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Review

The toxic Doppelganger: on the ionic and molecular mimicry of cadmium

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Cadmium is a toxic heavy metal which can cause numerous alterations in cell functioning. Exposure to cadmium leads to generation of reactive oxygen species, disorders in membrane structure and functioning, inhibition of respiration, disturbances in ion homeostasis, perturbations in cell division, and initiation of apoptosis and necrosis. This heavy metal is considered a carcinogen by the Agency for Toxic Substances and Disease Registry. At least some of the described toxic effects could result from the ability of cadmium to mimic other divalent ions and alert signal transduction networks. This review describes the role of cadmium mimicry in its uptake, reactive oxygen species generation, alterations in calmodulin, Wnt/β-catenin and estrogen signaling pathways, and modulation of neurotransmission. The last section is dedicated to the single known case of a favorable function performed by cadmium mimicry: marine diatoms, which live in zinc deficient conditions, utilize cadmium as a cofactor in carbonic anhydrase — so far the only described cadmium enzyme.

Keywords: cadmium, mimicry, signaling, carbonic anhydrase

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INTRODUCTION

Cadmium causes deleterious effects in all organisms. Exposure to this heavy metal leads to oxidative stress, lipid peroxidation, alterations in ion homeostasis, DNA damage, and initiation of apoptotic and necrotic processes (Belyaeva *et al*., 2008; Gonçalves *et al*., 2009; Kippler *et al*., 2010; Lehotai *et al*., 2011; Matović *et al*., 2011; Pytharopoulou *et al*., 2011; Wang *et al*., 2011 Arasimowicz-Jelonek *et al*., 2012; Filipič, 2012; Liu *et al*., 2012). It has also been shown to exhibit carcinogenic and, depending on the concentration and analyzed species, pro- or anti-inflammatory effects in mammalian cells (Joseph, 2009; Olszowski *et al*., 2012). In the case of plants cadmium toxicity manifests also in chlorophyll degradation, inhibition of photosynthesis and direction of the metabolism to the synthesis of protective compounds such as lignin or flavonoids (Küpper *et al*., 2007; Rascio *et al*., 2008; Pawlak-Sprada *et al*., 2011a; Pawlak-Sprada *et al*., 2011b; Sun *et al*., 2012). At least some of the toxic symptoms caused by cadmium stress could result from its ability to mimic essential ions.

Two types of mimicry can be distinguished at the cellular level: ionic and molecular. Ionic mimicry is the ability of unbound ions to mimic other ions or elements. An example of such a process is the entry of cadmium into the cell through transporters predestined for essential elements. Molecular mimicry, in turn, consists in replacing other metals in biological molecules (Bridges & Zalups, 2005). The cadmium molecular mimicry can alert signal transduction pathways and contribute to the Cd cytotoxicity in several ways. The substitution of essential ions by Cd^{2+} can lead to:

— release of the essential metals

The release of essential metals leads to an increase in their cellular concentrations. This phenomenon can have various consequences. Elevated levels of redox-active metals, such as iron and copper, can contribute to the generation of reactive oxygen species through Fenton and Haber-Weiss reactions. Release of calcium ions, in turn, can disrupt the cytoskeleton organization and Ca^{2+}-mediated signaling.

— alterations in target molecule structure

Examples of alterations in the target molecule structure resulting from cadmium mimicry include disruption of β-catenin/cadherin complexes leading to the release of β-catenin and activation of Wnt/β-catenin signaling, and replacement of Mg^{2+} in chlorophyll causing alterations in the structure and activity of photosystems.

— imitation of the action of the essential ion and activation of the target molecule

The binding of cadmium ions by a target protein can also mimic the action of other elements or molecules. Indeed, cadmium has been shown to imitate the function of Ca^{2+} in calmodulin and of estrogen in estrogen receptors.

The above examples of cadmium ionic and molecular mimicry and their influence on cellular signaling pathways are described in detail in the present review. The last section is dedicated to the so far unique example of a biological advantage of cadmium mimicry – the substitution for zinc ions in carbonic anhydrase in marine diatoms.

CADMIUM UPTAKE

Cadmium ions behave as "opportunistic hitch-hikers" — they enter cells through transporters and channels dedicated to essential divalent ions, such as Ca^{2+}, Fe^{2+} and Zn^{2+}. One of the candidates for Cd uptake are calcium channels. Treatment of plant or animal cells with calcium channel blockers, lanthanum and verapamil, caused augmentation in cadmium uptake (Braeckman *et al*., 1999; Kurtyka *et al*., 2011; Liu *et al*., 2012). Accord-

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Abbreviations: CA, carbonic anhydrase; GABA, gamma-aminobutyric acid; MLCK, myosin light chain kinase; ROS, reactive oxygen species; TCF/LEF-1, T-cell specific factors/lymphoid enhancer binding factor; Wnt, Wingless family.

ingly, Madin-Darby canine kidney cells subjected to the action of a calcium channel activator, maitotoxin, accumulated more cadmium than the untreated cells (Olivi & Bessler, 2000). Another putative route of cadmium cellular influx are transporters belonging to the ZIP family. A correlation between induced expression of ZIP10 and increased cadmium accumulation was observed in zebrafish (Chachene *et al.*, 2011). An enhanced cadmium uptake has also been shown in mouse fetal fibroblast over-expressing ZIP8 and ZIP14 (Dalton *et al.*, 2004; Girijashanker *et al.*, 2008). There is evidence that the protective role of glutathione against cadmium stress depends on the down-regulation of *ZIP8* gene expression (Aiba *et al.*, 2008). In plants the IRT1, ZNT1 and ZNT2 transporters belonging to the ZIP family have been shown to play a role in cadmium uptake (Connolly *et al.*, 2002; Mizuno *et al.*, 2005; Lee & An, 2009). The divalent cation transporters involved in cadmium uptake also include Nramp2 (alternative names: DCT1 or DMT1). *Xenopus* oocytes expressing human Nramp2 accumulated more cadmium than the control ones (Okubo *et al.*, 2003). Cadmium mimicry of essential ions could not only facilitate the uptake of this heavy metal but also its translocation and intracellular trafficking. Experiments performed with the use of six lines of *Arabidopsis thaliana* mutants showed that transporters belonging to heavy metal P_{1B}-ATPases (HMA proteins), namely HMA2 and HMA4, were involved in Cd root-to-shoot translocation (Wong & Cobett, 2003). Expression of AtHMA3 in a Cd-sensitive yeast strain, in turn, resulted in acquisition of tolerance to this heavy metal most probably through increased vacuolar sequestration (Gravot *et al.*, 2004). Also Nramp proteins are involved in Cd accumulation and vacuolar compartmentalization in plants. The Nramp3 and Nramp4 transporters have been shown to reside in the vacuole membrane in two cadmium hyperaccumulators, *Arabidopsis halleri* and *Thlaspi caerulescens*. Moreover, a double *nramp3nramp4* mutant of *Arabidopsis thaliana* was hypersensitive to Cd despite an unchanged intracellular Cd content (Oomen *et al.*, 2009; Takahashi *et al.*, 2011). These data show that mimicking divalent essential elements enables Cd^{2+} passing into animal and plant cells and its intracellular and long-distance translocation. In a cadmium-rich environment, Cd^{2+} can compete with other divalent elements for the transporters' binding sites. Therefore, the described ionic mimicry can lead to alterations in mineral homeostasis and distribution. Indeed, disorders in zinc, magnesium, calcium and potassium cellular balance have been reported in various organisms exposed to cadmium (Gonçalves *et al.*, 2009; Kippler *et al.*, 2010; Matović *et al.*, 2011; Liu *et al.*, 2012).

GENERATION OF REACTIVE OXYGEN SPECIES

One of the most common responses of organisms to cadmium exposure is generation of reactive oxygen species (Lehotai *et al.*, 2011; Pytharopoulou *et al.*, 2011; Vestena *et al.*, 2011; Wang *et al.*, 2011). Over-accumulation of ROS leads to oxidative stress which, in turn, causes lesions in various biological molecules such as peroxidation of lipids and oxidative damage of proteins and DNA. These lesions lead to membrane leakage, disturbed ion homeostasis, inactivation of enzymes, and increased rate of mutations (Scandalios, 2002). The reactive oxygen species generated in response to cadmium are also engaged in various signaling events (Chmielowska-Bąk & Deckert, 2012). The Cd-dependent over-production of ROS can result from disturbances in antioxidant systems, increased activity of NADPH oxidase, and alterations of mitochondria (Garnier *et al.*, 2006; Romero-Puertas *et al.*, 2007; Gzyl *et al.*, 2009; Ognjanović *et al.*, 2010; Chen *et al.*, 2011; Chou *et al.*, 2012). An important source of ROS are Fenton and Haber Weiss reactions catalyzed by redox-active metals, such as iron and copper (Kehrer, 2000). Cadmium has no reduction-oxidation activity, but it can replace the redox-active metals in biological molecules and, as a consequence, increase the metals' intracellular levels. This hypothesis was confirmed by experiments performed on living cells and artificial lipid bilayers — liposomes. In those experiments cadmium caused peroxidation of lipid membranes in living cells, but not in liposomes, implying that cadmium alone is unable to cause an oxidative stress. It was therefore suggested that the peroxidation of membranes observed in living cells resulted from a Cd-dependent release of Fe^{2+} from biological molecules. That hypothesis was confirmed by two facts. Firstly, application of Cd^{2+} caused release of iron from ferritin and rat liver microsomes. Secondly, exogenous application of Fe^{2+} induced peroxidation of lipids in liposomes (Casalino *et al.*, 1997). The ability of cadmium to substitute for iron has also been demonstrated in ferrodoxin (Bonomi *et al.*, 1994). Therefore, it is possible that Cd contributes to oxidative stress through the release of redox-active metals resulting from their substitution in biological molecules.

ACTIVATION OF WNT/β-CATENIN SIGNALING

In cells β-catenin can be found in membranes, cytoplasm and nucleus. In membranes this multifunctional protein forms complexes with E-cadherin and is engaged in cell-to-cell adhesion. The fate of cytoplasmic β-catenin strongly depends on the Wingless family (Wnt) ligands. As long as the Wnt signaling is switched off, cytoplasmic β-catenin is phosphorylated and directed for degradation. However, binding of the Wnt ligands to their receptors leads to the disruption of the complexes addressing β-catenin destruction. As a consequence, cytoplasmic β-catenin is translocated to the nucleus where it interacts with T-cell specific factors/lymphoid enhancer binding factor (TCF/LEF-1). This, in turn, leads to the activation of Wnt signaling target genes which are involved in regulation of numerous developmental processes (Berthon *et al.*, 2012). Exposure to cadmium can lead to abnormal activation of Wnt/β-catenin signaling. It has been shown that cadmium alters the distribution of N-cadherin, E-cadherin and β-catenin distribution in rat proximal tubule epithelium (Prozialeck *et al.*, 2003). A breakdown of adherens junctions and redistribution of β-catenin in cells has also been observed in chicken embryos (Thompson *et al.*, 2008). As E-cadherin has several Ca^{2+}-binding sites, it has been suggested that Cd^{2+} displaces the Ca^{2+} in E-cadherins, which in turn leads to deformation of the E-cadherin/β-catenin complexes and release of β-catenin to the cytoplasm and nucleus. This was confirmed by an experiment performed on rat proximal tubule cell cultures showing that the Cd-dependent increase in cytoplasmic and nuclear β-catenin levels was independent of transcription and translation (Chakraborty *et al.*, 2010). The increase of the β-catenin level in the nucleus in response to Cd administration leads to the activation of TCF4 transcription factor and induction of Wnt target genes, *c-Myc*, *cyclin D1* and *ABCB1*. The elevated expression of these genes can lead to enhanced cell proliferation and initiation of carcinogenesis (Chakraborty *et al.*, 2010).

SIGNALING MEDIATED BY CALMODULIN

Calmodulin is the main mediator of Ca^{2+} signaling. Binding of calcium ions to calmodulin causes changes in its conformation and exposure of hydrophobic residues in the central helix. The exposed residues are responsible for recognition and activation of various target proteins including kinases, ion channels, G-proteins, cytoskeleton elements, and transcription factors (Snedden & Fromm, 1998; Clapham, 2007). Calmodulin is highly conserved and regulates numerous processes in all eukaryotic cells. Perhaps the most spectacular example of calmodulin's role are the beak shapes in Darwin's finches shown to be partially determined by the level of calmodulin expression (Abzhanov *et al.*, 2006). Interestingly, calmodulin has also been shown to participate in the plant response to cadmium stress. Experiments on tobacco cell suspension culture show that activation of calmodulin is necessary for the Cd-dependent stimulation of NADPH oxidase and generation of H_2O_2 (Olmos *et al.*, 2003; Garnier *et al.*, 2006). There is evidence that calcium can be replaced in calmodulin by other divalent ions with an affinity dependent on the ionic radius (Ouyang & Vogel, 1998). Cadmium should be very efficient in substituting for calcium ions as the ionic radii of these elements are very similar (0.97 and 0.99Å respectively). Indeed, the ability of Cd^{2+} to bind to calmodulin has been shown by nuclear magnetic resonance (NMR), electrospray ionization mass spectrometry (ESI-MS), equilibrium gel filtration, flow microcalorimetry, and fluorescence techniques (Milos *et al.*, 1989; Ouyang & Vogel, 1998; Schirran & Barran, 2009). Importantly, it has been shown that cadmium ions binds to calmodulin in its C-terminal sites III and IV, which also show the highest affinity for Ca^{2+} (Milos *et al.*, 1989; Ouyang & Vogel, 1998). The Cd^{2+}-calmodulin complexes formed were able to activate a calmodulin target protein — myosin light chain kinase (MLCK) (Ouyang & Vogel, 1998). Moreover, cadmium stimulated calcium-dependent phosphorylation of several substrates in the cytosolic fraction of rainbow trout gonadal cells (RTG-2) (Behra & Gall, 1991). Interestingly, substitution of Ca^{2+} by Cd^{2+} leads to the inhibition of calmodulin activity in plants (Rivetta *et al.*, 1997). The signaling functions of calmodulin strongly depend on the concentration of cytosolic Ca^{2+}, which is strictly regulated by a complex machinery comprising of calcium channels, pumps and chelators (Clapham, 2007). The concentration of non-essential ions such as cadmium is not subjected to such a strict control, therefore the ability of Cd^{2+} to mimic Ca^{2+} functions in calmodulin can profoundly alter its signaling.

MIMICRY OF ESTROGEN PATHWAY

Recent research shows that Cd can modulate functioning of estrogen receptors (ERs) (Deegan *et al.*, 2011). The ERs are located in the nucleus and are involved in regulation of gene expression in response to female sex steroid hormones, estrogens, such as 17β-estradiol (E_2) (Brzozowski *et al.*, 1997). Estrogen receptors contain conserved structural and functional domains for ligand binding (LBD), DNA binding (BD), and transcriptional activation (Matthews & Gustafson, 2003). Several studies have demonstrated that cadmium is capable of mimicking the E_2 action at the ligand binding domain of the ER (Garcia-Morales *et al.*, 1994, Stoica *et al.*, 2000; Martinez-Campa, 2008; Rider *et al.*, 2009; Deegan *et al.*, 2011). Estrogen-like effects of cadmium have been reported both in cell culture and in experimental animals. In mammalian cell culture, cadmium causes activation of intracellular signaling similar to estrogen, induction of the expression of estrogen target genes, stimulation of estrogen-specific proteins, and proliferation of estrogen-dependent cells (Garcia-Morales *et al.*, 1994; Stoica *et al.*, 2000; Wilson *et al.*, 2004; Brama *et al.*, 2007; Martinez-Campa, 2008; Siewit *et al.*, 2010; Deegan *et al.*, 2011). *In vivo* studies in animal models, especially rats, have also provided strong evidence that Cd can mimic estrogen, specifically in organs and tissues known to be estrogen responsive. Exposure to cadmium increased uterine wet weight, promoted growth and development of the mammary glands and induced estrogen-regulated genes in ovariectomized animals (Johnson *et al.*, 2003; Alonso-González *et al.*, 2007; Höfer *et al.*, 2009; Liu *et al.*, 2010; Penttinen-Damdimopoulou *et al.*, 2010). The inhibition of those effects after the addition of antiestrogens further strengthens the conclusion that Cd^{2+} mimics estrogen signaling (Garcia-Morales *et al.*, 1994; Johnson *et al.*, 2003). Inappropriate stimulation of ERs activity by cadmium is believed to be an important factor contributing to the increasing incidence of cancer in industrialized countries. Recent epidemiological findings suggest an increased risk of hormone-dependent diseases, such as breast cancer, endometrial cancer, and endometriosis, after exposure to cadmium (Akesson *et al.*, 2003; Thomson & Bannigan, 2008; Strumylaite *et al.*, 2010).

CADMIUM AND NEUROTRANSMISSION

Exposure to cadmium is associated with various neurotoxic symptoms. This heavy metal causes damage of rat and rabbit cerebellar cortices, affects functioning of voltage activated calcium and sodium channels in neurons, inhibits adenylate cyclase activity in the cerebrum, cerebellum and brain stems, modulates the release of inhibitory and excitatory neurotransmitters and inhibits the NO generating enzyme nitric oxide synthase (Sadiq *et al.*, 2012). The involvement of cadmium in the modulation of nervous system functioning is recently becoming a subject of intense study. The neuromodulatory action of Cd^{2+} is based on its ability to replace Zn^{2+}. Zinc is directly and indirectly involved in neurotransmission: it functions as neurotransmitter in a specific type of neurons called zinergic neurons, as well as a regulator of gamma-aminobutyric acid (GABA) release in GABAergic neurons (Colvin *et al.*, 2003; Takeda, 2012). Zinc also modulates the activity of P2X receptors which function as ATP-dependent cationic channels in various cell types including brain and peripheral nerves (Lorca *et al.*, 2011). Experiments performed on *Xenopus* oocytes with injected $P2X_4$ receptors showed that, out of eight metals assayed, only cadmium was able to mimic the action of zinc on the $P2X_4$ receptors (Coddou *et al.*, 2005). It is possible that cadmium mimics also other zinc functions in the nervous system. This hypothesis could be further strengthened by the fact that both Zn^{2+} and Cd^{2+} inhibit the release of GABA (Sadiq *et al.*, 2012).

CADMIUM AS ESSENTIAL METAL: CARBONIC ANHYDRASE

Vertical profiles of Cd distribution in the ocean show that this metal, believed to be universally deleterious to organisms, has in fact a nutrient-like profile. Its concentration is extremely low in surface waters and increases in deep waters, similar to other biologically important

nutrients, such as phosphate. This profile reflects the uptake of elements by phytoplankton at the surface and regeneration in the depths by remineralization of sinking organic matter (Park *et al*., 2007; Xu *et al*., 2008). The high fractionation of cadmium in organic matter clearly indicates that there must be an active Cd uptake system in marine organisms (Morel & Price, 2003). Laboratory studies have established that Cd can be used as a co-factor in carbonic anhydrase (CA), particularly in the Cd-carbonic anhydrase found in the coastal diatom *Thalassiosira weissflogii* (CDCA1) (Lane *et al*., 2005) and categorized in a new zeta (ζ)-CA class (Lane & Morel, 2000; McGinn & Morel, 2008; Alterio *et al*., 2012). Carbonic anhydrase (EC 4.2.1.1) is a (primarily) zinc metalloenzyme that catalyses with an extremely high efficiency the reversible hydration of carbon dioxide, an essential reaction for many physiological processes such as respiration, ion transport, bone resorption, and photosynthesis (Ivanov *et al*., 2007; Supuran, 2010; Zhang *et al*., 2010). Diatoms, which are one of the most common types of phytoplankton and are responsible for 40% of the net marine primary production, use carbonic anhydrases (CAs) for acquisition of inorganic carbon (Park *et al*., 2007; McGinn & Morel, 2008). In the ocean, where zinc is nearly absent, these diatoms use Cd as the catalytic metal atom in CDCA1 (Lane & Morel, 2000; Park *et al*., 2007; Xu *et al*., 2008; Alteiro *et al*., 2012). This peculiar carbonic anhydrase is the first and hitherto the only known cadmium metalloenzyme and is responsible for the only known biologically beneficial cadmium-dependent reaction (Lane & Morel, 2000; Xu *et al*., 2008). Although CDCA1 was initially isolated as a Cd enzyme, it is actually a *cambialistic* carbonic anhydrase that can use either Zn (II) or Cd (II) for catalysis and spontaneously exchange the two metals at its active centre. Indeed, a kinetic analysis has demonstrated that both single CA repeats and the full length enzyme exhibit high CA activity with either Cd or Zn as the catalytic metal, with only a slightly higher catalytic efficiency for the zinc forms. The Cd form of CDCA1 can therefore satisfy a substantial fraction of the needs of the fast growing diatoms (Xu *et al*., 2008; Alteiro *et al*. 2012). Thus CDCA1 is an excellent example of adaptation to life in an environment containing a vanishingly small concentration of an essential metal. Furthermore, it has been suggested that the ability to use cadmium, an element known for its toxicity, probably gave a significant competitive advantage to diatoms in the ocean, which is poor in metals, and could have contributed to the evolutionary differentiation of diatoms during the Cenozoic Era and to the parallel decrease in atmospheric CO_2 (Xu *et al*., 2008).

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