].

The delayed type IV hypersensitivity reaction is a type of immune response where Th helper and cytotoxic Tc cells are also engaged. Th cells are responsible for the damage of infected tissues by macrophages and the activation of cytokines. Another type of cytotoxic Tc provides cell-mediates, which are responsible for cytolysis [26].

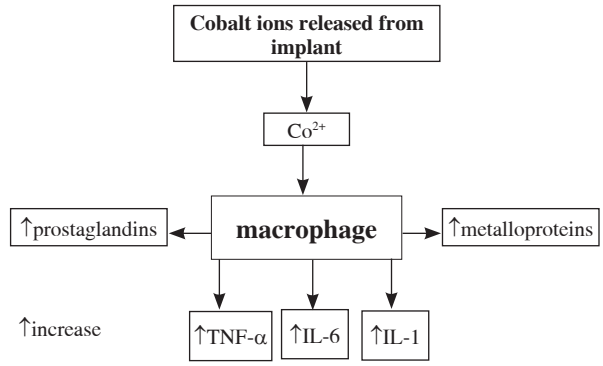

Fig. 4. Responses of macrophages on activity of cobalt ions released from implant surface

Summary

Cobalt is an essential trace element for the human body. This metal is very widespread in the natural environment and can be formed as an effect of anthropogenic activity. This metal occurrs in two forms: organic and inorganic. The organic form of cobalt is present in the green parts of plants, fish, cereals, and water, and it is a necessary component of vitamin B_{12}. Cobalt gets into the body in a few ways: firstly, with food; secondly by the respiratory system; thirdly, by the skin; and finally, as a component of biomaterials. Cobalt and its alloys are fundamental components in orthopaedic implants and have been in use for about 40 years. The use of biomaterials in medical practice should meet several conditions, e.g. toxicity, biological compatibility, healing of tissues, and mechanical properties. The corrosion of metal is the main problem in the construction of implants.

The condition of the individual patient should be considered when selecting the implant. Moreover, a patient with endoprosthesis MoM should be monitored, especially regarding the concentration of cobalt ions in the systemic liquid.

Inorganic forms of cobalt are toxic and can accumulate in tissues and evoke a chain of changes in cells. Cobalt ions released from the implant surface can cause toxic and immunological reactions.

Moreover, cobalt ions are released for a long time and the concentration of cobalt can develop into metalosis, and cobalt ions circulating with blood could accumulate in other organs, such as: heart, liver, spleen, lymph nodes, and kidneys, where the ions are excreted with urine and excrement. Also, these ions could induce cytotoxicity and genotoxicity effects in body cells. As illustrated, the influence of cobalt ions on the body should be studied deeply, as well as interactions between ions of cobalt and other metals occurring in body fluid. Perhaps these interactions have some specific and significant effects on the immunological system.

The authors declare no conflict of interest.

References

1. Daragó A, Sapota A (2011): Kobalt i jego związki nieorganiczne – w przeliczeniu na Co. Podstawy i Metody Oceny Środowiska Pracy 3: 47-94.
2. Pajdowski L (1982): Chemia ogólna. Wydawnictwo Naukowe PWN, Warszawa.
3. Ortega R, Bresson C, Fraysse A, et al. (2009): Cobalt distribution in keratinocyte cells indicates nuclear and perinuclear accumulation and interaction withmagnesium and zinc homeostasis. Toxicol Lett 188: 26-32.

4. Roesems G, Hoet PH, Dinsdale D, et al. (2000): In vitro cytotoxicity of various forms of cobalt for rat alveolar macrophages and type II pneumocytes. Toxicol Appl Pharmacol 162: 2-9.
5. Gault N, Sandre C, Poncy JL, et al. (2010): Cobalt toxicity: Chemical and radiological combined effects on HaCaT keratinocyte cell line. Toxicol Vitro 24: 92-98.
6. Bresson C, Lamouroux C, Sandre C, et al. (2008): An interdisciplinary approach to investigate the impact of cobalt in a human keratinocyte cell line. Biochimie 88: 1619-1629.
7. Patel E, Lynch Ch, Ruff V, Reynolds M (2012): Co-exposure to nickel and cobalt chloride enhances cytotoxicity and oxidative stress in human lung epithelial cells. Toxicol Appl Pharmacol 258: 367-375.
8. Unice KU, Monnot AD, Gaffney SH, et al. (2012): Inorganic cobalt supplementation: Prediction of cobalt levels in whole blood and urine using a biokinetic model. Food Chem Toxicol 50: 2456-2246.
9. Galanis A, Karapetsas A, Sandaltzopoulos R (2009): Metal-induced carcinogenesis, oxidative stress and hypoxia signaling. Mutat Res 674: 31-35.
10. Battaglia V, Compagnone A, Bandino A et al. (2009): Cobalt induces oxidative stress in isolated liver mitochondria responsible for permeability transition and intrinsicapoptosis in hepatocyte primary cultures. Int J Biochem Cell Biol 41: 586-594.
11. Karovic O, Tonazzini I, Robola N, et al. (2007): Toxic effects of cobalt in primary cultures of mouse astrocytes. Similarities with hypoxia and role of HIF-1alpha. Biochem Pharmacol 73: 694-708.
12. Simonsen LO, Harbak H, Bennekou P (2012): Cobalt metabolism and toxicology – a brief update. Sci Total Environ 432: 210-215.
13. Simonsen LO, Brown AM, Harbak H, et al. (2011): Cobalt uptake and binding in human red blood cells. Blood Cell Mol Dis 46: 266-276.
14. Lombaert N, Lison D, Van Hummelen P, Kirsch-Volders M (2008): In vitro expression of hard metal dust (WC-Co) – responsive genes in human peripheral blood mononucleated-cells. Toxicol Appl Pharmacol 2008; 227: 299-312.
15. Paduch DA, Niedzielski J (2005): Materiały biomedyczne. Część I: Pojęcie filmu biologicznego (biofilmu) i fizykochemiczne podstawy przyczepności substancji organicznych do biomateriałów. Chir Pol 7: 180-191.
16. Smith LJ, Holmes AL, Kandpal SK, et al. (2014): The cytotoxicity and genotoxicity of soluble and particulate cobalt in human lung fibroblast cells. Toxicol Appl Pharmacol 278: 259-265.
17. Gołąb J, Jakóbisiak M, Lasek W, Stokłosa T (2008): Immunologia. Wydawnictwo Naukowe PWN, Warszawa.
18. Filon FL, D'Agostin F, Crosera M, et al. (2009): In vitro absorption of metal powders through intact and damaged human skin. Toxicology 23: 574-579.
19. Granchi D, Cenni E, Tigani D, et al. (2008): Sensitivity to implant materials in patients with total knee arthroplasties. Biomaterials 29: 1494-1500.
20. Corsini E, Galli CL (2000): Epidermal cytokines in experimental contact dermatitis. Toxicology 142: 203-211.
21. Leggett R.W. (2008) The biokinetics of inorganic cobalt in the human body. Sci Total Environ 2008; 389: 259-269.
22. Simonsen LO, Harbak H, Bennekou P (2011): Passive transport pathways for Ca^{2+} and Co^{2+} in human red blood cells. 57 Co^{2+} as a tracer for Ca^{2+} in influx. Blood Cell Mol Dis 47: 214-225.
23. Gajski G, Jelčić Ž, Oreščanin V, et al. (2014): Physico-chemical characterization and the in vitro genotoxicity of medical implants metal alloy (TiAlV andCoCrMo) and polyethylene particles in human lymphocytes. Biochim Biophys Acta 1840: 565-576.
24. Papageorgiou I, Yin Z, Ladon D, et al. (2007): Genotoxic effects of particles of surgical cobalt chrome alloy on human cells of different age in vitro. Mutat Res 619: 45-58.
25. Lavicoli I, Falcone G, Alessandrelli M, et al. (2006): The release of metals from metal-on-metal surface arthroplasty of the hip. J Trace Elem Med Biol 20: 25-31.
26. Sargeant A, Goswami T (2006): Hip implant: Paper V. Physiological effects. Mater Des 27: 287-307.
27. Bauer S, Schmuki, Mark K, Park J (2013): Engineering biocompatible implant surfaces, Part I: Materials and surfaces. Progr Mater Sci 58: 261-326.
28. Świeczko-Żurek B (2009): Biomateriały. Wydawnictwo Politechniki Gdańskiej, Gdańsk.
29. Świeczko-Żurek B, Zieliński A, Ossowska A, Sobieszczyk S (2011): Biomateriały-Skrypt do przedmiotu. Politechnika Gdańska, Gdańsk.
30. Catelas I, Petit A, Zukorb DJ (2003): TNF-alpha secretion and macrophage mortality induced by cobalt and chromium ions in vitro-qualitative analysis of apoptosis. Biomaterials 24: 383-391.
31. Sargeant A, Goswami T (2007): Hip implant: Paper VI. Ion concentrations. Mater Des 28: 155-171.
32. Lin H-Y, Bumgardner JD (2004): In vitro biocorrosion of Co-Cr-Mo implant alloy by macrophage cells. J Orthop Res 22: 1231-1236.
33. Yang F, Wu W, Cao L, et al. (2011): Pathways of macrophage apoptosis within the interface membrane in aseptic loosening ofprostheses.Biomaterials 2: 9159-9167.
34. Papageorgiou I, Shadricka V, Davis S (2008): Macrophages detoxify the genotoxic and cytotoxic effects of surgical cobalt chrome alloy particles but not quartz particles on human cells in vitro. Mutat Res 643: 11-19.
35. Anderson JM, Rodriguez A, Chang DT (2008): Foreign body reaction to biomaterials. Semin Immunol 20: 86-100.
36. Bergschmidt P, Bader R, Mittelmeier W (2012): Metal hypersensitivity in total knee arthroplasty: revision surgery using a ceramic femoral component – a case report. Knee 19: 144-147.
37. Hallab NJ, Anderson S, Stafford T, et al. (2005): Lymphocyte responses in patients with total hip arthroplasty. J Orthop Res 23: 384-391.
38. Catelas I, Petit A, Zukorb DJ, et al. (2003): TNF-alpha secretion and macrophage mortality induced by cobalt and chromium ions in vitro-qualitative analysis of apoptosis. Biomaterials 24: 383-391.
39. Trindade MC, Lind M, Sun D, et al. (2001): In vitro reaction to orthopaedic biomaterials by macrophages and lymphocytes isolated from patientsundergoing revision surgery. Biomaterials 22: 235-259.
40. Catelas I, Petit A, Vali H, et al. (2005): Quantitative analysis of macrophage apoptosis vs. necrosis induced by cobalt and chromium ions in vitro. Biomaterials 26: 2441-2453.
41. Lhotka Ch, Szekeres T, Steffan I, et al. (2003): Four-year study of cobalt and chromium blood levels in patients managed with two different metal-on-metal total hip replacements. J Orthop Res 21: 189-195.
42. Thomsen P, Gretzer Ch (2001): Macrophage interactions with modified materials surfaces. Current Opinion in Solid State & Materials Science 5: 163-176.