REVIEW

The Influence of Size and Chemical Composition of Silver and Gold Nanoparticles on in vivo Toxicity with Potential Applications to Central Nervous System Diseases

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Abstract: The physicochemical and optical properties of silver nanoparticles (SNPs) and gold nanoparticles (GNPs) have allowed them to be employed for various biomedical applications, including delivery, therapy, imaging, and as theranostic agents. However, since they are foreign body systems, they are usually redistributed and accumulated in some vital organs, which can produce toxic effects; therefore, this a crucial issue that should be considered for potential clinical trials. This review aimed to summarize the reports from the past ten years that have used SNPs and GNPs for in vivo studies on the diagnosis and treatment of brain diseases and those related to the central nervous system, emphasizing their toxicity as a crucial topic address. The article focuses on the effect of the nanoparticle´s size and chemical composition as relevant parameters for in vivo toxicity. At the beginning of this review, the general toxicity and distribution studies are discussed separately for SNPs and GNPs. Subsequently, this manuscript analyzes the principal applications of both kinds of nanoparticles for glioma, neurodegenerative, and other brain diseases, and discusses the advances in clinical trials. Finally, we analyze research prospects towards clinical applications for both types of metallic nanoparticles.

Keywords: silver nanoparticles, gold nanoparticles, brain diseases, central nervous system, toxicity, in vivo applications

Introduction

Nanoscale particles have been intensively studied for a wide range of in vitro and in vivo biomedical applications, such as diagnosis (sensing), therapy, drug delivery, and imaging for different diseases, due to their unique physicochemical properties, such as small size, large surface area to volume ratio, high stability, carrier capacity, and optical properties.[1,2] Among the different nanoparticles used for biomedicine, silver nanoparticles (SNPs) and gold nanoparticles (GNPs)[1–3] have stood out from the rest, since they also show better performance of localized surface plasmon resonance (LSPR). This LSPR arises from a coherent oscillation of the surface conduction electrons excited by electromagnetic radiation of a wavelength much smaller than that of the nanoparticles.[4,5] This phenomenon is absent in small nanoparticles (d < 2.0 nm) and in bulk materials and is sensitive to changes in size, shape,[3] surface functionalization, solvent, core charge, and temperature.[1,3,6,7] As shown in Figure 1, the increase in SNPs and GNPs size shifts the LSPR to a greater wavelength.[8–11]

SNPs and GNPs of different sizes and morphologies exhibit high efficiency in the LSPR.[12,13] However, despite the wide use of SNPs, there are concerns regarding their adverse effects on human health.[14] On the other hand, GNPs possess some advantages, such as easy, reproducible synthesis and optoelectronic properties, which are related to GNPs size and shape, and the surface's functionalization/stabilization with a wide range of molecules (polymers, proteins, and oligonucleotides); therefore, GNPs have been the main nanoparticle studied for biomedical applications.[15–18]

Among the different biomedical applications of SNPs and GNPs, our particular interest was to review articles where these nanoparticles were used for in vivo diagnosis and treatment of diseases related to the central nervous system (CNS). Even though a diverse range of nanoparticles are able

reach the brain after systemic administration, most of them have a limited delivery to the CNS due to the blood-brain barrier (BBB).[19] The BBB is a defensive, highly selective, and low permeable barrier that isolates the CNS, protecting and generating a stable environment for neurons. The use of transport mechanisms present in the BBB allows the passage of essential molecules for the brain's correct function, and has been explored to deliver nanoparticles to the CNS.[20–22]

Nevertheless, a very crucial aspect to consider is the nanoparticle's potential neurotoxic effect, which must be addressed for their potential clinical use.[23,24] Physicochemical characteristics such as size, shape, chemical composition, surface charge, and aggregation state may affect the toxicological profile of the nanoparticles[25] and thus, condition their biomedical applications.[26] Several studies have indicated that small nanoparticles
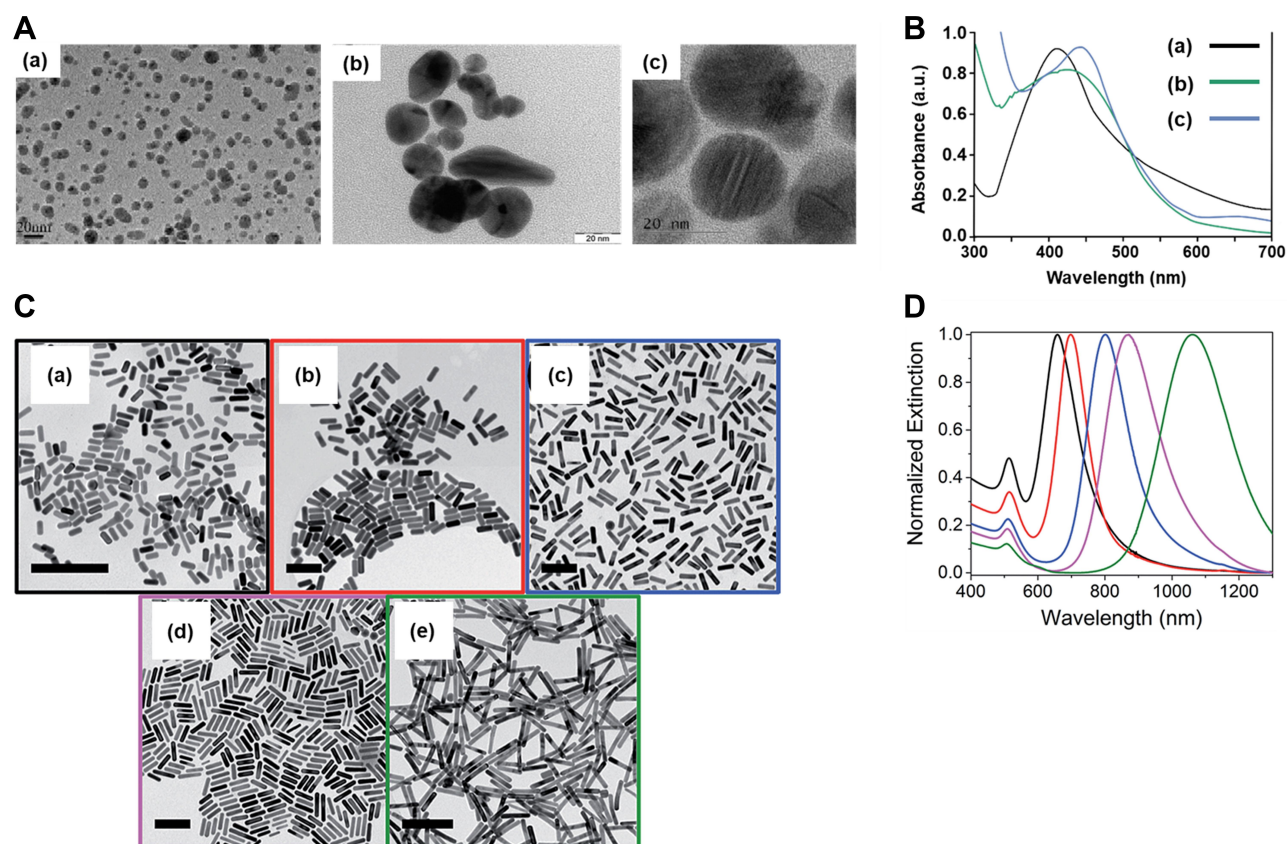

Figure 1 Effects of size on the plasmon band. (**A**) TEM images of SNPs of 10 (a), 20 (b), and 20–30 (c) nm. (**B**) UV-Vis spectra of the SNPs shown in (**A**). (**C**) TEM images of GNPs with aspect ratios of 2.4 (a), 2.7 (b), 3.6 (c), 4.4 (d), and 6.1 (e), scale bars are 150 nm. (**D**) UV-Vis spectra of the GNPs shown in (**C**). TEM images with their respective spectra were adapted with permission from: (**A**)(a) Adapted with permission from Liu P, Huang Z, Chen Z, et al. Silver nanoparticles: a novel radiation sensitizer for glioma? Nanoscale. 2013;5(23):11829. Copyright 2013, Royal Society of Chemistry[8]; (**A**)(b) Adapted from Nakkala JR, Mata R, Raja K, Khub Chandra V, Sadras SR. Green synthesized silver nanoparticles: catalytic dye degradation, in vitro anticancer activity and in vivo toxicity in rats. Mat SciEng C. 2018;91:372–381. Copyright 2018, with permission from Elsevier.[9]; (**A**)(c) Adapted from Khan I, Bahuguna A, Krishnan M, et al. The effect of biogenic manufactured silver nanoparticles on human endothelial cells and zebrafish model. Sci Total Environ. 2019;679:365–377. Copyright 2019, with permission from Elsevier[10]; and (**C**)(a-e) Adapted with permission from Hinman JG, Stork AJ, Varnell JA, Gewirth AA, Murphy CJ. Seedmediated growth of gold nanorods: towards nanorodmatryoshkas. Faraday Discuss. 2016;191:9–33. Copyright 2016, Royal Society of Chemistry[11] respectively.

can pass through the BBB; however, very small nanoparticles are vulnerable to renal and kidney excretion and clearance from target tissues. On the contrary, nanoparticles of bigger sizes (≥ 20 nm) can pass through the BBB, but larger nanoparticles (> 200 nm) can be susceptible to uptake by other organs.[26] Moreover, many studies have reported that even though the percentage of injected doses that reach the brain is relatively low, a high accumulation occurs in other vital organs, such as the liver and spleen.[6,7,27–29] This nanoparticle biodistribution and accumulation behavior may lead to an undesirable exposure to metal nanoparticles with potential toxic effects.

Regarding clinical trials related to applications of SNPs and GNPs for the treatment of central nervous system diseases, it is important to point out that, to date, no clinical studies have been conducted for CNS-related applications. Only general controlled clinical trials have been conducted for SNPs and GNPs, including the efficacy of SNPs as anti-cariogenic agents,[30] cariostatic efficacy[31] to prevent plaque accumulation,[32] and in wound healing after anorectal surgery in human patients.[33] In the case of GNPs, most of the trials have focused on the treatment and diagnosis of cancer using different shapes and coats.[34–39] Furthermore, GNP-based nanosystems have been clinically studied to treat and diagnose other diseases, such as atherosclerosis,[40] demyelinating neuromyelitis optica,[18] and pulmonary arterial hypertension.[41]

This review presents a comprehensive overview of in vivo studies that have used SNPs and GNPs for biodistribution and toxicity purposes during the last ten years. Emphasis is made on the size and chemical composition of these nanoparticles (also referred to as functionalization in this review), as the factors that may cause neurotoxicity. Of note, we excluded the articles that described in vitro studies, but these have been recently revised in Sawicki et al[42] and Ferdous et al.[43] Finally, we address the current clinical trials reported for both kinds of nanoparticles and discuss the challenges to be addressed and prospects to advance towards effective clinical use of SNPs and GNPs for nanomedicine.

General Toxicity and Distribution Studies

The size and functionalization of SNPs and GNPs change their physicochemical characteristics, affect their distribution and accumulation and thus, their toxicity. Related to the in vivo behavior, an essential step is to study how these factors affect the toxicity of SNPs and GNPs. Here, we refer to studies that elaborately evaluated nanoparticle biodistribution, accumulation, and clearance in animals, with a special interest in the CNS.

Silver Nanoparticles
Size

Exposure to SNPs and their persistence in the body can trigger undesirable effects.[44] However, it is still controversial if toxicity is associated with the nanoparticle itself or with the release of silver ions.[45] In this sense, Dąbrowska-Bouta et al[46] assessed prolonged and low-dose exposure to SNPs (10 nm, citrate-stabilized) or ionic silver (Ag$^+$) in male Wistar rats. Slight differences in behavior were found, but rats treated with SNPs increased their body weight and temperature, whereas after Ag$^+$ exposure, animals presented a weak depressive effect and hyperalgesia. Pathological changes in myelin were also observed in brain tissues from Ag$^+$ and SNPs-treated groups, compared to controls, and the levels of all examined proteins significantly decreased in the rats treated with Ag$^+$ and SNPs, suggesting that both treatments produce a neurotoxic effect. Hadrup et al[24] carried out a similar study, but compared the neurotoxic effects of 14 nm SNPs coated with polyvinylpyrrolidone (PVP) and ionic silver (Ag$^+$, the form of silver acetate). SNPs, and a filtered fraction (containing ionic silver, 12kDa), showed equal toxicity, whereas ionic silver was toxic at doses two or tenfold lower than those of SNPs. Female Wistar rats orally treated with SNPs-PVP or Ag$^+$ for 28 days did not show general appearance or behavioral changes, but presented alterations in neurotransmitter levels. The authors found a dose-response effect on neurotransmitter levels at the highest SNP concentration and the equimolar concentration for Ag$^+$ in the treatment. Both SNPs and Ag$^+$ also increased 5-hydroxytryptamine, noradrenaline and dopamine levels.[47,48] Moreover, the addition of SNPs to PC12 cells led to alterations in the mRNA levels of dopamine-related genes,[49] confirming that SNPs or ionic silver induce disturbances of neuronal cell differentiation and concomitant ability to produce dopamine. The results suggest that SNPs and ionic silver exert similar neurotoxic effects.

Soria et al investigated the toxic effects of commercial 10 nm SNPs on wild type and transgenic *Caenorhabditis elegans* (*C. elegans*) strains expressing two prototypic amyloidogenic proteins: b2-microglobulin and Aβ$_{3–42}$ peptide. The toxicity of SNPs could be detected in *C. elegans*

at lower concentrations than in in-vitro studies. In the N2 wild type *C. elegans*, the exposure to a range of SNP concentrations significantly affected the worm's body bends in a dose-dependent manner. In the absence of nanoparticles, both transgenic strains exhibited a significant defect in their locomotory function, compared to the ancestral nematodes, but SNPs severely worsened their motility. A dose-dependency was also observed with the highest concentration tested ($5.2\ \mu g/cm^2$), which reduced body bends by almost 50% in the transgenic strains; 50% in the strains expressing b2-m, and 30% in those expressing the Aβ peptide (versus 20% in N2 wild type *C. elegans*).[50]

The aforementioned studies suggest that small silver nanoparticles ranging from 10–20 nm possess in vivo toxicity effects, probably due to the induced production and release of silver ions from the nanoparticle's surface.

Functionalization

Surface modification of SNPs has been carried out for targeting and to improve their colloidal stability. Some studies, such as that of Khan et al,[10] have leaned towards a green synthesis of nanoparticles by studying the in vivo toxicity of Rumex acetosa SNPs of 20 to 30 nm (SNPs-RU) in a zebrafish model. Zebrafish embryos orally treated with SNPs-RU showed embryonic head and pericardial malformations, yolk sac edema, and a bent tail. Increased exposure to SNPs-RU also increased mortality and produced severe toxicity in a dose-dependent manner. SNPs-RU at 50 and 100 μg/mL concentrations produced pericardial edema and bent tail formation, indicating that SNPs-RU mediate apoptosis in a dose-dependent manner.

The previous report described an oral administration route. However, other vias, such as intravenous (IV) or intranasal (IN), may lead to distinct accumulation, retention, and elimination profiles and therefore, different toxic outcomes. Chrastina et al[28] developed radiolabeled 12 nm SNPs stabilized with polyvinylpyrrolidone (SNPs-PVP) and iodine-125 for in vivo tissue uptake tracking after IV administration in Balb/c mice. Biodistribution analysis revealed the highest uptake in the liver and spleen (24 and 41% ID/g, respectively), and minimal brain uptake (< 0.1% ID/g). Even though the authors indicated that SNPs strongly tend to produce hepatotoxic effects due to their higher accumulation in the liver, long-term exposure may trigger higher brain accumulation and retention, as described by Antsiferova et al,[27] who used a similar, but larger (34 nm)

nanosystem. Biodistribution, accumulation, and excretion after oral administration of SNPs-PVP in SHK mice and the ability of SNPs to penetrate the brain after a single- or multiple-administration (100 μg of SNPs) was reported. The highest concentrations were observed in the liver and blood in one-time exposure experiments (Figure 2Aa). However, redistribution was observed at two months, with the highest silver concentration in the brain and liver (Figure 2Ab). Even though the silver concentration was reduced in all organs after one month without treatment (Figure 2Ac), a shallow level of SNPs excretion from mouse brains (only 6% per month) was detected, compared to that observed in blood and the liver (about 80%), which may be due to accumulation of SNPs.[27] Similar results were described by Wen et al,[51] who studied intranasal instillation of SNPs-PVP (26 nm) or Ag^+ in neonatal Sprague-Dawley rats treated during a short-term study (4 weeks) or a long-term study (12 weeks + 4 weeks of recovery). No physiological alterations or behavioral changes were observed in rats treated with SNPs or Ag^+ at lower concentrations ($0.1\ mg\ kg^{-1}\ day^{-1}$). However, at $1\ mg\ Kg^{-1}\ day^{-1}$, Ag^+ exposure decreased body weight gain and caused 18.2% of mortality. The in vivo distribution and retention of SNPs or Ag^+ were evaluated by measuring total silver concentrations, which were found predominantly in the liver (Figure 2Ba) for both Ag^+ and SNP treatments after four weeks of exposure, similar to previous reports.[52] Interestingly, in the Ag^+ group, the second-highest silver level was detected in brain samples. The silver distribution analysis in specific brain regions, including the cortex, cerebellum, hippocampus, medulla, and olfactory bulb, is shown in Figure 2Bb, which depicts that all five regions contained equally high silver content in the Ag^+ and SNP groups. The tissue distribution profile varied distinctly after 12-weeks of exposure. The highest silver content was observed in brain tissue for both Ag^+ and SNPs, with a significant decrease in liver silver concentrations (Figure 2Bc). Finally, the silver retention study revealed that the elimination ratios of silver in serum and liver after 1- and 4-weeks of recovery were approximately higher than 60% for both treatments. However, and similarly to the study by Antsiferova et al,[27] silver concentrations in the brain remained unchanged or slightly elevated.

Therefore, despite the incorporation of molecules on the surface of 10 to 30 nm SNPs, the reported studies
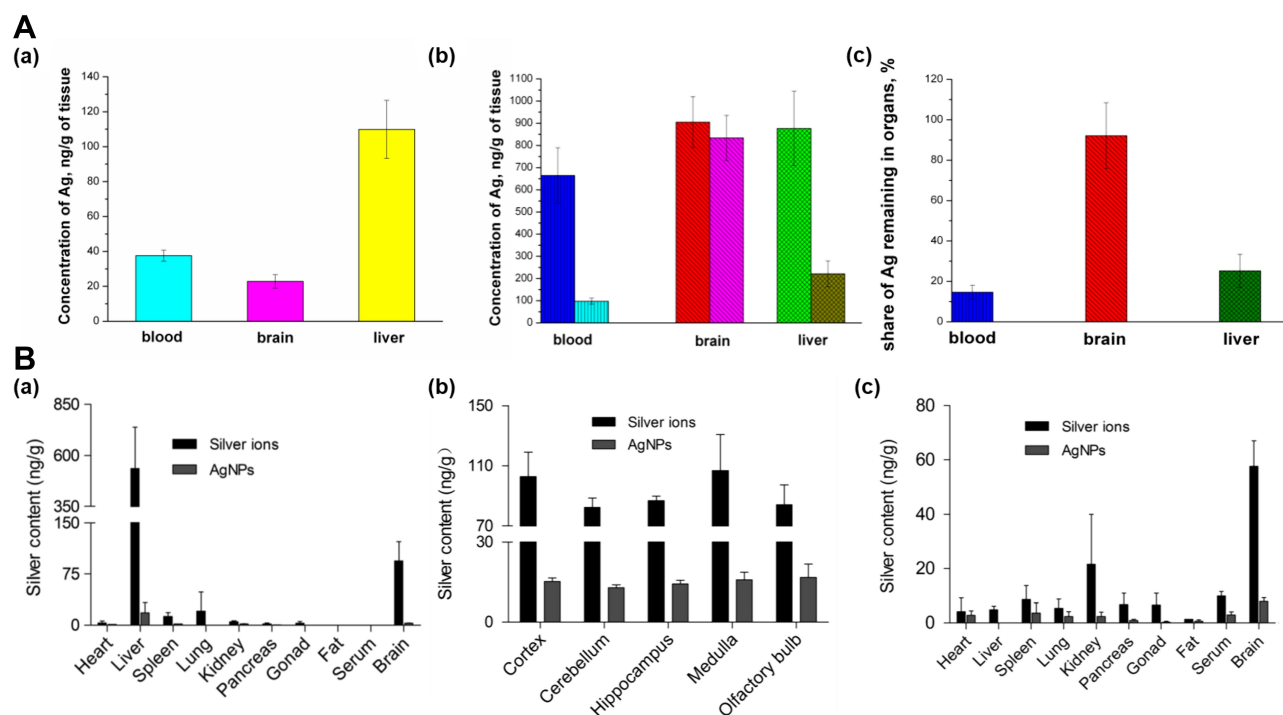
Figure 2 Biodistribution and toxicity of SNPs. (**A**) Silver concentrations in different organs (a) after one-time exposure to SNPs of size 34 nm, stabilized with PVP (b) after long-time exposure, (c) Fractions of silver remaining in mouse organs after 1 month of washing up. Adapted with permission from. Antsiferova A, Buzulukov Y, Demin V, Kashkarov P,Kovalchuk M, Petritskaya E. Extremely low level of Ag nanoparticle excretion from mice brain in in vivo experiments. IOPConference Series. 2015;98:012003. Creative Commons Attribution 3.0 licence (https://creativecommons.org/licenses/by/3.0/).[27] (**B**) Silver concentrations in main rat organs with daily intranasal administration of silver ions and SNPs of size 26 nm, at a dosage of 0.1mg kg^{-1} body weight day 1 (n = 4). (a) 4-week exposure (b) Silver distribution in different rat brain regions after 4-week exposure. (c) 12-week exposure. Adapted with permission from Wen R, Yang X, Hu L, Sun C, Zhou Q, Jiang G. Brain-targeteddistribution and high retention of silver by chronic intranasalinstillation of silver nanoparticles and ions in Sprague-Dawley rats. J Appl Toxicol. 2016;36(3):445–453. Copyright 2016 John Wiley and Sons.[51]

revealed the presence of toxic effects under the conditions studied,[10,49] suggesting that SNP hazard might be associated with the release of ionic silver from the nanoparticle's surface and its action. As described, SNPs exhibit a longer half-life in the brain than in other organs. Given this tendency, SNPs and the related dissolved ions exert considerable neurotoxic effects,[53] leading to an inflammatory response and oxidative stress, of which, the latter has been reported as one of the most important toxicity mechanisms related to silver exposure.[54–56]

Gold Nanoparticles
Size
Nanoparticle size may significantly influence the toxicological profile of gold nanosystems. Lee et al[20] determined the cytotoxicity of 5 and 100 nm GNPs in human neural precursor cells (NPCs) and Sprague Dawley rats via unilateral injection, stereotactically administered in the right cerebral cortex. The dose-dependency study on NPCs showed that 5 nm GNPs were more cytotoxic than 100 nm GNPs, reducing cell viability by up to 50%. Toxicity in the brain was determined in vivo by measuring nestin, whose expression is related to CNS injury. 5 nm GNPs showed higher nestin expression than 100 nm GNPs in immunoreactive cells, confirming that smaller GNPs were more toxic. On the other hand, other reports have demonstrated that the use of GNPs, with sizes ranging from 2 to 50 nm, does not produce significant neurotoxic effects.[6,57] Naz et al[6] reported a long-term in vivo study (90 days) of biokinetics, redistribution, and urinary excretion of 2 nm amine-terminated, citrate-capped 5 nm and 10 nm GNPs, administered by single IV injection in swiss albino mice at the dose of 1250 µg/Kg. The retention of GNPs in vital organs, such as the liver, spleen, lungs, heart, kidney, and brain, was estimated at different time points using inductively-coupled plasma atomic emission spectroscopy (ICP-AES). The maximum amount of GNPs found in the brain was 2.0, 2.2, 2.3 µg/g for 2, 5, and 10 nm GNPs, respectively, and corresponded to lower concentrations than

those found in other organs. The concentration of GNPs gradually decreased 1-day post-injection, and low concentrations were detected after 30 days. Brain tissue images showed occasional gold clusters in neurons and glial cells and confirmed very low sequestration of GNPs in the brain parenchyma. Moreover, the long-term study showed no effects of 2, 5, and 10 nm GNPs on mortality or abnormality of behavior, weight, necrosis, or fibrosis, compared with normal control mice. The authors indicated that ultra-fine GNPs did not produce systemic toxicity and may be considered safe for drug delivery.

Moreover, 50 nm GNPs obtained from phytosynthesis showed to be biocompatible.[58] In this study, Wistar rats were intravenously administered with GNPs every alternate day for 28 days. Brain sections of animals showed a normal cerebellum, no alterations in cerebrospinal fluid, and the brain tissue evidenced normal axons. No detectable abnormalities in the experimental animals were found, compared to the control group, and no significant differences were noticed in body weight or hematological and biochemical parameters. Rocha et al used 50 nm GNPs, but capped with citrate, to evaluate the daily activity and survival rates during two months, as
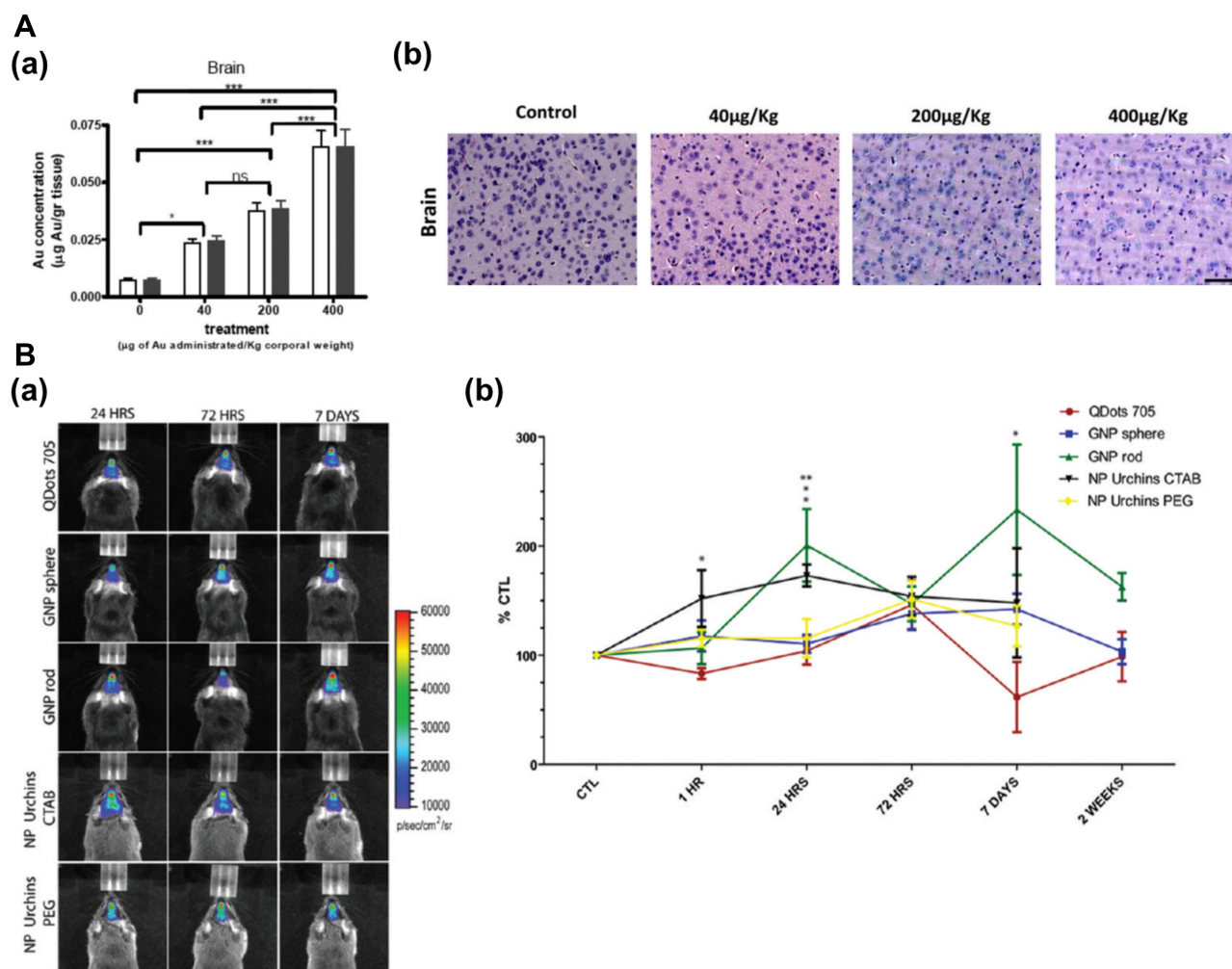

Figure 3 Distribution and toxicity of GNPs in the CNS. (**A**) Gold accumulation in the brain after intraperitoneal injection of GNPs (40, 200, and 400 μg/kg) for 8 consecutive days. (a) Gold accumulation was analyzed by GF-AAS (white bar) and ICP-MS (black bar). Bars represent mean ± standard error; data were analyzed by ANOVA with Bonferroni's post-test; ns: no significant difference, *$p < 0.05$, ***$p < 0.001$. (b) Histological analysis of the brain after treatment with GNPs to evaluate morphology and cellular damage. Tissues were stained with hematoxylin/eosin. Size of the bar corresponds to 75 μm. Adapted form Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, et al. Bioaccumulation and toxicity of gold nanoparticles after repeatedadministration in mice. Biochem Biophys Res Commun. 2010;393(4):649–655. Copyright (2010), with permission from Elsevier.[59] (**B**) In vivo bioluminescence imaging of microglia in transgenic mice after intranasal administration of GNPs. (a) Representative pseudocolor bioluminescence images (24 h, 72 h, 7 days, and 14 days) are shown for Quantum Dots 705, PEGylated gold spheres, rods, and urchins, and urchins capped with CTAB. At right, quantification scales of the total photon emission signal (photon/s/cm²/sr). (b) Time course of the change in bioluminescence signal intensity in mice. Note a biphasic activation of microglia caused by rod GNPs, and a small, but significant microglia response to gold spheres. Mean values and standard error of the means from four animals per group per time point are shown. Significant differences: *$p < 0.05$; **$p < 0.01$. Adapted with permission from Hutter E, Boridy S, Labrecque S, et al. Microglial response togold nanoparticles. *ACS Nano.* 2010;4(5):2595–2606. Copyright © 2010, American Chemical Society.[65]; Images were with permission from Refs.

parameters of neurotoxicity in cockroaches (*Blaberus discoidalis*).[57] GNPs affected insect behavior, but had no major impact on their life expectancy, which may be attributed to the encapsulation of GNPs inside the insect's brain (day 17).

Lasagna-Reeves et al evaluated the bioaccumulation and sub-acute toxicity of 12 nm GNPs in C57/BL6 mice.[59] Mice received daily intraperitoneal (IP) injections at doses of 40, 200, 400 µg/kg/day for 8 days. Results indicated that GNPs reached the CNS, and tissue accumulation was proportional to the administered doses (Figure 3Aa). At any of these doses, there were no alterations of behavior, animal weight, organ morphology, blood biochemistry, nor in histology examinations of the brain (Figure 3Ab) and other major organs.

Another interesting study evaluated the interaction of uncoated GNPs (40 nm) and gold microparticles (GMPs, 637 nm) with endothelial cell barriers.[60] Neither were genotoxic, mutagenic, nor affected the daily mortality and clinical behavior of ICR mice. However, when the authors examined the extravasation of Evans Blue dye in mice treated with GNPs, blue staining was evident in the brain at 24 and 48 h after dosing. These results suggested that GNPs alter the components of tight endothelial junctions, addressing the double-edged-sword effect of GNPs. Further studies are needed to investigate the direct/indirect toxicity caused by the opening of endothelial tight junctions induced by GNPs.

Functionalization

The incorporation of molecules on the nanoparticle's surface may improve biocompatibility of gold-based nanosystems and increase their delivery to the CNS. In this sense, 20 nm citrate GNPs were functionalized with a mixture of PEG and insulin (GNPs-INS) to determine their biodistribution pharmacokinetics for 48 hours.[19] GNPs-INS and control GNPs were injected into the tail vein of male BALB/c mice. Higher accumulation of GNP-INS was found in the liver and pancreas, whereas the %ID in the brain was 0.64%. Toxicological analysis revealed that the maximum injected dose (6 mg Au per mouse) had no toxic effects in vivo. Later, the same group explored the brain distribution of GNPs-INS.[7] Ex vivo and in vivo CT imaging and gold quantification studies revealed that particles migrated to the cerebral cortex, cerebellum, and hippocampus, which are structures involved in neurodegenerative and neuropsychiatric disorders. The authors mentioned that GNPs-INS were cleared from the brain by 48 h post-injection.

Improving the delivery of GNPs to the CNS is one of the main goals for the development of treatments or diagnostic tools. However, long retention in the brain may trigger toxic effects. Spinelli et al[61] studied the kinetics, distribution, and stability of Cy5.5-labeled GNPs in CD1 mice. Although GNPs showed low cytotoxicity in cultured hippocampal neurons, in vivo pharmacokinetic distribution in the brain showed particle retention up to 25 days after intravenous injection. Therefore, in order to assess the biocompatibility of these gold-based nanosystems, long-term studies should be carried out. Sousa et al[62] coated 15 nm citrate-GNPs with polyallylamine hydrochloride (PAH), polystyrene sulfonate (PSS), and human serum albumin to study the in vivo distribution of GNPs up to 7 days. The peak concentration in the head was detected between 19 and 24 h. GNPs were preferentially found in the hippocampus, thalamus, hypothalamus, and the cerebral cortex, brain areas of particular interest because they are related to Alzheimer´s (cerebral cortex), Parkinson´s (substantia nigra), and prion disease. No alteration in animal behavior was found over the complete experimental frame of 7 days.

Gold nanoclusters of about 3 nm have been radiolabeled with ^{64}Cu (^{64}Cu-GNCs) and complemented with the FUSIN technique to evaluate the ex-vivo spatial biodistribution in C57BL/6 mice.[63] Intranasal delivery of ^{64}Cu-GNCs and FUSIN to the brainstem enhanced the delivery to the brain region by 2.72-fold, compared with intranasal administration only, and histological studies indicated that these nanoparticles did not produce tissue damage in the nose, trigeminal nerve, nor in the brain. Other radioisotopes have also been used to modify GNPs and monitor their biodistribution.[29,64]

Even though most in vivo studies have used spherical nanoparticles, only a few reports have explored how shape might affect brain toxicity in vivo. Hutter et al[65] reported the interactions of microglia and neurons with GNPs of three different morphologies (spheres, rods, and urchins) and different coatings (PEG and CTAB). After intranasal administration of these GNPs in transgenic mice (Figure 3Ba), a real-time study showed a transient up-regulation of toll-like receptor 2 (TLR-2) in the microglial cells located in the olfactory bulb (Figure 3Bb). In conjunction with the in vitro results, the study demonstrated that the morphology and surface chemistry of GNPs strongly influence microglial activation and potentially produce toxic effects.

The in vivo toxicity and biodistribution studies of SNPs and GNPs have shown on one side, that small SNPs of 10–30 nm generate toxicity, probably due to the release of silver ions, which trigger biochemical alterations, abnormities in behavior, and neurotoxic effects. Moreover, although the
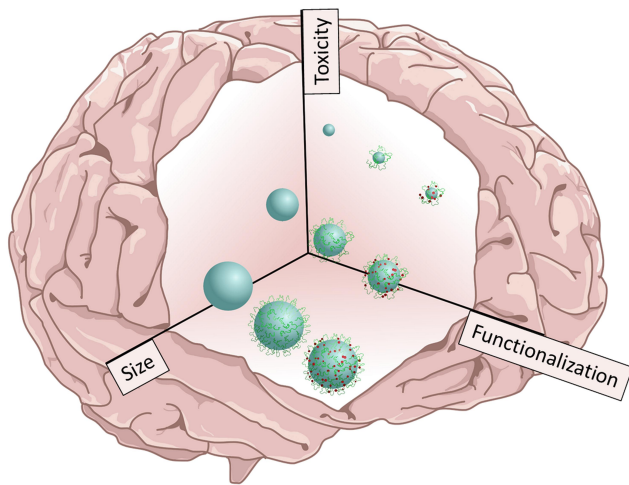
Figure 4 Effect of size and chemical composition on toxicity. Schematic illustration representing the size and chemical composition of either functionalized silver or gold nanoparticles as selected factors that produce toxicity effects in the CNS.

functionalization of SNPs diminishes their toxicity and they exhibit a more powerful radiosensitizing ability than GNPs, long-term accumulation of SNPs in the brain might be counter-productive. These results may be the cause of the limited number of articles on SNPs in the last decade.

On the other hand, the tendency to use GNPs has increased. While it is difficult to establish a correlation between size and toxicity for small GNPs ranging from 2 to 10 nm, it is clear that larger GNPs (from 5 to 100 nm) are less toxic. Moreover, the functionalization of GNPs plays a fundamental role, since it increases biocompatibility and exerts minimal or no abnormalities on animal behavior after the administration of GNP-based nanosystems. Figure 4 illustrates a general conclusion of the effect of size and functionalization on toxicity; while small and uncoated nano-particles increase toxicity, modified nanoparticles decrease it. However, there is a marked difference between the toxicity generated by GNP and SNP, since SNPs produce silver ions and generate a long-term accumulation in the brain and therefore, higher toxic effects than GNPs.

Delivery of Gold and Silver Nanoparticles to the Central Nervous System and Their Potential Biomedical Applications

Delivery of Nanoparticles to the CNS and BBB Permeability

The feasibility of developing bioapplications of SNPs and GNPs in the CNS relies on whether or not they reach the brain. One strategy to overcome the BBB is the use of ligands that recognize receptors highly expressed in the BBB, which act as shuttles from blood circulation to the brain.[26,66] The functionalization of nanoparticles with molecules such as transferrin,[67] L-Dopa,[68] Angiopep-2,[21] and HAI peptides,[69] or folic acid (in the case of gliomas),[70] has improved their delivery to the CNS. Another non-invasive and targeted strategy is to disrupt the BBB with ultrasound and micro-bubbles. Studies have demonstrated that GNPs with different sizes[71] and surface charges[72] can reach the CNS after sys-temic administration and ultrasound and microbubbles appli-cation. Another alternative route to overcome the BBB is to change the administration route. Intranasal administration is a promising and efficient therapeutic strategy for the non-invasive treatment of various CNS diseases since the nasal cavity is described as an open gate for the transport of nanoparticles to the CNS, due to the absence of the BBB.[73] However, until now, there are only a few studies that have addressed the intranasal route for the nose-to-brain delivery of nanoparticles, leaving a potential area to cover.[65,74–77]

Glioma

Gliomas are tumors that originate from glial cells and show a dismal prognosis. Glioblastoma multiforme (GBM) -a grade IV glioma- is the most prevalent and aggressive form.[78] Table 1 summarizes the studies in which SNPs and GNPs have been used for glioma applica-tions, where in vivo toxicity studies have been carried out.[79,80]

GNPs and SNPs may be used as radiosensitization agents to improve glioma treatment. Bobyk et al compared the radiosensitization efficacy of 1.9 and 15 nm GNPs in Male Fischer rats. Smaller GNPs showed to be toxic, so the combined therapy that the animals received was 15 nm GNPs + radiation. The treated group presented a mean increase in life span of 74%, in comparison to the untreated animals.[81] SNPs of 114 nm entrapped in PLGA nanoparticles and conjugated with chlorotoxin were used for glioma treatment in a mouse model of GBM. The application of radiation allowed SNPs to inhibit tumor growth and progression and, at the same time, led to a 4 to 6 fold increase of SNP accumulation in glioma.[82] Liu et al demonstrated in vivo that the radiosensitizing ability of SNPs was more powerful than that of GNPs.[83] Some authors have described that nanoparticles having high Z-elements can generate large amounts of free radicals after activation, which amplifies and prolongs the deleterious effects of radiotherapy.[84,85] Therefore, GNPs and SNPs of

Table 1 Overview of Silver and Gold Nanoparticles and Their Applications for Gliomas

NP (and Its Functionalization)	Mean Size (nm)	Application	Ref.
GNPs	1.9 15	Therapeutic radiotherapy	[81]
SNPs-PVP	10	Radiotherapy-imaging	[8]
GNPs-citrate and SNPs-citrate	15	Radiotherapy-imaging	[83]
GNP-PEG-FAL	60	Imaging-targeting and therapy	[93]
SNP-PLGA-CTX	114	Sensing and therapy	[82]
GNS-IR780-PEG	100	Imaging	[94]
GNPs-U2 aptamer	60	Therapy	[79]
GNP-miR-182	13	Therapy	[80]
GNP-DOX-An	40	Delivery-therapy	[87]
GNP-AK-citrate GNP-CABT-citrate	40	Treatment	[88]
GNPs-A&C-R, combined targeting with peptide R8-RGD	41	Treatment	[89]
GNP-^{131}I-PENPs-CTX	151 147	Imaging and therapy	[95]
CD–CS-T7-CD-CS-polyGION-miRNA	34	Theranostic	[86]
GNP-PP-polyI:C	13	Delivery-treatment	[17]
GNR-CD133 antibody	10 x 40 nm	Treatment	[92]
GNP-Cis	7	Treatment	[90]

Abbreviations: FAL, Phe-Ala-Leu-Gly-Glu-Ala; PLGA, poly-lactic-co-glycolic acid; CTX, chlorotoxin; DOX, doxorubicin; An, angiopep-2; AK, Ala-Ala-Asn-Cys-Lys; CABT, 2-cyano-6-aminobenzothiazole; PENP, polyethyleneimine; PP, methoxypolyethylene glycol-polyethyleneimine; polyI:C, polyinosinic-polycytidylic acid; Cis, cisplatin; CD–CS-T7-CD-CS-polyGION-miRNA, gold iron oxide nanostars loaded with miR-100 and antimiR-21 and T7 peptide functionalized β-cyclodextrin-chitosan.

different sizes, shapes, and functionalization should be evaluated to study and determine the differential radio-sensitizing efficacies.

GNPs with sizes ranging from 10 to 30 nm, and modified with mRNAs[80,86] and polymers[17] have also shown beneficial effects on GBM. Ruan et al have reported three systems based on 40 nm GNPs that improved the median survival time of C6 glioma-bearing mice.[87–89] Interestingly, the authors proposed a nanoparticle aggregation strategy based on GNPs modified with Ala-Ala-Asn-Cys-Lys (GNPs-AK) and 2-cyano-6-amino-benzothiazole (GNPs-CABT): GNPs-AK could be hydrolyzed in the presence of legumain, producing a cycloaddition with the cyano group on GNPs-CABT. Results indicated that the aggregated nanosystem blocked exocytosis of GNPs, which led to enhanced retention in vivo and reduced systemic toxicity of doxorubicin (DOX).[88] The same group improved the chemotherapeutic

effect of DOX by incorporating the R8-RGD peptide (Figure 5Aa),[89] in which the R8 portion improves cell penetration, and the RGD sequence allows the targeting of tumor cells. In vivo results showed that GNPs functionalized with the R8-RGD peptide were efficiently delivered to GBM (Figure 5Ab), improving DOX's chemotherapeutic effect (Figure 5Ac).[89] Coluccia et al used GNPs conjugated with cisplatin combined with MR-guided FU to intensify GBM treatment. MRgFUS led to increased BBB permeability of GNPs, allowing a significant reduction of tumor growth.[90] The animals did not show any neurological or behavioral sequelae from MRgFUS treatment, and the histology of organs showed no morphological alterations after a total administration of 7.5mg/kg GNP-UP-Cis over 8 days.

Furthermore, GNPs may be used for photothermal therapy, and these studies have demonstrated a significant improvement in animal survival.[91] Casanova-Carvajal et al used GNRs
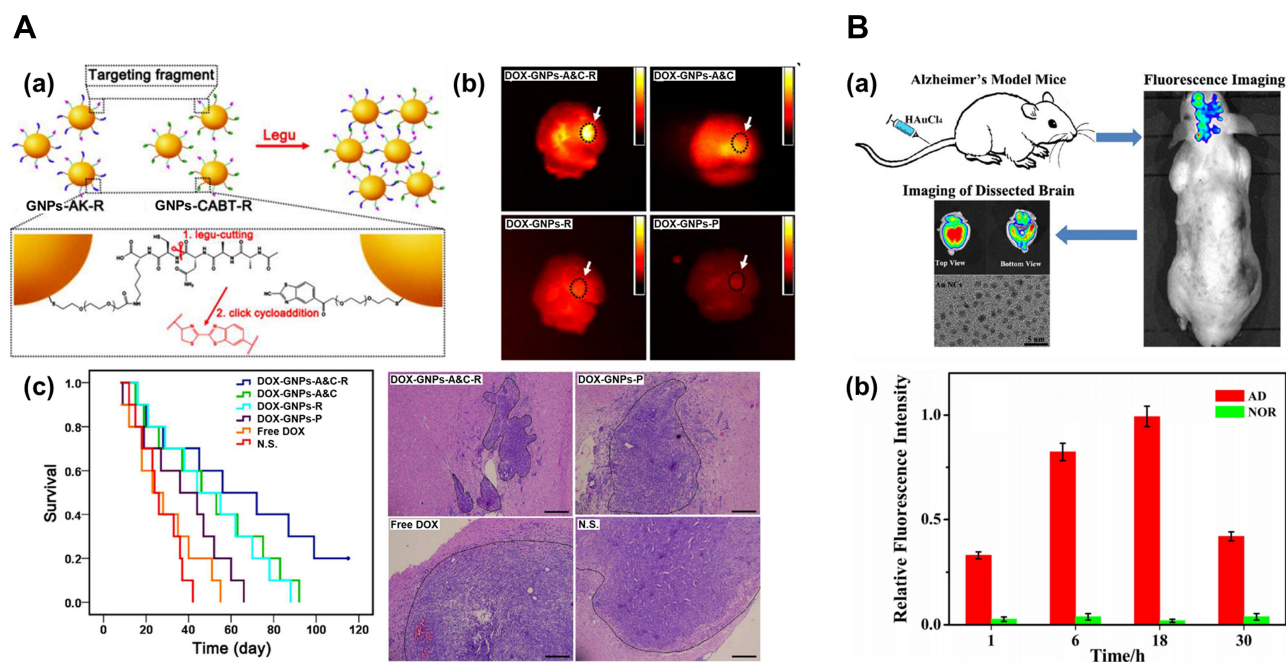
Figure 5 Applications of GNPs to brain diseases. (**A**) Targeting and retention of GNPs for enhanced treatment of glioblastoma. (a) Mechanism of legumain-activated click cycloaddition and aggregation process of GNPs-A&C-R. (b) Ex vivo imaging of the brain extracted from GBM-bearing mice treated with different formulations for 24 h. (c) Left: Kaplan-Meier plots of the survival in C6 GBM-bearing mice treated with different formulations. Right: H&E staining of GBM-bearing brain sections. The dotted region represents GBM cells, and bars represent 200 μm. Image A (a, b and c) adapted with permission from Ruan S, Xiao W, Hu C, Zhang H. Ligand-mediated andenzyme-directed precise targeting and retention for the enhancedtreatment of glioblastoma. ACS Appl Mat Interfaces. 2017;9 990(24):20348–20360. Copyright 2017, American Chemical Society.[88] (**B**) Fluorescent GNCs for in vivo imaging of AD. (a) Schematic illustration of fluorescence bio-marking of mouse brain with AD, which could be readily visualized by labeling the affected brain regions through intravenous injection of aqueous HAuCl₄ solutions. (b) The variations of mean fluorescence intensity in the AD mouse model and the normal control group (NOR) at various time points. Image B (a and b) adapted with permission from Royal Society of Chemistry Royal Society of Chemistry, Lai L, Zhao C, Li X, et al. Fluorescent gold nanoclusters forin vivo target imaging of Alzheimer's disease. RSC Adv.2016;6:30081–30088, permission conveyed through Copyright Clearance Center, Inc.[95]

Abbreviations: A&C, AK peptide and 2-cyano-6-aminobenzothiazole (CABT); R, R8-RGD dual peptide; P, PEG; DOX, doxorubicin; N.S., Saline group.

biofunctionalized with the CD133 antibody to treat mice with glioma. This treatment produced a notable deceleration in tumor size, compared to the untreated control groups.[92]

With respect to glioma diagnosis, GNPs-PEG of 60 nm modified with the Phe-Ala-Leu-Gly-Glu-Ala peptide have been used to demarcate tumor margins with pre-operative MRI; in this study, a higher distribution of nanoparticles was observed in glioma and in the invasive margin.[93] Tumors have also been visualized by using 100 nm GNPs[94] encapsulated in a silica layer and functionalized with PEG, and 150 nm GNPs radiolabeled with chlorotoxin and polyethyleneimine.[95]

Silver and Gold Nanoparticles for Neurodegenerative and Other Brain Diseases

Nanoparticles have also been studied as potential tools for treating and diagnosing neurodegenerative diseases,[26,97] such as Alzheimer´s disease (AD), ischemic stroke,[98,99] prion disease,[101] perinatal asphyxia,[100] and herpes-associated amyloid-β secretion.[102]

Neuronal death in AD is triggered by protein aggregates composed of the Aβ$_{1-42}$ peptide, its main therapeutic target. The use of nanosystems, which allow Aβ disaggregation or destruction, has emerged as a possible treatment for AD.[21,77,103,105] Morales-Zavala et al demonstrated that gold nanorods functionalized with the D1 peptide -which specifically recognizes the Aβ peptide- decreased in vivo toxicity of Aβ in the *C. elegans* model of AD.[104] Moreover, a study in the mouse AD model that used IV injection of 50 nm GNPs functionalized with anthocyanins proved a reduction of neuroinflammation and neurodegeneration induced by Aβ$_{1-42}$.[106] Lai et al developed in-situ gold nanoclusters (GNCs) of 3.3 nm for in vivo fluorescence imaging after IV injection of HAuCl₄ solution (Figure 5Ba). Since the redox environment of the AD brain is completely different from that of the normal brain, under these conditions HAuCl₄ forms Au salts, which subsequently self-assemble to form GNCs. While the presence of GNCs was confirmed in the brains of mice with AD, no GNCs were found in the brains of normal mice (Figure 5Bb).
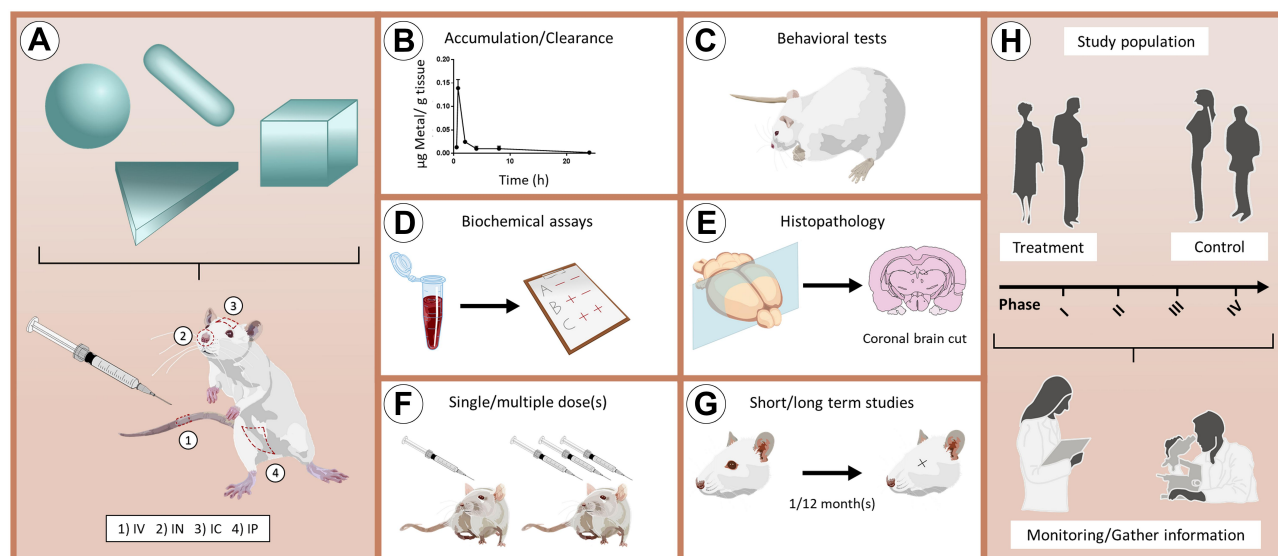
Figure 6 Ideal methodology to assess the toxicity of GNPs/SNPs for applications in brain-related disorders. (**A**) Schematic representation of in vivo administration of nanoparticles with different shapes through the main routes of administration. (**B–G**) Main in vivo experiments that can be performed to obtain a complete toxicological profile of nanoparticles. (**H**) Representation of a clinical trial that includes treatment and control groups. During the different phases of the clinical trials (I–IV), it is relevant to monitor individuals to gather essential information.
Abbreviations: IV, intravenous; IN, intranasal; IC, intracranial; IP, intraperitoneal.

After 4~6 weeks, neither AD nor normal mice showed histopathological abnormalities nor changes in their physical behavior.[96] In the case of SNPs, Zhang et al reported that IP administration of 12 nm SNPs obtained by green synthesis produced positive effects in preventing and reducing deficits in recognition and spatial memory in a rat model of sporadic AD.[107] However, the toxicity of the nanoparticles used in this study and their effect was not described.

Conclusions and Perspectives

SNPs and GNPs have provided endless opportunities for in vivo biomedical applications. However, like other xenobiotics, they are usually redistributed and accumulated in some vital organs and produce toxic effects, a crucial issue to consider for potentially advancing clinical trial designs. In the case of SNPs, a limited number of in vivo toxicity studies related to neurodegenerative and other brain-related diseases were found, probably due to their potential and major toxicity, especially in the case of bare SNPs, compared to GNPs. Currently, there is a consensus that the toxicity of SNPs is associated with the release of silver ions, which trigger biochemical alterations, abnormalities in behavior, and neurotoxic effects. As discussed previously, although in vivo functionalization of SNPs clearly diminishes their toxicity and SNPs exhibit a more

powerful radiosensitizing ability than GNPs, long-term accumulation in the brain might be counterproductive.

In contrast, a clear tendency for the use of GNPs has been observed during the last decade. While it is difficult to define a correlation between size and toxicity for small GNPs ranging from 2 to 10 nm, it was clear that larger GNPs (from 5 to 100 nm), are less toxic. Moreover, functionalization of the GNP surface increases biocompatibility, facilitates crossing the BBB, and enhances delivery to the brain, improving potential treatments and diagnostic methods. Moreover, all the studies that were reviewed showed minimal or no abnormality in animal behavior after administering GNP-based nanosystems. Therefore, GNPs are definitely less toxic than SNPs.

It is important to note that clinical trials lacked complete in vivo toxicity studies, and that most of the studies with SNPs and GNPs used spherical nanoparticles. It is well known that anisotropic shapes, such as rods, stars, and prisms, have interesting physicochemical properties; for example, plasmons centered at the near-infrared region can be exploited for biomedical research and clinical applications. In the coming years, more studies should thoroughly evaluate the in vivo toxicity of these nanosystems. As an approach, 3D human CNS in vitro models can be used as a perspective, especially when using human neural precursor cells (NPCs) derived from human-induced pluripotent stem cells (hiPSCs).[108] Depending on the developed 3D model, it

is possible to mimic specific aspects of human brain physiology, including the development of brain-related disease models. These 3D models could accelerate data acquisition related to SNP and GNP neurotoxicity, allow the development of high throughput screening methodologies, and reduce the time-consuming animal experimentation.

Figure 6 illustrates the main toxicity studies that should be performed to obtain a complete toxicological profile of SNP- and GNP-based nanosystems. The use of in vivo experiments, such as accumulation/clearance studies, behavioral tests, biochemical assays, histopathology examination, administration of single/multiple doses and short/long term studies will allow many of the interesting studies described in this review to get closer to the next step: clinical research. We hope that this review will stimulate further development of SNPs and GNPs of different sizes and shapes, and we encourage the scientific community to continue with in vivo experimentation, especially regarding the CNS and brain-related diseases.

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Disclosure

The authors report no conflicts of interest in this study.

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