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Effects of prenatal exposure to silver nanoparticles on spatial cognition and hippocampal neurodevelopment in rats

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ABSTRACT

Background: Silver nanoparticles (Ag-NPs) are among the most commonly used nanomaterials and may be exposed to human and ecosystem. Prior *in vitro* study showed that Ag-NPs compromised neurodevelopment of PC12 cells. This study aims to investigate the effect of prenatal exposure to Ag-NPs on spatial cognition and hippocampal neurodevelopment in rats.
Methods: Pregnant rats were exposed by intraperitoneal injection to various solutions during pregnancy, including vehicle (water and glycerol mixture, 1:1 in volume), uncoated Ag-NPs (0.427 mg Ag per g rat), polyvinylpyrrolidine (PVP)-coated Ag-NPs (0.407 mg Ag per g rat), silver nitrate (0.013 mg Ag^+ and 0.007 mg NO_3^+ per g rat) and sodium nitrate (0.007 mg NO_3^+ per g rat). Pregnant rats without any injection were used as blank control. Male offspring at postnatal day 35 (PND35) were randomly selected for Morris Water Maze (MWM) test. After the MWM test, the rats were decapitated and hippocampus were collected for analysis of tissue structure, silver content, GAP-43 mRNA and protein expressions.
Results: For the spatial learning in MWM test, since the third test day, the escape latency of rats in the uncoated Ag-NPs group was significantly higher than those in the other groups. The behaviors of rats were not significantly different among the other groups. The averaged silver content in the hippocampus of rats in the uncoated Ag-NPs group was 17.51 μg/g, significantly higher than those in the other groups. The hippocampal structure in rats of the uncoated Ag-NPs group was deformed as compared to those in the other groups. Compared with the rats in blank and vehicle controls, the levels of GAP-43 mRNA and protein in the uncoated Ag-NPs, PVP-coated Ag-NPs, silver nitrate and sodium nitrate groups were all significantly decreased. The levels of GAP-43 mRNA and protein of rats in the uncoated Ag-NPs group was significantly lower than those in the later three groups, while there was no significant difference among the later three groups.
Conclusions: Maternal exposure to uncoated Ag-NPs during pregnancy impaired spatial cognition in rat offspring. GAP-43 reduction might be involved in the cognitive impairment. The toxicity was mainly associated with release of silver ion. Coating with PVP reduced the toxicity of Ag-NPs.

1. Introduction

Silver nanoparticles (Ag-NPs) are among the most commonly used nanomaterials because of their strong antimicrobial activity (Morones et al., 2005; Nel et al., 2006; Pal et al., 2007). The widespread use of Ag-NPs may lead to increase in human and ecosystem exposures (Chen and Schluesener, 2008). This raises concern about the environmental and health consequences of exposure to Ag-NPs.

Ag-NPs have been reported to be among the most toxic nanomaterials in some studies (Bar-Ilan et al., 2009; Hussain et al., 2005; Soto et al., 2005, 2007). Prior *in vitro* studies of Ag-NPs toxicity revealed toxic effects of Ag-NPs through reduced cell viability, damage to the cell membrane and other biological effects on the organism (Hussain et al., 2006; Kvitek et al., 2009; Liu et al., 2009; Navarro et al., 2008; Lanone et al., 2009). Exposing rat embryonic cells to Ag-NPs suspension, a total of 279 genes up-regulated and 389 genes down-regulated were found by global gene expression analysis. KEGG pathway analysis showed that there were 23 signal pathways affected in the cells after exposure to Ag-NPs suspension. The most significant change concerned inflammatory signal pathways (Xu et al., 2014). The cytotoxicity of Ag-NPs has been associated with generation of reactive oxygen species (Hussain et al., 2005; AshaRani et al., 2009) and release of silver ions (Kvitek et al., 2009; Navarro et al., 2008). Compared to *in vitro* studies, *in vivo* studies were rare. Stebounova et al. (2011)

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reported that Ag-NPs induced minimal lung toxicity or inflammation in a subacute murine inhalation model (mice). However, longer term exposures with higher lung burdens of Ag-NPs are needed to ensure that there are no chronic effects and to evaluate possible translocation to other organs. Korani et al. (2011) studied the acute and subchronic dermal toxicity of Ag-NPs in guinea pig. In autopsy studies during the acute test, no significant changes in organ weight or major macroscopic changes were detected, but dose-dependent histopathologic abnormalities were seen in skin, liver, and spleen of all test groups. Subchronic test showed greater tissue abnormalities than acute test. It seems that Ag-NPs have the potential to provide target organ toxicities in a dose- and time-dependent manner.

Some studies showed that Ag-NPs exposure via inhalation or oral routes led to Ag accumulation in the adult rodent brain (Wijnhoven et al., 2009), altering the expression of genes involved in neuronal function (Rahman et al., 2009). Powers et al. (2011) showed that in PC12 cells (a well-established model of neuronal development) citrate-coated Ag-NPs impaired DNA synthesis and protein synthesis in undifferentiated cells, and in differentiating cells citrate-coated Ag-NPs evoked robust oxidative stress and impaired differentiation into the acetylcholine phenotype. The effects of polyvinylpyrrolidine (PVP)-coated Ag-NPs showed similarities to those of citrate-coated Ag-NPs, but there were significant differences in potencies and differentiation outcomes that depended both on particle size and coating. Liu et al. (2012) investigated the effects of the Ag-NPs on hippocampal synaptic plasticity and spatial cognition in adult rats. After two-week exposure to Ag-NPs through the nasal administration, compared with the control group, both long-term potentiation (LTP) and Morris water maze (MWM) results were abnormal in low-dose group (Ag-NPs, 3 mg/kg) and high-dose group (Ag-NPs, 30 mg/kg), and the quantity of reactive oxygen species (ROS) in hippocampal homogenate was increased significantly in low-dose group and high-dose group. Liu et al. (2013) studied the effects of Ag-NPs on spatial cognition and adult hippocampal neurogenesis in adult mice. The mice received intraperitoneal administration of Ag-NPs (10, 25, and 50 mg/kg body weight) or vehicle every day for 7 days. Compared with the control group, both reference memory and working memory were not impaired in Ag-NPs exposed groups, and no differences were observed in hippocampal progenitor proliferation, new born cell survival or differentiation. Clearly, the exposure effects might be influenced by many factors such as exposure way, time and dose. More studies were needed to further clarify this issue.

It's reported that Ag-NPs could be transferred from pregnant rat to offspring (Lee et al., 2012). Earlier study showed that silver could cross the placenta and concentrate in the human fetus, achieving higher concentrations than in the mother (Lyon et al., 2002). However, the effects of prenatal exposure to Ag-NPs on neurodevelopment in mammal animals have not been reported yet.

Growth-associated protein-43 (GAP-43) is a neuron-specific phosphoprotein that plays a major role in initial development and remodeling of neural connections (Benowitz and Routtenberg, 1997; Perrone-Bizzozero and Tanner, 2006). It's widely distributed in hippocampus which is a brain region responsible for learning and memory (Casoli et al., 2001). GAP-43 is vulnerable to prenatal stress. Several studies reported that alteration of the GAP-43 expression in the hippocampus correlated with changes in cognitive abilities in prenatally stressed rat offspring (Liu et al., 2005; Zhang et al., 2012).

The objectives of this study were to investigate the effect of maternal exposure to Ag-NPs during pregnancy on spatial cognition of rat offspring in MWM test, and to explore its neural developmental mechanisms including hippocampal structure, silver content, GAP-43 mRNA and protein expressions.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats served as subjects for this study. All animals were bred in our laboratory and underwent behavioral testing by postpartum day 35. Rats were housed under controlled conditions (ambient temperature 22 °C; 12-hour light/12-hour dark cycle with lights on at 7.00 am) with ad libitum access to food and water. All experiments were carried out in accordance with the guidelines of the Beijing Laboratory Animal Center and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

2.2. Prenatal treatment

At 3 months of age, female and male Sprague-Dawley rats (Vital River, a Charles River Laboratory in China) were mated. The first day after copulation was operationally defined as gestation day 0 (GD0). The pregnant rats were divided into six groups, i.e., blank control, vehicle control, uncoated Ag-NPs, PVP-coated Ag-NPs, silver nitrate and sodium nitrate. Each group contained six pregnant rats. Every two days from GD10 to GD18, the pregnant rats were injected intraperitoneally with experimental solutions (1 mL per time, 5 times, for each rat in the vehicle control, uncoated Ag-NPs and PVP-coated Ag-NPs groups). The vehicle solution was a mixture of water and glycerol (1:1, volume). The uncoated Ag-NPs solution contained 20.0 mg uncoated Ag-NPs per mL vehicle solution. The PVP-coated Ag-NPs solution contained 20.0 mg PVP-coated Ag-NPs per mL vehicle solution. Originally, the silver nitrate solution contained 20.0 mg silver ion (Ag^+) per mL vehicle solution. After one injection (1 mL), four of the six pregnant rats in the silver nitrate group died. After another injection (0.5 mL), the rest two pregnant rats in the silver nitrate group died. Therefore, the treatment was redone using lower Ag^+ concentration (2.0 mg Ag^+ per mL vehicle solution). Still, one pregnant rat died after one injection (1 mL, at GD10). The injection was adjusted, i.e., with longer injection interval (every four days, 3 times, 1 mL per time,). At GD12 and GD16, vehicle solution was injected into the rats instead of silver nitrate solution. Correspondingly, the sodium nitrate solution was injected in the same way as that for the silver nitrate solution (both contained 1.15 mg nitrate per mL vehicle solution). Uncoated Ag-NPs and PVP-coated Ag-NPs (purities over 99.5%) were purchased from Beijing De Ke Dao Jin Science and Technology Co., Ltd., China. Glycerol, silver nitrate and sodium nitrate (guaranteed reagents) were purchased from Sinopharm Chemical Reagent Co., Ltd., China.

After the offspring were born, the offspring stayed with their mothers till weaning (three weeks). Then, female pups were culled, and male pups were housed six per cage. These male pups were raised for another two weeks before testing.

2.3. Morris water maze test

2.3.1. Apparatus

Testing was conducted in a circular pool of 150 cm diameter, with water that was 22 cm deep (23 ± 2 °C). A circular Plexiglas platform (8 cm diameter) was placed 2 cm beneath the water level at different locations, depending on which test was currently employed. The water was made cloudy by adding black ink. A video camera was positioned above the water maze. The swim paths of the rats were tracked, digitized and stored for later behavioral analysis using Ethovision 3.1 (Noldus). The water maze was divided into four logical quadrants (north, south, east and west) that served as starting positions for the rats.

2.3.2. Spatial learning

At PND 35, for each group, 15 male pups were randomly selected for the spatial learning test (2 or 3 pups from each mother). The test consisted of 5 days of acquisition with the hidden platform, followed by a probe test on the sixth day without the platform. The platform was fixed in the middle of the west quadrant 45 cm from the maze wall. During the first 5 days, four swim trials were given per day in which each animal was released from a different quadrant in each trial. This process was performed in a pseudorandom manner, and the start quadrant varied for each day. A maximum of 60 s was allowed for each trial. If the rat did not find the platform within 60 s, it was guided to the platform and allowed to remain there for 15 s. At the end of the daily session, the rats were dried with paper towels and returned to their home cages. Both the latency to escape onto the platform and the distance traveled were recorded. On the sixth day, one trial was given in which each animal was released from the middle of the east quadrant. The time and traveled distance in the west quadrant where the platform had been set during the training were recorded.

The MWM test was consistently performed by the same person. The animals were transported to a testing room for the test. The animals rested one day before the test in the testing room. The test was performed from about 9 am to 12 pm each day. This related to the light time within the colony room. All animals were tested during the same time of the day. When performing the test, the room had only faint light from a red lamp.

2.4. Detection of silver in hippocampus by ICP-MS

After the MWM test, from each group, four rats were decapitated, brain tissues were removed from the skulls, and hippocampus were carefully dissected on ice. The hippocampus were weighted and placed in 50 mL digestion tubes. To each tube, 5 mL HNO_3 was added to digest the tissue for 3 h, and then 30 mL mixture of $HClO_4$ and HNO_3 (volume ratio, 5:1) was added and the tube was placed in a graphite digestion instrument digesting for 3 h; the obtained colorless liquid (about 3 mL) was diluted to 15 mL using 0.1 mol/L HNO_3. Blank and a series of silver standard solutions were prepared in 0.1 mol/L HNO_3. Silver was detected by ICP-MS with the detection limit of 0.05 μg/L.

2.5. Histological assessment of hippocampus by H&E staining

After the MWM test, from each group, three rats were decapitated and brain tissues were quickly removed from the skulls. The brain tissue of each rat was divided into two hemispheres, fixed in 4% paraformaldehyde solution and processed to prepare 5-μm-thick paraffin sections for H&E staining (Bancroft and Gamble, 2002). The stained tissues were observed under an optical microscope (Motic BA400, China).

2.6. Analysis of GAP-43 mRNA in hippocampus by real-time RT-PCR

After the MWM test, from each group, eight rats were decapitated and brain tissues were quickly removed from the skulls. The hippocampus were carefully dissected on ice and divided into two parts. The left hippocampus were used for real-time RT-PCR (fresh) and the right hippocampus were used for Western Blotting (snap-frozen in liquid nitrogen and stored at −80 °C for later use). The fresh tissues were homogenized in TRIzol® Reagent (Invitrogen, USA). Total RNA was extracted from Trizol homogenates following the manufacturer's instructions. RNA concentration and quality were evaluated by spectrometry. Reverse transcription (RT) of total RNA was performed following the procedure of the GoScript Reverse Transcription System (Promega, USA). The RT product was diluted 10-fold and stored at −20 °C. Real-time PCR reactions were carried out with a real-time PCR System (ABI 7500 Fast, USA). Each reaction (20 μL total volume) contained 0.5 μL cDNA solution, 0.5 μL 20 μmol/L forward primer, 0.5 μL 20 μmol/L reverse primer, 8.5 μL milli-Q water, and 10 μL SYBR® Green Realtime PCR Master Mix (TOYOBO QPK-201, Japan). No template controls (NTC) were run to determine the level of primer dimer formation. PCR program was as follows: an initial denaturation at 95 °C for 300 s followed by 40 cycles of 94 °C for 15 s, 58 °C for 25 s, and 72 °C for 25 s. For each sample, two sets of primers were used. One was for the growth associated gene GAP-43 (forward: 5′–3′ GAGGGAGATGGCTCTGCTAC; reverse: 5′–3′ CACATCGGCTTGTTTAGGC), and one was for the housekeeping gene β-Actin (forward: 5'-3' GTTGACATCCGTAAAGACC; reverse: 5′–3′ TGGAAGGTGG ACAGTGAG). Primer sequences were obtained from the literature (Liu et al., 2005). The level of GAP-43 mRNA was normalized by β-Actin mRNA. PCR products were run on 1% agarose gels to confirm that the correct band sizes were present.

2.7. Analysis of GAP-43 protein expression in hippocampus by western blotting

Frozen tissues were transferred to ice-cold buffer (containing 0.1 mol/L NaCl, 0.05 mol/L Tris–HCl (pH 7.6), 0.001 mol/L EDTA (pH 8.0), 0.1% Tween-20, aprotinin (1 μg/ml) and PMSF (100 μg/ml)) and homogenized on ice. The homogenate was centrifuged at 13,000 rpm for 10 min at 4 °C. Total protein concentrations were determined with a UV spectrophotometer using a modified Bradford assay. Equal amounts of protein from each sample (30 μg) were mixed with 15 μL sample buffer and boiled for 6 min. Samples were separated by electrophoresis (at 150 V) on 6–15% polyacrylamide gels. Separated proteins were transferred onto nitrocellulose (NC) membrane at 35 V for 12 h. The membrane was blocked with 5% dried, defatted milk in TBST buffer (25 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 7.5) for 1 h at room temperature. Blots were probed with specific antibodies against rabbit polyclonal GAP-43 and β-actin (1:1000, Abcam, UK). After washing with TBST, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated anti-rabbit antibodies (GAP-43 and β-Actin, 1:2000, Abcam, UK). Negative controls did not contain primary antibody. The optical densities of the specific bands were scanned and measured by image analysis software (Quantity One, Bio-Rad, USA).

2.8. Statistical analyses

The SPSS software (version 17.0) was used to perform the statistical analyses. The MWM test results were analyzed using two-way repeated measures analysis of variance (ANOVA) with drug injection as an independent factor and test day as a within-subject factor. Following significant analyses of variance, LSD (least significance difference) was used as the post-hoc test for the between-subject factor, and Bonferroni correction was used as the post-hoc test for the within-subject factor. The results of silver content, GAP-43 gene and protein expressions were examined using single factor ANOVA. The significance level was defined as $P < 0.05$.

3. Results

3.1. Spatial learning ability

The average escape latency traveled for the offspring in all the tested groups during the spatial learning were summarized in Fig. 1. Based on two-way repeated measures ANOVA, the main

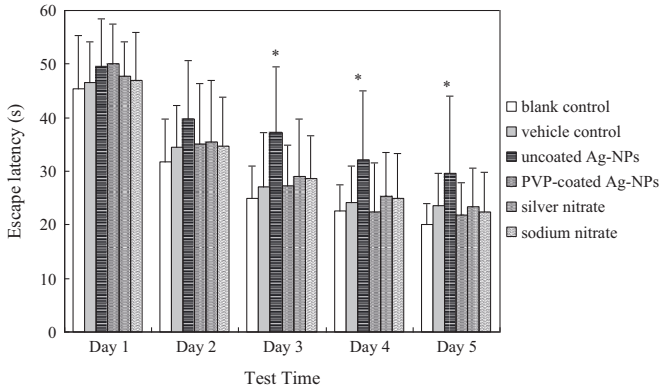

Fig. 1. Comparison of the average escape latency for the rat offspring in all the tested groups ($n=$ 15 per group, * denotes $P<0.05$).

effect of test day was significant ($F=129.951$, $P<0.05$), reflecting an overall cumulative learning process, and the main effect of treatment was also significant ($F=3402.717$, $P<0.05$), but the interaction between test day and treatment was not significant ($F=0.504$, $P>0.05$). Post hoc test analysis of treatment indicated that at the first two test days, there was no significant difference in escape latency among rats in all the groups tested. Since the third test day, treatment became a significant factor, mainly because the escape latency of rats in the uncoated Ag-NPs group was significantly higher than those in the other groups ($P<0.05$). Among the other groups, the escape latency was not significantly different. This indicated that uncoated Ag-NPs impaired the spatial learning ability of rat offspring.

Similar result was obtained in the probe test (shown in Fig. S1 in the supplemental materials). Rats in the uncoated Ag-NPs group had the lowest spatial preference for the target quadrant compared with those in the other groups ($P<0.05$), whereas the probe time was not significantly different among rats in the other groups. The recorded travel distance was not used in the statistical analysis because some debris floating on the water interfered with the recording of the data during the MWM test.

3.2. Silver content and hippocampus histology

The silver contents in the hippocampus of rat offspring were measured and shown in Fig. 2. The averaged silver content in the hippocampus of rats in the uncoated Ag-NPs group was 17.51 μg/g, significantly higher than those in the other groups ($P<0.05$). The silver contents of rats in the PVP-coated Ag-NPs (4.13 μg/g) and silver nitrate (5.31 μg/g) groups were not significantly different from those in the controls ($P>0.05$).

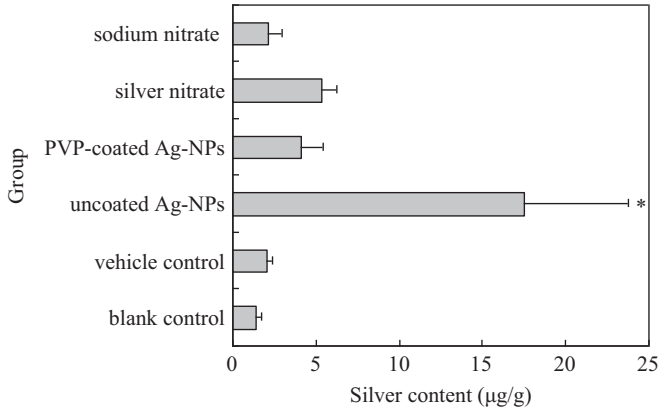

Fig. 2. Comparison of the silver content in hippocampus of rats in all the tested groups. ($n=$ 4 per group, ∗ denotes $P<0.05$).

The nerve cells of the hippocampus of rats in the blank control and vehicle control were well aligned, with distinct structure, normal intercellular space, clear outlines of the cells and plentiful Nissl's bodies. However, for rats in the uncoated Ag-NPs group, the intercellular space was enlarged due to shrinking of the cells and the number of Nissl's bodies seemed decreased. Fig. 3 compared the hippocampal structure of the rats in the vehicle control and the uncoated Ag-NPs group. Rats in the other groups had similar hippocampal structure to those in the controls (shown in Fig. S2 in the supplemental materials).

3.3. Measurement of GAP-43 mRNA in the hippocampus

There was no significant difference for GAP-43 mRNA levels in the hippocampus between rats in the blank control group and those in the vehicle control group. Compared with those in the blank and vehicle controls, the levels of GAP-43 mRNA in the other four groups were all significantly decreased ($P<0.05$). The level of GAP-43 mRNA in the hippocampus of rats in the uncoated Ag-NPs group was significantly lower than those in the PVP-coated Ag-NPs, silver nitrate and sodium nitrate groups ($P<0.05$). There was no significant difference in the GAP-43 mRNA level among the later three groups ($P>0.05$). Fig. 4 illustrated the levels of GAP-43 mRNA (normalized by β-Actin mRNA) in hippocampus of rats in all the tested groups.

3.4. Expression of GAP-43 protein in the hippocampus

There was no significant difference in GAP-43 protein expression in the hippocampus between rats in the blank control group and those in the vehicle control group. Compared with those in the blank and vehicle controls, the levels of GAP-43 protein in the other four groups were all significantly decreased ($P<0.05$). The level of GAP-43 protein of rats in the uncoated Ag-NPs group was significantly lower than those in the PVP-coated Ag-NPs, silver nitrate and sodium nitrate groups ($P<0.05$), but there was no significant difference in the GAP-43 protein level among rats in the later three groups ($P>0.05$) Fig. 5 compared the levels of GAP-43 protein (normalized by β-Actin protein) in hippocampus of rats in all the tested groups. The Western Blotting images were shown in Fig. S3 in the supplemental materials.

4. Discussion

This study aimed to investigate the effect of prenatal exposure to Ag-NPs on spatial cognitive in rats and explore its neural developmental mechanism. Two kinds of Ag-NPs, i.e., uncoated Ag-NPs and PVP-coated Ag-NPs, were exposed to the pregnant rats at similar doses. The two kinds of Ag-NPs had similar morphologies, as shown in Fig. S4 in the supplemental materials. Both had silver contents over 99.5% and size particles of 20–50 nm. The PVP-coated Ag-NPs contained about 0.2% (weight) PVP. Silver ion (using silver nitrate as a source) was applied to examine if the toxicity of Ag-NPs was due to release of silver ion. Compared to the Ag-NPs, silver ion displayed acute toxicity. After one injection of silver ion (dose at about 0.097 mg/g), four of the six pregnant rats were found dead in the following day, and after another injection of silver ion (dose at about 0.048 mg/g), the rest two pregnant rats were found dead in the following day. In the repeated experiment, even though the silver ion in the injection solution was ten times lower, one pregnant rat was found dead in the following day after one injection of silver ion (dose at about 0.009 mg/g). In comparison, no pregnant rat was found dead during the study period for the other groups.

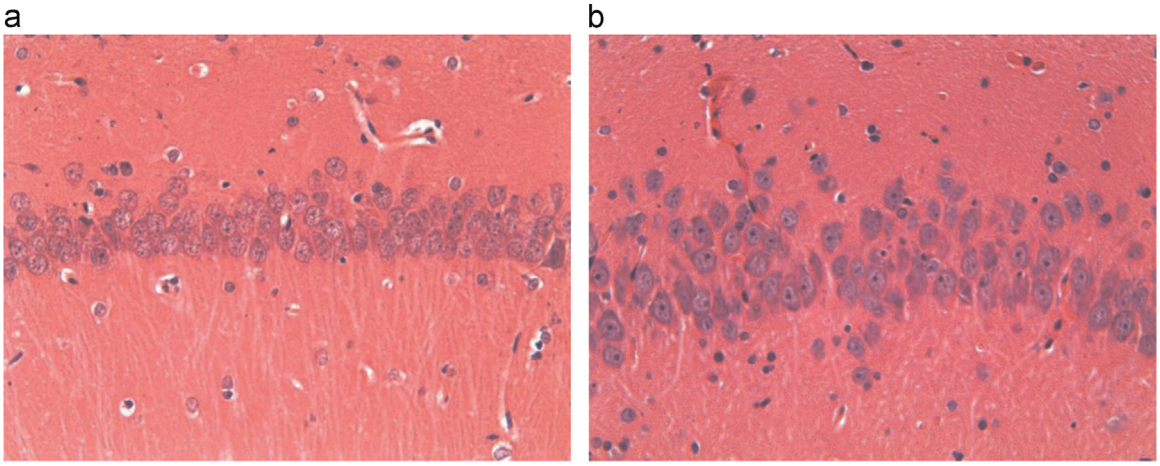

Fig. 3. Comparison of hippocampal structure of the rats in the vehicle control and the uncoated Ag-NPs group.

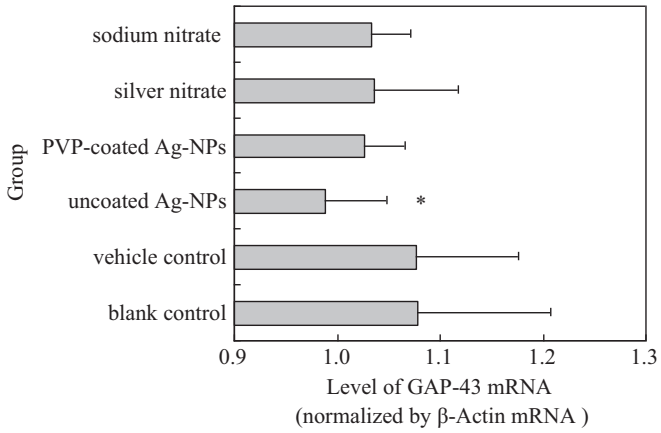

Fig. 4. Comparison of GAP-43 mRNA level in hippocampus of rats in all the tested groups. ($n=$ 8 per group, ∗ denotes $P<0.05$).

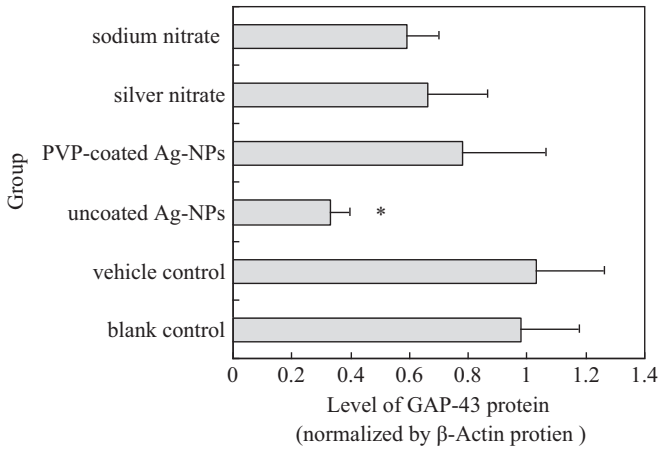

Fig. 5. Comparison of GAP-43 protein level in hippocampus of rats in all the tested groups. ($n=$ 8 per group, ∗ denotes $P<0.05$).

Ag-NPs displayed chronic toxicity. Prenatal exposure to uncoated Ag-NPs significantly impaired spatial cognition in rats. Both the spatial learning test and the probe test showed that offspring of rats exposed to uncoated Ag-NPs had lower learning and memory abilities than those in the controls. The impairment in spatial cognition was not observed in offspring of rats exposed to silver ion, probably because the exposure dose was low (about 0.013 mg/g, 30 times less than that for the Ag-NPs). Different from nitrate silver which exhibited acute toxicity at high dose, uncoated Ag-NPs could slowly and continuously release silver ion at a level that could sustain the rats. Prenatal exposure to PVP-coated Ag-NPs also did not cause impairment in spatial cognition, probably due to the low release of silver ion from the PVP-coated Ag-NPs. Tejamaya et al. (2012) compared the stability of three coated (i.e., citrate, PVP and polyethylene glycol coated) Ag-NPs in ecotoxicology media and found PVP-coated Ag-NPs was the most stable. No shape, aggregation, or dissolution change of PVP-coated Ag-NPs was observed during 21 days of exposure in standard OECD (Organization for Economic Co-operation and Development) media used for *Daphnia* sp. acute and chronic tests (in the absence of *Daphnia*). Huynh and Chen (2011) also reported PVP-coated Ag-NPs was quite stable, likely due to steric repulsion imparted by the large, non-charged polymers. Nguyen et al. (2013) compared the toxic effects of uncoated and PVP-coated Ag-NPs in J774A.1 macrophage and HT29 epithelial cells and found that uncoated Ag-NPs were more toxic than PVP-coated Ag-NPs. Uncoated Ag-NPs, at a concentration of 1 μg/ml, decreased cell viability by 20–40%. In exposures to PVP-coated Ag-NPs, cell viability dropped at 25 μg/ml or higher concentrations.

In this study, the silver content in the hippocampus of rat offspring in the uncoated Ag-NPs group was much higher than those in the other groups. The silver was possibly transferred from the pregnant rats. In a study performed by Lee et al. (2012), Ag-NPs (size: 7.9 ± 0.95 nm) were orally administered to pregnant rats (dosage: 250 mg/kg) and silver was found accumulated in the brain of the offspring (31.1 ± 4.3 ng/g, 5.4 fold as compared to the control). It remained unclear whether Ag-NPs could penetrate the blood-brain barrier (BBB). According to a study based on in vitro BBB model, Ag-NPs induced BBB inflammation and increased permeability in primary rat brain microvessel endothelial cells (Trickler et al., 2010). The measured silver in the hippocampus were likely silver ion, for Ag-NPs were not found in the hippocampus by Transmission Electron Microscope (TEM) analysis. Powers et al. (2010) reported that silver ion has the potential to evoke developmental neurotoxicity even more potently than known neurotoxicant such as chlorpyrifos (CPF). In undifferentiated PC12 cells, a 1-hr exposure to 10 μmol/L Ag^+ inhibited DNA synthesis more potently than did 50 μmol/L CPF; it also impaired protein synthesis. In differentiating PC12 cells, exposure to 10 μmol/L Ag^+ evoked even greater inhibition of DNA synthesis and more oxidative stress. Lowering the exposure to 1 μmol/L Ag^+ reduced the net effect on undifferentiated cells. However, in differentiating cells, the lower concentration produced an entirely different pattern, enhancing cell numbers by suppressing ongoing cell death and impairing differentiation in parallel for both neurotransmitter phenotypes.

Results from GAP-43 mRNA and protein analyses all showed that rat offspring in the uncoated Ag-NPs group were mostly affected, which was in agreement with the result from the MWM test. However, compared with those in the blank and vehicle controls, the levels of GAP-43 mRNA and protein in the PVP-coated Ag-NPs, silver nitrate and sodium nitrate groups were also significantly decreased. This demonstrated that GAP-43 expression was sensitive to prenatal stress. Change of GAP-43 expression by prenatal stress was also observed in other studies (Jutapakdeegul et al., 2010; Neeley et al., 2011). GAP-43 has been termed a "growth" or "plasticity" protein and is considered a crucial component of the axon and presynaptic terminal (Strittmatter et al., 1995; Benowitz and Routtenberg, 1997). It is associated with axonal growth during development, sprouting and regeneration. It is also involved in intracellular regulatory events allowing the growing axon to respond to a changing environmental framework (Gruart et al., 2006). Our study revealed a marked reduction in GAP-43 mRNA and protein expression in the hippocampus of rat offspring exposed to uncoated Ag-NPs, suggesting a disturbance in synaptic function and providing evidence for a relevance between synaptic plasticity and cognitive impairment in rats. It seems that when disorders in the development, regeneration and modulation of synapse are sufficiently large, pronounced impairment in cognition will ensue.

5. Conclusions

This study investigated the effect of prenatal exposure to Ag-NPs on spatial cognition and hippocampal neurodevelopment in rats. Maternal exposure to uncoated Ag-NPs during pregnancy impaired spatial learning and memory ability in rat offspring. GAP-43 reduction might be involved in the cognitive impairment. The toxicity was mainly associated with release of silver ion. Coating with PVP protected from the toxicity of Ag-NPs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.01.022.

References

AshaRani, P.V., Mun, G.L.K., Hande, M.P., Valiyaveettil, S., 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. Acs Nano 3, 279–290.
Bancroft, J.D., Gamble, M., 2002. Theory and practice of histological techniques, 5th ed. Churchill Livingstone, Philadelphia.
Bar-Ilan, O., Albrecht, R.M., Fako, V.E., Furgeson, D.Y., 2009. Toxicity assessments of multisized gold and silver nanoparticles in Zebrafish embryos. Small 5, 1897–1910.
Benowitz, L.I., Routtenberg, A., 1997. GAP-43: an intrinsic determinant of neuronal development and plasticity. Trends Neurosci. 20, 84–91.
Casoli, T., Stefano, G.D., Gracciotti, N., Giovagnetti, S., Fattoretti, P., Solazzi, M., Bertoni–Freddari, C., 2001. Cellular distribution of GAP-43 mRNA in hippocampus and cerebellum of adult rat brain by In Situ RT-PCR. J. Histochem. Cytochem. 49, 1195–1196.
Chen, X., Schluesener, H.J., 2008. Nanosilver: a nanoproduct in medical application. Toxicol. Lett. 176, 1–12.
Gruart, A., Munoz, M.D., Delgado-Garcia, J.M., 2006. Involvement of the CA3–CA1 synapse in the acquisition of associative learning in behaving mice. J. Neurosci. 26, 1077–1087.
Hussain, S.M., Hess, K.L., Gearhart, J.M., Geiss, K.T., Schlager, J.J., 2005. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol. In Vitro 19, 975–983.
Hussain, S.M., Javorina, A.K., Schrand, A.M., Duhart, H.M., Ali, S.F., Schlager, J.J., 2006. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. Toxicol. Sci. 92, 456–463.
Huynh, K.A., Chen, K.L., 2011. Aggregation kinetics of citrate and polyvinylpyrrolidone coated silver nanoparticles in monovalent and divalent electrolyte solutions. Environ. Sci. Technol. 45, 5564–5571.
Jutapakdeegul, N., Afadlal, S., Polaboon, N., Phansuwan-Pujito, P., Govitrapong, P., 2010. Repeated restraint stress and corticosterone injections during late pregnancy alter GAP-43 expression in the hippocampus and prefrontal cortex of rat pups. Int. J. Dev. Neurosci. 28, 83–90.
Korani, M., Rezayat, S.M., Gilani, K., Bidgoli, S.A., Adeli, S., 2011. Acute and subchronic dermal toxicity of nanosilver in guinea pig. Int. J. Nanomed. 6, 855–862.
Kvitek, L., Vanickova, M., Panacek, A., Soukupova, J., Dittrich, M., Valentova, E., Prucek, R., Bancirova, M., Milde, D., Zboril, R., 2009. Initial study on the toxicity of silver nanoparticles (NPs) against *Paramecium caudatum*. J. Phys. Chem. C 113, 4296–4300.
Lanone, S., Rogerieux, F., Geys, J., Dupont, A., Maillot-Marechal, E., Boczkowski, J., Lacroix, G., Hoet, P., 2009. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Part. Fibre Toxicol. 6, 14.
Lee, Y., Choi, J., Kim, P., Choi, K., Kim, S., Shon, W., Park, K., 2012. A transfer of silver nanoparticles from pregnant rat to offspring. Toxicol. Res. 28, 139–141.
Liu, H.X., Zhang, J.J., Zheng, P., Zhang, Y., 2005. Altered expression of MAP-2, GAP-43, and synaptophysin in the hippocampus of rats with chronic cerebral hypoperfusion correlates with cognitive impairment. Mol. Brain Res. 139, 169–177.
Liu, P., Huang, Z., Gu, N., 2013. Exposure to silver nanoparticles does not affect cognitive outcome or hippocampal neurogenesis in adult mice. Ecotox. Environ. Safe 87, 124–130.
Liu, R.T., Sun, F., Zhang, L.J., Zong, W.S., Zhao, X.C., Wang, L., Wu, R.L., Hao, X.P., 2009. Evaluation on the toxicity of nanoAg to bovine serum albumin. Sci. Total Environ. 407, 4184–4188.
Liu, Y., Guan, W., Ren, G., Yang, Z., 2012. The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats. Toxicol. Lett. 209, 227–231.
Lyon, T.D., Patriarca, M., Howatson, G., Fleming, P.J., Blair, P.S., Fell, G.S., 2002. Age dependence of potentially toxic elements (Sb, Cd, Pg, Ag) in human liver tissue from paediatric subjects. J. Environ. Monitor. 4, 1034–1039.
Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T., Yacaman, M.J., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16, 2346–2353.
Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., Sigg, L., Behra, R., 2008. Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. Environ. Sci. Technol. 42, 8959–8964.
Neeley, E.W., Berger, R., Koenig, J.I., Leonard, S., 2011. Prenatal stress differentially alters brain-derived neurotrophic factor expression and signaling across rat strains. Neuroscience 187, 24–35.
Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. Science 311, 622–627.
Nguyen, K.C., Seligy, V.L., Massarsky, A., Moon, T.W., Rippstein, P., Tan, J., Tayabali1, A.F., 2013. Comparison of toxicity of uncoated and coated silver nanoparticles. J. Phys. 429, 012025. http://dx.doi.org/10.1088/1742-6596/429/1/012025.
Pal, S., Tak, Y.K., Song, J.M., 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol. 73, 1712–1720.
Perrone-Bizzozero, N.I., Tanner, D., 2006. GAP-43 in neural development and plasticity. 3rd ed. In: Lajtha, A., Lim, R. (Eds.), The Handbook of Neurochemistry and Molecular Neurobiology, Vol. 9. Springer, Plenum Press, New York (Neuroactive Proteins and Peptides).
Powers, C.M., Wrench, N., Ryde, I.T., Smith, A.M., Seidler, F.J., Slotkin, T.A., 2010. Silver impairs neurodevelopment: studies in PC12 cells. Environ. Health Persp. 118, 73–79.
Powers, C.M., Badireddy, A.R., Ryde, I.T., Seidler, F.J., Slotkin, T.A., 2011. Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver ion, particle size, coating, and composition. Environ. Health Persp. 119, 37–44.
Rahman, M.F., Wang, J., Patterson, T.A., Saini, U.T., Robinson, B.L., Newport, G.D., Murdock, R.C., Schlager, J.J., Hussain, S.M., Ali, S.F., 2009. Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. Toxicol. Lett. 187, 15–21.
Soto, K., Garza, K.M., Murr, L.E., 2007. Cytotoxic effects of aggregated nanomaterials. Acta Biomater. 3, 351–358.
Soto, K.F., Carrasco, A., Powell, T.G., Garza, K.M., Murr, L.E., 2005. Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. J. Nanopart. Res. 7, 145–169.
Stebounova, L.V., Adamcakova-Dodd, A., Kim, J.S., Park, H., O'Shaughnessy, P.T., Grassian, V.H., Thorne, P.S., 2011. Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model. Part. Fibre Toxicol. 8, 5.

Strittmatter, S.M., Fankhauser, C., Huang, P.L., Mashimo, H., Fishman, M.C., 1995. Neuronal pathfinding is abnormal in mice lacking the neuronal growth cone protein GAP-43. Cell 1995, 445–452.

Tejamaya, M., Römer, I., Merrifield, R.C., Lead, J.R., 2012. Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. Environ. Sci. Technol. 46, 7011–7017.

Trickler, W.J., Lantz, S.M., Murdock, R.C., Schrand, A.M., Robinson, B.L., Newport, G. D., Schlager, J.J., Oldenburg, S.J., Paule, M.G., Slikker Jr., W., Hussain, S.M., Ali, S.F., 2010. Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicol. Sci. 118, 160–170.

Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Domen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., Van De Meent, D., Dekkers, S., De Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., 2009. Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3, 109–138.

Xu, L.M., Shi, C., Shao, A.L., Li, X.F., Cheng, X., Ding, R., Wu, G., Lee Chou, L.S., 2014. Toxic responses in rat embryonic cells to silver nanoparticles and released silver ions as analyzed via gene expression profiles and transmission electron microscopy. Nanotoxicology, 1–10. http://dx.doi.org/10.3109/17435390.2014.948942, Early Online.

Zhang, Z.Y., Zhang, H., Du, B.L., Chen, Z.Q., 2012. Neonatal handling and environmental enrichment increase the expression of GAP-43 in the hippocampus and promote cognitive abilities in prenatally stressed rat offspring. Neurosci. Lett. 522, 1–5.