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Role of oxidative stress in cadmium toxicity and carcinogenesis

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Abstract

Cadmium (Cd) is a toxic metal, targeting the lung, liver, kidney, and testes following acute intoxication, and causing nephrotoxicity, immunotoxicity, osteotoxicity and tumors after prolonged exposures. Reactive oxygen species (ROS) are often implicated in Cd toxicology. This minireview focused on direct evidence for the generation of free radicals in intact animals following acute Cd overload and discussed the association of ROS in chronic Cd toxicity and carcinogenesis. Cd-generated superoxide anion, hydrogen peroxide, and hydroxyl radicals *in vivo* have been detected by the electron spin resonance spectra, which are often accompanied by activation of redox sensitive transcription factors (e.g., NF-κB, AP-1 and Nrf2) and alteration of ROS-related gene expression. It is generally agreed upon that oxidative stress plays important roles in acute Cd poisoning. However, following long-term Cd exposure at environmentally-relevant low levels, direct evidence for oxidative stress is often obscure. Alterations in ROS-related gene expression during chronic exposures are also less significant compared to acute Cd poisoning. This is probably due to induced adaption mechanisms (e.g, metallothionein and glutathione) following chronic Cd exposures, which in turn diminish Cd-induced oxidative stress. In chronic Cd-transformed cells, less ROS signals are detected with fluorescence probes. Acquired apoptotic tolerance renders damaged cells to proliferate with inherent oxidative DNA lesions, potentially leading to tumorigenesis. Thus, ROS are generated following acute Cd overload and plays important roles in tissue damage. Adaptation to chronic Cd exposure reduces ROS production, but acquired Cd tolerance with aberrant gene expression plays important roles in chronic Cd toxicity and carcinogenesis.

Keywords

Cadmium; reactive oxygen species; spin-trapping technique; chronic toxicity; adaptation; malignant transformation

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Introduction

Cadmium (Cd) is a toxic heavy metal. In humans, acute Cd exposure *via* inhalation results in pulmonary edema and respiratory tract irritation, while chronic exposure often leads to renal dysfunction, anemia, osteoporosis, and bone fractures (Friberg et al., 1986). Cd is also a potent carcinogen in a number of tissues, and is classified by IARC as a human carcinogen (Waalkes et al., 2003).

Reactive oxygen species (ROS) are often implicated in Cd toxicology, either in a variety of cell culture systems (Liu et al., 1990; Hart et al., 1999; Liu and Jan, 2000; He et al., 2008), or in intact animals through all routes of exposure (Manca et al., 1994; Kayama et al., 1995; Yamano et al., 2000; Amara et al., 2008). It has been suggested that the mechanisms of acute Cd toxicity involve the depletion of glutathione and protein-bound sulfhydryl groups, resulting in enhanced production of ROS such as superoxide ion, hydrogen peroxide, and hydroxyl radicals (Manaca et al., 1994; Bagchi et al., 1997; Liu and Jan, 2000). Cd-increased ROS in turn produces lipid peroxidation, and results in DNA damage. However, little is known about direct evidence and mechanism for Cd-generated radicals until recently (Liu et al., 2008).

In contrast to acute toxicity, the roles of ROS in chronic Cd toxicity and carcinogenesis have been controversial depending on experimental conditions. For example, ROS has been implicated in chronic Cd nephrotoxicity (Shaikh et al., 1999), immunotoxicity (Ramirez and Gimenez, 2003), and carcinogenesis (Waisberg et al., 2003). Cd-induced ROS could overwhelm the antioxidant defenses, leading to increased lipid peroxidation and oxidative DNA damage (Bagchi et al., 1997; Waisberg et al., 2003). On the other hand, administration of Cd to animals at low levels for one year increases hepatic and renal glutathione levels, without elevations in tissue lipid peroxidation levels (Kamiyama et al., 1995). Cd-induced lipid peroxidation and increases in hepatic iron are evident 24 hr after a single oral dose, but these changes diminish after repeated Cd exposures (Djukic-Dosic et al., 2008). A biphasic ROS response to Cd exposure through the drinking water has also been proposed (Thijssen et al., 2007). ROS and ROS-related gene expression occur right after Cd exposure, but return to normal levels after 8 weeks of exposure (Thijssen et al., 2007). Similarly, ROS appears to play a minimal role in chronic Cd-induced malignant transformation in rat liver cells (Qu et al., 2005).

This minireview will first focus on studies using the spin-trapping technique in conjunction with electron spin resonance (ESR) for ROS detection following acute Cd overload, and discuss the roles of ROS in acute and chronic Cd toxicity and carcinogenesis, emphasizing Cd adaptation mechanisms.

Oxidative stress and acute Cd overload

ESR evidence for Cd-induced free radical generation

The spin-trapping technique involves a reaction of a short-lived radical with a paramagnetic compound (spin trap) to form a relatively long-lived free radical product (spin adduct), which can then be detected using conventional ESR. The intensity of the ESR signal is used

to measure the radicals trapped by the spin-trapping compound, and the hypertine couplings of the spin adduct are generally used to characterize the originally trapped radicals. Liver is the major target of Cd, and the trapped Cd-radical adducts can be excreted into the bile. Thus, biliary analysis of trapped radicals reflects the Cd-generated radicals in the liver (Kadiiska and Mason, 2000). The spin-trapping agent α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (POBN) is given immediately after Cd injection into bile-duct cannulated rats, and the POBN-radical adducts can be detected in the bile. Cd overload increases the POBN-trapped radical adducts (Fig. 1), including the generation of superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Using ^{13}C DMSO as an isotope substitution technique, the formation of POBN/methyl radical adduct (POBN/$^{\bullet 13}CH_3$) and POBN/lipid peroxides (POBN/$^{\bullet}$L) can also be identified in the bile of Cd overload animals (Liu et al., 2008).

The Cd-generated radical adducts can be detected in tissue extracts, using N-tert-butyl-alpha-phenylnitrone (PBN) as the spin-trapping agent (Kadiiska and Mason, 2002). Simultaneously injection of Cd with and PBN significantly increases the ESR signals of PBN-trapped radical adducts in the liver of mice (Liu et al., 2002). The extraction of PBN-trapped radicals from the liver in the presence of 2,2′-dipyridyl can prevent *ex vivo* radical formation with trace transition metals (Kadiiska and Mason, 2002). Cd-generated radicals can also be detected with the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) in cultured cells (Yang et al., 2007), in isolated mitochondria (Wang et al., 2004), or in the cell-free system in the presence of metallothionein (O'Brien and Salacinski, 1998).

Oxidative stress has been implicated in Cd toxicology, but the majority of studies used indirect measures for ROS. The ESR studies provide direct evidence to identify Cd-generated free radicals *in vitro* and *in vivo* as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydoxy radical ($^{\bullet}$OH) and lipid peroxides ($^{\bullet}$L), as illustrated in Fig. 3.

Factors modulating Cd-induced free radical generation trapped in vivo

Cd is a redox-stable metal; therefore, radical production by Cd must be mediated through some indirect mechanisms. One proposed mechanism by which Cd may generate free radicals is the disruption of the cellular antioxidant systems. Glutathione (GSH) is abundant in the liver and is thought to be the first line of defense against Cd hepatotoxicity as Cd binds tightly to thiol groups, and depletion of hepatic GSH by diethyl maleate significantly enhances Cd-induced hepatotoxicity (Dudley and Klaassen, 1984). Depletion of hepatic GSH by diethyl maleate also dramatically enhances Cd-generated POBN-radical adduct signals in the bile (Fig. 1B), suggesting that disruption of the cellular GSH system is a key element for Cd-induced oxidative stress in the liver.

Cd-induced inflammation in the liver is another important mechanism for Cd-induced oxidative stress (Kayama et al., 1995; Yamano et al., 2000). The activation of Kupffer cells, the resident macrophages of the liver, is an important source for Cd-induced inflammatory mediators such as IL-1β, TNF-α, IL-6, and IL-8 (Kayama et al., 1995; Yamano et al., 2000). Inhibition of Kupffer cell function with gadolinium chloride has been shown to decrease Cd hepatotoxicity in rats (Sauer et al., 1997) or in mice (Harstad and Klaassen, 2002). Pretreatment of rats with gadolinium chloride abolishes Cd-increased POBN-adduct signals

(Fig. 1C), suggesting that Kupffer cells could release inflammatory cytokines in response to Cd overload, which in turn contribute to Cd-generated free radicals in the liver.

The Fenton reaction driven with iron is an important source for free radical generation. The iron chelator Desferal has been shown to decrease the POBN-radical ESR signals in the bile of rats treated with formate (Dikalova et al., 2001) or aflatoxin B1 (Towner et al., 2003), presumably by inhibition of iron-driven Fenton reaction in the liver. Desferal can also abolish the Cd-generated POBN-radical adducts in the bile (Fig. 1D), clearly indicating the involvement of endogenous iron-dependent hydroxyl radical generation as a mechanism of Cd-induced oxidative stress.

Dimethyl sulfoxide (DMSO) has both pro-oxidant and antidote effects, depending on the dose and chemicals under investigation. For example, in plasmid pBR322 DNA, DMSO protects against Cd- and H_2O_2-induced DNA strands break (Badisa et al., 2007). DMSO treatment protects rats against the hepatotoxicity of acetaminophen, bromobenzene, and thioacetamide, but it fails to protect carbon tetrachloride-induced hepatotoxicity (Siegers, 1978), and even potentiates carbon tetrachloride-induced liver injury (Freston and Bouchier, 1967). DMSO administration decreases the formate-induced POBN-radical adducts in the rat bile (Dikalova et al., 2001), but increases Cd-induced POBN-radical adducts by 5-fold (Fig. 1E), and the formation of POBN-methyl radical adduct can be identified by ESR.

Mitochondrion is an important target of metal toxicity including Cd (Belyaeva et al., 2008). It has been proposed that Cd initially binds to protein thiols in mitochondrial membrane, affects mitochondrial permeability transition, inhibits respiratory chain reaction, and then generates ROS (Dorta et al., 2003). Cd is shown to inhibit mitochondrial complex III, resulting in accumulation of semiubiquinones at the Q_0 sites. The unstable semiubiquinones are prone to transfer one electron to molecular oxygen to form superoxide anion (Wang et al., 2004). Cd effects on mitochondrial electron transfer are the major origin for Cd generated ROS not only in mammalian cells but also in plants (Heyno et al., 2008). Selenium, an essential trace element in glutathione peroxidase, is shown to protect against Cd-induced apoptosis from ROS generated through mitochondrial dysfunction (Zhou et al., 2008).

Biological evidence for Cd-induced free radical generation

Lipid peroxidation is a major consequence of Cd-induced oxidative stress (Valko et al., 2006), and is correlated with ESR evidence in both *in vivo* (Liu et al., 2002) and *in vitro* (Yang et al., 2007). Consistent with Cd-induced free radical adduct formation and lipid peroxidation, a cascade of biological changes are evoked to overcome oxidative stress. The association of these biological changes further supports the ESR evidence for Cd-generated radical adducts.

The transcription factors such as AP-1 and NF-κB are sensitive to oxidative stress in the cell. The activation of these transcription factors by Cd has been shown in intact animals and in cultured cells (Hart et al., 1999; Liu et al., 2002; Qu et al., 2005; Yang et al., 2007). However, under different experimental conditions, a decrease in NF-κB has also been reported (Xie and Shaikh, 2006). The ROS sensitive nuclear factor E2-related factor 2

(Nrf2) is also activated by Cd-generated ROS (He et al., 2008), in an attempt to combat oxidative stress in the cell.

The mitogen-activated protein kinases (MAPK) pathways are important for signal transduction in response to oxidative stress. Mammals express at least three distinct groups of MAPKs, including extracellular signal-regulated protein kinase 1/2 (Erk1/2), c-Jun N-terminal kinase (JNK), and p38 MAPK. Cadmium is known to activate the MAPK pathway via generation of ROS (Qu et al., 2007, Chen et al., 2008). The activation of MAPKs by Cd is associated ROS production in intact animals (Liu et al., 2002; Regunathan et al., 2003) and in cultured cells (Qu et al., 2006; Chen et al., 2008), which in turn plays important role in Cd-induced apoptosis to eliminate oxidative damaged cells (Qu et al., 2007).

The expression of ROS-related genes in response to Cd-induced oxidative stress is often increased. In microarray analyses, the expression of heme oxygenase-1, oxidative stress protein A170, heat-shock proteins, and oxidative DNA damage responsive GADD45, GADD153, are increased following Cd overload; while the expression of genes encoding metabolism are generally suppressed in an attempt to switch the cellular energy to overcome oxidative stress (Liu et al., 2002; Regunathan et al., 2003; Zhou et al., 2004; Croute et al., 2005). In the cultured cells, Cd increases the expression of genes related to cellular redox status, such as heme oxygenase-1, GSH S-transferases, metallothionein (MT), NAD(P)H:quinone oxidoreductase, and other stress genes (Hart et al., 1999; Yang et al., 2007, He et al., 2008). It should be noted that MT-null mice are more sensitive than wild-type mice to Cd-induced ROS-related gene expression (Liu et al., 2002; Regunathan et al., 2003). Thus, oxidative stress defense mechanisms are activated in response to Cd overload in mammalian cells.

Oxidative stress and long-term, low-dose Cd exposure

ROS may not necessarily associate with chronic Cd exposure

Contrary to direct ESR evidence for ROS generation following acute Cd overload, ESR evidence for free radical generation following long-term, low-dose Cd exposure is often obscure. Mice are given a diet containing 100 ppm $CdCl_2$ for 6 months, followed by injection of POBN, and liver and kidneys are removed for extraction of POBN-trapped radicals. Chronic Cd exposure does not enhance the POBN-trapped radicals in the liver and kidneys, nor increase the hepatic and renal lipid peroxidation levels (data not shown). Thus, it is important to discuss the involvement of ROS production during the long-term exposure to Cd at environmentally-relevant low levels.

In chronic studies, the lack of ROS production following long-term Cd exposure is not uncommon, especially following oral Cd administration. For example, a prolonged Cd exposure (100 ppm, 23 weeks) through the drinking water does not produce overt changes in cellular redox status and lipid peroxidation levels (Thijssen et al., 2007). Dietary Cd exposure (up to 80 ppm) for one year even decreases lipid peroxidation levels in the liver and kidney of the bank vole (Wlostowski et al., 2000). A single oral dose of Cd (20 mg/kg) initially increases hepatic lipid peroxidation levels and iron concentrations 5 hr after Cd administration in mice, but repeated oral doses (10 mg/kg, daily for 14 days) produces no

change or a slightly decrease in hepatic lipid peroxidation levels (Djukic-Dosic et al., 2008). ROS tolerance is also seen with a long-term (one year) injection of Cd at low levels (0.3 mg/kg, 3 days/week), without increases in tissue lipid peroxidation levels (Kamiyama et al., 1995). In rats given chronic Cd injections (0.6 mg/kg for 12 weeks), kidney injury is evident with dramatic increase in expression of kidney injury molecule-1 and MT (Prozialeck et al., 2007), but the changes in the expressions of ROS-related genes and oxidative DNA damage genes are not appreciable.

Chronic Cd exposure, however, does increase ROS production under different experimental conditions. For example, oral administration of Cd to Sprague-Dawley rats at a daily dose of 4.4 mg/kg in drinking water increases lipid peroxidation (as determined by the formation of thiobarbituric acid reactive substances) in liver mitochondria and microsomes 15 days after administration, reaching peak between 60 and 75 days of treatment, with increased urinary excretion of lipid metabolites (Bagchi et al., 1997). Chronic injection of Cd (≈ 0.6 mg/kg, 5 days/week for 22 weeks) to Sprague-Dawley rats increases hepatic and renal lipid peroxidation levels, along with increases in tissue glutathione levels (Shaikh et al., 1999). Co-administration of antioxidants N-acetylcysteine or vitamin E prevents Cd-induced lipid peroxidation and protects animals from Cd toxicity to the liver and kidney (Shaikh et al., 1999). Many experimental variables could influence Cd-induced ROS production under chronic exposure conditions. It is hypothesized that during chronic Cd exposure, adaptation mechanisms are induced to offset Cd-induced ROS and oxidative stress. When excess ROS generation overwhelms the capability of the antioxidant defense system, lipid peroxidation and oxidative damage ensure.

Minimal role of ROS in Cd-induced malignant transformation

Cd-induced ROS is thought to be important in Cd carcinogenesis (Waisberg et al., 2003; Valko et al., 2006), either through oxidative stress or through the inhibition of oxidative DNA damage repair (Waisberg et al., 2003). However, little is known about direct evidence for ROS to be associated with Cd-induced cancers or Cd-induced malignant transformation. Exposure of liver cells to a low-dose (1.0 μM) of Cd that does not produce ROS, produces malignant transformation after 28 weeks of continuous exposure, as evidenced by forming highly aggressive tumors upon inoculation of cells into nude mice (Qu et al., 2005). To examine the role of ROS in Cd carcinogenesis, cellular ROS levels in control and Cd-transformed cells are determined using fluorescence probes, dihydroethidium for superoxide anion and 2,7′-dichlorofluorescin diacetate for hydrogen peroxide (Qu et al., 2005). Acute challenge exposure of liver cells to higher doses of Cd, ROS production is evident at doses above 10 μM Cd, and at 50 μM Cd, the fluorescence intensity is dramatically increased. In Cd-transformed cells, the intracellular levels of superoxide anion are significantly lower at basal conditions as compared to passage-matched control cells (Fig. 2), and these transformed cells were also highly tolerant to high dose of Cd (50 μM) induced ROS production, as evidenced by much weak fluorescence signals generated from the oxidation of dihydroethidium or 2,7′-dichlorofluorescin diacetate (Fig. 2). These data clearly indicate the minimal role of ROS in Cd-induced malignant transformation in rat liver cells, a target for Cd carcinogenesis (Waalkes, 2003). These Cd transformed cells also showed acquired apoptotic tolerance, with marked reduction of redox-sensitive SAPK/JNK signal

transduction pathways (Qu et al., 2006; 2007). Chronic exposure of human urothelial UROtsa cells to the low concentration (1.0 μM) of Cd produces malignant transformation, but not at higher concentrations (5 μM or 9 μM) which kill the cell (Somji et al., 2006). These Cd transformed cells showed increased resistance to Cd toxicity and oxidative DNA damage, notably, resistance to Cd-induced apoptosis (Somji et al., 2006). Similarly, acquired apoptotic resistance was also evident in chronic Cd-transformed human prostate epithelial cells, not only to Cd itself, but also to a number of anticancer agents such as etoposide and cisplatin (Achanzar et al., 2002). Thus, Cd at low, environmentally-relevant concentrations, can induce malignant transformation in rodent and human cells, and this effect is not necessarily associated with increased ROS levels in the cells. In fact, all these Cd transformed cells are tolerant to oxidative stress and show a high rate of cell proliferation (Achanzar et al., 2002; Qu et al., 2005; 2007; Somji et al., 2006). It is likely that Cd-induced apoptotic resistance is very important for Cd-induced malignancy, as the DNA damaged cells could escape from elimination through apoptosis, and proliferate with inherent DNA lesions, eventually progressing to malignant phenotype.

Cd-induced oxidative DNA damage is important for progression to neoplasia (Waalkes, 2003; Valko et al., 2006). As an epigenetic mechanism for Cd carcinogenesis, it should be noted that Cd can also induce alterations in DNA methylation status, including initially DNA hypomethylation following acute exposure (Takiguchi et al., 2003; Huang et al., 2008), and subsequently DNA hypermethylation following the long-term exposure at low doses/concentrations (Takiguchi et al., 2003; Benbrahim-Tallaa et al., 2007; Jiang et al., 2008). Cd-induced DNA hypermethylation in Cd-transformed prostate cells is associated with increases in DNA methyltransferases activity (Benbrahim-Tallaa et al., 2007) and decreases in oxidative stress and the redox-sensitive signal transduction pathways such as the JNK pathway, resulting in apoptotic resistance (Qu et al., 2007). Cd-induced DNA hypermethylation decreases the expression of tumor suppressor genes (e.g., *p16*, *RASSF1A*), which could be important in Cd-induced malignant transformation in human prostate cells (Benbrahim-Tallaa et al., 2007).

Adaptive mechanisms after chronic, low-dose Cd exposure

A number of cellular defense mechanisms are known to be activated in response to low dose of Cd exposures, among them are induction of MT, increase in cellular glutathione, activation of antioxidant transcription factor Nrf2 and other antioxidant components. These mechanisms are not mutually exclusive, but may function together in an integrated way to confer resistance to Cd-induced oxidative stress.

MT is a cysteine-rich, Cd-binding protein playing an important role in protecting Cd toxicity and in combating Cd-induced ROS through Cd sequestration or through its free radical scavenging activity (Dudley and Klaassen, 1984; Klaassen et al., 1999). Following chronic Cd exposure, tissue MT is markedly increased (Wlostowski et al., 2000; Prozialeck et al., 2007; Thijssen et al., 2007). Increased MT binds Cd in the cytosol, reducing Cd available amount to critical organelles. MT is also rich in sulfhydryl groups to trap Cd generated ROS in the cells (Klaassen et al., 1999). During chronic Cd carcinogenesis, marked elevations in MT is responsible for resistance to apoptosis and ROS in Cd transformed rat liver cells (Qu

et al., 2006), in Cd-tranformed human urothelial UROtsa35 cells (Somiji et al., 2006), or in Cd-transformed human prostate epithelial cells (Qu et al., 2007).

GSH is thought to be the first line of defense against Cd toxicity. In contrast to sharply depletion of GSH during acute Cd exposure (Dudley and Klaassen, 1984; Liu et al., 1990), chronic Cd exposure often result in elevations of tissue GSH levels (Kamiyama et al., 1995; Shaikh et al., 1999; Waisberg et al., 2003), which in turn will diminish oxidative damage from the metal. Differences in intracellular GSH level regulation between acute and chronic Cd exposure could be a major reason for differences in ROS accumulation in the cells.

Studies using supplement with N-acetyl cysteine, vitamin E, vitamin C, and selenium to enhance body anti-oxidant machinery have been shown to decrease Cd-induced oxidative stress in kidney, liver, and testes, with improved cellular and tissue functions in many *in vitro* and *in vivo* studies (Shaikh et al., 1999; Sen Gupta et al., 2004; Zhou et al., 2008). Thus, the adaptive mechanisms for Cd-induced oxidative stress would also likely involve the up-regulation of both enzymatic and non-enzymatic antioxidants in the liver to overcome Cd insults.

Nuclear factor erythroid 2-related factor 2 (Nrf2) has emerged as a key transcription factor in the transcriptional regulation of ARE-dependent gene expression in response to oxidative stimuli and a spectrum of toxic signals. Cd dramatically increased ROS production in Nrf2-null MEF cells, as evidenced by increased dihydroethium fluorescence intensity at as low as 2 μM, as compared to 50–100 μM in the wild-type cells, corresponding to increased cell death (He et al., 2008). The Nrf2-regulated genes, such as heme oxygenase-1 and NAD(P)H:quinone oxidoreductase 1, are markedly induced in wild-type cells, but not in Nrf2-null cells. Thus the activation of Nrf2 and related antioxidant components is another important adaptation mechanism in protecting against chronic Cd toxicity. In addition to the Nrf2 pathways, Cd activation of AP-1 and NF-κB pathways (Qu et al., 2005; Liu et al., 2002; Yang et al., 2007), as well as other cellular components, would contribute to adaptive response to oxidative stress.

Summary

As illustrated in Figure 3, acute Cd overload can generated free radicals, including superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydoxy radical ($^{\bullet}OH$) and lipid peroxides ($^{\bullet}L$). Cd is not a redox active metal, and free radical generation by Cd must be mediated through indirect mechanisms, including glutathione depletion, Kupffer cell activation, inflammation, and involvement of iron for the Fenton reaction. However, during long-term exposure to Cd at low doses, ROS accumulation is not necessarily associated with Cd-induced chronic toxicity and carcinogenesis. Adaptive mechanisms including induction of MT, GSH, and cellular antioxidants could diminish Cd-induced oxidative stress. Initial oxidative DNA damage and subsequent apoptotic resistance, epigenetic DNA methylation status changes, and aberrant gene expressions, all of which could play integrated roles in Cd carcinogenesis.

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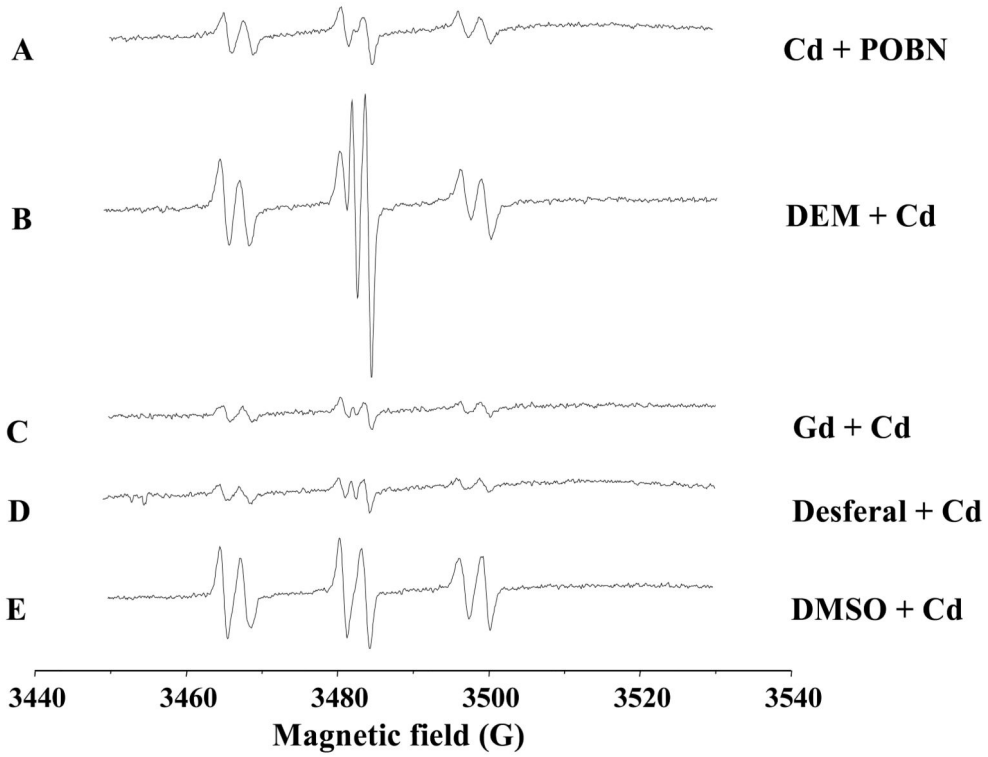

Figure 1.

Representative ESR spin-trapping evidence for cadmium-induced POBN radical adduct in bile duct-cannulated rats. After anesthesia, surgery was performed to cannulate bile duct. After the bile flow is table, rats were given CdCl2 (40 umol/kg, ip), followed by POBN (1 g/kg, ip). Bile samples were collected from 40–60 min after Cd administration and anaylyzed by ESR. (A) Cd + POBN; (B–E) Cd + POBN in rats pretreated with diethyl maleate DEM (0.85 ml/kg, 2 hr); gadolinium chloride (10 mg/kg, iv, 24 hr); Desferal (50 mg/kg, ip, 1 hr); and DMSO (2 ml/kg, ip 2 hr). Instrument settings of Burker EMX spectrometer; microwave power, 20 mW, modulation amplitude, 1G, scan time 660 s; and time constant, 1.3 s; and a significant scan 80G.

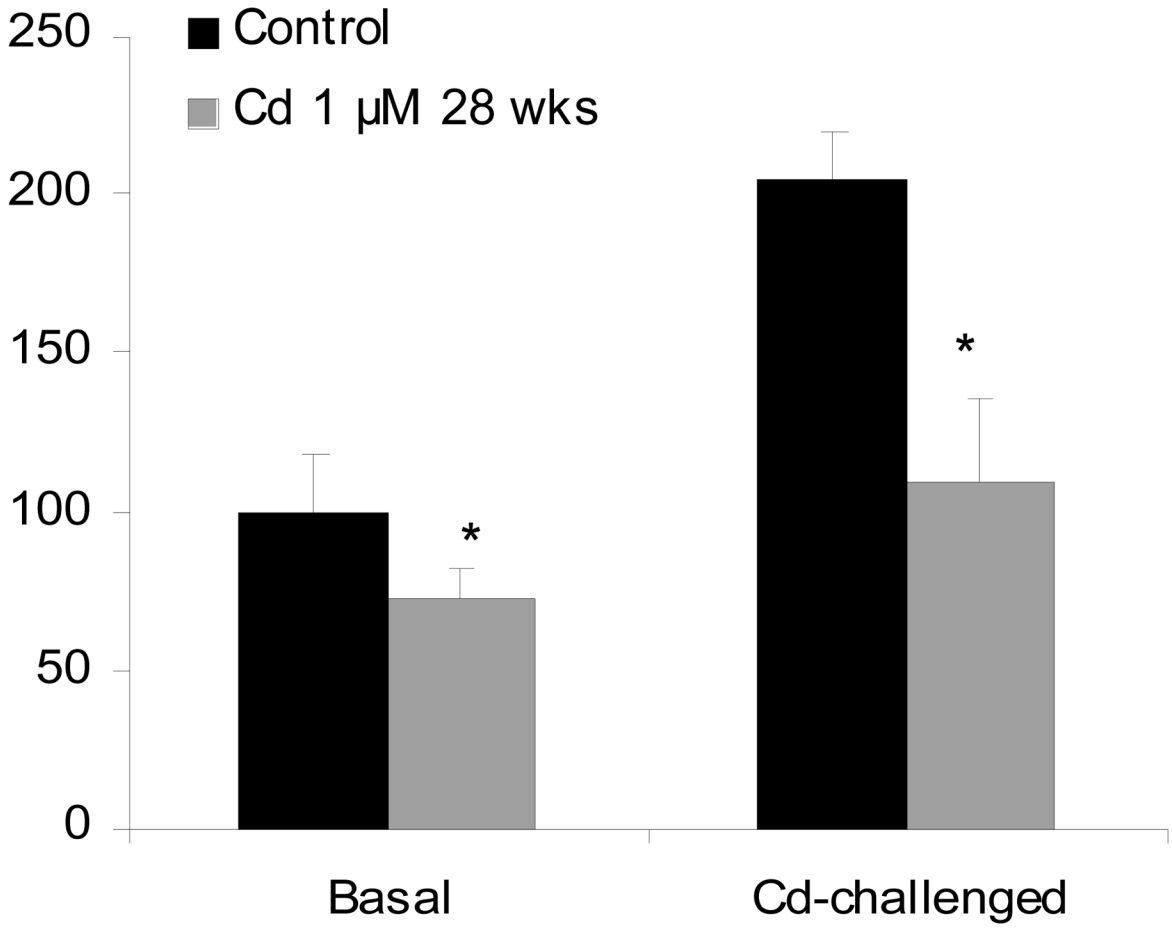

Figure 2.

Cadmium-induced ROS generation in control and Cd-transformed rat liver cells. Cells exposed to 1.0 μM Cd for 28 weeks and passage matched control cells were treated with 50 μM Cd for 60 min in the presence of 3 μM dihydroethidium for the production of superoxide anion O_2 for 20 min. The fluorescence images were captured with a confocal microscope. Data represent mean ± SEM of three determinations. *Significantly different from controls, $p<0.05$.

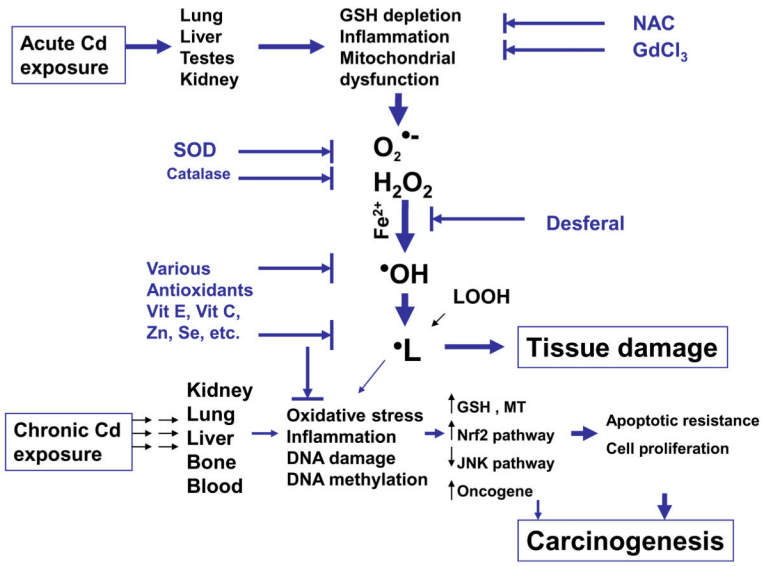

Figure 3.

Proposed pathways for ROS in Cd toxicology and carcinogenesis following acute and chronic exposures