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Vitamin E attenuates silver nanoparticle-induced effects on body weight and neurotoxicity in rats

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ABSTRACT

Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials; however, it remains unclear whether AgNPs induce neurotoxicity. Here, we investigated the potential neurological effects of AgNPs and the neuroprotective effect of vitamin E (VE). We found that intranasal instillation of AgNPs in neonatal Sprague–Dawley rats caused significant body weight loss. Moreover, histological examinations revealed activation of neuroglial cells with concomitant destruction of the granular layer of the cerebellum. Furthermore, western blot analyses showed an increase in the levels of the glial fibrillary acidic protein (GFAP), a marker of astrocyte activation. These observations suggest that AgNPs have significant neurotoxic effects on the rat cerebellum. Strikingly, oral administration of VE counterbalanced the toxic effects triggered by AgNPs. Taken together, our findings suggest that nasal administration of AgNPs may produce neurotoxicity in rats, and that VE supplementation attenuates these effects.

1. Introduction

Nanotechnology may be the most important engineering development since the industrial age. With the rapid development of nanotechnology and its applications, numerous nanotechnology-based consumer products have become available. Silver nanoparticles (AgNPs) are emerging as one of the most commonly used nanomaterials [1]. AgNPs exhibit strong antimicrobial and anti-inflammatory activity; thus, they have become widely employed in medical instruments, personal care products, building materials, food packaging, and textiles [2,3]. The large-scale use of AgNPs raises safety concerns, due to the considerable potential for high exposure in humans and the lack of sufficient information regarding their health risks.

Previous *in vitro* studies have shown that AgNPs are toxic in cells derived from a variety of tissues [4,5]. Furthermore, numerous studies have unequivocally demonstrated that AgNPs administered by inhalation, ingestion, or intra-peritoneal injection are capable of translocating into blood circulation and accumulating in several organs, thereby causing toxicity, in part via oxidative stress [6,7]. In contrast, AgNP-induced neurotoxicity remains relatively unstudied, particularly the one induced by long-term exposure to low AgNP levels. Nanoparticles can enter the brain through disruption of the blood–brain barrier (BBB) or directly via trans-synaptic transport [8,9]. Indeed, it has been shown that AgNPs administered to rats subcutaneously could enter the blood–brain barrier and accumulate in brain regions in the form of nanoparticles [10]. Furthermore, intranasal AgNP delivery can impair hippocampal functions, because inhaled nanoparticles can reach the brain through the nasopharyngeal system [9,11]. Thus, an adequate risk assessment of potential neurological effects in response to AgNP exposure is necessary.

Nanoparticle-induced oxidative stress is hypothesized to be the main mechanism regulating the biological effects of AgNPs [12–14]. Our previous study found that AgNP exposure could induce neurotoxicity *in vitro* through oxidative stress-induced apoptosis [15]. Under normal circumstances, the body is endowed with antioxidant systems to combat oxidative stress. However, under extreme oxidative challenge, such as those detected upon nanoparticle exposure [12–14], the antioxidant machineries can become overwhelmed. Vitamin E (VE), the most important lipid-

Abbreviations: AgNPs, silver nanoparticles; VE, vitamin E; GFAP, glial fibrillary acidic protein; TEM, transmission electron microscopy; DLS, dynamic light scattering; HRP, horseradish peroxidase; BCA, bicinchoninic acid; SD, standard deviation; BBB, blood–brain barrier; ROS, reactive oxygen species.

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soluble antioxidant, can decrease the rate of lipid peroxidation, and could protect the brain from oxidative hazards [16]. However, studies evaluating the neuroprotective effects of VE against AgNP-induced toxicity in animals have not been described. Thus, we investigated the protective properties of VE against AgNP-induced neurotoxicity. Our study provides a promising and simple approach to antagonize the potentially harmful effects of AgNPs.

2. Materials and methods

2.1. Silver nanoparticle preparation, characterization, and animal administration

AgNPs (1 mg/mL) were purchase from Sigma–Aldrich Co. LLC (St. Louis, MO USA), and coated with citrate, as suggested by the manufacturer. The suspension was stored at 4 °C, and mechanically mixed to ensure a uniform suspension prior to dilution with distilled water for *in vivo* studies.

Transmission electron microscopy (TEM) characterization was carried out to obtain the primary size, size distribution, and morphology of the AgNPs using a Hitachi H-7500 TEM (Japan). To characterize the size and zeta potential of the nanoparticles in suspension, dynamic light scattering (DLS) was performed on a Zetasizer Nano ZS instrument (Malvern, UK) at 25 °C. Both size and zeta potential were measured three times for accuracy.

Neonatal Sprague–Dawley rats, together with their mothers, were acquired from Peking University Health Science Center. They were housed in clear plastic cages in a room with a 12 h/12 h light/dark cycle at 25 °C and <70% humidity. After 28 days, the weaned animals were transferred to new cages, with male and female rats housed in different cages. All animals were maintained in accordance with the principles of care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee of Peking University. Neonatal rats, initially weighing 4–5 g, were used as a control or treated with 0.1, 0.2, 0.5, and 1 mg/kg/day AgNP (12–15 animals per group). AgNP nasal drops were given to rats via intranasal instillation once daily for 14 consecutive weeks, and body weight was measured weekly. A second group of neonatal rats was employed for the vitamin E (VE) antagonism study. The rats were divided into control (distilled water), VE treatment (100 mg/kg/day), AgNP treatment (2 mg/kg/day), and AgNPs-VE co-treatment groups (2 mg/kg/day for AgNPs and 100 mg/kg/day for VE). This experiment lasted 30 days, with AgNPs administered by intranasal instillation and VE administered orally once daily. Body weight was monitored every 3–4 days.

2.2. Histopathological analysis

Rats were sacrificed, and cerebellar tissues were harvested after cold PBS intracardiac perfusion. According to a previous protocol [17], the cerebellum samples were fixed with 4% paraformaldehyde (PFA), dehydrated with a series of ethanol solutions, and infiltrated and embedded in paraffin. Sections (5 μm) were cut and processed for immunohistochemistry (IHC) evaluation by deparaffinization, dehydration, antigen retrieval, and blocking. The sections were incubated overnight with a primary antibody against glial fibrillary acidic protein (GFAP, Abcam, Cambridge, UK, 1:1000), washed, incubated with a secondary horseradish peroxidase (HRP)-labeled antibody (goat anti-rabbit IgG, Santa Cruz, Santa Cruz, CA, USA, 1:5000) for 30 min, and washed again. Samples were developed with substrate (1% 3,3′-diaminobenzidine [DAB] and 0.3% H_2O_2, 1:1) for 5 min, and counter-stained with hematoxylin. Images were taken with a laser scanning confocal microscope, bright field (Leica, TSC SP5, Centennial, CO, USA).

2.3. Western blotting examination

For the western blot analysis of GFAP, cerebellar tissue samples were homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer (Sigma–Aldrich) containing Roche complete mini EDTA-free protease inhibitor cocktail tablets (Indianapolis, IN, USA). Protein concentration was determined by bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). Proteins (50 μg) were electrophoresed on 4–20% Mini-PROTEAN Precast Gels (Bio-Rad, Hercules, CA, USA) and transferred onto nitrocellulose membranes. Membranes were blocked in 5% skim milk for 1 h at room temperature, then blotted with primary antibody (rabbit anti-GFAP, 1:50000, ab7260, Abcam) overnight at 4 °C, washed with TBS plus tween-20 (0.1%), and incubated with an HRP-tagged secondary

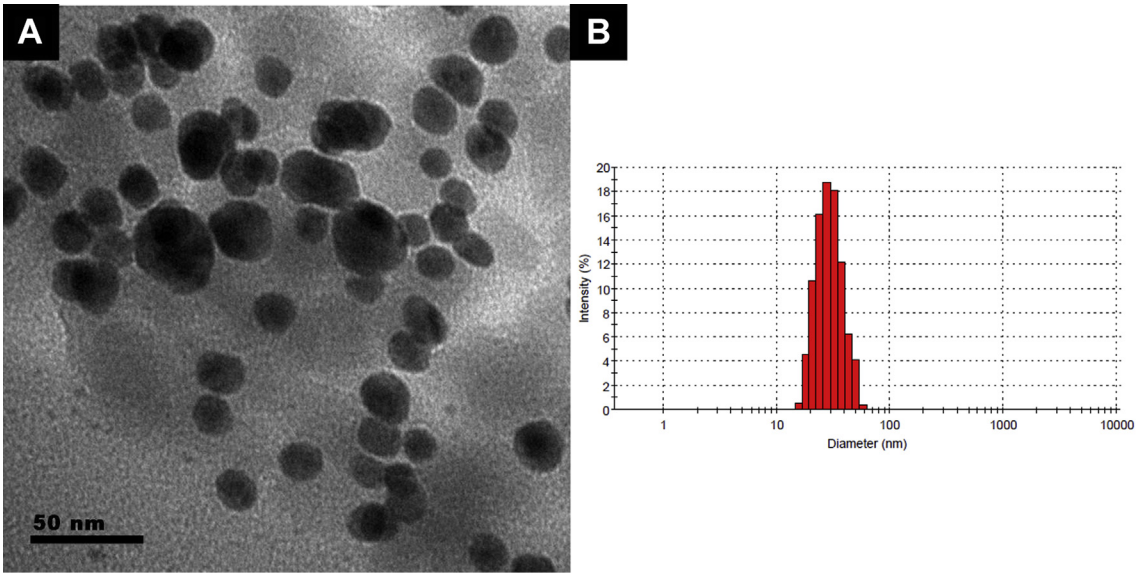

Fig. 1. Characterization of AgNPs. (A) TEM image of AgNPs. Scale bar is 50 nm. (B) The particle size distribution of AgNPs dispersed in distilled water by DLS.

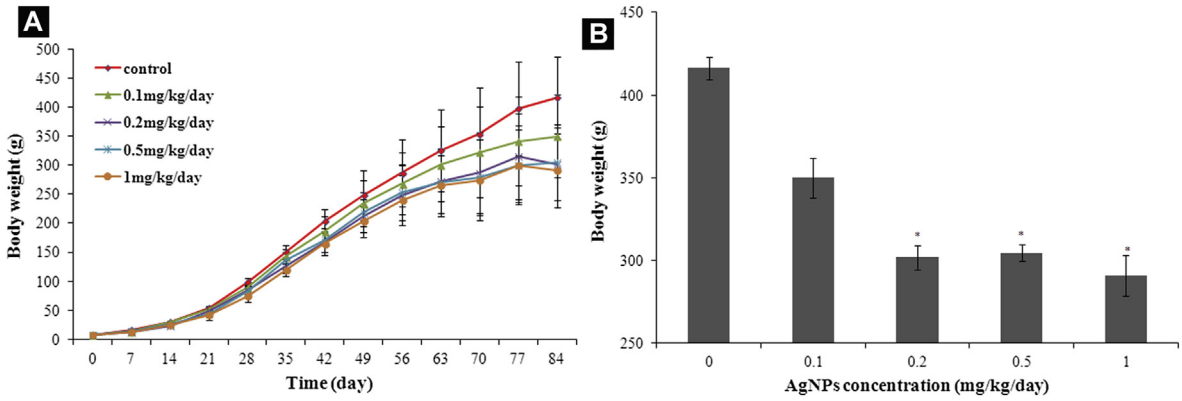

Fig. 2. Effect of AgNPs on body weight. (A) Body weight changes during 12 weeks of AgNP nasal administration. (B) The average body weight of the AgNP treated groups or the control group after cessation of AgNP administration (day 84).

antibody (1:1000, Santa Cruz) for 1 h at room temperature. Membranes were developed with enhanced chemiluminescence western blotting substrate according to the manufacturer's instructions (Pierce), and visualized by exposure to X-ray film (Kodak, Shanghai, China). Densitometric analysis using ImageJ software was used for quantification (http://rsbweb.nih.gov/ij).

2.4. Quantification of caspase-3 activity

The levels of activated caspase-3 in rat cerebellar tissues were measured using the Caspase-Glo kit (Promega). Briefly, tissue samples were homogenized in RIPA lysis buffer supplemented with a proteinase inhibitor cocktail. Equal concentrations of total protein extract (30 μg/mL) were loaded into a 96-well plate and caspase-3 levels measured according to the manufacturer's instructions. At least three replicates were used in each assay.

2.5. Statistical analysis

Results are shown as the mean ± standard deviation (SD). All results were analyzed by Student's *t*-test or ANOVA. A *P*-value less than 0.05 was considered statistically significant, and a *P*-value less than 0.005 was considered highly significant.

3. Results and discussion

3.1. Characterization of the AgNPs

AgNPs are found in diverse consumer products, medical devices, and pharmaceuticals, due to their excellent antibacterial and anti-inflammatory properties. Physical-chemical characterization of the nanomaterials used in toxicological studies is critical, because some parameters, such as size, surface charge, shape, aggregation, stability, and purity, may contribute to the toxicological responses

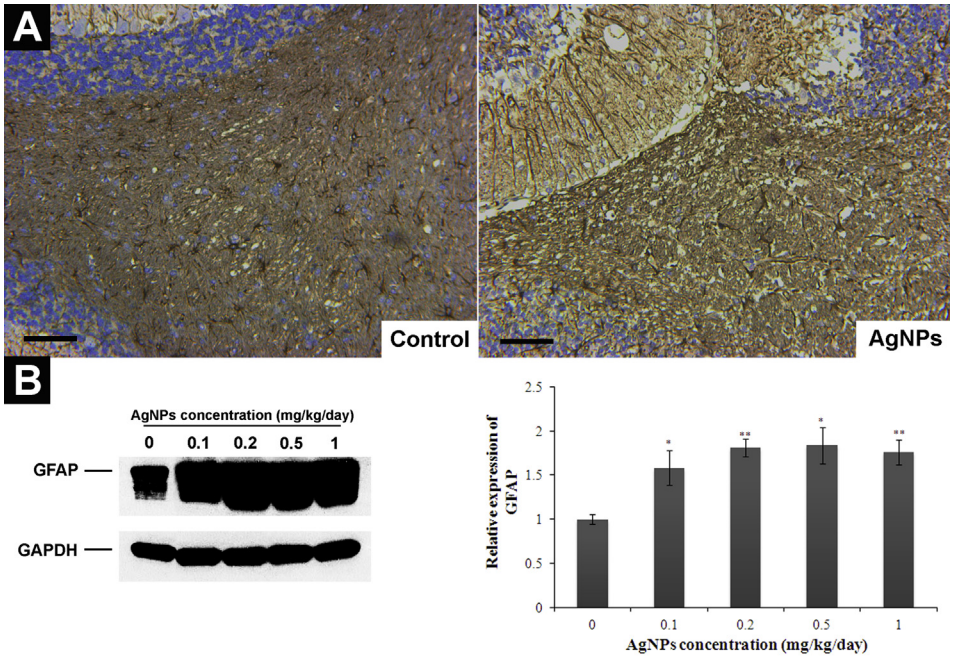

Fig. 3. Effects of AgNPs on GFAP. (A) Cerebellar tissue immunostaining with an anti-GFAP antibody and counterstaining with hematoxylin. Scale bar is 25 μm. (B) Western blot analysis of GFAP levels in rat cerebella. The protein was detected using specific antibodies, and GFAP levels were quantified based on grey levels of the band. Triplicate experiments were performed, and the data were presented as the mean ± SD. $^{*}P < 0.05$ and $^{**}P < 0.005$, versus the negative control.

[18]. For instance, the toxicity of AgNPs is related to their size, as lower concentrations of smaller particles produced more cytotoxic responses [19]. Therefore, we characterized our AgNPs by TEM to directly measure the primary size of the nanoparticles based on the projected area, and DLS to determine the hydrodynamic diameter of the nanoparticles based on the translational diffusion area. As shown in Fig. 1A, the AgNPs were approximately 20–25 nm in diameter, with a narrow size distribution and a uniform spherical morphology. Likewise, the behavior of our AgNPs in an aqueous environment was analyzed by DLS. These analyses revealed an average size of 22.3 ± 1.3 nm, with a polydispersity index (PDI) value lower than 0.2 (Fig. 1B). In addition, Zetasizer evaluation indicated that the surface charge (zeta potential) value of the AgNPs was −9.7 ± 1.1 mV, indicating that the particles did not have a tendency to form aggregates in solution.

3.2. AgNP administration decreased body weight gain in rats

Body weight is a sensitive indicator of toxic chemical effects [19,20]. Indeed, previous studies have shown that intravenous injections of AgNPs significantly decreased body weight in Wistar rats [21]. Similar phenomena were also reported in nickel and gold nanoparticles [22,23]. To investigate whether exposure to AgNPs could cause global health issues, we monitored the mortality rate, food consumption, water intake, and body weight of Sprague–Dawley rats over three months of AgNP administration. We did not observe any significant differences in survival or food/water intake between control and treated rats (data not shown). In contrast, although no disparity was witnessed in the first four weeks of treatment, the mean body weight of the AgNP treated groups decreased gradually from week 5, as compared to the

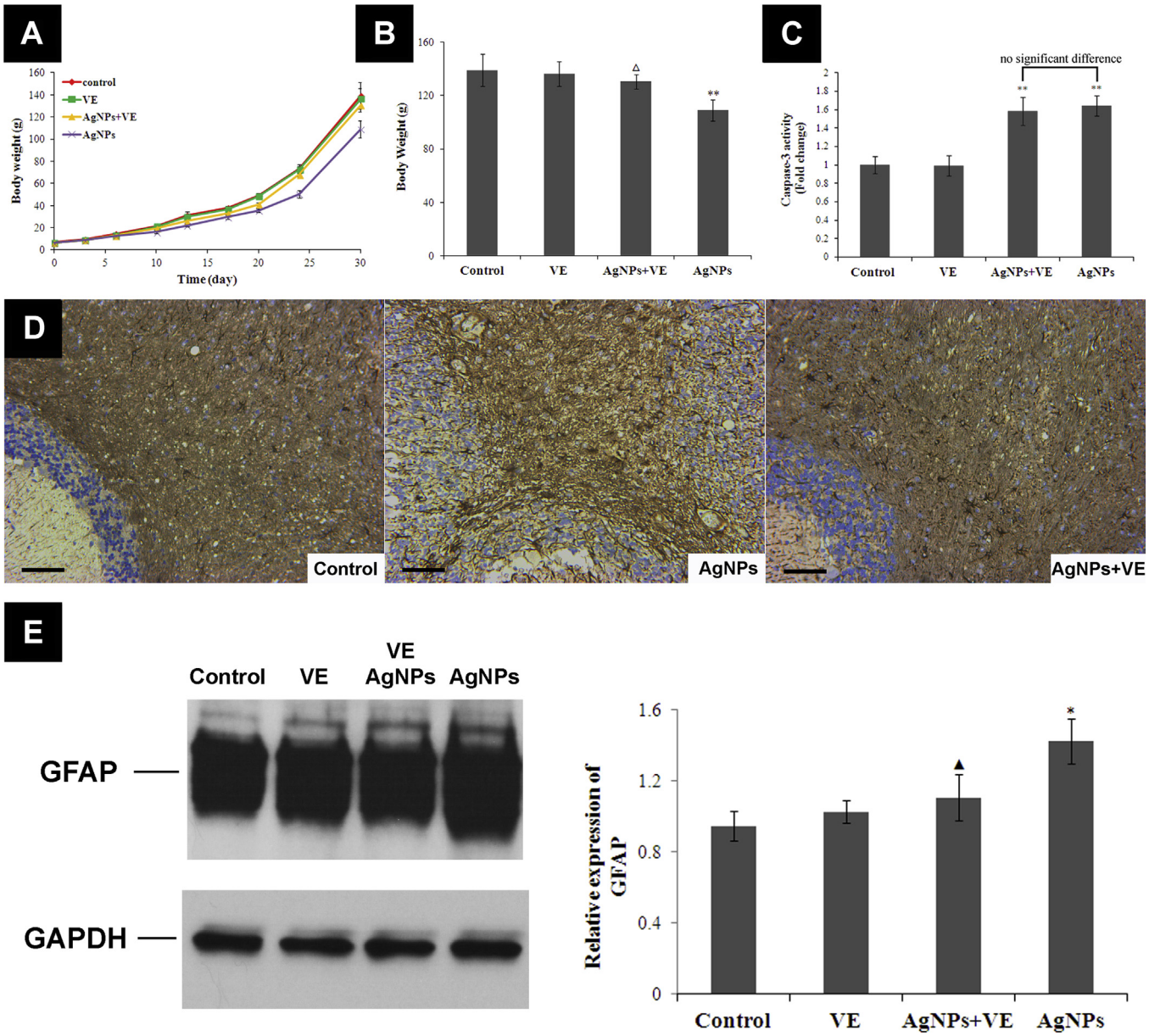

Fig. 4. VE attenuates AgNP-induced neurotoxicity. (A) Body weight changes during the administration period. (B) The average body weight of each group at the end of the exposure experiment (day 30). (C) Activated caspase-3 protein levels in rat cerebellar tissues as determined by the Caspase-Glo assay (D) Cerebellar tissue immunostaining with an anti-GFAP antibody. Scale bar is 25 μm. (E) Western blot analysis of GFAP levels in rat cerebella. The protein was detected using specific antibodies, and GFAP levels were quantified based on grey levels of the band. Triplicate experiments were performed, and the data were presented as the mean ± SD. $^{*}P < 0.05$ and $^{**}P < 0.005$, versus the negative control. ▲ $P < 0.05$ and △$P < 0.005$, versus the AgNP-treated group.

control group (Fig. 2A). The average body weight gain in the AgNP treated groups was significantly lower than the control group over 7 weeks of treatment when the exposure dose was higher than 0.2 mg/kg/day (Fig. 2A). At the end of the experiment (week 12), the average body weight of the rats administered 1 mg/kg/day AgNPs was 291.1 ± 12.1 g, as compared to 416.3 ± 6.8 g in control animals (Fig. 2B). The suppression of body weight gain clearly indicated that AgNPs have toxic effects in animals.

3.3. AgNPs induced glial-scar formation in rat cerebella

A previous study reported blood–brain barrier (BBB) inflammation and increased permeability in primary rat brain microvessel endothelial cells after AgNP exposure, providing evidence that *in vivo* exposure may also be neurotoxic [24]. It has been also shown that nanoscale particles are capable of permeating the BBB via carrier-mediated endocytosis or passive diffusion due to small particle size [8,25,26]. Moreover, inhalation exposure studies suggest that inhaled nanoparticles may reach the brain through the nasopharyngeal system [9]. Together, these results suggested that AgNPs induce neurotoxicity *in vivo*.

GFAP, an indicator of astrocyte activation, is considered a biomarker of early biological effects, including cell structure and movement, cell communication, and blood–brain barrier function. It is reported that GFAP expression is dramatically increased during acute infection or neurodegeneration [27]. To investigate whether AgNPs produce neurotoxicity *in vivo*, we examined glial cell overgrowth in the cerebellum by measuring GFAP levels. Immunohistochemistry analyses showed elevated levels of GFAP in rat cerebella upon nasal instillation of AgNPs (1 mg/kg/day), as compared to control animals (Fig. 3A), suggesting that AgNPs could stimulate astrocytes activation or proliferation. Due to their dense nucleus, granule cells could be characterized by their compact morphology, which produced blue staining when hematoxylin was used. Fig. 3A clearly revealed an obvious decrease in the intensity of blue-stained granule cells in AgNP-treated rats. We also examined GFAP protein levels in rat cerebella by western blot, and demonstrated that GFAP expression was elevated in rats treated with various concentrations of AgNPs (0.1–1 mg/kg/day, Fig. 3B, left panel), consistent with the histological findings. Quantification of the blots revealed that AgNPs enhanced GFAP expression 1.5–1.8 fold (Fig. 3B, right panel). These findings demonstrated that AgNPs can induce neuroglial cell activation with concomitant destruction of the cerebellum granular layer, suggesting that AgNPs are neurotoxic *in vivo*.

3.4. VE attenuated AgNP-induced toxicity

The mechanisms by which nanoparticles affect human health are still under investigation; however, growing evidence suggests that oxidative stress plays an essential role [28]. Previous studies using nanoparticles with different chemical components have shown that they are capable of causing oxidative stress [12–14]. Additionally, in our previous study, we found that AgNPs could attenuate rat cerebellum granule cell viability through apoptosis coupled to oxidative stress [15]. VE is regarded as the most important lipid-soluble antioxidant, and protects the brain from oxidative damage [16]. Thus, we hypothesized that VE could protect against reactive oxygen species (ROS)-induced toxicity following AgNP administration.

As shown in Fig. 4A and B, VE alone had no obvious effect on rat body weight gain. Nevertheless, oral administration of VE significantly improved body weight gain in rats administered with AgNPs. The average body weight of the AgNP treated group on day 30 was 109.0 ± 7.8 g, whereas the average body weight was 130.5 ± 5.5 g when VE was co-administered (Fig. 4B). Previously, we demonstrated that apoptosis was one of the key factors involved in AgNP-induced neurotoxicity *in vitro* and *in vivo* [15]. Therefore, to test the mitigating effects of VE we first looked at neuronal cell death via apoptosis. We examined the activation of caspase-3 in rat cerebella and confirmed that AgNPs could significantly increase the levels of activated caspase-3 (Fig. 4C). However, VE could not counterbalance those effects. Despite these results, immunostaining of cerebellum sections did confirm that VE administration attenuated the damage caused by AgNPs. Indeed, GFAP expression decreased and granule cell staining increased in rats co-treated with AgNPs and VE to levels similar to control rats (Fig. 4D). We also analyzed the expression of GFAP in rat cerebella by western blot. Treatment with VE alone resulted in no significant alterations in GFAP levels, as compared with the control group. However, a significant reduction in GFAP was detected in rats co-treated with AgNPs-VE, as compared with rats treated with AgNPs alone (Fig. 4E, left). Quantitative analyses revealed a 20–30% increase in GFAP expression when VE was co-administered (Fig. 4E, right), confirming the beneficial function of VE on AgNP-induced neurotoxicity *in vivo*. In our next study, we will further characterize the molecular mechanisms behind VE effects, and also screen additional antioxidants and/or anti-apoptotic compounds that would counteract AgNP-induced neurotoxicity.

In summary, we have shown that AgNPs are capable of suppressing body weight gain in Sprague–Dawley rats. Nanoparticle exposure also exerted significant neurotoxicity in the rat cerebellum by activating neuroglial cells and destroying the cerebellum granular layer. Interestingly, VE ameliorated AgNP-induced toxicity. To our knowledge, this is the first report of the use of a natural, side effect-free, compound (VE) that could counterbalance AgNP toxic effects.

Conflicts of interest

The authors disclose no conflicts of interest.

Acknowledgments

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Transparency document

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References

[1] M. Ahamed, M.S. Alsalhi, M.K. Siddiqui, Silver nanoparticle applications and human health, Clin. Chim. Acta 411 (2010) 1841–1848.
[2] H.Y. Lee, H.K. Park, Y.M. Lee, K. Kim, S.B. Park, A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications, Chem. Commun. (Camb) 28 (2007) 2959–2961.
[3] R. Gottesman, S. Shukla, N. Perkas, L.A. Solovyov, Y. Nitzan, A. Gedanken, Sonochemical coating of paper by microbiocidal silver nanoparticles, Langmuir 27 (2011) 720–726.
[4] N. Miura, Y. Shinohara, Cytotoxic effect and apoptosis induction by silver nanoparticles in HeLa cells, Biochem. Biophys. Res. Commun. 390 (2009) 733–737.
[5] S. Arora, J. Jain, J.M. Rajwade, K.M. Paknikar, Cellular responses induced by silver nanoparticles: in vitro studies, Toxicol. Lett. 179 (2008) 93–100.
[6] K.K. Comfort, E.I. Maurer, L.K. Braydich-Stolle, S.M. Hussain, Interference of silver, gold, and iron oxide nanoparticles on epidermal growth factor signal transduction in epithelial cells, ACS Nano 5 (2011) 10000–10008.
[7] Y.H. Hsin, C.F. Chen, S. Huang, T.S. Shih, P.S. Lai, P.J. Chueh, The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving

the mitochondrial pathway in NIH3T3 cells, Toxicol. Lett. 179 (2008) 130–139.
[8] P.H. Hoet, I. Bruske-Hohlfeld, O.V. Salata, Nanoparticles – known and unknown health risks, J Nanobiotechnology 2 (2004) 12.
[9] G. Oberdorster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox, Translocation of inhaled ultrafine particles to the brain, Inhal. Toxicol. 16 (2004) 437–445.
[10] J. Tang, L. Xiong, S. Wang, J. Wang, L. Liu, J. Li, F. Yuan, T. Xi, Distribution, translocation and accumulation of silver nanoparticles in rats, J. Nanosci. Nanotechnol. 9 (2009) 4924–4932.
[11] Y. Liu, W. Guan, G. Ren, Z. Yang, The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats, Toxicol. Lett. 209 (2012) 227–231.
[12] S. Klein, M.L. Dell'Arciprete, M. Wegmann, L.V. Distel, W. Neuhuber, M.C. Gonzalez, C. Kryschi, Oxidized silicon nanoparticles for radiosensitization of cancer and tissue cells, Biochem. Biophys. Res. Commun. 434 (2013) 217–222.
[13] S. Klein, A. Sommer, L.V. Distel, W. Neuhuber, C. Kryschi, Superparamagnetic iron oxide nanoparticles as radiosensitizer via enhanced reactive oxygen species formation, Biochem. Biophys. Res. Commun. 425 (2012) 393–397.
[14] M. Ahamed, M.A. Siddiqui, M.J. Akhtar, I. Ahmad, A.B. Pant, H.A. Alhadlaq, Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells, Biochem. Biophys. Res. Commun. 396 (2010) 578–583.
[15] N. Yin, Q. Liu, J. Liu, B. He, L. Cui, Z. Li, Z. Yun, G. Qu, S. Liu, Q. Zhou, G. Jiang, Silver nanoparticle exposure attenuates the viability of rat cerebellum granule cells through apoptosis coupled to oxidative stress, Small 9 (2013) 1831–1841.
[16] M. Pandolfo, Drug insight: antioxidant therapy in inherited ataxias, Nat. Clin. Pract. Neurol. 4 (2008) 86–96.
[17] B. Todorov, L. Kros, R. Shyti, P. Plak, E.D. Haasdijk, R.S. Raike, R.R. Frants, E.J. Hess, F.E. Hoebeek, C.I. De Zeeuw, A.M. van den Maagdenberg, Purkinje cell-specific ablation of Cav2.1 channels is sufficient to cause cerebellar ataxia in mice, Cerebellum 11 (2012) 246–258.
[18] D.B. Warheit, How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? Toxicol. Sci. 101 (2008) 183–185.
[19] H. Andersen, S. Larsen, H. Spliid, N.D. Christensen, Multivariate statistical analysis of organ weights in toxicity studies, Toxicology 136 (1999) 67–77.
[20] H.Y. Kim, S.B. Lee, K.T. Lim, M.K. Kim, J.C. Kim, Subchronic inhalation toxicity study of 1,3-dichloro-2-propanol in rats, Ann. Occup. Hyg. 51 (2007) 633–643.
[21] D.K. Tiwari, T. Jin, J. Behari, Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats, Toxicol. Mech. Methods 21 (2011) 13–24.
[22] L. Kong, M. Tang, T. Zhang, D. Wang, K. Hu, W. Lu, C. Wei, G. Liang, Y. Pu, Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats, Int. J. Mol. Sci. 15 (2014) 21253–21269.
[23] D.L. Xia, Y.F. Wang, N. Bao, H. He, X.D. Li, Y.P. Chen, H.Y. Gu, Influence of reducing agents on biosafety and biocompatibility of gold nanoparticles, Appl. Biochem. Biotechnol. 174 (2014) 2458–2470.
[24] W.J. Trickler, S.M. Lantz, R.C. Murdock, A.M. Schrand, B.L. Robinson, G.D. Newport, J.J. Schlager, S.J. Oldenburg, M.G. Paule, W. Slikker Jr., S.M. Hussain, S.F. Ali, Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells, Toxicol. Sci. 118 (2010) 160–170.
[25] C. Lasagna-Reeves, D. Gonzalez-Romero, M.A. Barria, I. Olmedo, A. Clos, V.M. Sadagopa Ramanujam, A. Urayama, L. Vergara, M.J. Kogan, C. Soto, Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice, Biochem. Biophys. Res. Commun. 393 (2010) 649–655.
[26] S. Ku, F. Yan, Y. Wang, Y. Sun, N. Yang, L. Ye, The blood-brain barrier penetration and distribution of PEGylated fluorescein-doped magnetic silica nanoparticles in rat brain, Biochem. Biophys. Res. Commun. 394 (2010) 871–876.
[27] N.L. Johnston-Wilson, C.D. Sims, J.P. Hofmann, L. Anderson, A.D. Shore, E.F. Torrey, R.H. Yolken, Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. The Stanley Neuropathology Consortium, Mol. Psychiatry 5 (2000) 142–149.
[28] X. Wang, Q. Tu, B. Zhao, Y. An, J.C. Wang, W. Liu, M.S. Yuan, S.M. Ahmed, J. Xu, R. Liu, Y. Zhang, J. Wang, Effects of poly(L-lysine)-modified Fe_3O_4 nanoparticles on endogenous reactive oxygen species in cancer stem cells, Biomaterials 34 (2013) 1155–1169.