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Effects of molybdenum and cadmium on the oxidative damage and kidney apoptosis in Duck☆

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ABSTRACT

Molybdenum (Mo) is an essential element for human beings and animals; however, high dietary intake of Mo can lead to adverse reactions. Cadmium (Cd) is one of the major transitional metals which has toxic effects in animals. To investigate the co-induced toxic effects of Mo and Cd on oxidative damage and kidney apoptosis in duck, 120 ducks were randomly divided into control group and 5 treatment groups which were treated with a commercial diet containing different dosages of Mo and Cd. Kidney samples were collected on the 60th and 120th days to determine the mRNA expression levels of ceruloplasmin (CP), metallothionein (MT), Bak-1, and Caspase-3 by quantitative RT-PCR. Additionally, we also determined the antioxidant activity indexes and contents of Mo, Cd, copper (Cu), iron (Fe), zinc (Zn), and selenium (Se) in serum. Meanwhile, ultrastructural changes of the kidney were observed. The results showed that glutathione reductase (GR) activity and CP level in serum were decreased in combination groups. In addition, the antioxidant indexes were decreased in co-treated groups compared with single treated groups. The mRNA expression levels of Bak-1 and Caspase-3 increased in co-treated groups. The mRNA expression level of CP in high-dose combination group was downregulated, while the mRNA expression of MT was upregulated except for low-dose Mo group. Additionally, in the later period the content of Cu in serum decreased in joint groups while the contents of Mo and Cd increased. In addition, ultrastructural changes showed mitochondrial crest fracture, swelling, deformed nuclei, and karyopyknosis in co-treated groups. Taken together, it was suggested that dietary Mo and Cd might lead to oxidative stress, kidney apoptosis and disturb homeostasis of trace elements in duck, and it showed a possible synergistic relationship between the two elements.

1. Introduction

Molybdenum (Mo) is an essential trace element for nearly all organisms and forms the catalytic centre of a large variety of enzymes such as nitrogenase, nitrate reductases, sulphite oxidase and xanthine oxidoreductases (Schwarz et al., 2009). Molybdenum ores and fuel containing Mo are widely used in human activities (such as coal combustion), which increases the circulation of Mo in the environment. Many studies have reported that mining and industrialization could lead to an increase in concentration of Mo in soil, water, and air which can be absorbed by terrestrial and aquatic organisms, such as fish and cattle, leading to chronic toxicity (Davies et al., 2005; Swan et al., 1998). High Mo ingestion leads to secondary copper (Cu) deficiency which is usually called molybdenosis (Khandare et al., 2005). The kidney is the target organ and the primary accumulation site of chronic molybdenum exposure (Mason, 1986). Mo is eliminated via kidney and it usually takes several weeks to be completely eliminated from the body. One study showed that a high dosage of Mo as an exogenous poison could induce oxidative damage, decrease antioxidant enzymes activities and decline in the antioxidant capacity of the organism (Markesbery et al., 2005). Some previous studies reported high Mo improved lipid peroxidation-induced oxidative stress, as well as decreased antioxidant enzyme activities (Raisbeck et al., 2006) and disarranged expression of cell apoptosis-related genes, which resulted in various degrees of cell injuries and apoptosis (Yang et al., 2011).

Cadmium (Cd) is an important heavy metal that of highly health concern by people because of its toxicity not only to humans but also to animals. Either acute or chronic Cd exposure could induce varying degree injuries to tissues, especially kidney and liver (Wallin et al., 2014). Once absorbed, Cd is efficiently retained in the organism and

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accumulates throughout life with a biological half-life of 10–30 years in humans (Shih et al., 2004). The kidney is the critical target organ for Cd-induced toxicity and is well documented by a number of studies in occupationally and environmentally exposed human subjects, as well as in various experimental models (Hagar and Al Malki, 2014). Cd accumulates mainly in the proximal tubules of the kidney and is known to cause renal dysfunction after chronic exposure (Johri et al., 2010). To date, the reported deleterious effects of Cd include increasing generation of reactive oxygen species (ROS), altering antioxidant enzymes activities, modulation of apoptosis, inhibition of DNA repair enzymes and initiation of various pathological conditions in humans and animals (Prozialeck and Edwards, 2012). Cd has the ability to alter the expression levels of a variety of genes such as metal protein genes and apoptosis genes (Lasfer et al., 2008). In addition, Cd competes for the same transmembrane carriers with other essential nutrients thus disturbing the homeostasis of trace elements (Martelli et al., 2006). Cadmium induces oxidative stress, but the molecular mechanisms involved in the cell damage from oxidative stress in cadmium-induced chronic kidney disease are not well understood (Gobe and Crane, 2010).

Apoptosis is a complex phenomenon, which involves multiple pathways including extrinsic and intrinsic pathways, and is a vital mechanism to maintain cellular development and homeostasis, and this programmed cell death is mediated by various genes. Intrinsic inducers involved intracellular signals in response to cellular stresses, like hypoxia and ROS, and extrinsic inducers may be certain metals, such as Cd and Mo (Ma et al., 2010; Su et al., 2013). Cd generates damages in humans via complex mechanisms involving interactions with other metals, induction of oxidative stress and apoptosis (Nemmiche et al., 2011). Following exposure to Cd, mitochondrial oxidative damage occurs via inhibition of Bcl-2 and Caspase-3 activation, leading to nuclear chromatin condensation, DNA fragmentation, and cell death (Jin et al., 1998). Oxidative stress pathway has a significant role in Cd-induced apoptosis (Wang et al., 2014).

Cerulolaplism (CP) is an oxidase with high antioxidative capacity which is involved in scavenging oxygen free radical and protecting various organs from lipid peroxidation and other oxidative attack in extracellular (Samsam and Alinejad, 2008). Metallothionein (MT) is low-molecular-mass cysteine-rich metal-binding protein with a high affinity for heavy metal ions and was found in a large variety of organisms. MT plays a crucial role in the detoxification of heavy metal ions, such as cadmium and mercury, as well as in the homeostasis of essential metals, like Zinc (Zn) and Cu, in addition to acting as scavengers of free radicals and reactive oxygen metabolites. Due to its higher metal affinity and inducibility by metals, MT was widely regarded as biomarkers of metal exposure (Ghedira et al., 2010).

In the mining and screening processes for tungsten ore, discharges containing Mo and Cd lead to a long-term accumulation, which pollutes the surrounding water, land, vegetation, and livestock in a large area which pose hazardously threats to animals and public health. The combined toxicity of Mo and Cd remains largely unexplored. Here, we aim to elucidate the toxicity of Mo and Cd on ducks through determining the contents of Mo, Cd, Cu, iron (Fe), Zn, selenium (Se) and the changes of antioxidant function in serum and the mRNA expression levels of CP and MT, cysteinyl aspartate-specific proteinase-3 (Caspase-3) and BRI1-Associated Receptor Kinase-1 (Bak-1), and ultrastructural changes of kidney tissue.

2. Materials and methods

2.1. Animals and treatments

All procedures used in this research were approved by the Institutional Animal Care and Use Committee of Jiangxi Agricultural University. Duck model of exposure to Mo and Cd was developed as described in our previous publication (Cao et al., 2016c). Briefly, 120 healthy 11-day-old ducks were randomly divided into 6 groups (20 ducks per group): control group (0 mg/kg Mo, 0 mg/kg Cd), low dietary of Mo group (LMo group, 15 mg/kg Mo), high dietary of Mo group (HMo group, 100 mg/kg Mo), Cd group (4 mg/kg Cd), LMoCd group (15 mg/kg Mo, 4 mg/kg Cd), and HMoCd group (100 mg/kg Mo, 4 mg/kg Cd). Hexaammonium molybdate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$), cadmium sulfate ($3CdSO_4{\cdot}8H_2O$) were used for Mo and Cd sources in this experiment respectively. The dosage was based on the weight of basal diet. Ducklings were fed with duckling basal diet and duck basal diet before and after 21-day-old respectively. All ducks were maintained in isolation cages at a constant temperature with good ventilation and light and were given free access to water and feed. The contents of Mo, Cd, Cu, Zn, Fe, and Se in the basal diet and water are shown in Table 1. The experimental period lasted for 120 days. All animals were handled and treated in accordance with the institutional ethic committee, and the experimental procedures were also complied with the criteria in Guide for the Care and Use of Laboratory Animals.

Table 1
Contents of Mo, Cd, Cu, Zn, Fe, and Se in the basal diet and water.

Trace elements	Duckling feed (μg/g)	Duck feed (μg/g)	Water (μg/mL)
Mo	4.151	4.729	0.010
Cd	0.247	0.476	0.008
Cu	191.351	109.330	0.021
Fe	747.835	709.081	0.188
Zn	210.741	189.874	0.152
Se	1.048	1.052	0.015

2.2. Sample collection

Ten ducks in each group were selected randomly on the 60th and 120th days of the experiment. The blood samples (10 mL) were taken from wing vein of each duck and packed in 5 mL Eppendorf Tubes for serum separation. The kidneys were removed from a random selection of 10 ducks from each group immediately after they were anesthetized with an overdose of an intravenous injection of sodium pentobarbital. Then, kidney tissues were placed into sampling tubes, which were transferred to liquid nitrogen immediately. In addition, the rest of each kidney specimen on the 120th day was collected for ultrastructural studies. After that, all samples were stored at −80 °C until analyzed.

2.3. Determination of antioxidant indexes

The activity of Glutathione reductase (GR) and the level of CP were determined according to the manufacturer's instructions. The kits for these assays were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). GR assay kit (Colorimetric method), catalog number: A005. CP assay kit (Colorimetric method), catalog number: A029.

2.4. Determination of trace elements

The trace elements including Mo, Cd, Cu, Fe, Zn, and Se in serum were analyzed using Agilent 240AA atomic absorption spectrophotometer (Agilent, America). All analyses were carried out according to the manufacturer's instruction by a trained technician.

2.5. RNA isolation and primer designing

Total RNA was isolated from kidney samples using Trizol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions and was then reverse transcribed. The resultant cDNA was synthesized using a PrimeScript™RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). The reverse transcription reaction (20 μL) was conducted in a mixture containing 2 μL of 5× DNA Eraser Buffer, 1 μL of gDNA Eraser, 1 μL of total RNA and 6 μL of RNase-free dH_2O and was