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Epigenetic Modifications Due to Heavy Metals Exposure in Children Living in Polluted Areas

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Abstract: The aim of the present article is to provide a summary of the epigenetic modifications that might occur in children exposed to heavy metals pollutants. It is known that children are more susceptible to environmental pollutants, because their detoxification enzymes are less competent, and this may lead to alterations in chromatin structure or of DNA causing, in turn, epigenetic modifications. Little is currently known about the long-term effects of these changes when occur early in childhood, nonetheless there are ethics and practical concerns that make the assessment of DNA modifications difficult to perform in large-scale.

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HEAVY METALS AS RISK FACTORS FOR CHRONIC DISEASES

Persistent organic polluttants (POPs) are a class of toxic compounds that negatively affect human health and the environment of the planet. POPs can be transported by wind and water, thus even if generated in one country POPs can reach people and wildlife far away. POPs persist for a long time in the environment where they can accumulate and be integrated into the local flora and fauna getting eventually into the food chain.

Among the most known POPs there are chemicals produced for being used in agriculture, disease control, manufacturing, or industrial processes - like PCBs and DDT. This latter is nowadays used only in some countries to reduce mosquitoes that transmit malaria. Another group of POPs includes those chemicals that are not deliberately produced, such as dioxins and heavy metals, which, are in some cases the result of industrial processes and combustion of waste products.

Unlike POPs, heavy metals are commonly found in either polluted and unpolluted environments, water, and soils, because most of them naturally occur all around the globe. Although heavy metals are commonly found in the Earth's crust, they are found in elevated amounts in agricultural soil because of outrageous use of commercial fertilizers, and of contamination caused by manufacturing plants. Monitoring of heavy metal concentrations in the environment and biological matrices is therefore extremely important [1, 2]. Some heavy metals have been recognized to be dangerous to health such as Mercury (Hg), Cadmium (Cd), Arsenic (As), Lead (Pb), Chromium (Cr); some may cause corrosion such as Zinc (Zn) and Lead (Pb). In particular, Cd is present in exceptionally high levels in cigarette smoke, because tobacco leaves accumulate high levels of cadmium from the soil, so that smokers have twice as much Cd in their blood than non-smokers [3]. This is relevant to the human health as cadmium is ranked as carcinogen [4], and furthermore smoke interferes either with epithelial wound healing [5] and the antioxidant/oxidant balance [6, 7]. Both these effects have been shown to be directly or indirectly affected by Cd exposure [8, 9].

As outlined by the European community, the chemical elements of highest concern for human health are As, Cd, Co, Cr, Cu, Hg, Mn, Ni, and Pb [10]. Several of these elements are essential nutrients of human's diet, as Co, Cu, Cr, and Ni. Other chemicals are instead carcinogenic or toxic, causing affections in the central nervous system (Hg, Pb, As), as well as in the kidney, the liver (Hg, Pb, Cd, Cu) or eventually skin, bones, or teeth (Ni, Cd, Cu, Cr). Some heavy metals have essential roles for human health, for instance copper is of fundamental importance in maintaining the activity of several enzymes including ferro-oxidase (ceruloplasmin), cytochrome c oxidase, superoxide dismutase and others. It is also known that Cu has a role in the metabolic processing of iron, in melanin synthesis, and in the central nervous system homeostasis. Iron is an essential component of a large number of enzymes and proteins, in particular hemoglobin, which is known to be vital for the transport of oxygen to tissues all over the body. Chromium widely occurs in two main oxidation states, +3 (III) and +6 (VI), Cr III is the most stable, and most likely is the form in the food supply because of the presence of food preservatives that act as reducing agents. Regardless of a positive effect of Cr in enhancing the action of insulin in patients with impaired glucose tolerance, the Cr VI, derived as a by-product of manufacturing stainless steel, chromate chemicals, pigments, and various other products, has a strongly

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oxidizing action, producing irritation by direct contact, and is carcinogenic when inhaled. Selenium protects protein from oxidant molecules as is a component of the enzyme glutathione peroxidase, acting on cell membranes, lipids, and nucleic acids. Zinc biological functions are linked to its presence as a component of a variety of enzymes, and in the preservation of the structural integrity of proteins, other enzymes, and in the regulation of gene expression.

Despite these positive effects, heavy metals might act as potent toxic substances and cause permanent damage to organs and at molecular level [11]. Heavy metal pollution can occur from numerous sources but most frequently arises from the refinement of metals, e.g., the smelting of copper and the preparation of nuclear fuels, while electroplating is the main source of Cr and Cd. Heavy metal pollutants can concentrate and lay inactive into soils and mud through precipitation of their compounds or by ion exchange. Unlike organic pollutants, heavy metals do not decompose and as a result cause a different sort of challenge for remediation. Nowadays, plants or even microorganisms are tentatively used to remove some heavy metals such as Hg. As a matter of fact, plants are able to accumulate metals over and above their needs, by concentration in their bio matter, and thus can be used to remove these toxicants from soils. Several intervention of mining tailings has taken place where the vegetation is then incinerated to recuperate the heavy metals. However, other environmental factors, like current or previous volcanic activity might also be responsible for the high amounts of heavy metals found in certain areas. In medical terms heavy metals are loosely distinct and take account of all toxic metals irrespective of their atomic weight. Indeed, the so called "heavy metal poisoning" can eventually include unnecessary amounts of aluminium, zinc, iron, mercury, manganese, or beryllium (which is a light metal) or of a semimetal as arsenic. Such description is made only on the assumption that increased levels of “metallic elements” in the human body can have unwanted toxic effects.

Historically heavy metals have been used also as health remedies, As was effective against protozoa, helminthes, amoeba, syphilis, and spirochetes; Cu was an emetic, and gold used in rheumatoid arthritis. The current uses include aluminium as anti-acid, iron for anaemia, Zn and Se as food supplement and for enrichment of functional foods. As a matter of fact the US Food and Drug Administration approved the use of arsenic trioxide (as an orphan drug) for the secondary treatment of acute promyelocytic leukaemia.

In 2011 the World Health Organization (WHO), filed a list of the 10 most toxic chemicals, which includes arsenic, cadmium, lead, and mercury [10]. The scientific literature concerning the risk for epigenetic modifications induced by heavy metals exposure implicated in the pathophysiology of cancer and endocrine/metabolic diseases, is mostly based on data obtained in adult subjects, our review will focus on the existing data in children living in heavy metals contaminated areas.

EPIGENETIC AND HEAVY METALS-RELATED EFFECTS

Despite the presence of single nucleotide polymorphisms (SNPs), to explain inter-individual differences, as well as insight into illness vulnerability and resistance, the activity of our genome is also relative to “beyond the genome” mechanisms which might include alterations of chromatin structure, concerning covalent alteration of the DNA itself, as well as the macromolecules that form chromatin. These modifications undergo to the term epigenetics and are studied to unveil gene-environment interactions, as these epigenetic mechanisms produce an additional level of transcriptional control that directs gene expression [12-15].

Epigenetics broadly refers to heritable alterations in gene function which do not involve variations in DNA sequence. Imprinted genes are vulnerable to genetic and epigenetic perturbation and have been tied to adverse health outcomes. As imprinted genes are monoallelically expressed with one of the copies of the gene silenced in a parent-of-origin dependent manner, only one copy is functional. As a result, mutations or epigenetic alterations on one allele that would normally have minimal impact for a biallelically expressed gene may lead to detrimental consequences for an imprinted gene. As it is a critical part of the epigenome, the inheritance and manifestation of traits associated with imprinted genes is regulated through epigenetic marks. Many imprinted genes are grouped in clusters and possess imprinting control regions (ICRs) or a central control region. These ICRs, as well as other regulatory regions associated with imprinted genes, are referred to as differentially methylated regions (DMRs) and display ~50% methylation, where one of the parental alleles is methylated and the other unmethylated in a manner based on parent of origin. These DMRs represent discrete DNA elements that hold a heritable epigenetic spot that is useful to differentiate the parental alleles.

Several evidences have linked epigenetic changes in imprinted genes to oncogenesis, progression and treatment of cancer [16], regulation of development and function of the nervous system [17], gene regulation, cellular stress events [18], nutrigenomics [19], aging and DNA repair [20]. Numerous studies are also directed towards identifying the dynamic functions of various modifications to DNA and proteins associated to genome. Alterations at epigenetic level can be also useful for identify specific markers to be used for cancer detection, diagnosis and prognosis. For this reason, we have to focus our attention on the chance that these toxic substances might join in food chain, increasing pollution and the possible exposure. For example, cadmium which is ubiquitous in the environment can be toxic to humans following exposure, that occurs mainly via a contaminated food chain or via tobacco smoking [21].

Nutritional epigenetics has also become known as a novel science that studies the underlying gene-diet interactions. Some nutrients are involved in DNA methylation, such as vitamin B12, vitamin B6, riboflavin, methionine, choline, and betaine. Several other nutrients and bioactive substances (for example retinoic acid, resveratrol, curcumin, sulforaphane, and tea polyphenols) can alter epigenetic patterns acting on S-adenosylmethionine, S-adenosylhomocysteine, and on the enzymes that are known to catalyze DNA methylation and histone modifications [19].

A variety of mechanisms have been studied, including the most widely documented, DNA methylation, as well as alterations in the organization of chromatin within the nucleus, post-translational histone modifications, and regula-

tion by non-coding small RNAs. Chromatin remodelling, for example, alters the accessibility of gene promoters and regulatory regions, thus influencing gene expression. While the permanence of these epigenetic "marks" is still in question, it is clear that most of these changes are quite stable, long-lasting, and likely have trans-generational effects [22].

Methylation of DNA is caused by the addition of a methyl (CH3) group at the carbon 5 position in the sequence 5'CpG3', to the cytosine ring, activated by the enzyme DNA methyltransferase. In case of hypermethylation in the promoter region, a reduction of gene expression is observed, in fact this event disallowes the bond of the transcriptional or repressor factors to their recognition sites [23]. These methyl groups interfere and inhibit transcription within the major groove of DNA. It has been estimated that in human DNA, approximately 1.5% of genomic DNA hold 5-methylcytosine [24], suggesting that many genes might be currently deactivated. In somatic cells, the presence of 5-mC is almost exclusively linked to paired symmetrical methylation at CpG site, where a cytosine is located right after a guanidine. Embryonic stem (ES) cells represent an exception to this, in fact in this case a substantial amount of 5-mC is also present in non-CpG sites. In the vastness of genomic DNA most CpG sites are extremely methylated, while the so called CpG islands (defined as sites of CpG clusters), remain unmethylated, especially when located in reproductive tissues and near the promoters of somatic cells, thus allowing gene transcription. When a CpG island located in the promoter region is methylated, the gene is completely repressed. The importance of methylation in turning on and off entire genes, make this process strictly controlled at several different levels. Methylation occurs by the activity of a family of enzymes called DNA methyltransferases (DNMTs). Three DNMTs (DNMT1, DNMT3a and DNMT3b) are necessary for the establishment and preservation of DNA methylation patterns. Two other enzymes (DNMT2 and DNMT3L) may also take part to the process. In particular, DNMT1 seems to be in charge for the maintenance of DNA methylation patterns previously present in the cells, while DNMT3a and 3b apparently mediate the establishment of novel DNA methylation patterns. Cancer cells, as well as those subjected to persistent stress (as in chronic diseases), may show a different DNMTs activity. In fact, DNMT1 alone is not responsible for preserving normal gene hypermethylation, and may cooperate with DNMT 3b for this function.

All these mechanisms responsible for DNA methylation, the consequent gene expression, the switching of genes during development, and that methylation could persist through cell divisions, being heritable, was first outlined in 1975 [23, 25]. Current epigenetics not only provides novel insights into gene regulation and heritage, but it radically changes the classic dogmas about evolution, genetics, and development. Most interestingly, it also suggests that environmental factors can modify genetic expression and cellular phenotypes, possibly occurring throughout a lifetime, beginning from intra-uterine life. Besides methylation of cytosine in CpG dinucleotides, most studied epigenetic modifications include post-translational modification of histones, above all changes in phosphorylation, acetylation, and ubiquitinylation status.

Adverse environmental exposures occurring in vulnerable periods, such as during the fetal period, development, and in early childhood alter the epigenome could be responsible for increased health risk in later life. Sources of exposure include air contaminants, persistent toxic molecules, and other pollutants found even in drinking water. Very few reports addressing the possible effects of chronic low environmental exposure to mixtures of heavy metals in the general population of industrialized countries, are available. Indeed, those considering the possible interactions with the epigenome are even less. Furthermore, there is a specific lack of data concerning children and adolescents, while this is actually a specific cause for concern. In fact, children are known to more readily absorb metals compared to adults because of their body composition and of defective detoxification systems, in addition they are particularly susceptible for biologic and developmental reasons [26]. Indeed, concerns have been raised that children may be more vulnerable to toxic exposure than adults as they have proportionally an increased intake of food additives, an active growth process, and multiple exposure pathways [27].

Most of our understanding concerning the health effects of toxic metals largely stems from studies performed in cell lines, animals, or populations with relatively high exposure. Persistent oxidative stress, mitochondrial dysfunction, elevated cytokine levels and epigenetic changes are among the mechanisms invoked to explain the toxic effects related to heavy metals.

However, recently published data demonstrates that the early exposure to cadmium is inversely associated with birth weight both in both newborns and 4.5-year old children [28]; it seems that the effects of Cd are related to the methylation of several CpG islands, and the effect was reported as sex-specific. In fact, the changes in methylation profile was described as positively related with development and bone mineralization in girls, while in boys the effects are responsible for alterations in cell death-related gene. Furthermore, cadmium exposure during pregnancy has been associated with future disease risk [29]. The mechanisms of toxicity of Cd during fetal growth is not yet understood, but probably the zinc transfer to the fetus [30], the interference with glucocorticoid balance [31], and the alteration of insulin-like growth factor (IGF) axis [32] are involved: all these factors may impair fetal growth in several ways. However, the crucial event is still DNA methylation, which confers long-term epigenetic silencing of particular sequences — transposons, imprinted genes and pluripotency-associated genes — in somatic cells [33]. The relationship between Cd exposure and involvement of fetus has been studied appreciating the level of the metal in the cord blood of the mothers exposed. Cadmium concentration in maternal blood was more strongly associated with cord blood DNA methylation, as compared to urine concentration. In addition, newborn boys showed hypermethylated repetitive sequences, while female newborn were mostly hypomethylated, indicating that low-level environmental Cd exposure during early pregnancy is associated with sex-specific alterations [34].

Epigenetic modifications due to environmental factors can also play an important role in neurological disorders. In recent years it has been observed an increase in these disor-

ders, in particular hyperactivity disorders and pervasive development disorder. Among environmental factors responsible of these alterations in nervous system, first of all it has been included the intoxication of the fetus by heavy metals (lead and mercury). Other substances of wide use, such as pesticides, polychlorinated biphenyls and now the recycling of electronic waste expose at risk infants and children, above all in the developing countries [35].

Moreover, neurodevelopmental disorder such as autism, attention deficit disorders, and mental retards were linked with early exposure to industrial pollutants such as lead and arsenic [36]; although the mechanisms through which these metals exert his action are not yet well understood, it is known that these pollutants impair the development processes also in context of relatively low exposure.

Lead and arsenic were proposed as responsible for microalbuminuria in a population of schoolchildren (12-19 years old) living in Hong Kong; the effects of these two metal on kidney function are probably mediated by alteration in miR-21 expression, which, in turn, acts as a protein expression regulator [37].

It was also demonstrated that a pre-natal lead exposure can cause a decreased long interspersed nuclear element-1 (LINE-1) methylation status which is a known biomarker for abnormal global DNA methylation. The effects of such kind of alteration in epigenomic-driven fetus development must be yet assessed, but this finding suggests that also the maternal cumulative lead burden could be responsible for alterations in fetus development process [38].

ISSUES RELATED TO THE DETECTION OF EPIGENETIC CHANGES IN CHILDREN

DNA hypermethylation is a commonly detected epigenetic modification, as afore mentioned, the addition of methyl groups to specific cytosines of the DNA, controls gene activity. In particular, the CpG islands, where methylation occurs in the CpG dinucleotides, are mostly present in the promoter regions and the introns of human genes [39], causing in turn gene silencing. Sampling, sample preparation and marker selection are important factors for achieving good results in epigenetic testing. Sampling biological matrices from children requires parents approval, and it is known that obtaining samples through invasive methods may have ethical and practical limitations. The potential use of biological matrices, as urine, serum, and plasma is limited by the inadequate level of methylated DNA found from total DNA extraction when collected from blood samples. A further drawback is represented by the technique used that is responsible for partial degradation of the methylated DNA due to bisulphite treatment, a treatment step required by many validated protocols. Thus, large whole blood samples are required for detecting epigenetic modifications in DNA, easily recovered in adequate amounts, from circulating blood cells. These issues, together with ethics considerations limit the identification of epigenetic modifications in children, especially those living in areas not included in biomonitoring campaigns.

FUTURE DIRECTIONS

As a matter of fact, a transgenerational epigenetic inheritance has been proposed to occur, at least in plants and insects [40-42], for many DNA changes related to the presence of heavy metals, thus implying the importance of monitoring these changes in the population to avoid health issues. As reviewed by us and other authors, the epigenetic changes induced by numerous environmental stressors, might accumulate over time. Independently of how marked are the changes caused to DNA, if these modifications are following acute or chronic exposure, if occur during development or after birth, the identification and mapping of these alterations may be useful in the next future for assessing health risks and the possible consequences for the offspring of exposed subjects [43].

CONCLUSION

The epigenetic mechanisms of transcriptional regulation are responsible for developmental and homeostasis processes, although the role played by these signaling network is not yet finely understood.

As environmental and industrial pollutants, the heavy metals negatively affect the healthy status of adult population as well as those of the children; in fact, several metals are known to be at least in part responsible for pathological conditions such as tumours, respiratory diseases, neurological diseases, metabolic and endocrinological disruption, and for the impairment of the development process also.

The mechanisms by which they exert these effects are not ever clarified; they may act affecting the functionality of several proteins, causing in turn an alteration of multiple cellular and organ functions, or determining an alteration in gene expression itself.

Considering the key role played by the epigenetic regulators, such as histone-modifiyng proteins, micro-RNAs, and CpG methylation, understanding the interaction between chemical pollutants and these mechanism of regulation is of primary importance nowadays. Several researches exploited this field of the environmental toxicology, contributing to a better understanding of the influence of heavy metals in the epigenetic mechanisms of transcriptional regulation. Nonetheless, there is a lack of data about susceptible population such as children, pregnant women, and elderly population; thus more studies are needed to better define the impact of these heavy metals in specific populations.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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