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Cadmium generates reactive oxygen- and carbon-centered radical species in rats: 3 Insights from in vivo spin-trapping studies

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Abstract

Cadmium (Cd) is a known industrial and environmental pollutant. In the present work, an in vivo spintrapping technique was used in conjunction with electron spin resonance (ESR) spectroscopy to investigate free radical generation in rats following administration of cadmium chloride (CdCl₂, 40 μmol/kg) and the spin trapping agent α-(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN, 1 g/kg). In Cd-treated rats, POBN radical adducts were formed in the liver, were excreted into the bile, and exhibited an ESR spectrum consistent with a carbon-centered radical species probably derived from endogenous lipids. Isotope substitution of dimethyl sulfoxide [(CH₃)₂SO] with ¹³C demonstrated methyl radical formation (POBN/*¹³CH₃). This adduct indicated the production of hydroxyl radical, which reacted with [(¹³CH₃)₂SO] to form *¹³CH₃, which then reacted with POBN to form POBN/*¹³CH₃. Depletion of hepatic glutathione by diethyl maleate significantly increased free radical production, whereas inactivation of Kupffer cells by gadolinium chloride and chelation of iron by desferal inhibited it. Treatment with the xanthine oxidase inhibitor allopurinol, the catalase inhibitor aminobenzotriazole, or the cytochrome P450 inhibitor 3-amino-1,2,4-triazole had no effect. This is the first study to show Cd generation of reactive oxygen and carbon-centered radical species by involvement of both iron mediation through iron-catalyzed reactions and activation of Kupffer cells, the resident liver macrophages.

Keywords

Cadmium; Free radicals; Rat; Spin-trapping; Electron spin resonance spectroscopy

Cadmium (Cd) is a widespread toxic environmental and industrial pollutant. It is listed by the U.S. Environmental Protection Agency as one of 126 priority pollutants. In humans, the primary route of exposure is via contaminated drinking water, food supplies, or tobacco. Acute Cd exposure via inhalation results in pulmonary edema and respiratory tract irritation, whereas chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis, and bone

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fractures [1,2]. Cd is carcinogenic for a number of tissues [3] and is classified by IARC as a human carcinogen [4]. In laboratory animals, acute Cd poisoning produces primarily hepatic and testicular injury, whereas chronic exposure results in renal damage, anemia, and immuno- and osteotoxicity [2,5].

It has been suggested that the mechanism of Cd toxicity involves production of reactive oxygen species and free radicals, although the mechanism is obscure [6–8]. Exposure of cultured hepatocytes to Cd causes enhanced lipid peroxidation and depletion of cellular glutathione (GSH) [9,10]. In vitro, Cd induces the production of superoxide anion, nitric oxide, and hydrogen peroxide [11–13]. Exposure of animals to Cd by inhalation [6,14], injection [15], or ingestion [16] has been shown to induce lipid peroxidation and oxidative damage within the lungs, liver, and kidneys.

The liver is a major target tissue for Cd toxicity following acute exposure [17,18]. Cd appears to be directly toxic to hepatocytes, but also indirectly acts on these cells via activation of Kupffer cells [19]. Suppression of Kupffer cell function significantly diminishes Cd hepatotoxicity [20,21]. Direct hepatotoxic effects of Cd occur in the rough endoplasmic reticulum and nucleus of hepatocytes [22]; these lesions likely result in both apoptosis and necrosis [23]. Efforts to define indirect mechanisms for Cd hepatotoxicity have centered on induction of Kupffer cell-derived cytokines and production of NO by inducible nitric oxide synthase (iNOS) [21,22]. However, no distinct pattern has emerged with regard to Cd-induced cytokine expression as a measure of Kupffer cell activation [22]. Similarly, iNOS-knockout mice are no more resistant to Cd-induced hepatotoxicity than wild-type mice [23].

There is an increasing body of evidence that the toxicity of Cd may be associated with the production of reactive oxygen species (ROS) [24–26]. Although detailed studies in the past two decades have demonstrated that metals like cadmium possess the ability to affect the activation of various signaling pathways and to produce reactive radicals resulting in DNA damage and lipid and protein oxidation, no direct evidence for the generation of free radicals by Cd in vivo has been reported.

In the study described here we investigated the formation of free radical metabolites by CdCl₂ using an ESR in vivo spin-trapping rat model with the goal of elucidating the mechanism of free radical production in the liver by a non-redox metal. The effect of depletion of GSH in vivo prior to Cd administration was examined as well by pretreating the rats with DEM, a universal sulfhydryl-blocking agent. In addition, the effects of a number of chemicals known to modify acute Cd toxicity were examined: gadolinium chloride, a Kupffer cell inhibitor; Desferal, a ferric ion chelator; allopurinol, a xanthine oxidase inhibitor; 3-aminotriazole, a catalase inhibitor; and the cytochrome P450 inhibitor aminobenzotriazole. These chemicals were used to explore the involvement of GSH, inflammatory cells, metal ions, and different enzyme activities in the generation of free radical metabolites by Cd.

Materials and methods

Chemicals

Cadmium(II) chloride (CdCl₂), diethyl maleate (DEM), gadolinium (III) chloride hexahydrate, allopurinol, 1-aminobenzotriazole (ABT), desferrioxamine mesylate (Desferal), 3-amino-1, 2,4-triazole (AT), 2,2'-dipyridyl, and bathocuproinedisulfonic acid disodium salt hydrate were all obtained from Sigma-Aldrich (St. Louis, MO, USA). The spin-trap agent α -(4-pyridyl-1-oxide)-*N*-tert-butyl nitron (POBN) was obtained from Alexis (San Francisco, CA, USA) and Sigma. [¹³C] Dimethyl sulfoxide ([¹³C]DMSO) was obtained from Isotech (Miamisburg, OH, USA). All other chemicals were of reagent grade.

Animals and treatments

The animal study protocol was approved by the National Institute of Environmental Health Sciences Animal Care and Use Committee, and all animals received humane care in compliance with the National Research Council's criteria for humane care as outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health.

Male Fischer rats (300–400 g; Charles River Laboratories, Wilmington, MA, USA) were used in all experiments. Rats were fed a standard rodent chow (NIH open formula, Ziegler Brothers, Gardner, PA, USA). Rats were anesthetized with Nembutal (50 mg/kg, ip) and given an intraperitoneal injection of Cd (40 μ mol/kg as CdCl₂, dissolved in 2 ml saline), immediately followed by an intraperitoneal injection of POBN (1 g/kg, freshly prepared in 2 ml deionized H₂O) to the other side of the abdomen. Bile was collected by bile-duct cannulation via PE-10 tubing. The bile samples (~300 μ l) were collected every 20 min over a 2-h period into plastic Eppendorf tubes containing a 50- μ l solution of 2,2'-dipyridyl (30 mM) and bathocuproinedisulfonic acid disodium salt hydrate (30 mM) to prevent auto-oxidation catalyzed by trace transition metals [27]. The samples were frozen in dry ice immediately after collection, and ESR analysis was performed on the same day.

In a separate experiment, groups of rats were given one of the following treatments: POBN only ($n=9$), Cd only ($n=3$), or Cd+POBN ($n=10$). Other groups of rats were given the following treatments before Cd+POBN administration: DEM (0.85 ml/kg in corn oil, 2 h) ($n=9$); gadolinium chloride (GdCl₃, 10 mg/kg, in saline, iv, for 24 h) ($n=7$); Desferal (50 mg/kg in saline, ip, for 1 h) ($n=6$); allopurinol (50 mg/kg, 24 and 5 h before Cd and POBN) ($n=7$); ABT (100 mg/kg, in saline, ip, for 2 h) ($n=4$), AT (1 g/kg, ip, for 1 h) ($n=7$); DMSO (2 ml/kg, ip, for 2 h) ($n=5$); and [¹³C]DMSO (2 ml/kg, ip, for 2 h) ($n=2$). Some rats were dosed with DMSO +POBN ($n=3$) and DEM+POBN ($n=8$).

Electron spin resonance

ESR spectra were recorded on a Bruker EMX (Billerica, MA, USA) ESR spectrometer equipped with a Super High Q cavity, using the following settings: microwave power, 20 mW; modulation amplitude, 1 G; conversion time, 0.6 s; and time constant, 1.3 s. Spectra were recorded on an IBM-compatible computer interfaced to the spectrometer. Hyperfine coupling constants were determined by using a computer spectral simulation program (<https://dir-apps.niehs.nih.gov/stdb/index.cfm>). Relative ESR signal intensity was measured from the height of the first line of recorded POBN radical adduct spectra.

Measurement of Cd and Fe concentrations in bile and liver

Samples from bile and liver were digested in nitric acid at 70°C overnight and diluted in distilled water. Cd concentrations in the bile were determined using an atomic absorption spectrometer (Perkin–Elmer, Norwalk, CT, USA) and expressed as micrograms per milliliter of 1 bile or micrograms per gram of liver.

Statistical analysis

Data were means $\pm$ SEM. Data were analyzed using one-way ANOVA, followed by Duncan's multiple range test or by Student's *t* test when appropriate. Differences were considered significant when $P<0.05$.

Results

Detection of Cd-induced radicals in rat bile

Acute Cd overload was induced in rats by injection of CdCl₂. When POBN was administered with CdCl₂, a six-line ESR spectrum of POBN radical adduct was detected in bile collected between 40 and 60 min after administration (Fig. 1A). In the absence of Cd exposure, only a residual signal of POBN radical adduct was observed (Fig. 1B), confirming the dependence on Cd of this POBN radical adduct. Likewise, when the spin-trapping agent was not included, there was no six-line ESR signal of a radical adduct, but only the ESR signal of the ascorbate semidione radical (Fig. 1C). A rat that had been pretreated with DEM and then treated with CdCl₂ showed a more intense six-line spectrum (Fig. 1D).

The Cd–POBN radical signal was detectable in the bile as early as 20 min after Cd administration and gradually increased, reaching a peak between 40 and 60 min and thereafter gradually decreasing for the next 3 h (data not shown). Thus, the ESR signals at the 40- to 60-min interval after Cd administration were used for further quantitative comparisons.

Because isotope substitution may allow rigorous identification of free radicals trapped in complex biological systems, experiments were performed using [¹³C]DMSO. The ¹³C-hyperfine splittings observed in the wings of each of the lines of the nitroxide triplet are proof that the radical adduct was derived from [¹³C]DMSO and could not have been derived from any other carbon source (Fig. 2A). The computer simulation of the 12-line spectrum (Fig. 2B) showed clearly that it contained two radical species. The major radical adduct with hyperfine coupling constants $a^N=16.06$ G, $a^H=2.87$ G, and $a^{13C}=4.86$ G (Fig. 2C) was assigned to POBN/¹³CH₃ based upon comparison of these splitting constants with the literature [28–31]. The unequivocal identification of the POBN/methyl radical adduct in the bile of rats 1 h after CdCl₂ administration indicates production of the hydroxyl radical. The hyperfine coupling constants of the second radical species (Fig. 2D) were $a^N=15.62$ G and $a^H=2.97$ G ($n=4$). Radical adducts with these coupling constants are formed during lipid peroxidation and by the one-electron reductive decomposition of lipid hydroperoxides [28–31]. Therefore, this radical was assigned as POBN/L.

Factors modulating Cd-induced POBN radical adducts in the bile

To study the mechanism of free radical generation by CdCl₂, we tested several agents and enzyme inhibitors known to alter Cd toxicity in animals. These were administered to the rats before treatment with CdCl₂ and POBN (Fig. 3). GSH has been suggested to be an important component of Cd-induced liver injury as prior depletion of hepatic GSH with DEM markedly enhanced Cd-induced hepatotoxicity in rats. Thus, experiments by Dudley and Klaassen [18] demonstrated that maximal hepatic depletion of GSH (>85%) occurs 2 h after DEM at a dose used in this study (0.85 mg/kg, ip). Pretreatment of rats with DEM in our study resulted in a significantly increased ESR signal intensity of the POBN radical adduct as compared with that of Cd alone (Fig. 1A, Fig. 3). It should be mentioned that DEM without Cd may increase the POBN radical signal by 1.6-fold (data not shown) as compared with a 3.5-fold increase in the presence of Cd.

Pretreatment of rats with gadolinium chloride, an inhibitor of Kupffer cell function that protects against Cd hepatotoxicity [32,33], significantly diminished Cd-induced POBN radical signals to control levels (Fig. 3). Similarly, pretreatment with Desferal, a ferric ion chelator that decreases Cd-dependent lipid peroxidation [34], significantly reduced the ESR signal intensity (Fig. 3). Quantitatively, Cd treatment doubled the basal ESR signal for POBN adducts, which was significantly increased (3.5-fold) by DEM pretreatment, but decreased by GdCl₃ (93%) and Desferal (75%) (Fig. 3). However, Cd induced POBN radical adduct formation was not

significantly changed when rats were pretreated with ABT, a suicide substrate of cytochrome P450s; with allopurinol, an inhibitor of XANTHINE OXIDASE; or with the catalase inhibitor AT (Fig. 3).

Cd and Fe concentrations in liver and bile

To further examine the factors influencing Cd-dependent POBN radical generation, Cd and Fe concentrations in the liver and bile were determined by atomic absorption spectrometry. Much higher liver concentrations of both Cd ($914.1 \pm 85.0 \mu\text{g/g}$, $n=3$) and Fe ($27.0 \pm 6.7 \mu\text{g/g}$, $n=3$) were detected in the liver of Cd treated rats than in the liver of the corresponding nontreated controls (11.6 ± 5.3 and $12.4 \pm 2.3 \mu\text{g/g}$, $n=3$ for each control group). At the 60-min time point, there were significant amounts of Cd excreted into the bile (Fig. 4). DEM decreased biliary Cd excretion by more than 80% to $\sim 5 \mu\text{g/ml}$. Desferal and gadolinium chloride had no effect on the levels of biliary Cd. In addition, Cd modestly increased the amount of Fe excreted into the bile (Fig. 4). Desferal significantly increased biliary Fe excretion to almost $90 \mu\text{g/ml}$, whereas DEM and gadolinium chloride had no effect on the level of biliary Fe (Fig. 4).

Discussion

Literature data from in vitro and in vivo studies demonstrate that Cd cations induce an oxidative stress that results in oxidative deterioration of biological macromolecules. Cadmium depletes glutathione and protein-bound sulfhydryl groups, resulting in enhanced production of reactive oxygen species such as superoxide, hydroxyl radicals, and hydrogen peroxide. These reactive oxygen species result in increased lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression, and apoptosis [35].

The present study demonstrates that acute Cd exposure induces in vivo hepatic free radical generation as evidenced by POBN-trapped radical metabolites formed in the liver and excreted into the bile. ESR analysis of the POBN adducts in a bile sample has been established as a valid measure of radical production in the liver of intact animals, as with formate intoxication [27] or aflatoxin B1-induced hepatotoxicity [36], among many other similar examples. Thus, the ESR analysis of the bile samples in this study provides evidence for Cd-induced radical generation in vivo. The data showing altered radical production after treatment with various chemical agents known to affect Cd hepatotoxicity also support the mechanistic relevance of radical formation in in vivo hepatotoxicity in rats.

Cd is a redox-stable metal; therefore, radical production by Cd must be mediated through some indirect mechanism(s). One proposed mechanism by which Cd may generate free radicals is disruption of the cellular antioxidant defense systems. 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 [37,38]. It has been shown that depletion of hepatic glutathione by DEM significantly enhances Cd-induced mortality and hepatotoxicity [18]. In the present study, GSH depletion by DEM markedly enhanced Cd-induced POBN radical adducts, consistent with its aggravation of Cd hepatotoxicity [18,38]. In addition, GSH depletion reduced biliary excretion of Cd, thereby increasing its accumulation in the liver, where it could continue to exert its hepatotoxic effects. Consistent with the role of glutathione in Cd-induced free radical generation, the glutathione precursor *N*-acetylcysteine is effective in the reversal of Cd-induced oxidative stress and protects against Cd-induced toxicity to the liver [39] and kidney [15]. The present work indicates that disruption of the cellular glutathione system is a key element in the mechanism of Cd-induced oxidative stress in the liver.

Another important mechanism of Cd-induced oxidative stress is thought to be mediated through the activation of Kupffer cells, the resident macrophages of the liver. Cd-activated Kupffer

cells and/or neutrophils will then release inflammatory cytokines, such as tumor necrosis factor α , interleukin-1, interleukin-6, and interleukin-8 [40–42]. Gadolinium chloride, an inhibitor of Kupffer cell function, protects against Cd hepatotoxicity in rats [32] and in mice [33]. The present study shows that gadolinium chloride decreases Cd-induced POBN radical adduct formation, suggesting that in vivo Cd-induced radical formation, or at least part of it, depends on Kupffer cell and/or neutrophil activation in the liver. The significant decrease in radical adduct formation from the reaction of hydroxyl radical with DMSO in vivo by GdCl_3 indicates that Kupffer cells participate as the primary source of free radicals. This is the most realistic interpretation of our data, as Kupffer cells are located in the sinusoidal space, and it is possible that these radicals could be transported to the bile. We detected radicals in the bile, which allows us to assume the role of Kupffer cells in their production and not the effect of other macrophages. Moreover, others have demonstrated that reduction of the inflammatory response by the inhibition of Kupffer cell activation by GdCl_3 plays a significant role in protecting against Cd hepatotoxicity [20].

The iron chelator Desferal has been shown to decrease formate-induced [27] and aflatoxin B1-induced [36] radical adducts in rat bile, presumably by inhibition of iron-driven Fenton reactions in the liver. In the present study, Desferal significantly decreased radical generation induced by acute Cd exposure, suggesting the involvement of endogenous iron-dependent hydroxyl radical generation. Desferal has been shown to decrease Cd-induced lipid peroxidation and oxidative damage to the rat adrenal gland [34]. Although Cd is not a redox-active metal and cannot directly participate in Fenton reactions, Cd could indirectly produce radicals by displacing iron or copper from their normal cellular sites of storage or use. In addition, released iron and/or copper could alter the functional status of Kupffer cells by enhancing their respiratory burst to produce oxidative stress [43].

Other cellular oxidant components such as xanthine oxidase, cytochrome P450 oxidase, and catalase have also been proposed to play a role in Cd-induced oxidative stress [44,45]. Indeed, the xanthine oxidase inhibitor allopurinol and the generalized cytochrome P450 oxidase inhibitor ABT have been shown to suppress formate-induced POBN radical adduct formation in formic acid-dosed rats [27]. However, ABT did not prevent alcohol-induced liver damage in rodents [46]. We attribute lack of effect of 3-AT on free radical generation by Cd to the fact that there are other enzymes such as glutathione peroxidases that are capable of metabolizing H_2O_2 . In the present study, none of these chemicals significantly altered Cd-induced generation of radical adducts in the bile, although they had a tendency to attenuate it. These results suggest that the roles of xanthine oxidase, cytochrome P450 oxidase, and catalase in Cd-induced hepatic radical formation are not significant.

Evidence for hydroxyl radical formation in vivo due to acute Cd exposure is provided by spin trapping of the methyl radical resulting from the scavenging of hydroxyl radical by DMSO ($\text{DMSO}/\cdot\text{OH}$). The experiment with $[^{13}\text{C}]\text{DMSO}$ confirmed that, indeed, a POBN/ $\cdot\text{CH}_3$ radical adduct, whose source was a result of the reaction between DMSO and $\cdot\text{OH}$, and an additional unidentified POBN adduct, whose source was probably one or more endogenous lipid biomolecules, were being detected after Cd exposure. With LC/ESR in another study, two other POBN adducts, $\cdot\text{CH}_2\text{OH}$ and $\cdot\text{CH}_2\text{S}(\text{O})\text{CH}_3$, were identified in vitro. Both radical species were formed from DMSO in addition to the $\cdot\text{CH}_3$ adduct [47]. The inhibitory effect of gadolinium chloride and Desferal on radical yields confirms a role for the hydroxyl radical in producing the species detected in the bile samples.

In this study, we propose that the formation of the POBN radical adducts is mediated by the hydroxyl radical, which, in turn, is generated through the reduction of hydrogen peroxide by a Cd-liberated transition metal, presumably iron. The present investigation demonstrates, for the first time, strong evidence for the formation of hydroxyl radical, through the detection of

the POBN/ $^{13}\text{CH}_3$ radical adduct from ^{13}C DMSO, and lipid-derived radicals from enhanced lipid peroxidation presumably initiated by $\cdot\text{OH}$ radical.

In vivo free radical formation by Cd requires involvement of both iron mediation through iron-catalyzed reactions and liver Kupffer cell activation, as both inhibitors, Desferal and GdCl_3 inhibited the radical yield (Scheme 1). In addition, our experimental evidence indicates that glutathione and/or protein sulfhydryl depletion triggers free radical production to a much greater extent as shown in Fig. 1D and Scheme 1.

In conclusion, in the ESR spectroscopic and spin-trapping studies presented here, we identify two radical species not known to be involved in the Cd stress response in vivo. Even though the mechanism for Cd-induced oxidative stress is complicated, it is at least, in part, closely correlated with depletion of cellular SH groups and/or glutathione. Another important factor is the minimization of POBN adduct formation in response to Kupffer cell inhibition and iron chelation, thereby suggesting a specific molecular mechanism involving stimulation of Kupffer cells by Cd and Fe participation. As discussed above, the identity of the POBN radical adducts generated from Cd in vivo seem to result from a combination of a methyl radical, from the reaction of ^{13}C DMSO with hydroxyl radical, and a lipid radical resulting from lipid peroxidation. These data support the suggested mechanism involving stimulation of Kupffer cells by Cd with Fe participation. One interesting issue brought up in this study is the question of how non-redox active metals may generate free radicals and induce oxidative stress. Even though Cd^{2+} is in a stable redox state, its ability to generate free radical species in an experimental animal model opens new avenues of research beneficial in medicinal applications to reactive oxygen species-associated environmental toxicity and diseases.

Abbreviations

iNOS, inducible nitric oxide synthase; ESR, electron spin resonance spectroscopy; POBN, α -(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone; DEM, diethyl maleate; ABT, aminobenzotriazole; AT, 3-amino-1,2,4-triazole; DMSO, dimethyl sulfoxide.

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Fig. 1.

ESR spin-trapping evidence for cadmium-induced, POBN radical adduct formation in vivo. Bile duct-cannulated rats were given CdCl_2 ($40 \mu\text{mol/kg}$, i), immediately followed by POBN (1 g/kg , ip). Bile samples were collected from 40 to 60 min after Cd administration and analyzed by ESR as detailed under Materials and Methods. (A) Cd+POBN. (B) POBN only. (C) Cd only. (D) pretreatment of rats with DEM (0.85 ml/kg , ip). Instrumental settings of Bruker EMX spectrometer: microwave power, 20 mW ; modulation amplitude, 1 G ; scan time 660 s ; time constant, 1.3 s ; and a single scan of 80 G .

Fig. 2.

Identification of Cd-induced, POBN radical adduct formation in vivo. Bile duct-cannulated rats were pretreated with [^{13}C]DMSO (2 ml/kg, ip) 1 h before receiving CdCl_2 (40 $\mu\text{mol/kg}$, ip) and POBN (1 g/kg, ip). Bile samples were collected from 40 to 60 min after Cd administration, and analyzed by ESR. (A) [^{13}C]DMSO+Cd+POBN. (B) composite computer simulation of the spectrum in (A). (C) computer simulation of the POBN/ $^{13}\text{CH}_3$ radical adduct. (D) computer simulation of the spectrum from endogenous POBN/lipid-derived radicals. Instrumental settings of Bruker EMX spectrometer: microwave power, 20 mW; modulation amplitude, 1 G; scan time 660 s; time constant, 1.3 s; and a single scan of 80 G.

Fig. 3. Quantitative analysis of ESR signal intensity in bile samples collected from 40 to 60 min after Cd (40 μ mol/kg, ip) and POBN (1 g/kg, ip). Rats were pretreated with DEM (0.85 ml/kg, ip), GdCl₃ (10 mg/kg, iv), Desferal (50 mg/kg, ip), AT (1 g/kg, ip), allopurinol (50 mg/kg, ip), and ABT (100 mg/kg, ip). Data represent means $\pm$ SEM. *Significant difference ($P<0.05$) from control not treated with Cd; #significant difference from rats treated with Cd alone. Number of rats (n) used in each group is given under Materials and Methods.

Fig. 4.

Biliary Cd and Fe excretion. Cd and Fe content in the bile was collected from 40 to 60 min in rats after Cd (40 µmol/kg, ip) and POBN (1 g/kg, ip) injections. Rats were variously pretreated with DEM (0.85 ml/kg, ip), GdCl₃ (10 mg/kg, iv), Desferal (50 mg/kg, ip), or Cd alone and untreated (Control). Total Cd and total Fe concentrations were determined by atomic absorption spectrometry. Data represent means±SEM. *Significant difference ($P<0.05$) from control; #significant difference from rats treated with Cd alone. Number of rats (n) used in each group is given under Materials and Methods.

Scheme 1.

Proposed pathway for free radical generation by Cd in an experimental rat model of cadmium overload.