Blood biomarkers: from nanotoxicity to neurodegeneration

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Various blood biomarkers were screened for their ability to monitor nanoparticle neurotoxicity by analyzing bound proteins on their surface.

Nanoparticles (NPs) are artificial or natural particles with diameters of 100nm or less. Unique and hidden physical, chemical, electrical, and optical properties of NPs have been observed as their use has expanded in electronic, chemical, and biological applications. Due to their high surface-to-volume ratio, NPs may be toxic to cells and tissues through endocytosis, in which cells engulf an NP as if it were a protein or similar molecule. NP accumulation in the body may cause pulmonary and cardiovascular disease, spleen injury, liver DNA cleavage, and the other problems. Newly developed NPs may require risk assessment and standardized protocols and guidelines for control and regulation.

NPs can enter the body through inhalation, injection, dermal penetration, or ingestion. Inhaled NPs may enter the circulatory system and spread to various organs by traveling deep into alveoli in the lungs, causing inflammation, or entering the bloodstream by crossing the air-blood barrier. Can we locate the NPs in the brain? If so, what would be the mechanisms of their translocation across the blood-brain barrier (BBB)? Even though the mechanisms and pathways of NP penetration into the brain are unclear, direct disruption or interactions with various blood proteins may enable them to cross the BBB.

To answer this question, many studies use protein corona analysis to examine the bound proteins on the surface of NPs (see Figure 1). Bound proteins are released from NPs and analyzed via gel electrophoresis and liquid chromatography tandem mass spectrometry. Visualization of the protein analysis network reveals potential mechanisms of NP toxicity. Adsorbed proteins on NPs may undergo conformational changes, causing rearrangement of critical domains or exposure epitopes.

Figure 1. Schematic diagram of a process to study neurotoxicity of nanoparticles. NP: Nanoparticle. CSF: Cerebrospinal fluid. LC-MS/MS: Liquid chromatography-tandem mass spectrometry. MDS: Multimer detection system. MRI: Magnetic resonance imaging. SPECT: Single-photon emission computed tomography. PET: Positron emission tomography.

Consequently, the fate of NPs could potentially be determined through interactions with associating proteins. For example, apolipoprotein E is one of the major components of the protein corona, and may act as a carrier protein for crossing the BBB.

Oxidative stress is an important neurotoxic mechanism. Reactive oxygen species could be generated through free-radical activity on the surface of NPs, using lysosome-associated and toll-like receptor-induced signaling pathways. NP interaction with toll-like receptors may activate nicotinamide adenine dinucleotide phosphate oxidase cascades, leading to...
reactive oxygen species generation and inducing inflammation (see Figure 1). After BBB penetration through interactions with carrier proteins, NPs could damage adjacent tissues through oxidative stress, leading to the expression of proinflammatory cytokines, chemokines, and apoptosis (programmed cell death)-related genes.\(^8\) Reactive oxygen species might also play a role in nuclear factor-κB (NF-κB)-dependent inflammation, and the activated NF-κB may initiate transcription of inflammation-promoting genes such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-8. Neuroinflammation from NPs could activate glial cells and astrocytes, chronically disturbing neuronal hemostasis and eventually leading to apoptosis or necrosis.\(^9\)

An NP-associated neurodegeneration study reported neural inflammation and damage in the hippocampus.\(^10\) Another study reported that nanosized carbon black caused increased levels of inflammation-related proteins, such as monocyte chemotactic protein-1, IL-6, and C-reactive protein.\(^11\) Moreover, mice exposed to silica NPs revealed increased levels of haptoglobin, C-reactive protein, and serum amyloid-A, supporting the hypothesis of neurodegeneration through chronic inflammation.\(^12\)

We are investigating the role of NPs in various neurodegenerative diseases, including Alzheimer’s disease, transmissible spongiform encephalopathies, Parkinson’s disease, and amyotrophic lateral sclerosis, by monitoring inflammation biomarkers. Neurodegenerative diseases such as these share a common similarity of misfolded proteins: β-amyloid, prion protein, α-synuclein, and superoxide dismutase, respectively.\(^13\) Since oligomers of these respective proteins may cause neurotoxicity through reactive oxygen species induction, oligomers could be an important biomarker for disease diagnosis. Oligomer-based techniques, such as oligomer detection of β-amyloid and α-synuclein, chemical techniques using thioflavin, real-time quaking-induced conversion assay, protein misfolding cyclic amplification, and multimer detection systems along with imaging analysis, could all be effective tools for diagnosis.\(^14,\,15\)

In Alzheimer’s disease, we have observed neuroinflammatory states in transgenic animal models. In these animal models there was slow but significant disease progression in later stages. In transmissible spongiform encephalopathies, activated microglia and astrocytes have been observed on transmission of infectious prion in vivo, including elevated levels of proinflammatory innate cytokines, such as TNF-α, IL-1β, and IL-6.\(^16\) The above studies suggest chronic neuroinflammation as the root of neurodegenerative diseases. Therefore, NP-induced chronic inflammation and related neurodegeneration should not be neglected in the risk assessment of NPs.

Due to diverse interactions between NPs and proteins, the mechanisms of neurotoxicity remain controversial, especially in long-term exposure. Since the neurodegeneration and neurotoxicity of NPs may progress slowly, the steady loss of neurons may not present any particular pre-symptoms until extensive neuronal damage has occurred. We are currently conducting neuro-nanotoxicity studies of zinc oxide and silica oxide NPs in 20 and 100nm sizes with both positive and negative charges by oral administration in rats. And we are continuing the verification of blood biomarkers, in parallel with other imagining techniques such as magnetic resonance and positron emission tomography, for various neurodegenerative diseases.

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