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Review

Potential neurotoxicity of nanoparticles

Yu-Lan Hu, Jian-Qing Gao*

Institute of Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, 388 Yuhangtang Road, Hangzhou, Zhejiang 310058, PR China

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ABSTRACT

With the rapid development of nanotechnology, there is a growing interest on the application of nanoparticles in various fields such as photonics, catalysis, magnetics, and biotechnology including cosmetics, pharmaceutics, and medicines. However, little is known about their potential toxicity to human health. Owing to their special properties, nanoparticles have the capacity to bypass the blood–brain barrier (BBB). However, the toxic effects of nanoparticles on central nervous system (CNS) function are still lacking. And the interactions of nanoparticles with the cells and tissues in CNS are poorly understood. Thus, neurotoxicity induced by nanoparticles is still a new topic that requires more attention. In this review, we summarized the pathways by which the nanoparticles could enter into the CNS and the recent investigations on the neurotoxicity of nanoparticles both *in vitro* and *in vivo*, as well as the potential mechanisms. Furthermore, the future direction in the neurotoxicity studies of nanoparticles is also discussed.

Contents

1. Introduction

The rapid development of nanotechnology has led to the wide application of nanoparticles (NPs) in various fields such as photonics, catalysis, magnetics, and biotechnology including cosmetics, pharmaceutics, and medicines (Donaldson, 2006; Kagan et al., 2005; Linkov et al., 2008; Medina et al., 2007). However, there is a lack of information concerning the impact of NPs on human health, as it was proved that the nanoparticles could be administered to human body by several routes including inhalation, ingestion, dermal penetration, and injection, followed by the distribution of these nanoparticles to various tissues through systemic circulation (Burch, 2002; Takenaka et al., 2001). Typically, after systemic administration, the nanoparticles are small enough to penetrate very small capillaries throughout the body, and therefore they could offer the most effective approach to target certain tissues (Braydich-Stolle et al., 2005) such as brain and can affect the physiology of any cell in an animal body (Brooking et al., 2001). Particularly, site-specific drug targeting using nanoparticle drug carrier systems have been developed, and nanoparticle-based drug brain-targeting delivery systems have been introduced in the treatment of brain diseases (Kreuter, 2001; Roney et al., 2005).

In the past few decades, the population of people older than 65 years has been increasing fast. They are at high risk of having brain disease such as Alzheimer's disease (Farrer, 2001). Also, the incidence of primary brain tumors has been increasing at an alarming rate (Basso et al., 2001; Orringer et al., 2009). Tremendous efforts have been focused on the chemotherapy of the CNS diseases. However, the existence of the blood–brain barrier (BBB), which limits the entry of many substances into the brain, makes it difficult to deliver drugs to lesions within the CNS. The nanocarriers appear to be a promising drug brain-targeting strategy, as evidenced by a number of studies (Borm and Muller-Schulte,

* Corresponding author. Tel.: +86 571 88208437; fax: +86 571 88208437.
E-mail address: gaojianqing1029@yahoo.com.cn (J.-Q. Gao).

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Table 1
Transport of nanoparticles to the brain.

Nanoparticle (particle size)	Animal model	Route of administration	Results	References
Non-degradable NPs				
Quantum dot (QDs) coated by hydroxyl group modified silica networks (21.3 ± 2 nm)	Mice	Intravenously	QDs were found to rarely distribute in the brain.	Chen et al. (2008)
Silica-overcoated magnetic nanoparticles (50 nm)	Mice	Intraperitoneally	Nanoparticles were detected in the brain.	Kim et al. (2006)
Nanosized TiO(2) particles (25, 80 and 155 nm)	Mice	Gastrointestinal administration	The mice had a slight brain lesion associated with exposure to TiO_2 particles.	Wang et al. (2007b)
Fe_2O_3 NPs (280 ± 80 nm)	Mice	Intranasally	A deep brain penetration of the NPs and its potential to disrupt the cellular morphology in the hippocampus were observed.	Wang et al. (2007a)
Fluorescent magnetic nanoparticles (FMNPs) (50 nm)	Mice	Inhalation	FMNPs were found to distribute in the brain.	Kwon et al. (2008)
MnO, Mn_2O_3 NPs (30 nm)	Rats	Inhalation	NPs were detected in olfactory bulbs and in deep brain structures such as the cortex and cerebellum.	Elder et al. (2006)
^{13}C NPs (about 36 nm)	Rats	Inhalation	A significant and persistent increase of ^{13}C NPs in the olfactory bulbs.	Oberdorster et al. (2004)
Degradable polymer NPs				
Long-circulating PEGylated cyanoacrylate NPs (100–200 nm)	Mice and rats	i.v. injection	PEGylated PHDCA nanoparticles penetrated into the brain to a large extent.	Calvo et al. (2001)
Dalargin absorbed on polysorbate-80 coated PBCA NPs (230 nm)	Mice	i.v. injection	Nanoparticle could deliver dalargin across the BBB.	Kreuter et al. (1995)
Dalargin adsorbed on polysorbate-80 coated PBCA NPs (200–300 nm)	NMRI mice	i.v. injection and oral administration	Polysorbate 85 (Tween 85) stabilized and dalargin-loaded nanoparticles are able to induce a central analgesic effect after i.v. application as well as after oral treatment.	Schroeder et al. (1998)
Doxorubicin bound to polysorbate-coated NPs (270 ± 20 nm)	Rat	i.v. injection	Doxorubicin bound to NPs could crossed the intact blood–brain barrier, thus reaching therapeutic concentrations in the brain.	Steiniger et al. (2004)
Lectin-conjugated PEG–PLA nanoparticles (70–80 nm)	Rats	Intranasally	There was a twofold increase in the brain uptake of WGA-conjugated nanoparticles.	Gao et al. (2006)
Delivery of nimodipine by methoxy-PEG–PLA NPs (76.5 nm)	Rats	Intranasally	Significant enhancement of nimodipine in the CSF and olfactory bulb was reported.	Zhang et al. (2006)

2006; Kreuter, 2001). Poly(butyl cyanoacrylate) (PBCA) nanoparticles coated with polysorbate 80 facilitate the brain delivery of a number of drugs that are unable to cross the BBB in their free form (Kreuter, 2001). Subsequently, different types of the nanoparticles, such as poly(alkyl cyanoacrylate) (Calvo et al., 2001), human serum albumin (Michaelis et al., 2006), and solid lipid nanoparticles (Goppert and Muller, 2005), employing polysorbate 80 as a coating surfactant, were proved to be effective in brain targeting. Moreover, some inorganic NPs are engineered to carry MRI contrast agents, fluorescent and visible dyes, chemotherapeutic agents and photosensitizers to brain for the diagnosis and positioning. For example, a large variety of colloidal dispersions of Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) have been developed and explored for a range of new biological, biomedical, and diagnostic applications with regard to their magnetic properties (Cengelli et al., 2006; Corot et al., 2004; Kircher et al., 2003).

However, due to their special physicochemical properties, such as large surface area, the nanoparticles may cause neurotoxicity after entering into the brain. Therefore, the evaluation of the potential neurotoxic effects of these nanoparticles on CNS function is required, as specific mechanisms and pathways through which nanoparticles may exert their toxic effects remain largely unknown.

The BBB is a specialized system that separates blood from cerebrospinal fluid. It consists of endothelial cells connected by complex tight junctions, which restrict the access of large or hydrophilic compounds to the brain (Begley, 1996). However, NPs made of different materials could cross the BBB (Kreuter, 2001). Also, NPs can move inside the brain from the nasal cavity (Oberdorster et al., 2004) (Table 1). As certain NPs are not easily eliminated by physiological clearance systems, they could accumulate within brain to elicit further cytotoxicity. Several reports showed that the NPs could enter into the brain and cause tissue injury (Medina et al., 2007; Sharma, 2007). As it is difficult for the therapeutic drugs to cross BBB, the treatment of this injury mostly depends on the self-regenerative ability of neurons within the CNS. However, the self-regenerative ability of neurons is limited. Therefore, the neurotoxicity of NPs should be carefully evaluated. However, there are very few studies investigating the neurotoxic effects of nanomaterials, and no guidelines are presently available to quantify these effects.

Here, we summarized the pathways by which the nanoparticles could enter into the CNS, and the recent investigations of the nanoparticle neurotoxicity both *in vitro* and *in vivo*, as well as the potential mechanisms. Lastly, we discussed the future directions in the neurotoxicity studies of nanoparticles.

2. The brain as a target for NPs

Generally, most molecules cannot cross the BBB, as BBB is a tight barrier to protect the brain from the penetration of xenobiotics. However, NPs made of certain materials and with varying particle sizes can overcome this physical barrier and enter into the brain, or enter into the brain by the nerve endings of the olfactory bulb (Koziara et al., 2006; Kreuter, 2001; Kreuter et al., 1995) (Table 1). NPs were capable of being administered to human body via several routes including inhalation, oral administration, and injection (Table 1) (Chen et al., 2008; Schroeder et al., 1998; Wang et al., 2007a).

Generally, the specific mechanisms of the most nanoparticle targeting to the brain have yet to be elucidated. So far, two different pathways have been proposed (Borm and Muller-Schulte, 2006). The first pathway for NPs to reach the brain involves the uptake of nanoparticles by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to CNS structures. In addition, nanoparticles can be taken up by the nerve

endings of the olfactory bulb and translocated to the CNS (Medina et al., 2007; Oberdorster et al., 2004). This pathway has been studied primarily using carbon, Au and MnO_2 nanoparticles in experimental inhalation models (Oberdorster et al., 2005). For example, the translocation of ultrafine ^{13}C particles (35 nm) was detected in the brain olfactory bulb after inhalation exposure. Other reports (Donaldson, 2006; Peters et al., 2004) also showed that inhaled carbon NPs could enter into the brain via the olfactory epithelium and its associated neurons that pass directly into the olfactory lobes of the brain. To demonstrate the transport pathway of nanoparticles within the olfactory region, rats were allowed to inhale poorly soluble salts of manganese (MnO, Mn_2O_3) applied as an aerosol. Manganese oxide NPs, with a diameter of 30 nm, were found to move to the brain by the olfactory route, proved by the existence of manganese in different parts of the brain (Elder et al., 2006). Moreover, the penetration of intranasally instilled fine Fe_2O_3 particles into the brain was demonstrated by Wang et al. (2007a). The micro-distribution map of iron in the olfactory bulb and brain stem showed an obvious increase of Fe contents in the olfactory nerve and the trigeminus of brain stem. The average content of Fe in the exposed mice was about 31% higher than in the control mice, suggesting that Fe_2O_3 particles (280 ± 80 nm) were possibly transported via uptake by sensory nerve endings of the olfactory nerve and trigeminus. In order to improve the availability of nanoparticles to the brain following nasal administration, conjugate biorecognitive ligands–lectins to the surface of poly(ethylene glycol)–poly(lactic acid) (PEG–PLA) nanoparticles was prepared. Wheat germ agglutinin (WGA)-conjugated nanoparticles, including the binding of WGA to N-acetyl-D-glucosamine and sialic acid, which were abundantly observed in the nasal cavity, were selected as a model lectin. There was a twofold increase in the brain uptake of WGA-conjugated nanoparticles (Gao et al., 2006). The distribution of NPs in the nasal passage increases not only their CNS targeting potential but also the potential for NPs toxicity to sensitive neurons within the CNS.

The second pathway is the uptake through the BBB via systemic distribution. It has been extensively studied in drug delivery, as an approach to deliver drugs to the brain (Kreuter, 2004). Especially, coating of the NPs with the polysorbate (Tween) surfactants improved the transportation of drugs across the blood–brain barrier (Kreuter, 2004). It was shown (Roney et al., 2005) that with appropriate surface modifications, NPs could deliver drugs of interest through the BBB for diagnostic and therapeutic applications in neurological disorders, such as Alzheimer's disease (AD). The intravenously injected doxorubicin-loaded polysorbate 80-coated nanoparticles were found to lead to a 40% cure in rats with intracranially transplanted glioblastomas (Steiniger et al., 2004), indicating these NPs could enter into the brain. Schroeder et al. (1998) also showed that polysorbate 85-coated poly(butyl cyanoacrylate) nanoparticles may even enable a brain targeting after oral administration. The physicochemical properties of the NPs at different surfactant concentrations, stabilizers, and amyloid-affinity agents could influence the transport mechanism.

3. Neurotoxicity studies of NP

The central nervous system is composed of two parts: the brain and the spinal cord. Both of them are delicate organs in human body which must be protected from the injury to xenobiotics. Several drugs could distribute into the CNS and thus cause unwanted neurotoxicity by themselves (Chow et al., 2003; Screnci and McKeage, 1999). Also, recent observations suggests that several NPs, such as polysorbate 80-coated PBCA NPs and pegylated PLA immunonanoparticles, are able to cross BBB (Olivier, 2005; Sharma and Sharma, 2007) through intravenous administration and followed by the accumulation in the brain. As these NPs have the capacity to penetrate the BBB, they may subsequently influence the BBB function and brain physiology and cause severe side effects. So far, there are already some reports, but not many, which observed the neurotoxicity of nanoparticles both *in vitro* and *in vivo* (Kim et al., 2008; Pisanic et al., 2007).

3.1. In vitro studies

Several laboratories have reported potential toxic effects of nanoparticles on different types of cells *in vitro* (Deng et al., 2009; Hussain et al., 2006; Long et al., 2007; Pisanic et al., 2007). The most widely used cell model for NPs neurotoxicity study is PC12 cells which are a cultured neuronal phenotype and was used as a paradigm for neurobiological and neurochemical studies (Greene and Tischler, 1976). Changes in cellular viability after exposure to nanoparticles are assessed using MTT method. For instance, Pisanic et al. (2007) showed that exposure to increasing concentrations of anionic magnetic nanoparticles (MNPs), from 0.15 to 15 nm of iron, resulted in a dose-dependent diminishing viability of PC12 cells using MTT method. Also, the capacity of PC12 cells to extend neurites was deceased in response to nerve growth factor (NGF). Hussain et al. (2006) reported that the exposure of PC12 cells to manganese oxide (Mn-40 nm) particles increased the production of reactive oxygen species (ROS) and could deplete dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in a dose-dependent manner, while the Ag-15 nm could produce cell shrinkage and irregular membrane borders. Wang et al. (2009) examined the expression changes of dopaminergic system-related genes in PC12 cells induced by manganese, silver, or copper nanoparticles, and found that Cu-90 nanoparticles induced dopamine depletion in PC12 cells, which was similar to the effect induced by Mn-40. The results suggested that Mn and Cu NPs could induce dopaminergic neurotoxicity and might share some common mechanisms associated with neurodegeneration.

Besides PC12 cell lines, primary culture cell lines were also used to assess the neurotoxicity of NPs. To examine the possible neurotoxicity of TiO_2, brain cultures of immortalized mouse microglia (BV2), rat dopaminergic neurons (N27), and primary cultures of embryonic rat striatum were exposed to Degussa P25, a kind of commercially available TiO(2) nanomaterial. BV2 microglia, which was exposed to P25 (2.5–120 ppm), responded with an immediate and prolonged release of ROS. Microarray analysis on P25-exposed BV2 microglia indicated up-regulation of inflammatory, apoptotic, and cell cycling pathways, and down-regulation of energy metabolism. These results indicate that P25 is nontoxic to isolated N27 neurons, but stimulates BV2 microglia to produce ROS and damages neurons at low concentrations in cultures of brain striatum, plausibly though microglial generated ROS (Long et al., 2007).

Nanosized zinc oxide particles were examined for their neurotoxicity in mouse neural stem cells (Deng et al., 2009). It was found that ZnO nanoparticles induced cell apoptosis and this toxicity comes from the dissolved Zn (2+) in the culture medium or inside cells.

Generally, the above mentioned NPs of different particle sizes, ranging from 20 to 300 nm, or produced by different materials could cause cell apoptosis *in vitro,* indicating there are potentially harmful effects of NPs to human health, and further studies on the acute and long-term effects of NPs *in vivo* is both warranted and necessary.

3.2. In vivo studies

In vivo studies are necessary to explore the bio-distribution pattern of NPs and provide vital information to assess the neurotoxic

Table 2
Neurotoxicity of nanoparticles.

Nanoparticle (particle size)	Animal model	Route of administration	Results	References
Cu, Ag or Al (about 50–60 nm)	Rats and mice	Intravenous, intraperitoneal or intracerebral	Nanoparticles induced brain dysfunction in normal animals and aggravating the brain pathology caused by whole-body hyperthermia.	Sharma (2007)
TiO_2 (5 nm)	Mice	Injected into abdominal cavity	The accumulation of TiO_2 nanoparticles in the mouse brain occurs and caused the oxidative stress and injury of the brain.	Ma et al. (2009)
Mn oxide (about 30 nm)	Rats	Inhalation	Mn oxide NPs results in the increase of macrophage inflammatory protein-2, glial fibrillary acidic protein, and neuronal cell adhesion molecule mRNA level in the brain region.	Elder et al. (2006)
Ag (25 nm)	Mice	Intraperitoneally	Mouse oxidative stress and antioxidant defense genes were significantly differentially expressed in the caudate, frontal cortex and hippocampus, suggesting Ag-25 NPs have the potential to cause neurotoxicity.	Rahman et al. (2009)
Fe_2O_3 particle (280 ± 80 nm)	Mice	Intranasal	The neuron fatty degeneration occurred in the hippocampus, implying an adverse impact of inhalation of fine Fe_2O_3 particles on CNS.	Kircher et al. (2003)
Ultrafine carbon black (ufCB) (14 nm or 95 nm)	Mice	Intranasal	Up-regulation of proinflammatory cytokines mRNA in brain olfactory bulb, not in the hippocampus of mice instilled with 14 nm ufCB intranasally.	Tin Tin Win et al. (2006)
nC60 (30–100 nm)	Fish	NA	Significant lipid peroxidation was found in brains. GSH was also marginally depleted in gills of fish.	Oberdorster (2004)
Titanium dioxide (21 nm)	Fish	NA	The increases of zinc level, decreases of Cu level, and inhibition of Na^+K^+-ATPase activity, in the brain.	Ramsden et al. (2009)
Neutral E. wax NPs (about 100 nm)	Rat	Brain perfusion	Neutral NPs and low concentrations of anionic NPs were found to have no effect on BBB integrity, whereas, high concentrations of anionic NPs and cationic NPs disrupted the BBB. The brain uptake rates of anionic NPs at lower concentrations were superior to neutral or cationic formulations at the same concentrations.	Lockman et al. (2004)

effects of NPs (Fischer and Chan, 2007; Maurer-Jones et al., 2009) (Table 2).

Studies by Sharma (2007) and Sharma and Sharma (2007) revealed that intravenous (30 mg/kg), intraperitoneal (50 mg/kg) or intracerebral (20 μg in 10 μl) administration of Ag, Cu or Al nanoparticles (~50–60 nm) disrupted the BBB to Evans blue albumin in rats and mice in a highly selective and specific manner. A recent study by Kim et al. (2008) also showed that silver nanoparticles produced a diverse set of toxicological changes and accumulated in various organs including kidney, live, brain, etc., when the nanoparticles were administered orally. Similarly, after intranasal instillation of fine Fe_2O_3 particle (280 ± 80 nm) suspension in the mice, it was found that the neuron fatty degeneration occurred in the hippocampus, implying an adverse impact of the inhalation of fine Fe_2O_3 particles on CNS (Kircher et al., 2003). A study by Ma et al. (2009) also showed that daily abdominal cavity injection for 14 days could result in an accumulation of TiO_2 nanoparticles in the mouse brain, which caused the oxidative stress and injury of the brain. It was suggested that 150 mg/kg body weight (BW) TiO_2 nanoparticulate appeared to trigger a cascade of reactions such as lipid peroxidation, the decrease of the total anti-oxidation capacity and activities of antioxidative enzymes, the excessive release of nitric oxide, the reduction of glutamic acid, and the down-regulated level of acetylcholinesterase activities. It was also found that the contents of titanium in the mouse brain from the 150 mg/kg body BW bulk TiO_2 group was significantly lower (349.0 ± 17.5 ng/g) than those of the 150 mg/kg BW nanoparticulate anatase TiO_2 group (500.2 ± 25.0 ng/g), suggesting that nanoparticulate anatase TiO_2 migrated into the brain more readily or was absorbed more from the circulation than the bulk TiO_2.

The neurotoxicity of NPs was also evaluated by the examination of the expression level of inflammation related cytokines in the brain. For instance, the exposure of rats to manganese (Mn) oxide NPs resulted in an increase of macrophage inflammatory protein-2, glial fibrillary acidic protein, and neuronal cell adhesion molecule mRNA level in the brain region, indicating that manganese oxide NPs may cause inflammation in the brain (Elder et al., 2006). Rahman et al. (2009) evaluated the effects of silver-25 nm (Ag-25) nanoparticles on gene expression in different regions of the mouse brain and revealed the expression of genes varied in the caudate nucleus, frontal cortex and hippocampus of mice when treated with Ag-25, suggesting that Ag-25 nanoparticles may produce neurotoxicity by generating free radical-induced oxidative stress and by altering gene expression. The distillation of ultrafine carbon black (ufCB) (14 nm or 95 nm) into the nostrils of mice was also found to induce the up-regulation of proinflammatory cytokines (interleukin-1 beta and tumor necrosis factor-alpha) and chemokines (monocyte chemoattractant protein-1/CCL2, macrophage inflammatory protein-1 alpha/CCL3), and monokine induced interferon-gamma/CXC chemokine ligand (CXCL9) mRNA in brain olfactory bulb, thus may influence the brain immune function (Tin Tin Win et al., 2006).

Using a fish model, Oberdorster (2004) investigated the toxicity of fullerenes NPs on the brain of bass through the evaluation of oxyradical-induced lipid and protein damage as well as total glutathione (GSH) levels. Significant lipid peroxidation was found in the brains of largemouth bass after 48 h of exposure to 0.5 ppm uncoated nC60 (30–100 nm). Therefore, the fullerenes NPs could cause cell damage in the brains of fish. Also, the dietary exposure to titanium dioxide nanoparticles in rainbow trout caused the subtle biochemical disturbances in the brain, such as the increase of zinc level, decrease of Cu level, and inhibition of Na^+K^+-ATPase activity (Ramsden et al., 2009).

The effect of neutral, anionic and cationic charged NPs on BBB integrity and NP brain permeability was also evaluated by *in situ* rat brain perfusion (Lockman et al., 2004). The results showed that neutral NPs and low concentrations of anionic NPs had no effect on BBB integrity, whereas high concentrations of anionic NPs and cationic NPs disrupted the BBB and may have an immediate toxic effect

on the BBB. Therefore, besides the materials that NPs are made up from, surface charges should also be considered as an important factor for nanoparticle neurotoxicity and their brain distribution profiling. In this study, it should be noted that concentration is also an important factor for NPs neurotoxicity. However, by i.v. administration, the concentration would be less and probably inferior to toxic concentration observed in this particular study.

4. The mechanisms of neuron injury by ambient and metal NPs

The mechanisms of neuron injury are diverse for such a wide variety of materials used (Donaldson, 2006). However, a common mechanism of oxidative stress (OS) caused by surfaces, organics and metals associated with the NPs has been identified (Nel et al., 2006; Oberdorster et al., 2004). This oxidative stress leads to inflammation (Donaldson et al., 2003) and then forms a link between the exposure to these particles and the types of adverse effects observed (Donaldson et al., 2005). As a number of manufactured NPs have already been demonstrated to cause oxidative stress, this may be a common mechanism of NP toxicity (Donaldson, 2006). For example, it was suggested (Rahman et al., 2009) that Ag-25 nanoparticles have the potential to generate reactive oxygen species (ROS) via a metabolic pathway and induce oxidative stress, including oxidative DNA damage. Also, the elevated levels of oxidative stress (OS) were found in the brains of Apo E-deficient mice exposed to concentrated particles, indicating nanoparticle could cause OS in the brain (Veronesi et al., 2005).

OS caused by free radicals generated by the interaction of NPs with cells may result in cell death. Evidence of mitochondrial distribution and oxidative stress response after endocytosis of nanoparticles was noted (Chan, 2006; Oberdorster, 2004). And it is thought that nanoparticles, such as 60 nm NH_2-labeled polystyrene (PS) nanospheres, may injure cells by gaining access to cell organelles (such as mitochondria) while larger particles (200 nm NH_2-labeled polystyrene (PS) nanospheres) may not (Xia et al., 2008). In the olfactory bulb, the particles were found to be located mostly within mitochondria. The mechanism for such an accumulation is unclear, but it is evident that the translocation of nanoparticles into the mitochondria can lead to cellular toxicity (Mistry et al., 2009; Xia et al., 2008).

ROS is reported to be associated with several neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and Huntington's disease (Mates et al., 1999). Evidence for the involvement of ambient air NPs in these disorders was presented by studies on biopsies from city dwellers. Alzheimer's-like pathology was demonstrated in brain sections by increased markers of inflammation and AB42-accumulation in frontal cortex and hippocampus in the presence of nanoparticles (Calderon-Garciduenas et al., 2004). Also inhalation exposure of BALB/c mice to particulate matter showed activation of proinflammatory cytokines in the brain (Campbell et al., 2005). The brain is especially vulnerable to OS damage because of its high content of easily peroxidizable unsaturated fatty acids, high oxygen consumption rate, and relative paucity of antioxidant enzymes compared with other organs (Skaper et al., 1999). NPs possess unique physical and surface properties and may cause oxidative stress. Therefore, brain could be continuously exposed to ROS generated by the NPs. In the CNS, OS is largely mediated by the microglia, a macrophage-like, phagocytic cell that is normally inactive unless confronted by potential damage and exogenous stimuli (e.g., xenobiotics, chemicals, particles) (Fig. 1). Their immediate response to such stimuli is known as the "oxidative burst" (Colton and Gilbert, 1987; Segal and Abo, 1993) and involves a rapid sequence of events that includes an increase in metabolic activity, a change in cell shape and size, and cytoplasmic

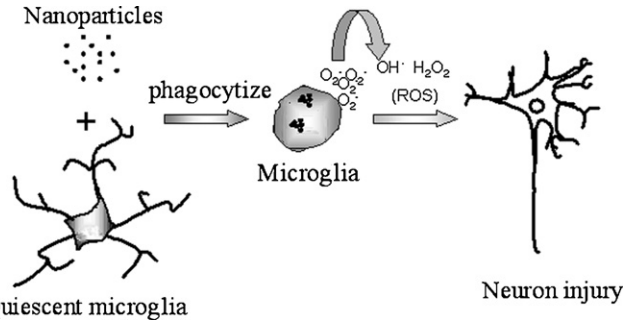

Fig. 1. The representative schematic of neuron injury caused by nanoparticles (NPs). The exposure of quiescent microglia to NPs results in the elaboration of numerous pseudopodia which engulfed NPs, and followed by the generation of ROS, which caused oxidative stress and induced neuron injury.

engulfment (i.e., phagocytosis) of the offending stimuli (Long et al., 2006, 2007).

Kleinman et al. (2008) found that inhaled ultrafine particulate matter could affect CNS inflammatory processes and they also demonstrated that such effect may be exerted via MAP kinase signaling pathways using apolipoprotein E knockout mice as a model. Increased nuclear translocation of two key transcription factors, NF-kappaB and AP-1, being involved in the promotion of inflammation, was found in the brains of mice exposed to the nanoparticles. Furthermore, several MAP kinases involved in the activation of these transcription factors were analyzed and it was found that JNK in the active form was significantly increased in the animals receiving a lower concentration of concentrated ambient particles (CAPs).

Therefore, the neurotoxicity of ambient and mental nanoparticles may be induced initially via the generation of ROS, which could cause oxidative stress, and followed by the up-regulation of MAP kinases to activate the MAP signaling pathway. Then, the inflammation related cytokines are highly expressed in the brain to cause brain injury.

5. Future perspectives

Although various kinds of inorganic nanomaterials, such as quantum dots (QDs), carbon nanotubes, and fullerenes, were under investigation for their toxicity (Borm and Muller-Schulte, 2006; Chan, 2006), fewer studies were concerned with the NPs produced by degradable polymer materials. As the polymer NPs and pollutant NPs share some common features, the study of pollutant NPs may provide us with useful information concerning the safety issue of polymer NPs. Several polymer materials were used for the preparation of nanocarriers for gene/drug delivery in our previous studies, and the results demonstrated that the degradable polymer/lipids could enhance the gene expression, cellular uptake, targeting or improve the solubility and stability of drugs (Chen et al., 2009; Han et al., 2008, 2009; Zhao et al., 2009).

As these nanomaterials possess unique physical and surface properties, they have inspired plans for a wide spectrum of applications, such as target-specific vehicles for *in vivo* sensing, diagnosis, and therapy (e.g., nanomedicine, drug delivery). However, after entering into the body, they may cause toxicity such as cytotoxicity and immune-toxicity because of the body distribution, change of cellular affinity, as well as the increase of cellular uptake (Maurer-Jones et al., 2009; Xia et al., 2008). For example, it was reported that the ability of treated mice to establish a specific immune response was markedly impaired when polybutylcyanoacrylate nanoparticles (PBCN) were administered at high doses. The degree of depression was in a dose-dependent manner (Simeonova et al., 1998). Besides, the cytotoxicity of chitosan NPs was evaluated

using tumor cell line and it was found that these NPs elicited dose-dependent inhibitory effects on the proliferation of tumor cell lines (Qi et al., 2005). Specifically, these unique properties may also induce neurotoxicity, damaging CNS *in vivo* and causing brain diseases. Thus, there is an immediate need for researchers to address the questions about the neurotoxicities of these nanoparticles, and the neurotoxicity studies could provide a foundation for the further design and development of the drug delivery system (DDS) using NPs. Unfortunately, till now, the evaluation of nanoparticle neurotoxicity is still limited. Thus, our understanding of the role of nanoparticles on the CNS function is poor, and requires further investigations.

In the evaluation of nanoparticle neurotoxicity, the morphology, surface area, surface charge, coating, purity, material solubility, and the materials which NPs are made up from are expected to play very important roles, and thus should be carefully considered. Also, the dosage, administration route, concentration in the target organ, duration of action, and the degradation time of the biodegradable materials are expected to be the most important and fundamental problems in the evaluation of nanoparticle neurotoxicology.

The intranasal application of nanoparticles has demonstrated the potential for direct nose-to-brain transport of NPs. These neurotoxicity studies were mostly carried out in relation to an evaluation of the toxicity of environmental nanoparticles/pollutants to the CNS (Mistry et al., 2009). From a drug delivery perspective, application of nanoparticles using polymer system has shown greater ability in delivering model drugs to the brain than a conventional formulation of the drug. However, several reports showed that the nanoparticles or nanospheres produced by nontoxic degradable polymers still could induce the cytotoxicity or immue-toxicity (Maurer-Jones et al., 2009; Xia et al., 2008).

The toxic effects of these nanoparticles after they cross the BBB have not been examined in detail. Therefore, new investigations dealing with the effects of several kinds of nanocarriers on the CNS with special regard to neurotoxicity are urgently needed.

The extent to which the effect of nanoparticles on CNS is mostly observed in mice, the extrapolation of these data to humans remains a big challenge, because the exposed dose, the genetic factors, and the underlined mechanism for the transportation of nanoparticles may differ in human and mice.

Further studies in the nanoneurotoxicity studies should be directed to the above mentioned questions and aimed at the establishment of a guideline for the evaluation of the nanoneurotoxicity.

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