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Nanoneurotoxicity to Nanoneuroprotection Using Biological and Computational Approaches

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Nanoparticles (NPs) that are ∼100 nm in diameter can potentially cause toxicity in the central nervous system (CNS). Although NPs exhibit positive aspects, these molecules primarily exert negative or harmful effects. Thus, the beneficial and harmful effects should be compared. The prevalence of neurodegenerative diseases, such as Alzheimer disease, Parkinson disease, and some brain tumors, has increased. However, the major cause of these diseases remains unknown. NPs have been considered as one of the major potential causes of these diseases, penetrating the human body via different pathways. This review summarizes various pathways for NP-induced neurotoxicity, suggesting the development of strategies for nanoneuroprotection using in silico and biological methods. Studies of oxidative stress associated with gene expression analyses provide efficient information for understanding neuroinflammation and neurodegeneration associated with NPs. The brain is a sensitive and fragile organ, and evolution has developed mechanisms to protect it from injury; however, this protection also hinders the methods used for therapeutic purposes. Thus, brain and CNS-related diseases that are the cause of disability and disorder are the most difficult to treat. There are many obstacles to drug delivery in the CNS, such as the blood brain barrier and blood tumor barrier. Considering these barriers, we have reviewed the strategies available to map NPs using biological techniques. The surface adsorption energy of NPs is the basic force driving NP

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gathering, protein corona formation, and many other interactions of NPs within biological systems. These interactions can be described using an approach named the biological surface adsorption index. A quantitative structural activity relationship study helps to understand different protein-protein or protein-ligand interactions. Moreover, equilibrium between cerebrovascular permeability is required when a drug is transferred via the circulatory system for the therapy of neurodegenerative diseases. Various drug delivery approaches, such as chemical drug delivery and carrier-mediated drug delivery, have been established to avoid different barriers inhibiting CNS penetration by therapeutic substances. Developing an improved understanding of drug receptors and the sites of drug action, together with advances in medicinal chemistry, will make it possible to design drugs with greatly enhanced activity and selectivity; this may result in a significant increase in the therapeutic index.

Keywords: Nanoparticles; central nervous system; neurodegenerative diseases; neurotoxicity; oxidative stress; blood brain barrier; QSAR; nanoneuroprotection; computational; bioinformatics

1. INTRODUCTION

Nanoparticles (NPs) are the particles less than 100 nm in diameter, which are typically classified in two categories: combustion-derived NPs (e.g., diesel exhaust particles and welding fumes) and engineered or synthetic NPs (e.g., carbon black, carbon nanotubes, copper oxide, etc.) [1]. The intratracheal uptake of synthetic NPs, such as carbon black (CB), results in pulmonary inflammation. These NPs translocate through the lymphatic system via the lymph. Studies in rats have shown that 13C NPs enter the brain through the olfactory nerves. Studies have also shown that the NPs penetrate the brain, leading to neurodegenerative diseases. Therefore, it eliminates certain NPs using clearance systems, as these molecules become concentrated in the brain over time, resulting in toxicity. Both in vivo and in vitro studies have demonstrated that combustion-derived NPs are neurotoxic [2]. When NPs of specific sizes are inhaled, these molecules penetrate the respiratory tract through diffusion. The uptake and translocation of NPs through epithelial and endothelial cells into the blood and lymph to target sites, such as bone marrow, heart, and lymph, is mediated by the small sizes of these molecules. Studies have shown that NPs also reach the central nervous system (CNS) via transfer through the axons and dendrites of neurons. NPs accessing the skin circulate via uptake into lymphatic channels [3]. In this review, we highlight research on NP-induced neurotoxicity and the mechanisms leading to nanoneuroprotection.

Brain and CNS-related diseases remain one of the most challenging causes of disability and disorder, despite a large number of advances in brain and CNS research. Additionally in the drug design a major challenge is the predicting the BBB permeation [90]. Furthermore, these challenges highlight the need for increased strategies toward the development of medications, treatment therapies, and potential cures. The blood-brain barrier (BBB), a unique

membranous barrier separating the brain from the circulating blood, is a major problem in drug delivery to the brain and CNS [4, 5]. Moreover, despite the estimated total surface area of 12 m^2 of capillaries in the human brain, the active BBB makes the brain practically inaccessible for lipid-insoluble compounds. As a result, the therapeutic value of many favorable or promising drugs is diminished, demonstrating that brain and CNS diseases or disorders are the most obstinate to therapeutic mediations. Drug delivery is most difficult when the target site is a CNS tumor, as genetic alterations in axin, b-catenin, the adenomatous polyposis coli (APC) gene, and the b-catenin-axin-adenomatous polyposis coli APC-glycogen synthase kinase (GSK) 3b multiprotein complex can lead to tumorigenesis [6]. Drug delivery to the neurons in a solid tumor is mediated through the nonuniform distribution of microvasculature throughout the tumor interstitium and might lead to irregular drug delivery [7].

The surface adsorption energy of NPs provides the foundation for nanoparticle gathering, protein corona formation, and many other interactions of NPs within biological systems, which are described using the biological surface adsorption index (BSAI) [8]. The complex interactions and dynamic changes of manufactured or engineered NPs in biological systems have hindered advances in bionanoscience [9–11].

Various drug delivery approaches using therapeutic agents have been established to avoid the different barriers inhibiting CNS penetration [12–14]. Prodrug formation can be used for the uptake of drugs in the brain [15]. Furthermore, chemical drug delivery systems (CDDS) can also be used to target specific sites or organs based on enzymatic activation. Moreover, many circulating nutrients, such as peptides, use intracellular transport pathways, such as carrier-mediated transport (CMT) and receptor-mediated transport (RMT). Receptor-mediated drug delivery to the brain utilizes chimeric peptide technology, in which a nontransportable drug is combined with a BBB transport vector. Liposomes, in which relatively large amounts of molecules can be integrated, are typically used to deliver the desired drug.

The regenerative medicine and tissue engineering utilize metallic ions, which have unique applications in these fields and benefits for therapeutic purposes, such as reduced cost, increased stability, and lower risks, compared with genetic engineering. Biological interactions with NPs, such as metallic ions, play a major role in various diseases and disorders, including cancer, CNS disorders, and so on [16]. Abnormal metallic ion metabolism might lead to pathological states, such as hemochromatosis, Wilson disease, and Menkes disease [17–19]. A novel technology, carbon nanotubes (CNTs), is the most effective technology in the field of nanotechnology due to the unique electrical, mechanical, and chemical characteristics of these molecules, which facilitates the development of a number of mini-sized devices with excellent properties [20, 21].

In this review, we highlight research on NP-induced neurotoxicity and different approaches leading to nanoneuroprotection. A precise overview of

nanotoxicological science and its current state is also included in this review. Furthermore, drug delivery approaches using metal and carbon nanoscale tubes will also be discussed in detail.

2. NPs CAUSING NEUROTOXICITY

Human exposure to NPs has always been prevalent, and increased exposure has resulted from industrialization. In addition to the developing field of nanotechnology, changes in the environment and increased sources of NPs, such as combustion and thermodegradation, have dramatically increased exposure to NPs [3]. Studies have shown that human membranes take up smaller sized and highly activated NPs (i.e., silica) without rejection. These NPs passively diffuse through human membranes. The absorption and enrichment of various poisonous materials (i.e., metals, dioxides, etc.) are the major health hazards that result from exposure to these particles [22]. Tetra-ethyl lead (PbC8H20), formed by lead petrol emitted from car exhausts, accumulates in the human brain when inhaled [23], subsequently leading to neurotoxicity. NPs of different chemicals are more toxic and inflammogenic compared with micro-sized particles of the same chemicals at an equal mass dose [24, 25]. However, in vivo and in vitro studies have demonstrated that combustion-derived NPs are neurotoxic [26].

2.1NPs: Penetration and Circulation into the Brain

The prevalence of neurodegenerative diseases, such as Alzheimer disease [27–29], Parkinson disease, Huntington disease [30], and primary brain tumors [31], has recently increased. The major cause of these diseases remains unknown, but NPs have been implicated as one of the major causes. NPs can penetrate the human body via different pathways (Figure 1), including inhalation, ingestion, injection, and skin penetration. After penetration, these particles may be dispersed via systemic circulation to various tissues or organs [31, 32], including the brain.

2.2 NPs, Oxidative Stress, and Gene Expression Analysis

Lipids, nucleic acids, and proteins are destroyed through oxidative stress (OS) or free radicals at particle concentration and translocation sites. Due to high-energy demands and low levels of antioxidants, the brain is vulnerable to OS [33]. Combustion-derived NPs damage dopaminergic neurons in CNS cultures through high levels of free radicals generated after microglial activation [34]. Experimental studies have implicated reactive oxygen species (ROS) and OS in the pathogenesis of various neurodegenerative disorders [35]. Studies of OS associated with gene expression analyses and immunological markers have

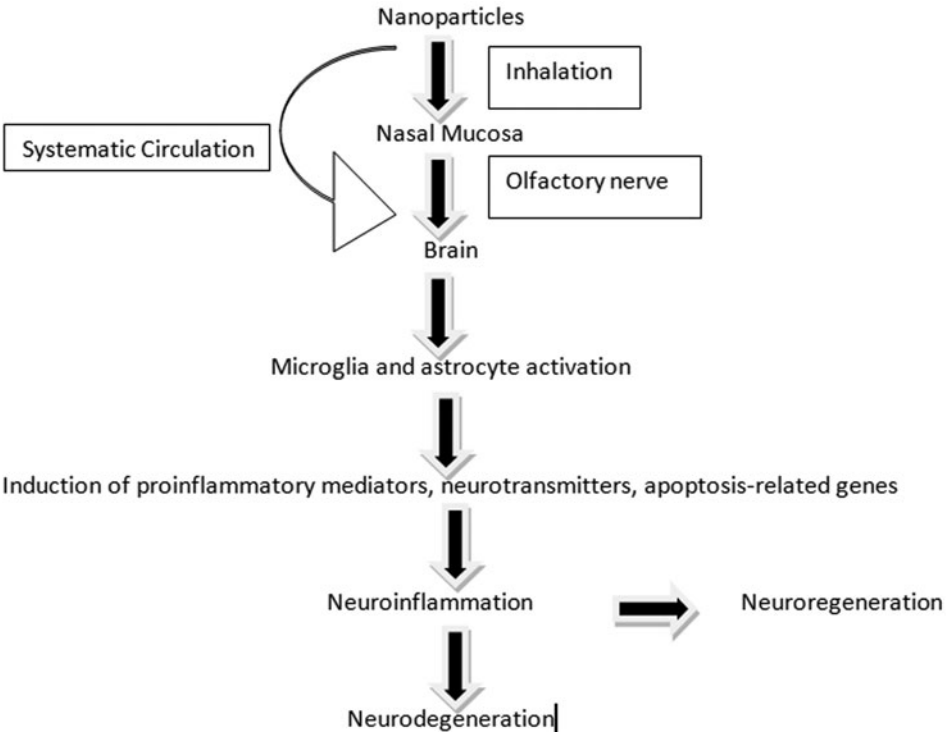

Figure 1: NPs penetration pathways that cause neurotoxicity; adapted from (2).

led to the greater understanding of the mechanisms underlying neuroinflammation and neurodegeneration. NPs (i.e., carbon nanotubes, quantum dots, and ultrafine particles) are a major source of ROS, particularly when exposed to UV light or transition metals [36, 37]. ROS have been associated with neurodegenerative disorders, such as Parkinson disease and Alzheimer disease [30]. Moreover, it has recently been shown that the expression of genes associated with apoptosis and OS changes in response to maternal exposure to titanium oxide (TiO2) NPs in newborn mice [38].

3. BRAIN AND CNS

The brain is a sensitive and fragile organ, and evolution has developed mechanisms to protect the brain from any injury. Unfortunately, these same protection mechanisms also hinder the methods used for therapeutic purposes. Many therapeutic drugs are inefficient in treating brain and CNS diseases because of the difficulties surrounding drug delivery into the brain. Brain and CNS-related diseases remain the most challenging causes of disabilities and disorders worldwide, as a variety of hindrances inhibit drug delivery to the brain

and spinal cord despite numerous advances in brain and CNS research. Thus, these challenges highlight the need for increased strategies toward the development of medications, treatment therapies, and potential cures [4, 5] through drugs that specifically target sites to reduce the harmful effects of toxicity and increase the efficacy of treatment.

3.1 Barriers to CNS Drug Delivery

The barriers to CNS drug delivery are discussed next.

3.1.1 Blood-Brain Barrier

The BBB, a unique membranous barrier separating the brain from the circulating blood, is the major problem for drug delivery to the brain and CNS [4, 5, 39], as shown in Figure 2(A). The structural differences in the blood capillaries of the CNS and the blood capillaries of other tissues generate a permeability barrier between the blood within brain capillaries and the extracellular fluid in brain tissue. The small pores of the capillaries of the brain and spinal cord mediate the translocation of solutes into other organs, but the capillaries of the vertebrate brain and spinal cord lack these pores. These capillaries are lined with unique endothelial cells lacking porous structures and are closed with tight junctions. Similar to this barrier, a tight epithelium is also present in other organs, such as skin, lungs, and so on [40, 41]. This permeability barrier, comprising the brain capillary endothelium, is called the BBB. The transmission of action potentials through salutatory conduction is realized in the myelin sheath (surrounds axons), which is formed and maintained though oligodendrocytes. A high transendothelial electrical resistance of 1500–2000 $\Omega.cm^2$ compared with the 3–33 $\Omega.cm^2$ resistance of other tissues due to the presence of tight junctions between endothelial cells, reduces the aqueous-based paracellular diffusion in other organs [42, 43]. Other nonspecific exchanges have been described in the capillaries of other organs or parts of the body. Moreover, with a total surface area of 12 m^2 of human brain capillaries, the active BBB makes the brain inaccessible for lipid-insoluble compounds. Thus, the therapeutic value of many favorable or promising drugs is diminished, and brain and CNS diseases or disorders remain the most difficult diseases to treat with medication. Furthermore, the BBB is supported through the enzymes that are present in large numbers inside endothelial cells, which contain large mass-to-volume ratios of mitochondria, and solutes passing through the cell membranes are exposed to these enzymes. BBB enzymes rapidly degrade most peptides, including naturally occurring neuropeptides [44, 45].

3.1.2 Blood-Cerebrospinal Fluid Barrier

The blood-cerebrospinal fluid barrier (BCB) is the second barrier encountered by a targeted drug before entering the CNS. The cerebrospinal fluid

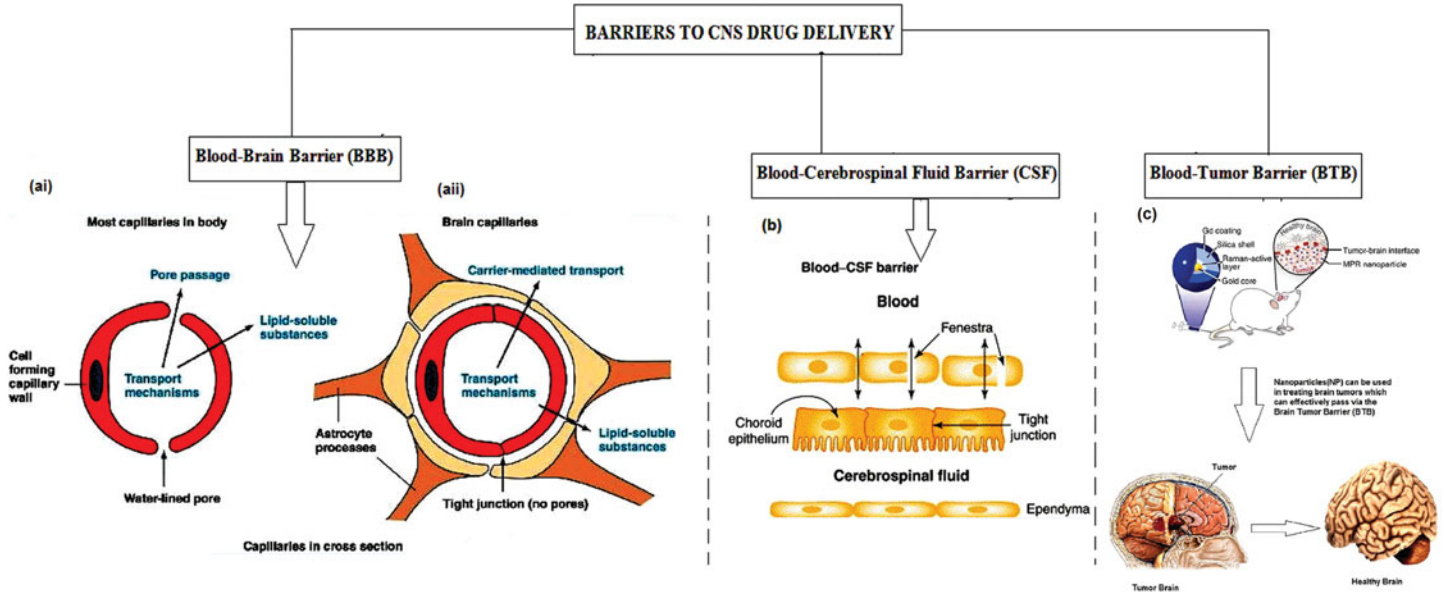

Figure 2: (A) Blood-brain barrier separating the brain from circulating blood. (B) Blood cerebrospinal fluid barrier presents in the epithelium of the choroids plexus that is organized to control the transition of molecules and cells into CSF. (C) Blood-tumor barrier (BTB) showing the mouse brain with a nanodrug that crossed the BTB. This approach can be used to treat a tumor in the human brain. Thus, a healthy brain is distinguished from a tumor-containing brain. (Color figure available online).

(CSF) exchanges molecules with the interstitial fluid of the brain parenchyma, and the transition of blood-borne molecules into the CSF is controlled through the BCB. The BCB is present in the epithelium of the choroids plexus, organized for controlling the transition of molecules and cells into the CSF. A double-layered structure forms on the external surface of the brain through the folding of ependymal cells, which are subsequently stabilized in the dura and pia to form the arachnoid membrane. The subarachnoid space between the double layers plays a role in CSF effluent. Tight junctions prevent the passage of substances from the blood through the arachnoid membrane [46].

Moreover, the BCB protects CSF-borne organic acids in the blood through an active organic acid transporter system in the choroids plexus. Consequently, various therapeutic organic acids, such as penicillin (antibiotic), methotrexate (antineoplastic agent), and zidovudine (antiviral agent), can be removed from the CSF, thereby inhibiting the diffusion of these agents into the brain parenchyma. Consequential incompatibilities typically occur between the composition of the CSF and the interstitial fluid of the brain parenchyma, indicating the presence of the CSF-brain barrier [47]. The equilibrium between the CSF and the brain interstitial fluid is achieved through the diffusion distance of the CSF-brain barrier (Figure 2(B)). Thus, entry into the CSF does not necessarily guarantee that a drug will enter the brain.

3.1.3 Blood-Tumor Barrier

Intracranial drug delivery is more complicated when the target site is a CNS tumor, as shown in Figure 2(C). The presence of the BBB in the microvasculature of CNS tumors has clinical benefits. In CNS malignancies, various physiological barriers that are common to all solid tumors inhibit drug delivery through the cardiovascular system in which the BBB is intact. Drug delivery is most difficult when the target site is a CNS tumor. Drug delivery to neurons in a solid tumor is mediated through the nonuniform distribution of microvasculature throughout the tumor interstitium, potentially resulting in irregular drug delivery. The vascular surface area is reduced, thereby diminishing the transvascular exchange of blood-born molecule(s) during tumor growth. In addition, large intracapillary distances increase the diffusion requirements of neoplastic cells for drug delivery. The hydrostatic pressure in normal brain parenchyma cells adjacent to the tumor is also increased, reflecting high interstitial tumor pressure and associated peritumoral edema. Consequently, the cerebral microvasculature in the regions adjacent to these tumors is less permeable to drugs than normal brain endothelium, leading to exceptionally low extratumoral interstitial drug concentrations [6]. Brain tumors can also disrupt the BBB, but this disruption is local and heterogeneous [48, 49].

4. MAPPING THE NPs IN BIOLOGICAL SYSTEMS

NPs have prompted developmental advancements in the fields of biomedicine and technology, for instance, diagnostics and treatment. NPs are small enough to penetrate every part of the body, including cells and organelles, causing biological hazards [50–53] and resulting in the emergence of a new approach to medicine (nanomedicine). Little is known about the interaction of NPs with biological systems, despite extraordinary developments in nanoscience. This lack of information is considered a serious obstacle in the development of nanomedicine and nanotoxicology. NPs are coated with proteins when they enter into biological fluid, which may transmit biological effects due to changes in the protein conformation. Knowledge of the equilibrium properties and kinetics of the proteins associated with NPs is required for understanding the biological effects of NPs. The isolation and identification of particle-associated proteins is not a simple task, as essential background information is required for nanobiology, nanomedicine, and nanotoxicology. The development of strategies for the identification of both major and minor particle-associated proteins and to characterize the competition between proteins for binding under kinetic or thermodynamic control is necessary. However, the separation of free protein from NP-bound protein presents an obstacle. Centrifugation may be used to achieve this separation, identifying the major serum proteins, albumin and immunoglobulin G (IgG), that are associated with a wide range of NPs [54]. Albumin is typically observed on particles, and because of its abundance, this protein may be retrieved, even if the binding affinity is relatively (Figure 3(A)) [55].

Nanoparticle gathering, protein corona formation, and many other interactions of NPs within biological systems are based on the surface adsorption energy of NPs, which are described using the BSAI [8]. The complex interactions and dynamic changes of manufactured or engineered NPs in biological systems interfere with the development of bionanoscience [9–11]. The accumulation of engineered NPs in media is one of the major issues in the biological studies of NPs [56, 57]. Adsorption of biomolecules onto the surfaces of NPs forming nanoparticle-protein coronas that change within the biological environment is another difficulty [9, 51, 58]. Studies show that the surface adsorption energy of NPs is the basic force behind the aggregation of NPs and the protein corona formation processes and the NP changes within biological systems. The surface adsorption energy is specific to the small size of NPs, with a high surface-to-volume ratio, where the unsaturated surface chemical bonds adsorb other chemicals or biomolecules to minimize their surface energy [9, 59].

4.1 Interaction of Super Paramagnetic Iron Oxide NPs with the Brain

Super paramagnetic iron oxide NPs (SPIONs) suggest a supplementary function for NPs, as the magnetic properties of these molecules may

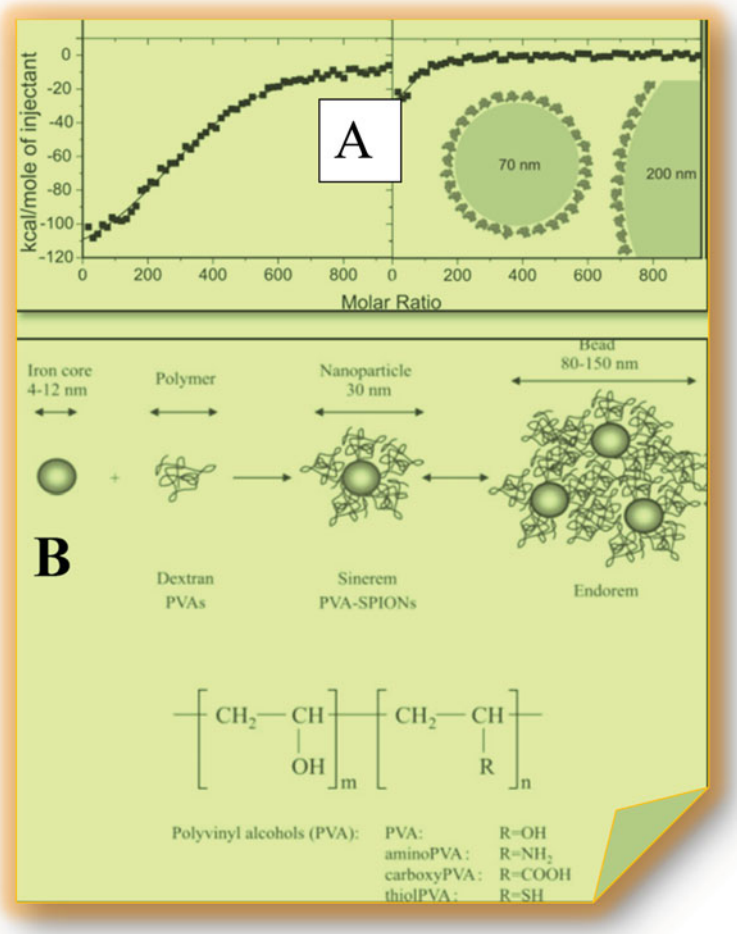

Figure 3: (A) Injection heat-map versus the molar ratio of protein to nanoparticle and a fit using a one-site binding model size comparison of albumin and particles of 70 or 200 nm diameters; adapted from (55). (B) Structures of the various SPIONs and aminopoly vinyl alcohols (PVA) adapted from (73). (Color figure available online).

facilitate a range of new biological, biomedical, and diagnostic mechanisms due to the development of various colloidal dispersions of SPIONs. The application of the different forms of iron oxides has been widely used for radiological diagnostic processes to verify vascular leakage, macrophage imaging, or cell tracking [60–64], but depending on their size or the expression of the scavenger receptors, these molecules are poorly taken up by cells [61–63].

The development and identification of the chemical and biophysical characteristics of biocompatible SPIONs that deliver therapeutic drugs without initiating deleterious cell reactions is the next challenge, particularly in neurodegenerative diseases. Under specific conditions, SPIONs cross the BBB either through direct transport [65–67] or through passive transport via the olfactory bulb [68, 69], leading to active lesions in neurodegenerative disorders [61]. Particles that can enter the brain and other organs act as a potential health risk [50, 68, 70–72].

SPIONs incorporated with magnetic resonance imaging are currently being studied to improve the detection methods of neurodegenerative diseases. Therapeutic drugs are linked to the SPIONs to achieve targeted drug delivery, either at the cell surface or intracellularly, without initiating deleterious cell reactions [73].

The high drug concentrations required to assess the efficiency of drug resistance mechanisms and the poor cell selectivity of therapeutic agents limit treatment mechanisms for various human disorders, particularly brain disorders. These characteristics are particularly important for neurodegenerative disorders. The brain is separated from the bloodstream by the BBB [74]. The presence of tight junctions between the cells of the BBB protects the brain from blood-borne pathogens. This barrier also prevents the access of therapeutic drugs. In various disorders, such as neurodegenerative disorders and brain cancer, the BBB inhibits drug delivery [75]. The size, surface area-to-volume ratio, and physiochemical and biochemical properties of the coating shell and cell type are factors that are involved in the development of biocompatible functionalized SPIONs capable of entering cells. No inflammatory reaction was observed, and when aminopoly vinyl alcohols (PVA) SPIONs entered brain structures, only a small concentration of these compounds were observed [73]. The structure of various SPIONs and PVA are shown in Figure 3(B).

5. NANOBIOLOGICAL INTERACTIONS

A coating of protein molecules is acquired by NPs within biological environments (i.e., the formation of protein coronas) [76, 77]. The biological relevance of the formation of the NP protein corona is important to understand the surface properties of NPs in biological environments. Understanding nano-bio interactions is important for applications of NPs in biomedical sciences, which could lead to a reduction of the adverse effects of NPs in biological systems [78].

5.1 NPs Interactions with CNS

Biological advantages, such as killing pathogenic bacteria and viruses, can be provided by NPs, but studies have also shown that NPs may produce toxic or

adverse effects in human cells. The hippocampal cells of the CNS are the most sensitive and delicate cells in the human body, and these cells are responsible for brain functions and emotions. Studies have shown that NPs might lead to functional and toxicological effects on these cells, reflecting the ability of these molecules to pass through cell membranes [79].

NPs are maneuverable and have superior mobility, potentially reflecting the transportation of NPs across the BBB through passive diffusion or through carrier-mediated endocytosis [71]. The brain can absorb NPs directly through trans-synaptic transport [80]. For example, the silver (Ag) nanoparticles can enter via the BBB [81] and accumulate in various regions of the brain [82], which is helpful for drug transmission but may be hazardous to the patient [83, 84]. Conventional neurons, if exposed to NPs, can induce neurons deterioration [85]. NPs can pass through biological membranes, which may have toxic effects on human neuronal cells [79].

In the CNS, the half-life of silver is longer than in other organs, implying that this NP may have compelling physiological functions, consequences, and hazards to the brain due to extended exposure. Neuronal decline may reflect the induction of the BBB destruction in response to silver nanoparticles [86]. Different studies and analyses have focused on PC-12 cells, a neuroendocrine cell line with the ability to secrete the neurotransmitter Dopamine (DA) and to participate in functional DA metabolism pathways (Figure 4). PC-12 cells are approximately 25–30 micrometers in length; however, the length of these cells increases to several hundred micrometers after cell division, which may increase when exposed to Ag particles. This exposure causes the cells to shrink and to exhibit uneven membrane borders [23].

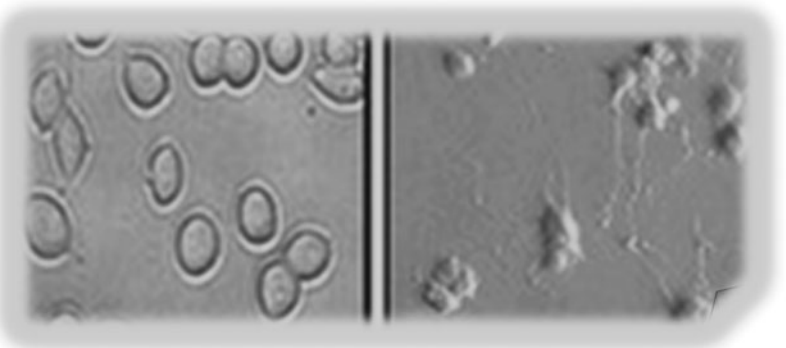

Figure 4: PC-12 cells used in toxicity studies; adapted from (23). A: Cells without exposure of Ag. B: Cells after exposure to Ag particles therefore cells have shrunk.

6. NANONEUROPROTECTION

Antioxidants and metal chelators are treatment options for the adverse health effects caused by the neuronal uptake of NPs. In neurodegenerative disease therapy, metal chelators traversed the blood-brain barrier, implying a promising approach [87]. Functionalized fullerenes [88] and NPs that are composed of compounds holding oxygen vacancies show great antioxidant properties [89]. Fullerols, or polyhydroxylated fullerenes, are excellent antioxidants with tremendous solubility, and the ability of these chemicals to cross the blood-brain makes them promising as neuroprotective agents [88]. Cerium oxide (CeO2) NPs have strong antioxidant properties on rodent nervous system cells [89], which aid cell durability under OS. Diseases, such as leukemia, which is not a neurodegenerative disease, affect the CNS, resulting in neurological symptoms. Selectively decreasing the availability of precursors for the *de novo* biosynthesis of purine nucleotides represents a potential cure for leukemia [141]. However, we can also use a nanoneuroprotection approach when leukemic cells attack the CNS.

7. DRUG DELIVERY APPROACHES

To avoid barriers restricting CNS ingression several drug-targeting approaches have been developed [12–14]. These strategies include manipulating drugs, eliminating the BBB or identifying alternative routes for drug delivery.

7.1 Drug Manipulations-Lipophilic Analogs

Entry into the CNS is recommended through the reduced molecular weight, deficiency of ionization at physiological pH, and lipophilicity [91]. Various mechanisms can be employed by poorly lipid-soluble compounds to pass the BBB, which may include transient osmotic breaching of the BBB, exploiting innate chemical transporters, increased dose chemotherapy, or biodegradable instillation. As lipophilicity is closely associated with cerebrovascular permeability, hydrophobic analogs of meager hydrophilic drugs should easily pass the BBB. Adequate examples of such attempts include lipophilic analogs of nitrosoureas. A quantitative structural activity relationship (QSAR) study suggested that the antineoplastic activity was inversely proportional to the lipophilicity of these compounds. This result suggests that more lipophilic analogs become less soluble in the aqueous plasma and more readily bind plasma proteins, thereby lowering the accessibility of drugs for the CNS. Therefore, equilibrium between cerebrovascular permeability and plasma solubility is required when a drug is transferred via the circulatory system for therapy in CNS diseases [92].

7.2 Prodrugs

The development of prodrugs may elevate the uptake of the drug into the brain [15]. Transient chemical modifications of active species result in the formation of prodrugs, which are pharmacologically inactive compounds. A chemical change occurs to enhance the physicochemical-deficient properties, such as membrane permeability or water solubility. After administration, the prodrug is applied near the receptor site for extended periods of time. Here, the drug is converted into the active form via an activating step. In the CNS, the active compound is released after the hydrolysis of the modifying group. However, prodrugs have limitations. A possible method of entry into the CNS for prodrugs is the attachment of the drug to a lipid moiety, such as fatty acids, glycerides, or phospholipids. These prodrug strategies were analyzed for acid-containing drugs, such as levodopa, GABA, niflumic acid, and valproate or vigabatrin coupled to diglycerides or modified diglycerides [93]. Moreover, prodrug approaches that target specific membrane transporters have also been recently analyzed [94].

7.3 Chemical Drug Delivery Systems

Chemical drug delivery systems (CDDS) present new and ordered ways of targeting active biological molecules to specific target sites or organs based on the anticipated enzymatic activation. The alteration of inactive chemical derivatives of a drug through a mechanism involving the addition of monomolecular moieties generates a site-enhanced delivery of the drugs through multistep enzymatic or chemical transformations. Two types of bioremovable moieties were added to transform the drug into an inactive precursor form. A target or (T) moiety is responsible for targeting and site-specificity. The CDDS is manufactured to proceed through step-by-step metabolic conversions, disconnecting the modifier functions and the target specificity [95].

CDDS are manufactured based on the concept that if a lipophilic compound migrating into the brain is transformed into a lipid-insoluble molecule, it will no longer be able leave the brain; for instance, this molecule will become "locked-in." Moreover, the lock-in mechanism works against the concentration gradient, yielding perpetuating effects. As a result, CDDSs retain lipophilic compounds within the brain. This has been successfully achieved in the case of steroid hormones. Recently, targeted drug delivery to the brain via phosphonate derivatives was also studied, and anionic chemical delivery systems were synthesized and evaluated for testosterone [96].

7.4 Carrier-Mediated Drug Delivery

Carrier-mediated transport (CMT) and receptor-mediated transport (RMT) pathways are applicable for circulating nutrients and peptides. The accessibility of endogenous CMT or RMT pathways suggests that access to

the brain for circulating drugs is possible. In the brain, many transport systems for nutrients and endogenous compounds are available [97, 98], including the hexose transport system, the neutral amino acid transport system for neutral amino acids; the acidic amino acid transport system for glutamate and aspartate; the basic amino acid transport system for arginine and lysine; the b-amino acid transport system for b-alanine and taurine; the monocarboxylic acid transport system for lactate and short-chain fatty acids, such as acetate and propionate; the choline transport system for choline and thiamine, the amine transport system for mepyramine; the nucleoside transport system for purine bases, such as adenine and guanine, but not pyrimidine bases; and the peptide transport system for small peptides, such as enkephalins and thyrotropin-releasing hormone [98].

7.5 From Nanodomains to Rafts

The evolutionary picture is consistent with a highly optimized and poised system that is in dynamic equilibrium and is capable of rapidly shifting from nanodomains to rafts (i.e., from ∼10 to ∼200 nm) in response to fluctuating local conditions [99]. The molecular basis of the system is the nonconformity of the cholesterol sterol structure with the rigid double bond of unsaturated lipids and the projecting amino-acid side chains of transmembrane proteins. These structural nonconformities induce the energetically favorable exclusion of cholesterol from proteins and unsaturated lipids, resulting in cholesterol and protein-enriched regions. In a cholesterol-enriched region, glycosphingolipids and sphingomyelin are rarely present in sufficient concentrations to create large, stable rafts; as a consequence, the region is dominated by nanodomains. However, it is not known whether these nanodomains are functional. An intriguing hypothesis [100] suggests that thermodynamically stable, cholesterol-enriched "lipid shells" of ∼7 nm in diameter comprising ∼80 lipid molecules encases a transmembrane protein and reduces its buoyant density, thereby increasing its affinity for sphingolipid-cholesterol rafts.

In neurons, putative raft involvement in several major disorders pathologically reflects the basic signal-platform functions that mediate external and cytosolic traffic and modulate the process of electrochemical signaling. Rafts may play significant regulatory roles in synaptic transmission, action potential propagation, and membrane signaling to the nucleus.

7.5.1 Parkinson Disease

Parkinson disease (PD) is a neurodegenerative movement disorder [101]. PD is a common age-related disorder that displays infrequent conditions and affects 1%–3% of the population 65 years and older. Many current PD therapies afford relief from clinical symptoms but do not provide neuroprotection [102]. This constrained efficacy has prompted the search for molecular therapies that cure or even prevent this disease by targeting PD

upstream of the pathophysiology. One major focus of this strategy is the small protein α-synuclein (α-syn), which is abundant in Lewy bodies and is possibly raft-associated and is a significant factor in PD etiology [103–107]. Alpha synuclein association with plasma-membrane rafts, which is clearly relevant to molecular therapies, has been insufficiently investigated. The use of raft-targeting lipid-based NPs, which could inhibit or even reverse α-syn fibrillation via endocytosis and the cytosolic release of an α-syn-targeting molecule, is also a possible therapeutic strategy.

7.5.2 Alzheimer Disease

Alzheimer disease (AD) is also a neurodegenerative disease affecting almost 28 million people worldwide. Due to adverse effects, such as toxicity and cancer, synthetic compounds are not widely used in the treatment of AD [107]. Neuroprotective compounds of a natural origin and synthetic derivatives present encouraging results with minimal adverse effects, and some of these molecules are currently in different phases of clinical trials [107]. Alkaloids are effective in alleviating the symptoms of neurodegenerative diseases, such as AD. Large numbers of natural alkaloids and synthetic derivatives have exhibited neuroprotective effects. Polyphenols are one of the most significant secondary metabolites, exhibiting natural antioxidant properties. These molecules are abundantly present in fruits, vegetables, herbs, and different drinks (e.g., tea, wine, and juices). These phenolic compounds have acquired increasing interest through multiple epidemiological studies. Polyphenols, both flavoids and nonflavoids, are effective in alleviating and protecting against the neurodegenerative diseases in various cell culture and animal models. Moreover, various polyphenyls and nutrients with respect to application of nanotechnology have recently reported as cancer chemotherapeutic agents in the literature [142]. Epidemiological evidence has shown that the Mediterranean diet, which is rich in antioxidants, is effective in the prevention of age-related diseases, such as AD [108]. Altered iron homeostasis has also been reported in AD, as indicated by changes in the levels of iron, ferritin, and transferrin receptor in the hippocampus and cerebral cortex [109]. Iron promotes both the deposition of Aβ and the induction of oxidative stress. Indeed, it has been demonstrated that amyloid deposits are enriched with zinc, iron, and copper [110]. Currently, researchers have been exploring on different aspects of nanotechnological approach in the management of AD and Type 2 diabetes [143].

7.6 Lipid-based Nanocarriers

Liposomes and NPs are large and complicated constructs made from different chemical components, ranging up to 500 nm in diameter. Greater amounts of drug can be added into these structures to deliver large amounts of drug to the CNS. The surface of the liposome or nanoparticle can be adjusted and various functional groups can be attached so that this structure may target the CNS via specific BBB mechanisms.

Pegylated immunoliposomes have been used to target and transiently transfect h-galactosidase (LacZ reporter gene) and luciferase to the brain [111]. The gene is integrated into the center of the liposome, and the surface of the liposome is coated with polyethylene glycol (PEG) to elongate the circulation time. Furthermore, 2% of the PEG strands have a mAb to the transferrin receptor (8D3mAb), which is attached to increase specificity.

7.7 Metallic Ions as Therapeutic Agents

Different metallic ions act as cofactors of enzymes and stimulate a chain of reactions, which is correlated with cell signaling pathways toward tissue balance [112, 113]. In metabolic disorders, such as cancer, CNS disorders, and infectious diseases, among others, interaction with metallic ions may play a significant role [16, 114]. Therefore, the effectiveness and selectivity of the therapeutic effect of metallic ions can be upgraded through controlling the level and location of ions in the body. A drawback of metallic ions is these molecules produce toxic effects when taken directly due to their unstable ionic states. Current metallic-ion-based drugs are susceptible to significant systemic toxicity. Therefore, the metallic ion metabolism can lead to pathological states, such as hemochromatosis, Wilson disease, and Menkes disease [17–19]. Certain metallic ions are involved in the pathogenesis of various chronic diseases, such as diabetes mellitus, coronary heart disease, epilepsy, and nephropathy [115]. The uncontrolled release of metal ions may produce adverse effects [116–118].

Nano-sized titanium dioxide (TiO2) is used in different consumer products [119], and the widespread use and potential entry of this compound though dermal, ingestion, and inhalation routes suggests that nano-sized TiO2 could pose an exposure risk to humans. Studies indicate that TiO2 is toxic to eco-relevant species, such as *Escherichia coli* and daphnia [120] and mammals [121].

Studies suggest that inhaled or injected NPs enter systemic circulation and migrate to various organs and tissues [122, 123] where they could accumulate and damage different organ systems that are particularly sensitive to OS, for instance, the brain, which is highly susceptible to OS because of energy demands, low levels of endogenous scavengers (e.g., vitamin C, catalase, superoxide dismutase), and high cellular concentration of OS targets (i.e., lipids, nucleic acids, and proteins). Experimental studies reveal that NPs can easily pass through the blood-brain barrier [75] and enter the CNS of exposed animals [68, 122].

To examine the possible neurotoxicity of TiO2, nerve cells, microglia were exposed to an available nanomaterial, Degussa P25. This material is an uncoated photo-active form of nano-sized TiO2. The BV2 microglia is an immortalized mouse cell line that responds to pharmaceutical agents, particulates, and environmental chemicals, with diagnostic signs of OS [124, 125]. Neuronal

populations, such as dopaminergic (DA) neurons in the brain striatum, are particularly vulnerable to OS [126]. The neurotoxicity of P25 was studied in N27, an immortalized rat DA neuronal cell line [127], and sophisticated CNS cultures of embryonic rat striatum, which has high numbers of DA neurons [128].

7.8 Cultured Neurons and Carbon Nanotubes

Carbon nanotubes (CNTs) have been an integral part of nanotechnology due to their unique properties, which allow for the development of various miniaturized devices [20, 21]. CNTs, which are carbon nanostructures, were discovered in the early 1990s [129]. The in-complex shape of a CNT is that of a single-walled nanotube (SWNT). SWNT diameters typically range between 0.8 and 2 nm. Multiwalled nanotubes, however, are composed of numerous concentric graphite cylinders and can reach diameters of up to 100 nm. Depending on its hexagonal lattice structure, the resulting electronic conduction within an SWNT has the properties of an insulating, conducting, or semiconducting material. CNTs can be systematically tailored by the addition of different chemical groups and can be functionalized to display a variety of surface charges, for instance, positive, neutral, or negative charges. The ease of such chemical

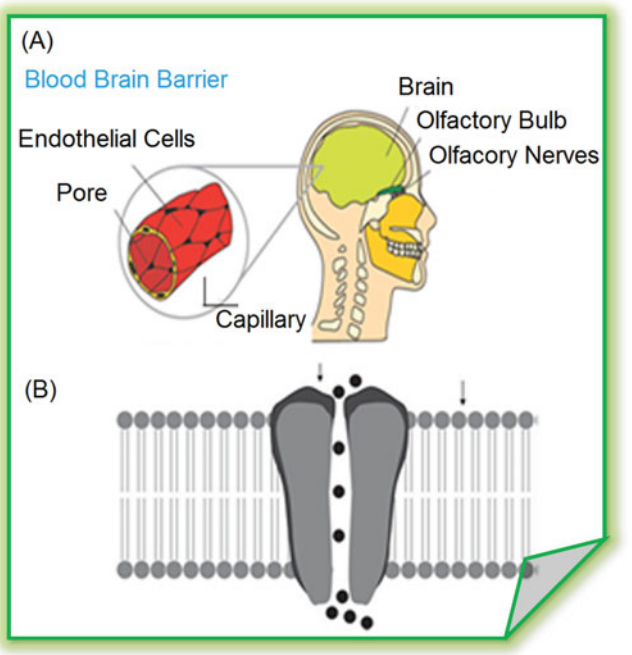

Figure 5: (A) Neuronal uptake of NPs via the blood-brain barrier; adapted from (140). (B) Neuron synaptic transmission and the region of the neuronal cell membrane where CNTs easily cross the BBB; adapted from (23). (Color figure available online).

modifications, combined with their natural characteristics, make CNTs an excellent candidate for interfacing with neural systems [130]. There have been recent developments of biocompatible, durable, and robust substrates and devices that affect neuronal growth and potentially provide therapies for CNS disorders [131].

Studies suggest stimulation of neurons via CNTs (Figure 5). In various cases, the electrical stimulation of neurons employed in a neural prosthesis and neurological therapies requires microelectrode arrays. Indeed, CNT-based microarrays have been successfully generated [132–137] through growing vertically aligned carbon nanofibers (VACNFs). Thus, VACNF arrays not only can be used for electrochemical applications but they can be used for repetitive stimulation, for example, in rat hippocampal neurons that are grown on the arrays [137]. Currently, these VACNF devices were also employed for extracellular recordings and stimulations of cultured hippocampal organotypic slices [136].

Taken together, CNT-based arrays offer a stage for interfacing with neurons and neural tissue and have great promise for studying networks and medical applications. Some of the initial concerns regarding the toxicity of these compounds [138, 139] have been alleviated by the fact that CNTs are less hazardous than carbon black, which is a different form of carbon that has been widely used and has defined exposure guidelines. Because extensive commercialization of CNTs will occur, we should be aware of the possible negative effects that these new nanomaterials could exert on human health, and we must employ additional toxicity testing.

8. CONCLUSION

NPs are useful against pathogens but cause neurotoxicity. NPs are small enough to cross membrane barriers in living organisms. Several studies regarding the interaction between CNS neuronal cells and NPs have used metal or metal oxides with selected neuronal cell lines. Neurologists have an interest in both the functionality and toxicity of NPs, with recent studies focusing on the interaction of NPs with hippocampal cell membranes regarding CNS drug toxicity. Neurologists have an equal interest in both the areas of positive functionality and negative toxicity of NPs on human neurons and the interactions that occur when NPs pass through the BBB. The range of applications for nanomaterials is increasing at a rapid rate such as application in cancer management [139]. The potential of individual NPs and carbon nanotubes as constituents of toothpastes, beauty products, sunscreens, coatings, drug delivery systems, sensors, building materials, and textiles are being explored. Using recent studies, this review summarizes various pathways for NP-induced neurotoxicity. Although numerous in vivo and in vitro studies have provided evidence of the toxic effects of various types of NPs, our understanding of the

potential health and safety issues regarding NPs lags behind the rapid commercialization of nanomaterials. The NPs have some positive aspects but exhibit negative or harmful effects. These beneficial and harmful effects should be weighed against one another. One major problem is the lack of information regarding the possible adverse health effects caused by exposure to different nanomaterials. Therefore, understanding of the neurotoxic effects of manufactured or engineered NPs would help to develop safety guidelines and would promote the safe use of nanotechnology applications. In the brain, NPs may induce inflammation, apoptosis, and OS by releasing various mediators from microglia and astrocytes [40]. Depending on the production of toxic (e.g., NO, excitatory neurotransmitters) or antitoxic mediators (e.g., anti-inflammatory cytokines, neurotrophins), neurodegeneration or neuroregeneration may occur. Figure 6 represents the overall process of neurotoxicity and shows the different types of barriers and nanoneuroprotections using different in silico and in vitro approaches.

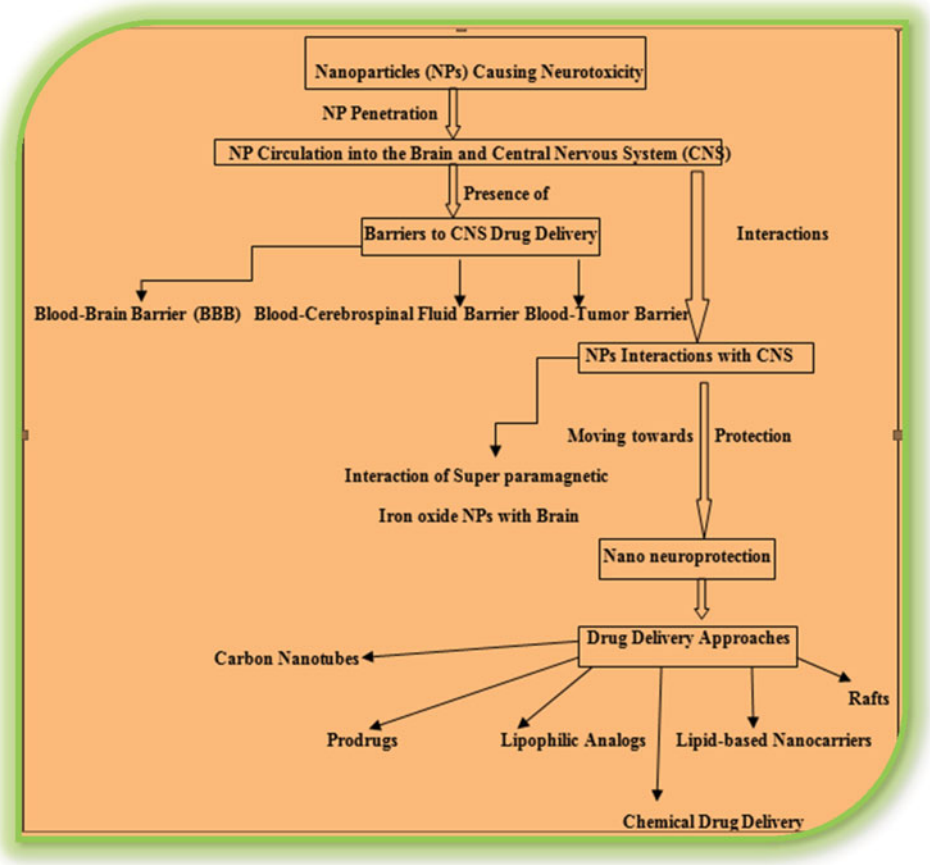

Figure 6: Flow chart of nanoneurotoxicity to nanoneuroprotection. (Color figure available online).

9. LOOKING TO THE FUTURE

The improved understanding of drug receptors and drug targets, together with advances in medicinal chemistry, make it possible to design drugs with enhanced activity and selectivity. Further physicochemical modifications of these enhanced drugs may provide a small but vital increase in their ability to enter the CNS, resulting in a significant increase in the therapeutic index. Furthermore, designing drugs with reactivity and an influx or efflux transport system in the BBB will facilitate the entry of drugs into or out of the CNS, as desired. The use of vectors employing a transporter or acting in a nonspecific manner may lead to increased application, especially when combined with a nanoparticle or liposome containing the drugs for delivery. Approaches that modify the properties of the BBB by increasing the permeability of tight junctions or inhibiting the activity of efflux transporters are most likely most suited to short-term treatments, where a single or infrequent exposure to a drug is required. We can now begin to appreciate the challenges presented by simple nanoscale materials, such as TiO2, ZnO, Ag, carbon nanotubes, and CeO2. However, these simple materials are merely the vanguard of a new era of complex materials, where novel and dynamic functionality is engineered into multifaceted substances. If we are to meet the challenge of ensuring the safe use of this new generation of substances, research efforts should move beyond "Nano" toxicology and toward a new toxicology of sophisticated materials. One can aim for either the modification of existing drugs to increase BBB penetration through existing promising strategies or through the development of a new chemical that possesses the desired permeability properties.

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