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RESEARCH ARTICLE

Lipid peroxidation of kidney of the turtle *Mauremys reevesii* caused by cadmium

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Abstract

This research was designed to investigate lipid peroxidation of the kidney of turtle (*Mauremys reevesii*) caused by cadmium. Turtles were injected intraperitoneally with cadmium at the concentration of 0 (control), 7.5, 15, and 30 mg/kg, and 5 turtles were taken from each group after exposure for 1 week (1 w), 2 weeks (2 w), and 3 weeks (3 w). Superoxide dismutase (SOD) and catalase (CAT) activities as well as glutathione (GSH) and malonyldialdehyde (MDA) contents in the homogenate of kidney tissue were analyzed. The results demonstrated that a short time of low dose of cadmium could stimulate the increase of SOD activity in the kidney of turtles, but a long time of high dose of cadmium could induce the decrease of SOD activity in the kidney of turtles. Cadmium could decrease CAT activity and GSH content in turtle kidney, but increased MDA content in turtle kidney. There were some other effects on the turtles, such as depression and diarrhea. The experimental results indicate that cadmium causes temporary oxidative stress on the kidney of turtles.

Keywords Cadmium · Turtles · Kidney · Temporary oxidative stress · Experimental effects

Introduction

Cadmium is a worldwide freshwater aquatic pollutant (Dieter et al. 2014; Helen et al. 2011). Because the biological half-life of cadmium is very long, the poisoning effects of cadmium are able to accumulate across the food chain and be biologically amplified (Mehinto et al. 2014; Rose et al. 2015). So, cadmium is able to cause biochemical changes, physiological dysfunctions, and morphological abnormalities (Lei et al. 2011; Mehinto et al. 2014; Helen et al. 2011). Researches of its mechanisms showed that cadmium induces the bioaccumulation of reactive oxygen species (ROS), which results in many injuries such as lipid peroxidation and DNA mutation (Lang et al. 2016; Lei et al. 2011).

Former researches have shown that cadmium has influences on the physicochemical capabilities of some turtles (Alexandra et al. 2013; Elodie and Krishna 2012; Heather et al. 2011; Huo et al. 2017A; Joanna et al. 2010; Ley-Quiñónez et al. 2011; Yu et al. 2011).

Cadmium poisoning mechanism is not very clear, and several interpretations are presented (Varoni et al. 2017). Cadmium toxicological studies indicate that it can cause an increase in reactive oxygen species (ROS), which is able to induce multiple structural and functional damage, such as cellular lipid peroxidation, protein destruction, and DNA mutation (Cuypers et al. 2010; Huo et al. 2017a, b, 2018, 2020a, b; Wu et al. 2015). One of the products of lipid is peroxidation malonyldialdehyde (MDA), which is able to bind to the amino acids of protein and trigger the internal or mutual connection of protein, leading to cell damage (Li et al. 2012). Therefore, the content of MDA can reflect the attack degree of ROS on cells and it can be referenced as one markers of membrane damage. There is an antioxidant system in the body, which includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Jiao et al. 2017; Koim-Puchowska et al. 2020; Laurent et al. 2017; Serdar et al. 2018; Yuan et al. 2016). ROS can be cleared by the

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antioxidant system. SOD can convert ROS into hydrogen peroxides (H_2O_2), and GSH-Px or CAT is able to degrade H_2O_2 (Afonso et al. 2007). Cadmium regulates the activities of SOD, CAT, and GSH-Px of the antioxidant system (Yuan et al. 2016).

Many studies of cadmium have been done on wild or laboratory animals. Some studies have been done on turtles with other metals (mercury, lead), in addition to cadmium (Adel et al. 2017; Lídia et al. 2017; Malik et al. 2013; Manuel et al. 2019; Rodriguez et al. 2020). However, there is lack of studies of cadmium on freshwater turtles. Thus, more studies on turtles ecotoxicology are required.

For a long time, turtles are used for food, pets, and traditional medicines in many regions of the world (Fordhama et al. 2007; Huo et al. 2020a, b; Mutalib et al. 2013; Xu et al. 2014). Mauremys reevesii is one of the most commercially cultured turtles for aquaculture and is widely distributed in China (Cheung and Dudgeon 2006; Du et al. 2007). The longevity and omnivorousness of this turtle make it suitable for monitoring the influence of chemical pollutants in terms of bioconcentration and bioaccumulation. Furthermore, it is easy to handle *Mauremys reevesii* turtle because it is moderate in sized and is not aggressive (Tada et al. 2004). *Mauremys reevesii* is located at the upper of the food web of aquatic system. Nevertheless, toxicological studies of turtles (*Mauremys reevesii*) exposed to cadmium are limited (Huo et al., 2017a, b, 2018, 2020a, b).

Previously, we have investigated the toxicokinetics of cadmium in *Mauremys reevesii* and we found cadmium slowly accumulated in the kidney and reached its peak in 8 weeks after treatment (36.66 μg/g ww) (Huo et al. 2017A).

Therefore, we carried out the research, aiming to study lipid peroxidation in the kidney of turtle *Mauremys reevesii* exposure to cadmium.

Materials and methods

Animals and treatment

Mauremys reevesii were purchased from one aquatic product market in Taiyuan City of Shanxi Province, China. *Mauremys reevesii* were acclimated in glass aquaria filled with tap water for 2 weeks before experiments. In the course of acclimation, aerated water was changed every 2 days and *Mauremys reevesii* were fed commercial turtle feed one time everyday.

After acclimation, healthy, similar weight (130 ± 10 g) *Mauremys reevesii* were selected for experiment. *Mauremys reevesii* randomly divided into one control group (5 individuals) and three experiment groups (45 individuals, 15 turtles in each group). The control group turtles were injected with 0.85 % sodium chloride solution, and the experiment group turtles were injected with 7.5, 15, and 30 mg/kg cadmium chloride (calculated with cadmium) with quantitative syringe respectively for once. During the experimental period, all other conditions were kept the same as those used for acclimation.

Samples collection

Five turtles were sacrificed from every group after 1 week (1 w), 2 weeks (2 w), and 3 weeks (3 w) of cadmium treatment. The turtles were beheaded for collecting kidneys. Kidneys were immediately excised, and tissues of the kidney were immediately collected. By weight (g), volume (ml) is equal to the proportion of 1:9, add 9 times medium (0.85% saline solution), homogenate, 2500 r/min, centrifuge for 10 min. The supernatant were stored at − 80 °C for detection oxidation index.

Biochemical assays

Under the manufacturer's protocols, the content of GSH, MDA, and the activities of SOD and CAT in supernatant were detected with the detection kits. All the detection was performed by a microplate reader.

The activities of SOD were measured by the method of xanthine/xanthine oxidase (Nishikimi 1975). One unit of SOD was defined as the amount of enzyme that inhibited superoxide-induced oxidation (monitored at 550 nm) by 50%. The activities of CAT were measured at 405 nm. One unit of CAT was defined as 1 μmol H_2O_2 decomposed one milligram of protein per second (Góth 1991). The content of GSH was quantified basing on the reaction between remaining glutathione after the action of GSH-Px and 5, 5′-dithio bis-(2-nitro benzoic acid) to form a complex that absorbed maximally at 412 nm (Rotruck et al. 1973). The content of MDA was determined basing on the reaction with thiobarbituric acid at 532 nm and 95 °C. Its result was expressed as nanomoles per milligram of protein (Ohkawa et al. 1979).

Statistical analysis

All the data represented mean values of five independent sets of experiments and were expressed as means ± standard deviations (SD). Differences between treatments of different exposure times and metal concentrations were statistically analyzed by two-way analysis of variance (ANOVA) and LSD multiple comparisons at a 0.05 probability level using SPSS 22.0.

Results

Effects of cadmium on SOD activities of kidney of treated turtles

As can be seen from Fig. 1, the activities of SOD in kidney tissue increased in the short-term exposure (1 week, 2 weeks) and this trend was more obvious in the low-dose exposure group. SOD activities in the 7.5 mg/kg group were significantly higher than that in the control group at 1 and 2 weeks after exposure ($P < 0.05$). The activities of SOD in the 15 mg/kg group was significantly higher than that in the control group ($P < 0.05$), but the increasing of SOD activities became less significant with the extension of the exposure time and the increasing of the exposure dose. SOD activities in the high dose group (30 mg/kg) were significantly lower than that in the control group ($P < 0.05$). The activities of SOD in the 15 mg/kg and 30 mg/kg exposed groups were significantly decreased in 3 weeks compared with that in 1 week ($P < 0.05$).

In a word, different doses and time of exposure had an impact on the activities of SOD. Low dose of exposure for a short time stimulated the increase of the activities of SOD, while high dose of exposure for a long time inhibited the activities of SOD.

Effects of cadmium on CAT activities of kidney of treated turtles

CAT activities in renal tissue of each exposed dose group at each exposure time (1 week, 2 weeks, 3 weeks) was significantly lower than that of the control group ($P < 0.05$) (Fig. 2).

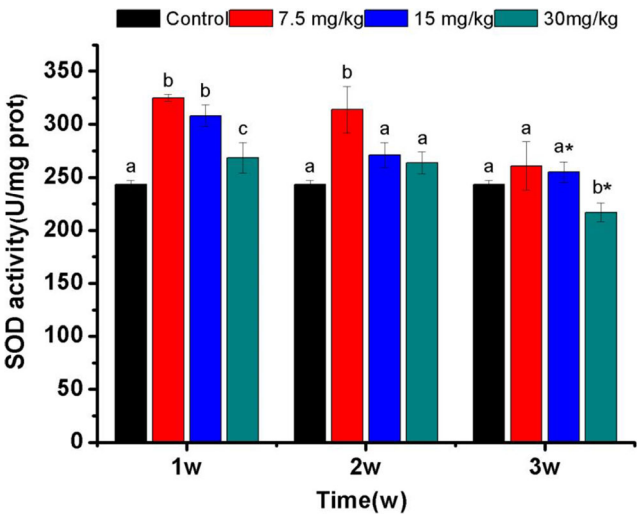

Fig. 1 Effects of Cd on the activities of SOD in kidney of the turtles. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$). Comparison of different exposure time in the group: *: compared with 1W ($P < 0.05$); #: compared with 2W ($P < 0.05$)

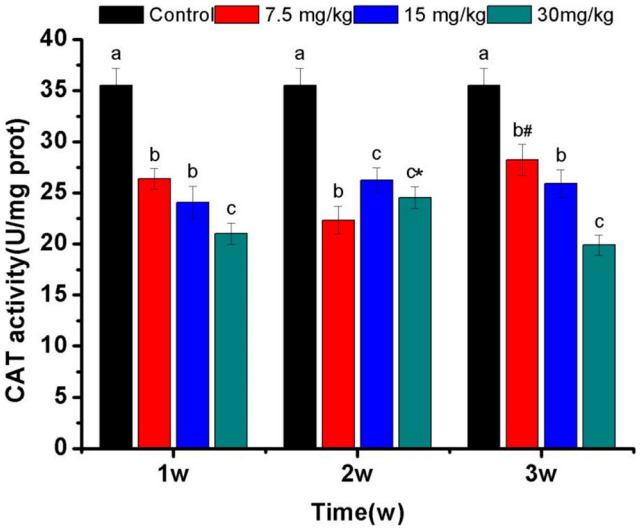

Fig. 2 Effects of Cd on the activities of CAT in kidney of the turtles. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$). Comparison of different exposure time in the group: *: compared with 1W ($P < 0.05$); #: compared with 2W ($P < 0.05$)

Comparison of different exposure time in the groups showed that the activities of CAT in the 30 mg/kg exposed group increased significantly in 2 weeks compared with that in 1 week ($P < 0.05$), and the activities of CAT in the 7.5 mg/kg exposed group increased significantly in 3 weeks compared with that in 2 weeks ($P < 0.05$). The results showed that different exposure doses and exposure time of cadmium had effects on CAT activity, which showed inhibition of CAT activity, but there was no significant time and dose dependence.

Effects of cadmium on GSH content in kidney of treated turtles

At each exposure time (1 week, 2 weeks, 3 weeks), GSH content in renal tissue gradually decreased with the increase of the exposure dose compared with the control group (Fig. 3). Compared with different exposure time in the groups, GSH content in renal tissue of the 30 mg/kg exposed group reduced significantly in 3 weeks compared with that in 1 and 2 weeks ($P < 0.05$). The results showed that the content of GSH in renal tissue was related to the dose and time of exposure, which was manifested as the decrease of GSH content in renal tissue.

Effects of cadmium on MDA content in kidney of treated turtles

MDA content in renal tissue increased significantly with the extension of exposure time and the increase of exposure dose compared with the control group ($P < 0.05$)

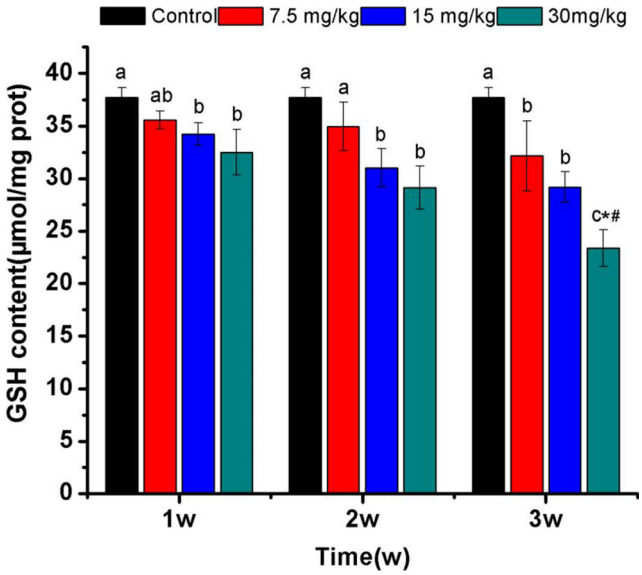

Fig. 3 Effects of Cd on the content of GSH in kidney of the turtles. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$). Comparison of different exposure time in the group: *: compared with 1W ($P < 0.05$); #: compared with 2W ($P < 0.05$)

(Fig. 4). Compared with different exposure time in the groups, MDA content in renal tissue of the 7.5 mg/kg exposed group increased significantly in 3 weeks compared with that in 1 week ($P < 0.05$). MDA content in renal tissues of the 15 mg/kg and 30 mg/kg groups increased significantly in 3 weeks compared with that in 1 and 2 weeks after exposure ($P < 0.05$).

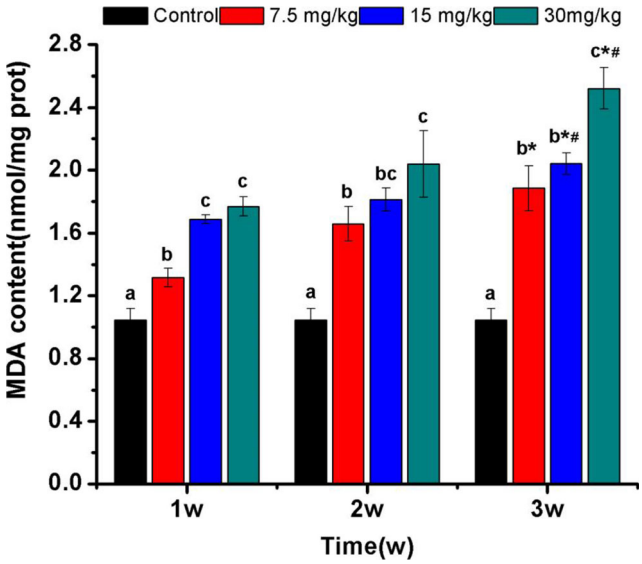

Fig. 4 Effects of Cd on the content of MDA in kidney of the turtles. Comparison between groups at the same exposure time: the difference of different letters was statistically significant ($P < 0.05$). Comparison of different exposure time in the group: *: compared with 1W ($P < 0.05$); #: compared with 2W ($P < 0.05$)

Discussion

Cadmium can not only induce excessive free radicals in many ways but also replace the metal (iron or copper) in antioxidant enzymes to decrease the activity of antioxidant enzymes and reduce the ability of the body to eliminate free radicals (Li et al. 2011). Cadmium also formed covalent connections with mercapto groups (such as mercaptol binding proteins), which destroyed the homeostasis of sulfhydryl groups in cells (Bharavi et al. 2011). Then cadmium induced lipid peroxidation by consuming reduced glutathione and inhibiting antioxidant enzyme activity. Cadmium can reduce GSH content in Nile tilapia (Almeida et al. 2009; Bharavi et al. 2011). The activities of GSH and SOD in kidney of trout exposed to cadmium were markedly decreased ($P < 0.05$), while MDA increased significantly (Topal et al. 2013).

Our study found that the activities of SOD in renal tissue were related to the dose and time of exposure. Low dose and short time of exposure stimulated the increase of the activities of SOD, which was especially obvious in the low dose group (7.5 mg/kg). With the extension of exposure time and the increase of exposure dose, the increase of SOD activities became no longer significant, and the activities of SOD in the 3-week high-dose exposure group (30 mg/kg) were significantly lower than that in the control group ($P < 0.05$). Compared with different exposure time in the groups, SOD activity of the 15 mg/kg and 30 mg/kg groups decreased significantly in 3 weeks compared with that in 1 week ($P < 0.05$), indicating that the duration of exposure also affected SOD activity. There were some studies on the effects of cadmium on the kidney of some animals. The activity of SOD in the mice kidney is reduced (Pang et al. 2010; Wang et al. 2016B). SOD activities in the rats' kidney are declined with increasing of exposure time (Wang et al. 2008; Wang et al. 2018; Wang et al. 2006; Zhang et al. 2002; Zhou et al. 2018). SOD activities in cadmium-induced human renal cell are declined (Cai et al. 2002). SOD activities in the Bufo gargarizans kidney are declined (Jia et al. 2004). SOD activities in the cock kidney are declined (Wang et al. 2007B). Interestingly, SOD activities in the Frog Rana nigromaculata kidney are first raised and declined afterwards with increasing of exposure time (Wang et al. 2006). The activity of SOD in grass carp's kidney tissues decreases first then increases and decreases once again when Cd^{2+} presents low concentration pollution, while the activity of SOD is inhibited obvious all along when Cd^{2+} presents high concentration pollution (Wang et al. 2007A). These reports are accordance with our results, indicating that cadmium exposure induced oxidative stress injury in the renal tissue. Xiang also repot that the reduction in SOD activity in the rats' kidneys occurred after morphological changes and mRNA transcription inhibition (Xiang et al. 2001). The inhibition of the expression of SOD gene is one factor of kidney injury caused by cadmium (Xiang et al. 2001). But Wang's report

that SOD activities in the Orechromis niloticus kidney are increased (Wang et al. 2016A). These are worthy of further study.

Our study found that different exposure doses and exposure time of cadmium had effects on CAT activity, and generally showed inhibition of CAT activity, but there was no obvious time and dose dependence. CAT activity of renal tissue in each exposure time and dose group was significantly lower than that in the control group ($P < 0.05$). Compared with different exposure time in the groups, CAT activity in the 30 mg/kg exposed group increased significantly in 2 weeks compared with that in 1 week ($P < 0.05$). CAT activity in the 7.5 mg/kg group was significantly higher in 3 weeks than that in 2 weeks ($P < 0.05$). The activities of CAT in the Bufo gargarizans kidney are declined (Jia et al. 2004). This report is consistent with our findings.

Our study found that GSH content in renal tissue gradually decreased compared with the control group with the increase of exposure dose at each exposure time, and this trend was especially obvious in 3 weeks of exposure. Compared with different exposure time in the groups, GSH content in renal tissue of the 30 mg/kg exposed group reduced significantly in 3 weeks compared with that in 1 and 2 weeks ($P < 0.05$). There are some reports about the effect of cadmium on the content of GSH. As the duration of exposure increases, GSH contents are reduced markedly in the kidneys of Frog Rana nigromaculata (Wang et al. 2006). The contents of GSH are negative correlation with the contents of MDA in Frog Rana nigromaculata kidneys (Wang et al. 2006). GSH contents are decreased in the Bufo gargarizans kidneys (Jia et al. 2004). These studies are consistent with our researches. But some studies are in other way. GSH contents in the rats' kidneys are increased with the prolonged time (Wang et al. 2008; Yu et al. 2006; Zhang et al. 2002). GSH contents are increased in the Orechromis niloticus kidneys (Wang et al. 2016A).

Our study found that different dose and time of cadmium exposure affected MDA content in renal tissue. MDA content in renal tissue increased significantly with the extension of exposure time and the increase of exposure dose compared with that in the control group ($P < 0.05$). Compared with different exposure time in the groups, MDA content in renal tissue of the 7.5 mg/kg exposed group increased significantly in 3 weeks compared with that in 1 week ($P < 0.05$). MDA content in renal tissue of the 15 mg/kg and 30 mg/kg exposed groups increased significantly in 3 weeks compared with that in 1 and 2 weeks ($P < 0.05$). There are some reports about the effect of cadmium on the content of MDA. The levels of MDA in the mice kidneys are increased with the prolonged time (Pang et al. 2010; Wang et al. 2008; Wang et al. 2018; Wang et al. 2006; Wang et al. 2016B). MDA contents in the rats' kidneys are increased with the prolonged time (Zhang et al. 2002). In the rats' kidney cortex the contents of MDA increased (Yu et al. 2006). As the duration of exposure increases, the contents of MDA in the Frog Rana nigromaculata kidneys are rapidly increased (Wang et al. 2006). The MDA contents in the Bufo gargarizans kidneys are increased (Jia et al. 2004). The MDA contents in the cock kidneys are increased (Wang et al. 2007B). These reports are consistent with our findings, indicating that cadmium exposure induced oxidative stress injury in the renal tissue.

Conclusions

A short time of low dose of cadmium could stimulate the increase of SOD activity in the kidney of turtles, but a long time of high dose of cadmium could induce the decrease of SOD activity in the kidney of turtles. Cadmium could decrease CAT activity and GSH content in turtle kidney but increased MDA content in turtle kidney. The experimental results indicate that cadmium causes temporary oxidative stress on the kidney of turtles.

Authors' contributions Aiguo Dong and Junfeng Huo designed the study, performed the research, analyzed data, and wrote the paper. Juanjuan Yan was a major contributor in performing the research. Ailing Dong was a major contributor in writing the manuscript. Biwang Liu was a contributor in performing the research. All authors read and approved the final manuscript.

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Data availability Not applicable.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval and consent to participate This study was approved by Shanxi University of Chinese Medicine (permit number: 2018LL054).

Consent to publish Not applicable.

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