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Selenium and zinc: Two key players against cadmium-induced neuronal toxicity

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ABSTRACT

Cadmium (Cd), a worldwide occupational pollutant, is an extremely toxic heavy metal, capable of damaging several organs, including the brain. Its toxicity has been related to neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. The neurotoxic potential of Cd has been attributed to the changes induced in the brain enzyme network involved in counteracting oxidative stress. On the other hand, it is also known that trace elements, such as zinc (Zn) and selenium (Se), required for optimal brain functions, appears to have beneficial effects on the prevention of Cd intoxication.

Based on this protective effect of Zn and Se, we aimed to investigate whether these elements could protect neuronal cells from Cd-induced excitotoxicity. The experiments, firstly carried out on SH-SY5Y catecholaminergic neuroblastoma cell line, demonstrated that the treatment with 10 μM cadmium chloride ($CdCl_2$) for 24 h caused significant modifications both in terms of oxidative stress and neuronal sprouting, triggered by endoplasmic reticulum (ER) stress. The evaluation of the effectiveness of 50 μM of zinc chloride ($ZnCl_2$) and 100 nM sodium selenite (Na_2SeO_3) treatments showed that both elements were able to attenuate the Cd-dependent neurotoxicity. However, considering that following induction with retinoic acid (RA), the neuroblastoma cell line undergoes differentiation into a cholinergic neurons, our second aim was to verify the zinc and selenium efficacy also in this neuronal phenotype.

Our data clearly demonstrated that, while zinc played a crucial role on neuroprotection against Cd-induced neurotoxicity independently from the cellular phenotype, selenium is ineffective in differentiated cholinergic cells, supporting the notion that the molecular events occurring in differentiated SH-SY5Y cells are critical for the response to specific stimuli.

1. Introduction

Cadmium (Cd) is the seventh most toxic heavy metal as per Agency for Toxic Substances and Disease Registry - ATSDR ranking (ATSDR, 2017) among the environmental pollutants with which humans and animals can potentially come in contact. Given that Cd is widely distributed in natural and industrial sources (Mead, 2010), exposure to cadmium can occur in occupations such as mining, electroplating or in the vicinity of Cd-emitting industries or incinerators where it is produced or used. In fact, Cd levels in ambient range from 0.1 to 5 ng/m^3 in rural areas, 2–15 ng/m^3 in urban areas, and 15–150 ng/m^3 in industrialized areas (ToxGuide™ for Cadmium, 2012). Nevertheless, numerous studies have reported health effects of daily cadmium exposure in the general population also in the absence of specific industrial exposure, the main source of exposure being food and tobacco smoke. It was reported that the average Cd intake from food generally varies between 8 and 25 μg per day (Bérglund et al., 1994; MacIntosh et al., 1996; Thomas et al., 1999; Ysart et al., 2000; Larsen et al., 2002; Olsson et al., 2002; Llobet et al., 2003; Egan et al., 2007), and that normal smokers present twice the levels in their body than non-smokers and

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this values are four times higher in heavy smokers (Järup and Akesson, 2009; ATSDR, 2017). The half-time for Cd in the whole body in humans is > 26 years and in general population the Cd normal human level in the blood (indicative for a recent exposure) is 0.315 μg/L, whereas the urine level (indicative for previous exposure) is 0.185 μg/L (ToxGuide™ for Cadmium, 2012).

Many evidences highlighted the correlation between environmental pollutant (in particular heavy metals) and chronic brain inflammation and neurodegeneration (Calderon-Garciduenas et al., 2002; Calderon-Garciduenas et al., 2003). In particular, Cd is included among the etiopathogenetic factor of some neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and autism spectrum disorder (ASD) (Thatcher et al., 1982; Marlowe et al., 1983; Panayi et al., 2002; Barnham and Bush, 2008).

Cadmium-induced neurotoxicity involves the generation of reactive oxygen species (ROS) and free radicals, disturbances in calcium/zinc-dependent processes, dysregulation of cell repair systems, epigenetic modifications and oestrogen-mimicking effects (Wang et al., 2004; Bertin and Averbeck, 2006; Monroe and Halvorsen, 2009; Kim et al., 2013; Yuan et al., 2016). Indeed, Cd is known to block calcium channels in mitochondria, inducing a membrane potential decrease and the consequent release of cytochrome *c*, eventually leading to the activation of the apoptosis cascade (Fern et al., 1996; Xu et al., 2011; Yuan et al., 2013). Furthermore it has been demonstrated that Cd induces ER stress (Chen et al., 2015), leading to cell death by a non-mitochondrial dependent pathway (Hitomi et al., 2004).

The most commonly used therapeutic strategy for heavy metal poisoning is chelation therapy to promote metal excretion. However chelators are reported to have a number of different safety and efficacy concerns, and none of these therapies have yet been approved for clinical use (Goyer and Clarkson, 2001; McCarty, 2012). Recent studies have shown that essential metals dietary supplements play important roles in protecting against Cd even because they are expected to have very few side effects compared to the chelators (Zhai et al., 2015).

One of the most well studied essential metal is zinc (Zn), possessing similar chemical and physical properties to Cd, competing for the binding sites of metal absorptive and enzymatic proteins (Bridges and Zalups, 2005). Moreover, Zn induces the synthesis of the CNS specific metallothionein III (Suzuki et al., 1990; Aschner et al., 1997; Jin et al., 1998; Hidalgo et al., 2001), a low molecular weight, cysteine-rich protein that has high affinity for Cd and causes detoxification by binding Cd (Nordberg and Nordberg, 2000; Hartwig, 2001). Moreover, Zn intake has been reported to alleviate the oxidative stress caused by Cd and lead exposure (Amara et al., 2008; Prasanthi et al., 2010).

On the other hand, a considerable number of studies have shown that selenium (Se) administration is protective against Cd toxicity within a range of different organs of mice, including the brain (Newairy et al., 2007; Cardoso et al., 2015). Selenium is a cofactor of the antioxidant enzyme glutathione peroxidase (GPx) and it contributes to the antioxidant defence system, reducing the Cd-induced oxidative stress and enhancing the antioxidant capacity of the host (Luchese et al., 2007; Liu et al., 2013).

Therefore, the first aim of this study was to investigate the neuroprotective properties of Zn and Se against Cd-induced neurotoxicity in SH-SY5Y neuroblastoma cell line, a widely used catecholaminergic *in vitro* model for studies on neurotoxicity of compounds affecting the nervous system (Faria et al., 2016; Heusinkveld and Westerink, 2017). However, in addition to the catecholaminergic system (Gupta et al., 1990), Cd has been shown also to affect glutamatergic (Borges et al., 2007; Borisova et al., 2011), monoaminergic (Ali et al., 1990; Gutierrez-Reyes et al., 1998; Abdel Moneim et al., 2014), as well as cholinergic system where it blocks the cholinergic transmission inducing a more pronounced cell death (Del Pino et al., 2014). Furthermore, many studies have evidenced significant degree of interplay between catecholaminergic and cholinergic system in the regulation of CNS activity (Raevskii et al., 1993). Since undifferentiated dopaminergic SH-SY5Y human neuroblastoma cells can be differentiated by retinoic acid (RA) in mature cholinergic neurons (Presgraves et al., 2004; Lopes et al., 2010; Kovalevich and Langford, 2013), the second aim of the present study was to evaluate if the treatments with Zn and Se show different efficacy against Cd-induced neurotoxicity in undifferentiated catecholaminergic cells with respect to the cholinergic neuronal phenotype.

2. Materials and methods

2.1. Cell line and treatments

Human neuroblastoma SH-SY5Y cell line, was purchased by Istituto Zooprofilattico dell'Emilia e della Romagna (Brescia, Italy). Cells were routinely cultured in DMEM High Glucose/Ham's F12 Mixture Medium (1:1) supplemented with 10% foetal bovine serum (FBS), 2 mM L-Glutamine (EuroClone S.p.a., Milano, Italy) at 37 °C, 5% CO_2 in humidified atmosphere. The growth medium was changed every 2–3 days.

In order to reproduce *in vitro* conditions that could mimic a chronic human Cd intoxication, we decide to use a concentration of 10 μM of $CdCl_2$ (Sigma Aldrich, Milano, Italy) and a time of exposure of 24 h as reported by Del Pino (Del Pino et al., 2014) and further confirmed by dose-response curves (Supplementary Fig. S1 – Panel A).

Aimed to evaluate the effect of Zn and Se supplementation at doses corresponding to the human physiological levels, the concentration of 100 nM Na_2SeO_3 and of 50 μM $ZnCl_2$ (Sigma Aldrich, Milano, Italy) were chose on the basis of previously reported data (Szuster-Ciesielska et al., 2000; Barayuga et al., 2013; Hendrickx et al., 2013) and of dose-response curves performed for both essential metals (Supplementary Fig. S1 – Panels B and C).

All treatments were performed in starvation medium because manipulating Se and Zn content of culture medium is impaired by the presence of these essential elements in FBS. The timeline with the entire experimental procedures were reported in Supplementary Table S1.

2.2. SH-SY5Y differentiation

Human neuroblastoma SH-SY5Y cell line was differentiated with 10 μM all-*trans* RA (Sigma Aldrich, Milano, Italy) for 48 h in their appropriate medium (DMEM High Glucose/Ham's F12 Mixture Medium (1:1), 2 mM L-Glutamine) supplemented with 1% FBS. Briefly, in all the experiment reported below, the cells were seeded in each support for 24 h in their complete growth medium. The day after, cells were starved in 1% FBS medium for 48 h and differentiated by adding RA 10 μM. After two days of differentiation, the cells were starved in 0% FBS medium for 24 h and then stimulated for 24 h in starved medium (0% FBS) as reported above. The stimuli and the different time of each treatment, was the same described for each experiment both for undifferentiated and differentiated SH-SY5Y.

2.3. Cell viability assay

Cell viability was evaluated by the reduction of 3-(4,5-di-methylthiozol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as an index of mitochondrial functional activity. Briefly, SH-SY5Y cells were seeded into 96 well plates at a density of 20,000 cells/well in complete growth medium for 1 day. Differentiated and undifferentiated cells were