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Interaction of nanoparticles with central nervous system and its consequences

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ABSTRACT

Nanotechnology has gained a non-replaceable role in a host of applications in biomedical, agricultural, consumer, military, and in industrial sectors. However, when observed through the perspective of the central nervous system (CNS) particularly, studies evidently provide data on both beneficial and detrimental effects of nanoparticles (NPs). While there exist a milieu of beneficial applications like axonal regeneration, CNS imaging, neurological surgeries etc., NPs open the doorway for an array of toxic reactions in CNS centers because of their effortless passage through brain barriers. Even though literature endow with generalized toxic mechanisms mediated by NPs in varying tissues, specific toxic reactions in CNS are still lacking. Present review mainly focuses on different routes through which NPs get access into the brain and certain modes of toxic mechanisms exhibited by NPs in CNS. A number of applications of NPs in the diagnosis and treatment of CNS disorders also will be reviewed in concise.

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Introduction

Nanotechnology refers to the manipulation of matter at the atomic or molecular levels. Nanoparticle (NP) is the most fundamental constituent in the fabrication of nanomaterials which exhibits a size range of 1 billionth of a meter (1 nm = 10^{-9} m). Conceptually, NPs possess far smaller size than that of the conventional objects whose properties obey Newton's law of motion; however, it is larger than an atom and those particles which behave in agreement with quantum mechanics. Globally recognized and accepted magnitude of an NP falls in between 1 and 100 nm. Expansion in the fields of biology, chemistry, and material science gave support to the parallel advancements in the development of a wide variety of nanomaterials with unique advantageous properties. Nevertheless, the emerging advances in nanotechnology ground, there exist only constrained knowledge regarding the toxicity aspects of NPs in a living system. Nanotoxicity mechanisms vary widely in accordance with different physicochemical properties of NPs as well as different bodily locations of exposure. Among the susceptible organs, the central nervous system (CNS) (which is the principal member controlling whole bodily functions in harmony) is comparatively needed to be emphasized further [1].

Nervous system is a network of interconnected cells which are playing the principal role in the control and regulation of intact bodily functions. It consists of two major categories: the CNS and peripheral nervous system (PNS). PNS includes both sensory neurons and motor neurons; both are on stage with different roles. Sensory neurons are responsible for the primary perception of various stimuli and transferring them to the CNS where further processing occurs. Motor neurons carry the processed instructions from the CNS towards the peripheral tissues in order to modulate activities. Nervous system includes excitable nerve cells or neurons which carries information in the form of electrical signals all the way through the body. Nerve cells meet together at specialized structures called "synapses" which connects neurons together and also with other centers. Although neurons fluctuate in their size and complexity,

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the primary mechanism and function remain in identity [2].

Brain and spinal cord constitute the fundamental parts involved in CNS. Spinal chord arises from the brain stem, further runs through the spinal cavity whilst the brain is kept inside the cranial cavity. Brain is the chair for numerous specific vital functions of the body including consciousness, memory, speech, vision etc. Different specialized cell types are seen in CNS owing to their functioning [3].

There exist a number of reasons behind increased receptiveness of CNS towards diseases. Some of them are high energy demands to meet unrestrained metabolic reactions, high intracellular concentration of oxidative stress targets like proteins, lipids, nucleic acids etc., minor levels of endogenous scavengers like superoxide dismutase, catalase etc. Commonly observed reasons for CNS disorders comprise various types of traumas and infections; degeneration of cells, structural abnormalities and tumors, stroke etc. Unlike many other tissues, CNS has inferior levels of regenerative capacity and is therefore considered to be very delicate [4]. Surveys proved that approximately 1 billion people around the world are suffering from CNS related complications with varying intensities ranging from migraines to lethal neurodegenerative diseases including Alzheimer's and dementia, parkinsonism, multiple sclerosis, epilepsy, cerebrovascular, and other infectious diseases. In addition, ~12% death around worldwide is primarily due to CNS diseases which eventually affect aged people [5,6].

Cerebrovascular diseases imply disturbances associated with the vasculature lining the CNS which ultimately results either in hemorrhage (rupturing of blood vessels causing leakage of blood into CNS tissues) or ischemia (insufficient supply of blood into CNS tissues). Both of these conditions often lead to another noxious situation like stroke [7]. Upon taking potential exogenous substances into consideration, NPs imply one of the major toxic representatives causing CNS toxicity. Despite the protective barriers in the brain, most of the known NPs are able to enter into the brain and associated areas thereby causing chronic and dreadful consequences. Unlike the rest of the tissues, the magnitude of nanotoxic effects in CNS is often unpredictable and hazardous. Therefore, a better understanding of the alarming toxic effects caused by NPs in CNS must have to be discussed in depth. Possible ways of NP interaction with CNS and consequences are narrated in the following sections.

Nanotoxicity Associated with CNS

Unpredictable beneficial effects due to the increased surface area to volume ratio exhibited by NPs drives their use as an effective ingredient in a wide range of commercial as well as medical products. Manufactures are nowadays introducing nano components in their mainstream products so as to exploit the beneficial aspects residing in them [8]. However, highly activated surfaces enable the NP to induce carcinogens and mutagens in living systems along with enhanced cyotoxicity. Coupled with these surface properties, size effects are also equally relevant. Seeman et al. [9] compared the size of NPs with red blood cells (RBCs) as they are about 100 times smaller than that of RBCs [9]. This tiny size range increases the potential of interaction with nucleic acids, proteins, and other relevant biomolecules along with certain organs like lungs. As a wide range of metallic as well as non-metallic NPs are used because of their antimicrobial properties in consumer products and other devices, more care should be taken as far as the toxicity level is concerned.

Although there are evidence of nanotoxicity in several biological systems, present review mainly focuses on the effects of NPs on CNS. One of the greatest challenges that researchers met in the 21st century is that; the ability of NPs to cross the biological barriers is the primary reason in the wake of their functional and toxic effects in neural cells. Intentionally and unintentionally exposed NPs gain entry through ingestion, inhalation, and dermal routes very well. Furthermore, they enter into systemic circulation, migrate to different regions on the body and thereby gaining residence in such sites; ultimately exhibit the particular toxic actions [10].

NPs which are well known for their potent neurotoxic effects include single-walled and multi-walled carbon nanotubes, metal oxides like SiO_x, TiO_2 etc., which cause worse effects in both CNS and PNS similar to that of neurotoxins [11]. It was reported that one of the manufactured carbon NPs; fullerenes also caused oxidative stress in the brain of largemouth bass in juvenile stage [12]. The outcome of such NP interactions is that a large number of pathological conditions with varying intensities arise in CNS; most of which are incurable. Even if there exist an array of therapeutic preparations available for such CNS related complications, most of them fail to reach the target site due to the presence of some specialized barriers in the brain. Blood–brain barrier (BBB) constitutes the

principal representative of such barriers which selectively prevents the passage of foreign substances from blood to the brain.

Association of Nanoparticles with BBB

BBB is the decisive interface between brain and systemic circulation which is dynamic in nature. BBB is the major entrance way for most therapeutic drugs administrated to CNS. As the name implies, it is the "barrier" for loads of foreign substances as well as drug candidates entering into the circulation via their way to the brain. Highly specialized organization attributes to their uniqueness among other blood capillaries of the rest of the body parts. BBB is supplied with fewer levels of pinocytic vesicles with no fenestrations while having more mitochondria to meet high energy demands. A cross link between adjacent cells (through tight junctions) makes up a functional unit which is crucial for the maintenance for BBB. They are found to be surrounded by pericytes and astrocytes along with neurons and phagocytic microglia [13]. The tight junctions are covered with large fatty deposits which effectively prevent the passage of hydrophilic moieties along with it. But this part of BBB must be analyzed carefully as it demonstrates the possible way of hydrophobic substances to pass through. For example, metallic NPs like TiO_2, SiO_2 etc., which are poorly water soluble with sufficient nano-size enters into the brain in a much easier manner [14]. A schematic comparison between brain capillaries and general capillaries is shown in Figure 1.

A peculiar efflux transporter protein present on the endothelial compartment of BBB called P-glycoprotein (P-gp) plays a significant role in the strengthening of the protective effect of BBB. It is an important cell protein effectively regulating the flow of drugs and other molecules into and out of the brain. Increased affinity of P-gp protein towards hydrophobic molecules denotes the probability of NP admission into the brain [15]. Metabolic products like glucose and smaller ions like Na^+, K^+, Cl^- etc move across the BBB which is aided by specific transport channels present [16].

BBB presents only diminished level transport of drugs and other moieties across it, particularly because of the presence of junctions instead of deep fenestrations. As a rule, there are three types of junctions present in BBB: Tight junction, adherent junction, and gap junction. The transendothelial resistance of BBB is mainly provided by tight junctions [17]. Moreover, a high electrical resistance provided by the pericytes and astrocytes (~1,500–2,000 Ω cm^2) surrounding the endothelial cells in BBB provides additional blockage to different components to pass through. These difficulties make drug delivery into the brain a more complicated task. However, different strategies have been developed in order to overcome this. Surface functionalization protocols including chemical modification of drugs and prodrugs, employing neurosurgeries and usage of NPs to aid local administration as well as temporary disruption of BBB etc., have already been accepted worldwide [18].

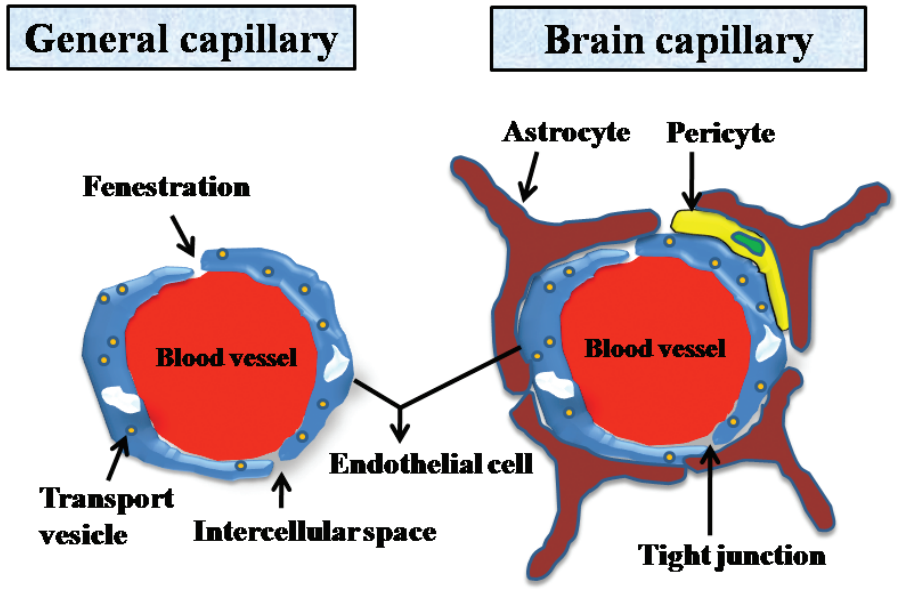

Figure 1. A comparison between general capillary and brain capillary.

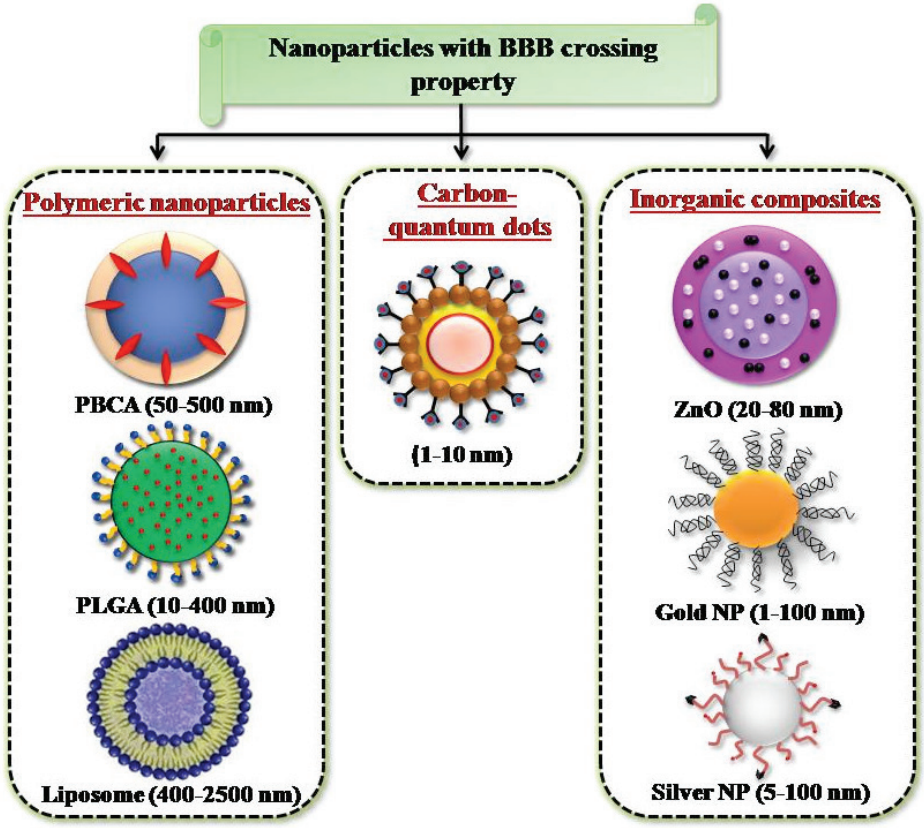

Figure 2. NPs developed for targeted drug delivery into the brain.

Numerous NPs have purposefully developed and identified to have the exclusive property of crossing BBB to establish targeted drug delivery into the brain. Some of them include inorganic composites of gold, silver, and ZnO NPs, polymeric NPs including Polybutyl cyanoacrylate (PBCA), PLGA [Poly (lactic-co-glycolic acid)], liposomes as well as photo-luminescent quantum dots (QDs) (Fig. 2).

Main Routes of Nanoparticles into Brain

The major transport routes towards brain adopted by a broad range of NPs remains in unity. They include BBB pathway, olfactory nerve translocation pathway, and placental barrier pathway; each of which involves varying cellular components and courses.

BBB pathway

There has been a parallel advancement in the field of nanomedicines and recognition of the role played by BBB in their applications. BBB brings out a dual role by both providing a key protection to CNS from toxins and also by mediating molecular exchange between neurons, capillaries, and neuroglia. Being a "neurovascular unit" controlling and normalizing sustained supply of essential nutrients, glucose, and oxygen, BBB plays an inevitable task in managing optimal CNS functioning. In return, neurons also have proven to be providing nutritional factors for survival of BBB. This mutual interaction results in the restricted transport of molecules across BBB. Greater surface area of BBB turns it to be the major barrier in the brain even though additional minor barriers like cerebrospinal fluid (CSF) (Blood-CSF), CSF-brain barrier is present in the brain.

The major pathways through which molecules get ahead of BBB include passive diffusion, carrier- and receptor-mediated transport, and adsorptive transcytosis. Unlike other cellular membranes, passive mode of diffusion by molecules is relatively lower in the case of BBB since tight junctions are there in between adjacent endothelial cells. Certain smaller lipid soluble molecules including barbiturate drugs (e.g. Methohexital, Amobarbital etc) are able to cross BBB effortlessly primarily by means of passive diffusion. Receptor- and carrier-mediated transport mechanism is usually preferred by molecules to gain access into the brain. Carrier-mediated transport, on the other hand, regulates the bidirectional transport of molecules by the way of cerebral homeostasis. Certain efflux transporters present on the BBB selectively expels moieties out of the

barrier into the bloodstream; especially toxic ones. Indeed, this enforces some of the NPs to revisit bloodstream from the brain parenchymal side. This clearly emphasizes a possible risk for drug delivery into the brain (e.g., P-glycoprotein or P-gp on BBB) [19]. Being the foremost transcytosis routes preferred by NPs, only adsorptive and receptor-mediated transport mechanisms are briefly shown in the following sections:

Adsorptive transcytosis

Adsorptive mode of transcytosis occurs chiefly on the basis of electrostatic manner of attraction between positively charged extracellular macromolecules like proteins and NPs with that of the negatively charged cellular membrane. Interaction of mentioned particles with plasma membrane triggers a specific endocytic pathway which involves either clathrin-coated pits or caveolin protein in association with the invaginated vesicle. Clathrin-mediated pathway presents coated invaginations containing the particles to be transported towards the abluminal side of the brain province. Even though both clathrin and caveolin are present in the luminal side of the endothelial cells, clathrin is abundant in luminal than in the abluminal area. This is the fact behind the occurrence of "unidirectional" clathrin-mediated transcytosis of NPs from bloodstream to brain capillary. In fact, the caveolin pathway also embraces the same mode of transport. However, the relative concentration of caveolin in brain capillary is poorer than peripheral capillaries. Molecules such as albumin and chitosan could be used to surround NPs for transport. Transcellular passage of NPs has been under research as it is an effective mean of drug delivery into the brain. In the meantime, the role of adsorptive transcytosis in the transport of endogenous substances is still under exploration [20].

Receptor-mediated transcytosis

In the receptor-mediated mode of transcytosis, definite ligands are being incorporated onto the NP surface which has an affinity towards the endothelial receptors on the cell surface. These ligands include insulin, lactoferrin, transferrin, and certain antibodies. Surfactant molecules like polysorbate 80 can also be used to functionalize NPs since it selectively unite with lipoproteins in circulation (such as apolipoprotein E) and hence, enable binding with lipoprotein receptor-related proteins on the endothelial cell surface. Ligand interaction with specific surface receptors elicits plasma membrane to invaginate to form vesicles which ultimately aids in their delivery into the abluminal side of brain parenchyma. A representation of various mechanisms of NP delivery into brain abluminal side is shown in Figure 3. NPs mostly failed to retain in the brain neighborhood

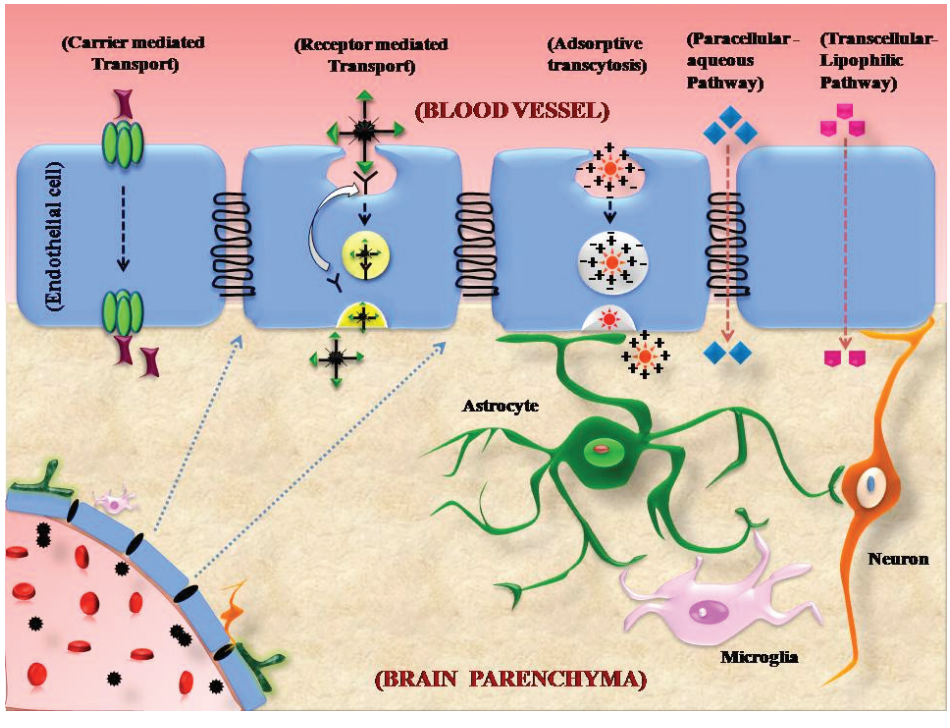

Figure 3. Various mechanisms of NP transport into brain parenchyma.

because of the presence of efflux pumps on the cell surface [21].

Nose to brain pathway

Intranasal administration of therapeutic agents has been a trustworthy mean of drug delivery into brain since it provides an opportunity to circumvent BBB. The olfactory or trigeminal neurons constitute the underlying components involved in this pathway and these neurons arise in the brain and end up in the nasal cavity. High surface area abounding high rate of blood flow, porous nature of the endothelium, instant accessibility make nasal mucosa an apposite route for NP preparation into brain regions [22]. The nose to brain transport of NPs is achievable primarily through three pathways: Olfactory pathway, trigeminal pathway, and systemic pathway.

Olfactory pathway

Once the NPs gain entry through the nose, they travel along the nasal mucosa or otherwise termed as nasal epithelium. Nasal epithelial cells possess an intimately arranged stuffing along with tight junctions, adherent junctions, and desmosomes. This arrangement allows a paracellular mode of particle transport through the spacing in between the nearby cells or via transcellular way through the cells. Preceding this entry, NPs soon interact with cilia residing at the terminal portion of olfactory receptor neurons. This interaction initiates the transduction process on the road to the brain. Captured NPs then acquire a voyage through axon of receptor neurons and arrive at olfactory bulb situated at the forebrain and the CSF. From the CSF, NPs integrate into the brain by mixing with interstitial fluid [23].

Olfactory pathway possesses two sub-divisions namely: Intraneuronal pathway and extraneuronal pathway. Predictable axonal transport through olfactory receptor neurons constitutes intraneuronal pathway; whereas passage across perineuronal channels is termed as an extraneuronal pathway. Intraneuronal route consumes several hours to days to get access into the brain; while few minutes are adequate for the other. NPs and materials get accomplished into the deeper areas of the brain like cerebrum, cerebellum etc. by this olfactory pathway of transport [24].

Trigeminal pathway

Trigeminal nerve is the fifth and largest among the cranial nerves and is largely accountable for the sensations from face to the brain. It is located within the brain and has three major branches named: Ophthalmic, maxillary, and mandibular. It manages the motor functions like biting and chewing. Among these, ophthalmic and maxillary play major roles in transport of NPs towards the brain. Portions of these neuronal branches found to diffuse through the nasal mucosa and also possess connections with an olfactory bulb of the brain. Hence, those NPs which have entered into the mucosal layer in the nasal cavity pass through the trigeminal neuron in the respiratory and olfactory regions via axonal transport and arrive at tail regions of the brain including spinal cord, medulla, and pons [25]. There are reports proving prompted delivery of insulin-like growth factor-1 into forebrain after intranasal administration by means of a trigeminal pathway. Following absorption through the nasal cavity, NPs diffuses via mucosal membrane by different transport mechanisms like carrier-mediated transport, receptor-mediated transport and transcytosis etc. by means of paracellular and transcellular mode. Studies suggest that specific cation transporters, amino acid transporters, dopamine transporters etc. are at hand for carrier-mediated NP transport through nasal mucosal membrane [26].

Systemic pathway

Blood circulation comprises an added passageway for NPs entering into the nasal cavity. Augmented supply of vasculature of respiratory epithelium than olfactory mucosal membrane unlock doorway of NPs into systemic circulation. This in turn induces broadened release towards the brain and CNS. Specialized organization of respiratory endothelium comprising uninterrupted as well as fenestrated portions allow liberated passage of both small and large NPs into bloodstream and ultimately into cerebral tissues. In contrast to large hydrophilic particles, smaller lipophilic ones exhibit effortless entry into blood and BBB. Distributed NPs from systemic circulation rapidly enter into nasal blood vessels from where they come into carotid blood supply towards the brain and spinal cord by a unique counter current mechanism [27]. The three

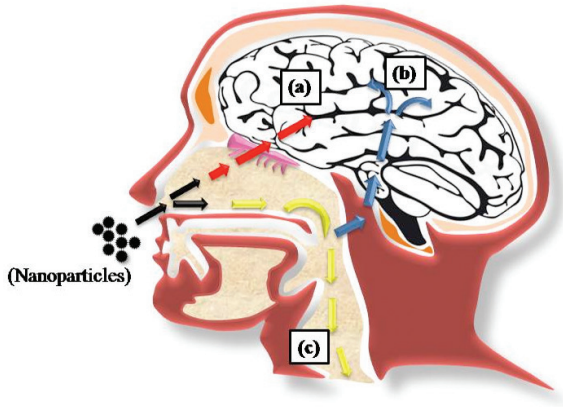

Figure 4. Major nose to brain pathways for NP transport into the brain. (a) Olfactory pathway, (b) trigeminal pathway, and (c) systemic pathway.

local noses to brain pathways for NP entrance into the brain are shown in Figure 4.

Placental barrier pathway

Placental barrier constitutes another vital internal interface that shelters developing embryo. Besides, it provides protection to the fetus from the detrimental effects of exogenous substances which may pull in from maternal circulation at some stages during development. At the same time, it acts like a better platform for the transport of nutrients and oxygen from the maternal blood supply and thereby playing a decisive role in the proper development and maturation of embryo. Meanwhile, recent studies evidently provide data demonstrating the transfer of potentially harmful moieties including NPs from the maternal body and their subsequent gathering in the fetal brain exclusively. This was confirmed during experiments using NP exposed pregnant mice; which showed relocation towards the fetal brain in subsequent time periods. Further studies with this affected progeny reveal the fact that transferred NPs results in neuronal death which was preceded by impaired homeostasis inside fetal brain tissue [28].

Several psychiatric disorders like schizophrenia, autism, mental depression etc. have been demonstrated to be in close association with these maternally transferred hurtful consequences in developing fetus [29]. Certain outcomes observed in the brain tissue of rat neonates after the exposure of pregnant mice with TiO_2 NPs, in general, are shown in Figure 5.

Cellular Mechanisms of Nanoneurotoxicity

In order for the proper manufacturing of nanomedicines, a precise knowledge about cellular mechanisms of NP uptake is mandatory. Well-known mechanisms behind NP uptake include phagocytosis, macropinocytosis, clathrin, and caveolin-mediated endocytosis [30]. Cells largely vary in their NP uptake mechanism. Moreover, NPs exhibit different

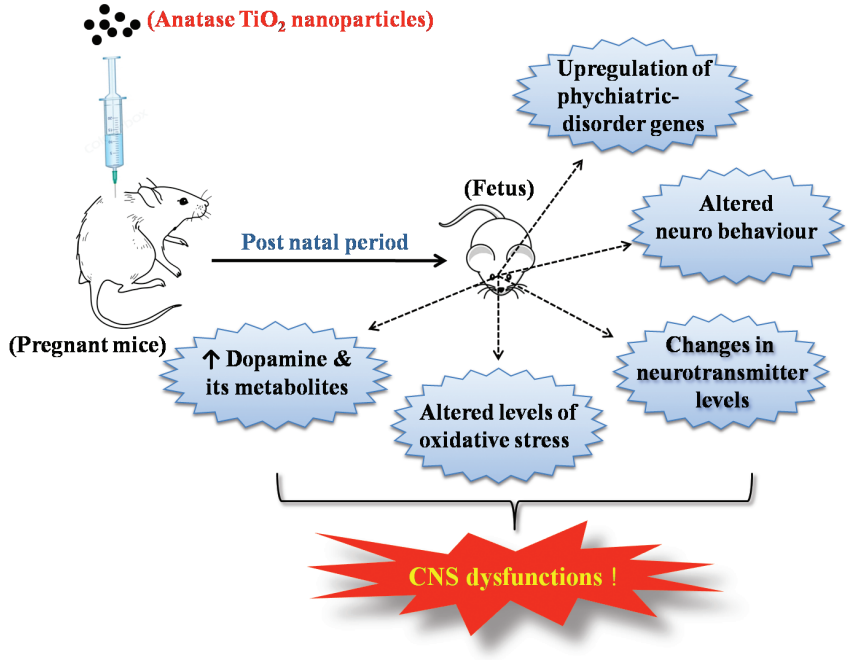

Figure 5. General consequences of anatase TiO_2 NPs in rat neonate's brain after maternal transport through the placenta.

intracellular location for each cell types. NPs doorway into the cells is analogous to that of other cell types; in which initially they enter into a monolayer of endothelial cells followed by accumulation along the conventional endolysosomal pathway (includes a set of interconvertable membrane-bound intracellular compartments which are under regulation of Rab family of proteins). Finally, they enter into lysosome as a part of the delivery of other intracellular components for further processing. Because of the minute size, NPs remain all along this pathway and hence affecting the normal morphology and functioning of BBB directly. This prolonged intracellular residence of NPs lead to undesirable interactions with biomolecules including proteins, nucleic acids, carbohydrates as well as lipids. Ultimately, these nano-biomolecular interactions results in harmful consequences like conformational changes in proteins, increased permeability of cell membranes, genetic mutations, induction of signaling pathways, oxidative stress, lipid peroxidation, disturbed ion exchange equilibrium, expression of additional protein epitopes or vanishing of existing epitopes, or even variations with enzymatic activities [31].

Upon interaction with NPs, cells of the immune system (especially astrocytes and microglia) get forced to induce apoptosis, inflammatory reactions, and oxidative stress also. Unfortunately, poor capacity of the brain to regain its lost cells (lack of tissue regeneration) makes it difficult to treat most of the brain damages. This drawback is the major obscurity in the assessment of specific nano-neurotoxic mechanisms in tissues. Moreover, different NPs (from lighter liposomes to harder metal oxides) exhibit different cellular toxicity mechanisms; therefore, scientists are busy with finding out the exact reasons about what the NPs are causing during cellular interaction.

Geraets et al. studied the biodistribution as well as blood and tissue kinetics of micro- and nano-sized cerium oxide NPs upon inhalation exposure in rats during and after 28 days. Ten percent of exposed NPs was found to be accumulated in pulmonary regions after single exposure wherein no significant effect of particle size upon tissue accumulation was observed. Similar trend was reported for extra-pulmonary distribution of the NPs also after 6 hours of single exposure. Following exposure for the proposed period, the study focused on body clearance of cerium oxide NPs as well. Results showed that the NPs showed only an insignificant level of elimination even after 48 and 72 hours of circulation. However, the study concludes the fact that further effects of cerium oxide NPs in pulmonary as well as extra-pulmonary regions are needed. Also, the oral exposure studies are yet to be done in future [32]. Some of the neurotoxicity mechanisms of NPs are discussed in the following sections.

Oxidative stress

Mitochondrial Adenosine triphosphate (ATP) generation is the major source of energy generation in cells. It releases an array of reactive chemical species as byproducts including reactive oxygen species (ROS), reactive nitrogen species, and sulfur- and carbon-centered radicals, hydrogen peroxide (H_2O_2) etc. [33]. For proper functioning, development, and maintenance of CNS, there should be an optimal level of reactive chemical species in action. However, if these species exceed their optimal range, potential damage occurs. During most of the circumstances, the potential ROS produced is effectively eliminated by brain's own antioxidant systems including superoxide dismutase, peroxidase, catalase etc. Even though such protective mechanisms are present for proper oxygen consumption and redox generation capacity, scavenging capacity of brain antioxidant systems falls short if the amount of potential chemical species is beyond the optimal range. The role of ROS like nitric oxide (NO) in long-term differentiation and synaptic plasticity was analyzed by O'dell et al. The study proved that injection of inhibitors to the postsynaptic cell or perfusion blocks long-term potentiation and exogenously produced NO increases presynaptic release of the transmitter in culture [34].

CNS is well known for their susceptibility to ROS. This is generally attributed to the reasons of high oxygen demands (high metabolic rates), lower levels of antioxidant systems, unique terminal differentiation properties of neuronal cells etc. Studies have proved that oxidative stress contributes to toxicities of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and dementia. Associated DNA damages occurring during these complications were proved to alter neuronal cell viability.

Regulation of oxidative stress in CNS by NOX enzymes

Although mitochondria is believed to be responsible for the chief source of ROS generation, another category of transmembrane proteins called Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) enzymes is also known to be contributing for this. Emerging investigations are focusing mainly on the involvement of NOX enzymes in inflammatory

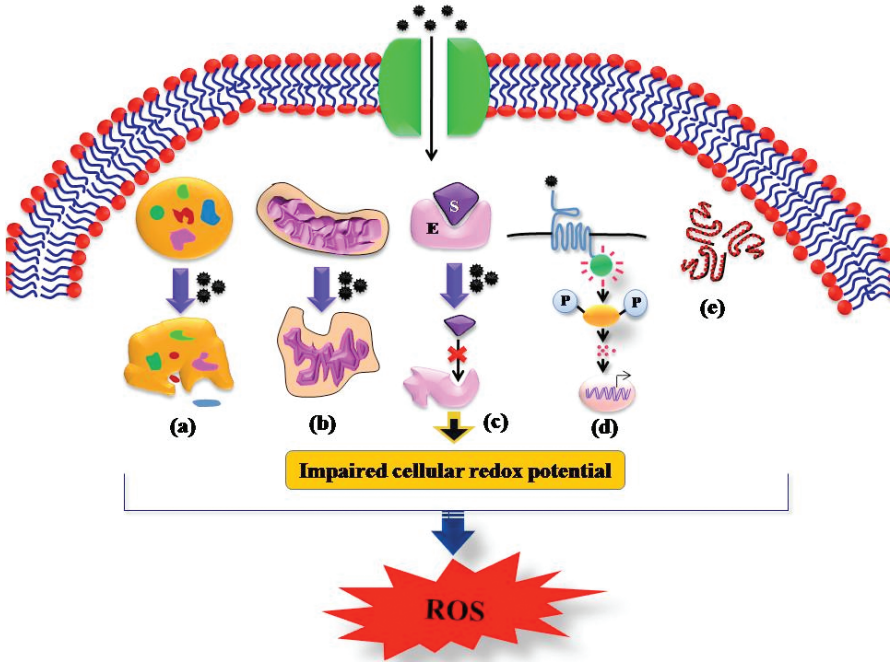

Figure 6. Modes of interactions of NPs with cellular components and emergence of ROS. (a) Impaired lysosomal integrity, (b) mitochondrial damage, (c) structural damage to enzymes, (d) disturbed signaling pathway, and (e) distorted protein strands (e.g., NADPH).

diseases and numerous other complications in CNS. The major contributor of the inflammatory response of microglia in the brain is NOX2 genes which get transcribed during the invasion of a foreign antigenic substance into the brain or any other cerebrovascular diseases [35].

Among the identified NOX genes so far, seven classes are known to be most prominent. They include NOX1, NOX2, NOX3, NOX4, NOX5, Dual oxidase -1 (DUOX)-1 , and DUOX2. NOX2 is also known as gp^{91phox} ("phox" meaning phagocytic oxidase) and is the first identified NOX enzyme in phagocytic glial cells. NOX2 produces ROS upon interaction with another transmembrane protein, p^{22phox} and some other cytosolic proteins called p^{47phox}, p^{67phox}, and p^{40phox}. In addition to these, either one of the two Rho-Guanosine triphosphate (Rho-GTP) binding proteins (Rac 1 or 2) has also been found to be bind with NOX for ROS generation. Other categories including NOX1, NOX3, and NOX4 have the same transmembrane component in association with but show difference in activation cascade [36]. Among these discovered proteins, NOX4 is constitutively expressing without any interaction with cytosolic components while its interaction with Rac is still under investigation [37].

Besides the alterations in membrane structure and function, lipid peroxidations etc., ROS mostly affects mitochondrial DNA system since it is devoid of any DNA repair enzymes like conventional nucleic acids in the nuclear compartment. Insoluble protein accumulation due to oxidative reactions is one of the underlying reasons behind several neurodegenerative pathologies. High surface area of NPs mainly contributes to the generation of ROS, the main factor directing cellular pathogenesis. Moreover, different cell types exhibit varying degree of susceptibility for toxicity towards the same NP. Hanley et al. demonstrated the varying susceptibility of different human immune cells towards ZnO NPs of about 20 nm size. According to the study, ZnO induced a significant level of cytotoxicity depending on the NP-cell membrane association, phagocytic ability, and inherent ROS generating capabilities. Among the cells studied, monocytes were greatly underwent cytotoxic actions of ZnO, whereas natural killer cells (NK cells) lymphocytes showed moderate and highest susceptibility, respectively. Study also confirmed that ROS generation was the primary cytotoxic mechanism exhibited by ZnO which in turn depends on NP size. The major inflammatory mediators produced by ZnO NPs were interferon (IFN)-γ, tumor necrosis factor (TNF)-β, and interleukin (IL)-12 [38]. Some modes of interaction of NPs with cellular components and the emergence of ROS are shown in Figure 6. However, even in this

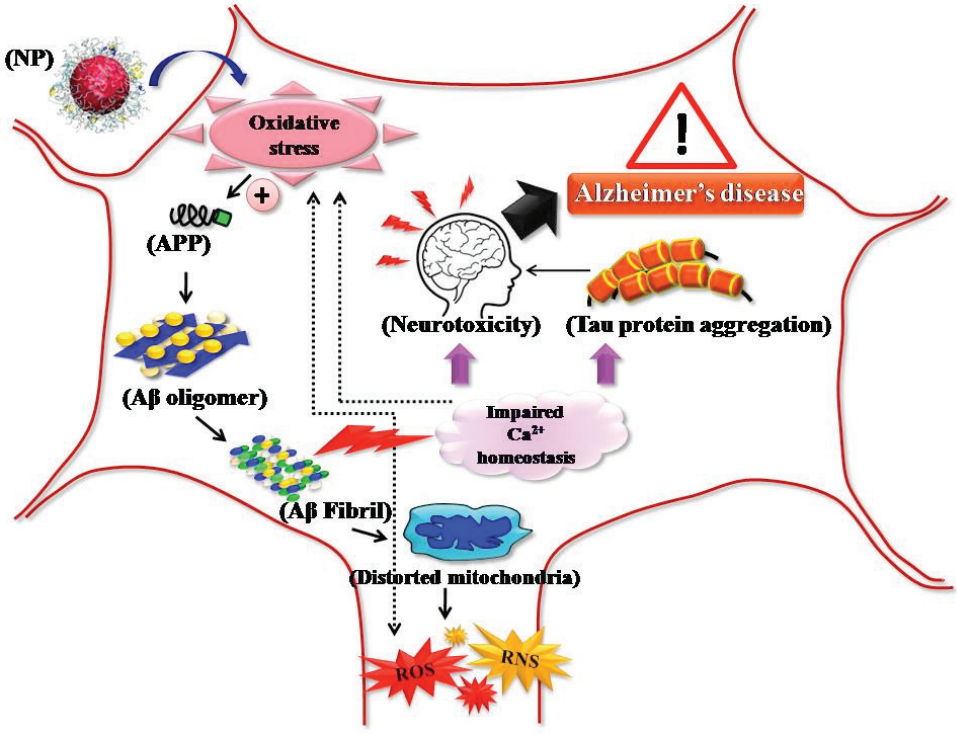

Figure 7. NP-induced oxidative stress, mitochondrial damage, and corresponding aggregation of destabilized protein strands.

century, a detailed evidence on NP interactions and oxidative stress in incomplete. Hence, the data drawn here are only based on the current available literature.

Mitochondrial interaction of nanoparticles

ATP is a minuscule chemical that is well known as the "energy currency" of the cell. They are responsible for the incessant energy supply to meet the whole energy demands of biochemical reactions. Mitochondrion is the site where ATP is generated through electron transport chain (ETC) across their specially organized membrane [39]. NPs upon entrance into the cytoplasm accumulate in mitochondria; thereby disturbing the ETC. As this respiratory chain functioning is directly coupled with ROS generation, NP influence on this ETC results in elevation of oxidative stress to an atypical level. A noteworthy level of neuropsychiatric diseases is proven to be happening due to these mitochondrial membrane dysfunctions and oxidative stresses. Oxidative stress and associated mitochondrial damage sometimes cause aggregation of destabilized protein strands. Also, non-specific post-translational modifications of proteins accompany the hurdles in neurodegenerative disorders like Parkinson's disease (PD) and Alzheimer's disease (AD) (Fig. 7).

An *in vivo* study by Bressan et al. confirmed the fact that silver NP (Ag NP) interaction with human dermal fibroblast results in specific accumulation in mitochondrial compartments despite their presence inside the cytoplasm. Also, it was observed that Ag NPs moved towards the nuclear compartment and surrounded the nucleus to act as a physical barrier from ROS and associated defects. It was interesting that neither of the cellular organelles except mitochondria were badly affected. The disintegrated mitochondria caused the release of antioxidant enzymes including mitochondrial superoxide dismutase, catalase, glutathione peroxidase, thioredoxin peroxidase etc. into the cytoplasm and concomitant protection of nucleus from ROS attack [40].

An elevation of Bax/Bcl-2 levels was sometimes obtained with a prior declining in mitochondrial membrane potential, ultimately causing cellular apoptosis. This concludes the fact that mitochondrion plays a mediator role in ZnO induced apoptosis [41]. During the investigation of toxicity of TiO_2 NPs in rat and human glial cells (C6 and U373, respectively), it was observed that a significant induction in strong oxidative stress happened in both the glial cell lines. Specifically, the researchers focused on certain events taking place concomitantly with oxidative stress like the assessment of the oxidation level of a redox potential marker

compound; 2',7'-dichlorodihydrofluorescien diacetate, lipid peroxidation study by cis-parinaric acid, real-time PCR analysis of antioxidant enzyme expression, and Rh123 and MitoTracker Green FM staining for mitochondrial membrane damage detection. Gene expression analysis confirmed an elevation in the expression of genes for glutathione peroxidase, superoxide dismutase, and catalase [42]. Consequent studies revealed caspase-3 activation, chromatin condensation, and necrosis after mitochondrial membrane damages in C6 and U373 cell lines [43].

Interaction of NP with cytoplasmic enzymes regulating redox potential

A collection of antioxidant enzymes and certain compounds like NADPH play decisive roles in maintaining overall redox potential of the cell. Catalases, peroxidases, and superoxide dismutase are some of the prominent antioxidant enzymes present which protect against detrimental health effects in the cell. Once entered into the cytoplasm, NPs interact with the cellular enzymes, especially antioxidant enzymes, by this means disrupting the redox potential of the cell. In this way, NPs induces elevation of ROS production [44]. Nanostructures ceria and cerium oxide (CeO_2) is one of the industrially relevant NP which has wide applications for being a suitable automobile exhaust catalyst, ion oxide conductor in fuel cells, electrode component in gas sensors, UV absorbent etc. Even though ceria is a weak luminescent material; hence, having limited biomedical applications; recently, it was observed that after intravenous administration in rats, they unpredictably altered both brain oxidative stress indicators and antioxidant enzymes. This study sticks to the fact that metal NPs possess the potential neurotoxic ability. Hardas et al. found that a single dosage of 30 nm ceria NPs altered expression of Phase-II proteins in the hippocampal region of the rat. Since these proteins are a set of enzymes essential for the protection from certain mutagens and even carcinogens entering into the brain, trouble in their production and maintenance markedly causes invasion of above-mentioned toxic agents and lead to harmful diseases [45].

Wilhelmi et al. investigated the toxicity level of ZnO NPs (RAW 264.7). The study indented to focus on the apoptotic and necrotic damage of macrophage since it has significant roles in clearance of particulates entering via inhalation and maintenance of immune response during inflammation. It was observed that ZnO NPs significantly induced nuclear condensation, DNA fragmentation, and formation of apoptotic remnants and hypodiploid DNA fragments. ZnO caused oxidative DNA damage owing to their ability to cause DNA strand fragments in RAW 264.7 cells and $p47^{phox}$ NADPH oxidase depended on superoxide generation in macrophages derived from bone marrow. Study concludes that ZnO NPs cause apoptotic cell death chiefly via NADPH oxidase and Nrf-2 independent pathways and it is also reported to be prompted by some other alternative routes as well [46].

In 2010, a panel of researchers analyzed the effect of industrially pertinent cerium oxide NPs (doses significant in chronic inhalation or contact with skin) in murine 3T3 fibroblast cells. It was observed that there occurred a dose depended release of superoxide dismutase and catalase into the culture medium together with a decline in cell viability, induction of apoptosis in early stages, and declination in antioxidant capability. Study came into a conclusion that the observed cytotoxicity is due to the commencement of both mitochondrial as well as Nox2- and Nox4-dependent NADPH oxidase systems [47]. These studies clearly emphasize the possibility of application of efficacy of NPs in the inhibition of ROS generating enzymatic pool in cells for treatment of oxidative stress-related cardiovascular and neurodegenerative disorders. However, the exact mechanism of action of nanomaterials in CNS by the way of ROS generation is still unclear and need to get explored more.

Stimulation of ROS generation by nanoparticle exposure

As with other signaling cascades, NP (mimics signaling molecule) once binds with the specific cellular receptor; induces a series of reactions inside the cell ultimately causing abnormal ROS genesis. An ideal cell responds to oxidative stress via the activation of ROS produced during NP exposure initiates a series of intracellular reactions chiefly by means of microtubule associated protein kinase (MAPK) pathway. Normally, Phase II category of drug metabolizing enzymes is regulated by nuclear factor erythroid 2 related factor 2 (Nrf2) on the nucleus. This Nrf2 factor binds with antioxidant response element consensus sequence. The major transcriptional factors involved during this gene expression are Activator protein-1 (AP-1) or Nuclear factor κβ (NF-κβ). These factors further fallout in the generation of antioxidant enzymes for the exclusion of stress-related ROS. Conversely, NP exposure leads

to an alteration in this gene expression causing over-production of ROS [48].

Wang et al. studied the effect of ZnO NPs in primary astrocytes culture. They found that, in the vicinity of the particular target cell, nano ZnO produces ROS which in turn stimulates an intracellular signaling cascade chiefly by means of the (JNK) pathway. Egr-1 expression was sharply elevated during this ZnO exposure which is one of the membrane-associated protein stimulating further occurrence of the gene expression pathway. Scientists confirmed the inhibitory action of the antioxidant compound N-Acetyl cysteine on this expression of phosphorylated Egr-1 and (ERK). Treatment of the JNK pathway inhibitor SP600125 decreased ZnO induced caspase-3 expression and cleavage of Poly ADP – ribose polymerase (PARP) protein. Whereas there was no inhibitory action of ERK pathway; confirming the involvement of JNK pathway for the particular toxicity reaction of ZnO NPs in primary astrocytes [49].

In spite of the varying chemical composition, size, and surface properties, the capabilities of NP to elicit an intracellular signaling pathway, specific genetic mechanisms, nuclear reactions etc. depend on the level of ROS produced either intracellularly or extracellularly. Moreover, it is a well-accepted fact that the cellular/tissue response of the biological system depends on the specific types of protein corona surrounding the NPs immediately after entrance. The difference in shape, size, functional group, hydrophilicity, hydrophobicity etc., of the particles determines the type of proteins covering on them. This corona of proteins further influences the type of signaling mechanism they impart [50].

It has been increasingly known that upon degradation of coated NPs inside lysosomes, the prospect of ROS generation is comparatively high due to the interaction of degraded products form the particles and the acidic lysosomal environment. Once get internalized by the lysosome inside the target cell, NPs disrupts the phospholipids bilayer and enhances lysosomal membrane permeabilization. The hydrolytic enzymes including caspases and cathepsins contained inside the lysosome get released into the cytoplasm due to the increased permeability of the lysosomal membrane. Oxidative stress triggered by the NPs also leads to lysosomal degradation ultimately causing DNA fragmentation and cell death. Iron oxide NPs release ferric ion into the cytoplasm from lysosome and late endosomes via divalent metal transporters. Susceptibility of cell functionality is greatly elevated by this release of ferric ions [51].

Effects in immune function

Microglia cells (20% of total glial cells in the brain) represent the primary line of defense mechanism in CNS which share loads of properties and functions to that of macrophages in other tissues. However, during a resting stage or in non-activated form, they exhibit a "ramified" morphology which is normally absent in other resident macrophages in the body. They inhabit in parenchymal cells of CNS. Although microglia denotes the major candidate for immune function, there are some additional immune cells which are not part of parenchymal cells in CNS. They comprise perivascular and meningeal macrophages, pericytes etc. These are found to be enveloped by basement cells of the perivascular region inside blood vessels [52]. Once encountered with a foreign antigen like NPs, immediately they are able to change their morphology and form in order to present a better platform for defense properties.

Choi et al. did a first attempt to study the effect of silicon-based NPs in primary astrocytes *in vitro*. They observed using TEM and confocal fluorescence microscopy that even low concentrations of Si NPs were able to elicit a notable change in astrocyte morphology and gene expression levels. Genes coded for cytokine release and pro-inflammatory responses were markedly increased, hence demonstrated the involvement of microglial cells in the immune response of CNS [53].

An optimal level of activity of microglial cells is mandatory for the proper functioning of brain immune mechanisms. However, overstimulation sometimes leads to neurotoxicity by the release of surplus amounts of proinflammatory mediators and cytokines. Major inflammatory mediators released during the commencement of a brain immune response include IL 1-β, TNF-α, IFN-γ, prostaglandin E2 etc.

Several NPs have been proved to be capable of inducing inflammatory mediators from brain cells upon exposure. For example, TiO_2 and hydroxyapatite (HAP) NPs triggered the expression of genes encoded for NO synthesis during treatment with astrocytes *in vitro*. Xue et al. then collected the cell-free supernatant from microglial cells which contained the NP-induced soluble factors like proinflammatory mediators and given to PC12 culture *in vitro.* In contrast to expectations, the applied supernatant altered the gene expression

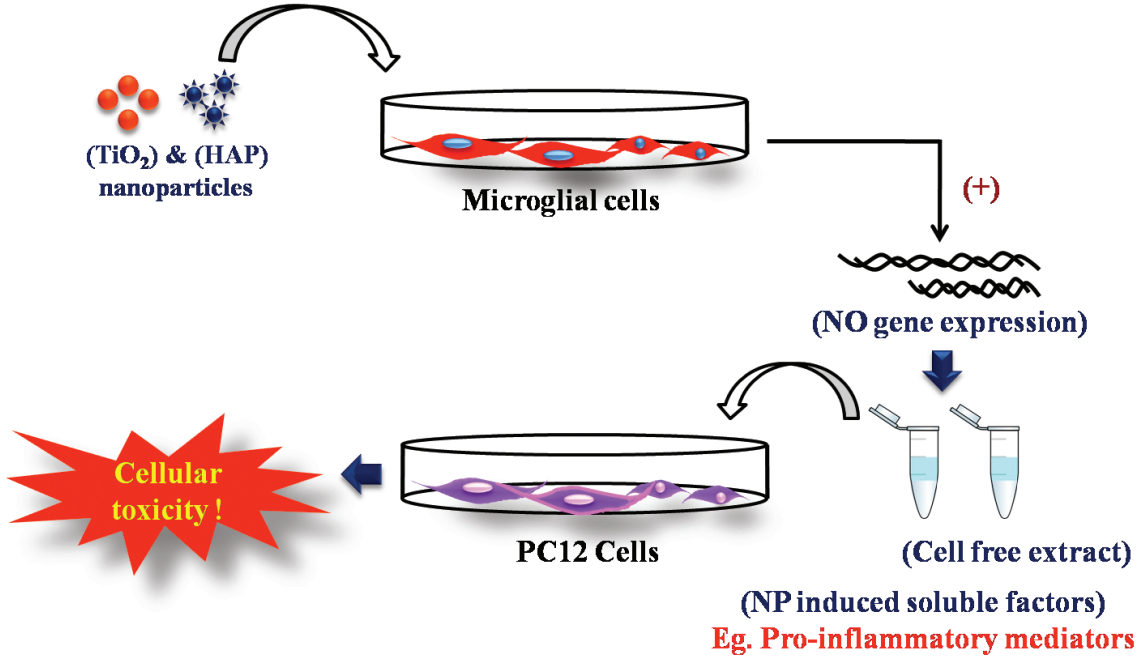

Figure 8. Possible mechanism of action of pro-inflammatory mediators produced in microglial cells exposed to TiO2 and HAP nanoparticles on PC12 cells.

in PC12 cells causing cellular toxicity. From this result, researchers concluded that NPs are capable of inducing microglial cells and consequently release pro-inflammatory compounds which alter PC12 functioning and finally cause cytotoxicity. Figure 8 illustrates possible mechanism of action of pro-inflammatory mediators produced in microglial cells exposed to TiO_2 and HAP NPs on PC12 cells [54].

Deregulation of autophagy and apoptosis in neuronal cells

Autophagy refers to an evolutionarily conserved cellular mechanism for the expulsion of damaged cellular organelles and other debris, proteins which have lost their lifespan and functionality through a lysosome-mediated elimination mechanism. The mentioned components migrate towards lysosome for degradation either as endosomes or as autophagosomes. Neuronal cells are comparatively susceptible more to changes with these interactions, principally with aging. Any type of mutations in genes involved in autophagy indisputably leads to neurodegenerative disorders like AD, PD, Huntington disease (HD), amyotrophic lateral sclerosis etc. Mutations occur at various stages of autophagy and hence pathophysiology and therapeutic strategies differ from disease to disease. One of the common symptoms seen in a majority of neurodegenerative diseases is the accumulation of protein aggregates which failed to degrade through autophagic pathways in a conventional manner. In addition to this, dysfunctionalized cell organelles like mitochondria are often present [55]. Examples for protein aggregates observed in neurodegenerative disorders include hyperphosphorylated tau-containing neurofibrillary tangles in AD, α-synuclein aggregates (lewy bodies) in PD, N-terminal fragments of mutated Huntington (htt) in HD etc.

In an attempt to study the role of autophagic genes in potential protein aggregation associated with neurodegenerative disorders, Cheung et al. did a neuron-specific knockout experiment in mice. Study evidently proved that genes mainly involved in autophagy are Atg5, Atg7, and beclin-1 [56]. Numerous inducers have been identified for their role in autophagy in brain cells. NPs are one among the prominent autophagy modifiers which contribute to potential neurotoxicity. Kenzaoui et al. evaluated the response of brain-derived endothelial cells to different NPs of different nature and size. They used uncoated and citrate-coated iron oxide NPs (core size = 8–9 nm), 25 and 50 nm sized fluorescent Silica NPs, PLGA-PEO or [poly(lactic-co-glycolic acid)]-[poly(ethylene oxide)], 21 nm TiO_2 NPs. The study showed that immediately after cellular internalization, all of the tested NPs migrated toward lysosome and subsequently prompted lysosomal hydrolytic enzymes; especially proteases.

Iron oxide and TiO_2 NPs caused DNA strand breakages which accompany oxidative stress damages. A sharp elevation in autophagic gene expression was also observed [57].

ZnO treatment of macrophages caused induction of oxidative stress which is accompanied by apoptosis and autophagy. Oxidative stress was happened primarily due to dysfunctioning of antioxidant enzymes which additionally included lipid peroxidation and increased protein carbonyl contents in the macrophage. Apoptosis markers like capase-3, 8, and 9 were cleaved upon ZnO exposure and autophagy marker proteins like microtubule-associated protein-1 and beclin-1 were also found to be elevated after 0.5–24 hours of treatment. PI3K/mTor/Akt signaling pathway was picked up by ZnO to confer its autophagic effects in macrophage [58].

Studies claim that autophagic processes are supposed to play either cytoprotective or cytotoxic roles after NP interaction. Their action is closely related with apoptosis or necrosis of cellular components. Hyperstimulation or hypostimulation of autophagy often result in cytotoxicity, unfortunately. Treatment of different neuronal cell lines with TiO_2 NPs-induced apoptosis via varying mechanisms including altered redox potential of the cell, depolarization of mitochondrial membrane, and poor level of antioxidant enzyme activity. For example, human astrocyte cell-like astrocytoma U87 showed high-level death even at 1 μg/ml concentration of TiO_2 NPs [59].

Being a major mechanism for the removal of large-sized foreign particles entering the cell, autophagy is the chief method by which cells depend on for the effective elimination of potential NPs. Numerous physio-chemical characteristics including surface components, charge, size, and shape together with biological nature like the interaction with different biomolecules determine the interaction with cellular components including autophagy and apoptosis.

Factors Affecting Toxicity of Nanoparticles in CNS

Different routes used by the cell to uptake NPs, intracellular processing mechanisms, cytotoxicity events etc. are determined by the different surface characteristics of the NPs entering the biological system. Key factors influencing extend of cellular toxicity of NPs in CNS is reviewed in the following section.

Effect of nanoparticle shape

There is only limited data available regarding the consequence of NP shape on cytotoxicity so far; whereas innumerable literature exist which describe the generalized toxicity mechanisms adopted by NPs in a biological system. The fact is cells show a different level of response to NPs of different shapes like round, rod, spiral, wire etc. Meng et al. proposed that cancer lines habitually exhibit a nature of unusual level sensory mechanism for the acceptance of NPs gaining entry into them. The study analyzed the particular property using A549 and HeLa cell lines using mesoporous silica NPs (MSNPs) of different aspect ratios. MSNPs of aspect ratios ranging in between 2.1 and 2.5 were taken up in comparatively high amounts. MSNPs with intermediary aspect ratio effectively stimulated the emergence of filopodia in maximal level. Also, the particles induced actin polymerization and activation of small GTP-binding proteins like Rac 1 and CDC42 mediating actin filament assembly and hence perfect cytoskeletal arrangement. The study identified the involvement of macropinocytosis in the whole uptake process [60].

HAP NPs have been a subject of interest from several years because of some exceptional properties of them similar to that of human bone tissues. They are extensively used as a filling material in defected tissues, as a coating material covering tissue implants, in drug-releasing processes, also, as a purifying agent in column chromatographic procedures etc. However, HAP was shown to be the reason for certain inflammatory reactions in cells. Xu et al. proposed the cytotoxic effect of HAP NPs with different morphologies like short and long rod-shaped, spherical, and needle-shaped with varying size ranges (10–20, 10–30, and 20–40 nm) in primary osteoblast cells of the rat. The study confirmed the growth inhibition of osteoblasts in a dose depended manner. Also, it was observed that particles bearing relatively smaller surface area stimulated apoptosis in a lower propensity compared to those with the higher surface area. Needle-shaped and short rod-like particles caused greater level of cellular damages than spherical and long needle-shaped ones. p53 and cytochrome c expression were also elevated together with apoptotic cell death. Mechanical trauma caused by the sharp edges of the particles along with greater surface area is the main contributing factors behind these cellular deformities [61].

Effect of nanoparticle size

To date, numerous studies are focusing their attention towards the consequences of NP size on cellular systems. Studies proved varying toxic effects as the NP size differ. Thus, careful scruitinization is necessary during designing of NP preparations for biomedical applications. Kim et al. analyzed the effect of Ag NP size on the cytotoxicity levels in MC3T3-E1 and PC12 cell lines *in vitro.* Ag NPs of three different sizes (~10, 50, and 100 nm) were used for the study. Size and dose depended alterations in cellular viability, ROS production, lactate dehydrogenase production, and stimulated expression of genes controlling stress reactions were observed. Interestingly, it was striking that Ag NP of 10 nm showed improved cytotoxicity than 50 and 100 nm sized ones; confirming the inverse proportionality of NP size and respective cytotoxicity. Moreover, MC3T3-E1 cell line was stimulated to be undergone apoptotic cell death while necrotic cell death was predominant in PC12 cell line during Ag NP treatment [62].

Meanwhile, *in vivo* studies proving the effect of NP size on animals; especially in CNS is relatively deficient. As a short-term remedy, Sharma et al. conducted an experiment involving chronic administration of engineered NPs from metals like Ag, Cu, and Al in mice. Different size ranges of the particles were used for the study (50–60 nm, 50 mg/kg, intraperitoneal administration on a daily basis for 1 week). This chronic administration resulted in a significant influence on BBB in the way of neurotoxicity; proving harmful consequences in CNS during chronic NP exposure. BBB was deeply disrupted and severe pathological lesions seen in the case of old mice (18–22 weeks aged). In addition to particle size, the age of mice was also contributing to the nanotoxicity levels. Compared to young and old aged mice, middle-aged ones are far more susceptible to NP-induced neurotoxicity. Since toxicity of Al NP was lesser compared to those of Cu and Ag, study further sticks onto the fact that apart from size, inherent properties of NPs also strongly influence on toxicity levels. BBB breakdown was accompanied by brain edema, improved gene expression of the glial fibrillary acidic protein, and vesicle formation in myelin sheath around neuronal cells [63].

Effect of Zeta potential of nanoparticle

Overall charge distribution on the surface of NP influences greatly on cellular uptake and distribution. Since the bilayered membrane of cells are organized in a manner that the average charge is largely anionic. Consequently, NPs having a cationic charge distribution are likely to enter through this bilayered membrane more easily. This electrostatic interaction between cellular membrane and NP surface in turn influences on NP toxicity levels. Tarn et al. proposed the improved production of ROS by the exposure of cationic NPs than anionic and neutral ones [64]. Negatively charged or anionic NPs are generally believed to be lesser toxic because of their repulsive interaction with cell membrane components. Marked changes in membrane integrity and charge distribution of neuronal cellular membrane by NP exposure cause adverse impacts on signaling cascades and impulse transmission along the neuron. Obviously, any disturbances in the membrane integrity and potential by charged NPs cause harmful consequences such as neurological disorders.

Effect of aggregation and dispersion status of nanoparticle

The actual initial cellular response towards potent NPs primarily depends on the physio-chemical identity of the particle; generally termed as "synthetic identity." Furthermore, the dynamic composition of the extracellular matrix with which the NP comes into contact will then induces a change in shape, size, surface characteristics (modify by "protein corona") etc. to generate a "biological identity." This biological identity differs from previous synthetic identity and this new identity further determines the type of cellular response including endocytic and other signaling pathways along with cellular uptake. Immediately after getting the "signal" of biological identity, cells usually undergo a process known as "cell conditioning" in which the composition of the extra cellular matrix (ECM) changes enormously consequently altering protein corona on the NP. As a part of cell conditioning, cells are forced to release certain proteins and other metabolites into ECM and influences further particle aggregation. Cell's phenotypes, conditions adopted for culturing, surface characteristics etc. are the main factors influencing biological identity and cell conditioning. The dynamic nature of the ECM should be considered while dealing with the analysis of NP-cell interactions. The aggregation potency of NPs is found to be necessary for the cellular uptake of smaller NPs [65].

Chithrani et al. proposed that clustering of cell membrane receptors is necessary for getting enough potential energy for the membrane wrapping and further endocytosis in the way of particle

internalization (especially smaller monodispersed particles) [66]. Another study recommends that before the execution of NP-cellular interaction, there occurs the generation of "pre-aggregation" of NPs which induces the gathering of membrane receptors. This receptor aggregation greatly improves the amount of energy required for particle uptake and processing than their monodispersed counterparts [67]. Inherent particle design and properties influence greatly on this pre-aggregation.

Monodispersed NPs have been the choice of interest for the study of the influence of particle size on cellular toxicity. However, it was also observed that inorganic NPs tend to aggregate in biological media with high intensity compared to organic one. Sadhukha et al. demonstrated well the effect of aggregated superparamagnetic iron oxide NPs in the treatment of cancer cell lines by means of magnetic hyperthermia. The study clearly brings out the fact that monodispersed NPs induce apoptosis-mediated cell killing mechanism like that of conventional hyperthermia treatment whereas micron-sized aggregates depended on oxidative stress mechanism via temperature dependent autophagy. Also, in the micron size range, NPs caused rapid membrane damage, hence achieving acute cell killing. Lower doses of NPs present more active surfaces to cause cellular toxicity because of peculiarity in their nano size range rather than that observed in bulk forms. In contrast to this dispersed particles, high doses of NPs show aggregation tendency (probably in the absence of any surface modifiers) due to the close proximity of adjacent particles. These nanoaggregates effectively mask the reactive counterparts of the dispersed particles in suspension. Hence, as a whole, aggregated NPs in their higher concentrations are less toxic than in their dispersed stages [68].

Effect of surface modification of nanoparticles

In order to obtain a stable suspension, NPs should be subjected to surface modification using suitable agents. This improves not only their dispersion ability but also the extent of biocompatibility. Improved repulsive force raised during surface modifying agent is the contributing factor behind this phenomenon. In this way, the modified surface of NPs effectively change the biological response they are indent to bring out. Modification of metal NPs with thiol group is a perfect example for this which incorporates sulfur atoms on the particle surface and prevents aggregation [69]. Relevance of surface modification of NPs was further confirmed by the investigation by Oberdörster et al. using fullerene NPs in the brain of juvenile largemouth bass. There occurred declining of glutathione peroxidase (GSH) along with lipid peroxidation after exposure of 0.5 mg/l of an aqueous suspension of fullerene C60. On contrary to this finding, another study proved the antioxidant action of fullerenes modified in their C3 and C5 positions with carboxylic groups against iron oxide NP-induced oxidative stress. This protective response evidently reveals the peculiarity of surface modification of potent NPs during interaction with biological systems [70].

In an attempt to study the effects of four different types of NPs on the brain of mice, Zhang et al. found that along with size effect and solubility properties, surface modification using suitable agents remarkably persuade on cellular toxicity levels. Surface modification of nano-TiO_2 with silica significantly improved its solubility in circulation and entered into cerebral cortex as well as to brain striatum via the intranasal route. Whereas non-coated hydrophobic nano TiO_2 particles of comparable size could transport only up to striatum. Also, it was observed that micron-sized TiO_2 particles failed to transport into sub-brain regions; hence be a sign of deprived translocation ability of NPs raised due to size effect. In addition to evaluating pathological changes in the brain, the influence of nano TiO_2 in monoamine neurotransmitter was also studied. Monoamine neurotransmitter plays a significant role in the regulation of autonomic functions, motor activities, sleep-wake cycle etc. Quite a lot of studies are there proving the onset of psychiatric abnormalities including schizophrenia, depression, anxiety, hyperactivity disorders etc. caused due to the deficit in monoamine neurotransmitter. The researchers proved that hydrophilic nano TiO_2 altered the levels of monoamine neurotransmitter release indicating the probability of occurrence of deficiency disorders [71]. Depending on the data from loads of toxicity studies reveals that surface modified or coated NPs cause elevation in the inflammatory cellular responses than uncoated particles. For instance, surface modification of nano TiO2 using vanadium pentoxide exhibited improved expression of genes controlling the release of pro-inflammatory mediators and genetic responses.

When looking through the perspective of gold NP, they hold a great level of potential for future use in nanomedicine due to their enormously high surface specific volume ration as well as unusual surface properties. Sousa et al. evaluated the uptake and distribution of surface functionalized

gold NPs *in vivo* using mice model. Modifier used was a polyelectrolyte multilayer created in such a way to be utilized as a moiety for drug release in prion diseases. After intravenous injection of NPs, investigators followed brain distribution of the particle using near-infrared time-domain imaging up to 7 days. Maximal concentration of the particle was obtained in brain regions between 9 and 24 hours of injection. Different microscopic techniques and X-ray analyses showed that the coated gold NPs were able to cross BBB and then entered into specified regions of the brain including the hippocampus, thalamus and hypothalamus, and in the cerebral cortex also. Peak level concentrations were seen in areas where protein aggregates specific to neurodegenerative disorders normally appears (like β-amyloid and prion proteins). One of the advantages using gold NPs in this scenario is that easy detection of them using different analytical tools in the diagnosis of neurological imperfections is possible [72]. There are studies proving that the unique optical properties of gold NPs can be successfully be used for visualization and quantification of synthesis of neurochemicals in biological systems. This works entirely on the basis of colorimetric methods which hold gold promise for future in nanoscience [73]. However, possible health hazards of gold NPs are still under exploration and highly recommended because of the increasing demands.

Applications of Nanoparticles in Diagnosis and Treatment of CNS Disorders

The field of analytical neurochemistry is endowed with a milieu of techniques for the screening of neurochemicals and neuronal activities. Studies claim that simultaneous analysis of a large number of neurochemicals always seems to be challenging. This necessitates the categorization of analytical techniques into imaging techniques, sampling and separation techniques, and electrochemical techniques. Major imaging techniques include fluorescence, functional magnetic resonance imaging (MRI), positron emission tomography, and mass spectroscopy. Sampling and separation techniques include microdialysis, push–pull perfusion, high-performance liquid chromatography, microfluidics and capillary electrophoresis. Electrochemical techniques include exocytosis measurements, fast scan cyclic voltammetry, and electrode development. Most of these techniques exploit the unique properties of NPs to perform [74].

Nanoparticles in neuronal cell imaging

Apart from the toxicity aspects, the foremost advantage of using NPs is the improved ability to monitor and track molecules present in CNS locality. Number, type, development, and physiological activities can also be identified for desirable time periods. Fluorescent NPs like QDs attached with specific antibody molecules complementary to that of cellular components open door towards these possibilities with successful results [75]. Such NPs provide a dual purpose of both imaging and improvement of metabolic functions via changing cellular responses.

In an imaging study, researchers conjugated such fluorescent QDs with nerve growth factor (NGF); which is an essential protein for the proper development, functioning, and survival of neurons. Upon exposure of this QD-conjugated NGF with potent tumorigenic neuronal cell line for a proposed time period, there occurred significant alterations in cellular responses including changes in cell signaling pathways and development of neurites from neuronal cell bodies [76]. Investigators claimed that use of fluorescent QDs to a large extend could reduce both deprived photostability (i.e., poor ability to provide sustained fluorescence) and signal-noise ratio (i.e., undesirable background fluorescence masking the actual signal fluorescence) than the conventional fluorescent probes. Such a system was reported to be useful in the tracking of lateral movement of glycine receptors (a well known inhibitory neurotransmitter in human nervous system); while conventional techniques were a failure in proper monitoring of synaptic development and neurotransmitter functioning since the neurotransmitter receptors is not static in their sites on the cell surface [77].

Nanoparticles as neuroprotective agent

One of the difficult tasks during the treatment of neurological injuries like cerebral infraction and traumas is the generation of a number of chemical species including oxygen free radicals, superoxides, and peroxides. Over accumulation these compounds perhaps lead to impairment of mitochondrial functioning, alterations associated with transport proteins, elevation in levels of intracellular calcium, sometimes induction of apoptosis. An array of pharmacological approaches has been developed to brush off these difficulties; but exhibited limited efficacies. Meanwhile, use of NPs offers an excellent alternative for this crisis of undesirable

secondary reactions during CNS injuries. For example, fullerenes; a well-known carbon NP has been studied in detail as a free radical scavenger. Results indicated it as an effectual antioxidant agent [78].

Nanoparticles for stem cell migration studies in CNS

It is believed among neurobiologists and clinicians that stem cell therapy is an excellent opportunity to battle with numerous CNS diseases since it aids in the regeneration of vanished cells in damaged brain tissue [79]. Currently, stem cells are administrated by direct intravenous injection including for enzymatic disorders. Iron oxide NPs have been for a long time for the monitoring of particle-tagged cells in damaged tissues by the aid of MRI technology. For analyzing the effect of particle tagged stem cells, investigators induced cerebral infarction in mice and provided iron oxide coupled stem cells in order to study further fate of them in CNS. Scientists depended on MRI to locate transplanted cells and obtained successful results [80].

Nanoparticles as CNS sensors

Localized physiological changes within CNS have been monitored by researchers using NPs which mimic the actions of magnetic nanoswitches. Engineered iron oxide NPs are mainly used for the intention of *in vivo* analysis of dynamic changes within CNS. It is mainly based on the relative concentrations of certain molecules (sugars, proteins, amino acids etc.) in the local area of interest. Assembly and disassembly of the particles in accordance with the concentration of these molecules cause indications in the form of magnetic relativity using MRI. Fluctuations in the levels of molecules symbolize corresponding physiological changes existing within CNS [81]. All of the above-mentioned studies emphasize ongoing applications of NPs in CNS therapy.

Conclusion

Scientific collaboration with nanotechnology began with the advent of NPs and their exciting traits to be used with high potentials. However, concern about their alarming harmful outcomes originated recently leaving gaps to be filled even in this century. Concerning certain toxicity mechanisms, NPs display varying intensities from one organ to another. Being a more susceptible organ, nanotoxicity of CNS has to be emphasized more since it is the "control center" of every living system. Major pathways employed by NPs to pierce into the brain include BBB pathway, Nose to brain pathway and through the placental barrier; each of which presents different components and pathways. Upon entering the brain, NPs results in various neurotoxic mechanisms in accordance with their physicochemical properties. Moreover, after interaction with NPs, cells standing for CNS immune functions will get forced to induce toxic reactions like oxidative stress, apoptosis, and other inflammatory pathways. Oxidative stress and associated mitochondrial damage in turn contribute to aggregation of protein strands and non-specific post-translational modifications; leading to AD and PD. In addition, NPs also have identified to be one among the modifiers of cellular autophagic response leading neurodegeneration. Major characteristics driving these toxic effects include NP size, shape, zeta potential as well as aggregation and dispersion status.

In summary, although NPs have been under extensive research for recent years; precise toxic reactions and underlying mechanisms for those actions in specific tissues and organs are not yet elucidated in detail. Particularly, the role of NPs in originating neurological disorders and possible mechanisms for preventing these abnormalities is still behind the veil. Future research has to be polished to bring innovative strategies to elucidate the molecular mechanism of neurotoxicity in CNS along with promising approaches for circumventing it.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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