Cognitive impairment in rats induced by nano-CuO and its possible mechanisms

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HIGHLIGHTS

► We examine cognitive deficits of rats induced by nano-CuO in vivo. 
► We use MWM test, LTP recording and ELISA methods. 
► Nano-CuO has toxic effects on hippocampus, induced learning and memory deficits. 
► Mechanisms may be impairments of synaptic plasticity and oxidative damage.

ARTICLE INFO

Article history: 
Received 7 March 2012 
Received in revised form 11 June 2012 
Accepted 10 July 2012 
Available online xxx

Keywords: 
Nano-CuO 
Spatial cognition 
Long-term potentiation 
ROS 
Apoptosis 
Rats

ABSTRACT

Several studies have reported the adverse effects of nano-CuO on hippocampal CA1 neuron, whereas little has been known about nano-CuO neurotoxicity in vivo. In the present study, we investigated the effects of nano-CuO on spatial cognition and electrophysiological alterations in rats. In addition, histological and biochemical changes in rat's hippocampus were measured as well. Morris water maze (MWM) test showed that learning and memory abilities in nano-CuO-treated group were weakened significantly. The long-term potentiation (LTP) test exhibited that field excitatory postsynaptic potentials (fEPSPs) slopes were significantly lower in nano-CuO-treated group compared to that in control group. Furthermore, the levels of ROS and malonaldehyde (MDA) in hippocampal homogenate of nano-CuO-treated group were considerably enhanced while the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were statistically reduced. Moreover, the enhanced 4-hydroxynonenal (HNE) and caspase-3 implied the progression of apoptosis in the hippocampus. The results suggested that the neuronal damage, induced by impairing oxidation–antioxidation homeostasis, led to the impairment of hippocampal LTP, which was associated with the poor performance of animals in behavior tests.

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1. Introduction

Copper oxide (CuO), a semi-conducting compound with a monoclinic structure, has attracted particular attention and exhibits a range of potentially useful chemical and physical properties. Because it is cheaper than silver, easily mixed with polymers, and relatively stable characteristic of chemical and physical properties (Cava, 1990; Tranquada et al., 2004), CuO nanoparticles have widely been used nowadays. For example, they have potential to facilitate carbon monoxide oxidation instead of noble metal catalysts (Fan et al., 2006). The suspension of them (nanofluid) has been used as a heat transfer fluid in machine tools due to its excellent thermal conductivity (Kulkarni et al., 2006). Similarly, several evidences suggested that the bacteriostasis function of CuO nanoparticles was on a range of bacterial pathogens, such as Staphylococcus aureus and Escherichia coli, even though in a low bactericidal concentrations condition (Allaker, 2010; Kahru and Dubourguier, 2010; Kasemets et al., 2009). Surprisingly, when the size of particle decreases and the proportion of the surface area increases to nanoscale particle it tends to show abnormal biological activity, particularly biochemical toxicity compared to micron-sized one (Oberdorster et al., 2005). Thus, along with the increase of nanometer material applications, it becomes more and more essential to examine its possible adverse effects to human health.

So far, several investigations have been done and the toxicity of nanoparticle metal and metal oxides was carefully evaluated (Gojova et al., 2007; Jeng and Swanson, 2006; Park et al., 2007). Among the nanoparticles, nano-CuO induced the most serious health risk and logically received broad attention (Heinlaan et al., 2008; Karlsson et al., 2008; Yokohira et al., 2008). Lately, there were several studies that reported the deleterious effects of nano-CuO. For instance, nano-CuO was able to induce ROS generation in mouse pulmonary microvascular endothelial cells (Yu et al., 2010) and cause the ecotoxicological effects toward crustaceans (Heinlaan et al., 2007).

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0378-4274/5 – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2012.07.007
et al., 2008) and algae (Aruoja et al., 2009). In particular, the previous studies from our lab showed that nano-CuO could regulate the delayed rectifier potassium current in hippocampal CA1 pyramidal neurons of rat (Xu et al., 2009) and alter the action potential of hippocampal CA1 neurons by impairing the functional properties of voltage-gated sodium channels (Liu et al., 2011). The modification of the functional properties of hippocampal ion channels would be expected to cause dysfunction of hippocampus. Importantly, it was reported that nano-CuO was able to pass through the blood–brain barrier (BBB) and enter central nervous system (CNS) (Kreyling et al., 2002; Lockman et al., 2004; Oberdorster et al., 2004). Thus, the potential neurotoxicity was expected to be induced after long-term exposure to nano-CuO in vivo.

Nanoparticles of CuO have adverse attributes toward neurons (Liu et al., 2011; Xu et al., 2009), which may induce hippocampal dysfunction and further affect abilities of learning and memory. In order to test such a particular hypothesis, Wistar rats were treated with nano-CuO via intraperitoneal injection for 2 weeks. Subsequently, the Morris water maze (MWM) test and electrophysiological examinations were performed to examine the possible differences between normal rats and nano-CuO-treated rats. It is well known that oxidative damage is often used to explain toxicity associated with particle exposure (Stone et al., 2007). The ability of CuO nanoparticles to generate oxidative stress in vitro has been demonstrated (Ahamed et al., 2010; Fahmy and Cormier, 2009), however, little is known about the neurotoxicity of the CuO nanoparticles in vivo. Additionally, oxidative damage was sometimes associated with cognitive dysfunction (Kucukatay et al., 2007; Lima et al., 2008; Rahman et al., 2009). Thus, in the present study, it will also be focused on hippocampal oxidative damage and morphological alterations induced by nano-CuO. Its potential mechanisms have subsequently been discussed as well.

2. Materials and methods

2.1. Materials and chemicals

Reactive oxygen species (ROS), superoxide dismutase (SOD) and malondialdehyde (MDA) assay kits were purchased from the Nanking Jiangchen Bioengineering Research Institute (Nanking, China). Glutathione peroxidase (GSH-Px) ELISA kit, 4-HNE ELISA kit and Caspase-3 ELISA kit were bought from R&D Systems (USA). Other reagents were of A.R. grade.

2.2. Nano-CuO particles and solutions

In this study, the nano-CuO was provided by the Research Institute of Science and Technology (RSTI), University of Hertfordshire, Hampshire, UK. The nano particles of CuO were compounded at Queen Mary, University of London using the raw materials initially got from QinetiQ Nanomaterials Ltd., produced through Plasma (QLN Tesima™) Technology, UK.

The main characteristics of nano-CuO particles, including the size, surface area and particle diameter, have been described in our previously reports (Liu et al., 2011; Xu et al., 2009). Briefly, it shows that nano-CuO particles size distributes from 10 to 70nm. Its surface area used in this research is 15.6931 m2/g and the average particle diameter is given by 60.6 nm. SEM-EDS analysis shows that the weight percents of copper and oxygen are 80.65% and 16.96% correspondingly. And the atomic percents of copper and oxygen are 54.18% and 45.26%, respectively. Furthermore, the particle size of nano-CuO suspension in saline was characterized by dynamic light scattering (DLS) using a Zeta-PALS + BI-90Plus (Brookhaven Instruments Corp., USA) at a wavelength of 659 nm. They had a wide range from 52.16 to 370.02 nm due to the aggregation. The zeta potential of the nanoparticles was measured in the saline with a combination of laser Doppler velocimetry and phase analysis light scattering (PALS) using Zeta-PALS + BI-90Plus. It was 29.67 ± 21.91 nmV, which suggests that the physicochemical characteristic of nano-CuO in the saline was generally stable.

2.3. Animal model

Adult male, specific-pathogen free (SPF) Wistar rats, 280–300 g, were obtained from the Laboratory Animal Center, Academy of Military Medical Science of People’s Liberation Army, and reared in the Animal House of Medical School in Nankai University. All experiments were performed according to the protocols approved by the Committee for Animal Care at Nankai University and in accordance with the practices outlined in the NIH Guide for the Care and Use of Laboratory Animals. Food and water were freely available during all phases of the experiment.

Animals were randomly divided into two groups, nano-CuO group (n = 8) and control group (n = 8). In the nano-CuO group, rats were treated with nano-CuO at a dose of 0.5 mg/(kg.day) via intraperitoneal injection and given once a day over a period of 14 consecutive days. The nano-CuO solution (0.75 mg/ml) was prepared in 0.9% saline and then sonicated in suspension for at least 30 min to assure that it was completely dissolved, while the animals in control group were only received the same dose of 0.9% saline solution.

Previous study (Yokohira et al., 2008) reported that fourteen rats, instilled with nano-CuO at a dose of 2 mg/kg body weight, died rapidly and there were only three rats that survived 1 day after treatment. It also reported that exposure to 2 mg/kg body weight nano-CuO for a consecutive 28 days was severely fatal due to affect normal physiological metabolism. In a previous study (Yokohira et al., 2009), the nano-CuO was shown to induce toxicities at a dose of 0.5 mg/kg body weight for a phase of 14 successive days. Therefore, an appropriate dose for subchronic experiment at 0.5 mg/kg body weight was chosen in the current study.

2.4. Physical observation

From day 1 to day 14, the weight of each rat was recorded everyday at the same time. During the period of treatment, animals were examined for neurological deficits, such as head tilt and hemiparesis, and emotional abnormality including stress and anxiety. While other physical changes including spontaneous activity, accumulation of liquid, depilation and piloerection were measured as well.

2.5. Behavioral experiments in water maze

24h after the last treatment, all rats were trained and tested in Morris water maze (MWM, RB-100A type, Beijing, China) to monitor their spatial learning and memory behaviors. The water maze consists of a circular tank (150 cm in diameter, 60 cm in height) divided into 4 equal quadrants (I–IV) by two imaginary perpendicular lines crossing in the center of the tank, and there is a 10-cm-diameter platform submerged 2–3 cm below the water surface in the center of quadrant III. The water was made opaque using non-toxic black ink and maintained at 25 ± 1 °C. Movement of rats in the maze was monitored by a computerized video tracking system (Ethovision 2.0, Noldus, Wageningen, Netherlands) connected to a personal computer, through which data were collected for off-line analyzing.

The task consisted of two phases, place navigation phase and spatial probe phase. In the place navigation phase, animals were subjected to 10 sessions of training (2 sessions per day and each session consisted of 4 trials) for 5 consecutive days. In each session, subsequent starting positions proceeded in a clockwise manner in the trials and rats were located in the same position on every trial at one of 4 starting quadrant points. Animals were allowed to swim freely and trained to find a hidden platform in a circular water tank. The times, required to find the platform (escape latency) and the swimming speed, were recorded. If an animal failed to locate the platform within 60s, it was placed on it for 10s, and its escape latency was recorded as 60s.

The interval between two trials was approximately 5 min, and the time between sessions was approximately 8h. In the spatial probe phase, rats were subjected to the probe trial test 24h after the place navigation phase. The platform was removed from the tank. Rats were released individually into water from the starting point of quadrant 1 and allowed to swim for 60s as probe test. Quadrant dwell time (the percentage of time spent in the target quadrant) and platform crossings (numbers passing platform area) were collected. Only one session was tested in this phase (An et al., 2011).

2.6. In vivo electrophysiological test

Electrophysiological tests were performed after the MWM assessment. The animals were anesthetized with 30% urethane (Sigma-Alrich, St. Louis, MO, USA, 4 ml/kg) by intraperitoneal injection. And then they were placed in a stereotactic frame (SN-3, Narishige, Japan) for surgery and recording as described previously (An et al., 2011). Briefly, at the rat left head side, two small holes were drilled in the skull for the recording and stimulating electrodes inputting respectively. According to the rat brain in stereotaxic coordinates, the bipolar stimulating electrode was implanted into the hippocampus Schaffer collaterals region (4.2 mm posterior to the bregma, 3.5 mm lateral to midline, 2.5 mm ventral below the dura), and the recording electrode was implanted into hippocampus CA1 region (3.5 mm posterior to the bregma, 2.5 mm lateral to midline, 2.0 mm ventral below the dura). The optimal depth of the electrode was determined by electrophysiological criteria (Leung, 1980). For LTP experiments, test stimuli were delivered to the Schaffer collaterals every 30 s at an intensity that evoked a response of 70% of its maximum (range 0.5–0.7 times threshold), and then the field excitatory postsynaptic potentials (fEPSPs) were amplified (100×), filtered at 5 Hz–5 kHz, digitized and collected at 20kHz sample frequency (Scope software from Compusphere, Australia) every 60 s for 60min. Initial data measurement was performed in Clampfit 9.0 (Molecular Devices, Sunnyvale, CA, USA). The fEPSPs slope (20–80% level of the falling phase) was used to measure synaptic efficacy.
2.7. Preparation of tissue samples

After the electrophysiological experiments, all animals were deeply anesthetized and the brains were removed individually. Subsequently, the left side of the hippocampus was isolated on an ice-cold operation table. After weighed and digested, it was rinsed in 0.1 M phosphate buffer (pH 7.4) and homogenized with ice-cold saline to be 10% (w/v) homogenates. The mixtures were homogenized using a glass homogenizer for 5 min on ice and centrifuged at 3000 rpm at 4 °C for 15 min. The supernatant was collected and stored at −70 °C for the biochemical tests. In addition, the other parts of the brain, such as cortex, striatum and amygdale, were dissected out and preserved as the above method.

2.8. Elemental content assay

The left side of the hippocampus was analyzed for copper content by the method of the previous study (Hu et al., 2010). Briefly, prior to elemental analysis, the tissues of interest were digested in nitric acid (ultrapure grade) overnight. After adding 0.5 ml H2O2, the mixed solutions were heated at about 160 °C using high-pressure reaction container in an oven chamber until the samples were completely digested. After that, the solutions were heated at 120 °C to remove the remaining nitric acid until the solutions were colorless and clear. At last, the remaining solutions were diluted to 3 ml with 2% nitric acid. Inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Electron Co., Finland) was used to analyze the copper concentration in the samples.

2.9. HE staining

When the left hippocampus was isolated from the killed rat brain, the right side of brain was removed and immersed in 4% paraformaldehyde fixed at 4 °C for at least 24 h, and then was dehydrated and embedded in paraffin for tissue sectioning. The coronary slices (5 μm) were obtained and used for hematoxylin/eosin (HE) staining in accordance with the standard procedure. The slides were observed viewed on a Leica microscope (Wetzlar, Germany) and photographed.

2.10. Measurement of biochemical indexes in hippocampus

The levels of superoxide anion radical, hydroxyl free radical, MDA, T-SOD, GSH-Px, 4-hydroxy-2-nonenal (HNE) and caspase-3 in hippocampus were determined according to the methods described in the references using commercial kits. ELISA kits procedures briefly: firstly, add prepared samples and standards, antibodies labeled with enzyme, reacting 60 min at 37 °C. Secondly, plate washed 5 min, adding chromogen solution, reacting 10 min at 37 °C. Finally, add stop solution and measure the OD value with 10 min. The protein levels of samples were measured by the Coomassie Brilliant Blue G-250 method with bovine serum albumin as standard.

2.11. Data acquisition and statistical analysis

All data were presented as the mean ± S.E.M. Escape latencies and swimming speeds in the acquisition phase of the MWM were analyzed with repeated measures ANOVA. The LTP recordings and data for biochemical tests were compared using independent-samples t-test. The probability value of less than 0.05 was considered to be statistical significance. The analyses were performed using SPSS 16.0 software.

3. Results

3.1. Copper content in hippocampus

The level of copper in the hippocampus was determined by ICP-MS and the results are shown in Fig. 1A. After administrating nano-CuO for a consecutive 14-days, the copper accumulation in hippocampus was significantly increased compared to that in control group (P < 0.01). The abnormal level of copper in hippocampus supported the notion that nanoparticles was able to pass through the blood–brain barrier (BBB) and affected the physiological function of CNS (Kreyling et al., 2002; Lockman et al., 2004; Oberdorster et al., 2004).

Additional experiments were performed for measuring the levels of copper in other parts of the brain. The results showed that the levels of copper in cortex, striatum and amygdala were 18.01 ± 1.36 μg/g, 31.51 ± 4.77 μg/g, and 10.21 ± 2.19 μg/g, respectively. It can be seen that the accumulation of nano-CuO was not selective for the brain regions of hippocampus, cortex, striatum and amygdala.

3.2. Physical findings

From day 1 to day 4, there were no significant differences of body weight between these two groups. There was lower body weight in nano-CuO group than that in control group (Fig. 1B, day 7, 10 and 13: P < 0.05). Moreover, no significant differences of hippocampus weight (92.19 ± 8.91 mg vs. 83.10 ± 7.39 mg, P > 0.05) between nano-CuO group and control group were found (figure not shown). The symptoms of piloerection, depilation and accumulation of liquid in peritoneal cavity were observed in the rats treated by nano-CuO. There was no observable stress or anxiety in the rats behavior with nano-CuO treatment; however, spontaneous activity was significantly reduced in nano-CuO group compared to that in control group.

3.3. Morris water maze test

During the five days of place navigation, the performance of all rats was improved progressively after circling the periphery of the tank in the first few trials, which was demonstrated by their decreasing escape latencies over trials. Overall analysis (n = 8 for control and n = 8 for nano-CuO group) showed that there was a significant effect of nano-CuO on average escape latency, which was statistically increased in nano-CuO group from day 3 to 5 (Fig. 2A, day 3 and day 4: P < 0.05, day 5: P < 0.01). In addition, the swimming

![Fig. 1. Copper content in hippocampus and the effects of nano-CuO on body weight. (A) The concentration of copper in the hippocampus. (B) Body weight changes of rats in nano-CuO group and control group over treatment days. Data are presented as mean ± S.E.M. *P < 0.05, **P < 0.01, n = 5 for each group.](image-url)
speeds of each group remained constantly throughout testing, with no significant difference to be found between these two groups on each day (Fig. 2B, \( P > 0.05 \)).

On the sixth day, the spatial probe test was performed. It was found that there was a marked effect of the nanomaterial on the nano-CuO-treated rats. Statistical results revealed that both the indexes of platform crossings (Fig. 2C, \( P < 0.05 \)) and quadrant dwell time (Fig. 2D, \( P < 0.01 \)) were decreased in nano-CuO group compared to those in control group.

3.4. In vivo LTP test

In LTP test, stimulation of Schaffer collaterals evoked a basal fEPSPs in hippocampal CA1 and high-frequency stimulation induced the LTP of the stimulated synapses for at least 1 h. Results representing the time course of fEPSPs slopes normalized to the 20 min baseline period were shown in Fig. 3A. The fEPSPs slopes increased immediately after high frequency stimulation and stabilized to a level above the baseline period. Moreover, it was found that fEPSPs
slopes were significantly smaller in nano-CuO group than those in control group (Fig. 3B, P < 0.01).

3.5. Histopathological observation

It was found that the number of neurons in hippocampal CA1 area was obviously reduced in nano-CuO group (Fig. 4A), compared to that in control group (Fig. 4B). As seen in Fig. 4A, there are clearly morphological changes in the hippocampus in nano-CuO group. The nuclei are side-moved and dark-stained. The cytoplasm of neurons is shrunken, while necrotic neurons are observed everywhere in nano-CuO group. In control group, it can be seen that the neurons are full and arranged tightly and the nuclei are light-stained (Fig. 4B).

3.6. Oxidative damages induced by nano-CuO

Fig. 5A and B shows the effects of nano-CuO on the level of superoxide anion radical and hydroxyl free radical. It can be seen that superoxide anion radical (P < 0.05) and hydroxyl free radical (P < 0.01) levels in nano-CuO group are significantly increased compared to that in control group. The effect of nano-CuO on the level of MDA was determined and the result was presented in Fig. 5C. It was found that MDA level was significantly increased in nano-CuO.
group \(P < 0.01\) compared to that in control group. Furthermore, Fig. 6A and B shows the results that there were the effects of nano-CuO on the activities of T-SOD and GSH-Px. It was found that T-SOD \(P < 0.01\) and GSH-Px \(P < 0.05\) activates in nano-CuO group were significantly decreased in the hippocampus compared with that in control group.

3.7. Effects of nano-CuO on 4-HNE and caspase-3 levels in the hippocampus

The effect of nano-CuO on the activity of 4-HNE was determined and the result was presented in Fig. 7A. It can be seen that 4-HNE level is significantly increased in nano-CuO group \(P < 0.05\) compared to that in control group. Furthermore, the level of caspase-3 was measured and showed in Fig. 7B. It was found that the caspase-3 content was significantly increased in nano-CuO group compared to that in control group in the hippocampus \(P < 0.05\).

4. Discussion

Recently, the toxic effects of nanosized CuO on human and mammal have been reported broadly, such as respiratory diseases, asthma, cancer and cardiopulmonary diseases (Heinlaan et al., 2008; Karlsson et al., 2008). Previous studies highlighted the in vivo and in vitro toxicities of CuO nanoparticles (Aruoja et al., 2009; Yokohira et al., 2008). Our recent studies showed that there was a correlation between exposure to nano-CuO and the potential danger of nervous system dysfunction (Liu et al., 2011; Xu et al., 2009). Remarkably, it was demonstrated that the high toxic nano-CuO was able to pass through the blood–brain barrier (BBB) (Kreyling et al., 2002; Lockman et al., 2004; Ober dorster et al., 2004), suggesting that CuO nanoparticles might have potential toxic effects on the CNS after exposure to this toxin. In the present study, we examined the effect of nano-CuO on the hippocampus in rats. Several experiments, including behavioral test, electrophysiological experiments, histological analyses and biochemical tests, were performed accordingly.

During 14 consecutive treatment days, the reduction of bodyweight gain and spontaneous activity, the symptoms of piloerection and accumulation of liquid in peritoneal cavity revealed the abnormal status of rats in nano-CuO-treated group. Histological analyses showed that the outline of CA1 pyramidal neurons was greatly altered in nano-CuO treated group compared to that in control group. It suggested that the nano-CuO treatment induced nuclear shrinkage and necrotic neurons in the hippocampus, which were similar to those findings obtained from recent studies (Fahmy and Cormier, 2009; Karlsson et al., 2008; Seiffert et al., 2012). To evaluate whether nano-CuO affected the cognitive functions, the Morris water maze test, one of the most effective behavioral test for examining learning and memory in rodents (D’Hooge and De Deyn, 2001), was performed. The data showed that there was a significant prolongation of escape latency during 3–5 training days in nano-CuO group compared to that in control group, suggesting that nano-CuO-treated rats were associated with their disruption of learning and memory. Furthermore, it was found that the swimming speed remained constant throughout testing, suggesting there was no evidence that motor function was the underlying determinant for the prolonged latencies. In spatial probe period, rats took less time to perform in target quadrant, while the number of crossing target quadrant was reduced considerably in nano-CuO group compared to that in control group. All of the above data suggest that the impairment of learning and memory performance are induced by nano-CuO treatment. In fact, several previous reports showed that the other nanoparticles, such as zinc oxide and titanium oxide, had deleterious effects on cognition as well (Han et al., 2011; Hu et al., 2010).

Since we have found deficits of learning and memory in nano-CuO treated rats, we want to further explore whether hippocampal CA1 synaptic plasticity is changed. The decreased eEPSPs slope in nano-CuO treated rats was consistent with the impaired LTP, which supported our behavioral findings, and further indicated that synaptic projections from CA3 to CA1 might have participated in the regulation of spatial learning and memory. Interestingly, the level of copper (21.36 ± 3.36 μg/g), detected in nano-CuO treated rats in the study, was really close to the concentration of nano-CuO (5 × 10^{-5} g/ml) used in the extracellular solution in our previous investigations, suggesting nano-CuO impaired the functions of potassium channels and sodium channels in hippocampal CA1 neurons (Liu et al., 2011; Xu et al., 2009). It is well known that potassium channels play an essential role in regulating synaptic integration and plasticity, such as shaping synaptic integration through their NMDAR and Ca^2{+}-dependent internalization and inducing LTP (Kim and Hoffman, 2008; Ramakers and Storm, 2002). Moreover, the back-propagating sodium action potentials may play a key role in regulating synaptic strength and inducing LTP in CA1 neurons (Takagi, 2000).

The study indicated that oxidative damage was proposed as a common mechanism of cell damage induced by many types of nanoparticles (Stone et al., 2007), while it was also reported that nano-CuO generated oxidative stress and produced apoptosis (Ahamed et al., 2010; Fahmy and Cormier, 2009). Accordingly, a
The hypothesis was proposed that the neurotoxicity induced by nano-CuO might be mediated through generation of oxidative damage. In the current study, it was revealed that ROS of nano-CuO group, such as superoxide anion radical and hydroxyl free radical levels, was markedly increased comparing with that of control group. The data were consistent with the previous study, suggesting that ROS generated by nano-CuO induced oxidative stress in airway epithelial cells (Fahmy and Cormier, 2009) and human lung epithelial cell line A549 (Karlsdon et al., 2008). Generally, cells respond to oxidative burden by fortifying their antioxidant defense mechanisms in order to protect themselves from any oxidative damage. However, if the defense mechanisms fail to neutralize the oxidative burden, oxidative damage occurs (Fahmy and Cormier, 2009). It was found that the activities of T-SOD and GSH-Px were decreased after exposing to nano-CuO. These results suggested that one of the mechanisms was attributed to the decrease of endogenous antioxidants. Lipid peroxidation was the classic result of oxidative damage and its byproduct was often formed when free radicals attacked cellular membranes (Chen et al., 2010). In the present study, the excessive level of MDA, a stable metabolite of lipid peroxidation, would further confirm the generation of oxidative damage in the hippocampus. All the above data suggest that nano-CuO affects the oxidation–antioxidation homeostasis of hippocampus via increasing ROS and reducing antioxidant enzymes.

The high content of 4-HNE caused by nano-CuO treatment obtained from this study showed that nano-CuO induced the progression of neuronal apoptosis, which was consistent with our morphological observation and the findings of other researches (Liu et al., 2011; Seiffert et al., 2012). Cleaved caspase-3 appeared to be the most abundant of the caspases and was involved in the convergence of all caspase-mediated pathways related to apoptosis (Widlak and Garrard, 2006). Previous studies reported that 4-HNE triggered changes in cellular redox status related to apoptotic cell death through activation of the caspases-3 (de Villiers et al., 2007; Li et al., 2006; Ramachandran et al., 2001). Our findings showed that nano-CuO treatment resulted in apoptosis associating with increased cleaved caspase-3 level. It suggests that caspase-3, activated by 4-HNE, is involved in the regulation of apoptosis in this study.

It is well known that one neuron can be connected to a lot of other neurons by either dendrites or axons, but with so many neuron apoptosis, the connection would be weakened tremendously, and this might account for the impairment of LTP. Moreover, a recent study showed that lipid peroxidation affected oxidative phosphorylation, maintenance of mitochondrial membrane potential and mitochondrial Ca2+ buffering capacity (Ott et al., 2007), suggesting there was a potential reason that the LTP was significantly impaired in the present study. Therefore, as the hippocampus is one of the critical regions for learning and memory (Eichenbaum, 2004; Geinisman et al., 2004), the occurrence of hippocampal neural apoptosis via affecting oxidation–antioxidation homeostasis provided supportive evidence for causative role in the impairment of synaptic plasticity.

In conclusion, in the present study it was found that nano-CuO had toxicity effect on the cognitive functions of rats. The mechanisms of nano-CuO neurotoxicity, at least partly, lay in the impairments of synaptic plasticity. From the result of LTP, it can be clearly seen that nano-CuO depressed LTP from Schaffer collaterals to CA1 region in the hippocampus, and then the learning and memory impairment emerged. Considering the occurrences of imbalance of oxidation–antioxidation homeostasis and neuronal damages in the hippocampus, it suggested that oxidative damage and neuronal apoptosis were induced by nano-CuO treatment. It could be considered the evidences of synaptic plasticity impairment and spatial cognition deficits. However, these results only provide the preliminary data for the neurotoxicology of nano-CuO. The future experiments will be done to explore in cellular signal transmission.

Conflict of interest

None.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31171053) and Tianjin Research Program of Application Foundation and Advanced Technology (12JCZDJC23300, 10JCZDJC19100).

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