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Drinking water disinfection byproduct iodoacetic acid interacts with catalase and induces cytotoxicity in mouse primary hepatocytes

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HIGHLIGHTS

- The work offers a combined cellular and molecular toxicity evaluation method.
- CAT activity increased due to the stimulation of CAT production in the hepatocytes.
- IAA triggered conformational changes in CAT and inhibit CAT activity.
- IAA binds to CAT with (4.05 ± 1.98) sites mainly via van der Waals and hydrogen bonds.
- IAA preferentially binds to the interface of CAT rather than its active site.

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ABSTRACT

Disinfection byproducts (DBPs) are produced during the disinfection of drinking water and pose a hazard to human health. As a typical type of DBPs, iodoacetic acid (IAA) exhibits prominent cytotoxicity in mammalian cell systems which links with oxidative stress. However, little is known about the relationship of catalase (CAT) with the cytotoxicity of IAA and the adverse effects of IAA to CAT. This study investigated the effects of IAA on the cell viability and CAT activity in the mouse primary hepatocytes. It was shown that IAA exposure induced the loss of cell viability and the increase of intracellular CAT activity. Intracellular CAT activity significantly increased due to the stimulation of CAT production under IAA exposure. The molecular CAT activity was inhibited due to the direct interaction of IAA with HIS 74 and TYR 357 around the active sites of CAT. IAA binds to CAT with (4.05 ± 1.98) sites via van der Waals and hydrogen bonding interactions, resulting in the loosening of protein skeletons and the change of protein size.

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1. Introduction

The disinfection of drinking water is greatly beneficial for preventing against infectious diseases spread by water. But the products generated in the disinfection process, known as disinfection byproducts (DBPs), have raise considerable concerns for their toxicity to human health. DBPs are generated from the reaction of highly reactive disinfectants with organic constituents, bromide, and iodide in raw water (Fabbricino and Korshin, 2009; Farré et al., 2013). As an important class of environmentally hazardous pollutants, adverse effects of DBPs are confirmed by research in test animals, in vitro studies and epidemiological studies (Richardson et al., 2007; Du et al., 2013; Liu et al., 2016; Zuo et al., 2017; Ding et al., 2018).

Iodoacid DBPs are found in chloraminated drinking water with high level of iodide (Plewa et al., 2004; Liu et al., 2016). Chlorine/chloramine residuals in water could react with the iodide to form hypoiodous acid and then react with remaining natural organic matter to generate iodoacid DBPs (Pan et al., 2016; Zhu and Zhang, 2016; Gong et al., 2018). Recent findings suggested that iodinated aromatic DBPs are generally significantly more toxic than iodinated aliphatic DBPs (Yang and Zhang, 2013; Liu and Zhang, 2014; Pan et al., 2016). While, some research has suggested that iodoacetic acid (IAA) is the most toxic one when evaluated in a mammalian

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cell system (Plewa et al., 2004; Cemeli et al., 2006). Previous research has shown that IAA is cytotoxic, genotoxic, mutagenic, teratogenic, and potentially carcinogenic, which are all linked with oxidative stress(Komaki et al., 2009; Escobar-Hoyos et al., 2013; Procházka et al., 2015; Zhou et al., 2015). The exposure of IAA induced oxidative stress through the excessive generation of reactive oxygen species (ROS). Pals et al. have found that antioxidants could mitigate IAA toxicity in human intestinal epithelial cell line, suggesting the involvement of oxidative stress (Pals et al., 2013). Wang et al. have found that IAA activates Nrf2-mediated antioxidant response in vitro and in vivo and that oxidative stress plays a role in IAA toxicity (Wang et al., 2014).

Catalase (CAT, EC 1.11.1.6) is a common antioxidant enzyme existing in nearly all aerobic organisms (Chelikani et al., 2004). Most living organisms utilize CAT in every organ, especially with high concentrations in the liver in mammals (Ilyukha, 2001). CAT is essential for maintaining ROS level by catalyzing H_2O_2 and used as a biomarker for indicating ROS production. Inactivation of CAT could allow the generation of excessive ROS and then induce oxidative stress in organisms (VI, 2011). Few studies have been conducted to examine the response of CAT under IAA exposure. Whitaker et al. have found that CAT activity significantly decreased in porcine oocytes when exposed to 2 mM IAA for 48 h (Whitaker and Knight, 2008). To date, little is known about the relationship of CAT with the cytotoxicity of IAA. Different results might be obtained under other experimental conditions. Besides, IAA could directly interact with CAT and then elicit toxicity due to the structural and functional changes of CAT (Liu et al., 2009). Therefore, it is of great value to investigate the interactions of IAA with CAT for better understanding the potential toxicity of IAA and revealing the mechanism of IAA toxicity. Yet, the adverse effects of IAA to CAT remains unknown, including the mechanism of CAT activity change, the structural and functional changes of CAT molecule and the binding mode of the interaction between CAT and IAA.

It was reported that the liver is the major organ of CAT distribution and hydrocarbon detoxification (Livingstone, 1991; Halliwell and Gutteridge, 1999; Rodés, 2007). The primary cell of target organs is used as an important complementary method for toxicity studies of animal testing and helpful for improving the simulation of the in vivo physiological state. Therefore, the isolated primary hepatocytes were chosen to investigate the relationship of CAT with the cytotoxicity of IAA and the mechanism of CAT response under IAA exposure. The cell viability and CAT activity change of mouse primary hepatocytes were examined in the absence and presence of antioxidant N-acetyl-l-cysteine (NAC) or CAT molecule inhibitor 3-amino-1,2,4-triazole (3-AT) under IAA exposure. And then the activity assay of purified CAT molecule was performed to explore the mechanism of intracellular CAT activity change under IAA exposure and the effects of IAA on enzyme function. The underlying mechanism of the interaction between CAT and IAA was investigated by multi-spectroscopic methods, isothermal titration calorimetry and molecular modelling studies. This study provides a combined cellular and molecular project to explore the relationship of biomacromolecules with the adverse effects and the underlying mechanism when evaluating the toxicity of hazardous chemicals. Also, this study provides valuable information to fill in a gap in the adverse outcome for assessing the risk of DBPs to human health.

2. Materials and methods

2.1. Materials

CAT from bovine liver, IAA, NAC and 3-AT were purchased from Sigma–Aldrich (St. Louis, MO, USA). IAA (AR, 98%) was obtained from Shanghai Aladdin Bio-Chem Technology Ltd (Shanghai, China). Ca^{2+} and Mg^{2+}-free Hank's balanced salt solution (HBSS) was purchased from Beijing Solarbio Ltd (Beijing, China). Dulbecco's Modified Eagles Medium (DMEM), fetal bovine serum and penicillin/streptomycin were obtained from Thermo Fisher Scientific (Waltham, Massachusetts, USA). H_2O_2 was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China). Protein quantification kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ were obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Phosphate buffer (0.02 M, pH = 7.4) was applied to control pH in the molecular experiments.

2.2. Cell isolation and treatment

The following tests were conducted according to the Guiding Principles outlined in the Use of Animals in Toxicology published by the Society of Toxicology in 1989. The isolation of mouse (C57BL/6 J) primary hepatocytes was performed as described in our previous study (Wang et al., 2015). IAA was dissolved in HBSS and diluted as required before use. The isolated hepatocytes were cultured in DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin and planted in 96-well plates for CCK-8 assays or 24-well plates for intracellular CAT activity assays. The hepatocytes were pretreated with 0.1 mM NAC or 20 mM 3-AT for 1 h in a 5% CO_2 humidified atmosphere at 37 °C. Then the hepatocytes were treated with different concentrations of IAA (0, 1, 5, 10, 50, 100, 500, 1000, 2000, 400, 6000, 8000 μM) for 24 h. Neither 0.1 mM NAC nor 20 mM 3-AT had any significantly obvious effects on the cell viability of the hepatocytes without IAA treatment. For the positive control, the hepatocytes were treated with 100 μM H_2O_2 for 15 min (data not shown) (Wang et al., 2015).

2.3. Cell viability assay

The cell viability was conducted using CCK-8 assay (Dojindo Laboratories, Kumamoto, Japan) (Tominaga et al., 1999): 10 μLof CCK-8 solution was added to each well after 24 h IAA treatment. The hepatocytes were then incubated at 37 °C for 2 h. The absorbance at 450 nm was recorded on a microplate reader (GF-M3000, Rainbow, China).

2.4. CAT activity assay

For intracellular CAT activity assays, the hepatocytes were collected in centrifuge tube and then ultrasonically lysed 20 times (4s each) on ice using microson ultrasonic cell disruptor (VCX150PB, SONICS & MATERIALS INC.). After centrifuging at 10000 g for 15 min at 4 °C, the supernatants were used for the subsequent assays. The activity was determined by measuring the decreasing ratio of absorbance at 240 nm (A_{240}) in 3 mL HBSS containing 200 μL supernatants and 10 mM H_2O_2 (Góth, 1991). The protein amount of every samples was determined using the Bradford method by recording the absorbance at 595 nm (A_{595}) (Bradford, 1976). Both A_{240} and A_{595} were measured on a UV-2450 spectrophotometer (Shimadzu, Japan). Results were expressed as relative activity of the blank control.

For molecular CAT activity assay, the assays were performed based on the same methods as described above, except that the supernatants were substituted for the CAT-IAA mixture.

2.5. Isothermal titration calorimetry (ITC)

ITC experiments were carried out with a Microcal ITC200 microcalorimeter. Approximately 40 μL of IAA (20 mM) was titrated into a buffer-matched CAT solution (200 μL, 100 μM) with stirring

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