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Review

Nano-interventions for neurodegenerative disorders

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ABSTRACT

With an increase in lifespan and changing population demographics, the incidence of central nervous system (CNS) diseases is expected to increase significantly in the 21st century. Contrary to common belief, it is recognized that neurodegenerative diseases may be multisystemic in nature and this presents numerous difficulties for the potential treatment of these disorders. This review focuses on applications in the nano-delivery of therapeutic agents across the blood–brain barrier. We explore various types of nanoparticles, ranging from polymerics to liposomes. A brief discussion of the pharmacokinetic parameters and specific targeting strategies of these nanoparticles follows, presenting suggestions for the mechanisms of cellular and intracellular uptake and possible toxicity considerations of nanoparticles.

Contents

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1. Introduction

With an increase in lifespan and changing population demographics, the incidence of central nervous system (CNS) diseases is expected to increase significantly in the 21st century. The most challenging of the CNS diseases are neurodegenerative diseases, characterized by age-related gradual decline in neurological function, often accompanied by neuronal death. Alzheimer's disease, Parkinson's disease and Huntington's disease are some examples of neurodegenerative diseases (ND) and have been well described in terms of disease mechanisms and pathology. However, successful treatment strategies for neurodegenerative diseases have so far been limited [1].

Contrary to common belief, it is recognized that neurodegenerative diseases may be multisystemic in nature and this presents numerous difficulties for the potential treatment of these disorders. The death of specific types of neurons in neurodegenerative diseases is provoked by a cascade of multiple deleterious molecular and cellular events rather than a single pathogenic factor. Complicating the situation, further, is the constraint of the blood–brain barrier (BBB) which prevents 98% of potential neuropharmaceuticals and the drug release kinetics causing peripheral side-effects [2].

Briefly, BBB, a dynamic interface composed of brain endothelial cells separates the brain from systemic circulation and is the major entry route for therapeutics to the CNS [2]. It is estimated, that the total length of human brain capillaries is 650 km, with a total surface area of 10–20 m^2 [3,4]. The primary role of the BBB is to create ionic homeostasis for neuronal functions [5], supplement the brain with nutrients and protect it from toxic insults by sophisticated transport systems [6]. The low level of paracellular flux and transendothelial vesicular trafficking result in a transport barrier for drugs which are hydrophilic and have a molecular mass bigger than 400 Da, while the presence of effective efflux transporters at the luminal membrane of brain endothelial cells limits the brain penetration of lipophilic xenobiotics and drugs [7].

Most pharmaceutical agents have primary targets within cells and tissues; ideally, these agents may be preferentially delivered to these sites of action within the cell. Selective subcellular delivery is likely to have greater therapeutic benefits. In general, cytosolic delivery, for instance, is desirable for drugs that undergo extensive exportation from the cell via efflux transporters such as multidrug resistance proteins and P-glycoproteins [8]. These efflux mechanisms continuously reduce therapeutic intracellular drug concentrations. An intracellular nanoparticle, consequently, may act as a drug depot within the cell. This is achieved by enabling the engineering of the particle backbone structure and the size and shape of the nanoparticle core, providing yet another dimension of physical control that can be exerted toward the specific tailoring of function. Thus, nanotechnology may be used to achieve therapeutic dosing via targeted therapies, establish sustained-release drug profiles, and provide an intracellular sanctuary to protect therapeutic compounds from efflux or degradation [9].

Considering this fact, the use of nanoparticles to deliver drugs to the brain by infiltrating blood–brain barrier (BBB) may provide significant strategy to break this impasse. The primary advantage of nanoparticle carrier technology is that it can cross blood–brain barrier entrapping the original characteristics of the therapeutic drug molecule. As reiterated earlier, this system may reduce drug leaching in the brain and decrease peripheral toxicity [10]. Therefore, nanotechnology may provide a possible solution to overcoming many of these challenges for the treatment of Alzheimer's Disease and Parkinson's Disease by affording targeted drug delivery and enhancing the bioavailability and/or efficacy of various drugs and other bioactive agents used in NDs.

This review focuses on applications in the nano-delivery of therapeutic agents across the blood–brain barrier. We explore various types of nanoparticles, ranging from polymerics to liposomes. A brief discussion of the pharmacokinetic parameters and specific targeting strategies of these nanoparticles follows, presenting suggestions for the mechanisms of cellular and intracellular uptake and possible toxicity considerations of nanoparticles.

2. The blood–brain barrier: a structural impediment to CNS drug delivery

Complex and highly regulated, the BBB screens the biochemical, physicochemical and structural features of solutes at its periphery, thus affording barrier selectivity in the passage of desired molecules into the brain parenchyma. The dual purpose of the BBB is to ensure a constant internal milieu within the CNS and to provide essential nutrient supply. Unlike peripheral capillaries that allow relatively free exchange of substances across/between blood and tissue parenchyma, the BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers.

2.1. Structural components of BBB

The BBB is a unique, selective barrier formed by the endothelial cells that line cerebral capillaries, together with perivascular elements such as closely associated astrocytic end-feet processes, perivascular neurons and pericytes. Pericytes and endothelial cells are unsheathed by the basal lamina; indirectly involved in the establishment and maintenance of the BBB: these various cell types and basal lamina collectively constitute the 'neurovascular unit' (NVU), a concept recently proposed to highlight the functional interactions which control BBB integrity [11]. Because of the presence of the BBB, circulating molecules gain access to brain interstitial fluid via one of two processes: (i) lipid-mediated transport of micromolecules by free diffusion, and (ii) facilitated (catalyzed) transport of micro- and macromolecules. For facile understanding of the complexity of the BBB, this section enlists the various cellular components of BBB.

2.1.1. Endothelial cells

Brain endothelial cells (ECs) differ significantly from non-brain ECs by (i) the absence of fenestration correlating with the presence of intercellular tight junctions (TJs), (ii) the low level of non-specific transcytosis (pinocytosis) and paracellular diffusion of hydrophilic compounds, (iii) a high number of mitochondria, associated with a strong metabolic activity and (iv) the polar-

ized expression of membrane receptors and transporters which are responsible for the active transport of blood–borne nutrients to the brain or the efflux of potentially toxic compounds from the cerebral to the vascular compartment [12,13]. Besides, they are enriched with enzymes; solutes crossing the cell membrane are subsequently exposed to degrading enzymes present in large numbers inside the endothelial cells that contain large densities of mitochondria, metabolically highly active organelles. Enzymes and receptors found in the BBB include, among others, adenylate cyclase, guanylate cyclase, Na–K ATPase, alkaline phosphatase, catechol O-methyl transferase (COMT), monoamine oxidase (MAO), GABA transaminase, DOPA decarboxylase.

2.1.2. Basal lamina

It is a 30–40-nm thick membrane composed of collagen type IV, heparin sulfate proteoglycans, laminin, fibronectin, and other extracellular matrix proteins. The basal lamina of the cerebral endothelium is constituted by 3 apposed layers, one produced by ECs and containing laminin-4 and -5, second being astrocyte-derived, containing laminin-1 and -2 and third, the collagen IV-containing middle one, contributed by both cell types [14]. All three layers are also made of various types of collagen, glycoproteins and proteoglycans [15,16]. Although its contribution to BBB integrity has been often underestimated, the basal lamina is now being considered as a key component of the NVU [11]. Multiple basal lamina proteins, matrix metalloproteases (MMPs) and their inhibitors, the Tissue Inhibitor of Metalloproteases (TIMPs), are involved in the dynamic regulation of the BBB in physiological as well as inflammatory conditions [17].

2.1.3. Glial cells, astrocytes

Though the role of astrocytes in the induction and maintenance of BBB integrity has been well documented for more than two decades [18], the molecular mechanism mediating their action still remains unclear. Astrocytes enclose more than 99% of the basal capillary membrane, and play a prominent role in the BBB induction of high paracellular electrical resistance. A gap of only 20 nm separates the astrocytes from the EC and the pericytes. Indeed, a number of astrocyte-released and more generally glial-released factors have been suggested to contribute to BBB integrity, including glial-derived neurotrophic factor (GDNF), angiopoietin-1 [19,20] and more recently angiotensin II [21]. Astrocytes, generally classified into fibrous and protoplasmic, represent the major component (90%) of the brain mass. Fibrous astrocytes have a star-like morphology and often present many long processes, known as "end-feet", that end on the basal membrane of the BBB. These cells have a multitude of functions important for the brain homeostasis (maintenance of K+ levels, inactivation of neurotransmitters, regulation and production of growth factors and cytokines), many of which are related to the production of apolipoprotein E (ApoE).

2.1.4. Pericytes

Pericytes are present along brain and non-brain microvessels, within the basal lamina surrounding ECs; interestingly, brain microvessels are notably rich in pericytes and the pericytes/ECs ratio has been correlated with the barrier capacity of the endothelium. Pericytes lie along the outer axes of cerebral capillaries and perform in contractility. This close association (and function) helps to monitor blood flow, and thus, the adhesion of pericytes with the microvasculature indirectly regulates EC activity and BBB transport. Pericytes could also manage endothelial growth and development by inhibiting cell proliferation.

2.1.5. Neurons

Brain endothelium, perivascular astrocytes and pericytes are in close contact with neuronal projections, allowing neuronal mediators to affect cerebral blood flow and vessel dynamics. However, the precise physiological or pathophysiological consequences of neuronal input onto the BBB still remain largely unknown.

2.1.6. Intercellular junctions

Tight junctions provide significant transendothelial electrical resistance (TEER) to bone marrow microvascular endothelial cells and impede the penetration of potential therapeutic agents such as oligonucleotides, antibodies, peptides and proteins. Tight junctions, between brain endothelial cells, are elaborate structures composed of integral membrane proteins, linker or adaptor proteins connecting them to the actin cytoskeleton and signaling molecules enabling the dynamic regulation of the paracellular transport. They are constituted by three major transmembrane proteins (or protein families), occludin, claudins and Junction Associated Molecules (JAMs), and several cytoplasmic proteins including Zonula Occludens (ZO)-1, ZO-2, ZO-3, which interact with these transmembrane proteins in multi-protein complexes linked to the actin cytoskeleton.

2.1.7. Efflux transport systems

Efflux transport systems at the BBB and the B–CSF-B greatly reinforce the barrier properties by removing substances from the brain or the CSF and transferring them to the systemic circulation, respectively [22]. To date, several classes of transporters have been implicated in the efflux of P/P drugs from the brain like multidrug resistance (MDR) transporters, monocarboxylate transporters (MCT) and organic anion transporters/organic anion transporting polypeptide (OAT/OATP) [23]. Due to their polarized localization MCT and OAT/OATP are also implicated in the carrier-mediated influx. Each of the three classes comprises multiple transporters, each having multiple substrates, and the combined substrate profiles of these transporters include a large number of commonly used drugs. Usually small drugs are preferred targets, though P/P drugs can also be involved. However, it should be noted, that the primary pathway for removal of intracytoplasmic proteins is still degradation rather than efflux pumping. Nonetheless, the activity of these efflux transporters at the BBB is often associated with limited effectiveness of P/P drugs targeted at CNS disorders.

Therefore, modulation of these efflux transporters, by designing inhibitors and/or compounds that have minimal affinity for these transporters could prove to be an effectual strategy to treat intractable CNS disorders [24]. Following section briefly elucidates the roles of different type of transporters in drug disposition across the BBB.

2.1.7.1. Transporters. Drug transporters belong to two major superfamilies, ABC (adenosine triphosphate binding cassette) and SLC (solute carrier) transporters. Another non-ABC, non-SLC protein, RLIP76, has been associated with drug resistance in patients with epilepsy [25], but its localization and function remains controversial [26].

2.1.7.1.1. Transporters of the adenosine triphosphate binding cassette superfamily. ABC transporters are primary active transporters, which couple ATP hydrolysis to active efflux of their substrates against concentration gradients. The 49 human ABC transporter genes are classified into seven subfamilies designated A through G [27]. The most extensively studied BBB transporter of the ABC family is P-glycoprotein (P-gp), but members of the MRP family (ABCC) and breast cancer resistance protein (BCRP; ABCG2) have also been identified in brain endothelial cells and Choroid Plexus (CP) epithelial cells.

i. P-glycoprotein (P-gp)

P-gp is the most widely studied member of the adenosine triphosphate (ATP)-binding cassette family of efflux drug trans-

porters. P-gp is known for is critical role in mediating cellular resistance to many chemotherapeutic agents and in limiting the tissue penetration of a broad range of chemically diverse substrate drugs at many blood–tissue barriers. Since its discovery in 1976 in multidrug resistant tumor cell lines [28], numerous studies have shown its expression in the small intestine, the BBB, liver and kidney.

In the brain, P-gp is expressed on the luminal, abluminal and intracellular membrane of capillary endothelial cells as well as the epithelium of the choroid plexus [29,30]. It is principally responsible to expel substrates (drug) back into the circulation after they initially diffuse into the endothelial cell membrane, thereby restricting their penetration into the brain and cerebrospinal fluid (CSF). With the on-going research, the presence of P-gp has also been demonstrated in blood vessels that supply human gliomas and metastatic brain tumors, but at reduced levels, compared to those at the BBB. Compared to the BBB, the localization of P-gp at the blood–CSF barrier is less well established. P-gp expression (by immunostaining) in the CP of human adults, neonates and in rats has been detected by some investigators, but others have reported it to be undetectable. When detected in native CP and cultured CP epithelial cells, P-gp is mainly located at the apical (CSF-facing) membrane and in sub-apical cell compartments. This apical membrane localization is thought to allow P-gp to transport substrates into the CSF.

Typically, the substrates for this receptor (ranging in size from less than 200 to almost 1900 Da) are organic amphipathic molecules. The list includes the antiretroviral agents indinavir, nelfinavir and saquinavir (Kim et al., the immunosuppressants cyclosporine A (cyclosporine) and tacrolimus, the cardiac agents digoxin and verapamil and the opioid loperamide). However, many commonly prescribed drugs from various chemical and pharmacological classes are now known to be P-gp substrates.

ii. Multidrug resistance-associated proteins

Members of the second ABC superfamily, the multidrug resistance-associated proteins (MRPs), are predominantly organic anion transporters but in addition transport neutral organic compounds. While they are also ATP-dependent transporters, some require the presence of co-factors for transport. For most MRP isoforms, data on subcellular localization in humans, as well as level of expression and substrate recognition are inconsistent, but it seems that MRP4 and MRP5 (and possibly MRP2, in epileptogenic brain tissue from humans and rodents) are located on the luminal membrane of brain endothelial cells are present in the blood-facing membrane of the human CP epithelial cells. MRP1, MRP4 and MRP5 were also identified in endothelial cells from brain tumors. MRP3 (ABCC3) has been detected in glioma capillaries, but not in normal human brain endothelial cells. The substrate and inhibitor selectivity of individual MRPs may partially overlap with that of other ABC transporters; P-gp, ABCG2, and organic anion transporters.

In MRP2-deficient TR-rats with induced seizures, phenytoin extracellular concentrations and anticonvulsant activity were two-fold greater than in rats that do not lack MRP2. Breast cancer resistance protein (BCRP, ABCG2, or MXR) is an ABC half transporter. BCRP is expressed at the luminal membrane of human microvessel endothelium and on the CSF side of murine CP epithelial cells. Together with MDR1, BCRP is the main ABC transporter expressed in human brain microvessels. Unlike P-gp, BCRP seems to be upregulated in tumor capillaries relative to those of the normal brain. The substrate specificity of BCRP partially overlaps with that of P-gp and includes zidovudine, lamivudine, prazosin, pantoprazole, and the chemotherapeutic agents methotrexate, doxorubicin, daunorubicin, mitoxantrone, topotecan, irinotecan, imatinib (Gleevec) and gefitinib (Iressa). Recent studies in BCRP (−/−) mice have shown that this transporter contributes only to a moderate extent to the brain distribution of dantrolene, prazosin and triamterene [31].

2.1.7.1.2. Transporters of the solute carrier superfamily proteins [30,101–104]. Transporters of the solute carrier (SLC) superfamily proteins of the SLC family include facilitated transporters and ion coupled transporters and exchangers that do not require ATP. Over 360 human SLC transporters have been identified so far and more than 40 SLC transporter families are included in the Human Genome Organization (HUGO) Nomenclature Committee Database. Among these, members of the organic anion transporting polypeptides (SLCO) and organic anion/cation/zwitterions (SLC22) transporter families are of special interest in terms of drug transport across the BBB. Additional transporters which can potentially contribute to DDIs across the BBB include monocarboxylate transporters, system L, and nucleoside transporters.

i. Organic anion transporting polypeptides

Organic anion transporting polypeptides (OATPs) are sodium-independent, multispecific anion exchangers, i.e. they exchange a drug for another ion or molecule. OATP-mediated transport can be bidirectional and depends on local substrate gradients. Among OATP family members, four transporters have been identified at human blood–brain interfaces. OATP1A2 and OATP2B1 are localized at the luminal membrane of brain endothelial cells, whereas OATP3A1 is expressed in the CP. The thyroid hormone transporter, OATP1C1 has also been identified in human brain endothelial cells, but its precise localization is currently unknown. OATP1A2 and 2B1 have been detected in the blood–tumor barrier in gliomas and may affect the availability of chemotherapeutic drugs to tumor cells. OATP substrates are anionic amphipathic molecules with molecular weights greater than 450 Da and a high degree of albumin binding. They include a broad range of drugs, such as fexofenadine.

ii. Organic anion transporters

OATP drug transporters mediate the sodium-independent uptake of a broad range of substrates, including the drugs fexofenadine, levofloxacin, methotrexate, and ouabain, benzylpenicillin, valacyclovir, zidovudine, mercaptopurine, methotrexate and valproic acid. Along with bile acids and the synthetic peptides deltorphin II and D-penicillamine (2,5)-enkephalin (DPDPE). Recent studies suggest that in addition to its importance to intestinal drug absorption, the organic anion transporters (OATs) of the SLC22 gene family, in common with OATPs, are anion exchangers. The localization of most OATs in the brain is unclear, although OAT3 and OAT1 are found in epithelial cells of the human CP. The contribution of individual OATs to the brain disposition of their substrates is currently unknown.

iii. Organic cation transporters

Organic cation transporters (OCTs), like OATs, belong to the SLC22 family. They include the potential-sensitive OCTs and the proton gradient-driven OCTNs. OCTs are expressed in rodent and human brains, but so far have been localized in humans mainly to neurons and glial cells and not to endothelial cells. OCTs mediate the bidirectional transport of small, hydrophilic, positively charged compounds, such as cimetidine, desipramine, metformin, amantadine, memantine. OCTN2 (SLC22A5) is expressed in brain endothelial cells of various species, including humans, and has been recently localized to the abluminal membrane in bovine brain capillary endothelial cells. OCTN2 mediates carnitine uptake into the brain and recognizes several cationic drugs, but its involvement in drug uptake into the CNS has yet to be assessed. System L transporters are heterodimers composed of a catalytic subunit (LAT1 or LAT2) covalently linked with the

glycoprotein 4F2hc. System L transports bidirectionally large neutral amino acids with branched or aromatic side chains, such as L-phenylalanine, L-tyrosine, L-tryptophan and L-leucine and aminoacid mimicking drugs, including levodopa, α-methyldopa, baclofen, melphalan, gabapentin and pregabalin. LAT1 is the predominant isoform at the BBB of humans and rodents and in general has greater affinities to system L substrates than LAT2. It is expressed in both membrane domains of endothelial cells and normally participates in uptake of substrates from blood to brain.

iv. Monocarboxylate transporters

MCT1 is expressed on the luminal membrane of endothelial cells at the blood–brain barrier. Its primary role is in the transport of monocarboxylate solutes, such as lactate and pyruvate, into the brain, although more recently, drug substrates of MCT1 and other members of the MCT family have been studied. MCTs potentially contribute to enhanced brain uptake of HMG-CoA reductase inhibitors that contain a carboxylic acid moiety, such as simvastatin, and of the drug of abuse gamma-hydroxybutyrate. The most widely studied drug associated with MCT1 is γ-hydroxy butyrate (GHB), a controlled substance that has been used clinically to treat insomnia, cataplexy, and narcolepsy. On the other hand, they may restrict brain distribution of probenecid. Valproic acid is taken up into the brain by a transport system for medium-chain fatty acids and has been shown to be a MCT substrate and inhibitor. Other drugs that contain a carboxylic group in their chemical structure are also potential MCTs substrates.

v. Nucleoside transporters

The nucleoside transporters are encoded by the SLC28 (concentrative nucleoside transporter, CNT) and SLC29 (equilibrative nucleoside transporter, ENT) gene families. CNTs mediate Na+-dependent uptake of nucleosides into cells whereas ENTs are Na+-independent transporters. In humans, nucleoside transporters are present in the brain, but have not been localized to the BBB. However, a sodium-dependent CNT3-like system was demonstrated in CP from humans and monkeys. It is recently shown that the brain-to-plasma concentration ratio of ribavirin is 2.1-fold lower in Ent1(−/−) mice, compared to Ent1(+/+) controls, indicating an important role for Ent1 in the uptake of ribavirin into the mouse brain. The involvement of nucleoside transporters in the distribution of other nucleoside analog drug into the CNS is currently unknown.

2.2. Physiological function of the BBB

Unlike leaky endothelia, the endothelial cells at the tight junctions (TJ) demonstrate a high electrical resistance of 8000 V/cm^2, making the transcellular uptake of the solute across the BBB practically absent. Inspite of this, there is uptake of essential nutrients, hormones and vitamins, through the brain endothelium by a variety of membrane transporters, notably the large family of Solute Carrier transporters (SLC). Besides uptake mechanism, the BBB is enriched with enzymes responsible for enzymatically degrading many peptides and neurotransmitters and the energy-dependant toxin efflux mechanisms to maintain cerebral vitality by disallowing injurious substances. Another feature of the BBB, the direction of flow is from the plasma to the brain, or vice versa, with these two parameters defining influx and efflux; this highlights a unidirectional, concentration-dependent movement of the solutes. Thus, net flux is the difference between the two unidirectional rates, and is greatly influenced by the nature of the BBB. Importantly, this flux is a determinant in drugs reaching therapeutic concentrations within the CNS [32].

2.2.1. Mechanisms of solute transport across the blood–brain barrier

There are four basic mechanisms by which solute molecules move across membranes. First is *simple diffusion*, which proceeds from low to high concentrations. Second is *facilitated diffusion*, a form of carrier-mediated endocytosis, in which solute molecules bind to specific membrane protein carriers, also from low to high concentration. Third is *simple diffusion through an aqueous channel*, formed within the membrane. Fourth is *active transport through a protein carrier* with a specific binding site that undergoes a change in affinity Fig. 1.

2.2.1.1. Paracellular (aqueous) diffusion. Diffusion of substances between the cells is termed as paracellular diffusion. It is non-saturable and non-competitive. In brain, however, it does not occur to any great extent at the BBB, due to the “tight junctions”. Only small water-soluble molecules can diffuse through the BBB by apparently passing through the tight junctions.

2.2.1.2. Transcellular (lipophilic) diffusion. Diffusion of substances across the cells is termed as transcellular diffusion. Similar to paracellular diffusion, it is also non-saturable and non-competitive In the case of transcellular diffusion, the general rule is the higher the lipophilicity of a substance along with a molecular weight less than 450, the greater the diffusion into the brain [33]. If two substances, identical on all other fronts, vary in molecular weight, the smaller substance will penetrate more rapidly; consequently small inorganic molecules (i.e. O_2, CO_2, NO, and H_2O) are highly permeable across the endothelial cells by dissolving in their lipid plasma membrane. Additionally, hydrogen bonding property is also a major determining factor. Since hydrogen bonding is primarily associated with oxygen and nitrogen moieties in a molecule, then, if the sum of the nitrogen and oxygen atoms in the molecule is five or less, then the molecule has a high probability of entering the CNS.

2.2.1.3. Saturable (carrier-mediated) transport. Other substances exchanged between the blood and the brain interstitial fluid, including endogenous substances and nutrients, are actively transported by highly selective membrane bound-carrier systems. The expression of these carriers is often polarized (co-localized on both the luminal and abluminal membranes of the brain microvessel endothelia) to optimize substrate (endogenous substances and nutrients) transport into the brain. Several carrier systems have been described in brain capillaries including those specific for small-molecule peptides, hexoses, monocarboxylic acids, amino acids, organic anions and cations, neurotransmitters and nucleosides. Although the exact mechanisms of carrier-mediated influx of many substrates are unknown, this process probably involves the formation of transient narrow pores induced by binding of the respective substrate to the carrier, which then allows only the passage of the specific substrate molecule. Utilization of these carrier systems expressed at the BBB might be an attractive strategy for therapeutic delivery of other peptides and proteins that would otherwise have minimal access to the CNS.

2.2.1.4. Receptor-mediated endocytosis. The transport of peptides and proteins across cellular barriers has been documented in a number of systems like insulin [34]. Insulin-like growth factors (IGF-I, IGF-II), angiotensin II, atrial and brain natriuretic peptide (ANP, BNP), IL-1 and transferrin. However, receptor-mediated endocytosis across the BBB in vivo has been shown for few peptides and proteins like insulin, transferrin, certain cytokines and leptin while angiotensin II and ANP may exert their effects by binding on the luminal cytoplasmic membrane of brain microvessel endothelia, and may even be involved in the regulation of BBB permeability for other substances.

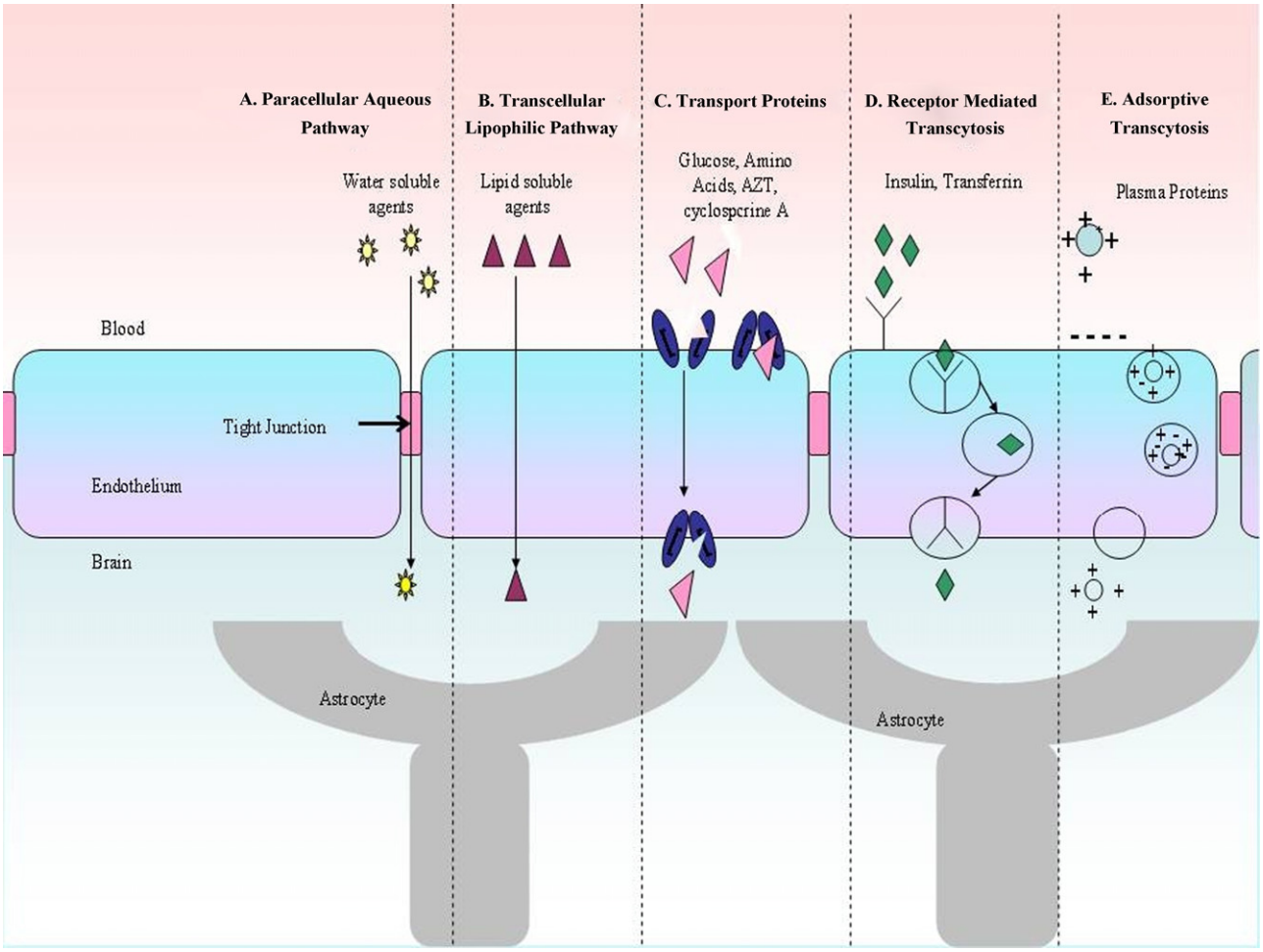

Fig. 1. Solute transport mechanisms across the blood–brain barrier.

3. Uptake mechanisms of nanoparticles across the blood–brain barrier

There are several possible endocytic pathways for internalizing nanoparticles, such as clathrin-mediated endocytosis, caveolae-mediated endocytosis, and clathrin–caveolae-independent endocytosis. Together, these mechanisms represent transcytosis or transcellular pathways that are predominant in all mammalian cells. Broadly, transcytosis is distinguished into three types; fluid mediated (FMT), adsorptive transcytosis which entails non-specific binding of solutes to the cell membrane and receptor-mediated endocytosis wherein dilute solutes are captured by specific high-affinity receptors which are concentrated into specialized endocytic transport vesicles. However, in the brain, there is virtually no fluid-phase (i.e. nonselective) transcytosis.

The brain capillary ECs contain two kinds of vesicles that are open to the luminal blood capillary space: the caveolae, also called plasmalemmal vesicles, and the clathrin-coated pits/vesicles, which are majority in number. Because the clathrin-coated pits that open at the luminal brain endothelial surface are negatively charged, they repel anionic molecules. In contrast to FMT, adsorptive transcytosis requires the interaction of a ligand with moieties expressed at the luminal surface of cerebral EC. Based on the interaction, it is further divided into specific (receptor-mediated transcytosis; RMT) and non-specific (adsorptive-mediated transcytosis; AMT) processes [35]. For a detailed understanding of endocytosis at the blood–brain barrier, the reader is referred to a review by Mark et al. [36].

3.1. Adsorptive-mediated transcytosis

The stage of transcytosis at the BBB starts with uptake either through clathrin-coated pits or caveolae. Transcytosis of molecules at the BBB is an energy requiring/ATP-dependent transport process, both for the endocytosis of the transported molecule at the luminal side of the EC and for its transport across the EC as well as for its exocytosis at the basolateral side. The density of mitochondria in cerebral EC is roughly five times greater than in peripheral endothelia, increasing the energy potential of the BBB as well. This enhanced cerebral capillary work capacity may be related to energy-dependent transcapillary vesicular transport. AMT may not involve specific plasma membrane receptors and that endocytosis is initiated through charge–charge interaction between polycationic substances and negative charges on the endothelial surface.

3.1.1. Caveolae-mediated endocytosis

Caveolae are characteristics flask-shaped, non-coated membrane invaginations having a size in the lower end 50–100 nm range. They are characterized by their association with caveolin, a dimeric protein that binds cholesterol, inserts as a loop into the inner leaflet of the plasma membrane, and self-associates to form a striated caveolin coat on the surface of the membrane invaginations. They are enriched in cholesterol and glycosphingolipids. Molecules found within caveolae, such as glycosyl phosphatidyl inositol (GPI)-anchored proteins, are not present in the coated pits. Caveolae may also contain an abundance of membrane receptors and transporters, as well as signaling molecules, which suggests

their possible involvement in various important cellular processes, in addition to their role in the endocytosis/transcytosis of specific molecules. The caveolae mediate the transcytosis of different sets of molecules across endothelial barriers.

The endocytosis via this mode is a highly regulated process involving complex signaling, which may be driven by the cargo itself. After binding to the cell surface, particles move along the plasma membrane to caveolae invaginations, where they may be maintained through receptor–ligand interactions. Fission of the caveolae from the membrane, mediated by the GTPase dynamin, then generates the cytosolic caveolar vesicle, which does not contain any enzymatic cocktail [37]. In some cases, this pathway is employed by many pathogens to escape degradation by lysosomal enzymes. However, in the brain, the molecules internalized can traffic through the brain ECs to allow for accumulation in lysosomes for degradation. It is to be noted, even after activation, caveolae are only slowly internalized (half-time, t 1/2, .20 min) and the small vesicles (~50–60 nm in diameter) carry little fluid-phase volume. Thus, it is unlikely that this process contributes significantly to bulk fluid-phase uptake.

3.1.2. Clathrin-mediated endocytosis [105,106]

Clathrin-mediated endocytosis (CME) involves the concentration of high-affinity transmembrane receptors and their bound ligands into 'coated pits' on the plasma membrane, which are formed by the assembly of cytosolic coat proteins, the main assembly unit being clathrin. Coated pits invaginate and pinch off to form endocytic vesicles, CCVs, which are encapsulated by a polygonal clathrin coat and carry concentrated receptor–ligand complexes into the cell. CCVs are very abundant in brain tissue and are relatively easily isolated, allowing identification of the main coat proteins. Clathrin is a three legged structure, called a triskelion, formed by three clathrin heavy chains, each with a tightly associated clathrin light chain. Under non-physiological conditions (low salt and high calcium concentrations), clathrin triskelions spontaneously self-assemble into closed polygonal 'cages'. However, clathrin-cage assembly under physiological conditions requires the other main coat constituents, the assembly proteins (APs). Two classes of structurally and functionally distinct APs were identified based on their ability to assemble clathrin: the monomeric assembly protein AP180, and heterotetrameric adaptor protein complexes. There are four structurally related adaptor protein complexes (AP1–4), each mediating vesicle formation at distinct subcellular localizations; however, only AP2 is involved in endocytic CCV formation. It consists of two large, structurally related subunits called a- and b2-adaptins, a medium subunit, m2, and a small subunit, j2. AP2 complexes have a barrel-shaped core comprising the amino termini of the adaptin subunits and the two smaller subunits, and two protruding appendages that are reminiscent of 'ears' formed by the carboxy termini of the a- and b2-adaptins, respectively.

3.1.3. Clathrin- and caveolin-independent endocytosis [105,106]

The mechanisms that govern caveolae- and clathrin-independent endocytosis remain poorly understood, as illustrated by the fact that these pathways are described only in negative terms. Nonetheless, it is likely that each of these pathways fulfils unique functions in the cell and varies mechanistically not only in how the vesicles are formed, but in terms of which cargo molecules they transport, to what intracellular destination their cargo is delivered, and how their entry is regulated. It is likely that these different pathways have evolved so that pinocytosis can be coordinated with more complex aspects of cell physiology, such as signal transduction, development and modulation of the cell's responses to and interaction with its environment.

3.1.4. Factors influencing adsorptive-mediated endocytosis

i. Particle size

The basic parameters such as particle size strongly influence the initiation of certain endocytic mechanisms over others. The research group incubated fluorescently labeled polystyrene nanoparticles of 50, 100, 200, 500 and 1000 nm diameter with murine melanoma cells (B16–F10). The cells were treated with selective endocytic inhibitors to reveal the pathways used to internalise the nanoparticles. Confocal microscopy revealed that <200 nm diameter nanoparticles were involved with clathrin-coated pits. However, caveolae-mediated endocytosis became more apparent as the nanoparticles increased in size (200–1000 nm). Moreover, 50–100 nm diameter particles were more rapidly internalized into cells than 200 nm nanoparticles; the mechanism for this was unclear.

ii. Surface charge

The surface charge does influence the internalisation pathway of nanoparticles. Both 90 nm diameter, anionic PEG–PLA nanoparticles and a cationic version which incorporated the cationic lipid stearylamine were incubated separately with MDCK (Canine Kidney Epithelial) cells and their uptake was studied using confocal microscopy, immunofluorescence and Western blotting [38]. It was found that the cationic nanoparticles avoided the downstream lysosomal pathway as opposed to the anionic particles.

3.2. Receptor-mediated transcytosis

Large molecules which are necessary for the normal function of the brain are delivered to the brain by specific receptors. These receptors are highly expressed on the endothelial cells forming the BBB. These include the insulin receptor, transferrin receptor, LDL receptor and its related protein, and others. Research is still on-going to identify new receptors. The receptor-mediated transcytosis occurs in 3 steps: (1) Receptor-mediated endocytosis of the compound at the luminal (blood) side. (2) Movement through the cytoplasm of the endothelial cell. (3) Exocytosis of the drug at the abluminal (brain) side of the brain capillary endothelium. The precise mechanism of transcytosis across polarized endothelial cells has not been determined. Additional molecules may be involved in the transcytosis across the BBB and bypassing of lysosomes in the cytoplasm which could degrade the molecules being transported. The physiologic approach comprises targeting these receptors at the BBB by specific ligands, modified ligands and antibodies. Therapeutic compounds are able to cross the BBB after association/conjugation to these specific ligands forming molecular Trojan horses (MTH) [33]. To delivery larger amounts of therapeutics, liposomes decorated with specific ligand have also been developed.

3.2.1. Transferrin receptor (TR) [107]

The function of the TR is to provide iron to cells. Drug targeting to the TR can be achieved by using the endogenous ligand transferrin, or by using antibodies directed against the TR. For transferrin (Tf) the in vivo application is limited due to high endogenous concentrations of Tf in plasma. Transferrin is an essential protein needed for iron delivery to cells and is found at mg/ml amounts in plasma.

3.2.2. Insulin receptor

Pardridge et al. have extensively documented the use of the insulin receptor for the targeted delivery of drugs to the brain using specific antibodies directed against the IR.

3.2.3. Low-density lipoprotein receptor related proteins 1 and 2 (LRP-1 and 2)

LRP is a multifunctional endocytic receptor that mediates the internalization and degradation of multiple ligands involved in

Table 1
Recent Examples of surface modified nanoparticles.

Sr. no.	Nanoparticles	Ligand	Pathway	References
1.	Modified DNA loaded cationic dendrimer-based nanoparticles	Lactoferrin	Clathrin-dependent endocytosis, caveolae-mediated endocytosis, and macropinocytosis	[40]
2.	Azidothymidine loaded pegylated albumin nanoparticles	Transferrin	Receptor-mediated transcytosis	[41]
3.	Daunorubicin liposomes	p-Aminophenyl-α-D-manno-pyranoside and transferrin	Receptor-mediated transcytosis	[42]
4.	Protamine-oligonucelotide nanoparticles	Apolipoprotein A-I	LDL receptor-mediated transcytosis	[43]
5.	Human serum albumin nanoparticles	Apolipoprotein E	LDL receptor-mediated transcytosis	[44]

diverse metabolic pathways. LRP is a multiligand lipoprotein receptor which interacts with a broad range of secreted proteins and resident cell surface molecules (eq. apoE (apolipoprotein E), á2M (á2 macroglobulin), tPA (tissue Plasminogen Activator), PAI-1 (Plasminogen Activator Inhibitor 1), APP (Amyloid Precursor Protein)), Factor VIII, Lactoferrin, mediating their endocytosis or activating signaling pathways through multiple cytosolic adaptor and scaffold proteins. LRP contains four putative-ligand binding domains (LBD) labeled with numerals I, II, III and IV. LRP, a type I transmembrane protein, is synthesized as a 600 kD precursor protein cleaved in the trans Golgi compartment by furin, to generate a large 515 kD subunit and a smaller 86 kD that remain non-covalently linked. The shorter cytoplasmic tail of LRP contains NPxY motifs and two dileucine-based motifs, and interacts with a number of cytoplasmic adaptor and scaffold proteins. LRP is expressed in many tissues and in the CNS. In the cerebellum, LRP expression was observed in neurons diffusely scattered throughout the granular cell layer. LRP expressed on neuronal cells functions similar to that of other cell types (i.e. hepatocytes) in both binding and endocytosis of ligand. Expression of LRP in astrocytes is detectable with moderate expression. LRP is over-expressed in malignant astrocytomas, especially in glioblastomas. LRP 1 and 2 have been exploited to target drugs to the brain in a similar fashion as TR and IR pathways through multiple cytosolic adaptor and scaffold proteins [39]. LRP contains four putative-ligand binding domains (LBD) labeled with numerals I, II, III and IV. LRP, a type I transmembrane protein, is synthesized as a 600 kD precursor protein cleaved in the trans Golgi compartment by furin, to generate a large 515 kD subunit and a smaller 86 kD that remain non-covalently linked. The shorter cytoplasmic tail of LRP contains NPxY motifs and two dileucine-based motifs, and interacts with a number of cytoplasmic adaptor and scaffold proteins. LRP is expressed in many tissues and in the CNS. In the cerebellum, LRP expression was observed in neurons diffusely scattered throughout the granular cell layer. LRP expressed on neuronal cells functions similar to that of other cell types (i.e. hepatocytes) in both binding and endocytosis of ligand. Expression of LRP in astrocytes is detectable with moderate expression. LRP is over-expressed in malignant astrocytomas, especially in glioblastomas. To illustrate the relevance of these pathways scientists have explored the utility of surface modification to enhance the uptake of nanoparticles. As depicted in Table 1, these modifications have been met with considerable success in comparison to the conventional delivery systems.

4. Nanoparticulate strategies to delivery drugs

Formerly, approaches for brain delivery included invasive superficial and ventricular application of chemical or the application of chemicals to brain parenchyma; making them less patient friendly, more laborious and requiring skill with possible damage permanent to the brain. In view of these considerations, novel drug delivery systems such as the nanoparticles are being explored for their suitability for targeted brain delivery. The clinical success of nanotechnology could be ascribed to their ability to deliver drugs in the optimum dosage range, often resulting in increased therapeutic efficacy of the drug and weakened side-effects. Blood circulation residence, maximal tolerated dose (MTD), and selectivity are the most important factors for achieving a high therapeutic index and corresponding clinical success. Typically, the drug is conjugated to the surface of the nanoparticle, or encapsulated and protected inside the core. Moreover, the delivery systems can be designed to provide either controlled release or a triggered release of the therapeutic molecule. The existing nanoparticulate systems can be broadly classified into two categories; lipid-based nanoparticles and polymeric nanoparticles.

4.1. Lipid-based nanoparticles

4.1.1. Liposomes

Liposomes were the first generation of novel drug delivery systems. Conventional liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer composed of biocompatible and biodegradable lipids similar to biological membranes. Cholesterol, an important constituent of many cell membranes is frequently included in liposome formulations because it reduces the permeability and increases the stability of the phospholipid bilayers. Thus, they can be classified according to their size and preparation method as follows: (i) small unilamellar vesicles (diameter between 20 and 50 nm; SUV), (ii) large unilamellar vesicles (100 nm; LUV), (iii) reverse phase evaporation vesicles (0.5 mm; REV) and (iv) multilamellar large vesicles (2–10 mm; MLV). Though, liposomes are hydrophobic in nature the exact mechanism by which they traverse the BBB is not fully understood. However, the transport is presumably achieved by passive diffusion through the lipophilic endothelial cells, by endocytosis or by fusion with brain capillary endothelial cells. The endocytic pathway represents an important means of transport for smaller liposomes with a diameter not larger than 80–100 nm, as their size is comparable with that of the brain endothelial cell vesicles which presumably transport them [45,46].

Gershon et al. [47] demonstrated a two-fold higher uptake of i.v. administered serotonin liposomes as opposed to drug solution; this was ascribed to the transport by monocytes following phagocytosis of liposomes. The serotonin liposomes were composed of distearoylphosphatidylcholine (DSPC), distearoyl phosphatidyl glycerol (DSPG) and cholesterol [DSPC:DSPG:CHOL, 3:1:2 molar ratio], having a size of 169.32 ± 36.32 nm, zeta potential of -29 ± 1.9 mV and a 10% encapsulation yield of the added serotonin concentration (50 mM) [47].

4.1.1.1. Cationic liposomes. Recent advances in liposomal formulations include cationic liposomes used to entrap genetic material. Encapsulation of genetic material into cationic liposomes confers a protection from the extracellular environment and provides a mechanism for genetic material transfer to target cells. The ability of cationic liposomes to mediate transfection was attributed

to certain properties such as spontaneous electrostatic interactions between the positively charged liposomes and the negatively charged DNA, which results in an efficient condensation of the nucleic acids. Through the interactions between cationic liposomes and nucleic acids, true liposomal structures are not formed but hexagonal structures, which are called "lipoplexes" [48].

A variety of mono or multivalent cationic lipids are currently available for gene transfer, such as DOTMA (*N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride) or DOTAP (1,2-dioleoyl-3-trimathylammonium-propane). These cationic lipids are frequently mixed with the neutral lipid dioleoyl phosphatidylethanolamine (DOPE), which is known to enhance transfection efficacy due to its ability to form hexagonal phases that may contribute to the destabilization of the endosomal membrane. The cholesterol also increases the levels of transfection and can potentially reduce the destabilization of the liposomes in the presence of serum [49]. Unlike liposomes, the cationic liposomes are thought to undergo adsorptive-mediated endocytosis, however, it is yet to be confirmed.

In a recent study, it was demonstrated that chlorin *m*-tetrahydroxyphenylchlorin (*m*-THPC) loaded mixed cationic liposomes, composed by different ratios of dimyristoyl-*sn*-*glycero*phosphatidylcoline (DMPC) and a cationic Gemini surfactant (Gemini 1), resulted in a high grade of laser-mediated (652 nm) cytotoxic effect on glioblastoma cells [50]. Shinji Takeoka et al. [51] reported there was increased in neuronal transfection of lipolexe formed by electrostatic interaction between amino acid, Arg-Glu2C16, liposomes and pDNA when applied to neuronal SH-SY5Y cells, maximum expression of the exogenous gene (16% of the transfected cells expressing GFP) was obtained with the lipoplexes with a lipid-to-DNA ratio of 15 at pDNA much higher than commercially available transfection reagent Lipofectamine 2000, with which 4% of cells were GFP-positive [51].

4.1.2. Nanoemulsions

Nanoemulsions are nanometric-scale emulsions, typically displaying droplet diameters in the range of 20–200 nm [52]. In contrast to microemulsions that are thermodynamically stable systems that form spontaneously, nanoemulsions are only stable kinetically. Two fundamental processes may be applied for the preparation of nanoemulsions, either by high-energy emulsification methods (e.g. high-pressure homogenizers) or ultrasound generators or by low energy methods (e.g. spontaneous emulsification) or the phase-inversion temperature (PIT) [53–57].

For brain delivery, the choice of oil component of nanoemulsion plays an important role. Several brain uptake studies have illustrated the selective uptake of essential polyunsaturated fatty acids, omega-6 fatty acids such as pinolenic and linoleic acids.

This was corroborated by research undertaken by Edmond 2001, wherein it was observed that linoleic acid with 18-carbon monocarboxylic acids with two cis-double bonds was imported in the brain, while oleic acid containing one cis-double bond was not [58]. In addition, nonessential fatty acids, including palmitic and stearic acids, were not found in the brain. The beneficial role of dietary supplements of omega-3 and omega-6 fatty acid containing oils has shown to reduce the tumor burden significantly. This was exemplified by the research work undertaken by Amiji et al. [59], combination of paclitaxel (PTX) and the apoptotic signaling molecule, C6-ceramide (CER), was administered in oil-in-water nanoemulsion (200 nm) formulated with pine-nut oil, which has high concentrations of essential polyunsaturated fatty acid (PUFA). The developed nanoemulsion showed significant enhancement in cytotoxicity due to an increase in apoptotic activity following treatment with combination PTX and CER therapy in the U-118 glioblastoma cells resulted in enhancement in cytotoxicity. Interestingly, when the cells were exposed to blank nanoemulsion, there was a slight increase in apoptotic activity (13%) while with PTX and CER at 100 and 10 mM dose, respectively, as single agents, the percent of cell undergoing apoptosis values were 12% and 10% for aqueous solution and 18% and 16% for the nanoemulsion formulations. Moreover, combination of PTX and CER led to substantial increase of 22% in cellular apoptosis with aqueous solution and more than 32% with the nanoemulsion formulation [54].

4.1.3. Nanocapsules

Nanocapsules are colloidal-sized, vesicular systems in which the drug is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating [59]. There are two variations possible, depending on the core and the structure of the surrounding polymer. Frequently, the core is an oily liquid, the surrounding polymer is a single layer of polymer, and the vesicle is referred to as a nanocapsule/lipid nanocapsule. Alternatively, if the core of the vesicle is an aqueous phase and the surrounding coating is a polymer bilayer, the particle is referred to as a polymersome [60]. Generally, they are prepared by nanoprecipitation, emulsion–diffusion, double emulsification, emulsion–coacervation, polymer coating and layer-by-layer [61].

Recently, etoposide loaded lipid nanocapsules (LNC) as drug delivery device was developed and evaluated for the drug release and their efficiency to reduce cell growth in cell culture for C6, 98 and 9L glioma cell lines. The developed LNC exhibited a very small size (mean diameter 25–100 nm) that facilitates their intracellular uptake. Additionally, the developed LNC was hypothesised to reverse MDR owing to the presence of P-gp inhibiting surfactant PEG–HS (polyethylene glycol–660 hydroxystearate), one of the LNC constituents [62,63].

Abattastini et al. developed indomethacin-loaded nanocapsules using indomethacin, poly(e-caprolactone), capric/caprylic triglyceride and sorbitan monostearate. Although indomethacin is not an agent used in the treatment of brain tumors, the study in rats with implanted C6 glioma demonstrated the effectiveness of sub-therapeutic (1 mg/(kg day)) dose of indomethacin-loaded nanocapsules to avert mortality and toxicity, while improving the body weight of treated animals, compared to the control group [64].

4.1.4. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are a comparatively stable colloidal carrier system in which melted lipid is dispersed in an aqueous surfactant by high-pressure homogenization, solvent injection, solvent emulsification–diffusion, solvent emulsification–evaporation or microemulsification. They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed [65]. Smaller size (around 10–200 nm) and narrow size range (100–200 nm) allows them to cross tight endothelial cells of the blood–brain barrier (BBB), escape from the reticuloendothelial system (RES), and bypass liver. They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix, and provide a controlled release lasting up to several weeks [66]. On the other hand, since SLNs are prepared from biocompatible materials, triglycerides, fatty acids, or waxes, they are bound to release natural occurring degradation byproducts. Moreover, the phagocytosis of SLN can be controlled modifying their surface properties as has been done for liposomes and polymeric micro- and nanoparticles. In this way, it is possible to target molecules to the brain by limiting RES uptake [67].

Manjunath and Venkateshwarlu made SLNs of a lipophilic drug nitrendipine for improving its bioavailability upon i.v. administration. Nitrendipine loaded SLNs were made using different triglycerides (tripalmitin, trimyristin and tristearin), emulsifiers—soy lecithin, poloxamer 188 and charge modifiers (dicetyl phosphate; DCP and stearylamine, SA). Upon i.v. administration of nitrendipine suspension and nitrendipine SLNs,

nitrendipine SLNs were found to be taken up to a greater extent by the brain and maintained high drug levels for 6 h as compared to only 3 h with nitrendipine suspension. The C_{max} of 3.2, 7.3 and 9.1 times was achieved with nitrendipine tripalmitin, nitrendipine tripalmitin dicetyl phosphate and nitrendipine tripalmitin stearylamine SLNs when compared with nitrendipine suspension [68]. Wang et al. have reported the synthesis of 3′,5,-dioctanoyl-5-flouro-2,-deoxyuridine to overcome the limited access of the drug 5-flouro-2,-deoxyuridine (FUdR) and its incorporation into solid lipid nanoparticles (DO–FUdR). The brain area under the concentration/time curve of DO–FUdR–SLN and DO–FUdR were 10.97- and 5.32-fold higher than that of FUdR, respectively. These results indicated that DO–FUdR–SLN had a good (2 times the free drug) brain targeting efficiency in vivo. These authors report that SLN can improve the ability of the drug to penetrate through the blood–brain barrier and is a promising drug targeting system for the treatment of central nervous system disorders [69,70].

4.2. Polymer-based nanoparticles

4.2.1. Polymeric nanoparticles

The polymeric nanoparticles may be defined as a matrix type, solid colloidal particles in which drugs are dissolved, entrapped, encapsulated, chemically bound or adsorbed to the constituent polymer matrix [71–73]. These particles are typically larger than micelles having diameters between 100 and 200 nm and may also display considerably more polydispersity [74]. The mechanisms by which polymeric nanoparticles pass through the BBB are not completely understood. Recent studies attribute a central role to endothelial cells in the process of nanoparticle adhesion (e.g. by recognition of a specific blood protein adsorbed on the particle surface) and subsequent endocytosis, transcytosis, tight junction modulation and P-glycoprotein inhibition. It has also been reported that the size of the carriers, polymer type, as well as their surface characteristics could induce steric stabilization of nanoparticles, thus increasing blood circulation time and accumulation in the solid tumor [60].

Khuller et al. evaluated the potential of orally administered poly-lactide-co-glycolide (PLG, a synthetic polymer) nanoparticle encapsulated antituberculosis drugs (ATDs) (rifampicin, isoniazid, pyrazinamide and ethambutol) for cerebral drug delivery in a murine model. The authors reported a single oral dose of the formulation to mice could maintain sustained drug levels for 5–8 days in the plasma and for 9 days in the brain. There was a significant improvement in the pharmacokinetic parameters such as mean residence time and relative bioavailability as compared with free drugs. The pharmacodynamic parameters such as the ratio of area under the curve to minimum inhibitory concentration (AUC/MIC) and the time up to which MIC levels were maintained in plasma (TMIC) were also improved. In Mycobacterium tuberculosis H37Rv infected mice, five oral doses (as opposed to 46 doses of conventional free drugs) of the nanoparticle formulation administered every 10th day resulted in undetectable bacilli in the meninges, as assessed on the basis of colony forming units and histopathology [75]. George et al. demonstrated the use of polybutylcyanoacrylate nanoparticles for delivery of intact, functional proteins into neurons and neuronal cell lines. Uptake of these particles is primarily dependent on endocytosis via the low-density lipoprotein receptor [76].

4.2.2. Polymeric micelles

Polymeric micelles as drug delivery systems are formed by amphiphilic copolymers having an A–B diblock structure with A, the hydrophilic (shell) and B, the hydrophobic polymers (core). The polymeric micelles are thermodynamically and kinetically stable in aqueous media [66]. Their stability depends upon strong cohesive force between drug and core polymer segments as well as cross-linking of the shell or core, which is performed by radical polymerization. Prolonged circulation and targeted delivery of PMs is possible by designing of environment-responsive polymeric micelles (pH, light, temperature, ultrasound, etc.) [77].

Earlier studies by Kabanov et al. [77] have shown that poloxamer (Pluronic™) micelles conjugated with antibodies may improve brain distribution of haloperidol, a neuroleptic agent; this approach has resulted in dramatic improvement of drug efficacy. This result indicates that Pluronic™ micelles provide an effective transport of solubilized neuroleptic agents across the BBB [78]. However, recent investigations made by the same group demonstrated that only Pluronic™ unimers allowed cell penetration in bovine BMEC monolayers of molecules such as rhodamine 123, digoxin or doxorubicin by inhibition of the P-gp mediated drug efflux system [79–81]. Other studies performed by Witt et al. have shown an increased analgesic effect [82].

4.2.3. Dendrimers

Dendrimers are globular, nanoscaled macromolecules with a particular architecture constituted of three distinct domains: (i) a central core that is either a single atom or a group having at least two identical chemical functionalities, (ii) branches emanating from the core, composed of repeat units having at least one junction of branching, whose repetition is organized in a geometric progression that results in a series of radially concentric layers called generations (G), and (iii) many identical terminal functional groups, generally located in the exterior of the macromolecule, which play a key role in their gene-complexing or drug-entrapping ability. They possess exceptional structural properties such as monodispersity (∼1), high density of peripheral functional group and well-defined globular shape and multivalency [83].

Patrice Hildgen et al. studied the uptake and permeation of rhodamine B labeled polyether-copolyester (PEPE) dendrimers across the blood–brain barrier model and explored the underlying mechanisms. They observed saturation in the uptake of PEPE dendrimers brain vascular endothelial cells at high concentrations. Clathrin and caveolin inhibitors produced partial inhibition of the dendrimer uptake, signifying contribution of both pathways in the uptake process. The results of this study suggested that architecture of dendrimers plays a major role not only in influencing the extent and mechanism of uptake by brain vascular endothelial cells but also permeation across the BBB model [84].

Dhanikula et al., reported enhanced anti-tumoral efficacy of methotrexate (MTX)-loaded polyether-copolyester (PEPE) dendrimers against U87 MG and U 343 MGa cells. Furthermore, there was reduction in IC50 of MTX after loading in dendrimers than that of the free MTX, suggesting that loading MTX in PEPE dendrimers increased its potency. In addition, the amount of MTX-transported across BBB was three to five times more after loading in the dendrimers. Moreover, these MTX-loaded dendrimers were able to kill even MTX-resistant cells highlighting their ability to overcome MTX resistance [85].

5. Toxicity considerations of nanoparticles

The lack of toxicology data on nanocarrier systems hinders governmental regulation [86,87,89,90,92,93]. Currently, no regulatory requirement to test nanoparticles for health, safety, and environmental impacts has been formalized [65]. Toxicity studies are critical to establish the full in vivo potential of nanotechnology and nanomedicine in particular [85–91,94–96]. Understanding the physicochemical, molecular, and physiological processes of nanoparticles is imperative for nanomedicine to become a reliable and sustainable treatment modality [96]. Many aspects of nanopar-

ticle architecture and composition influence systemic toxicity [97]. Nanoparticles enter cells via endocytotic processes including clathrin-mediated endocytosis, potocytosis, pinocytosis, and patocytosis [1,8,10]. Following endocytosis, the engulfed material is delivered to the endosome and subsequently ends up in a degradative compartment, the lysosome [1]. In the lysosome, materials are exposed to hydrolytic enzymes that are active on proteins, polysaccharides, and nucleic acid components [86]. Due to their size, nanoparticles have a large specific surface area [89,90] which may translate into increased biological activity, due to different contact interactions with cells and its components, and variable biokinetics [89]. Physicochemically, nanoparticles vary widely from the properties of bulk materials [85–87], making it pertinent to investigate the stability of nanoparticles since there is always a possibility of Oswalt ripening and agglomeration [98,99]. There have been incidence of nanoparticles inducing the formation of pro-oxidants, especially under exposure to light, ultraviolet (UV) light, or transition metals; thereby, destabilizing the balance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify or repair the system [87,100]. It is well known that toxic effects brought about by exposure to nanoparticles are related to the ability of these nanoparticles to catalyze the production of reactive oxygen species and to bind irreversibly to membranes or DNA. This causes interference at multiple levels of cellular metabolism, signalling and genetic alterations. Studies, so far, point towards a majority of intracellular rather than extracellular interferences, posing the question of how nanoparticles enter the cells of utmost importance.

As described above, nanoparticles may trigger an inflammatory process resulting in the release of cytokines and chemokines, such as IL-6, (IL)-1b, TNF-α, reactive oxygen species, C-reactive protein, and transcription factors. This cascade results in the activation of mitogen-activating protein kinase (MAPK), redox sensitive transcription factors, nuclear factor kappa B (NF-jB), and activating protein-1 (AP-1). By analogy, the etiology of atherosclerosis and coronary heart disease is thought to be inflammatory, as patients display similar pro-inflammatory markers. These inflammatory mechanisms can lead to cardiopulmonary events. Studies using genetically susceptible mice exposed to long-term nanoparticles air pollution showed an acceleration of atherosclerosis and vascular inflammation. It may be inferred that these nanoparticles may promote, if not trigger, low-level systemic inflammation at distant organs and tissues, depending on nanoparticles access to the vasculature via penetration of small blood vessels and capillaries [97].

It has been hypothesized that dermal exposure might be the most significant route of exposure; however, few literature reports are available that refer to the absorption and effects of nanoparticles in the skin [86–88]. As the skin is easily accessible, the transdermal absorption is well studied in recent vaccine and drug delivery research projects. These targeted studies involve delivery of nanoparticles to the dermis by penetration of the epidermis. The studies reported to date indicate that nanoparticles migration through the skin is possible, especially when mechanical flexion is applied to the skin. The migration of nanoparticles through the dermis suggests that systemic circulation can be reached. However, quantitative data confirming this absorption process is lacking. Almost all experiments are performed on healthy human or porcine have reported the penetration of negatively charged latex particles (50 and 500 nm), while positively charged and neutral particles were not able to penetrate the epidermis at all. They concluded that also the charge of nanoparticles is one of the important factors in the transdermal absorption process. In addition, quantum dots (spherical: 4.6 nm and ellipsoid: 12 nm by 6 nm) showed penetration through the intact skin (dermis) [104]. This suggests that the skin is permeable to nanomaterials with distinct physicochemical properties (size, shape, charge, material). Once in the epidermis, nanoparticles reach the lymphatic system and regional lymph, and from there they can translocate to the systemic vasculature [86].

Another portal of entry includes the respiratory system; wherein particle deposition in the respiratory tract is often governed by particle size, breathing force and the structure of the lungs. The respiratory tract can be divided into three regions: nasopharyngeal, tracheobronchial, and alveolar regions [88]. Significant amounts of certain particle size ranges can deposit in each region, for example, 90% of nanoparticles of 1 nm in diameter deposit in the nasopharyngeal region, whereas only 10% of these nanoparticles deposit in the tracheobronchial region and almost none reach the alveolar region [89]. Owing to the small diameter of the nanoparticles and associated Brownian diffusion, nanoparticles are able to penetrate into the deeper regions of the lungs and diffuse to the high lung surface area presented in the alveolar region. It has also been demonstrated that nanoparticles in the low nanometer region deposited in the upper airways due to strong diffusion prior to their transportation into the deep lung [3,25]. It has been shown that 15% of nanoparticles of 20 nm in diameter deposit in the nasopharyngeal region, 15% in the tracheobronchial region, and approximately 50% in the alveolar region [89]. Eventually, the nanoparticles get absorbed across the lung epithelium into the blood and lymph to reach cells in the bone marrow, lymph nodes, spleen, and heart [86]. Nanoparticles can even reach the central nervous system and ganglia following translocation (i.e. the transport of dissolved materials within the body); which is one of the mechanisms proposed for nanosized particles to reach extrapulmonary sites and then other target tissues [86,95]. Nanoparticles can access the systemic vasculature directly or via lymphatic transfer by transcytosis, crossing the epithelia of the respiratory tract into the interstitium, phagocytosis, endocytosis or some other transmembrane process [86,95]. A second target after translocation is suggested to be the sensory nerve endings embedded in the airway epithelia, followed by translocation to ganglia and the central nervous system via axons [86,93].

GI tract is also another medium for nanoparticles; it could be through ingestion directly in food, water, cosmetics, drugs, and drug delivery devices or possibly after mucociliary clearance from the respiratory tract through the nasal region. Though there is not much data to expound on the toxicity of lipid/polymeric-based nanoparticles via this route [86,96], there are studies which have shown the possibility of nanoparticles to enhance the immunogenic response especially in parasitic infections. Since, in gastrointestinal tract most of the materials are broken down or modified by the action of enzymes as well as pH, it could plausibly modify the toxicological proclivity of the systems. Further studies on gastrointestinal lymphatic uptake and transport, and direct toxicological effects on the GI tract are required to ascertain the safety of the nanosystems.

Treatment of neurodegenerative disorders is an inexhaustible pursuit which has translated into a tremendous advancement in the outlook of the researchers worldwide. This has rendered in the exploitation of nanotechnology using different materials with different characteristics. The purpose of all the innovations has always been to obliterate the side effect associated with the long-term use of medications. Though nanoparticles are portending to be an effective delivery system, their toxicology needs to be thoroughly investigated to achieve an effective therapy.

6. Summary

The blood–brain barrier (BBB) is the most important limiting factor for the development of new drugs and drug delivery for the central nervous system. With unprecedented increase in the population afflicted by neurodegenerative disorders, it has become

increasingly important to develop a dosage form capable of surmounting the challenges imposed by the anatomical barrier of the brain. Physiologically, blood–brain barrier is designed in such a manner that it can only permit the transport of molecules essential for functional activity of brain. It efficiently prevents flow of water-soluble molecules from blood circulation into central nervous system, and can also decrease concentration of lipid-soluble molecules by the action of enzymes or efflux pumps. As discussed, the use of nanoparticles to deliver drugs to the brain by infiltrating blood–brain barrier (BBB) may provide a significant strategy to break this impasse. As illustrated in numerous literatures, nanoparticle cross blood–brain barrier without altering the original characteristics of the therapeutic drug molecule. Furthermore, this system may reduce drug leaching in the brain and decrease peripheral toxicity. However, in-depth studies need to be undertaken for better comprehension of the likely toxicity potential of these nanosystems. Nevertheless, this expanding realm with appropriate improvisations could be touted as an effective tool to tackle the brain disorders.

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