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Silver nanoparticles affect on gene expression of inflammatory and neurodegenerative responses in mouse brain neural cells

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ABSTRACT

Silver nanoparticles (AgNPs) have antibacterial characteristics, and currently are applied in Ag-containing products. This study found neural cells can uptake 3–5 nm AgNPs, and investigated the potential effects of AgNPs on gene expression of inflammation and neurodegenerative disorder in murine brain ALT astrocytes, microglial BV-2 cells and neuron N2a cells. After AgNPs (5, 10, 12.5 μg/ml) exposure, these neural cells had obviously increased IL-1β secretion, and induced gene expression of C-X-C motif chemokine 13 (*CXCL13*), macrophage receptor with collagenous structure (*MARCO*) and glutathione synthetase (*GSS*) for inflammatory response and oxidative stress neutralization. Additionally, this study found amyloid-β (Aβ) plaques for pathological feature of Alzheimer's disease (AD) deposited in neural cells after AgNPs treatment. After AgNPs exposure, the gene expression of amyloid precursor protein (*APP*) was induced, and otherwise, neprilysin (*NEP*) and low-density lipoprotein receptor (*LDLR*) were reduced in neural cells as well as protein level. These results suggested AgNPs could alter gene and protein expressions of Aβ deposition potentially to induce AD progress in neural cells. It's necessary to take notice of AgNPs distribution in the environment.

1. Introduction

In recent years, nanotechnology grows rapidly, and nanoparticles are produced and widely utilized in diverse areas of different industrial applications because of its high interfacial reactivity and unique physicochemical properties (Loo et al., 2013). As to antibacterial/antifungal characteristics, silver nanoparticles (AgNPs) have been used in clothes, cosmetics, wound dressing, air-freshener sprays, water disinfectant, sunscreens, hygiene products and food containers, which increases the release of nanoparticles to environment and may cause exposure to human (Ribeiro et al., 2013). The exposure route for AgNPs happens via ingestion, inhalation or dermal contact. Kulthong et al. (2010) indicated that the antibacterial fabric from six commercial fabrics releases silver of AgNPs when is immersed in artificial sweat as a model to represent the human skin environment. In addition, AgNPs may have an access to systemic circulation through broken skin when we use the AgNP-containing products such as bandages or wound dressings (Singh and Ramarao, 2012). After injection different particles size of Ag (nanosized and microsized) in rats (62.8 mg/kg), AgNPs can translocate to the blood circulation and distribute throughout the main organs, especially in the kidney, liver, spleen, lung and brain, and induce blood–brain barrier (BBB) destruction and astrocyte swelling to cause neuronal degeneration (Tang et al., 2009).

Ag is one of the most toxic metals for the marine systems (Tappin et al., 2010), and the monovalent silver ion is considered as the most toxic silver species in aquatic systems and causes intracellular accumulation in phytoplankton (Lee et al., 2005). However, the AgNPs (< 100 nm, 0.5 and 1 μg/ml) cause nuclear condensation and induce higher dramatically cytotoxicity than Ag ions in human lymphoma cells (Eom and Choi, 2010). In addition, a proteomic analysis showed that 20 nm AgNPs interfere with protein regulations of mitochondrial translation, RNA processing, tRNA metabolism and cell proliferation more than Ag ions and larger size AgNPs (100 nm) in human colon adenocarcinoma LoVo cells (Verano-Braga et al., 2014). The diameter 139 ± 37 nm AgNPs trigger dose-dependent effect of decreased cell viability on human lung carcinoma A549 cells in exposure to 5, 10 and 15 μg/ml AgNPs (Foldbjerg et al., 2011). Besides, the cell deaths in apoptosis and necrosis all increase after exposure to AgNPs (2.5, 5, 10 and 15 μg/ml). Moreover, Gaiser et al. (2013) pointed that 20 nm

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diameter nanoparticles can cause toxicity, inflammation and oxidative stress after exposure to human C3A hepatocytes and female Wistar rats. Besides, the inflammatory cytokines, e.g., IL-8, MIP2, IL-1RI and TNF-α, are both increased on in vitro and in vivo models after AgNPs-induction. Overall, the Ag and Cu nanoparticles can easily enter the mice brain to disrupt BBB permeability and induce neurotoxicity, which alters brain sensory, motor and cognitive functions (Sharma and Sharma, 2012). AgNPs (20 nm; 1, 5, 10 and 50 μg/ml) can reduce cell viability in primary rat cortical cells, and inhibit the sprouting of neuronal branches and elongation of neuritis for fragmentation and degeneration of mature neurons (Xu et al., 2013).

Brain is composed of endothelial cells, neurons and glial cells. Astrocytes are known as reactive astrogliosis cells to regulate metal homeostasis, supply nutrients to neurons and protect other brain cells against oxidative stress and metal toxicity (Sofroniew and Vinters, 2010). Microglias are a type of glial cell major brain-resident macrophage-like cells in the central nerve system (CNS) to defense against microorganism invasion and injury, and release some cytokine factors to mediate neuroinflammatory processes (Wang et al., 2011). The inflammatory response, a tissue reaction to injury or an antigen, releases cytokines, chemokines, reactive oxygen species (ROS) and nitric oxides (NO) (Wei et al., 2013). Nerve cells connect to each other to form neural networks. Neurons are electrically excitable brain endothelium to transmit information through electrical and chemical signals via synapses and contact with perivascular astrocytes and pericytes (Weiss et al., 2009). Tang et al. (2010) indicated that AgNPs can cross through the BBB of rat brain to influence brain cells through transcytosis of capillary endothelial cells detectable by transmission electronic microscopy (TEM) and inductively-coupled plasma mass spectrometry (ICP-MS). Thus, the highest concentration of silver is observed in the kidneys and brain 28 days after injection a dose 5 mg kg^{-1} bw AgNPs (20 and 200 nm) in Wistar rats. Dziendzikowska et al. (2012) found that AgNPs increase ROS generation and heme oxygenase 1 (HO-1) protein expression to cause neuronal oxidative damage and directly interfere with calcium responses in primary mixed neural cells. Increased levels of ROS occurred chronically in the early disease, which is relevant to neurodegenerative disorders, such as Alzheimer's and Parkinson's disease (Smith and Cass, 2007). Moreover, glutathione metabolism plays an important role of protecting cell from oxidative stress, and their gene expression related to oxidative stress are significantly altered in the caudate, frontal cortex and hippocampus of male C57BL/6N mice after administered 25 nm AgNPs (Rahman et al., 2009).

The C-X-C motif chemokine 13 (*CXCL13*) play a role in the B-cell recruitment and distribution, associated with chronic inflammatory process (Nakajima et al., 2008). Macrophage receptor with collagenous structure (*MARCO*) is important for immune responses to bacterial infections by mediating the binding and phagocytosis of pathogens (Komine et al., 2013). Accordingly, studies have indicated that AgNPs can induce ROS and cytokines increasing and then cause inflammatory response. As *CXCL13* and *MARCO* genes are immune mediators in response to inflammation, exposure to AgNPs may change their gene expression. Moreover, glutathione synthetase (GSS) can synthesize glutathione (GSH) potentially to inhibit oxidative stress and prevent cellular damage from free radicals and peroxides (Koike et al., 2013), and AgNPs exposure probably alters *GSS* gene expression.

Amyloid beta (Aβ) is a peptide of amino acids that is processed from amyloid precursor protein (APP). Aβ protein is considered the main responsible for neurodegenerative disorder such as Alzheimer's disease (AD). The up-regulation of *APP* gene expression interferes with Aβ metabolism underlying the pathogenesis of AD (Dong et al., 2012). Low-density lipoprotein receptor (LDLR) enhances Aβ uptake and degradation through binding Aβ and Aβ/ApoE complex (Basak et al., 2012). Thus, the down-regulation of *LDLR* gene leads Aβ deposition. Besides, neprilysin (NEP) is a major Aβ-degrading enzyme in brain to degrade Aβ protein (El–Amouri et al., 2007). Sequentially, it is important to investigate the receptors and gene expression regulating Aβ amyloid internalization in neural cells for understanding the AD pathogenesis.

According to previous studies, the information until now is not well known that whether the AgNPs-induced neuroinflammation cause the changes in gene expression related neurodegenerative disorder such as AD. In this study, we investigated whether the 3–5 nm AgNPs can pass through mouse brain neuronal cells and induce Aβ amyloid generation underlying the potential effect of AgNPs on gene expression of inflammatory response, oxidative stress, and Aβ deposition.

2. Material and methods

2.1. Cell culture and exposure

This study used three types of neural cells, murine brain ALT astrocytes (BCRC 60581), murine microglial BV-2 cells (ICLC ATL03001) and mouse neuroblastoma Neuro-2a (N2a) cells (BCRC 60026). N2a cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM; CORNING, New York) supplemented with 10 percent fetal bovine serum (Invitrogen, Carlsbad, Canada), 1 percent antibiotic (Biowest, Loire Valley, France), 1 percent L-glutamine (Invitrogen), 1 percent sodium pyruvate (Invitrogen) in a cell incubator with 5 percent CO_2 at 37 °C. ALT cells and BV-2 cells were cultured in the similar medium with N2a cells except for the lack of 1 percent sodium pyruvate. When N2a cells grew to 70–80 percent confluence of a culture plate, the growth medium was removed and replaced with differentiation medium for two days. The differentiation medium containing forskolin and isobutylmethylxanthine (IBMX) was added to N2a cells for 24 h differentiation. N2a cells can be differentiated into a neuron-like morphology with expression of several neuronal markers. The 3–5 nm AgNPs were produced by a physical method without surfactants or stabilizers (Gold Nanotech Inc., Taiwan). AgNPs (0.5, 1, 5, 10 and 12.5 μg/ml) and lipopolysaccharides (LPS; 0.2 and 2 μg/ml; Invitrogen) were respectively added into the medium to treat ALT, BV-2 and N2a cells for 24 h exposure.

2.2. Polarizing microscope

N2a cells were cultured on glass coverslips with the treatment of AgNPs (5 nm, 12.5 μg/ml) for 24 h. After treatment, the cells on coverslips were fixed in 4 percent paraformaldehyde (PFA) for 10 min at 4 °C, then washed with phosphate buffered saline (PBS) and mounted with slides in mountain medium. Images of AgNPs location were captured under the polarizing microscopy (IX71, Olympus, Tokyo, Japan).

2.3. Cell proliferation of neural cells

The neural cells 1×10^4 cm^{-2} were seeded in 96-well plates for cell viability analysis. After exposure to AgNPs or LPS, the suspensions were discarded, alamarBlue® reagent (DMEM/10 percent FBS 1:10; Invitrogen) was added as a cell viability indicator followed by a 2 h incubation at 37 °C, and the absorbance was monitored at 570 nm using 600 nm as a reference wavelength. The cell viability was calculated as [cell number of exposure samples]/[cell number of control] × 100. Cell numbers were derived from a standard curve, which was obtained after seeding serially diluted cells (from 5×10^4 to 1.56×10^3 cells/ml) in a 96-well plate.