Research article

The neuroprotective effects of taurine against nickel by reducing oxidative stress and maintaining mitochondrial function in cortical neurons

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HIGHLIGHTS

• Taurine diminished nickel-induced cytotoxicity in primary cultured neurons.
• Taurine attenuated the oxidative stress and mitochondrial dysfunction of neurons exposed to nickel.
• Taurine may have pharmacological potential in treating the adverse effects of neurotoxins that target the mitochondria.

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ABSTRACT

Previous studies have indicated that oxidative stress and mitochondrial dysfunction are involved in the toxicity of nickel. Taurine is recognized as an efficient antioxidant and is essential for mitochondrial function. To investigate whether taurine could protect against the neurotoxicity of nickel, we exposed primary cultured cortical neurons to various concentrations of nickel chloride (NiCl₂; 0.5 mM, 1 mM and 2 mM) for 24 h or to 1 mM NiCl₂ for various periods (0 h, 12 h, 24 h and 48 h). Our results showed that taurine efficiently reduced lactate dehydrogenase (LDH) release induced by NiCl₂. Along with this protective effect, taurine pretreatment not only significantly reversed the increase of ROS production and mitochondrial superoxide concentration, but also attenuated the decrease of superoxide dismutase (SOD) activity and glutathione (GSH) concentration in neurons exposed to NiCl₂ for 24 h. Moreover, nickel exposure reduced ATP production, disrupted the mitochondrial membrane potential and decreased mtDNA content. These types of oxidative damage in the mitochondria were efficiently ameliorated by taurine pretreatment. Taken together, our results indicate that the neuroprotective effects of taurine against the toxicity of nickel might largely depend on its roles in reducing oxidative stress and improving mitochondrial function. Taurine may have great pharmacological potential in treating the adverse effects of nickel in the nervous system.

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1. Introduction

The high consumption levels of nickel-containing products in modern society have raised considerable concerns about their potential hazardous effects on human health. Nickel proves to be a potential carcinogenic agent and has multiple toxic effects in various systems [6,7]. The nervous system is one of the main target organs of nickel toxicity. Previous evidence showed that nickel exposure resulted in a variety of neurological symptoms, including headaches, giddiness, tiredness, lethargy and ataxia [6]. In different types of nerve cells in vitro studies, nickel
exposure decreased mitochondrial activity, inhibited cell proliferation, disturbed neuronal calcium homeostasis and displayed developmental neurotoxicity through changing the gene expression of the glutamate receptors [21,26,34]. Among the many mechanisms involved in nickel-mediated neurotoxicity, oxidative stress has been proposed to play a pivotal role [6,23]. Substantial recent attention has focused on the use of various antioxidants to prevent the adverse effects of nickel on the nervous system.

Mitochondria are the major sites that produce reactive oxygen species (ROS). Excess ROS induce oxidative damage to unsaturated fatty acids, proteins and DNA in the mitochondria [20,30]. With unique structural and functional characteristics, mitochondria are often the targets of a number of environmental toxins, especially those that cause mitochondrial oxidative stress [10]. Mitochondrial dysfunction induced by oxidative stress could disturb the electron transferring and amplify the ROS generation, which exacerbate the oxidative stress and form a vicious cycle. Thus, oxidative damage to the mitochondria is typically considered as the primary or secondary etiology of toxicity of various environmental toxins including nickel [2]. Indeed, our previous study indicated that the nickel exposure induced obvious oxidative stress and mitochondrial dysfunction both in vitro and in vivo [17,33,34].

Taurine, a sulfur-containing amino acid, presents at high concentrations in the mammalian brain. Taurine plays significant roles in neurotransmission, neuromodulation, osmoregulation, detoxification and calcium homeostasis [9,11]. As a type of antioxidant, taurine has been found to be neuroprotective against oxidative damage under various pathological conditions including hypoxia, hypoglycemia, ischemia, excitotoxicity, metabolic poisons and β-amyloid-induced neurotoxicity [14,24]. Particularly, taurine could interact with mitochondria and maintain mitochondrial homeostasis. In the mitochondria, taurine could reduce oxidative stress, stimulate mitochondrial anti-oxidative enzymes (Mn-SOD), regulate mitochondrial calcium homeostasis and preserve mitochondrial function [9,31]. Because oxidative stress and mitochondrial dysfunction are recognized as an adverse effects of nickel exposure, the purpose of our study was to investigate the neuroprotective roles of taurine against the neurotoxicity of nickel.

2. Materials and methods

2.1. Cell culture and treatment

The primary cultured neurons were prepared as previously reported [34]. On day 8, cortical neurons were pre-treated with 10 mM taurine (Sigma, St Louis, MO, USA) for 2 h, a procedure based on the previous study [5] and our pre-experiment. After taurine pretreatment, neurons were exposed to nickel chloride (NiCl2, Sigma, St Louis, MO, USA) for the various experiments. In the LDH release assay, neurons were incubated with various concentrations of NiCl2 (0.5 mM, 1 mM and 2 mM) for 24 h or with 1 mM NiCl2 for various periods (0 h, 12 h, 24 h and 48 h), which was according with our previous study. In other experiments, neurons were incubated with NiCl2 (1 mM and 2 mM) for 24 h.

2.2. Neurotoxicity measurement

Lactate dehydrogenase (LDH) release was evaluated to assess the neurotoxicity of nickel in cortical neurons and was measured with the cytotoxicity detection kit (Roche, Mannheim, Germany). Results were expressed as the percentage of maximum LDH release obtained by lysing the cells in 1% Triton X-100.

2.3. Assay of intracellular reactive oxygen species (ROS)

The determination of intracellular oxidant production was based on the oxidation of DCFH-DA (Beyotime Company, China). Briefly, 1 × 10⁴ cortical neurons were seeded in 96-well plates. After treatment, cells were incubated with DCFH-DA (1:1000) for 1 h. The fluorescence was read at 485 nm for excitation and 530 nm for emission with the Infinite™ M200 Microplate Reader (Tecan, Mannedorf, Switzerland). The experiment was repeated four times and cellular fluorescence intensity was expressed as the fold increase compared to the controls.

2.3.1. Assessment of oxidative stress within mitochondria

Oxidative stress within the mitochondria was determined by using MitoSOX™ Red (Invitrogen Corp., Carlsbad, CA), a mitochondria-targeted fluorescent probe for the highly selective detection of mitochondrial superoxide. After treatment with taurine and nickel, cortical neurons were incubated with culture medium containing 5 µM MitoSOX for 10 min at 37°C. Fluorescence intensity was analyzed with the microplate reader.

2.3.2. Superoxide-dismutase (SOD) activity determination

The total (Cu–Zn and Mn) superoxide-dismutase activity was determined with the total superoxide dismutase assay kit (Beyotime Company, China) based on the ability of SOD to inhibit xanthine/xanthine oxidase-derived superoxide anion reduction in WST-1 formazan.

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**Fig. 1.** Taurine protected cortical neurons against nickel-induced cytotoxicity. After cortical neurons were pretreated with 10 mM taurine (Tau) for 2 h, the effects of taurine on LDH release in nickel-treated neurons, were examined by exposing neurons to NiCl2 at the desired concentrations (0.5 mM, 1 mM and 2 mM) for 24 h (A). Time course analysis was performed by incubating the neurons with 1 mM NiCl2 for 0 h, 12 h, 24 h and 48 h (B). After treatment with nickel and taurine, cell-free culture supernatants were collected and incubated with LDH assay solution at 25 °C for 30 min. Optical density was measured at 490 nm and the reference value at 620 nm was subtracted out. Results are expressed as the percentage of maximum LDH release which was obtained by lysing the cells in 1% Triton X-100. **p < 0.01 versus sham-exposed control group, #p < 0.05, ##p < 0.01 versus groups treated with nickel at the same concentration or at the same time point. Values are means ± SE, n = 4.
2.4. GSH content determination

GSH content was measured with a commercially available kit (Beyotime Company, China) following the manufacturer’s instructions. Total glutathione (GSH plus GSSG) content was determined by the method of DTNB–GSSG recycling assay spectrophotometrically at 412 nm. GSSG content was measured by the same method after the supernatant was pretreated with 10 mM 2-vinylpyridine to remove the reduced GSH. The reduced GSH content was calculated as the difference between total glutathione and GSSG, which was expressed as μmol/g protein.

2.5. ATP content determination

ATP content was measured with the ATP determination kit (Invitrogen Corp., Carlsbad, CA). The luminescence generated by cell lysis and determination of buffer mixtures was analyzed with the luminometer (Turner Designs, Sunnyvale, CA). ATP content was calculated from the ATP standard curve and expressed as the percentage relative to control group.

2.5.1. Measurement of mitochondrial membrane potential (ΔΨm)

Mitochondrial membrane potential assay kit with JC-1 probe (Invitrogen Corp., Carlsbad, CA), was applied to measure the ΔΨm in cortical neurons. For confocal microscopy, neurons were incubated in 1 × JC-1 in growth medium at 37°C for 20 min. The coverslips were visualized under a Leica confocal laser scanning microscope (TCS SP2, Germany). An uncoupling agent, carbonyl cyanide m-chlorophenylhydrazone (CCCP), was served as positive controls. To quantify the fluorescence ratio, cortical neurons were seeded into a 96-well plate. The fluorescence intensity was analyzed with the microplate reader with emissions at 530 and 590 nm, respectively. The ΔΨm was calculated by the ratio of red fluorescence divided by green fluorescence.

2.6. Detection of mtDNA content

Quantitative real-time PCR was applied to detect the content of mtDNA as previously described [34]. The −ΔΔCt (mtDNA to β-actin) represented the mtDNA content in a cell.

2.7. Statistical analysis

The experimental data were expressed as the means ± SE. Each experiment was carried out at least three times. Data comparisons were completed using one way ANOVA or paired t-test to compare the means of the different treatment groups. *p < 0.05 was considered statistically significant.

3. Results

3.1. Taurine protected cortical neurons against cytotoxicity induced by nickel

Nickel has been shown to induce significant neurotoxicity in different types of nerve cells in vitro. In the present study, NiCl₂ was applied to cell cultures in a series of concentrations (0.5 mM, 1 mM,
Fig. 3. Taurine improved mitochondrial dysfunction in cortical neurons exposed to nickel. (A) Images of green (JC-1 monomer) and red (JC-1 aggregate) fluorescence within individual cells were taken with a Leica confocal laser scanning microscope. Colocalization appeared as an orange–red color due to the mixing of the red and yellow signals. Depolarized mitochondria, which served as positive controls, were established by treating cells with 50 μM CCCP at 37 °C for 5 min. For fluorescence ratio quantification (B), the fluorescence intensity was analyzed with the Microplate Reader. The △Ψm was calculated by the ratio of red fluorescence (590 nm) divided by green fluorescence (530 nm). (C) ATP content was measured with the ATP determination kit. Two specific fragments of mtDNA were designed for mtDNA content quantification: the gene of COX I of the mtDNA (D) and the gene of ND6 (E) of the mtDNA. The amounts of mtDNA were normalized to the internal control, /H9252-actin. 

3.2. Taurine reduced oxidative stress in cortical neurons exposed to nickel

Because the toxicity of nickel is tightly related with oxidative stress, we tested whether taurine would reduce the oxidative stress...
in nickel-treated neurons [6,7]. We found that the 24 h exposure to 1 mM and 2 mM NiCl₂ caused an increase in the production of ROS by 2.0 and 3.7 fold, respectively, as compared to controls. However, these increases in ROS production were significantly reduced by taurine pretreatment (Fig. 2A). Accordingly, mitochondrial superoxide concentration was significantly elevated after neurons were exposed to 1 mM and 2 mM NiCl₂, which corresponded to 1.57 and 1.85 fold, respectively, as compared to controls. Taurine pretreatment also significantly reversed these trends in mitochondrial superoxide concentration (Fig. 2B). In addition, SOD activity and GSH content were reduced by different degrees after neurons were exposed to 1 mM and 2 mM NiCl₂. However, taurine pretreatment reversed these decreases as expected (Fig. 2C and D). These results suggest that taurine could reduce oxidative stress in nickel-treated neurons.

3.3. Taurine improved mitochondrial dysfunction in neurons exposed to nickel

Mitochondrion is the main point that ROS generates, whose function is susceptible for the oxidative attack. Thus, we analyzed the mitochondrial membrane potential ($\Delta \Psi_{m}$), ATP content and mtDNA content to explore the protective effects of taurine against nickel on mitochondrial function. As shown in Fig. 3A, the JC-1 probe stained both green depolarized mitochondria and red polarized mitochondria in control cortical neurons, which represents the membrane potential under normal physiological conditions. After exposed to NiCl₂ (1 mM and 2 mM) for 24 h, cortical neurons showed a decrease of the red fluorescence and a significant increase of green fluorescence, which indicated that nickel exposure damaged the $\Delta \Psi_{m}$. However, taurine pretreatment efficiently preserved the $\Delta \Psi_{m}$ in nickel-treated cortical neurons. These findings were supported by the quantification of red fluorescence divided by green fluorescence (Fig. 3B), which showed that NiCl₂ (1 mM and 2 mM) caused a marked loss (46% and 58%, respectively) in $\Delta \Psi_{m}$ compared to controls, and pretreatment with taurine preserved the $\Delta \Psi_{m}$. ATP content was significantly decreased by 34% and 41% after neurons were treated with NiCl₂ (1 mM and 2 mM, respectively) for 24 h. As expected, taurine pretreatment efficiently reversed these declines (Fig. 3C). To evaluate the mtDNA content, which is important for the mitochondrial function [30], we compared relative amounts of mtDNA with nuclear DNA copy numbers. The mtDNA amplicons were generated from two different segments of mtDNA: the cytochrome c oxidase subunit I (COX1), which is encoded by the heavy chain of mtDNA and NADH dehydrogenase subunit 6 (ND6), which is encoded by the light chain of mtDNA. Both COX I and ND6 were significantly decreased compared with control cortical neurons after exposed to nickel (Fig. 3D and E). However, taurine pretreatment increased the mtDNA to different degrees in neurons treated with nickel. These results reflected that taurine could efficiently maintain the mitochondrial function in nickel-treated nerve cells.

4. Discussion

The nervous system is one of the susceptible organs of nickel toxicity [6,7]. Nickel exposure was reported to induce cytotoxicity in different kinds of nerve cells in vitro and result in various neurological symptoms in vivo [16,17,34]. Even worse, the chelating agents, the conventional therapy for the accidental nickel exposure, make the body burden of nickel redistribute and raise the concentration of nickel in the brain [32]. Numbers of efforts have been done for exploring the protective compounds that prevent the neurotoxicity of nickel [16,19,34]. In present study, we found that taurine diminished the nickel-induced LDH release in primary culture cortex neurons, suggesting the pharmacological potential of taurine to cure the adverse effects of nickel in the nervous system.

Oxidative stress is typically considered to be a significant mechanism underlying the neurotoxicity of nickel [6,7]. As a transition metal, nickel lead to disturbing the iron homeostasis and accumulation of free iron ion, which in turn generates ROS through Haber–Weiss and Fenton reaction [22,29]. Additionally, nickel inactivates a serial of oxidation-reduction enzymes, like aconitase, NADH dehydrogenase, succinate dehydrogenase etc., which promotes the ROS generation through the abnormal electron transferring [4,15]. Exposure to nickel was found to generate ROS, which lead to increase lipid peroxidation, trigger nitric oxide (NO) production and alter the cellular antioxidant system [8,12,22]. The oxidative stress could cause oxidation of proteins, unsaturated fatty acid and mtDNA in mitochondria, which arises the morphologic and functional abnormal of mitochondria. Then, the damaged mitochondria could further exacerbate ROS production and aggravate oxidative stress. In the present study, we found that nickel exposure not only significantly increased oxidative stress in neurons, but also induced the mitochondrial dysfunction. The capability of taurine that attenuated the nickel-induced oxidative stress and mitochondrial dysfunction might contribute to its protective effects against the neurotoxicity of nickel.

Taurine was essential for mitochondrial homeostasis. Recent studies reported that taurine transporter knockout (taur—/−) mice exhibited severe lower respiratory control ratio in isolated mitochondria of liver tissue [31]. In our study, the beneficial effects of taurine against nickel-induced neurotoxicity largely depended on its activity in reducing oxidative stress and maintaining mitochondrial function. On one hand, as an efficient antioxidant, taurine scavenged ROS production and stimulated anti-oxidative enzymes directly or indirectly [13,25]. Importantly, on the other hand, taurine efficiently enhances mitochondrial function. Taurine protected against the impairment of downstream ATP-dependent processes and maintained the cell viability of neurons, such as synaptic function and integrity, DNA and RNA synthesis and signaling transduction pathway [3]. In addition, taurine maintained the $\Delta \Psi_{m}$. This effect might be beneficial for improving mitochondrial oxidative phosphorylation and ATP generation under nickel-induced oxidative stress [30]. These protective effects of taurine on mitochondria are very important for the brain which is extremely susceptible under the oxidative stress or energy crisis. Indeed, the level of taurine in the brain is rather high compare to the other organs [3]. Because de novo taurine synthesis is limited in humans or murine, taurine supplements are tested to treat vary of nerve diseases, like traumatic brain injury, ischemic stroke, diabetic encephalopathy, neurodegeneration [1,18,27,28] etc. Our study indicates the pharmacological potential of taurine for exogenous neurotoxin exposure.

5. Conclusions

Taurine protected against nickel-induced oxidative damage in cortical neurons. The neuroprotective effect of taurine may closely relate with its roles in reducing oxidative stress and maintaining mitochondrial function. As having multiple roles in reducing oxidative stress and maintaining mitochondrial function, taurine could also be used to potentially protect against the deleterious effects of other neurotoxins targeting mitochondria.

Conflict of interest

The authors declare that there are no conflicts of interest.
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