研究简报

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PROTECTIVE EFFECTS OF SELENIUM NANOPARTICLES ON OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES ACTIVITIES INDUCED BY MICROCYSTINS IN THE LIVER OF MICE

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纳米硒对微囊藻毒素致小鼠肝氧化损伤的保护作用

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The occurrence of cyanobacterial blooms has been reported in fresh water all over the world [1]. Cyanobacterial bloom in ponds and reservoirs are associated with adverse effects on organisms, including acute toxicity in animals and cases of illness in humans when the toxins released into the aquatic environment after cyanobacterial cell lysis [2]. The most commonly found cyanobacterial toxins are microcystins (MCs) in blooms and the major toxic effect of MCs is its hepatotoxicity [2, 3]. Microcystins exert their toxic effects mainly by inhibiting protein phosophatase type-1 and 2A [4]. In addition, it has been reported that oxidative stress also involve in the toxicity of MCs [5, 6]. Several studies indicated that antioxidants and free radical scavengers such as Vitamin E or selenium have protective roles in MCs-induced hepatotoxicity [7, 8].

Selenium is one of essential elements for organism and it plays important role in the health of mammalian animals [9]. It was reported that selenium could reduce carcinogenesis in experimental animals, and could be effective in cancer prevention in humans [10, 11]. However, toxic effect of selenium was also known in humans and animals at a level not much higher than the beneficial requirement [12].

Nanotechnology has a great promise for medication and nutrition because materials at the nanometer dimension show novel properties different from their larger counterparts [13]. Selenium nanoparticles (Nano-Se) can be synthesized by reducing selenite in an environment containing bovine serum albumin [14]. It has been reported that Nano-Se has a similar bioavailability in rat [15] and much less acute toxicity in mouse compared with selenium [16]. In addition, Huang *et al*. [17] found that Nano-Se has the capacity of scavenging free radical in vitro. Considering the potential protective role of Nano-Se on hepatotoxicity induced by MCs, we have great interest in investigating whether management with Nano-Se has any protective effect on lipid peroxidation and antioxidant system in MCs-treated mice.

1 Materials and methods

1.1 Chemicals

Nano-Se in the particle size range of 10—80 nm was obtained from Guangzhou Bosar Biochemical Technology Research Co., Ltd., China. All other reagents obtained from various commercial sources were of analytical grade.

1.2 Microcystins preparation

Microcystis aeruginosa used in this experiment was obtained from FACHB (Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences) and its CCTCC No. was FACHB-1125. They were cultured in MA medium at the temperature of (25±1°)C [18]. The *Microcystis* cell extracts were made using the modified method based Metcalf and Codd [19] and Harada *et al*.[20]. Briefly, the cells were incubated in boiling water for 15 min, and then samples were removed, cooled on ice, and centrifuged at 10, 000 r/min for 10 min. After centrifugation, the supernatant was pooled together and applied directly to C18 cartridges. The cartridge containing the toxin was washed with 100% methanol. The

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methanol elute was collected and evaporated in rotary evaporator to dryness. Finally, they were dissolved in 10 mL deionized water. HPLC assay showed that the components of *Microcystis* cell extracts were microcystins-LR and microcystins-YR. The concentrations of microcystin-LR and microcystin-YR were 948.60 μg/mL and 83.96 μg/mL, respectively. Therefore, the total concentration of toxin in *Microcystis* cell extracts was expressed as 1032.56 μg microcystins/mL.

1.3 Animals

Male Kunming mice in weight range of 20—22 g were bought from Experimental Animal Centre of Henan Province, China. They were maintained in the animal house for at least 1 week before the experiment under controlled condition of illumination (12h light/12h dark) and temperature (20—25°C). They were allowed free access to a standard requirement diet and water libitum. The animals' treatment protocol was complied with the guidelines in the China Law for Animal Health Protection and Instructions for Granting Permit for Animal Experimentation for Scientific Purposes (ethics approval No. SCXK(YU)2005-0001).

1.4 Experimental protocol

The mice were randomly divided into four groups of 10 animals each and received daily intraperitoneal (i.p.) injections for 10 consecutive days as follows: Control group with 0.9% saline solution; Nano-Se group with Nano-Se solution at a dose of 100 μg / kg and the dosed based on previous literature [7]; Microcystins group with microcystins solution at a dose of 9 μg / kg and the dose was based on our previous result [21] which was approximately 1/4 LD_{50} for microcystins in mice. Microcystins plus Nano-Se group with microcystins solution at a dose of 9 μg / kg and mice of this group also with Nano-Se solution at a dose of 100 μg / kg.

At the end of the treatments, all the animals were fasted for 12h before being anesthetized and sacrificed by cervical dislocation. The mouse livers were quickly removed, and the blood immediately washed out with ice-cold 0.9% saline solution. The livers were weighed and stored at −70°C.

1.5 Biochemical assays

Liver homogenates were prepared at 10% (*w*/*v*) in 4°C PBS (pH 7.4). The homogenate was centrifuged at 9000 r/min for 15 min, the supernatant was collected and used for enzyme activity and soluble protein assays.

Total superoxide dismutase (SOD) activity was determined according to Giannopotitis and Ries [22]. One unit of SOD activity was defined as a 50% inhibition in the rate of nitro blue tetrazolium reduction measured at 560 nm. Catalase (CAT) activity was measured by the methods of Cakmak and Marschner [23], calculating the rate of decomposition for H_2O_2 at 240 nm. The concentration of thiobarbituric acid reactive substances (TBARS), expressed as MDA concentration (nmol/mg protein), was assayed using the method of Ohkawa *et al*. [24]. Glutathione (GSH) level was determined colorimetrically as protein-free sulfhydryl content using 5,5-dithiobis-2-nitrobenzoic acid (DTNB) Ellman reagent [25]. The activity of glutathione peroxidase (GPx) was measured following the procedure of Paglia and Valentine [26]. Total protein content in liver was determined by the method of coomassie protein assay dye assay using bovine serum albumin as standard [27].

1.6 Statistics

All results were expressed as mean ± standard errors (S.E.) (n =10). Differences between groups were calculated using a one-way analysis of variance (ANOVA), followed by the Tukey – Kramer multiple comparison test. $P < 0.05$ was considered to be statistically significant. SPSS 11.5 for windows was used for statistical analysis.

2 Results

2.1 General observation

No mortality was found throughout the experimental period in any groups. An increased liver/body weight index (LBI % = liver weight×100/body weight) was observed in the MCs-treated group compared to the control. No change was found in Nano-Se-treated group. However, decreased level of LBI was recorded in the Nano-Se plus MCs-treated group when compared with the MCs-treated group (Fig. 1).

2.2 Effects of Nano-Se on MCs-induced activity changes of antioxidant enzymes

The activities of antioxidant enzymes in mouse livers were given in Fig. 2. The results showed the activities of SOD, CAT and GPx were significantly decreased in the liver of the MCs-treated group as compared with control group. Nano-Se treatment caused a significant increase in GPx activity and no any significant change in the activities of SOD and CAT when compared with control group. However, administration of Nano-Se significantly promoted the activities of these enzymes in the MCs-treated mice when compared to the MCs-treated group without Nano-Se.

2.3 Effect of Nano-Se on the MCs-induced change of GSH level

The content of GSH in the MCs-treated mouse livers significantly decreased compared to the control group. In contrast, tissue content of GSH was significantly increased in the Nano-Se plus MCs-treated group when compared to the MCs-treated group without Nano-Se. However, Only Nano-Se treatment did not change GSH content when compared to control group (Fig. 2).

2.4 Effect of Nano-Se on the MCs-induced lipid peroxidation

MDA is a secondary product of lipid peroxidation and its level was used to determine lipid peroxidation induced by

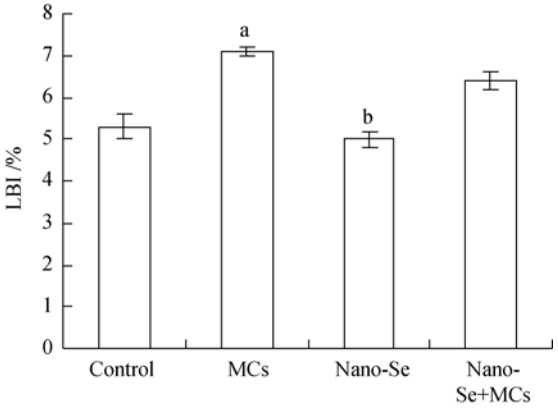

Fig. 1 Effect of Nano-Se on MCs-induced change in LBI. The date are expressed as mean ± S.E. (n=10)
a: $P < 0.05$ as compared with control group. b: $P < 0.05$ as compared with microcystins group

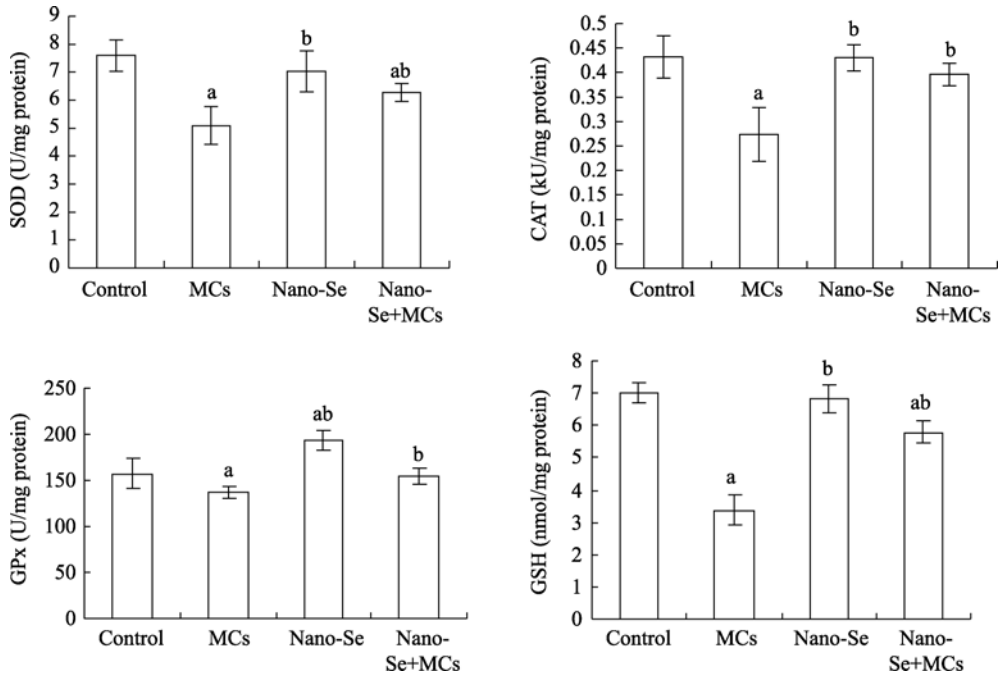

Fig. 2 Effects of Nano-Se on antioxidant enzymes and level of GSH in the liver of MCs-treated mice
a: $P < 0.05$ as compared with control group. b: $P < 0.05$ as compared with microcystins group

toxicant/toxin. In this study, microcystins treatment resulted in a significant increase of MDA level when compared with control group. But Nano-Se treatment significantly decreased the MCs-induced MDA level. In addition, only Nano-Se treatment did not induced any significant change in MDA level when compared with control group (Fig. 3).

3 Discussions

3.1 Lipid peroxidation

The exact mechanisms of MCs-induced toxicity and tumor promotion have not been fully elucidated. Many researchers reported that oxidative damage was involved in the toxicity of MCs in animals [5, 6]. Lipid peroxidation has been suggested to be a contributing factor to the development of oxygen radicals-mediated tissue damage. Some studies found that MCs could induce the formation of MDA in the liver of mouse or rat [28, 29]. In the present study, MDA level significantly increased in the liver when mice exposed MCs at the dose of 9 μg / kg. This result indicated that a significant oxidative stress was induced in the liver tissues of the MCs-treated mice. The level of MDA in the MCs plus Nano-Se group significantly decreased when compared with the group treated only by MCs. These results revealed that Nano-Se clearly decreased the lipid peroxidation in liver induced by MCs. The mechanism of inhibition of lipid peroxidation by Nano-Se probably includes the direct scavenging of the radical and increased antioxidant capacity in cells [17].

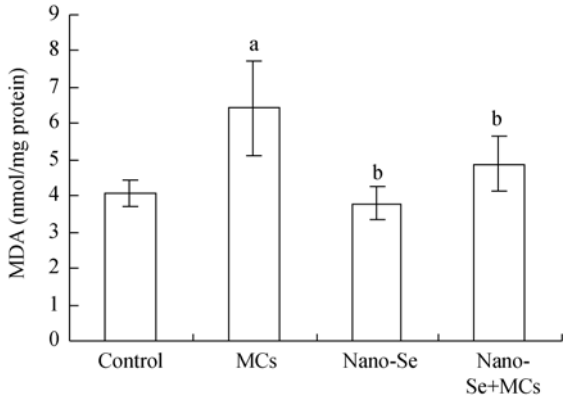

Fig. 3 Effect of Nano-Se on the level of MDA in the liver of MCs-treated mice
a: $P < 0.05$ as compared with control group. b: $P < 0.05$ as compared with microcystins group

3.2 Activities of antioxidant enzymes

Significant decreases in SOD and CAT activities of mouse liver treated by MCs were observed in this study. Our results are similar with the report of Moreno et al. [29] who found that acute exposure to microcystin-LR resulted in a decrease in these antioxidant enzymes in rats' liver. The decrease of SOD and CAT might predispose the liver to increased free radical damage because SOD is responsible for dismutation of highly reactive and potentially toxic superoxide radicals to H_2O_2 [30]. CAT has been considered to be the primary scavengers of H_2O_2 and its activity decreases can reduce the protection against free radicals and lipid peroxidation. Nano-Se supplementation maintained a level of SOD and CAT similar to that of control, indicating Nano-Se involved in different phases of free radicals detoxification.

In addition to increasing direct antioxidant enzyme activity, Nano-Se can provide indirect protection against free radical injury by improving the activities of selenium-dependent antioxidant enzyme, GPx. GPx is one of important enzymes protecting tissues from oxidative damage and various organic hy-

droperoxides that form an important group of toxic compounds produced in oxygen metabolism [31]. In the present study, GPx activity in Nano-Se group was significantly higher than that of the control group. This result suggests that Nano-Se has an obvious protection on this biomarker. The GPx activity in the MCs plus Nano-Se group significantly increased when compared with the MCs group without Nano-Se. Increasing GPx activity could be due to the higher selenium concentration above the nutritional optional activities for these enzymes and for the detoxification of MC.

3.3 GSH level

Glutathione plays an important role in the maintenance of intracellular redox status and antioxidant enzyme function [32]. In this study, GSH level was significantly decreased by MCs treatment compared to the control group. The decrease in GSH level may be on account of it's consumption in the prevention of free radical mediated lipid peroxidation. On the other hand, GSH could be consumed in the detoxification of MCs. It has been reported that GSH can conjugate with MCs under cell-free conditions or enzymatically via glutathione S-transferase in several biological system [33]. The present findings indicate that mouse received Nano-Se treatment can inhibit GSH consumption in liver during MCs intoxication.

In conclusion, these results of the present study suggest that MCs cause liver toxicity by inducing lipid peroxidation and decreasing antioxidant system of mouse liver. Nano-Se supplementation effectively reversed these changes and Nano-Se could be a hopeful chemoprotective agent against MCs-induced toxicity.

References:

[1] Codd G A, Morrison L F, Metcal F J S. Cyanobacterial toxins: risk management for health protection [J]. *Toxicology and Applied Pharmacology*, 2005, **203**: 264—272

[2] de Figueiredo D R, Azeiteiro U M, Esteves S M, *et al*. Microcystin-producing blooms-a serious global public health issue [J]. *Ecotoxicology and Environmental Safety*, 2004, **59**: 151—163

[3] Falconer I R. An overview of problems caused by toxic blue-green algae (cyanobacterium) in drinking and recreational waters [J]. *Environmental Toxicology*, 1999, **14**: 5—12

[4] Mackintosh C, Beattie K A, Klumpp S, *et al*. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants [J]. *FEBS Letters*, 1990, **264**: 187—192

[5] Weng D, Lu Y, Wei Y, *et al*. The role of ROS in microcystin-LR-induced hepatocyte apoptosis and liver injury in mice [J]. *Toxicology*, 2007, **232**: 15—23

[6] Zhang B J, Li X Y. Prolong sub-chronic exposed to *Microcystis* cell extract results in oxidative stress in mice liver [J]. *Acta Hydrobiologica Sinica*, 2009, **33**: 1088—1094

[7] Gehringer M M, Govender S, Shah M, *et al*. An investigation of the role of vitamin E in the protection of mice against microcystin toxicity [J]. *Environmental Toxicology*, 2003, **18**: 142—148

[8] Gehringer M M, Downs K S, Downing T G, *et al*. An investigation into the effect of selenium supplementation on microcystin hepatotoxicity [J]. *Toxicon*, 2003, **41**: 451—458

[9] Tinggi U. Selenium: its role as antioxidant in human health [J]. *Environmental Health and Preventive Medicine*, 2008, **13**: 102—108

[10] Lü J, Cheng J. Antiangiogenic activity of selenium in cancer chemoprevention: metabolite-specific effects [J]. *Nutrition and Cancer*, 2001, **40**: 64—73

[11] Rayman M P. Selenium in cancer prevention: a review of the evidence and mechanism of action [J]. *Proceedings of the Nutrition Society*, 2005, **64**: 527—542

[12] Hilton J W, Hodson P V, Slinger S J. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*) [J]. *Journal of Nutrition*, 1980, **110**: 2527—2535

[13] Ross S A, Srinivas P R, Clifford A J, *et al*. New Technologies for Nutrition Research [J]. *Journal of Nutrition*, 2004, **134**: 681—685

[14] Zhang J S, Gao X Y, Zhang L D, *et al*. Biological effects of a nano red elemental selenium [J]. *Biofactors*, 2001, **15**: 27—38

[15] Jia X, Li N, Chen J. A subchronic toxicity study of elemental Nano-Se in Sprague-Dawley rats [J]. *Life Sciences*, 2005, **76**: 1989—2003

[16] Zhang J, Wang H, Yan X, *et al*. Comparison of short-term toxicity between Nano-Se and selenite in mice [J]. *Life Sciences*, 2005, **76**: 1099—1109

[17] Huang B, Zhang J, Hou J, *et al*. Free radical scavenging efficiency of Nano-Se in vitro[J]. *Free Radical Biology and Medicine*, 2003, **35**: 805—813

[18] Ichimura T. Media for blue-green algae [A]. In: Nishizaua M, Chihara M (Eds.), Methods in Algology [C]. Kyoritsu Tokyo. 1979, 294

[19] Metcalf J S, Codd G A. Microwave oven and boiling water bath extraction of hepatotoxins from cyanobacterial cells [J]. *FEMS Microbiology Letters*, 2000, **184**: 241—246

[20] Harada K I, Suzuki M, Dahlem A M, *et al*. Improved method for purification of toxic peptide produced by cyanobacteria [J]. *Toxicon*, 1988, **26**: 433—439

[21] Ding X S, Li X Y, Duan H Y, *et al*. Toxic effects of *Microcystis* cell extracts on the reproductive system of male mice [J]. *Toxicon*, 2006, **48**: 973—979

[22] Giannopotitis C N, Ries S K. Superoxide dismutase in higher plants [J]. *Plant Physiology*, 1977, **59**: 309—314

[23] Cakmak I, Marschner H. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase,

ascrobate peroxidase, and glutathione reductase in bean leaves [J]. *Plant Physiology*, 1992, **98**: 1222—1227

[24] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction [J]. *Analytical Biochemistry*, 1979, **95**: 351—358

[25] Beutler E, Duron O, Kelly B M. Improved method for the determination of blood glutathione [J]. *The Journal of Laboratory and Clinical Medicine*, 1963, **61**: 882—890

[26] Paglia D E, Valentine W N. Studies on the qualitative characterization of erythrocyte glutathione peroxidase [J]. *The Journal of Laboratory and Clinical Medicine*, 1967, **70**: 159—169

[27] Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding [J]. *Analytical Biochemistry*, 1976, **72**: 248—254

[28] Guzman R E, Solter P F. Hepatic oxidative stress following prolonged sublethal microcystin-LR exposure [J]. *Toxicologic Pathology*, 1999, **27**: 582—588

[29] Moreno I, Pichardo S, Jos A, *et al*. Antioxidant enzyme activity and lipid peroxidation in liver and kidney of rats exposed to microcystin-LR administered intraperitoneally [J]. *Toxicon*, 2005, **45**: 395—402

[30] Kumar O, Sugendran K, Vijayaraghavan R. Oxidative stress associated hepatic and renal toxicity induced by ricin in mice [J]. *Toxicon*, 2003, **41:** 333—338

[31] Helmy M H, Ismail S S, Fayed H, *et al*. Effect of selenium supplementation on the activities of glutathione metabolizing enzymes in human hepatoma Hep G2 cell line [J]. *Toxicology*, 2000, **144**: 57—61

[32] Arteel G E, Sies H. The biochemistry of selenium and the glutathione system [J]. *Environmental Toxicology and Pharmacology*, 2001, **10**: 153—158

[33] Pflugmacher S, Wiegand C, Oberemm A, *et al*. Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication [J]. *Biochimica et Biophysica Acta* (*BBA*)-*General Subjects*, 1998, **1425**: 527—533