Biometals (2010) 23:927–940
DOI 10.1007/s10534-010-9329-x

Cadmium stress: an oxidative challenge

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Received: 5 December 2009 / Accepted: 19 March 2010 / Published online: 2 April 2010
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Abstract At the cellular level, cadmium (Cd) induces both damaging and repair processes in which the cellular redox status plays a crucial role. Being not redox-active, Cd is unable to generate reactive oxygen species (ROS) directly, but Cd-induced oxidative stress is a common phenomenon observed in multiple studies. The current review gives an overview on Cd-induced ROS production and anti-oxidative defense in organisms under different Cd regimes. Moreover, the Cd-induced oxidative challenge is discussed with a focus on damage and signaling as downstream responses. Gathering these data, it was clear that oxidative stress related responses are affected during Cd stress, but the apparent discrepancies observed in between the different studies points towards the necessity to increase our knowledge on the spatial and temporal ROS signature under Cd stress. This information is essential in order to reveal the exact role of Cd-induced oxidative stress in the modulation of downstream responses under a diverse array of conditions.

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Keywords Cadmium · Glutathione · Oxidative stress · Signaling · Thiol · Antioxidative defense · Reactive oxygen species

Introduction

Cadmium (Cd) is an earth's crust natural element and is usually found as a mineral in combination with other elements such as oxygen, chlorine, or sulfur. Over the past two centuries, anthropogenic and industrial activities have led to high emissions of Cd into the environment at concentrations significantly exceeding those originating from natural sources (Nriagu 1988; Vangronsveld et al. 1995). Since Cd can not be degraded, the risk of environmental exposure is constantly increasing because of accumulation via the food chain (ATSDR 2005).

At the cellular level, Cd induces oxidative stress in many organisms (Bertin and Averbeck 2006; Thévenod 2009), which might result in physiological damage to different organs among which kidneys, liver, lung, pancreas, testes, placenta, and bone (Jarup et al. 1998; Nawrot et al. 2008; Jarup and Åkesson 2009). Cadmium is a bivalent cation and unable to generate free radicals directly, nevertheless the production of reactive oxygen species (ROS) after Cd exposure has been reported in multiple studies (Hassoun and Stohs 1996; Hart et al. 1999; Szuster-Ciesielska et al. 2000; Thévenod et al. 2000; Galán et al. 2001; Wang et al.

2004, 2009; Valko et al. 2005; Belyaeva et al. 2006; Oh and Lim 2006; Pathak and Khandelwal 2006; Zhou et al. 2009). Cadmium-induced oxidative stress effects in animals and plants, cells and tissues are outlined in several reviews (Waisberg et al. 2003; Bertin and Averbeck 2006; Joseph 2009; Cuypers et al. 2009; Thévenod 2009). Since our current understanding on ROS related signaling is increasing, the present review summarizes Cd-induced ROS production and anti-oxidative defense and focuses on how the Cd-induced oxidative challenge leads to damage and/or signaling. Nevertheless, one should keep in mind that the obtained results should always be presented in relation with the experimental set-up used, as exposure time (acute–chronic), the way of administration (food, drinking water, intraperitoneal…), in vitro and in vivo experiments,… will have their impact on the research outcome.

Cd and ROS production

Exposure to Cd, originating from different sources—air, water, food—may produce effects in organs such as kidneys, liver, lungs, cardiovascular, immune and reproductive systems (Fowler 2009). Moreover, Cd is identified as a human carcinogen. Due to complex interactions between Cd ions and metabolism, a diverse range of cellular responses is found in different organs after Cd-exposure. Oxidative stress, however, has been mentioned and demonstrated in multiple studies as a part of early biological responses that involve characteristic molecular changes in organ systems prior to the onset of clinical diseases or the development of cancer (Bertin and Averbeck 2006; Thijssen et al. 2007; Fowler 2009; Thévenod 2009). Oxidative stress is a disturbance of the cellular redox balance in favor of the pro-oxidants, and can lead to disruption of cellular macromolecules (e.g., degradation of proteins, cross-links in DNA, and membrane fatty acid peroxidation). Nevertheless, elevated ROS concentrations can also act in signal transduction (Thévenod 2009).

As Cd shows a high affinity for thiols, the major thiol antioxidant, glutathione (GSH) that is highly abundant in cells, is a primary target for free Cd-ions. Therefore Cd-induced depletion of the reduced GSH pool (Lopez et al. 2006) results in a disturbance of the redox balance leading to an oxidative environment. Whereas the majority of published articles have focused on metal-induced effects on the antioxidative defense mechanisms, it is clear that sources of ROS production are currently under investigation. Under natural conditions ROS are produced in organelles with a highly oxidizing metabolic rate or those possessing electron transport chains, such as peroxisomes and mitochondria. Because Cd is a non redox-active, non-essential element, it cannot induce ROS production directly. This is relevant in terms of ROS production that is discussed in the following subsections.

Replacement of redox-active elements

Cadmium is unable to catalyze redox reactions in biological systems under physiological conditions. It has been shown, however, that Cd increases the free Fe-concentration possibly by its replacement in various proteins and hence increases the cellular amount of free redox-active metals (Casalino et al. 1997; Dorta et al. 2003). Free redox-active metals directly enhance the production of $^{\bullet}OH$ (hydroxyl) radicals through the Fenton reaction. Reduction of the oxidized metal ion can be achieved by the Haber–Weiss reaction with superoxide radicals ($O_2^{\bullet -}$) as a substrate (Fig. 1), but also other reducing agents, such as ascorbate can catalyze this reaction (Winterbourn 1979).

Mitochondrial ROS production

Mitochondria are a major cellular site of ROS production, but the quantity produced under normal and stress conditions is unknown (Fleury et al. 2002; Kehrer 2000; Ježek and Hlavatá 2005; Gao et al. 2008). The physiological activity of the respiratory chain (complexes I to V) present in the inner mitochondrial membrane produces ROS at complex I (NADH/ubiquinone oxidoreductase) and complex III (ubiquinol/cytochrome c oxidoreductase) (Boveris et al. 1976; Takeshige and Minakami 1979; Turrens

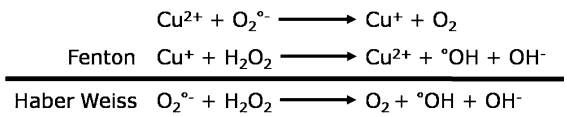

Fig. 1 Fenton and Haber Weiss reaction with Cu as an example of a redox-active metal

et al. 1985; Kehrer 2000, Gao et al. 2008). Whereas the production of radicals by mitochondria in healthy tissues is rather low, ROS production substantially increases in the presence of xenobiotics (Kehrer 2000). As a result, these ROS might lead to membrane lipid peroxidation, mtDNA cleavage and impaired ATP generation with resulting mitochondrial damage and induction of apoptosis during stress situations and aging (Huang et al. 1999; Takahashi et al. 2004).

Different studies point toward the generation of oxidative stress and mitochondrial dysfunction during metal-induced cytotoxicity (Stohs and Bagchi 1995; Valko et al. 2005; Belyaeva et al. 2008). It has long been known that exposure to Cd induces specific alterations in mitochondrial structure and function in animals. As soon as in 1971, Mustafa and Cross reported Cd-induced effects on electron and energy transfer reactions in the mitochondria of pulmonary alveolar macrophages. More recent studies confirmed the general view of mitochondria as one of the primary cellular targets of metals such as Cd (Müller 1986) and discussed potential mechanisms of Cd-induced cytotoxicity (Cameron et al. 1986; Early et al. 1992; Koizumi et al. 1994; Al-Nasser 2000; Tang and Shaikh 2001; Belyaeva et al. 2002, 2004, 2008; Belyaeva and Korotkov 2003; Lee et al. 2004; Pathak and Khandelwal 2006). More specifically, Belyaeva et al. (2006) found that Cd-induced effects in AS-30D rat ascites hepatoma cells, manifested by cell necrosis and/or apoptosis, involve ROS generation. This increased ROS production probably occurs at the level of complex III and is related to the opening of the mitochondrial permeability transition (MPT) pore (Belyaeva et al. 2006). Wang et al. (2004) investigated the inhibitory effects of Cd with respect to the individual complexes of the respiratory chain in mitochondria of guinea pig liver, brain and heart. They showed that complex II and III are more sensitive to Cd than complexes I, IV and V in all three tissues studied and demonstrated Cd-induced ROS generation only at the level of complex III. Results of kinetic studies and electron turnover experiments suggest the accumulation of unstable semiubiquinones, prone to transfer one electron to molecular oxygen and thereby forming superoxide, as a possible mechanism of Cd-induced ROS generation in mitochondria (Wang et al. 2004). This coincides with the observations of Poliandri et al. (2006), who found that Cd-induced ROS production in mitochondria of anterior pituitary cells is electron-transfer-chain-dependent since it was inhibited by rotenone. ROS production was shown to be one of the first steps in Cd-mediated cytotoxicity, preceding mitochondrial damage characterized by the loss of the mitochondrial membrane potential. This further leads to the activation of caspases and consequently cell death by apoptosis (Poliandri et al. 2006; Chatterjee et al. 2009). Dorta et al. (2003) defined two sets of Cd-induced responses in mitochondria isolated from rat liver. In a first phase Cd interacts with specific protein thiols leading to membrane permeability transition. This is followed by ROS generation in a second, slower phase together with Fe mobilization leading to mitochondrial membrane lipid peroxidation. In accordance with most animal-based studies, Cd-induced cellular processes in plants also include mitochondrial ROS production (Yeh et al. 2009).

Induction of NADPH oxidases

NADPH oxidase was originally identified as a key component of human innate host defense (Bokoch and Knaus 2003; Quinn et al. 2006). NADPH oxidases function as multi-component enzymes, and use electrons derived from intracellular NADPH to generate $O_2^{\bullet -}$ from O_2. Superoxide production generally is followed by its dismutation to H_2O_2 spontaneously or through e.g. SOD activity. The NOX family of ROS-generating NADPH oxidases consists of seven members and their role and tissue distribution was previously described by Krause (2004). All NOX members share a core structure consisting of six transmembrane domains and a long cytoplasmic C-terminus. Furthermore, consistent with their additional Ca^{2+} binding N-terminal EF domains, the NOX members NOX5, DUOX1 and DUOX2 are Ca^{2+} activated enzymes.

They participate in important cellular processes, related to signaling, cell proliferation and apoptosis.

Although ROS derived from NADPH oxidase play an essential role in normal functioning, these molecules have also been implicated in multiple stress and pathological conditions (Li and Shah 2003). Thijssen et al. (2007) found that NOX4 gene expression was upregulated in mice kidneys following chronic exposure to low levels of Cd. NOX4, unlike the other NOX proteins, functions independently of cytoplasmic cofactors and is regulated purely on the

gene expression level rather than on the level of enzyme activity (Krause 2004; Quinn et al. 2006). Increased NOX4 gene expression thus may have led to increased NOX activity in mice kidneys following Cd exposure in this tissue. The exact role of NOX4 in Cd toxicity has not been described, but may be linked to the production of free radicals for signal transduction to activate the antioxidative defense system or adaptive mechanisms (Fig. 2). NADPH-dependent ROS production is also described in Cd-exposed hepatocytes (Fotakis et al. 2005). This ROS production could trigger signaling leading to protective measures, as reported by Souza et al. (2009) in HepG2 cells. Alternatively, production of excess ROS may lead to Cd-induced toxicity, as shown by Rockwell et al. (2004) in mouse neuronal cells. These authors describe a cyclooxygenase-2 (COX-2) upregulation induced by NADPH oxidase dependent ROS, culminating in cell death after Cd exposure. Also multiple cancer cell lines are capable of constitutively releasing large amounts of ROS, whose enzymatic sources are unknown. A role for NOX proteins in this process is reasonable to suggest because nearly all of these cancer cell lines express several NOX isoforms, and their antioxidant production is sensitive to the inhibitor DPI (Bokoch and Knaus 2003; Quinn et al. 2006). Since NADPH oxidase activity can be induced by Cd, this could form a molecular link between Cd and cancer or other disease symptoms in different organs that accumulate Cd and express NOX genes.

In summary, potentially all NOX expressing cells can be targeted by Cd and the influence of Cd on

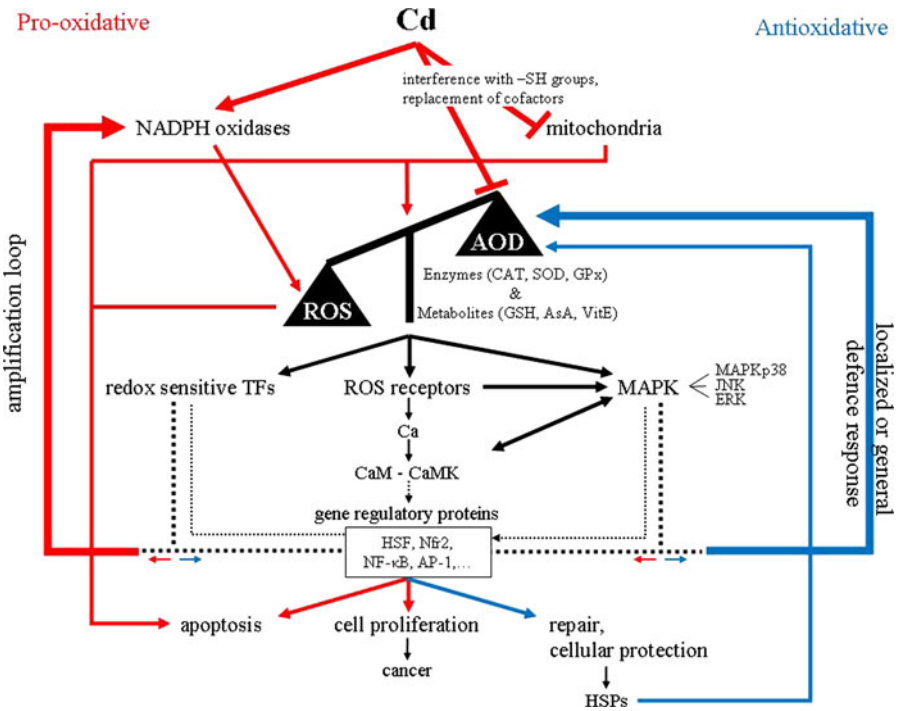

Fig. 2 Redox-related pathways during cadmium stress. Cadmium induces oxidative stress via indirect pathways such as through the induction of NADPH oxidases, via binding with thiol groups and by replacing Fenton metals from their active site. A disturbed redox balance influences both damaging (apoptosis and uncontrolled cell proliferation) as well as repair processes, via the activation of several signaling cascades. Both MAPK and Ca-dependent signaling pathways are important during cadmium stress, although exact interactions are not known. From this point, both amplification (pro-oxidative) as well as inhibitory (anti-oxidative) loops are hypothesized. - - - -: pathway not exactly known. AP-1: activation protein-1; AsA: ascorbic acid; CaM: calmodulin; CaMK: calmodulin kinase; CAT: catalase; ERK: Extracellular Signal-Regulated Kinase; GSH: glutathione; HSF: heat shock factor; HSP: heat shock protein; JNK: Jun N-terminal Kinase; MAPK: Mitogen-Activated Protein Kinase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; Nfr2: nuclear factor erythroid 2 related factor 2; ROS: reactive oxygen species; TF: transcription factor; VitE: vitamin E

NADPH oxidase activity can result in signaling leading to the onset of cellular protection mechanisms or, alternatively, in cell death (Fig. 2). Controlled levels of ROS production (via NADPH oxidase or other sources) are therefore necessary to ensure correct ROS levels for signaling or defense, hence a large network of antioxidative mechanisms is described.

Cadmium and antioxidative defense

Both the production of oxidants as well as the protection against them is intrinsic to every living cell. To minimize oxidative damage, organisms developed antioxidative mechanisms triggered by an increased ROS production. Oxidants such as ROS are balanced against this antioxidative defense system that consists of enzymes and metabolites in all subcellular compartments (Halliwell 2006). In stress conditions, however, normal capacities of these mechanisms are insufficient, triggering cells to increase and expand their antioxidative network.

The effects of Cd on antioxidative capacity are dual: on one hand, Cd can induce oxidative stress via the inhibition of antioxidants (cf. supra), but on the other hand it also activates several antioxidative components as a result of a disturbed redox balance and a consecutively induced signal transduction cascade (Fig. 2). The most important effects are summarized in the following paragraphs.

Antioxidative enzymes

Superoxide dismutases (SOD) are metallo-enzymes that catalyze the dismutation of $O_2^{\bullet -}$ to H_2O_2 with remarkably high reaction rates. Total SOD activity during Cd exposure has been studied intensively and both increases as well as decreases are described in literature (Jurczuk et al. 2004; Lopez et al. 2006; Yalin et al. 2006). These apparent discrepancies can be attributed to different exposure conditions as well as the organ system studied, leading to a different outcome. Jurczuk et al. (2004) exposed rats in vivo to 50 mg Cd/l in their drinking water during 12 weeks and noticed an increase in total SOD activity in kidneys, whereas a decrease was observed in the liver. Acute exposure (24 h), however, resulted in a decrease of both liver and kidney SOD activities after an intraperitoneal administration of a single dose of 5 mg/kg (Yalin et al. 2006). In vitro study where neuronal cortical cells were exposed for 24 h to different Cd concentrations showed an increase in total SOD activity (Lopez et al. 2006). Besides exposure conditions (acute versus chronic, in vivo versus in vitro, administration…) also the involvement of specific isoforms localized in different cell compartments should be taken into account. SODs are classified in different groups according to their metal cofactor (Halliwell 2006). Thijssen et al. (2007) demonstrated that a chronic exposure up to 23 weeks to low Cd-concentrations via the drinking water led to reversible inhibition of the gene expression of cytosolic Cu/ZnSOD and an unchanged mitochondrial MnSOD-transcript level in mice kidneys. The temporary Cd-induced inhibition of CuZnSOD, confirmed at both protein and metabolic level, may be due to a Cd/enzyme interaction which causes perturbations in the enzyme topography critical for its catalytic function (Casalino et al. 2002). Casalino et al. (2002) compared different ways of Cd administration (intraperitoneal, acute versus via drinking water, chronic) in relation to the sensitivity of SOD isoforms in liver and kidneys of rats. Acute exposure led to an immediate decrease in Mn- and CuZnSOD activities in both liver and kidneys. The overall decrease of MnSOD activity was also observed after Cd-exposure via the drinking water, but CuZnSOD activity showed an immediate decrease that was alleviated in a later phase.

Hydrogen peroxide detoxification can be accomplished by both enzymes, catalases (CAT) and glutathione peroxidases (GSH-Px). Catalases are heme-containing enzymes catalyzing H_2O_2 breakdown to water and divalent oxygen without using any cellular reducing equivalents (Mates 2000). In most organisms, CAT activity is mainly located in the peroxisomes and to a lesser extent in the cytoplasm of erythrocytes, in the nucleus and mitochondria (Schrader and Fahimi 2004). In mice, an increased liver CAT activity was detected after 6 days through a daily intraperitoneal exposure to Cd (Gong et al. 2008). These results were confirmed by experiments in rats that were exposed to Cd for 5 days via gastric gavage, where an increased blood CAT activity was observed (Tandon et al. 2003). On the other hand, Casalino et al. (2002) observed reduced CAT activities in both kidney and liver of rats after acute intraperitoneal administration (24 h) as well as via Cd-

exposure through drinking water (10–30 days). Waisberg et al. (2003) reported increased/decreased CAT activities obtained from several studies where different exposure conditions were used. Possible underlying mechanisms for decreased activities are postulated. Wronska-Nofer et al. (1999) hypothesized an interaction between Cd and the catalytic subunit of CAT as a possible underlying mechanism of the reduced CAT-activity. Jurczuk et al. (2004) observed Fe deficiency in liver and kidney of rats exposed to Cd during 12 weeks via the drinking water, which might be the cause of a decreased CAT activity, since it has Fe as an essential element in its active center.

Whereas CAT are active in severe stress situations, peroxidases (Px) are suggested to protect the cell against low levels of oxidative stress (Mates 2000), possibly indicating a role for this enzyme in the fine-tuning of ROS levels important in signal transduction. In contrast to CAT, the detoxification of H_2O_2 by peroxidases occurs via the oxidation of other organic substrates. Glutathione peroxidase (GSH-Px), which uses GSH as a substrate, appears in five isoforms in most mammals and has a selenocysteine in its active site (de Haan et al. 1998; Brigelius-Flohe 1999). As for CAT, activation of these isoforms is probably differentially regulated in different tissues or organisms, and also dependent on exposure conditions, which may explain differences in reported results. In rats, an increased activity of GSH-Px in blood cells was found after acute (24 h) intraperitoneal exposure to Cd (Ognjanovic et al. 2003), whereas decreased GSH-Px activities were reported after chronic Cd exposure in liver and kidney of mice and rats, with a possible underlying mechanism for selenium (Se) depletion through Cd–Se–cys complex formation that forms the core of the active site of the GSH-Px (Newairy et al. 2007; Hispard et al. 2008; Ognjanovic et al. 2008; Jihen et al. 2009; Renugadevi and Prabu 2009). Furthermore, Ognjanovic et al. (2008) suggested competition between GSH-Px and metallothioneins for S-aminoacids as a potential cause for GSH-Px activity decreases during Cd-stress.

Antioxidative metabolites

Whereas antioxidative enzymes are specifically involved in ROS-scavenging, several metabolites are essential for diverse metabolic processes, but also possess antioxidative properties. These metabolites can be classified into two groups: (1) water-soluble or hydrophilic metabolites such as glutathione (GSH) and ascorbic acid (AsA) that react with Cd-induced oxidants in the cell and blood plasma and (2) lipid-soluble or hydrophobic metabolites such as vitamin E (vit E) that protect cell membranes from lipid peroxidation caused by Cd-induced oxidative damage (Sies 1997).

The widely distributed tripeptide GSH (L-γ-glutamyl-L-cysteinyl-glycine) is one of the most important metabolites dealing with Cd-induced oxidative stress. Glutathione plays a predominant role as a substrate for GSH-S-transferases (GST) in order to protect cells against xenobiotic compounds and oxidants. Its antioxidant properties are attributed to the thiol (SH-) group on the cysteine residue (Dickinson and Forman 2002; Noctor and Foyer 1998). As such, GSH is able to transfer its reducing equivalents to several enzymes/components such as GSH-Px, GSTs, glutaredoxins (Grx) and ascorbate. In cells, GSH is maintained in its reduced form by glutathione reductase. In Cd-induced cellular responses, GSH has a dual role as it neutralizes ROS but also detoxifies Cd directly (cf. infra).

Ascorbic acid (AsA) can directly neutralize Cd-induced ROS. However, humans, primates and some other species, must obtain AsA from the diet, as they lost the enzyme that oxidizes the final step in AsA biosynthesis during evolution (animal L-gulono-1,4-lactone oxidase) (Linster and Van Schaftingen 2007). Reduced AsA is diminished under Cd stress, as shown by Brandao et al. (2009) in mouse testes, although no effects on renal AsA content were detected (Thijssen et al. 2007). Several animal studies indicate that AsA supplementation reverses the adverse effects of Cd like decreased SOD activity, increased lipid peroxidation, apoptosis, and necrosis, possibly through scavenging the ROS generated by Cd administration (Sen Gupta et al. 2004; Erdogan et al. 2005). This is probably related to its antioxidative properties, although AsA also influences Cd absorption and distribution (Grosicki 2004).

Vitamin E (tocotrienol) is part of the tocochromanol family which refers to a family of eight molecules: α-, β-, γ-, and δ-tocopherol and -tocotrienol. This vitamin is only synthesized by photosynthetic cells, so animals have to obtain it through their diet. α-tocopherol is the most abundant and active

isoform in human and animal tissues (Packer et al. 2001). As a hydrophobic antioxidant that incorporates into lipid environments, vit E significantly decreases Cd-induced lipid peroxidation in different organs and body fluids of rats (El-Demerdash et al. 2004; Kara et al. 2008; Karabulut-Bulan et al. 2008). Exact mechanisms of uptake, distribution and cellular effects still have to be elucidated.

Cd and the thiol metabolism: an intense relationship

Cellular redox reactions are involved in metabolic, signaling and transcriptional processes with thiols in the form of cysteine residues as vital players in redox sensing and regulation (Jones et al. 2004). During Cd stress, thiols or organic sulfhydryl compounds are primarily involved in mobilizing and detoxifying Cd through the formation of Cd–thiol complexes inside the cell (Vairavamurthy et al. 2000), but their antioxidative capacities are also important in Cd-induced oxidative stress.

Thiols: a role in Cd complexation and detoxification

Cadmium can form covalent attachments with various cellular thiols such as GSH, metallothioneins (MT), other small peptides as well as proteins (Zalups and Ahmad 2003; Hansen et al. 2006). If the Cd is enterally absorbed, it is taken up primarily by the liver, where it binds with GSH and MT. Subsequently, it is either excreted into the bile, or released into the blood stream in the form of Cd–GSH (Ercal et al. 2001), or Cd–MT complexes (Thévenod 2009). As such, it can then be absorbed by different organs. Due to the numerous thiol groups in MTs, sulfhydryl reactive metals such as Cd are easily bound to them. This is an important transport from within the body as well as an important intracellular form for the Cd-storage, explaining its long biological half life in different organs like kidney, liver, pancreas and intestine—organs with high MT content (Thévenod 2003; Klaassen et al. 2009). Complexation by MT and GSH makes free Cd unavailable for the cell metabolism, thus blocking the mechanisms leading to Cd-induced oxidative stress. Reactive oxygen species, however, can be produced by the replacement of Fenton metals from MT by Cd, or via the depletion of GSH (Thévenod 2003, cf. supra).

Thiols: a role in the Cd-induced antioxidative defense

Metabolites containing thiol groups have a strong antioxidant capacity through their ability to oxidize and form disulfide bridges that in their turn can be recycled to the reduced state. Peroxiredoxins function as thiol peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides as well as peroxynitrite (Rhee et al. 2005). They are found in bacteria, plants and mammals and are shown to be important in normal ageing as mice lacking Prx 1 or 2 are viable and fertile, but have shortened life span owing to the development of severe hemolytic anemia and several malignant cancers (Neumann et al. 2003). Peroxiredoxins possess Cys residues in their active site that can be oxidized to sulphenic acid. The recycling of sulphenic acid is accomplished by thiols coming from GSH, thioredoxin and glutaredoxin. Hyperoxidation of the sulphenic acid to sulphinic acid was thought to be an irreversible modification until the discovery of the sulphinic acid reductase sulfiredoxin was discovered (Biteau et al. 2003; Salmeen et al. 2003; van Montfort et al. 2003; Barranco-Medina et al. 2009). Based on these antioxidant interactions, the involvement of Prx in Cd-induced oxidative stress is often suggested. Sheader et al. (2006) confirm this hypothesis as they detected a strong upregulation of Prx after Cd exposure in *Platichthys flesus*. The oxidized form of Prx-3, on the other hand, seems to be involved in Cd-induced apoptosis in human neuroblastoma cells (Oh et al. 2009). Thioredoxin (Trx) and glutaredoxin (Grx) are important compounds in the maintenance of the intracellular redox homeostasis and are kept in their reduced state via NADPH dependent thioredoxin reductases (TrxR) or GSH respectively (Meyer et al. 2008). Most studies indicate an enhanced oxidation of Trx1 and Trx2 redox states after Cd exposure (Hansen et al. 2006; Oh et al. 2009), resulting in apoptosis. This is probably due to an increased binding of Cd although a reduction in TrxR activity was also detected during Cd stress (Hodkova et al. 2008).

Thiols under focus-glutathione and metallothionein

Glutathione is a primary intracellular antioxidant and conjugating agent (Kidd 1997) that accounts for up to 90% of the total low molecular weight cellular thiols (Hansen et al. 2006). Cadmium shows a high affinity for GSH which is abundant in most organ systems, especially in the liver, where Cd induces GSH depletion (Dudley and Klaassen 1984). Glutathione acts by scavenging Cd to prevent its interaction with critical cellular targets (Kamiyama et al. 1995; Rana and Verma 1996; Waisberg et al. 2003). During acute Cd exposure, GSH contents undergo a sharp depletion. In contrast to these findings, chronic exposure leads to an elevation in tissue GSH levels, which in turn diminishes the oxidative damage by Cd. This can be explained by an initial Cd-induced GSH depletion which is immediately counteracted via an increased GSH synthesis. During prolonged Cd exposure, the system probably fails to respond to the rising demands (Ercal et al. 2001). Differences in intracellular GSH level regulation between acute and chronic Cd exposure are a possible underlying cause for the distinct redox signatures observed in both situations (Liu et al. 2009). The observed effects on GSH metabolism are, however, not unambiguous as only few effects of Cd on the GSSG/GSH ratio were described (Hansen et al. 2006; Picaud and Desbois 2006; Thijssen et al. 2007).

Metallothioneins are low molecular weight (6 kDa) proteins rich in cysteines (20–30%). The induction of MT by Cd has been mentioned as a "double edged sword". MTs bind and detoxify Cd, but increased oxidative stress produces a concomitant decrease in metal binding stability, hereby promoting the dissociation of metal ions from MT (Jiménez et al. 1997). Nevertheless, they also act as a scavenger for $^{\bullet}OH$ and $O_2^{\bullet-}$ in vitro (Klaassen et al. 1999). The multiple cysteine residues of MT are known to be oxidized during oxidative stress, and the subsequent release of zinc has been proposed to be vital in the protection against oxidative damage (Maret and Vallee 1998). There are several studies pointing out that the sensitivity of tissue specific and species specific variation to Cd toxicity is related to the amount of MT (IARC 1993; Suzuki et al. 1998; Liu et al. 2001). The high amount of MT ensures protection against Cd induced lung carcinogenesis to mice while the relative lack of MT induction is responsible for the high susceptibility to Cd-induced pulmonary carcinogenesis in rats (IARC 1993). Based on several similar studies, it has also been concluded that the protective effect of MT depends on the distribution of Cd, inducibility of MT genes, and finally the levels of MT in various tissues and species (Waisberg et al. 2003).

Cadmium: an oxidative challenge

Cadmium interferes with the structure and function of several molecules with marked endpoints such as cell death and (uncontrolled) cell proliferation. Milder forms of Cd stress, however, can also result in the activation of cellular repair mechanisms (Fig. 2). In general, Cd induces both damaging as well as protective signaling pathways, but the exact underlying mechanisms remain to be resolved. At the cellular level, a common mechanism in both Cd-induced damage and repair processes is oxidative stress. The cellular responses against oxidative stress balance between cell death and cell proliferation, and signaling molecules such as p38-MAPK (Mitogen-Activated Protein Kinase) and JNK (Jun N-terminal Kinase) are involved in both stress-induced processes (Seifried et al. 2007). What the exact role of ROS in the activation of signal transduction pathways involved in defense mechanisms during Cd stress is, still needs to be clarified.

The mechanisms of Cd-induced cytotoxicity are under intense investigation. Several studies focus on apoptosis as an important process mediating Cd-toxicity in different organs (Hamada et al. 1997; Shih et al. 2004; Oh and Lim 2006; Coonse et al. 2007; Lasfer et al. 2008; Hossain et al. 2009; Wang et al. 2009; Yeh et al. 2009; Zhou et al. 2009), but non-apoptotic cell death is also apparent (Kim et al. 2010; Belyaeva et al. 2006, 2008; Sancho et al. 2006). When applied in low to moderate concentrations under in vivo and in vitro conditions, Cd mainly causes apoptosis (Sancho et al. 2006). The exact mechanisms of execution (extrinsic, intrinsic or caspase-independent pathways), however, remains controversial as several investigations using different cell types yield diverse outcomes (Li et al. 2000; Kondoh et al. 2002; Lemarié et al. 2004; Shih et al. 2004; Coutant et al. 2006; Lee et al. 2006; Oh and Lim 2006; Sancho et al. 2006; Hossain et al. 2009). However, exposure to Cd also causes necrotic cell

death, characterized by cell membrane disintegration followed by dissemination of intracellular contents (Ishido et al. 2002; Sancho et al. 2006). Both apoptosis and necrosis can be induced by increased accumulation of ROS (Fleury et al. 2002; Hossain et al. 2009), and increased lipid peroxidation after Cd-exposure has been shown several times (Hussain et al. 1987; Yiin et al. 2000; Jurczuk et al. 2004; Babu et al. 2006; Kotelnikova et al. 2008; Slyuzova et al. 2008). A disturbed redox balance can, however, also induce uncontrolled cell proliferation and Cd carcinogenicity seems to be crucially mediated by the production of ROS (Waisberg et al. 2003). Overall, different opinions exist regarding the accelerating and/or inhibitory effect of ROS on cell proliferation mechanisms (Kawata et al. 2009).

Besides damaging, Cd-induced ROS formation also exert a positive role as ROS influence cell growth and induce biological repair mechanisms for Cd- and ROS-induced damage. Reactive oxygen species play crucial roles in normal physiological processes and are ideal signaling molecules as they are small and able to diffuse over short distances (Halliwell 2006; Seifried et al. 2007).

Cadmium-induced ROS can interact with the cellular defense via the activation of MAPKs and other signaling pathways (Fig. 2). As a consequence, genes coding for molecules involved in the biological defense and cellular repair, including antioxidants such as MT, heme oxygenase 1 (HO-1) and TrxR but also heat shock proteins (HSP) and other chaperones, are markedly upregulated (Li et al. 2008; Nishitai and Matsuoka 2008; Valbonesi et al. 2008; Yamada et al. 2009). The exact mechanisms of activation remain to be elucidated, but the involvement of redox-sensitive transcription factors, such as Nfr2 (nuclear factor erythroid 2 related factor 2), AP-1 (activation protein-1) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), is suggested (Hart et al. 1999; Liu et al. 2002; Qu et al. 2005; Yang et al. 2007; Chen et al. 2008; Li et al. 2008). The antioxidative defense system, for example, is activated via Nfr2 binding to antioxidant responsive elements (Chen et al. 2008). Some researchers suggest a direct involvement of Cd, as HO-1 (heme oxygenase 1) and HSP70 are shown to be regulated via Cd- and metal-responsive elements (CdRE/MRE) (Koizumi et al. 1994, 2007).

Among the various signaling pathways involved in Cd stress, MAPK and Ca^{2+} signaling play an important role (Thévenod 2009 and references herein). The activation of the p38-MAPK signal cascade during Cd stress leads to both pro- and anti-apoptotic events as it can trigger both caspase-3 and HSP70 (70 kD Heat Shock Proteins) in different organisms (Kefaloyianni et al. 2005; Valbonesi et al. 2008; Souza et al. 2009). Cd increases JNK signaling via changes in $[Ca^{2+}]_{cyt}$ fluxes and/or changes in the cellular redox balance (Matsuoka and Igisu 1998; Haberstroh and Kapron 2006; Chen et al. 2008). Neither the exact sequence of events nor its specific role is clear although several studies indicate the involvement of the JNK signaling pathway in Cd-induced apoptosis (Papadakis et al. 2006; Qu et al. 2006). Reactive oxygen species produced in response to Cd also contribute to the cellular defense via the activation of the STAT3 (Signal Transducer and Activator of Transcription 3) - ERK (Extracellular Signal-Regulated Kinase) signaling pathway (Souza et al. 2004; Nishitai and Matsuoka 2008). They are plausibly also involved in the Cd-induced changes in cytosolic Ca-fluxes and CDK (Cyclin-Dependent Kinase), CaM (Ca^{2+}/calmodulin) and CaMK (Ca^{2+}/Calmodulin-dependent Protein Kinases) activities. The influence of Cd on cellular signaling cascades was reviewed by Thévenod (2009). The sequential kinetics of the MAPK–ROS–$[Ca^{2+}]_{cyt}$ interactions during Cd stress, however, still have to be elucidated. Both sequential and spatial interactions of these signaling pathways probably depend on the strength and duration of the Cd-exposure as well as on the influence of the surrounding environment. These interaction differences, as well as the activation of different isoforms, clarify the involvement of similar signaling cascades in the induction of distinct processes such as cell growth and cell death (Nishitai and Matsuoka 2008). Moreover, we suspect a central role for the cellular redox signature herein, as small differences in ROS specificity, concentration and location can lead to such diverse responses. Earlier research on different organisms indicates an immediate ROS production after Cd-exposure and knock-down-experiments confirm the above hypothesis (Chen et al. 2008; Yokouchi et al. 2008). Nevertheless, ROS production can be maintained and/or inhibited via the presence of positive and negative amplification loops (Fig. 2) (Mittler et al. 2004).

In conclusion, Cd is not redox-active, but it anyway clearly imposes an oxidative challenge to cells in organisms exposed to mild or severe Cd stress. Although oxidative stress is a general phenomenon occurring in different organisms under many stress conditions, attention should be given to the specificity of the ROS signature, spatial and temporal, in order to understand and to reveal the exact role of Cd-induced ROS production in the modulation of downstream responses, i.e. damage and/or signaling.

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