Toxicology Letters 237 (2015) 112–120

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Silver nanoparticle exposure induces rat motor dysfunction through decrease in expression of calcium channel protein in cerebellum

Nuoya Yin[a], Yang Zhang[b], Zhaojun Yun[a], Qian Liu[a], Guangbo Qu[a], Qunfang Zhou[a,*], Ligang Hu[a], Guibin Jiang[a]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China
[b] Department of Pharmacy, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

HIGHLIGHTS

- Silver nanoparticles cause cerebellar ataxia-like symptom in rats.
- Silver nanoparticles induce rat motor dysfunction.
- Silver nanoparticles decrease calcium channel protein expression in rat cerebellum.

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 29 May 2015
Accepted 5 June 2015
Available online 9 June 2015

Keywords:
Silver nanoparticles
Neurotoxicity
Motor coordination dysfunction
Locomotor activity impairment
Calcium channel protein

ABSTRACT

Silver nanoparticles (AgNPs) are currently used widely, however, their impact on central nervous system still remains ambiguous and needs to be elucidated. This study is performed to investigate the neurotoxicity of AgNPs and illustrate the potential molecular mechanism. Neonatal Sprague–Dawley (SD) rats are exposed to AgNPs by intranasal instillation for 14 weeks. It is demonstrated that AgNPs exposure causes cerebellar ataxia like symptom in rats, evidenced by dysfunction of motor coordination and impairment of locomotor activity. Observation of cerebellum section reveals that AgNPs can provoke destruction of cerebellum granular layer with concomitant activation of glial cells. AgNPs treatment decreases calcium channel protein (CACNA1A) levels in cerebellum without changing potassium channel protein (KCNA1) levels. The levels of silver in rat cerebellum tissue are correlated with exposure doses. *In vitro* experiments reveal that AgNPs treatment significantly reduces the protein and mRNA levels of CACNA1A in primary cultured cerebellum granule cells (CGCs). These findings suggest that AgNPs-induced rat motor dysfunction is associated with CACNA1A expression decrease, which reveals the underlying molecular mechanism for the neurotoxicity of AgNPs. Possible counteractions may accordingly be suggested to attenuate the unexpected harmful effects in biological applications of AgNPs.

1. Introduction

Due to silver nanoparticles' excellent antibacterial activity, they are becoming more and more popular in various fields (Prucek et al., 2011). Despite their wide application, information on their environmental and human health effect is limited. Previous studies revealed that silver nanoparticles (AgNPs) were able to be translocated by blood circulation and distributed throughout the main organs in the form of particles (Tang et al., 2009). Given that AgNPs are capable of permeating the tight blood–brain barrier (BBB) and entering the brain tissue (Tang et al., 2010), the issue on assessing the specific responses of neurons to AgNPs is of great

Abbreviations: AgNPs, silver nanoparticles; SD, Sprague–Dawley; CACNA1A, calcium channel protein; KCNA1, potassium channel protein; CGCs, cerebellum granule cells; BBB, blood–brain barrier; CNS, central nervous system; ICP-MS, inductively coupled plasma mass spectrometry; TEM, transmission electron microscope; H&E, hematoxylin–eosin; IHC, immunohistochemistry; PFA, paraformaldehyde; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; DLS, dynamic light scattering; PDI, polydispersity index.

* Corresponding author. Fax: +86 10 62849339.
E-mail address: zhouqf@rcees.ac.cn (Q. Zhou).

http://dx.doi.org/10.1016/j.toxlet.2015.06.007
0378-4274/

relevance. Animal studies proved that intravenous injection of AgNPs in Sprague–Dawley (SD) rats caused the functional injury of central nervous system (CNS) by decreasing their locomotor activity (Zhang et al., 2013), showing the potential neurotoxicity of AgNPs.

AgNPs containing nasal drops have been utilized for the treatment of coryza in clinic (Damiani et al., 2011), which introduces a special route for AgNPs exposure. Nasal administration may deliver therapeutic agents preferentially to the brain, which has gained significant interest (Graff and Pollack, 2005). This technique provides a practical, non-invasive method of bypassing the BBB and allows agents to be delivered to the CNS within minutes. The intranasal delivery of AgNPs was demonstrated to induce impairment of hippocampus function in rats (Liu et al., 2012). Clinical case study showed slight sign of cerebellar ataxia in argyria patient using nasal drug administration (Aaseth et al., 1981). Characterized by the impaired coordinate balance, cerebellum ataxia is a non-specific clinical manifestation implying partial dysfunction of the nervous system that coordinates movement, such as the cerebellum (Schmahmann, 2004). Mounting evidences have given some important clues to pathogenic mechanisms in cerebellum ataxia, such as disruption of voltage-gated potassium and calcium channels (Jen et al., 2007; Mori et al., 1991). Our previous study found that AgNPs exposure could attenuate the viability of rat cerebellum granule cells (CGCs) through apoptosis (Yin et al., 2013). Consequently, uncovering *in vivo* neurological deficits induced by AgNPs and understanding the underlying mechanism are of much significance for finding out the solution to their potential harmful effects.

Our objectives in this study were to evaluate neurobehavioral dysfunction in AgNPs exposed rats by nasal administration, examine their neural pathological effects and elucidate the potential molecular mechanism.

2. Materials and methods

2.1. AgNPs characterization

AgNPs coated with citrate (1 mg/mL) was purchased from Sigma–Aldrich Co. LLC (St. Louis, MO, USA). Characterization of AgNPs was performed by morphological observation through transmission electron microscope (TEM, Hitachi H-7500, Japan) as well as particle size distribution and zeta-potential measurement by Malvern Zetasizer Nano ZS (Malvern, UK) at 25 °C. The stock solution was kept at 4 °C and freshly diluted with distilled water for *in vivo* studies or cell culture medium for cell experiments after 30-min sonication when used.

2.2. Animals

Neonatal SD rats with their mother rats were purchased from Peking University Health Science Center. All animals were maintained in accordance with the principles of care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Peking University. The whole animal room was maintained at 25 °C, <70% of humidity and 12 h/12 h light/dark cycle. Neonatal animals were housed in clear plastic cages with their mother for normal breastfeeding. Twenty eight days later, the weaned animals were transferred to new cages with male and female rats housed in different cages.

2.3. Exposure and sacrifice protocols

The neonatal rats (initially weighting 4–5 g) were set as control, 0.1, 0.2, 0.5 and 1 mg/kg/day of AgNPs exposure groups, respectively. The exposure dose selection in animal studies was based on the preliminary experiments (data not shown) and the guidance on Dose Level Selection for Regulatory General Toxicology Studies for Pharmaceuticals. The number and gender of the rats in each group were listed in Table 1. The total exposure period lasted for 14 consecutive weeks. AgNPs working solutions were administered by intranasal instillation once a day. Equivalent volume of distilled water was used as the vehicle control. The animal body weight was monitored. Rotarod and open field test were performed in the last 2 weeks before exposure termination. The rats were sacrificed by CO_2 and the cerebellum tissues were harvested after 60 mL of cold PBS intracardiac perfusion. The cerebellum were either fixed in 4% paraformaldehyde or frozen at −80 °C for further analysis.

2.4. Rotarod test

The motor coordination of rats was evaluated by rotarod (Panlab Le8500 Harvard Apparatus, Spain). We performed the tests in both the constant speed mode (5, 10, 15 and 20 rpm) and acceleration speed mode (increasing from 4 rpm to 40 rpm during 10 min). The maximum test time of 5 min was set for the constant speed mode. The rats were habituated on the horizontal rotating rod and pre-trained for 3 trials before the formal tests. All the rats were tested 3 trials per day for 7 consecutive days. The apparatus automatically recorded the elapsed time when the rat fell to the base of the apparatus from the spindle.

2.5. Open field test

The open field test was performed for all the rats according to the protocol suggested by Zhang et al. (2013). The dimension of

Table 1
The behavior evaluation for the control and AgNPs exposed rats in open field test.

Behavior parameters	AgNPs concentration (mg/kg/day)				
	0[a]	0.1[b]	0.2[c]	0.5[d]	1[e]
Total moving distance (m)	162.5 ± 21.9	132.6 ± 11.1	118.2 ± 13.1*	88.0 ± 7.9**	48.8 ± 8.4**
Moving velocity (cm/s)	27.9 ± 5.3	25.1 ± 1.9	22.6 ± 2.7	18.1 ± 0.9*	11.1 ± 1.4**
The number of entries into center (times)	20.7 ± 3.1	13.5 ± 4.9	9.3 ± 2.5*	4.8 ± 1.1**	0.6 ± 0.9**
Resting time (s)	82.9 ± 9.4	97.7 ± 2.7	105.1 ± 12.7	127.6 ± 15.6*	172.8 ± 28.1*
Rearing frequency (times)	68.9 ± 20.7	53.5 ± 17.5	45.9 ± 19.7	30.0 ± 10.7*	19.2 ± 3.4*

[a] The number of the rats in each group. $n = 13$ (male: 7, female: 6).
[b] The number of the rats in each group. $n = 12$ (male: 8, female: 4).
[c] The number of the rats in each group. $n = 14$ (male: 9, female: 5).
[d] The number of the rats in each group. $n = 13$ (male: 6, female: 7).
[e] The number of the rats in each group. $n = 14$ (male: 7, female: 7).
* $p < 0.05$ *versus* negative control.
** $p < 0.005$ *versus* negative control.