Heavy Metals Acting as Endocrine Disrupters

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Abstract
Last years researches focused on several natural and synthetic compounds that may interfere with the major functions of the endocrine system and were termed endocrine disrupters. Endocrine disrupters are defined as chemical substances with either agonist or antagonist endocrine effects in human and animals. These effects may be achieved by interferences with the biosynthesis or activity of several endogenous hormones. Recently, it was demonstrated that heavy metals such as cadmium (Cd), arsen (As), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) may exhibit endocrine-disrupting activity in animal experiments. Emerging evidence of the intimate mechanisms of action of these heavy metals is accumulating. It was revealed, for example, that the Zn atom from the Zn fingers of the estrogen receptor can be replaced by several heavy metal molecules such as copper, cobalt, Ni and Cd. By replacing the Zn atom with Ni or copper, binding of the estrogen receptor to the DNA hormone responsive elements in the cell nucleus is prevented. In both males and females, low-level exposure to Cd interferes with the biological effects of steroid hormones in reproductive organs. Arsen has the property to bind to the glucocorticoid receptor thus disturbing glucocorticoids biological effects. With regard to Hg, this may induce alterations in male and female fertility, may affect the function of the hypothalamo-pituitary-thyroid axis or the hypothalamo-pituitary-adrenal axis, and disrupt biosynthesis of steroid hormones.

Keywords: arsen, cadmium, endocrine disrupter, heavy metal, mercury, nickel.

1. Introduction

Endocrine disrupters are defined as chemical substances with either agonist or antagonist endocrine effects in human and animals [1]. These effects may be achieved by interferences with the biosynthesis or activity of several endogenous hormones. Recently, it was demonstrated that heavy metals such as cadmium (Cd), arsen (As), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) may exhibit endocrine-disrupting activity in animal experiments. Hence, a new class of hormone-active substances was characterized, that of endocrine-disrupting metals.

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2. Arsen (As)

Arsen is widely distributed in the natural environment, both as pure element and within complex compounds. Arsen represents the twentieth most prevalent metal in the earth crust, with a soil concentration of 1-2 ppm. This heavy metal is part of a large number of pesticides widely used in intensive agriculture. The digestive tract and the skin represent the main ways of contamination with As, which is excreted through urine, sweat and milk, in both animals and human. Milk excretion significantly contributes to contamination of youth in animal husbandry. Likewise, breastfeeding may represent a significant way of contamination with As in human. Concerning environmental pollution, it was reported that As can accumulate in groundwater and well water from natural sources. It has a well-known mutagenic role and, hence, it

is associated with increased disease risks including bladder, lung, skin and other cancers. Moreover, pilot data suggest diabetogenic, neurological and reproductive effects, likewise. Increased health risks may occur at levels as low as 10-50 ppb, while biological effects have been observed in experimental animal and cell culture systems at much lower levels.

Arsen is the first metal to be linked with endocrine disruption through binding to the glucocorticoid receptor and interfering with glucocorticoid hormones activity in several biological processes. In fact, instead of activating the glucocorticoid receptor, as for example organochlorine pesticides do after binding to the estrogen receptor, As rather inhibits glucocorticoid receptor-mediated gene activation. In a similar manner, this metal interferes with the estrogen receptor. Experiments have shown that As markedly suppressed estrogen receptor-dependent gene transcription of the 17ß-estradiol-inducible vitellogenin gene in chicken embryo liver [2]. In cell cultures, non-cytotoxic concentrations of As of 2-225 ppb significantly inhibited estradiol receptor-regulated effects in human breast cancer MCF-7 cells [2].

3. Cadmium (Cd)

The main sources of contamination with Cd are represented by industrial aerosols, water wastes from extraction mines, phosphate-based fertilizers, Cd-containing pesticides etc. It has been documented that in the industrial area, Cd concentration in the air peaks to 0.03 mg Cd/ m^3. Fodder plants are the main constituents through which Cd enters the food chain. It was shown that Cd levels in fodder may attain on average 0.6 ppm. Moreover, through the process of bioaccumulation, it reaches even higher concentrations in animal products and subproducts. Interestingly, the accumulation process is age-dependent, due to the long half-time of the metals, with up to 20 years needed for Cd to be completely metabolized in human. Significantly higher Cd concentrations were demonstrated in males compared to females, in various tissues and organs such as liver, kidney, muscle, blood, hair and wool. A particular high affinity is described for the kidney cortex and the testes. Milk levels of Cd are about 2-10 mcg/l, however, they increase about tenfold in cases of abnormal exposure. The metal is excreted in feces and urine but also milk and bile. The free fraction is rapidly metabolized, whereas the protein-bound fraction accumulates for years as a metaloprotein (metallothionein). Metallothioneins are cysteine-rich, low-molecular weight proteins, that have the capacity to bind various heavy metals (selenium, zinc, copper, cadmium, mercury, arsen, nickel, silver) through the thiol group of their cysteine residue. However, it appears that the formation of metallothioneins has no impact on heavy metals toxicity. International guidelines restrict Cd concentration to 1 mg/l water. By Romanian guidelines, these levels are 0,01 ppm for milk, salted fish and oils; 0.05 ppm for cheese, meat and meat products; 0.03 ppm for vegetables. Despite its presence in very low concentrations in water, Cd may accumulate in the phytoplancton and enter the aquatic trophic chain, thus bioaccumulating in molluscs, crustaceans and fish.

Several data indicate that even low-level exposure to Cd interferes with the activity of steroid hormones in both male and female reproductive organs. Cd disrupts steroidogenesis by interfering with the biosynthesis of androgens, estrogens and progesterone in vivo and in vitro experiments, thus, leading to disturbed sex differentiation and altered gametogenesis. On the other hand, it may bind both the estrogen and androgen receptor. In utero animal exposure to Cd induces histological changes in the breast linked with breast cancer [3, 4]. Recently, a Lithuanian study brought evidence of significantly higher Cd levels in breast tissue and biological media from women with breast cancer compared to controls, thus, suggesting that exposure to Cd could be interpreted as a potential risk factor for breast cancer [5].

4. Mercury (Hg)

Organic mercury fungicides are widely used in agriculture. In addition, mercury is used as a catalyser in industrial processes. In the form of aerosols it is largely spread in the environment. Mercury polluted water forms sediments further metabolized by microorganisms into methyl-mercury, which is a stable but highly neurotoxic and teratogenic compound. Some organic mercury derivates accumulate in plants by absorption from soil and water through the plants roots. For example, use of organic mercury fungicides in agriculture resulted in contamination of potatoes tubercles reaching a concentration of 0.03-0.2 ppm mercury. A direct correlation was found between the Hg content in fodder and the

concentration of the heavy metal in tissues originating from foddered animals. Animals may be contaminated by water and fodder supply, through the skin, by inhalation of vapors and aerosols, or transplacental. Mercury compounds are transported by blood and the lymph and diffuse in practically all tissues but are preferentially stored in the liver, kidney, spleen, skeleton, lymph nodes, brain and the muscles. In patients with chronic intoxication, mercury exerts neurotoxic, teratogenic, mutagenic and endocrine-disrupting effects. It is metabolized and excreted through milk, feces, kidneys and saliva. Completely, mercury is eliminated after minimum 90 days post-exposure. Previous reports indicated that both organic and inorganic mercury compounds highly accumulate not only in the liver and the kidneys but also in major endocrine glands, for example, the hypothalamus, the pituitary gland, the thyroid, the testes, the ovaries and the adrenal cortex. Mercury-based compounds disrupt steroidogenesis, including sex hormones synthesis, male and female fertility as well as the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-adrenal axis [6]. Most data available indicate the fact that mercury may act as a major endocrine disrupter [7, 8].

5. Nickel (Ni)

The heavy metal nickel originates from natural and artificial sources and can be found in practically all environmental compartments: air, water, soil and living organisms. In the air it is distributed in the form of aerosols that contain various nickel concentrations, depending on the primary source of metal contamination. Transportation and distribution of Ni particles between different compartments of the environment is strongly dependent on the particles size and the climatic conditions. Generally, the particles originating from artificial resources are smaller compared to those derived from natural sources. Water contamination results by sedimentation of metal particles form the atmosphere, of residual industrial waste and city waste as well as by erosion of the soil and natural rocks. In running water, Ni is mainly transported in precipitated form; in lakes, it is found in ionic form, predominantly in association with organic matter. Part of Ni compounds may reach by the way of running surface waters the planetary ocean. The amount of these compounds plunged yearly into water is estimated around 135 x 10^7 kg. According to the type of the soil it contaminates, Ni shows diverse mobility, eventually reaching groundwater. Acid rain shows great ability to mobilize Ni from soil. The amount of metal that is absorbed by particles from soil depends on the physical and chemical properties of the soil, including the type of the soil, the pH, the soil humidity and the organic matter content of the soil. Terrestrial plants absorb the metal from soil, mainly by their roots and Ni levels above 50 mg/kg dried substance are toxic for most plants.

High Ni levels were reported for aquatic plants. In unpolluted waters, Ni levels in fish may vary between 0.02-2 mg/kg. Nevertheless, these values may increase about tenfold in fish from contaminated water. Nickel concentrations commonly found in the air vary between 5-35 ng/m^3, with a 0.1-0.7 μg daily human contamination. Drinking water contains less than 10 μg/l Ni, whereas its concentration in fresh food is less than 0.5 mg/kg product. Soy, some dried vegetables, nuts and oat may contain high quantities of the metal. The daily Ni intake from food largely varies, in dependence of food habits, between 100-800 μg/day with a medium intake of 100-300 μg/day. Pulmonary contamination is about 2-23 μg/day. Nickel may be asorbed by respiration, ingestion or transdermal. The most frequent way of contamination is the respiratory way, whereas gastro-intestinal contamination is of secondary relevance. Transdermal absorption is neglijible. The absorption rate is related to the compound solubility; as carbonyl, Ni is most rapidly and completely absorbed in animals and human. It circulates bound to albumin. The digestive absorption rate is highly variable and dependent on diet composition. In human, the absorption percent from water is about 27% in contrast to the absorption percent from food which is less than 1%. All body secretions represent potential pathways for Ni excretion, including urine, and bila, sweat, tears and milk. Unabsorbed Ni is excreted through the feces. Nickel chloride contamination by ingestion or inhalation decreased iodine uptake by the thyroid gland. Given orally (0.5-5.0 mg/kg per day, for 2-4 weeks) or by inhalation (0.05-0.5 mg/m^3) to rats, nickel chloride significantly decreases iodine uptake by the thyroid, the effect being more pronounced with inhaled Ni.

In mice, parenteral administration of nickel chloride and subsulfide results in intrauterine death of the fetus and severe intrauterine growth restriction in neonates. Respiratory exposure to Ni carbonyl results in fetal death, intrauterine growth restriction and congenital anomalies in rats and hamsters. Nickel carbonyl is associated to dominant letal mutations in rats. In early studies, it was reported that nickel chloride or nickel suphate given parenterally to dogs and rabbits, induce insulin resistance and hyperinsulinemia. Latter on, the authors observed that i.v. or i.p. injection in rabbits, rats or chicken or p.o. administration in rabbits resulted in an rapid peak of plasma glucose levels which returned to normal after about 4 hours. Histological examination of pancreatic cells reported distruction of α cells and degranulation and vacuolization of ß Langerhans cells. In rats fed with nickel acetate, marked lysis of exocrine pancreatic cells and inhibition of amylase release [9] was noticed. In hypophysectomized or adrenalectomized rats, the hyperglycaemic effect was markedly reduced but not completely abolished. Indeed, pretreatment with insulin will reduce nickel-induced hyperglycaemia. It appears that Ni combinations may inhibit the release of prolactin, most probably due to hypothalamic dopamine agonistic effects. Other endocrine effects related to Ni contamination were inhibition of growth hormone secretion [9], although stimulatory effects on growth hormone release from the pituitary were also demonstrated.

6. Lead (Pb)

Lead is largely found in nature, in rocks, under several combinations. The terrestrial crust contains on average 1-2 ppm lead. However, due to important pollution, lead is largely spreaded in the environment in air, water, soil, flora and fauna. Hayfields and animal fodder cultured in the vicinity of industrial buildings using Pb in the fabrication process are at high risk to be contaminated with Pb resulted from vapor precipitation or the sedimentation of Pb particles. Aerosols may be dispersed on a distance of 2-3 km (0,1 mg/m^3) and even 10 km (0.01 mg/m^3) around the contamination source. Spontaneous vegetation and fodder fields around intensively trafficked autorays contain 50-60-fold higher concentrations of Pb.

Contamination of the human or animal with Pb is made by ingestion, respiration or transdermal. It has been shown that digestive absorption is highly species specific, as from the intake about 90% is absorbed in bovine, 1-2% in ovine and 0.8-1% in rabbits. In human, the absorption percent is about 50%. In bovines, ingestion of contaminated fodder leads to a 20-fold increase of Pb levels in the liver and a 3-4-fold increase of Pb levels in milk and meat. If fodder contains 1-6 mg/kg Pb, then the milk content persists at 0.05-0.15 mg/kg for about 120 days.

Lead toxicity consists of inhibition of cellular enzymes, binding of sulfhydril groups or dissociation of biological active metal ions from metaloenzymes. Additionally, it affects the membrane stability of erythrocytes, induces functional disturbances in peripheral nerves and disturbances in the development of the skeleton. Lead is a powerful disruptor of adrenal and ovarian steroidogenesis, inhibiting synthesis and activity of progesterone, 17-hydroxyprogesterone, 17,20-dihydroxyprogesterone, deoxycorticosterone, corticosterone and 21-deoxycortisol in a dose-dependent manner. Interestingly, its effects on 17β-estradiol, testosterone and cortisol are biphasic, with stimulatory effects after low-levels exposure and inhibitory effects after high-leve exposure [10]. Lead exposure results in disturbed fertility in females, as revealed by an in vitro study that examined the consequences of Pb exposure on cytochrome P-450 aromatase (P-450 ARO) and ß-estradiol receptors, two key proteins in the function of the pituitary-ovarian axis. It was shown that the activity of both P-450 ARO and ER-β in the granulosa cells of the ovarian follicles was strongly inhibited in women exposed to Pb [11]. In summary, Pb contamination may alter endocrine-regulated processes such as longevity, development, sexual receptivity, fertility and locomotion. For example, in Drosophila, Pb interferes with the expression of about 122 genes involved in locomotion. In the larvar state, these genes regulate the level of intracellular calcium associated with neuronal activity at the neuromuscular jonction sinapses [12].

7. Zinc (Zn)

Zinc is found in several combinations both in the earth crust and vegetal and animal cells. In fact, Zn is one of the most important natural, biologically active constituents, indispensable to life itself. In all tissues, Zn levels are 2-fold higher compared to iron levels. It was revealed that the

Zn atom from the Zn fingers of the estrogen receptor can be replaced by several heavy metal molecules such as copper, cobalt, Ni and Cd. By replacing the Zn atom with Ni or copper, binding of the estrogen receptor to the DNA-hormone-responsive elements in the cell nucleus is prevented.

Zinc contamination results from industrial smoke, with the most relevant compounds represented by Zn chloride, Zn chromate, Zn phosphur, Zn sulphate and Zn oxide. Contamination is also possible by use of zincate containers to heat milk and foods. Moreover, Zn is an important substance used in the fabrication process of several pesticides. It may enter the body either by enteral or respiratory way. It is easily absorbed in all tissues and rapidly diffuses. Zinc is excreted by feces, the bila etc.; it is co-secreted with insulin by the pancreas. In the presence of As, Zn toxic effects are 3-4-fold increased.

The maximum admitted limit of Zn in our country is of 25 ppm in vegetables, 40 ppm in dry beans, 50 ppm in meat, meat subproducts and conserves, 30 ppm in eggs, 5 ppm in milk, beverage, oil and tomato juice. Besides direct toxic effects, Zn salts act as endocrine disrupters. In a recent study, copper, Cd, Pb, Hg and Zn at 95.4 pM-1 mM, alone or in combination with the natural estrogen, 17ß-estradiol, have been tested using the yeast estrogen screen, an estrogen receptor-dependent transcriptional expression assay. No direct trans-activation of the estrogen-responsive element could be measured with any of the concentration of the metals tested. However, Zn, Cd and copper were able to potentiate the estradiol-induced response in a dose-dependent manner, thus indicating that Zn can act as a potential endocrine disrupter by modulating the estrogenic activity of endogenous hormones (xenoestrogen) [13].

Conclusions

Besides several man-made chemicals, most of them major components of widely used pesticides, recent studies strongly suggest that some heavy metals may exert endocrine-disrupting activities in animals and human. Of these metals, Zn, Pb and Hg and As interfere with sex hormones and adrenal cortex hormones steroidogenesis to alter reproduction and sex differentiation, Cd is involved as a risk factor for breast cancer and Ni appears to induce pancreatic cell lesions, possibly increasing the risk of diabetes mellitus. Nevertheless, further studies are needed to establish a dose-effect relationship. Future strategies in agriculture, animal husbandry and environmental health should aim limitation of the endocrine disruption phenomenon induced by heavy metals.

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