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Presence of nano-sized chitosan-layered silicate composites protects against toxicity induced by lead ions

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ABSTRACT

Protecting cells from toxicosis even apoptosis induced by a variety of toxic heavy metals stimulus has drawn more and more attentions. This study was designed to elucidate whether chitosan-organic rectorite (CS-OREC) composites exhibited any protective effects on altered oxidative stress parameter in PC12 cells exposed to lead ions (Pb^{2+}). The cells were exposed to Pb^{2+} either alone or in combination with CS-OREC composites for designated time to evaluate the efficacy of the composites on Pb^{2+}-induced toxicity. The MTT assay results showed that the cell viability of PC12 was remarkably decreased when exposed to Pb^{2+}, but significantly retained after adding CS-OREC composites compared to that of the control. The beneficial effect of CS-OREC composites on cytotoxicity was related, at least in part, to its ability to protect against apoptosis in PC12 cells exposed to 50 μM Pb^{2+}. Their protective effect was also associated with the inhibitory effect on Pb^{2+}-induced activation of Bax/Bcl-2, P-38, and caspase-3 pathways, while was independent on JNK pathway.

1. Introduction

Lead, a non-biodegradable heavy metal (Goyer, 1990), as one of the metals most commonly encountered in environment (Shotyk et al., 1998), continues to pose serious health risks worldwide (Tong, Schirnding, & Prapamontol, 2000; Wakefield, 2002), in spite of several efforts being made for reducing its levels in environment. The widespread industrial applications have made lead to be a ubiquitous toxicant and serious threat to human health (Juberg, Kleiman, & Kwon, 1997). Nearly all the organs and systems can be affected by lead, including gastrointestinal, hematopoietic, cardiovascular, nervous, immune, reproductive and renal systems (Tiwari, Flora, Gupta, Jatawa, & Gupta, 2013), in which developmental central nervous system (CNS) is the most susceptible target to lead induced toxicity (Cory-Slechta, 2003).

Recently, lots of efforts have been made directly toward overcoming this metal toxicity problem. Unfortunately, up to now, there is no satisfied therapeutic tools or treatment measures against lead-induced neurotoxicities. Adsorption therapies are regarded as the major measures most widely implemented in treating lead poisoning. However, all the adsorbents have their potential risks. British antilewisite (BAL), as a chelating agent, has been applied in lead poisoning treatment for a long time, but its side effects affect can be evidenced in 50% of patients after taking it (Gurer & Ercal, 2000). Considering that, new chelating or adsorption agents which can reduce even eliminate lead from human body may pave new ways for the prevention and treatment of lead-induced neurotoxicities.

The most popular adsorbent for the adsorption process is activated carbon, which possesses a high surface area, high adsorption capacity and degree of surface reactivity. But safe and◦C reliable activated carbon is expensive and regeneration is needed after each adsorption process (Özcan & Özcan, 2004). In order to overcome the above problems, many scientists have attempted to investigate inexpensive, efficient and easily available adsorbents. Herein natural materials including polysaccharides and clay materials such as rectorite have been applied to remove lead ions by adsorption manner (Farrag, Khalıl, & Mehrım, 2009).

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Besides, as an adsorbent used *in vivo*, its safety and toxicity should be considered firstly. Chitosan (CS), a polysaccharide with non-toxicity, good biocompatibility and biodegradability (Yu et al., 2014), has increasingly been studied for the adsorption of various metal ions from dilute solutions or waste water (Jin & Bai, 2002). It has been reported that CS could be used as an effective adsorbent for lead ions in waste water treatment (Ng, Cheung, & McKay, 2003). Although CS has been used in different forms such as 2D films, hydrogels, sponges, and fibrous scaffolds in tissue engineering and drug delivery, there is no report regarding its potential inhibition effect against lead-induced cytotoxocity.

Interestingly, polymer/layered silicate composites, which can combine the physical and chemical properties of both inorganic and organic materials, have become more and more popular for providing polymeric materials with desirable properties for practical applications. Recently, the drug-controlled abilities, antibacterial activity, gene transfection efficacy, and thermal stability of these kinds of composites fabricated from CS and layered silicate have been reported (Deng et al., 2012; Tu et al., 2015; Wang, Du, Luo, Lin, & Kennedy, 2007; Wang, Tang, Li, Du, & Yumin, 2010). However, till now, there is no previous study on the neuroprotective effect of CS/layered silicate composites against neuronal damage caused by lead.

As we know, the layered silicates possess high adsorption power, attributed to its large specific surface area, high structural stability, chemical inertia, and strong cation exchange capacity (He, Guo, & Xie, 1999; Hu, Lu, Chen, Gu, & Zhang, 2002). It was reported that the addition of bentonite with levels 1 or 2% to alleviate the toxic effects of lead oxide contaminated dietary for O. niloticus fish (Farrag et al., 2009). Based on our recent reports, rectorite (REC), especially organic rectorite (OREC) modified from REC, had larger interlayer distance, better separable layer thickness and layer aspect ratio than montmorillonite (MMT), and the toxicity of OREC was little (Liu, Deng, Xiao, Xie, & Zhou, 2014). Since CS chains could insert into the interlayer of REC and OREC, resulting much larger interlayer distance (Wang et al., 2007). Hence, it is reasonable to hypothesize that the combination of CS and OREC could enhance the absorption capacity of lead and reduce even eliminate lead poisoning.

To confirm the above hypothesis, the pheochromocytoma derived cell line PC12, considering its wide application in investigating neuronal differentiation, signal transduction and neuronal cell death (Itano, Kitamura, & Nomura, 1994; Sombers, Colliver, & Ewing, 2002), has been chosen as the suitable neuron model in this study. We aimed to (1) investigate the potential effects of CS-OREC composites on lead-induced apoptosis in PC12 cells and (2) elucidate whether the novel intercalated composites had any protective abilities against such cytotoxicity. Therefore, the present study was designed to evaluate the protective efficacy of CS-OREC composites and compare them to neat CS or OREC in lead-induced toxicity in PC 12 cells. The cells were exposed to lead acetate or in combination with CS-OREC (CS or OREC alone) for designated time to evaluate the protective efficacy of the composites on lead-induced toxicity.

2. Materials and methods

2.1. Materials

The starting materials included chitosan (CS, $M_w = 2.1 \times 10^5$ D, DD = 92%, Yuhuan Ocean Biochemical Co., China) and calcium rectorite (REC, Mingliu Inc. Co., China). Lead acetate was purchased from Sigma-Aldrich (Sigma-Aldrich, USA). All the other chemicals were of analytical grade. Cetyltrimethyl ammonium bromide (CTAB) was supplied by Xinrui Science and Technology Inc. Co. (Wuhan, China). All aqueous solutions were prepared using purified water with a resistance of 18.2 MΩ cm.

2.2. Treatment suspensions preparation for cell culture experiments

The organic rectorite (OREC) and the intercalated CS-OREC composite suspensions were prepared according to our previous reports (Deng, Li et al., 2011; Deng, Wang et al., 2011; Li et al., 2012). The average diameter of the REC and OREC was 458.3 ± 26.2 and 243.7 ± 16.9 nm according our previous investigation (Liu et al., 2014). In CS-OREC composites, the initial weight ratio of CS: OREC was adjusted at 6:1.

CS-OREC stock suspensions and treatment suspensions for cell culture were prepared as following: First, 1% (wt/wt) acetic acid was used to prepare CS-OREC stock suspensions with the concentration of 2% (wt/wt). After that, take 0.1 mL of the as-prepared CS-OREC suspensions (2% wt%) and add into 9.9 mL cell culture medium to prepare the treatment suspensions for the next lead-induced toxicity experiment. The cells were synchronized by medium with 1% fetal bovine serum (FBS) for 12 h, and then treated with medium containing 50 μm $Pb(Ac)_2$ stock solutions or CS-OREC with 1% fetal bovine serum for 48 h and collected, respectively. Here, the concentration of the treatment suspensions was fixed at 1% (Volume/Volume). Hence, 1% (V/V) herein means the 10 mL obtained treatment suspensions contained 0.1 mL 2% (wt/wt) CS-OREC stock suspensions. According this method, a series of treatment suspensions with different concentrations (0%-n%, V/V) were prepared using 2% (wt/v) CS-OREC stock suspensions. CS or OREC treatment suspensions for cell culture were prepared according the same method.

2.3. Characterizations

Fourier transform infrared (FT-IR) spectra were recorded on Nicolet FT-IR 5700 spectrophotometer (Nicolet, Madison, USA). The specific surface area to volume and pore size distribution of the samples were determined by surface area analyzer (Micromeritics, ASAP 2010, USA). The surface areas of all specimens were calculated as single point from the BrunauereEmmetteTeller (BET) isotherm using adsorption points. The small angle X-ray diffraction (SAXRD) was performed on type D/max-rA diffractometer (Rigaku Co., Japan) with Cu target and Ka radiation (λ = 0.154 nm) at 40 kV and 50 mA. The scanning rate was 0.5°/min and the scanning scope of 2θ was 1–10° at room temperature. Ultrathin films for transmission electron microscopy (TEM) were prepared by cutting from the epoxy block with the embedded nanocomposite sheet at room temperature using an LKB-8800 ultratome, the TEM micrographs were obtained by a transmittance electron microscope (JEM-2010 FEF (UHR), JEOL, Japan) at an accelerating voltage of 200 kV.

2.4. Cell line and cell culture

Pheochromocytomaderived cell Line (PC12 cells) was selected for investigating the cytotoxic effect of Pb^{2+} and CS-OREC composites. Differentiated PC12 cells were obtained from Cell Bank of Chinese Academy of Sciences, maintained and cultured in High-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics(100 units/ml of penicillin, and 100 μg/mL of streptomycin)at 37 °C in humidified air with 5% CO_2.

The medium was replenished every 48 h, and the cells were plated at a density of 5×10^4 cells/mL on microtiter plates. Only cells in exponential growth were used for the experiments. Cells were exposed to lead acetate alone or pretreated with different