Are synapses targets of nanoparticles?

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Abstract

The last few years have been marked by real breakthroughs in the field of nanotechnology. Application of nanoparticles was proposed for diagnosis and treatment of different central nervous system diseases. Exposure to nanoparticles in vivo increases the risk of onset of neurodegenerative diseases and nanoparticles are apparently able to kill neurons in vitro. We suggested that presynaptic terminals of neurons are another target for nanoparticles, beyond the already established microglial cells. Ferritin was chosen as a prototypic nanoparticle model. We found that even a high concentration of ferritin, 800 μg/ml, was not able to induce spontaneous release of [14C]glutamate. In contrast, [14C]glutamate uptake was inhibited by ferritin in a dose-dependent fashion. As a next step, the influence of ferritin on the formation of reactive oxygen species was monitored using the fluorescent dye DCFH-DA (2′,7′-dichlorofluorescein diacetate). It was shown that ferritin leads to a dose-dependent formation of free radicals. We found that the ferritin-mediated changes in glutamatergic neurotransmission at presynaptic endings can result in neuronal damage and finally neurodegeneration.

What are nanoparticles?

The last few years have been marked by significant breakthroughs in the field of nanotechnology. Application of NPs (nanoparticles) was proposed for the diagnosis and treatment of different central nervous system diseases [1–4]. Generally, NPs are recognized as particles of 1–100 nm in diameter, having different shapes and chemical origins [5,6]. They are able to penetrate the blood–brain barrier and can enter the brain following inhalation of aerosols through the olfactory epithelium [5,7–9]. However, it should be noted that many studies into the action of NPs were conducted using mouse and rat laboratory models. Rodents rely on their olfactory sense to explore and recognize the world, and the surface area of their olfactory epithelium is correspondingly large. In contrast, olfaction is less important for higher primates, and human olfactory epithelium is less developed. There is little evidence that inhalation is as effective for NP entry into the human brain as it is for rodent brain [5].

The ability of NPs to penetrate the blood–brain barrier allows them to be used as contrast agents for different methods of vital diagnosis, as well as vehicles for the target end delivery of genetic materials and drugs [2–4].

Nanoparticles are able to damage the brain

To what extent is the presence of NPs safe for brain, if the blood–brain barrier is so easily permeable to them? It was shown that all NPs are more or less toxic and the brain can serve as a target for their neurotoxic action [3–5]. Exposure to NPs in vivo increases the probability of neurodegenerative disease onset [5,9,10] and NPs are able to kill neurons in vitro [11,12].

Unfortunately, our knowledge of the cellular and molecular mechanisms of the damaging influence of NPs on the brain is restricted. Generally, NPs can kill cells by three main pathways: (i) ROS (reactive oxygen species) formation; (ii) mechanical damage of intracellular organelles; and (iii) an increase in cytosolic Ca2+ concentration [4,5].

At least two of these mechanisms are involved in nanotoxicity in the brain. It has been shown that free radicals can mediate neuronal damage induced by NPs originating from diesel exhaust fumes. They are able to induce NADPH oxidase in microglial cells, which leads to superoxide anion formation and the death of dopaminergic neurons [11,12]. Inhalation of ferric oxide-containing NPs in vivo induces oxidative stress in the brain [9]. Quantum dots in vitro are able to increase intracellular Ca2+ in hippocampal neurons [13].

Two hallmarks of the brain are special mechanisms of damaging of cells and cell death. (i) Misfolding of proteins followed by formation of insoluble intra- and extra-cellular aggregates. Most commonly known examples are amyloid plaques in Alzheimer’s disease and intracytoplasmic fibrils of synuclein in Parkinson’s disease [14,15]. (ii) Excessive glutamate release with subsequent receptor overactivation and neuronal death. Neuronal death is mediated by Ca2+ overload, caspase activation and ROS formation. This mechanism is termed excitotoxicity [16].

Amyloid plaques contain iron oxide NPs [17]. Moreover, it was recently shown that NPs containing titanium oxide are able to increase amyloid peptide aggregation, and can serve as ‘crystallization centres’ for senile plaques [18]. It might be suggested that NPs are able to damage the brain by a mechanism similar to that observed in Alzheimer’s disease; however, this hypothesis requires additional testing.

Key words: ferritin, free radical, glutamate release, glutamate uptake, nanoparticle, synaptosome.

Abbreviations used: NP, nanoparticle; ROS, reactive oxygen species.

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Even less is known about the involvement of excitotoxicity in NP-induced neuronal death. It has been shown that zinc oxide NPs can trigger Na\(^+\) influx and plasma membrane depolarization in hippocampal neurons [19]; however, there is no evidence that these changes ultimately lead to glutamate release and excitotoxicity.

We suggested that the presynaptic terminals of neurons are another target for NPs beyond the already established microglial cells [11,12]. Neurotransmitter release is carried out by exocytosis, therefore the presynaptic ending is a part of the neuron which is specialized for vesicle recycling and, as a consequence, has a high recycling rate. Generally NPs become trapped in the cell following endocytosis [4,5]. Thus a high NP-uptake rate can be expected for this region of the neuron. Supporting this suggestion, it has been shown that hippocampal neurons are able to capture NP quantum dots via synaptic vesicle recycling. Quantum dots are concentrated in presynaptic endings, as sites specialized for synaptic vesicle recycling [20].

We used isolated presynaptic endings termed synaptosomes in our investigation. They have many of the properties of intact presynaptic endings, including the protein machinery and ion channels required for synaptic vesicle recycling [21,22]. Synaptosomes display Na\(^+\)-dependent neurotransmitter uptake and Ca\(^{2+}\)-dependent neurotransmitter release [23,24].

**Ferritin as a prototypic nanoparticle**

It was necessary to choose any prototypic NPs for the investigation of synaptic mechanisms of nanotoxicity. Some of their properties can be valid for all types of NP. We chose ferritin because this compound can be considered to be a model nanoparticle. Ferritin NP s consist of a hydrous ferric oxide phosphate particle having a diameter of 7 nm and are coated by a protein shell. The estimated size of the protein shell is approx. 12 nm [5].

Ferritin was chosen as a model for several reasons. Ferritin is readily available commercially compared with other NPs. This protein serves also as a good tool for investigation of possible toxicity of metal NPs coated by polymer or dextran shells. These particles are widely used as supramagnetic agents for magnetic resonance imaging, for labelling of different cells and tissues and for transfection of neurons [3,25]. It should be noted that ferritin contains Fe\(^{3+}\), which in contrast with Fe\(^{2+}\) is unable to participate in the Fenton reaction with strong ROS formation [26,27]. Finally, it should be taken into account that insoluble iron particles have been found in amyloid plaques in Alzheimer’s disease [17] and inside neurons in Parkinson’s disease [28]. The involvement of iron deposits in the pathogenesis of neurodegenerative diseases is still unclear. Investigation of ferritin’s influence on different neuronal subfractions will help to resolve this question.

We found that even a high concentration of ferritin, 800 \(\mu\)g/ml, was unable to induce the spontaneous release of \(^{14}\)C glutamate. In contrast, \(^{14}\)C glutamate uptake was inhibited in a dose-dependent manner [29]. Inhibition of glutamate uptake results in more prolonged exposure of neurons to glutamate and might thus potentially modify synaptic transmission, leading to neuronal damage [30]. For instance, a mutation in the glutamate transporter GLT-1, which decreases glutamate uptake, can contribute to amyotrophic lateral sclerosis, a neurodegenerative disease [31].

The influence of ferritin on ROS formation was monitored using the fluorescent dye DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate). It was shown that NPs lead to dose-dependent formation of free radicals. This increase in free radicals develops after a 1 min lag phase, which is probably required for entry of NPs into presynaptic endings [29]. We found, that in contrast with microglial cells, NP-induced oxidative stress was insensitive to DPI (diphenyleneiodinium chloride), an inhibitor of NADPH oxidase. This allows the exclusion of the involvement of NADPH oxidase [11,29]. We also found that the mitochondrial uncoupler CCCP (carbonyl cyanide m-chlorophenylhydrazone) was ineffective in inhibiting the DCF (2′,7′-dichlorofluorescein) fluorescence increase [29]. However, it should be noted that the connection between membrane potential and ROS formation in intrasynaptosomal mitochondria is not straightforward [32], therefore the contribution of mitochondria cannot be completely ruled out. Free radical synthesis in neuronal presynaptic endings can be particularly important in the pathogenesis of different neurodegenerative diseases owing to the high sensitivity of the exocytosis machinery to local ROS [33,34]. Imbalance in neurotransmitter release leads to the death of neurons [16].

So, we found two events induced by ferritin which can ultimately lead to neurodegeneration. We do not know exactly which part of ferritin, the protein shell or the Fe\(^{3+}\) ion, is responsible for these changes; however, it is very unlikely that the protein part is capable of inducing such dramatic changes.

**Conclusions**

It should be noted that a hallmark feature of many neurodegenerative diseases is a slow developmental onset. Often the first clinical symptoms can be monitored only after the death of a defined population of neurons. A larger number of neurons have to die before the first symptoms appear. Sometimes, for instance in prion infection, the development of infection can be very slow. Therefore it is very important to use more informative tests for the estimation of neuron survival in vivo and nanoparticle cytotoxicity in vitro. Tests for biochemical and morphological markers of neurodegeneration after prolonged contact with NPs are required for the characterization of new nanomaterials.

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References


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