BIOCELL
2015, 39(3): 15-18

ISSN 1667-5746
ELECTRONIC

Adverse effects induced by chromium VI, cadmium and arsenic exposure on hypothalamus-pituitary physiology

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Key words: metalloid, heavy metals, cell death, oxidative stress, hormone release

ABSTRACT: Environmental contamination with some metalloids and heavy metals (M/HM) raises concern due to well known adverse effects on health. Among these pollutants, chromium VI (Cr VI), cadmium (Cd) and arsenic (As) are frequently present as a result of natural sources or due to industrial activities. They are able to easily enter the organism and negatively affect many organs and systems. *In vivo* (exposure to Cr VI, Cd or As through drinking water) and *in vitro* experiments (primary pituitary cell cultures) were performed in male Wistar rats to address their actions on hypothalamus-pituitary axis. All the M/HM accumulated in hypothalamus and pituitary gland and decreased pituitary cell viability and prolactin release mostly by generation of reactive oxygen species, since it was partially prevented by antioxidant treatment. In the pituitary, they increased lipid peroxydation and the expression of several oxidative stress markers. Cell death was mainly due to caspase-dependent apoptosis. Lactotrophs (prolactin-secreting cells) were the most affected pituitary population. Cd-driven cell death was also partially calcium- and calpain-dependent. Parallely, Cd stimulated the production of low levels of nitric oxide which exerted cytoprotective actions. These results showed that these M/HM display deleterious actions in hypothalamic-pituitary physiology by altering hormone release and promoting cell death.

Exposition due to environmental contamination with man-made chemicals as well as metalloids and heavy metals (M/HM) has increased along the last decades. Many of these pollutants exert well known adverse effects on health in humans and wildlife. Chromium VI (Cr VI), cadmium (Cd), and arsenic (As) are frequently present as a result of natural sources or due to industrial activities. It has been widely reported their toxic actions on many organs and systems but little was known about their effects on the hypothalamus-pituitary axis.

The importance of hypothalamus-pituitary axis in reproduction and the poor knowledge about the effects of these M/HM on it led us to investigate some of the most relevant contaminants such as Cd, Cr VI and As. Our hypothesis stated that these M/HM affect cell viability and pituitary hormone release by oxidative stress mechanisms. The current report reviews our studies on the effects and mechanisms by which these pollutants affect hypothalamus-pituitary axis in male Wistar rats, both *in vivo* and *in vitro*.

Chromium (Cr)

This is a transition metal that is present in environment as a trace element. Cr III and Cr VI are the most stable species. While Cr III forms stable compounds that are component of rocks and minerals and is an essential nutrient involved in lipid and carbohydrates metabolism, Cr VI is a highly oxidizing, potent toxic whose main source is anthropogenic activity. It enters the organism by ingestion, skin contact and inhalation. In physiological conditions, Cr VI occurs as dichromate ($Cr_2O_7^{2-}$) which is able to trespass cell membranes through phosphates and sulphates transporters. Once inside the cell, Cr VI is reduced through a Fenton-like reaction and consequently, reactive oxygen species (ROS) and Cr

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Received: October 30, 2015. Accepted: April 16, 2016

intermediates up to Cr III are generated (U.S. Environmental Protection Agency, 1998).

In vivo studies performed through administration of Cr VI in drinking water for 30 days showed that the metal accumulated in hypothalamus and pituitary and decreased prolactin (PRL) serum levels (Quinteros *et al.,* 2007). In both tissues, Cr VI increased lipid peroxydation and oxidative stress markers expression, such as metallothionein-1 and -3 (MT-1 and -3) and hemooxygenase-1 (HO-1). Besides, it augmented the activity of some enzymes of the cellular antioxidant system such as superoxide dismutase and glutathione reductase (Nudler *et al.,* 2009).

In vitro studies showed that Cr VI reduced pituitary cell viability and decreased PRL release. Lactotrophs resulted to be the most affected pituitary cell type. Cr VI-induced toxicity involved induction of apoptosis through augmented proapoptotic factors expression as Bax and mitochondrial production of ROS, which in turn led to caspase-3 activation.

This metal affected cellular antioxidant system by decreasing the activity of glutathione peroxidase and catalase activities. Oxidative stress generation constituted a key mechanism in cell death process, since treatment with an antioxidant (N-acetyl-cysteine) prevented Cr VI cytotoxicity (Quinteros *et al.,* 2007; Quinteros *et al.,* 2008).

Cadmium (Cd)

This metal is an environmental toxic naturally found in rocks, but the most important source of contamination comes from antropogenic activities. It is highly bioaccumulated with an average half-life of 20 years. Ingestion and inhalation are important routes of entry being tobacco smoking the main source of intoxication (Agency for Toxic Substances and Disease Registry, 1998). After entering the body, it is rapidly uptaken through divalent metals transporter (DMT-1) coupled to protons and through calcium channels (Sarkar *et al.,* 2013). *In vivo* studies were performed where Cd was given to male rats in drinking water for 30 days. In these conditions, the metal is highly accumulated in pituitary gland and in a lesser extent in hypothalamus. Cd increased lipid peroxydation, HO-1, nitric oxide synthases-1 and -2 and MT-1 expression in anterior pituitary while it did not alter oxidative markers expression in hypothalamus. Besides, it reduced PRL, luteinizing hormone (LH) and thyroid-stimulating hormone serum levels (Poliandri *et al.*, 2006; Miler *et al.,* 2010). Remarkably, melatonin treatment -simultaneously or after Cd exposure- or removal of the contamination source are all able to revert Cd toxic effects (Miler *et al.,* 2010). *In vitro* studies in anterior pituitary primary cell cultures have shown that Cd increased ROS generation, lipid peroxydation and activation of caspases. The antioxidants N-acetyl-cysteine and Trolox (a hydrosoluble derivative of vitamin E), but not ascorbic acid, reversed both Cd-mediated cytotoxicity and the inhibition of PRL release, supporting the involvement of oxidative stress in the mechanism of Cd action (Poliandri *et al.,* 2003). Cd-induced oxidative stress caused apoptosis being lactotrophs the most affected cell type (Poliandri *et*

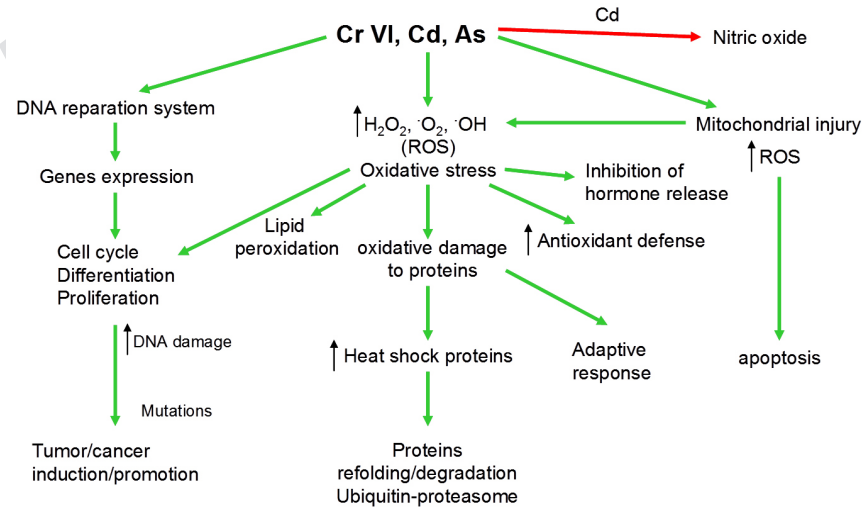

FIGURE 1. An outline of cytotoxic mechanisms of Cr VI, Cd and As. The mechanisms involved in deleterious actions -mainly through oxidative stress generation- are depicted in green arrows. Cytoprotective mechanism (in red arrow) involves nitric oxide synthesis which at low levels contributes to decrease reactive oxygen species concentration at steady state (modified from Bertin and Averbeck, 2006).

al., 2004). Another mechanism by which Cd induces cell death involved the raise in intracellular calcium levels and calpains activation (Poliandri, 2006). On the other hand, Cd augmented the synthesis of nitric oxide (NO) which at low doses exerts cytoprotective effects by inducing MT-1 expression and by decreasing both, ROS production and caspase-3 activity (Poliandri *et al.,* 2004).

Arsenic (As)

This metalloid is ubiquitously present in the environment with potent toxic and genotoxic effects. About one third of the As in the atmosphere comes from natural sources, such as volcanoes, and the rest comes from man-made sources. Once inside the body, As is uptaken by aquaporin-9 and phosphate transporters and it is metabolized to methylated forms thus facilitating its excretion but shielding more toxicity than inorganic As (Agency for Toxic Substances and Disease Registry, 2007).

In vivo studies on male rats exposed to As in drinking water for 30 days showed that Cd accumulated in anterior pituitary and caused a decreased in PRL and LH serum levels. Furthermore, As exposure increased HO-1, MT-1 and thioredoxin-1 expression in anterior pituitary gland, but did not modify their expression in hypothalamus (Ronchetti, 2014).

In vitro studies in anterior pituitary cells in culture confirmed that As is able to induce oxidative stress by increasing ROS levels and oxidative stress markers expression. This metalloid decreased PRL release and induced cell death mainly by apoptosis in anterior pituitary cells. Similarly to what has been observed with other studied metals, lactotrophs are by far the most affected cell type in the pituitary. ROS generation is involved in As-mediated cytotoxicity given that antioxidant treatment prevented the increase of oxidative stress markers expression, PRL decrease and cell death caused by As (Ronchetti *et al.,* 2012; Ronchetti, 2014).

In summmary, Cr VI, Cd and As affect anterior pituitary physiology. They cause oxidative stress which is directly involved in both PRL release inhibition and cell death. An outline of the M/HM actions reviewed here and by other authors (Bertin and Averbeck, 2006; Jomova and Valko, 2011; Kim *et al.,* 2015) is depicted in Fig. 1. The lactotrophs, the most numerous cell type among pituitary population, seems to be particularly sensitive to the deleterious actions of the studied M/HM. Unlike Cd and As, Cr VI induces oxidative stress at hypothalamic level. This differential action might be due to Cr VI higher toxicity. These studies underline the importance of addressing the M/HM effects on pituitary physiology to improve our understanding of the M/HM-induced dysfunction on the endocrine system.

Further experiments must be carried out in order to elucidate the mechanisms of M/HM-induced cytotoxicity and investigate their effects at lower concentrations as endocrine disruptors or xenoestrogens.

Acknowledgement

This study was funded by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT Nº 32311, 08603, 1668 and 2013-1324), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 5536) and Universidad de Buenos Aires (UBA M025).

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