

Toxic effects of silver nanoparticles in mammals – does a risk of neurotoxicity exist?

Joanna Skalska, Lidia Strużyńska

Laboratory of Pathoneurochemistry, Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

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Abstract

Over the last decade, silver nanoparticles have become an important class of nanomaterials utilized in the development of new nanotechnologies. Despite the fact that nanosilver is used in many commercial applications, our knowledge about its associated risks is incomplete. Although a number of studies have been undertaken to better understand the impact of silver nanoparticles on the environment, aquatic organisms and cell lines, little is known about their side effects in mammalian organisms. This review summarizes relevant data and the current state of knowledge regarding toxicity of silver nanoparticles in mammals, as well as the accumulated evidence for potent neurotoxic effects. The influence of nanosilver on the central nervous system is significant because of evidence indicating that it accumulates in mammalian brain tissue.

Key words: silver nanoparticles, neurotoxicity, mammals, nanotoxicology.

Introduction

Silver is well known for its many industrial applications such as soldering, electrical conduction and plating applications. Additionally, this metal is used in the production of jewellery, cutlery, coins, medical instruments and photographic materials. In medical applications silver is included in wound dressings, urinary catheters and other medical devices, because of the ability of silver ions to inhibit growth of bacteria and fungi. Known for millennia, the antimicrobial effect of ionic silver arises from its ability to generate reactive oxygen species (ROS) and to inactivate microbial enzymes [19,75,88].

In addition to antibacterial activity, silver (particularly in the form of soluble silver compounds) exerts toxic effects in animals and humans. Acute symptoms of over-exposure to silver ions in humans include damage to the gastrointestinal tract, abdominal pain, diarrhea and convulsions [113]. The most common adverse effects associated with chronic exposure to silver in humans are discoloration of eyes (argyrosis) and pigmentation of the skin and mucous membranes, which turn irreversibly gray or bluish-gray (argyria). Argyria has been reported mainly in workers associated with mining, manufacturing or packing of silver [8,28,96]. Moreover, animal studies have revealed that prolonged administration

Communicating author:

Lidia Strużyńska, PhD, Laboratory of Pathoneurochemistry, Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego St., 02-106 Warsaw, Poland, e-mail: lidkas@imdik.pan.pl

of silver ions in low doses leads to accumulation of silver granules in eyes, heart enlargement, anemia and pathological changes to the liver and kidneys [28,113].

Recently, a resurgence in commercial applications of silver has occurred due to the development of nanotechnologies which make extensive use of silver in the form of nanoparticles (NPs). NPs are defined as materials having at least one dimension below 100 nm. NPs have unique properties useful in many applications. The most important features include a large surface area per unit mass and the potential to generate surface modifications which alter their properties. As a result, NPs have enhanced chemical reactivity, improved cell penetration and specific influences on biological systems. Moreover, the surface of NPs can be modified with various chemical groups which allow them to be conjugated to ligands or drugs [23,90,120]. As a result of their enhanced reactivity, NPs may generate toxic effects which differ from the bulk materials from which they are produced.

The number of NP-based applications is significantly increasing, and many products containing NPs are commercially available. There is great excitement about the potential benefits of NPs in medical applications. NPs have been tested as vehicles for gene therapy and drug delivery and as tools in diagnostic imaging and targeting systems for recognition of cancer cells [90]. Metal NPs in general and silver nanoparticles (AgNPs) in particular are among the

most important nanomaterials used in a wide range of industrial applications. According to the Woodrow Wilson inventory, about 30% of all NP-based products in the marketplace contain AgNPs [114]. The list of AgNP-based consumer products includes cleansers for disinfecting hard surfaces, laundry and dish-washing detergents, bath and sports towels, clothing, socks, underwear, water and air filters, personal cleansers, deodorants, cosmetics, cleansing soaps, toothbrushes, toothpastes, health supplements, nursing bottles and associated nipples, children's toys, and nanosilver-coated devices such as mobile phones and laptops. Appliances such as refrigerators and washing machines include interior coatings with AgNPs. Additionally, silver nanoparticles are included in paints used to cover walls in hospital rooms and in food storage containers (a complete listing of AgNP-based products is available at: http://www.nanotechproject.org/process/assets/files/7039/silver_database_fauss_sept2_final.pdf). The medical applications of AgNPs are summarized in Table I.

This particular interest in AgNPs relates to their antimicrobial activity. The rapid development of bacterial resistance against conventional antimicrobials and the challenges involved in development of new drugs have led to searches for promising alternatives. The smallest AgNPs, which have sizes within a range of a few nanometers, exhibit particularly strong antibacterial effects [61,82,94]. It has been found that in addition to size, the specific types of surface-coating agents have a significant effect on the biocidal potency of AgNPs [27]. AgNPs are also effective against fungi and viruses [40,42].

Although the commercialization of AgNPs has led to great excitement about potential benefits of their strong antimicrobial activity, it has simultaneously created a risk of hazardous interactions with biological systems [69,76].

There is a potential hazard to the environment and human health when AgNPs present in commercially available products, such as clothing, towels, socks, underwear, and toys, are released to the environment when these items are washed. AgNPs present in personal care products, cleaning supplies, detergents or cosmetics can be directly introduced into the environment during use and/or disposal [10,11]. Applications such as health supplements containing AgNPs, as well as food and drink storage containers, may also be a source of AgNPs [24]. Moreover, increasing

Table I. Selected medical applications of silver nanoparticles

Medical applications	References
Bone prostheses	[17]
Contraceptive devices	[91]
Gloves	[58]
Medical catheters	[117]
Orthopedic implants	[110]
Prosthetic devices	[21]
Endotracheal tubes	[83]
Surgical instruments	[31]
Wound dressings	[142]
Dental restorative materials, endodontic cements, dental implants	[43]

use of silver nanoparticles is expected to raise occupational exposure mainly through inhalation [18].

AgNPs released from consumer products are expected to enter aquatic and terrestrial ecosystems, but their fate after long-term accumulation and their impact on the environment are not fully known. There is significant concern regarding aquatic organisms in locations where AgNPs accumulate [87,115,140]. Unfortunately, our knowledge about the environmental and human risks remains at a very low level. It is therefore challenging to assess the long-term health consequences of environmental contamination. This problem is currently in the center of interest for scientists and various national agencies as well as public and private organizations.

Routes of exposure and biodistribution of AgNPs in mammalian organisms

Since AgNPs are found in a wide variety of products, exposure to them may occur via different routes of entry into the body. AgNPs from consumer products or medical applications may gain access to systemic circulation via oral or intravenous exposure as well as via inhalation or through the skin.

The gastrointestinal tract is the most likely route of entry for silver nanoparticles, directly through intentional ingestion (medical or dietary supplements, toothpastes) or indirectly via dissolution of AgNPs from products (food and drink containers, toothbrushes) [24]. Moreover, increasing environmental contamination may further lead to indirect and unintentional ingestion via consumption of water or fish. Inhalation of dust and fumes containing AgNPs or skin contact occurs mainly in occupational settings. Furthermore, certain products such as cosmetics, clothing, underwear, socks or wound dressings may allow AgNPs to penetrate the skin, primarily under conditions of concomitant presence of skin diseases such as allergic dermatitis, atopic eczema, psoriasis or simply during skin damage [92]. Medical or diagnostic compounds can also cause entry of AgNPs into the circulatory system by intravenous administration.

Information about absorption of AgNPs is incomplete. Park and co-workers observed that the bioavailability of AgNPs (7.9 nm) after oral administration to rats was very low, in the range of 1.2% to 4.2% based on a single dose [101]. Following entry into the systemic circulation, AgNPs can become distributed

among a number of mammalian organs, most notably liver and spleen [73,145]. Furthermore, silver nanoparticles have been found in blood, lungs, kidney, brain, heart and genital organs [67,70,73,81,101,131,145]. The results of research on the biodistribution of AgNPs indicate that most organs are able to remove AgNPs over time, with the exception of brain and testes [70,138]. Numerous studies have shown that AgNPs can be distributed within the brain of mammals, regardless of the route of exposure. Selected studies on biodistribution of AgNPs in mammalian brain are listed in Table II.

Lee and co-workers observed that after a single intravenous injection of citrate-coated AgNPs (7.9 nm), they become distributed in serum, liver, kidney, spleen, lungs, brain, testes and thymus of rabbits. Significantly, the presence of silver nanoparticles was observed at time points 1, 7 and 28 days after the injection [73]. Silver was also detected in brain of rats at 24, 96 and 168 h after an intraperitoneal injection of bovine serum albumin-coated 2 nm AgNPs [45].

Studies using the model of oral exposure to nanosilver indicate distribution among many organs of animals, including brain, after 90 days of repeated administration [67]. In organs of rats chronically exposed to AgNPs (10, 25 nm) by the oral route, nano-sized granules were observed in liver, kidney, spleen, brain, testes and ovaries [70]. Moreover, the oral exposure of rats to uncoated AgNPs (< 20 nm) or PVP-coated AgNPs (< 15 nm) showed a very similar pattern of biodistribution. The nano-sized granules were detected in liver, kidney, lungs, heart, spleen, brain, bladder, testes, blood, intestine and stomach [138]. Subchronic inhalation of AgNPs may also cause them to enter systemic circulation. Studies in which rats were exposed to AgNPs (18-19 nm) via inhalation for 13 weeks revealed that the lungs and liver are targeted organs. Additionally, nano-sized granules were identified in kidney, the olfactory bulb, blood and brain tissue [131]. The results of a study on the biodistribution of AgNPs (25 nm) after a single dose via intranasal administration demonstrated that silver accumulates in spleen, lungs, kidney, the nasal cavity and brain tissue [46].

Additionally, the assessment of the level of silver in urine and feces of treated animals suggests that excretion of AgNPs occurs mainly via feces, indicating that AgNPs are secreted in bile [63,73,101].

Table II. Studies on the biodistribution of AgNPs in mammalian organisms

Surface coatings and/or sizes of AgNPs	Animal model	Route of administration and dosage	Time of AgNP level measurement after last administration and time of administration	Organs/tissues examined	References
AgNPs: 20 and 200 nm	Rats	<i>i.v.</i> : 5 mg/kg b.w.	1, 7 and 28 days after single injection	Liver, spleen, kidney, lungs and brain	[29]
Citrate-coated AgNPs: 7.9 nm	Rabbits	<i>i.v.</i> : 0.5 or 5 mg/kg b.w.	1, 7 and 28 days after single injection	Serum, liver, kidney, spleen, lungs, brain, testes and thymus	[73]
BSA-coated AgNPs: 2 nm	Rats	<i>i.p.</i> : 50 mg/kg b.w.	24, 96 and 168 h after single injection	Liver, spleen, kidney, heart, lungs and brain	[45]
AgNPs: 50-100 nm	Rats	<i>s.c.</i> : 62.8 mg/kg b.w.	2, 4, 8, 12, 18 and 24 weeks after single injection	Brain	[135]
AgNPs: 25 nm	Mice	Intranasal: 100 or 500 mg/kg b.w.	1 and 7 days after single treatment	Spleen, lungs, kidney, nasal cavity and brain	[46]
AgNPs: 56 nm	Rats	Oral: 30, 125 or 500 mg/kg b.w./day	90 days of repeated exposure	Blood, liver, kidney, lungs, testes and brain	[67]
AgNPs: 10 and 25 nm	Rats	Oral: 100 or 500 mg/kg b.w./day	28 days of repeated exposure with measurement of silver level after a wash-out period of 1, 2 and 4 months	Blood, brain, kidney, spleen, liver, testes and ovaries	[70]
PVP-coated AgNPs: 14 ± 4 nm	Rats	Oral: 9 mg/kg b.w./day	28 days of repeated exposure	Plasma, liver, kidney, stomach, lungs, muscle, brain and small intestine	[81]
AgNPs: 22, 42 and 71 nm	Mice	Oral: 1 mg/kg b.w./day	14 days of repeated exposure	Liver, kidney, brain, lungs and testes	[99]
Uncoated AgNPs: < 20 nm, PVP-coated AgNPs: < 15 nm	Rats	Oral: 90 mg/kg b.w./day	28 days of repeated exposure; the measurement of silver level 1 day, 1 week and 8 weeks after previous administration	Liver, kidney, lungs, heart, spleen, brain, bladder, testes, blood, intestine and stomach	[138]
AgNPs: 18-19 nm	Rats	Inhalation: 49, 133 or 515 µg AgNPs/m(3)	Exposure for 6 h/day, 5 days/week, for 13 weeks in a whole-body inhalation chamber	Liver, kidney, olfactory bulb, brain, lungs, and blood	[131]

AgNPs – silver nanoparticles, BSA – bovine serum albumin, PVP – polyvinylpyrrolidone, *i.v.* – intravenous, *i.p.* – intraperitoneal, *s.c.* – subcutaneous, b.w. – body weight

Toxicity of AgNPs

There has been significant progress in silver-based nanotechnology in recent years. The commercialization of nanoproducts is increasing each year, and studies on the toxicological potential of such products are needed. Currently available information about hazards associated with AgNPs requires further verification.

The existing data suggest that the size of AgNPs is highly correlated with their toxicity. In many studies with mammalian cells, it was found that smaller AgNPs are more toxic than larger ones in equivalent dosages. The role of the size of AgNPs in toxicity was confirmed in *in vitro* experiments comparing

cytotoxicity and genotoxicity of 20, 80 and 113 nm AgNPs [102]. Furthermore, cytotoxic effects were exhibited by 10 nm AgNPs, but not 40 and 75 nm citrate-coated silver nanoparticles [48]. Size-dependent toxicity of AgNPs was also confirmed in studies on the influence of 15, 30 and 55 nm AgNPs on viability and oxidative stress induction in alveolar macrophages [15]. Research was also designed to evaluate size-dependent cytotoxic effects of AgNPs of different sizes (5, 20 and 50 nm) on various types of human cells. In all toxicity endpoint studies (cell morphology, viability, cellular membrane integrity and oxidative stress) it was observed that 5 nm AgNPs induce the most severe damage, with larger particles inducing less damage [78].

The surfaces of silver nanoparticles are often coated with various types of compounds to provide stability and prevent agglomeration. For example, polysaccharide-coated AgNPs do not agglomerate, in contrast to uncoated AgNPs [1]. The type of capping agent may also play a crucial role in stabilization of AgNPs. Polyvinylpyrrolidone (PVP)-coated AgNPs were found to be stable over a 1-week period in water, whereas citrate-coated AgNPs are unstable [136].

The most popular types of nanoparticle coatings are citrate, chitosan, PVP, polysaccharides, peptides and carbon. Different types of superficial agents may generate coating-specific behavior of AgNPs in solution [66] or in physiological fluids [13], and consequently may cause different antimicrobial or toxic effects.

Only a few studies have compared the influence of various coating agents on AgNP-induced toxicity; therefore this issue is still not fully understood. Among other effects, it was demonstrated that carbon-coated silver nanoparticles influence cell viability to a lesser extent than uncoated AgNPs of similar size [95], whereas polysaccharide-coated AgNPs cause more severe effects than uncoated AgNPs [1]. Moreover, it was shown that PVP-coated [66] or peptide-coated [51] AgNPs are more toxic than citrate-coated AgNPs with similar particle core sizes.

It is also claimed that all observed AgNP-mediated toxic effects are a consequence of silver ions released from the surface of nanoparticles inside cells through the “Trojan Horse effect” [48,100,126]. Moreover, it is considered that smaller AgNPs are able to release silver ions from their surfaces more efficiently than larger ones, because of the larger surface area per unit mass [48].

Ag⁺ ions are released after surface oxidation of AgNPs. Notably, it was shown that the intracellular solubility of AgNPs is 50 times greater than their solubility in pure water [126].

The most effective cellular conditions for dissolving endocytosed AgNPs are found in the acidic environment of lysosomes, which has a pH of about 4.8 [26,122]. However, it has been suggested that the toxic effects are a combined result of both AgNPs and released silver ions [16,44,108,127].

It was observed that PVP-coated AgNPs and Ag⁺ ions both affect cellular pathways involved in oxidative stress and homeostasis of Na⁺, K⁺ and H⁺ ions. Toxic effects of AgNPs on fish were found to be mediated by activation of a few nuclear recep-

tors and inhibition of ligand binding to the dopamine receptor. In contrast, in tissues of Ag⁺-exposed fish, ligand binding to adrenergic receptors α 1 and α 2 and cannabinoid receptor CB1 were found to be inhibited [44]. Powers and co-workers showed that ascorbate protects cells against Ag⁺-induced oxidative stress, but does not act as an effective antioxidant with respect to stress induced by AgNPs [108].

Other studies indicate that the pattern of expression of stress-related genes in liver of AgNP- or Ag⁺-treated fish (Japanese Medaka) is different [16]. Silver ions induce an inflammatory response in the liver of exposed fish, whereas AgNPs increase expression of genes implicated in DNA damage, carcinogenesis and oxidative stress.

Toxic effects of AgNPs in microorganisms

It was observed in a variety of studies that AgNPs exhibit antimicrobial activity against gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) [94,129,133]. Thus it was proposed that AgNPs may constitute an attractive alternative to antibiotics. It has been proposed that silver nanoparticles could play a major role in solving the serious public health problem caused by the presence of multidrug-resistant bacteria, which are resistant to most antibiotics [4]. In addition, AgNPs are also useful in disrupting the formation of bacterial biofilms, wherein bacteria aggregate into complex invasive structures. These structures provide the basis for a natural survival strategy used by microorganisms after invasion of a host and provides resistance to a lot of commonly used anti-microbial agents. Strains of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* have been found to be susceptible to the anti-biofilm properties of AgNPs [65,98].

Even though the antibacterial effects of AgNPs have been extensively examined, their mechanisms of action have been only partially elucidated. Although one hypothesis emphasizes the role of silver ions released from the surfaces of AgNPs inside bacterial cells [53,82], direct action of AgNPs on microorganisms has also been proposed [64].

Many studies indicate that AgNPs or Ag⁺ released from their surfaces may directly damage the cell membranes. Silver structures are known to adhere to the microbial cell wall and cause structural changes in the cell membrane proteins, such as cis-trans

isomerization of unsaturated fatty acids. The changes in the membrane components lead to increased membrane fluidity and decreased resistance to environmental factors [53]. Structural changes in membrane proteins cause them to become inactivated and released, causing degradation of membrane structure [129]. These abnormalities lead to a significant increase in permeability, resulting in cell death.

Structural proteins and enzymes with thiol groups are highly sensitive to inactivation by AgNPs or released Ag⁺ ions [125]. Interactions of AgNPs with the thiol groups of the L-cysteine residue have been found to disturb the function of several enzymes of *Staphylococcus epidermidis* [50]. Moreover, both forms of silver may interact with bacterial DNA and prevent DNA replication and cell division, leading to cell death [34,125]. Another mechanism of antibacterial action induced by AgNPs is generation of reactive oxygen species, which damage all components of the cell, including cell membranes and DNA [64,72].

The biocidal potency of AgNPs is also effective with respect to fungi and viruses. Antifungal activity against *Cladosporium cladosporioides*, *Aspergillus niger* [109], *Trichophyton rubrum* [104] and *Candida* sp. [93] has been demonstrated. AgNPs have been found to be a potential weapon against a wide range of viruses. Due to the low likelihood of resistance, AgNPs may provide effective antiviral therapies against HIV-1 [68], hepatitis B virus [84], herpes simplex virus types 1 [9] and 2 [97] and influenza virus [143]. The mechanism of antiviral potential of AgNPs is still being investigated. It is thought that AgNPs interact with glycoprotein receptors [68], the viral envelope [143] and double-stranded DNA/RNA [84], thereby preventing the replication of viruses, or block the binding of viruses to the host cell.

Toxic effects of silver nanoparticles in mammals

Following entry of AgNPs into the systemic circulation, they may migrate into many organs and induce toxicity. Table III lists studies demonstrating the negative influence of AgNPs in mammalian organisms. A series of studies has shown that systematically administered AgNPs cause inflammatory and cytotoxic effects including pulmonary toxicity after prolonged inhalation [132] and hepatotoxicity after prolonged oral [30,67,103], intravenous [25] or intraperitoneal [32] administration. Moreover, histo-

pathological changes in kidneys and increasing levels of creatinine have been reported [33,119], indicating that AgNPs may cause nephrotoxic effects. Impairment of spermatogenesis in rats exposed to AgNPs was also observed [128].

Toxic effects of AgNPs in liver of rodents have been intensively investigated. Adverse effects were observed including marked pathological changes in liver morphology [32,67,71], changes in liver enzyme activities [2,56,99], changes in the level of plasma lipids [30,67], generation of ROS [30,103] and inflammatory response [25,99]. Autophagy and apoptosis have been confirmed to play roles in mediating hepatotoxicity [71].

Mechanisms of AgNP-induced toxicity

In recent years, the mechanisms of AgNP-induced toxicity have been intensively investigated. It remains unknown if observed toxic effects are caused only by direct interaction of AgNPs with biological systems, or if silver ions released from the surfaces of the AgNPs inside the cell are also involved. In any case, the contribution of individual forms of silver has been found to induce toxicity, and a number of *in vitro* and *in vivo* studies have provided strong evidence for a connection between AgNP-mediated production of reactive oxygen species, oxidative stress, DNA damage, inflammation and cell death. A schematic representation of mechanisms of toxicity of AgNPs is shown in Figure 1.

It is likely that AgNP-mediated ROS production is related to physical (size, shape, surface charge) and chemical (surface coating, solubility, elemental composition) properties which create chemical conditions to induce an oxidative environment inside the cells. These conditions cause an imbalance in the cellular energy system, which depends on redox potential, leading to initiation of the inflammatory response or cell death. However, there is evidence that the mechanisms of toxicity of AgNPs towards neurons are much more complex [148].

AgNP-induced oxidative stress and disruption of mitochondria

Reactive oxygen species are chemically reactive molecules produced as natural byproducts during the mitochondrial electron transport process in aerobic respiration or by oxidoreductase enzymes and have an important role in cellular processes, such as

Table III. Selected studies demonstrating toxic effects of silver nanoparticles in mammals

Type of AgNPs	Mammalian model	Administration method, dosage, time of exposure	Observed toxic effects	References
AgNPs: 20, 100 nm	Rats	<i>i.v.</i> : 28 days of repeated administration, dose 6 mg/kg b.w./day	(Only 20 nm toxic) – Decreased body weight – Enlargement of spleen and liver – Histopathological changes in liver, spleen and lymph nodes – Increased activity of liver enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase) – Changes in red blood cell parameters – Changes in immune parameters (suppression of natural killer cells; changes in production of cytokines: IL-10, IL-1 β , IL-6, TNF- α ; decrease in interferon- γ production; increase in IgM and IgE immunoglobulin levels in serum)	[25]
AgNPs: 20 nm	Rats	Oral: 81 days of repeated administration, dose 500 mg/kg b.w./day	– Decreased body weight – Increased level of total cholesterol and LDL-cholesterol and decreased level of triglycerides – Increased plasmatic alanine transaminase activity – Increased liver and cardiac superoxide anion (O ₂ ⁻) production – Increased level of IL-6 and TNF- α in liver – No changes in liver SOD activity, liver lipid peroxidation and plasma antioxidant capacity	[30]
AgNPs: 56 nm	Rats	Oral: 90 days of repeated administration, dose 30, 125 or 500 mg/kg b.w./day	– Decreased body weight of male rodents – Increased alkaline phosphatase activity (dose 500 mg/kg b.w.) – Increased level of cholesterol (doses: 125 and 500 mg/kg b.w.) – Histopathological changes in liver tissues (bile-duct hyperplasia, fibrosis, pigmentation inflammatory cell infiltration) and intestines (pigmentation) – No changes in hematological parameters, except for a significant increase in the number of monocytes (dose 500 mg/kg b.w.)	[67]
AgNPs: 10-30 nm	Rats	<i>i.p.</i> : single injection, dose 500 mg/kg b.w./day	– Low level of ATP content in liver tissue – Induction of autophagy and apoptosis in liver – Histopathological changes in liver tissues (piecemeal necrosis and chronic inflammatory cell infiltration)	[71]
AgNPs: 22, 42 and 71 nm	Mice	Oral: 14 days of repeated administration, dose 1 mg/kg b.w./day or 28 days of repeated administration, dose 1 mg/kg b.w./day (42 nm)	– No changes in body weight of rodents – Increased level of TGF- β in serum after 14 days of administration (other cytokines not investigated) – Increased level of cytokines: IL-1, IL-6, IL-4, IL-10, IL-12 and TGF- β in serum after 28 days of exposure – Increased distribution of NK cells and B cells after 14 and 28 days – Increased activity of liver enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase) – Increased IgE production after 28 days of administration – No histopathological changes in organs (liver, kidney and small intestine)	[99]

Table III. Cont.

Type of AgNPs	Mammalian model	Administration method, dosage, time of exposure	Observed toxic effects	References
AgNPs: 10 nm	Rats	Oral: 5 days of repeated administration at doses of: 5, 25, 50 and 100 mg/kg b.w./day	(Effects observed for doses of 50 and 100 mg/kg b.w.) – Induction of ROS production in liver – Increased activity of liver enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase) – Increased lipid peroxidation in liver tissue – Morphological alterations in liver tissue (hepatocyte disruption, hepatocellular vacuolization, degeneration of liver, central vein injury and areas of necrosis) – DNA damage in liver	[103]
AgNPs: size < 100 nm	Rats	<i>i.p.</i> : two injections of AgNPs in a dose of 2 mg/kg b.w./day	– Histopathological alterations in liver and renal tissues – Increased number of white blood cells and increased hemoglobin level – Increased serum creatinine, urea, and aspartate and alanine aminotransferases	[119]
AgNPs: 18 nm	Rats	90 days of 6 h/day exposure via inhalation at concentrations of 0.7×10^6 , 1.4×10^6 and 2.9×10^6 particles/cm ³	– Significant decrease of tidal volume and minute volume – Histopathological changes in lungs (mixed inflammatory cell infiltration, chronic alveolar inflammation and small granulomatous lesions)	[132]
AgNPs: 8.7 nm	Rats	<i>i.p.</i> : 28 days of repeated administration at doses of 1, 2, and 4 mg/kg b.w./day	– No significant changes in the body weight of rodents – Histopathological changes in liver tissue (bile-duct hyperplasia, cholangiofibrosis, hepatocellular necrosis and leukocytosis) – Increased lipid peroxidation in liver tissue after dose of 2 and 4 mg/kg b.w. – No change in GSH level in liver tissue – Chromosomal aberrations after 4 mg/kg b.w.	[32]
AgNPs: 35-45 nm	Mice	Oral: 14 days of repeated administration at a dose of 50 µl of AgNP solution at concentration of 20 or 50 ppm	– Increased activity of liver enzymes (alanine transaminase and aspartate transaminase) – No change in blood parameters (values of red blood cells, hemoglobin and hematocrit) – Histopathological changes in liver (cytoplasmic vacuolization of hepatocytes with necrosis, inflammation and degeneration of hepatic cells)	[56]
AgNPs: 21 ± 8 nm	Rats	<i>i.v.</i> : single injection, dose 10 mg/kg b.w.; intratympanic injection: 40 µl of 0.4 % of AgNP was injected into the middle ear cavity	– Glycosaminoglycan accumulation in the basement membrane associated with up-regulation of production of hyaluronic acid in kidney and cochlea (after <i>i.v.</i> injection) leading to renal failure and hearing loss (a significant hearing loss over a broad range of frequencies after intratympanic injection) – Increased concentration of urea and creatinine in the serum (after <i>i.v.</i> injection) – Presence of proteins in the urine (after <i>i.v.</i> injection)	[33]

Table III. Cont.

Type of AgNPs	Mammalian model	Administration method, dosage, time of exposure	Observed toxic effects	References
AgNPs: 43.6 ± 6.4 nm	Mice	<i>i.p.</i> : single injection in doses of 26, 52 and 78 mg/kg b.w.; animals were sacrificed 24 and 72 h after injection	<ul style="list-style-type: none"> – Increased activity of liver enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase) – 24 and 72 h after injection in all doses – Oxidative DNA damage in lymphocytes – 24 and 72 h after injection in all doses – Induction of apoptosis in liver tissue – mainly after 78 mg AgNPs/kg b.w.) – Histopathological changes in liver (lymphocyte infiltration in the hepatic portal space, necrosis, vacuolization of hepatocytes and edema around the blood vessels) 	[2]
AgNPs: 20 nm	Rats	<i>i.v.</i> : 28 days of repeated administration at doses of 0.0082, 0.025, 0.074, 0.22, 0.67, 2, and 6 mg/kg b.w.	<ul style="list-style-type: none"> – Decreased body weight – Reduced thymus weight and increased spleen weight, no effect on liver and kidney weights – Decrease of NK cell activity – Changes in red blood cell, hemoglobin and white blood cell parameters – Decreased IgG and increased IgM levels – Changes in the level of cytokines 	[139]
AgNPs: 20 nm	Rats	<i>i.v.</i> : single injection, dose: 238-263 µg/kg b.w.	<ul style="list-style-type: none"> – No changes in GSH level in liver – Increased mRNA expression of IL-8, macrophage inflammatory protein 2, IL-1 receptor and TNF-α; and no changes in mRNA level of IL-1β, IL-10 	[39]

b.w. – body weight, *GSH* – reduced glutathione, *IgE* – immunoglobulin E, *IgG* – immunoglobulin G, *IgM* – immunoglobulin M, *IL* – interleukin, *i.p.* – intraperitoneal, *i.v.* – intravenous, *SOD* – superoxide dismutase, *TGF- β* – transforming growth factor β , *TNF- α* – tumor necrosis factor α

growth, proliferation, apoptosis, differentiation and activation of cell signaling cascades [55]. Moreover, phagocytic cells are able to produce ROS while participating in induction of host cell defense mechanisms [111,121].

ROS are generated mainly in mitochondria during oxidative phosphorylation. Physiologically, cells defend themselves against ROS damage with antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase, as well as using non-enzymatic factors such as glutathione to reduce ROS. An imbalance between the level of destructive ROS and the availability of biological systems for detoxification of the reactive species leads to oxidative stress [12]. Pathologically increased free radicals cause oxidative damage to all cell components, including oxidation of polyunsaturated fatty acids in lipids, oxidation of amino acids in proteins and DNA damage. Significant damage to cell structures leads to apoptotic cell death if cellular repair mechanisms are ineffective [37]. Brain tissues are highly sensitive to abnormal levels of ROS, because their

defensive mechanisms are limited. Moreover, reactive oxygen species are involved in the development of the inflammatory response, which is an important element of the pathogenesis of neurodegenerative diseases such as Alzheimer's disease [107] or Parkinson's disease [41].

Presumably, AgNP-mediated ROS production is associated with intracellular oxidation of AgNPs to Ag⁺ ions. This chemical process creates a pro-oxidant environment which interferes with mitochondrial functions and may lead to overproduction of ROS and mitochondrial damage [126]. A deleterious influence of silver on mitochondrial functions has been observed *in vitro* in rat liver cells [60], human liver cells [106,130] and human colon cancer cells [118]. Moreover, AgNPs were shown to decrease the activity of mitochondrial respiratory chain complexes I, II, III and IV, leading to a drop in ATP levels and increased rates of ROS production [7,22].

Furthermore, it has also been proposed that AgNPs may interact, directly or via released Ag⁺ ions, with amino acid thiol groups, disrupting the function of structural proteins in mitochondrial membranes

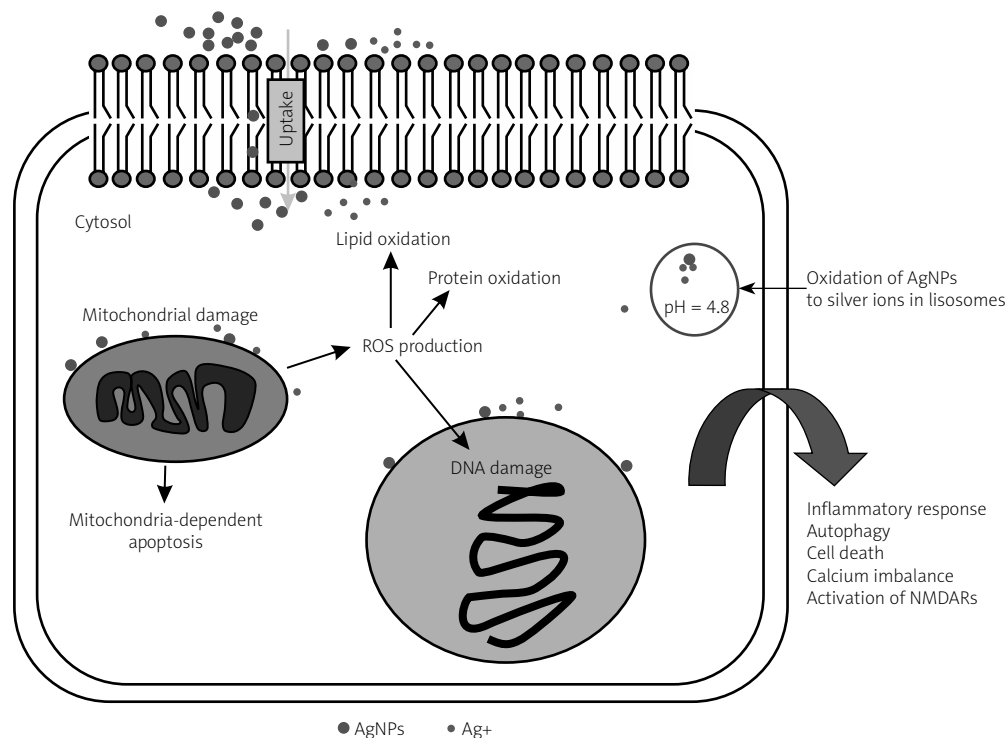


Fig. 1. Schematic representation of the mechanisms of AgNPs toxicity.

and/or mitochondrial enzymes [62]. For example, Ag⁺ ions were observed to interfere with thiol groups in the mitochondrial inner membrane, increasing its permeability [5]. This mechanism of AgNPs toxicity may be confirmed by the fact that weak antioxidants with -SH groups such as 2,3-dithiopropanol [26], N-acetylcysteine (NAC) [62], methionine and cysteine are more effective against AgNP-induced cytotoxicity than the most potent antioxidants without thiol groups such as Trolox (water soluble vitamin E analog) or Tempol [126].

Overproduction of ROS during exposure to AgNPs has been proven directly in several *in vitro* investigations [15,26,78,141] and also *in vivo* [30,103]. Among the oxidative stress-related changes caused by AgNPs, depletion of reduced glutathione (GSH) has been observed in human skin carcinoma cells [6], rat liver cells [60], mouse macrophage cells [100], human liver cells [106,141] and mouse embryonic fibroblasts [74]. Results of studies on aquatic organisms strongly support these *in vitro* observations [3,38,86], whereas limited *in vivo* studies demonstrate a lack of changes in GSH levels in liver of exposed rodents [32,39].

Furthermore, oxidative stress-related lipid peroxidation was demonstrated *in vitro* [6,106] and *in vivo* in liver of exposed rats [32,103]. DNA damage induced by ROS has been detected *in vitro* as increased DNA fragmentation in human alveolar cells [35], human liver cells [106], human epithelial embryonic cells [116], and *in vivo* in rodents [2,32,103] and aquatic organisms exposed to AgNPs [3,38].

Moreover, changes in the activity of antioxidant enzymes have been suggested to occur under the influence of AgNPs. Both decreased [6,130] and increased [141] SOD activity has been observed. It was also found that exposure to AgNPs leads to a decrease in the activity levels of glutathione peroxidases in a human liver cell line [130].

AgNP-induced inflammation and cell death

Several *in vitro* studies provide evidence of an inflammatory response in cells exposed to AgNPs. The connection between increased ROS levels and the release of inflammatory mediators such as interleukin-6, tumor necrosis factor-alpha [95], interleukin-1β and macrophage inhibitory protein (MIP-2) in macro-

phages [15] has been observed. The AgNP-mediated inflammatory response was also observed in rodent liver [30,56,67,71] and lungs [132], and increased expression of cytokines was observed in serum [25,39,99].

As described above, AgNP-induced changes in the mitochondrial membrane potential disrupt mitochondria and lead to reduction of ATP content. This may activate the protective process of autophagy. This conservative intracellular protein degradation system promotes cell survival by allowing the use of misfolded proteins as well as injured and unnecessary cellular components as alternative energy sources. However, it has been shown that prolonged autophagy may induce cell death through excessive autolysis or apoptosis [47,89].

There have been limited studies demonstrating that AgNPs may induce autophagy. Correlations between decreasing ATP content, autophagy and apoptosis have been observed in liver of rats exposed to AgNPs [71]. Additionally, relationships between oxidative stress, autophagy and apoptosis have been demonstrated in mouse embryonic fibroblast cells [74]. Induction of apoptosis by AgNPs has been demonstrated *in vitro* in many types of mammalian cells, for example in THP-1 monocytes [36], human lung cancer cells [35], human liver cells [106], human colon cancer cells [118], fibroblast cells [57], mouse embryonic fibroblasts [74], HeLa cells (human cervical carcinoma), A549 cells (human lung carcinoma) [26], baby hamster kidney (BHK21) and human colon adenocarcinoma cells (HT29) [49]. It was suggested that exposure of cells to AgNPs promotes ROS- and JNK-dependent apoptotic pathways [57]. Activation of p53 [49], down-regulation of the anti-apoptotic protein Bcl-2, up-regulation of pro-apoptotic protein Bax [49,106], activation of caspase-3 [49,74,106,118], release of cytochrome c from mitochondria into the cytosol, translocation of Bax to mitochondria [57,106], formation of DNA adducts and DNA fragmentation [26,35,36,118] have also been reported.

Current evidence of AgNP-induced neurotoxicity

The available data introduced in the section entitled “Routes of exposure and biodistribution of AgNPs in mammalian organisms” suggest that after entering bodily fluids, AgNPs can penetrate the brain tissues and be deposited there for long periods of time. There is a lack of information on the long-lasting effects

of accumulation of AgNPs in brain parenchyma, and thorough studies on this subject are required. In this chapter we concentrate on the analysis of the side effects of AgNPs in cultured cells of cerebral origin and in brain tissue of exposed mammals.

Certain data demonstrate that AgNPs may enter the brain along the olfactory nerve when administered via inhalation or intranasally [46]. AgNPs may also penetrate the brain through the blood-brain barrier (BBB) during systemic or oral administration, as indicated in Table II. The evidence that AgNPs cause neurotoxic effects is demonstrated in Table IV.

The influence of AgNPs on blood-brain barrier function

The blood-brain barrier is a highly specialized brain endothelial structure which separates components of the circulating blood from brain parenchyma. The BBB is composed of a basement membrane and microvascular endothelial cells (BMVECs) which interact with pericytes, perivascular astrocytes and neurons. Transport of substances across the BBB is strictly regulated by both physical and metabolic barriers. The physical barrier is created by tight junctions between the BMVECs, whereas the metabolic barrier is provided by specialized enzymes and diverse transport systems [105]. It is quite likely that AgNPs influence the function of endothelial cells and increase the permeability of the BBB by direct toxic effects or by induction of a cascade of events leading to disruption of tight junctions. Since the tight junctions maintaining the integrity of the BBB have a gap of 4-6 nm, it is very likely that nanoparticles pass through the endothelial cell membrane rather than through the inter-endothelial junctions. This is supported by the observation that endothelial cell membranes are undamaged [135].

It was demonstrated using an *in vitro* BBB model that AgNPs can pass the BBB mainly by transcytosis and accumulate inside endothelial cells of microvessels [134]. Moreover, there is evidence that AgNPs induce the release of IL-1 β and TNF- α in rat brain microvessels and that this leads to inflammation and a subsequent increase in the permeability of the BBB [137].

The influence of silver nanoparticles on the BBB was also demonstrated *in vivo*. Tang and co-workers observed that subcutaneous injections of AgNPs (50-100 nm) to rats in a dose of 62.8 mg/kg b.w. induce astrocyte swelling outside the blood-brain

Table IV. Studies demonstrating neurotoxic effects of AgNPs in mammalian cells and mammals

Type of AgNPs	Mammals/ Cell lines	Time of exposure, dose	Observed neurotoxic effects	References
<i>In vitro</i> studies				
AgNPs: 32.74-380.25 nm	Rat hippocampal slices	Final concentrations: 10^{-6} , 5×10^{-6} , 10^{-5} g/ml	<ul style="list-style-type: none"> – Decreased amplitude of voltage-gated sodium current (I_{Na}) of hippocampal CA1 neurons (observed within 2 min after AgNP treatment, 10^{-5} g/ml) – Extension of the recovery time of I_{Na} from inactivity (10^{-5} g/ml) 	[80]
AgNPs: 25, 40 and 80 nm	Primary rat brain microvessel endothelial cells (rBMECs)	Time of incubation: 8 or 24 h Final concentration: up to $50 \mu\text{g}/\text{cm}^3$	(Toxic effects observed for 25 nm AgNPs) <ul style="list-style-type: none"> – Induction of release of IL-1β, TNF-α and PGE$_2$ in rBMECs – Increased BBB permeability – Cellular damage with the appearance of large perforations in the monolayers – Decreased cell viability (mainly for 25 and 40 nm AgNPs) 	[137]
Peptide-coated AgNPs: 20 and 40 nm, AuNPs: 20 nm	Mixed primary cortical neural cell culture	Time of incubation: up to 24 h Final concentrations: 5, 10, 20, 30, 50 and $100 \mu\text{g}/\text{ml}$	(Toxic effects observed for 20 nm AgNPs) <ul style="list-style-type: none"> – Increased ROS production, reduced by antioxidants (NAC) – Formation of protein carbonyls – Induction of heme oxygenase-1 expression – Acute calcium response – Dose-dependent decrease of cell viability 	[52]
PVP-coated AgNPs: 75 ± 20 nm	Astroglia-rich primary cultures	Time of incubation: 4 h and further cultured in AgNP-free medium for up to 7 days Final concentrations: 10, 30 and $100 \mu\text{M}$ silver	<ul style="list-style-type: none"> – Upregulation of metallothioneins in cells – Unchanged total glutathione level and the GSSG/GSH ratio – No changes in cell viability – No changes in ROS generation – No changes in glucose consumption and lactate production – No changes in extracellular concentration of glucose and lactate 	[85]
polyethylene glycol-coated AgNPs: 5 ± 2 nm	Neuroendocrine cells (chromaffin cells)	Final concentrations: 13, 16, 43, $130 \mu\text{M}$ and 1.3 mM	<ul style="list-style-type: none"> – Dose-dependent reduction of the amplitude of sodium currents – Induction of local changes in network activity 	[14]
AgNPs: 20 nm	Rat cortical cells	Time of incubation: 2 or 3 days Final concentrations: 1, 5, 10 and $50 \mu\text{g}/\text{ml}$	<ul style="list-style-type: none"> – Degeneration of cytoskeletal components (β-tubulin, F-actin) – Inhibition of axonal outgrowth, reduction of the intensity of neuronal branches and overlaps, and reduction of cell viability of premature neurons and glial cells – Decreased cell viability of neurons and glia at mature stages of development – Mitochondrial dysfunction leading to mitochondria-dependent cell death – Synaptic degeneration in cortical neurons (reduction of the level of synaptic proteins: synaptophysin and PSD-95) 	[144]
PVP-coated AgNPs: from 21.7 ± 1.1 nm to 24.4 ± 0.6 nm	Rat cerebellar granule cells <i>In vivo</i> : neonatal rats	Time of incubation: 4 or 24 h Final concentration: up to $50 \mu\text{g}/\text{ml}$ <i>In vivo</i> : intranasally, 21 days of repeated exposure; dose: 0.2 or 1 mg/kg b.w.	<ul style="list-style-type: none"> – Decreased cell viability (after 24 h incubation with AgNPs at a concentration of $0.05 \mu\text{g}/\text{ml}$) – Increased ROS production after 4 h exposure – Depletion of reduced glutathione after 4 h incubation – Induction of apoptosis after 24 h of incubation – Increased intracellular calcium concentration <i>In vivo</i> observations: <ul style="list-style-type: none"> – Histopathological changes in cerebellum (alterations in the morphology of the granular layer: granule cells with abnormal shape and shrinking nucleus, degeneration granular layer with loss and separation of structure, edema and necrotic areas) – Activation of caspase-3 	[146]

Table IV. Cont.

Type of AgNPs	Mammals/ Cell lines	Time of exposure, dose	Observed neurotoxic effects	References
AgNPs: 20 nm	Human cerebral cells: neuroblastoma (SH-SY5Y), astrocytoma (D384); human lung epithelial cells (A549)	Short-term exposure: 4, 24 and 48 h; final concentration: 1-100 µg/ml; prolonged exposure: 7 and 10 days; final concentrations: 0.5-50 µg/ml	<ul style="list-style-type: none"> – Dose- and time-dependent changes in mitochondrial metabolism and cell membrane damage leading to decreased cell viability – observed for cerebral cell lines after short-term exposure – No significant changes in cell viability of A549 cells after short-term exposure – Dose-dependent reduction of proliferation ability and capacity to form colonies after long-term exposure of human cerebral cells and A549 cells to AgNPs 	[20]
PVP-coated AgNPs: < 100 nm	Rat cerebellar granule cells	incubation times: 10, 30 and 60 min, final concentrations: 2.5-75 µg/ml	<ul style="list-style-type: none"> – Decreased cell viability after 24h incubation (50 µg/ml) – Dose-dependent increase in the uptake of radioactive calcium after 10 min of incubation with the effect abolished by an antagonist of NMDAR (MK-801) – Dose-dependent increase in the intracellular calcium concentration – Activation of glutamatergic N-methyl-D-aspartate receptors – Increased ROS production after 30 min of incubation with 75 µg/ml – Decreased mitochondrial potential after 60 min of incubation 	[148]
AgNPs: 3-5 nm	Mouse brain neural cells: murine brain ALT astrocytes, murine microglial BV-2 cells, mouse neuroblastoma Neuro-2a cells	time of incubation: 24 h, final concentration: 5, 10, 12.5 µg/ml	<ul style="list-style-type: none"> – Increased IL-1β secretion in microglial cells exposed to AgNPs (dose: 12.5 µg/ml) – Increased gene expression of C-X-C motif chemokine 13 and macrophage receptor with collagenous structure in all types of neural cells – Increased expression of glutathione synthetase in microglial cells and decreased in astrocytes – Generation and deposition of amyloid-β (Aβ) protein in neuroblastoma cells (dose: 12.5 µg/ml) – Increased gene expression of amyloid precursor protein (APP) in all neural cells and APP protein level in Neuro-2a cells – Decreased gene expression of LDLR (all types of neural cells) and neprilysin (for neuroblastoma cells), decreased LDLR protein level for Aβ uptake in Neuro-2a cells exposed to AgNPs – Decreased cell proliferation of astrocytes and Neuro-2a cells with no change in microglial cells 	[59]
<i>In vivo studies</i>				
AgNPs: 14 nm	Rats, neuronal-like PC12 cells	Oral: 28 days of repeated exposure; doses: 4.5 and 9 mg/kg b.w., <i>in vitro</i> : 4-48 h of incubation; final concentrations: 0.5, 5 and 10 µg/ml	<p><i>In vivo</i>:</p> <ul style="list-style-type: none"> – Increased dopamine concentration in the brain (dose: 4.5 and 9 mg/kg b.w.) – Increased 5-hydroxytryptamine (5-HT) concentration in the brain (dose: 9 mg/kg b.w.) – Unchanged noradrenaline concentration in brain <p><i>In vitro</i>:</p> <ul style="list-style-type: none"> – Decreased viability of PC12 cells – Induction of necrosis in PC12 not observed – Induction of the mitochondrial and the death receptor pathways 	[54]
AgNPs: 50-100 nm	Rats	<i>s.c.</i> : single injection; dose: 62.8 mg/kg b.w.	– Histological changes (astrocyte swelling outside the blood–brain barrier, presence of pyknotic and necrotic neurons)	[135]

Table IV. Cont.

Type of AgNPs	Mammals/ Cell lines	Time of exposure, dose	Observed neurotoxic effects	References
AgNPs: 29.3 ± 12.5 nm	Mice	<i>i.p.</i> : single injection, dose: 100, 500, 1000 mg/kg b.w.	– Alterations in expression of oxidative stress-related genes in various regions of the brain (caudate nucleus, frontal cortex and hippocampus)	[112]
AlNPs, AgNPs, CuNPs: 50-60 nm	Rats, mice	<i>i.v.</i> : single injection, dose: 30 mg/kg b.w.; <i>i.p.</i> : single injection, dose: 50 mg/kg b.w.; intracarotid: single injection, dose: 2.5 mg/kg b.w.; and intracerebroventricular: 20 µg/10 µl	Effects observed after administration of AgNPs or CuNPs: – Increased BBB permeability leading to brain edema formation and decrease of local cerebral blood flow – Glial cell activation – Increased level of heat shock protein (HSP) – Loss of myelinated fibers	[123]
AlNPs, AgNPs, CuNPs: 50-60 nm	Rats	<i>i.v.</i> : single injection, dose: 30 mg/kg b.w.; <i>i.p.</i> : single injection, dose: 50 mg/kg b.w.; and intracerebroventricular: 20 µg/10 µl	– Increased BBB permeability leading to brain edema formation caused by AgNPs, CuNPs	[124]
AgNPs: 32.68-380.21 nm	Rats	Nasal administration: once every two days for 14 days; dose: 3 and 30 mg/kg b.w.	– Deterioration of space learning and memory ability, mainly in the group of animals exposed to 30 mg AgNPs/kg b.w. – Increased ROS production in hippocampal homogenate (3 and 30 mg/kg b.w.) – Histological changes of pyramidal neurons in the PP and DG regions of hippocampus (edema and nuclear shrink phenomenon as well as neurobiosis among the neurons)	[79]
AgNPs: 36.3 ± 1.2 nm	Mice	<i>i.p.</i> : 7 days of repeated exposure; dose: 10, 25 and 50 mg/kg b.w.	– No influence on spatial learning and memory – Unmodified adult hippocampal neurogenesis (no changes in hippocampal progenitor proliferation, new born cell survival and differentiation)	[77]
citrate-coated AgNPs: 10 ± 4 nm	Rats	Oral: 14 days of repeated exposure; dose: 0.2 mg/kg b.w.	– Ultrastructural changes in synapses, mainly in hippocampus (e.g. swelling of nerve endings, blurred structure of synaptic cleft, enhanced density of synaptic vesicles, disturbed synaptic membrane with free synaptic vesicles located in neuropil, myelin-like bodies and multi-vesicular bodies) – Decreased level of synaptic proteins: synaptophysin, synapsin I and PSD-95	[127]
citrate-coated AgNPs: 20-25 nm	Neonatal rats	Intranasal instillation: 14 weeks of repeated exposure; dose: 0.1, 0.2, 0.5 or 1 mg/kg b.w.	– Significant body weight loss – Histological changes (neuroglial cell activation with destruction of the granular layer of the cerebellum) – Increased level of glial fibrillary acidic protein (a marker of astrocyte activation) – Activation of caspase-3	[147]

BBB – blood-brain barrier, b.w. – body weight, GSH – reduced glutathione, GSSG – oxidized glutathione, IL – interleukin, *i.p.* – intraperitoneal, *i.v.* – intravenous, LDLR – low-density lipoprotein receptor, NAC – N-acetylcysteine, NMDAR – N-methyl-D-aspartate receptor, PGE₂ – prostaglandin E₂, PSD-95 – postsynaptic receptor density protein, PVP – poly(N-vinylpyrrolidone), ROS – free radicals, TNF-α – tumor necrosis factor-alpha

barrier, and produce pyknotic and necrotic neurons [135]. The influence of nanoparticles on the integrity of the BBB after single intravenous (30 mg/kg b.w.), intraperitoneal (50 mg/kg b.w.) and intracerebroventricular (20 µg/10 µl) administration to rodents was investigated. Increased BBB permeability and brain edema formation [123,124], a marked decrease in local cerebral blood flow, glial cell activation and loss of myelinated fibers [123] were identified.

These limited studies have shown that AgNPs can induce BBB dysfunction and cause neuronal degeneration.

AgNP toxicity towards neuronal cells

The influence of AgNPs on neurons and glial cells has been predominately investigated using *in vitro* models. Many of the results show that exposure to AgNPs causes decreased cell viability, mainly of neurons [20,52,54,59,137,144,146,148]. In the case of astroglia-rich primary cultures, the results are generally inconsistent with either no changes in viability observed after incubation with AgNPs [85] or observations of high sensitivity [52]. Such differences are likely related to both the incubation time and the size of the AgNPs. Moreover, it was observed that AgNPs may negatively influence proliferation of human cerebral cells [20] and axonal outgrowth of premature neurons and glial cells [144].

Additionally, histopathological analysis of the cerebellum of neonatal rats exposed intranasally to AgNPs for 21 days showed many abnormalities including degeneration of the granular layer with loss of structure, edema and necrotic areas [146,147]. Similar changes were also identified in regions of rat hippocampus [79].

AgNPs can also cause changes in action potential, because they may lead to the reduction of the amplitude of voltage-gated sodium currents [14,80]. Xu and co-workers identified degeneration of cytoskeletal components (β -tubulin and F-actin) and synaptic degeneration in cortical neurons [144]. Additionally, ultrastructural changes in synapses were observed in hippocampus of exposed rats together with decreased levels of two presynaptic proteins (synaptophysin and synapsin I) and one postsynaptic protein (PSD-95) [127].

The contribution of oxidative stress to the mechanisms of AgNP-mediated neurotoxicity has been considered. In *in vitro* studies of activation of ROS generation [52,146,148], protein carbonylation [52],

induction of heme oxygenase-1 expression [52], and depletion of reduced glutathione concentration [146] were observed. However, loading of primary astrocytes with AgNPs did not cause significant alterations in total and reduced glutathione or ROS production [85]. Mitochondrial dysfunction caused by AgNPs [144,148] and activation of caspase-3 [54,146], which may result in mitochondria-dependent neuronal cell death [54,144,146], have been reported. Limited *in vivo* studies also confirm the connection between AgNPs, oxidative stress and apoptosis in the central nervous system. It was demonstrated that AgNPs may cause up-regulation of oxidative stress-related genes in the brain tissues of mice after a single intraperitoneal injection [112], increased ROS production in rat hippocampus after 7 days of intranasal administration [79] and activation of caspase-3 [147]. However, the mechanisms of neurotoxicity of AgNPs may be more complex in the brain than in other tissues. Recently, new lines of research have indicated an influence of AgNPs on intracellular calcium homeostasis [52,146,148]. Moreover, it was suggested that calcium imbalance, resulting in neuronal cell death, may be connected to activation of glutamatergic receptors (NMDARs) [148]. This observation is in line with data showing impairment of cognitive functions of rats after prolonged intranasal exposure [79]. In contrast to the effect on neurons, protective mechanisms are induced in astrocytic cells. During exposure of primary astrocytes to AgNPs, metallothioneins (metal-binding proteins involved in cell protection against metal-induced toxicity) were shown to be upregulated [85].

Based on these findings, it is apparent that exposure to AgNPs exerts neurotoxic effects in mammals. However, the more subtle or permanent effects should be further investigated. Because our knowledge of the neurotoxic effects of AgNPs is based mainly on *in vitro* studies, future investigations should focus on animal studies. With increasing recognition of the dangers related to extensive usage of AgNPs and potential environmental hazards, we will be able to limit human health risks.

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