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Hydroxyl radical production and oxidative damage induced by cadmium and naphthalene in liver of *Carassius auratus*

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Abstract

Freshwater goldfish (*Carassius auratus*) were exposed to cadmium (Cd) from 0 to 5 mg/L, and naphthalene (NAP) from 0 to 50 mg/L. Twenty-four hours after the exposure, reactive oxygen species (ROS) was trapped by phenyl-*tert*-butyl nitrone and detected by electron paramagnetic resonance (EPR). Protein carbonyl (PCO) and lipid peroxidation (LPO) content were determined. The activities of superoxide dismutase (SOD) and catalase (CAT) were also measured. The EPR spectra signals were characterized by prominent six-line spectra, which were defined as hydroxyl radical (·OH). As compared to the control group, Cd and NAP significantly induced ·OH production marked by the intensity of the prominent spectra at higher concentrations. Both xenobiotics also increased LPO content and PCO content, depending on the concentrations. Either LPO or PCO content showed significant relation with ·OH production. Cd increased the activity of SOD and decreased that of CAT at 5 mg/L, and NAP increased the activities of SOD and CAT at 5 mg/L. The results clearly indicated that these two structurally different non-redox cycling xenobiotics could induce ·OH generation and result in oxidative damage in liver of *C. auratus*, and these effects were concentration-dependent.
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Keywords: Cadmium; *Carassius auratus*; Electron paramagnetic resonance; Hydroxyl radical; Naphthalene; Non-redox cycling; Oxidative damage; Reactive oxygen species

1. Introduction

An increasing variety of foreign chemicals (xenobiotics) released by urban communities, industries and agricultures is entering the aquatic environment and bringing potential long-term adverse effects on aquatic organisms (Livingstone, 1998). These xenobiotics include organic trace pollutants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and dibenzo-*p*-dioxins (PCDDs), and many metals such as iron (Fe), cadmium (Cd) and lead (Pb). Such a diverse array of chemicals can have many different mechanisms of toxicity, and numerous xenobiotics may also involve the common mechanisms of toxicity (Stohs and Bagchi, 1995; Livingstone, 2001).

Xenobiotic agents through redox cycling have the potential to produce reactive oxygen species (ROS) that overcome the protection afforded by antioxidant defense mechanisms, thereby leading to oxidative damage which is manifest by damage to tissue macromolecules including DNA, proteins and lipids (Di Giulio et al., 1989). Oxidative stress has been proven to involve neurotoxicity, hepatotoxicity and nephrotoxicity, and even to result in disease such as carcinogenesis in mammalian systems (Stohs and Bagchi, 1995; Kasprzak, 2002). ROS production and resulting oxidative damage may be an important mechanism of toxicity in organisms exposed to a wide range of man-made xenobiotics (Livingstone, 2001). Possible xenobiotics of enhanced ROS include redox cycling ones such as quinones and transitional metals, and non-redox cycling ones such as PAHs and dioxins.

With the exception of some classic redox cycling xenobiotics, more widespread contaminants are non-redox

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cycling ones (Stohs and Bagchi, 1995; Livingstone, 2001; Suntres, 2002). Cd is a relatively abundant nonessential element, which is widely used in modern industry and has been found in foods, water and tobacco leaves (Satarug and Moore, 2004). A variety of adverse effects have been attributed to Cd-induced toxicity. Though Cd can not induce production of ROS through a Fenton-like reaction directly, an increasing body of evidence indicates that it can induce oxidative stress by enhanced lipid peroxidation (LPO), depletion of GSH and alterations in activities of antioxidant enzymes (Siraj Basha and Usha Rani, 2003; Wang et al., 2004). Naphthalene (NAP) is a bicyclic aromatic hydrocarbon that is widely used commercially. In addition, it is a common component of industrial products and waste materials. NAP exposure is associated with several toxic manifestations in humans and laboratory animals (Schreiner, 2003). Lots of experiments have indicated that NAP toxicity are closely related to ROS and oxidative stress (Bagchi et al., 2002; Stohs et al., 2002; Vijayavel et al., 2004).

In previous studies of contaminant-mediated oxidative stress, the evidence of ROS production has been focused primarily on mammalian systems exposed to redox cycling xenobiotics (Stohs and Bagchi, 1995; Livingstone, 2001; Suntres, 2002). Though much work has indicated that Cd and NAP can induce oxidative stress in aquatic organisms, ROS production are usually deduced through the changes of antioxidant systems such as activities of enzymes (Almeida et al., 2002; Vijayavel et al., 2004). There is still relatively little direct evidence available to prove ROS generation and oxidative damage in aquatic organisms, especially exposed to non-redox cycling xenobiotics, including Cd and NAP.

In the present paper, we exposed *Carassius auratus* to Cd and NAP at different concentrations. ROS production was detected by electron paramagnetic resonance (EPR), lipid peroxidation (LPO) and protein carbonyl (PCO) content were determined, and activities of superoxide dismutase (SOD) and catalase (CAT) were also measured. Our aim was to determine if these two structurally different non-redox cycling xenobiotics could induce ROS production and result in oxidative stress in liver of *C. auratus*, and if these effects were concentration-dependent.

2. Materials and methods

2.1. Reagents

Cyclohexane, phenyl-*tert*-butyl nitrone (PBN) and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). All other chemicals used in this study were analytical grade.

2.2. Fish treatment

C. auratus with mean mass of 40–50 g were purchased from a local breeder. Before experimentation, the fish were acclimated for 10 days and fed once daily. Fish were separated into 11 groups randomly and exposed to Cd and NAP. Concentrations of Cd were 0.0005, 0.005, 0.05, 0.5 and 5 mg/L, and those of NAP were 0.005, 0.05, 0.5, 5 and 50 mg/L. Six fish per group were killed 24 h after exposure. The livers were quickly taken out and separated for different sample preparation at 4 °C.

2.3. ROS trapping and EPR measurement

About 0.1 g of liver tissue was homogenized for 2 min after addition of 1.0 mL 50 mM PBN in cyclohexane. Then 0.4 mL supernatant of homogenized tissue was taken into a quartz tube and stocked in liquid nitrogen for EPR measurement. EPR spectra were recorded with Bruker EMX 10/12 X-band spectrometer at room temperature. The settings for samples were as follows: center field, 3470 G; scan range, 200 G; modulation frequency, 100 kHz; modulation amplitude, 0.5 G; receiver gain, 5×10^4; scans, 5 times; microwave power, 20 mW. The EPR signal obtained with these parameters was used to calculate the height of the central peak and indicative of the intensity of ROS.

2.4. LPO and PCO content assays

LPO and PCO content was measured using a UV-220 spectrophotometer. LPO was measured by analyzing thiobarbituric acid reactive substances (TBARS) as described by Utley et al. (1967). Briefly, the tissues were homogenized in chilled 0.1 M KCl. The assay mixture contained 0.67% thiobarbituric acid, 10% chilled trichloroacetic acid, and 10% homogenate in a total volume of 3.0 mL. The mixture was heated at 95 °C for 60 min, and centrifuged for 15 min at 1500×*g* followed by cooling. The absorbance of the pink color adduct in the supernatant was determined at 532 nm. The amount of malondialdehyde formed was calculated using molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$. Each sample was assayed in triplicate.

PCO content was estimated by 2,4-dinitrophenylhydrazine (DNPH) assay (Levine et al., 1994). About 0.1 g of liver was rinsed in 10 mM HEPES buffer (pH 7.4) and homogenized with addition of 2.0 mL PBS (pH 7.4). After centrifugation at 10,000×*g* for 10 min, 0.5 mL of tissue supernatant was taken into the tubes. In each tube, 0.5 mL of DNPH in 2.0 M HCl was added. The blank contained 2.0 M HCl only. Tubes were vortexed every 10 min in the dark for 1 h. Proteins were then precipitated with 30% trichloroacetic acid and centrifuged at 1000×*g* for 10 min. The pellet was washed three times with 1.0 mL of ethanol: ethyl acetate (1:1 v/v). The final pellet was dissolved in 1.0 mL of 6.0 M guanidine HCl in 20 mM potassium dihydrogen phosphate (pH 2.3). The difference in the maximum absorbance (360–370 nm) of the sample treated with DNPH in HCl was determined vs. the one with HCl

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