Chapter 15
Brain-Targeted Drug Delivery with Surface-Modified Nanoparticles

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Abstract Medical treatment of CNS disorders remains unsuccessful as most of the drugs could not penetrate through the blood brain barrier (BBB). Although several strategies were developed to overcome these problems, still the treatment remains ineffective. To overcome these problems, nanomedicines which are based on noninvasive strategies are an emerging trend for brain-targeted drug delivery. The advantages of nanoparticles such as small size, lipophilicity, target specificity, and controlled delivery of drug satisfy the requisites for brain targeting. However, it suffers from opsonization and phagocytosis, which can be bypassed by surface modification of nanoparticles. The carrier/transporter-mediated transcytosis, adsorptive-mediated transcytosis, receptor-mediated transcytosis are the different mechanism followed by surface-modified nanoparticles to cross the BBB. However, nanoparticles may cause neurotoxicity due to its accumulation, oxidative stress and protein aggregation. Still nanoparticles are a promising carrier for drug targeting to the brain. The present chapter highlights the significance and recent development of drug targeting to the brain with surface-modified nanoparticles, the mechanism of transport and nanotoxicity.

Keywords Brain-targeted nanoparticles · Noninvasive nanomedicines · Surface-functionalized magnetic nanoparticles · Carrier-mediated transcytosis · Receptor-mediated transcytosis · Low-density lipoprotein receptor · Neurotoxicity of surface-functionalized nanoparticles · Nanotoxicity

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1 Introduction

According to World Health Organization (WHO) report, neurological disorders ranging from epilepsy, Alzheimer disease, brain tumor, HIV encephalopathy, cerebrovascular diseases, and neurodegenerative disorders affect up to one billion people worldwide. About 6.8 million people die of neurological disorders every year. It signifies an inefficient delivery of CNS drugs to the brain. In the nineteenth century, a German physician Paul Ehrlich found out the existence of a physical barrier between brain and blood, and then it was not until 1960s that the researcher could find out the existence of BBB [1]. BBB provides the most distressing fact about the drug delivery to the brain. The other barrier present is the blood–cerebrospinal fluid barrier (BCSFB) and CSF–brain barrier (CSFB). The BBB is considered to be the major barrier due to its large surface area which is considered to be the main site for crossing of endogenous substances into the CNS [2]. BBB have a tendency to impair the drug distribution and refers to the major challenge for the development of CNS drugs. In spite of the complexity of the BBB, the lack of efficient technologies also limits the development of CNS drugs [3]. Although conventional therapies are available, yet the treatment fails. Briefly, BBB located at the choroid plexus epithelium controls the exchange of molecules between the blood and the cerebrospinal fluid. It is composed of tight junctions of protein complexes of endothelial cells, the capillary basement membrane, astrocytes end feet present over the basal lamina and pericytes present in the abluminal side of the endothelial cells, in the perivascular space, between the capillary wall and astrocytes end feet. The tight junctions of the endothelial cells are nonfenestrated, contains low number of endocytic vesicles, high electrical resistance, higher mitochondrial volume and specialized transport system. BBB restricts the entry of 98% of small molecules and 100% of macromolecules. Only lipophilic molecules, small molecular size (<500 Da) could penetrate through the BBB [4]. BBB restricts the penetration of most of the large-sized, hydrophilic drugs such as oligonucleotides, peptides, and antibiotics. The presence of the tight junctions between the endothelial cells at the BBB promotes a very high electrical resistance of around 1500–2000 Ω cm^2 in the brain compared with 3.33 Ω cm^2 in other body tissues. Still BBB allows the transport of chemical and biological endogenous substances to cross the membrane [5]. However, several endogenous substances are transported to the brain and toxic compounds are excreted from the cerebral and vascular compartment by the influx transport system in the endothelial cell membrane [6]. The passive transport is responsible for the influx of molecules having low molecular weight, good lipophilicity and low protein binding through the BBB. The small molecules such as hormones, O_2, and CO_2 are transported by passive transport mechanism [7], whereas active transport comprises transporter-mediated transcytosis and receptor-mediated endocytosis. The transporter-mediated transcytosis is for the influx of small hydrophilic molecules through the carriers present on the endothelial cell membranes. As such, glucose carrier GLUT1 and amino acid carrier LAT1 are responsible for the transport of glucose and amino acids through the BBB, whereas the membrane receptors present

on the endothelial cells are responsible for the transport of transferrin, insulin, and lipoprotein through the BBB by receptor-mediated endocytosis [8]. The drug delivery through the BBB remains the most challenging area of research, which attracts researchers to investigate on several strategies for an efficient CNS drug delivery. Basically, two strategies are studied—invasive and noninvasive. Invasive strategies involve either disruption of the BBB to allow drug delivery or direct injection of the drug into the brain. The disruption of the BBB involves an intra-arterial injection of hyperosmotic solution of mannitol which causes shrinkage of endothelial cells, resulting in opening the tight junctions for few hours, and thus facilitating delivery of the drug to the brain. Other substances such as high-dose ethanol, DMSO, alkylating agents like etoposide and melphalan, immune adjuvants, and cytokines have all been used to disrupt the BBB. No doubt such techniques can deliver drug to the brain, but as invasive strategies, they suffer from side effects such as seizures, bradycardia, and hypotension [9]. Recently, focused ultrasound with microbubbles is reported to be nondestructive delivery of drug to the brain. In this technique, microbubbles, 1–10 μm sized diameter, are introduced into the blood circulation prior to ultrasound administration. The microbubbles disrupt targeted areas of BBB without causing any neural damage, which reduces the intensity of the ultrasound needed to open the tight junctions. The second invasive CNS drug delivery based on injecting the drug through injection or catheter requires opening of the skull [10]. The major drawback is the penetration to the nontarget brain tissue, brain tissue damage, bleeding, and chance of infection. Alternatively, wafers such as Gliadel wafers which are biodegradable impregnated with chemotherapeutics can be implanted. Reliable on the diffusion of the drug from the injection and implant sites, the concentration of drug distribution at the site of action cannot be controlled due to an exponential decrease in the concentration of the drug with distance from the injection or implantation site [11]. On the other hand, convection-enhanced delivery developed with positive hydrostatic pressure to deliver drug at farther distances from the site of administration did not show a significant result with Gliadel wafers, as reported [12]. The invasive technique facilitates an increase in permeability of the BBB which is reported to be harmful. As reported, similar permeability enhancement and disruption of the BBB occurs during cerebral ischemia and hypoxia. It leads to the leakage of the serum proteins into the brain which triggers the activation of astrocytes and brain immune system, leading to neuronal hyperexcitability and neurodegeneration. Similar results may be observed with multiple sclerosis and encephalitis where the disruption of the BBB leads to the leakage of plasma components into the blood vessels and surrounding tissues results neuronal damage and other disabling cerebrovascular conditions—lacunar stroke, leukoaraiosis, and dementia [13]. The drawbacks associated with the invasive techniques are based on high cost and chemotherapy either by osmotic agents or direct injection or Gliadel wafers or convection enhanced delivery may lead to BBB dysfunctioning and multiple brain diseases [14]. Alternatively, noninvasive strategies were developed to overcome the potential disadvantages of invasive strategies. In addition to the passive transport of small-sized lipophilic molecules, other transport mechanism constitutes paracellular aqueous pathway for water-soluble agents, carrier or transporter-mediated

transcytosis that relies on the transport mechanism of glucose and amino acid, receptor-mediated transcytosis that mimics the endogenous molecules such as insulin and lipoproteins to act on the specific receptor in the endothelial cell membrane and adsorptive-mediated transport that allows polycationic substances such as cationized albumin to attach to the negatively charged plasma membrane [3]. Another noninvasive strategy follows the conversion of water-soluble/polar drugs into lipid soluble ones by linking with some lipid/nonpolar moiety. Esterification or amidation of the hydroxyl, amino, and carboxylic groups of the drug enhances the lipid solubility and membrane permeability of the drug. This concept is known as prodrug. Prodrug as such is pharmacologically inactive compounds which crosses the BBB and metabolizes into the parent drug. The receptor-mediated transport or carrier-mediated transport have been exploited significantly in delivering the prodrug across the BBB. One example is the antiparkinsonism drug, L-DOPA acting on the L-amino acid transporter system. The prodrug suffers from the drawback of adverse pharmacokinetics, increase molecular weight of the drug [15]. Another noninvasive strategy, nanomedicine has gained lots of attention in the development of CNS-targeted drug delivery due to its ability for targeted drug delivery and sustained release of drug [16]. This chapter highlights the significance and recent development of drug targeting to the brain with surface-modified nanoparticles, the mechanism of transport and nanotoxicity.

2 Nanoparticles for Brain Drug Delivery

2.1 Nanoparticles Technology

Nanoparticles can be defined as colloidal particles of size range 1–100 nm which adsorb the drug to their surface or entrap the drug within their matrix. Other noninvasive techniques could deliver an inadequate drug to the brain and also affect the nontarget sites causing toxicity, whereas nanoparticles are target specific and deliver only the required quantity of the drug to the site of action without affecting the nontarget sites and thus reduces toxicity like other noninvasive techniques. In order to target drugs to the brain, nanoparticles should be nontoxic, should be biodegradable, have small particle size (<100 nm), have prolonged blood circulation without agglomeration, target specificity, and good drug loading [17]. A prolonged blood circulation is essential to recognize the therapeutic site of action so as to have an efficient drug release. However, the opsonization or removal of nanoparticulate drug carriers from the body by the mononuclear phagocytic system (MPS), also known as the reticuloendothelial system (RES), obstructs the efficient drug delivery at the site. When nanoparticles enter systemic circulation after intravenous administration, they undergo opsonization and phagocytosis, leading to an inadequate distribution in the brain and poor drug availability in the brain, thus making the therapy inefficient.

As such opsonization can be described as the process of attachment of opsonin to the surface of undesirable particles during systemic circulation, thereby making it visible to phagocytic cells. Without the presence of opsonin on their surface, phagocytes may not be able to recognize the foreign particles. Thus, opsonization and phagocytosis are the two methods of clearance of foreign particles from the bloodstream. The common opsonin present in the blood serum constitutes immunoglobulins and components of the complement system such as C3, C4, and C5 [18]. Other blood serum protein includes laminin, fibronectin, C-reactive protein, type I collagen, and many others. As such, opsonins are inactive proteins, but when it comes in contact with foreign particles, it undergoes conformational changes from inactive protein to active proteins which in turn are easily identified by phagocytes. Phagocytes' surface contains receptors which can easily identify the modified conformation of the opsonin and thus can easily alter the functioning of foreign bodies. The ingestion of nanoparticles by phagocytes takes place. The breakdown of the phagocytosed materials takes place due to the secretion of enzymes and other oxidative-reactive chemical factors, such as superoxides, oxyhalide molecules, nitric oxide, and hydrogen peroxide. Nanoparticles are taken rapidly by RES present in liver, spleen, bone marrow and distributed rapidly into liver (60–90)% and spleen (2–10)% and to a minor degree into bone marrow [19]. A low concentration of nanoparticles can enter the brain due to their uptake by RES following intravenous administration. In order to avoid opsonization and phagocytosis, several techniques are hypothesized to modify the surface of nanoparticles such as coated nanotechnology and ligand-based nanotechnology [19]. Surface-modified or -functionalized nanoparticles could not adsorb opsonin on the surface and may not be recognized by phagocytes. Thus, avoidance of opsonization and phagocytosis by the RES or MPS prevents its clearance from the bloodstream, leading to the prolonged circulation of nanoparticles in the blood, adequate delivery of the drug to the brain and maintaining the therapeutic concentration of the drug in the brain. Thus, nanocarriers as a noninvasive strategy are significant for brain-targeted delivery of drugs [20].

3 Types and Significance of Surface-Functionalized Nanocarriers

3.1 Liposomes

Liposomes are small vesicles composed of one or more phospholipid bilayers surrounding an aqueous space. Both hydrophilic and hydrophobic drugs can be incorporated in liposomes and their physicochemical characteristics can be manipulated to control drug delivery and tissue uptake of the drug. Zhao et al. reported that RDP peptide-conjugated liposome (RCL) could deliver curcumin to the intracranial glioma mice model transplanted with U251MG cells. RCL could enter the cells by

acetylcholine (Ach) receptor-mediated, energy-dependent endocytosis [21]. In another study, transferrin-conjugated PEGylated-liposome could internalize anti-cancerous drug, resveratrol to the U-87 glioblastoma cells by the receptor-mediated endocytosis through the transferrin receptor present on the endothelial cell [22] as reported by Jhaveri et al. In another study, Kim et al. followed transferrin mediated transcytosis pathway for successful targeting of temozolomide using transferrin grafted nanoliposomes in glioblastoma multiform tumor model in mice [23].Qu et al. reported that rabies virus glycoprotein (RVG29)-functionalized liposomes efficiently enhanced the entrapment efficiency of dopamine-derived *N*-3,4-bis(pivaloyloxy)-dopamine (BPD) in murine brain endothelial cells and dopaminergic cells and high penetration efficiency across the blood brain barrier (BBB) in vitro. RVG acts on the Ach receptor present on the brain endothelial cells and dopaminergic cells to facilitate the transport of BPD across the BBB [24]. Sonali et al. developed transferrin-conjugated theranostic D-alpha-tocopheryl polyethylene glycol 1000 succinate monoester (TPGS) liposomes which successfully targeted docetaxel and quantum into brain cancer cells [25]. T7 (a seven-peptide ligand of transferrin receptors) and ^{D}A7R (a D-peptide ligand of vascular endothelial growth factor receptor 2) dual peptides-modified PEGylated liposomes were able to code-liver doxorubicin (DOX) and vincristine (VCR) to C6 tumor mice model by receptor-mediated endocytosis as reported by Zhang et al. [26]. However, liposomes suffer from drawbacks such as low solubility, oxidation and hydrolysis of the phospholipids, leakage of the encapsulated drugs, and instability which limits its use in targeted drug delivery.

3.2 Polymeric Nanoparticles

Polymeric nanoparticles, made from biodegradable and nonbiodegradable polymers, are spherical, branched, or shell structure colloidal solid particles with a size range of 10–1000 nm in which drugs are incorporated by dissolution, entrapment, adsorption, and attachment or by encapsulation. The polymeric nanoparticles are an ideal platform for targeted and controlled drug delivery. Biodegradable polymeric nanoparticles have got a wide application in brain-targeted delivery of drugs, as it can be manipulated to fulfill the criteria needed for brain-targeted delivery due to small size, nontoxicity, biodegradability, etc. Poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(aspartic acid), poly(butylcyanoacrylate) (PBCA), poly(glycolic acid) (PGA), poly(D,L-lactide-*co*-glycolide) (PLGA), and poly(amino acids) are the most commonly used polymers in CNS delivery [27]. As such surface modification is essential to avoid opsonization and phagocytosis by macrophages. Till now, several research works have been carried out which successfully target different drugs across the BBB using biodegradable polymeric nanoparticles. In 1995, Kreuter et al. were the first to develop dalargin-loaded PBCA nanoparticles coated with polysorbate 80 that successfully targeted dalargin to the brain and also enhanced the penetration by threefold than the nanoparticles without surface

coating [28]. Later on, polysorbate 80 was further used to enhance drug transport of several drugs like loperamide and doxorubicin. Later PEGylated poly(hexa-decyl cyanoacrylate) (PHDCA) nanoparticles were found to penetrate the brain to a greater extent than the P80 formulation which might be due to passive diffusion or intake by macrophages [29]. PLA, PGA, and their copolymer, PLGA are extensively used in brain-targeted delivery of different drugs. In a study, H102 peptide, an antialzheimeric drugs was targeted successfully. A tocopherol PEGylated PLGA nanoparticles targeted oxcarbazepine, an antiepileptic drug, across in vitro models of the blood–brain barrier (hCMEC/D3 cells) and human placental trophoblast cells (BeWo b30 cells) [30]. Glutathione–PEG conjugate-coated PLGA nanoparticles showed higher permeation through the coculture of rat brain endothelial (RBE4) and C6 astrocytoma cells. The glutathione on the surface of the nanoparticles are found to bind to the glutathione transporters present on the BBB and deliver the drug by carrier or transporters mediated transcytosis [31]. Another report, by Ahmed et al., showed the efficient targeting of Rutin in rat model, an antioxidant, to the brain using chitosan nanoparticles through intranasal administration [32]. Although, FDA approved polymers are recommended for nanoparticulate drug delivery, yet some drawbacks exist during nanoformulation which are to be considered for further modification. Other than these, polymeric nanoparticles suffer from other disadvantages such as high cost, inability of autoclave sterilization, low-scale production, and presence of organic solvent residue. But, despite its drawbacks, it is still a potential drug delivery vector for targeting drugs to the brain [33].

3.3 Solid Lipid Nanoparticles (SLNs)

Later on, SLNs came into play for drug targeted delivery system. It has a size range of 1–100 nm, composed of a monolayer of phospholipid surrounding a solid hydrophobic core of lipids, such as monoglycerides, diglycerides, and triglycerides or fatty acids. They are stable and biodegradable under physiological conditions with high encapsulation efficiency both for hydrophilic and hydrophobic drugs [33]. Solid lipid nanoparticles can be applied for targeted drug delivery, controlled drug delivery and also can be surface-functionalized with polymeric coating or ligand grating for targeting drugs significantly. Other advantages include large-scale production, long-term stability, avoidance of organic solvents, easy scale-up and sterilization, less cost than polymeric/surfactant-based carriers, and easy validation and regulatory approval [34]. These make solid lipid nanoparticles an attractive approach for targeted drug delivery. The anticancer drug, camptothecin was the first drug delivered using solid lipid nanoparticles which showed stronger inhibition of melanoma cell proliferation than the free drug after a 24 h incubation period. It was hypothesized that solid lipid nanoparticles endocytosed by the cancer cells, leading to greater drug uptake and thus presenting SLNs as an attractive option for cancer therapy [35]. Later surface coating by polysorbate 80 on solid lipid nanoparticles carried out by Kreuter et al. [36] also showed satisfactory results. Several lipophilic

drugs, peptides, or proteins were delivered using solid lipid nanoparticles. Other antiepileptic drugs successfully brain-targeted include rizatriptan benzoate. An anticancer drug, carmustine (BCNU), loaded in transferrin (Tx) and lactoferrin (Lf)-functionalized PEGylated solid lipid nanoparticles could penetrate human microvascular endothelial cells (BMECs) ten times more than that of PEGylated solid lipid nanoparticles. Transferrin (Tx) prevents the efflux transporter system, while lactoferrin (Lf) undergoes receptor-mediated endocytosis (Kou et al. [37]). Another drug, resveratrol, a neuroprotective compound could penetrate in the hcmec/D3 monolayers 1.8-fold higher when incorporated in apolipoprotein E-conjugated solid lipid nanoparticles by endocytosis (Neves et al. [38]). Similarly, the concentration of drugs in multiple sclerosis-induced mice was 4–8 times higher when loaded in PEGylated solid lipid nanoparticles surface-modified with anticontactin 2 or antineurofascin than that of unmodified solid lipid nanoparticles [39]. Bruun et al. encapsulated *si*RNA in cationic angiopep-functionalized SLNs with >95% efficiency for delivery to glioma cells [40]. However, several limitations of SLNs due to poor drug loading capacity, drug expulsion after polymeric transition during storage, relatively high water content of the dispersions, the low capacity to load hydrophilic drugs due to partitioning effects during the production process does not limits its use as a drug delivery system [34]. In spite of these drawbacks, SLNs have got good potential for CNS-targeted drug delivery.

3.4 Magnetic Nanoparticles

In the recent years, magnetic nanoparticles (MNPs) gained special interest in brain-targeted delivery, since brain cells are quite sensitive to MNPs, compared to, liver and heart cells. At present magnetic nanoparticles have lots of applications: as a contrast agent for magnetic resonance imaging (MRI), to induce hyperthermia in cancer therapy, for cell labeling and cell separation, in targeted therapeutics, in magnetofection, etc. As a contrast agent in MRI and targeted therapeutics, superparamagnetic iron oxide nanoparticles (SPIONs) have been focused on with a wide range of applications. Magnetic nanoparticles under the influence of an externally applied low frequency magnetic field can elevate the physiological temperature, 38–39 °C, which facilitates the penetration into the blood–brain barrier. SPIONs degrade to Fe^{3+}in the body, which undergoes cell metabolism and ultimately eliminated from the body and also the particles due to very small size <30 nm, will not be attracted to each other, and so the risk of agglomeration in a medical setting is minimized. SPIONs can diagnose and directed to the diseased cell under magnetic field, can also generate radiation to treat the cells [41]. Anti-IL-1β monoclonal antibody (mAb)-functionalized SPIONs were used to render MRI diagnoses and simultaneously provide targeted therapy with the neutralization of IL-1β overexpressed in epileptogenic zone of an acute rat model of temporal lobe epilepsy. Similarly, they are also used to target metastases cells in human [42]. The dual application of MNPs as a diagnostics and treatment agent can be called a theranostics. A wide range of

applications of magnetic nanoparticles with their mechanism of drug delivery are discussed in the next section.

4 Mechanism of Surface-Functionalized Nanoparticle Drug Delivery

Surface-functionalized nanoparticles can noninvasively deliver neurotherapeutics to the CNS by modifying the endogenous molecules transport mechanism. Basically, three types of transport mechanisms for CNS drug delivery exist—adsorptive-mediated transcytosis, receptor-mediated transcytosis, and transport or carrier-mediated transcytosis. These transport mechanisms can be manipulated by the nanoparticles with surface modification either by coating with polymers or attachment of ligands to deliver drug to the brain [19].

4.1 Carrier/Transporter-Mediated Transcytosis (CMT)

Carrier-mediated transcytosis is based on the conformational change of membrane transport proteins add direct energy conversion such as ATP hydrolysis to move endogenous solutes such as glucose and amino acids along their concentration gradient. These transport proteins are present on the luminal and abluminal side of the brain endothelial cell membrane in the BBB. GLUT1and GLUT3 are the transporter protein for the intake of D-glucose and glucose analogs from the blood into the brain while LAT1 is the neutral amino acid transported protein in the membrane. There are two drug transport mechanism in CMT, either by chemical modification of the drug into a "pseudonutrient" to resemble these endogenous substances as in the transport of L-DOPA or the conjugation of the nanocarriers with a natural substrate to allow endogenous transport mechanism for the drug [43].

Glucose Transporter-Mediated Transcytosis The glucose transport to the brain involves the interaction of solutes, transporters, enzymes and cell signaling processes in the brain. The GLUT1 and GLUT3 are the sodium independent facilitative glucose transporters which are involved in the catabolism of the glucose to create a concentration gradient for the transport of glucose by GLUT1 from the blood toward the brain interstitial fluid [44]. Other glucose transporter (GLUT) and sodium-dependent transporters (SGLTs) also contribute in the transportation of glucose across the BBB [45]. It is found that the endothelial cells at the blood-brain barrier could transport around ten times their weight of glucose per minute to support the glucose requirements of the brain [46]. This provides good potential to mediate glucose transporter mechanism in drug delivery system. Till now, several neuroactive drugs conjugated with glucose to target GLUT1 are efficiently transported across the BBB. These drugs include neuroactive enkephalin peptides, antidepressant

drug—7-chlorokynurenic acid, and anti-inflammatory drugs (NSAIDs) —ketoprofen and indomethacin. Also, glucose conjugated to nanocarriers could deliver several drugs to the brain [47]. In one study, biodistribution of the fluorescent model drug, coumarin-6 loaded liposomes composed of phospholipids and glucose-derived cholesterols with different linker lengths (GLU200-LIP, GLU400-LIP, GLU1000-LIP, and GLU2000-LIP) were evaluated in vivo in mice brain. The liposomes exhibited the strongest brain delivery potential with GLU1000-LIP [48]. Another drug, docetaxel was delivered significantly when loaded in glucose-modified liposomes than control liposomes as observed in mice brain [49]. Another reported that GLUT1 and GLUT3 responsible for the cellular uptake of liposomes modified with *p*-aminophenyl-α-D-mannopyranoside by transporter-mediated transcytosis [50]. Similarly, dehydroascorbic acid-derivatized micelles have been developed for treating the highly aggressive cancer malignant glioma and have shown accumulation within tumor cells and therefore potential for delivering drugs to cancer sites in the brain and central nervous system via GLUT1 [47]. Similarly, doxorubicin, an anticancerous drug, was brain-targeted to GLUT1 and accumulated in glioma cells when loaded in Pluronic P105 polymeric micelles [51]. Nanoparticles of poly(ethylene glycol)-co-poly(trimethylene carbonate) functionalized with 2-deoxy-D-glucose were dual targeted to GLUT1 for drug delivery in glioma treatment [52].

Large Neutral Amino Acids Transporters Large neutral amino acid transporters (LAT1) are an endogenous nutrient transporter present in the luminal and abluminal cell membrane of the brain capillary endothelial cells (BCECs). The brain uptake of neutral amino acids such as phenylalanine, leucine, and tyrosine are regulated by LAT1. LAT1 has gained popularity for brain-targeted drug delivery either as "prodrug" or substrate conjugated to the drug delivery system resembling the endogenous neutral amino acids [43]. One example of "prodrug concept" is the delivery of L-DOPA. Dopamine as such cannot cross the BBB, but when delivered in the form of L-DOPA, LAT1 facilitates the uptake of L-DOPA, where dopamine is released by decarboxylation. Drugs such as baclofen, α-methyl-DOPA, and gabapentin are also transported by this technique [53]. Technique based on Trojan horse is used to deliver drug through LAT1. LAT1 substrate such as L-cysteine conjugate 6-mercaptopurine increases the lipophilicity of the drug, otherwise a polar molecule could not target to the LAT1 into the brain [54]. Similarly, LAT1 substrate tyrosine is coupled with the NSAID ketoprofen to form a zwitterionic prodrug which facilitates the release of conjugate drug by the action of esterase enzyme present in the brain parenchyma [47]. In another study, phenylalanine-coupled solid lipid nanoparticles were found to be capable for increased accumulation of efavirenz in the brain and cerebrospinal fluid to inhibit viral loads in neurodegenerative disorders which could be attributed to the presence of LAT1 transporters which facilitate transport of phenylalanine to the brain via carrier or transporter-mediated transcytosis [55]. Fernandez et al. reported that saxagliptin (SAX), a dipeptidyl peptidase-4 enzyme inhibitor molecule used in the therapy of Alzheimer disease is hydrophilic and not permeable across the BBB. An attempt of incorporating the drug in chitosan

nanoparticles, conjugated with L-valine showed a higher accumulation of 53 ng/mL SAX from the nanoparticles than the pure SAX after 24 h, as obtained after in vivo study in rat [56].

SMVT/SLC5A6 (Sodium-Dependent Multivitamin Transporter) Sodium-dependent multivitamin transporter (SMVT/SLC5A6) is a significant transporter requisite for the uptake of the vitamins—biotin and pantothenate, which are highly expressed in placenta, intestine, brain, liver, lung, kidney, and heart [57]. Thus exploiting the SMVT mechanism may transport several drugs to the brain. Biotin-labeled solid lipid nanoparticles penetration in an in vitro blood–brain barrier model hCMEC/D3 brain endothelial cell was compared to biotinylated glutathione-labeled nanoparticles. Biotin as a ligand increased the uptake and the transfer of nanoparticles across brain endothelial cells by SMVT supporting its use as a brain targeting vector [58].

Thiamine Transporter Thiamine (a water-soluble vitamin B1), a micronutrient essential for normal cell growth and development is reported to transport to the brain by thiamine transporter-mediated transcytosis. Thiamine was used as a surface ligand conjugated with solid lipid nanoparticles, composed of emulsifying wax and Brij 78, were reported to transport to the brain as tested in situ by rat perfusion technique [33]. The mechanism involves an interaction with the thiamine transporter, which is responsible for a facilitated transport or an increased passive diffusion of the nanoparticles toward the BBB.

ChT/SLC5A7 (Choline Transporter) Choline is an endogenous compound required in the synthesis of the neurotransmitter acetylcholine and the membrane phospholipid phosphatidylcholine. Choline transporters (ChT) are responsible for the cellular uptake of acetylcholine and the membrane phospholipid phosphatidylcholine [59]. Based on the sodium dependence and the affinity for choline, there are two choline transporter systems. The sodium-dependent and choline low-affinity transporter is expressed widely in the body, whereas the sodium-independent and choline high affinity transporter is expressed in the presynaptic cholinergic nerve ending. The sodium independent transporter is needed for the choline transfer across BBB [60]. Herein, a choline derivative was used as a ligand in the formulation of doxorubicin and gene complexed nanoscale codelivery system showed higher cellular uptake efficiency and cytotoxicity than unmodified codelivery system in U87 MG cells [61].

SVCT2/SLC23A2 (Sodium-Coupled Vitamin C Transporter 2) Sodium-dependent transporter for vitamin C (SVCT2), expressed by neuroepithelial cells of the choroid plexus are involved in the transport of the reduced form of ascorbic acid or vitamin C. Modification of the drugs in a form to target SVCT2 may facilitate the drug delivery to the brain. Recently, the anticholinesterase galantamine used for the treatment of neurodegenerative disorder, Alzheimer disease was incorporated in ascorbic acid grafted PLGA-*b*-PEG nanoparticles to increase the cellular uptake of nanoparticles in SVCT2 expressing NIH/3 T3 cells. A significantly higher

therapeutic and sustained action by drug-loaded PLGA-*b*-PEG-Asc NPs than free drugs and drug-loaded plain PLGA as well as PLGA-*b*-mPEG NPs was observed in an in vivo pharmacodynamic study [62]. The result also showed a higher biodistribution of the drug to the brain than other formulations.

MCT1/SLC16A1 (Monocarboxylate Transporter 1) MCT1 is a proton-coupled transporter expressed in endothelial cells in the BBB responsible for the transport of monocarboxylates lactate as well as the ketone body β-hydroxybutyrate across the BBB [63]. Venishetty et al. [64] studied the β-hydroxybutyrate-grafted docetaxel-loaded solid lipid nanoparticles to increase the drug distribution to brain. The result showed an increased uptake and cytotoxicity of β-hydroxybutyrate-grafted nanoparticles in brain endothelial cells as compared to unmodified nanoparticles as β-Hydroxybutyrate-grafted nanoparticles could effectively increase docetaxel distribution across the BBB by Monocarboxylate Transporter 1 (MCT1).

OCTN2/SLC22A5 (Novel Organic Cation Transporter 2) Another transporter OCTN2 is highly expressed in blood–brain barrier capillary endothelial cells. This transporter is overexpressed in glioblastoma multiforme T98G cells. Therefore, nanocarriers modified to target OCTN2 offers a potential platform for the brain-targeted delivery of chemotherapeutics. Kou et al. reported that the conjugation of L-carnitine significantly increased the uptake of paclitaxel loaded nanoparticles in BBB endothelial cell line hCMEC/D3 and glioma cell line T98G improving its antigliomic activity [65].

4.1.1 Inhibition of Efflux Transporter System

P-Gp/ABCB1 (P-Glycoprotein/ATP-Binding Cassette Transporter Family, Member B1 ATP-binding cassette (ABC) transporters are membrane transporters, which bind and hydrolyze ATP to drive the efflux of various compounds out of cells. Several drugs are moved out of the cells by efflux transport such as paclitaxel, docetaxel and doxorubicin. One of the ABC membrane transporters is *P*-glycoprotein (*P*-gp/ABCB1/MDR1). As involved in efflux transport, *P*-gp is doubtful in facilitating drug transport to the brain [66]. However, investigation is being done using *P*-gp as a substrate coupling with nanoparticles which reported to increase significantly the uptake of nanoparticles by brain. Such substrates are azithromycin, clarithromycin [67]. An increase in drug delivery to the brain can be obtained by using *P*-gp inhibitors, which inhibits *P*-gp overexpressed cells. Based on this strategy, doxorubicin uptake and transfection efficiency were significantly enhanced in rat brain endothelial by using *si*RNA-chitosan nanoparticles. It may be due to the *si*RNA-mediated silencing of the *P*-gp gene to improve the delivery of drug to the brain [68]. Another report showed that amisulpride when coadministered with A-cyclosporine showed an increase and prolonged antipsychotic effect as observed in vivo, which is due to the inhibition of *P*-gp efflux transport. Several polymers which are *P*-gp inhibitors are used for surface modification of nanocarriers. Natural

polymers such as xanthan and gellan gum, as well as alginates, could inhibit the action of the *P*-gp efflux pump at concentrations of 0.05% and 0.5 mg/mL, respectively. An increased concentration of *P*-gp substrates such as vinblastine and doxorubicin was shown in the presence of xanthan gum, whereas an increased intracellular concentration of doxorubicin was obtained in everted gut sac cells. Other synthetic polymers inhibitors of *P*-gp efflux transporter such as PEG 400, g-Pluronic P85, and D-α-tocopheryl polyethylene glycol 1000 succinate could enhance the digoxin concentration in the brain, whereas Pluronic P85 inhibits the *P*-gp transporter, causing reduction in ATP and inhibition of ATPase enzymes as well as lipid membrane fluidization. Another polymer, poloxamer 188 reported to transport doxorubicin across the BBB when coated on PBCA nanoparticles against intracranial glioblastoma in rat. Other drug transported was acyclovir [69]. Glutathione, an antioxidant, when used as a coating material of PLGA nanoparticles could efficiently delivered paclitaxel across the brain due to the inhibition of *P*-gp as observed by ATPase assay. Similarly, doxorubicin was also transported by the same mechanism using glutathione-coated PLGA PEG nanoparticles [19].The carrier or transporter-mediated transport system can deliver several therapeutics or neuroactive agents to the brain by manipulating the endogenous transport. However, this strategy suffers from drawbacks due to an increase molecular size of the moiety as the required criteria is that the drug/ligand must be very small and similar in structure to the nutrient [70].

4.2 Adsorptive-Mediated Transcytosis (AMT)

The adsorptive-mediated transcytosis relies on the electrostatic interaction between a positively charged molecule and the negatively charged brain endothelial cell membrane. Originally, Pardridge et al. demonstrated the capability of cationized albumin nanoparticles in delivering drugs and peptides to the cerebral parenchyma [71]. Later, the cell-penetrating peptides (CPPs) developed as positively charged peptides were developed, which could penetrate the brain endothelial cell membranes by adsorptive-mediated transcytosis [72]. Another example is the HIV-1 trans-activating transcriptor (TAT) peptide. TAT-derived CPPs bind to the surface of the cell, induce macropinocytosis, allow large molecules such as large chained peptides to transport across the BBB [73]. In another study, Liu et al. incorporated ciprofloxacin–HCl in the PEGylated nanoparticles conjugated with TAT peptides was found to cross the BBB [74].The application of AMT in drug delivery is limited due to its lack of tissue or site specificity which lead to an accumulation of undesired drug in the nonspecific or nontargeted tissue causing toxicity as well as low therapeutic concentration of the drug in the brain. Overall, it makes the treatment ineffective [75].

4.3 Receptor-Mediated Transcytosis (RMT)

Receptor-mediated transcytosis mainly responsible for the transport of large molecules such as insulin, lipoproteins, and transferrin to the brain. Surface-modified nanoparticles by coating or natural ligand conjugation mimic the normal endogenous substances, which acts on the specific receptor to deliver drugs to the brain. Nanoparticles surface-functionalized with polymers are based on RMT to deliver several therapeutics to the brain.

Low-Density Lipoproteins (LDL) Receptor Widely distributed in the brain endothelial cell membrane, LDL receptors (LDLr) are proven to be an effective tool for the delivery of several drugs. As such, coated nanotechnology based polysorbate 80-coated nanoparticles are proven to be efficiently acted on LDLr to deliver drugs to the brain. Polysorbate 80 coating covalently couple with apolipoprotein E, A-I, or B-100 in the bloodstream, which mimics the endogenous lipoprotein and act on the LDLr present in the brain endothelial cell membrane and deliver the drug by receptor-mediated transcytosis. Polysorbate 80-coated nanoparticles are able to deliver several drugs to the brain such as dalargin, gemcitabine, nerve growth factor, gallic acid, doxorubicin, and rivastigmine [19]. As a part of our research, based on the receptor-mediated transcytosis technique, an attempt has been made to investigate the ability of polysorbate 80-coated 6-carboxyflourescein (6CF) tagged kokum butter solid lipid nanoparticles (P806CFNvKLNs) in delivering Nevirapine (Nv), an antiretroviral drug to the brain in Swiss Wistar rat model. Conventionally, Nevirapine, a nonnucleoside reverse transcription inhibitor, suffers from the drawbacks of poor aqueous solubility, hepatotoxicity, and patient incompliance on frequent dosing, and also undergoes first pass metabolism and enzymatic degradation when orally administered in highly active antiretroviral therapy (HAART). The P806CFNvKLNs was administered in the tail vein of the rat, which were sacrificed by cervical dislocation and the cryosection of rat brain was taken to check the distribution of the P806CFNvKLNs at different time intervals of 1 h, 2 h, 4 h, 6 h, and 24 h under confocal laser scanning microscopy (CLSM) as shown in Fig. 15.1. Initially, at 1 h, the P806CFNvKLNs were in the surroundings of the BCECs. At 2 h, it undergoes receptor-mediated endocytosis and entered the BCECs and remained in the BCECs for 24 h showing higher intensity at 4 h. After 24 h, the P806CFNvKLNs moved out of the BCECs into the brain parenchymal cells indicating receptor-mediated transcytosis into brain parenchyma. The result showed that the P806CFNvKLNs may have acted on the LDLr and underwent receptor-mediated endocytosis showing uniform distribution of nanoparticles in the BCECs and parenchymal cells.

Other study showed that polysorbate 80-coated chitosan nanoparticles could target doxycycline HCl, antipsychotic drug to the brain as observed in brain micro vascular endothelial cells by Yadav et al. [76]. Curcumin showed its antidepressant activity in in vivo pharmacophore model when administered in polysorbate 80-coated PLGA nanoparticles [77]. Similarly, curcumin showed cytotoxicity on U87MG brain tumor cells, when loaded in P80-coated PEGylated PLGA

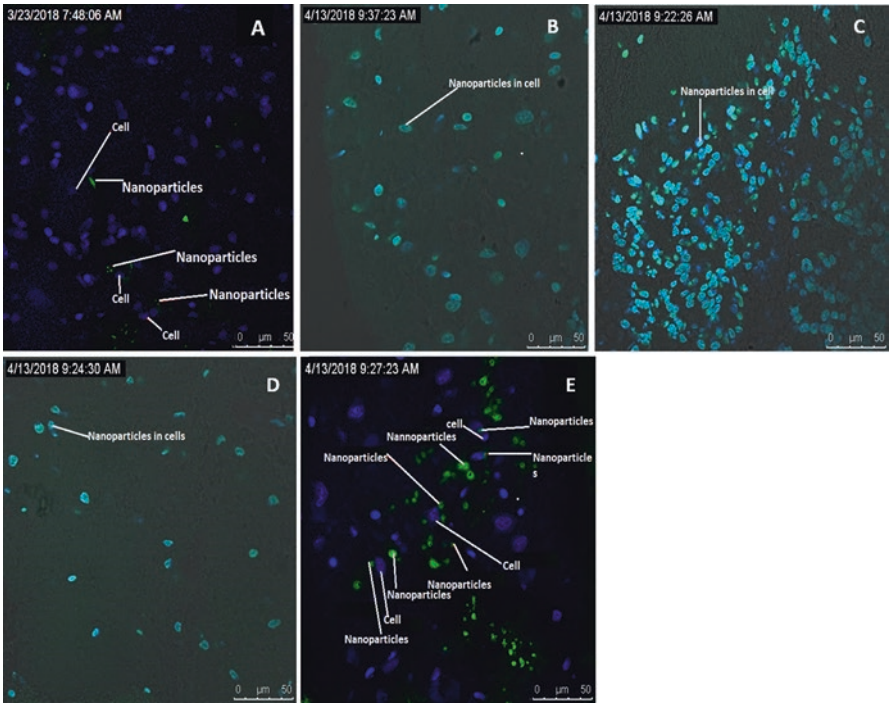

Fig. 15.1 (**a**) P806CFNvKLNs surrounding BCECs in Swiss Wister rat brain 1 h post injection, (**b**) LDLr mediated endocytosis of P806CFNvKLNs in Swiss Wister rat BCECs 2 h post injection, (**c**) P806CFNvKLNs in Swiss Wister rat BCECs 4 h post injection, (**d**) P806CFNvKLNs in Swiss Wister rat BCECs 6 h post injection, and (**e**) LDLr-mediated transcytosis of P806CFNvKLNs into the brain parenchyma after 24 h post injection

nanoparticles showing effective drug delivery to glioblastoma multiforme (GBM) tumor cells [78]. Acetylpuerarin is an acetylated derivative of puerarin could permeate through the BBB and showed its brain protective activity in rat when incorporated in P80-coated PLGA nanoparticles [79]. P80-coated PLGA nanoparticles also proven to deliver Bacoside, a neuroprotectant, to the brain for the treatment of neurodegenerative disorders as observed in Wister albino rats [80].

Angiopeptides Another receptor that has recently been targeted for brain drug delivery is the low-density lipoprotein receptor-related protein (LRP), a member of the low-density lipoprotein receptor family. It is highly expressed on BBB and involved in BBB transcytosis of several proteins and peptides, including lactoferrin, melanotransferrin and receptor associated protein. Angiopep-2, a 19 amino acid peptide, was reported as a ligand targeted to this receptor [81]. Still, a peptide conjugated with three molecules of paclitaxel linked to Angiopep-2 called ANG1005 showed to have activity against glioblastoma and to lengthen the survival of mice with intracerebral tumors [82]. Angiopep-conjugated poly(ethylene glycol)-copoly(3-caprolactone)nanoparticles (ANG-PEG-NP) enhanced

significantly the uptake by brain capillary endothelial cells (BCECs) compared with that of PEG-NP which might be due to LRP receptor-mediated transcytosis process [83].

Leptin Receptor Leptin is a 146-amino-acid polypeptide secreted into the bloodstream by adipocytes. Leptin, a specific receptor, ObR is overexpressed in the hypothalamus and other parts of the brain. Leptin, secreted in the bloodstream undergoes passive diffusion across the BBB by receptor-mediated transcytosis as it acts on the receptor, ObR, present on the luminal side of the brain capillary endothelial cells. Endogenous leptin binds to the ObR receptor only after meal, is responsible to reduce obesity by decreased food intake and retarding weight gain. This particular characteristic of leptin provides an ample potential to utilize leptin-mediated transcytosis pathway for brain-targeted delivery of drugs [84]. On the other hand, leptin-modified nanocarriers may not be effective in obese individuals due to its impaired activity. Till now, researchers have developed several leptin derived peptides for drug targeting to the brain. The gene transfection efficiency of Dendrigraft poly-L-lysine (DGL) plasmid DNA nanoparticles conjugated with leptin 30 were studied on brain capillary endothelial cells (BCECs), which express leptin receptors. The result showed the nanoparticles could transport across in vitro BBB model effectively by ligand-receptor-mediated endocytosis leading to enhanced gene transfection ability of DGLePEGeLeptin30/DNA NPs with low cytotoxicity. Hence, these observations would support the potential of the ObR receptor to serve as a transport system in brain delivery of drugs [85].

Transferrin Receptor Another receptor highly expressed in brain capillary endothelial cells is the transferrin receptors. It provides a wide scope of research for RMT. The transferrin or an antibody against transferrin is either coated or attached to nanocarriers to allow for the delivery of large molecules to the brain [19]. Nevirapine, an antiretroviral drug was successfully targeted to the brain using transferrin-conjugated PLGA nanoparticles [86]. Similarly, lactoferrin, a protein of the transferrin family, also induced uptake in an in vitro and in vivo model, when attached to PEG-PLA nanoparticles [87]. The disadvantages with transferrin are that the endogenous transferrin present in the blood competes with the transferrin-functionalized nanocarriers for the same receptor site in BBB, which may inhibit the uptake of nanocarrier reducing the efficacy of the drug. Thus, antibody against transferrin is in use to overcome its drawback. One such antibody is ox26 reported to bind to an extracellular epitome of transferrin receptor preventing the competition with natural endogenous transferrin for binding the same site. Ox26 nanocarrier conjugation can be done by covalent chemical linkages. One such method is sulfhydrylmaleimide coupling method [19]. Tempol was targeted to the brain by conjugation of ox26 antibody to the maleimide-grafted PLGA nanoparticles by NHSPEG 3500 maleimide cross-linker [88]. Ox26-conjugated PEGylated cationic solid lipid nanoparticles could enhance the penetration of baicalin, a flavone glycoside 1.12-fold higher than unmodified solid lipid nanoparticles as observed in vivo [89].Transferrin receptors are overexpressed in tumor cells. This concept was

proven by the enhancement of cellular internalization of temozolomide in glioblastoma cells, U215 and U87, when incorporated in ox26-conjugated PLGA nanoparticles [90]. Similarly, amphotericin B, a drug for candidal meningitis, got a low therapeutic level in the brain due to poor penetration. However, delivery attempt were made by modifying the transferrin receptor-mediated transcytosis. An anti-TfR antibody (OX26)-modified amphotericin-loaded PLA (poly[lactic acid])–PEG (polyethylene glycol)-based micellar drug delivery system was constructed which showed significant reduction of CNS fungal burden and an increase of mouse survival time [91]. In another study, loperamide, an antinociceptive drug showed significant antinociceptive effects in the tail-flick test in ICR (CD-1) mice after intravenous injection when incorporated in human serum albumin nanoparticles covalently bound with ox26, monoclonal antibody [92].

Melanotransferrin Receptors Another receptor known as melanotransferrin, is a high-level homolog of human serum transferrin and lactoferrin was first observed in melanoma cell membrane. Basically, it is an iron-bound protein crucially involve in the transport of iron from the blood plasma across the BBB by RMT, which is irrelevant to transferrin and transferrin receptor. Due to less plasma concentration of the endogenous melanotransferrin, it favors its utility in RMT CNS drug delivery. Melanotransferrin antibody and tamoxifen-conjugated solid lipid nanoparticles were able to release etoposide to the human-brain microvascular endothelial cells (HBMECs) and to restrain the proliferation of malignant U87MG cells [93]. Other drugs such as paclitaxel, an anticancer drug accumulation in the brain was 10 times higher than the free drug when conjugated with melanotransferrin [41].

Mannosyl Receptors Some receptors are distributed widely on the cell wall of the macrophages, which is used by macrophages for phagocytosis and endocytosis such as fibronectin, mannosyl, lectin, and galactosyl. Manipulation of these receptors by nanocarriers conjugated with ligands such as mannosyl, immunoglobulin, fibronectin, and galactosyl can target and deliver several drugs to the brain by RMT [19]. Mannan-coated gelatin nanoparticles could recognize the mannosyl receptors predominantly present in the macrophages cell of the brain and successfully target didanosine, an anti-HIV drug to the brain [94].

Insulin Receptors Insulin is transported to the brain by the transcytosis mechanism by acting on the insulin receptors present on the brain endothelial cell membrane. Insulin is not a suitable ligand for RMT due to the existence of competition with the natural endogenous insulin present in the blood circulation may reduce the therapeutic concentration of insulin in the brain and/or changes in receptor activity, of which the latter may have negative consequences for glucose metabolism. Thus, antibody against insulin receptors is in use for RMT, which act on different epitome of the insulin receptor other than the endogenous insulin binding site. One such is 83–14 mouse monoclonal antibody (mAb) for receptor-mediated endocytosis performed in primates (Rhesus monkey) [19]. Another report showed that the human anti-insulin receptor monoclonal antibody (HIRMAb) was

able to transport a TNFα decoy receptor (TNFR), which would neutralize the effects of TNFα in inflamed brain regions, into the brain of Rhesus monkeys following intravenous administration [95]. Similarly, loperamide-loaded human serum albumin nanoparticles with covalently bound insulin or the anti-insulin receptor monoclonal antibody, 29B4 stimulated significant antinociceptive effects in the tail-flick test in ICR (CD-1) mice after intravenous injection, showing that insulin or the antibodies covalently coupled to human serum albumin nanoparticles are able to transport loperamide across the blood–brain barrier (BBB), which the drug usually is unable to cross [96].

Rabies Virus Glycoprotein (RVG) RVG, a short peptide, is found on the surface of rabies virus. Rabies virus follows a decisive pathway to enter CNS. It enters the CNS by acting on the α1 subunits of nicotinic acetylcholine receptors (nAcR), which is widely distributed in CNS as reported by Lentz et al. Rabies virus glycopeptide is responsible for cellular entry and virus fusion. Modification of rabies virus CNS pathway can be a promising tool for the targeted drug delivery to the brain. One of the widely used RVG peptide is a 29-amino-acid peptide derived from rabies virus glycoprotein (RVG29). CNS targeting peptide such as RVG 29 is a promising strategy, underlined by its wide application as a targeting peptide for CNS-targeting strategies. It is reported to inhibit the binding of snake-venom toxin α-bungarotoxin (BTX) to the AchR in solution [97]. The RVG29 peptide has been exploited as a brain-targeted ligand to deliver gene into the brain by conjugation on the surface of polyethylene glycol-polyamidoamine (PEG-PAMAM) [98]. Another study showed the RVG29 peptide-conjugated albumin nanoparticles could deliver itraconazole to the immortalized mouse brain endothelial cells [99]. Zou et al. reported that rabies virus glycoprotein (RVG) peptide conjugated paclitaxel loaded PLGA nanoparticles could deliver anticancer drug, paclitaxel, to the human glioma of mice model [100]. RVG-peptide-linked trimethylated chitosan could efficiently delivered *si*RNA to the mouse neuroblastoma Neuro2a cells with increased serum stability, negligible cytotoxicity, and higher cellular uptake than the unmodified *si*RNA/TMC—mPEG complexes in acetylcholine receptor positive Neuro2a cells [101].

Diphtheria Toxin Receptor (DTR) A membrane-bound precursor of heparin-binding epidermal growth factor (HB-EGF), also known as the diphtheria toxin receptor (DTR) is a well-characterized endogenous transport receptor on the BBB for the targeting of drugs. Usually, it is based on endogenous transport mechanism of molecules by receptor-mediated endocytosis. Based on receptor-mediated endocytosis, several molecules of proteins, essential nutrients, etc. are transported through it. The absence of any endogenous ligands expected to prevent the competition with DTR and the blockage of transport of essential nutrients to the brain. As it is predominantly expressed on the BBB, neurons, and glial cells, it is overexpressed during diseased conditions, providing an opportunity for targeted drug delivery [102]. Pharmacologically active compounds (like heparin and protease

inhibitors) can modulate its biological activity. A nontoxic mutant of diphtheria toxin known as CRM197 is used as the receptor-specific carrier protein because of its use as a safe and effective carrier protein in human vaccines for a long time [103]. A study by Tosi et al. showed the BBB crossing efficiency of polymeric poly-lactide-co-glycolide (PLGA) NPs modified with a mutated form of diphtheria toxin (CRM197). CRM197 PLGA nanoparticles are able to reach the CNS by receptor-mediated endocytosis after interaction with diphtheria toxin receptor (DTR) [104]. In another study, CRM197-linked PEGylated polyethylenimine small interference RNA (CRM197-PEG-PEI/*si*RNA) could deliver therapeutic *si*RNA in glioblastoma cells [105].

Tetanus Toxin Tetanus toxin is a 150,000 molecular weight protein produced by the anaerobic bacterium *Clostridium tetani.* Tetanus toxin is transported to the brain as it binds to the gangliosides, a component of the neuronal membranes. Van Heyningen identified that tetanus toxin bound to the membrane glycolipids, gangliosides, and demonstrated that it bound best to certain specific disialogangliosides and trisialogangliosides. Later on, it was found that a retrograde transport from distal axon terminals to the neuronal cell body can carry both heavy and light chains of tetanus toxin in motor nerves. It is evident that retrograde axonal transport of several substances from the peripheral neurons to motor neurons can penetrate the CNS effectively bypassing the BBB. Hence, exploitation of retrograde neuronal transport may enhance the possibility to target nanoparticles to the CNS [106]. Out of the two fragments, the C-terminal heavy chain fragment is nontoxic, attached with the light chain which is endowed pathogenic enzymatic characteristics. The heavy chain fragments are transported along axons as it acts on the GT1b, the gangliosides receptor for tetanus toxin. As reported, an intramuscular injection of superoxide dismutase tetanus toxin into the mouse tongue reached higher order motor neurons. Also, an enhanced CNS uptake was obtained for intraperitoneal injection of tetanus toxin protein hybrid. Also, tetanus toxin-conjugated PLGA PEGylated nanoparticles showed six fold increase in binding to N18-RE-105 neuroblastoma cells. However, its immunogenicity due to vaccination is a major concern [107]. To overcome this problem, peptides analogous to tetanus toxin having similar binding efficiency and subsequent cellular processing was identified, Tet1. Tet1 is transported in a retrograde manner. Another peptide, G23, with a sequence identical to Tet1 was identified that revealed effective targeting by a transcytotic pathway in human brain capillary endothelial cells and mediates efficient transport of G23-coated polymersomes across an in vitro BBB model [108]. Following intracarotid artery injection in mice studies provided an evidence for in vivo transfer across the BBB of intact peptide-targeted drug delivery into brain parenchyma. Further research confirmed the binding of G23 peptide and Tet1 to both GM1 and GTb1 gangliosides indicating that both gangliosides involve in transcytotic transport of G23-coupled polymersomes across the BBB [109].

5HT Receptors 5-hydroxytryptamine or 5-HT or serotonin receptors, a group of G protein coupled receptor and ligand gated ion channels are located both in the CNS

and peripheral nervous system. Serotonin receptor modulates the secretion of several neurotransmitters such as acetylcholine, epinephrine/norepinephrine, dopamine, glutamate, GABA as well as many hormones such as oxytocin, prolactin, vasopressin, cortisol, corticotropin, and substance P. The natural endogenous serotonin is responsible for the activation of these receptors, which is transported across the BBB by receptor-mediated transcytosis. Several pharmaceutical drugs such as antidepressants, antipsychotics, anorectics, antiemetics, and antimigraine drugs are successfully targeted to these receptors [110]. Another subtype of 5HT or serotonin receptor is serotonergic 1B receptor subtype (S1BRS; 5-hydroxytryptamine (1B) receptor) expressed by brain endothelial cells.S1BRS could play a crucial role in the generation of and treatment for depression, regulation addictive drugs responses. The major concern is the reuptake of S1BRS by the CNS. Thus S1BRS antagonism could extend SSRI induced effect on 5-hydroxytryptamine levels in the frontal cortex. Using this strategy, Carmustine (BCNUs) solid lipid nanoparticles modulated with S1BRSA could target S1BRS expressed on the human brain microvascular endothelial cells (HBMECs) for BBB penetration [111]. Other drugs such as etoposide, antitumor drug were successfully transferred across BBB using 5-HT moduline-grafted cationic solid lipid nanoparticles. It acts on the 5-HT_{1B} receptors present on the brain endothelial cells and transported the drug by receptor-mediated endocytosis [112]. Similarly, an antimigraine drug, sumatriptan succinate, loaded in chitosan solid lipid nanoparticles could be successfully targeted to brain via oral delivery [113].

Other peptide used to target drugs based on ligand nanotechnology are trans-Golgi network (TGN) and QSH. Based on this technique, Zhang et al. developed a dual-functional nanoparticulate drug delivery system based on a PEGylated poly(lactic acid) polymer containing two targeting peptides, TGN and QSH, conjugated to the surfaces of the nanoparticles. TGN specifically targets ligands at the BBB, while QSH has good affinity for $A\beta_{1–42}$, which is the main component of amyloid plaques, in Alzheimer disease. These nanoparticles were delivered to amyloid plaques with enhanced and precisely targeted delivery in the brains of Alzheimer disease model mice [114].

4.4 Surface-Functionalized Magnetic Nanoparticles

As discussed in the previous section, magnetic nanoparticles [i.e., SPIONs or iron oxide nanoparticles (IONs)] have gained lots of attention as a vector in drug delivery. The dual functioning of magnetic nanoparticles both as a diagnostic agent and targeting got significance in brain-targeted drug delivery. Magnetic nanoparticles follow three mechanisms to cross the BBB. Firstly, natural ligands such as peptides, antibody-conjugated magnetic nanoparticles target to a specific receptor in the brain. Secondly, application of an external magnetic field directs the nanoparticle-incorporated drugs to the brain. Thirdly, application of low radiofrequency waves in the presence of an external magnetic field generates heat to open the tight junction

of the brain capillary endothelial cells which allow the diffusion of the drug across the BBB. The second and third strategies differentiate the magnetic nanoparticles from other nanoparticles while the first strategy based on attachment of ligands on the surface is similar with those other nanoparticles. Whereas combination of ligand-based strategies with the application of magnetic field may be useful for drug targeting to the brain [41].

Ligand-Functionalized Magnetic Nanoparticles Transport Across BBB The feasibility of drug delivery using natural ligand-conjugated or -coupled magnetic nanoparticles is investigated and reported. One such is the modification of magnetic nanoparticles surface by peptides. Angiopep-2(ANG) conjugated to the surface of Pluronic F127 water-dispersible poly(acrylic acid)-bound iron oxide complex could cross the BBB due to its dual targeting ability, one to recognize the low density lipoprotein receptor protein, which is overexpressed in both BBB and glioblastoma cells and secondly, it acts on the clathrin-mediated receptor on the U87 surface. Thus, ANG-conjugated iron oxide complex undergoes receptor-mediated transcytosis to cross BBB. Another peptide, trans-activator of transcription protein (TAT) when used to functionalize liposomes was able to increase permeability to the brain and also accumulated significantly in the spinal cord site [41]. Kaluzoba et al. reported that cetuximab, an epidermal growth factor inhibitor, when conjugated with IONs resulted in a significant antitumor activity in rat against glioblastoma (GBM), GBM stem like cells (GSCs) that was greater than with cetuximab alone due to more efficient, CD133-independent cellular targeting and uptake, EGFR signaling alterations, EGFR internalization, and apoptosis induction in EGFR-expressing GSCs and neurospheres [115].

Transport of Magnetic Nanoparticles Across the Brain Cell by External Magnetic Field Application The application of external magnetic field is directly proportional to the particle size of magnetic nanoparticles. So an increase in particle size and strong magnetization are essential for attractive forces to exist. Most of the anti-HIV drugs not able to cross the BBB provoke the occurrence of diseases called neuroAIDS [41]. Jain et al. reported to modify the transport of endogenous macrophage system based on the monocytes/neutrophils mediated delivery of Arg-Gly-Asp (RGD) anchored magnetic nanoparticles transport to an in vitro BBB model by an externally applied magnetic field [41]. Thomsen et al. studied the capacity of fluorescent IONs to pass through human brain capillary endothelial cells facilitated by an external magnetic field. In another study, transferrin-conjugated magnetic liposomes passage to the BBB was studied, both in the presence and absence of an external magnetic field. In the presence of external magnetic field, transferrin-conjugated magnetic liposomes showed an increase in transmigration due to a synergistic effect than that in the absence of magnetic field [116].

Magnetic nanoparticles therapy increased the survival of glioma-bearing rats by enhancing the brain concentration of paclitaxel, an antigliomic drug [117]. Kong et al. reported that polystyrene nanospheres encapsulated magnetic nanoparticles in the presence of an external magnetic field showed accumulation of nanospheres in

an in vitro brain model as observed by confocal laser scanning microscopy [118]. Another report, demonstrated that the application of the external magnetic field could target magnetic liposome to the glioma multiforme in vivo, quantitative determination by MRI, electron spin resonance spectroscopy to determine the magnetic liposomes crossing the BBB, confocal laser scanning microscopy showed enhanced accumulation of magnetic liposomes in brain parenchyma [119].

Heating by Low Radiofrequency to Increase of BBB Permeability Application of an external altering magnetic field in the presence of radiofrequency generates heat energy, which induce hyperthermia. A moderate heat is required to open up the BBB tight junction which is reversible. Tabatabaei et al. initially observed the permeability of magnetic nanoparticles in rat brain due to generation of moderate heat in the presence of an externally applied magnetic field [41]. Dan et al. also observed the that the SPIONs cross linked with IONs were able to permeate through an in vitro BBB model (bEnd.3 and Madin-Darby canine kidney II cells) which was activated by hyperthermia dissipated by externally applied magnetic field in a controlled manner and in a specific area [120]. Thus, it can be concluded that the hyperthermia generated due to an external applied magnetic field could have the potential to deliver SPIONs across the BBB for diagnostic and therapeutic activity.

Strategies Following Both Attachment of Ligand and Application of External Magnetic Field Recently, Nair showed that magnetic nanoparticles loaded with AZTTP (the triphosphate, active form of AZT) and encapsulated in liposomes could migrate across an established BBB model (primary human brain microvascular endothelial cells and astrocytes) when induced by application of an external magnetic field. Moreover, it showed the phagocytosis of the magnetic liposomes by human monocytes, and its transmigration across the BBB in the presence of an externally applied magnetic field in vitro [121]. A list of drugs/moiety delivered across the BBB is given in Table 15.1. Before implementing magnetic nanoparticles for drug delivery, toxicity studies need to be carried out in the specific cell type of interest. On a study, it was found that the unmodified SPIONs are responsible for cell death in dermal fibroblasts while lung cells appeared not to be affected. Secondly, it is the strength of applied magnetic field to control the concentration at the site of action, as the magnetic gradient decreases with an increase in distance from the site of application. Next is the small size, which reduces the magnetic strength making it a challenge for the particle to maintain its concentration at or near the site of action while withstanding the resistance of blood flow. Also, the effect of differences in physiological conditions (such as weight, cardiac output, blood volume), before extrapolating from animal study to human are to be considered. Thus, drug delivery using magnetic nanoparticles for the treatment of metastatic neoplasm or small tumors remains a challenge [122]. However, proper modification of magnetic nanoparticles has a significant potential or recognition as well as efficient drug targeting to the sites where the conventional dosage cannot reach.

Table 15.1 Drugs delivered across the BBB using magnetic nanoparticles

Strategies	Magnetic nanoparticles	Mechanism	Drugs/moiety	Application	References
Ligand conjugation	Lactoferrin-conjugated graphene oxide Iron oxide nanocomposites	Acting on lactoferrin receptor overexpressed in glioma cells and brain endothelial cells	Doxorubicin	Efficacy and stronger cytotoxicity against C6 glioma cells	[123]
	Transferrin-conjugated fluorescein magnetic nanoparticles	Acting on the transferrin receptors in brain endothelial cells	Fluorescein (FITC)	Wide distribution in brain parenchyma as observed by TEM and CLSM	[124]
	Anti-IL-1β monoclonal antibody (mAb) SPIONs	Neutralization of IL-1β overexpressed in epileptogenic zone of temporal lobe epilepsy (TLE)	Anti-IL-1 monoclonal antibody	Diagnosis and treatment of TLE by MRI	[42]
	Chlorotoxin loaded methotrexate PEGylated iron oxide nanoparticles	Receptor-mediated endocytosis through lysosomes in brain cytoplasm	Methotrexate	MRI diagnosis, accumulation and cytotoxicity in tumor cells in vivo	[125]
Application of magnetic field	Hybrid chitosan–dextran SPIONs	Application of magnetic field	Chitosan-dextran SPIONs	Magnetic resonance contrast enhancing properties for the delineation of the brain tumor	[126]
	cmHsp70.1 monoclonal antibody-conjugated SPIONs	SPIONs	Targeting membrane Hsp70 expressed in tumor cells	MRI diagnosis of tumor	[127]
	pEGFP/p53-loaded SPIONs	P53 gene	Targeting Tp53 to glioblastoma (U87) cells across a simulated BBB model that comprised KB cells	Induction of apoptosis in the cancerous cells	[128]

(continued)

Table 15.1 (continued)

Strategies	Magnetic nanoparticles	Mechanism	Drugs/moiety	Application	References
Application of heat and magnetic field	Doxorubicin graphene oxide-PEGylated iron oxide hybrid nanocomposite	Photothermal therapy induced magnetic field	Doxorubicin	MRI diagnosis, drug targeting,cytotoxic effect	[129]
	Hydroxyapatite (HAP)-conjugated SPIONs	Magnetic hyperthermia	SPIONs	Magnetic hyperthermia induced destructive effects onto cancer cells only while minimizing the risks imposed onto healthy ones observed in U87 human brain cancer cells and human mesenchymal stem cells (MSCs)	[130]

5 Neurotoxicity of Surface-Functionalized NPs

Drug delivery using nanoparticles as a novel carrier gained popularity as it can target the drug to the site generally unreachable by conventional dosage forms as well as control the drug delivery. As such, it can be used for targeting the drugs across the BBB due to small size, surface alteration characteristics, stability, target specificity, etc. Surface modification or functionalization is responsible to bypass the endogenous opsonization and phagocytosis in the bloodstream, which otherwise would lead to an inefficient drug delivery to the brain. As discussed in the previous section, several strategies adopted for surface functionalization of nanoparticles are successful in targeting several drugs, peptides, hormones across the BBB [19]. Still, chances of potential neurotoxicity exist during its passage to the brain. One such is the neuron injury due to oxidative nanoparticles upon metabolism releases free radical, reactive oxygen species (ROS), which causes oxidative stress including DNA damage. ROS are associated with neurodegenerative diseases like Alzheimer disease, Parkinson disease [131]. A study by Yuan et al. reported the neurotoxicity due to polymeric nanoparticles. Polysorbate 80-modified chitosan nanoparticles showed apoptosis and necrosis of neurons, slight inflammatory response in the frontal cortex and decrease of GFAP expression in the cerebellum when injected in rat with no obvious changes observed for oxidative stress. Oxidative stress due to generation of free radicals may cause cell death [132]. Smaller sized NH_2 polystyrene nanospheres gained access to the cell organelles (mitochondria) causing cell death due to free radical generated as reported. Another study showed that high dose

administration of poly-butyl cyanoacrylate nanoparticles caused depression in mice. Magnetic nanoparticles are also responsible for neurotoxicity due to the release of ROS [133] while neurotoxicity due to iron oxide magnetic nanoparticles is due to iron accumulation, oxidative stress and protein aggregation in the brain. Iron accumulation in the neurons releases ROS, which causes oxidative stress. Iron accumulation and the consequent oxidative stress leads to protein aggregation including Aβ and α-synuclein, which play a critical role in Alzheimer and Parkinson diseases, respectively. Ultimately, iron accumulation, oxidative stress and protein aggregation leads to cell death. A study carried out by Borysov et al. showed that the unmodified ferritin magnetic nanoparticles significantly reduced L-[14C] glutamate transport in synaptosomes and acidification of synaptic vesicles with no change in the membrane potential of synaptosome. While coated magnetic nanoparticles with polysaccharides such as dextran, had no significant effect on synaptic vesicle acidification, the initial velocity of L-[14C] glutamate uptake, ambient level of L-[14C] glutamate and the synaptosomes plasma membrane potential [134]. The neurotoxic potential for iron oxide nanoparticles was observed in rat brain striatum by incubating dopaminergic neurons with radioactive iron oxide nanoparticles. The result showed that it leads to neuron viability, trigger oxidative stress and caused apoptosis [135].For lipid nanoparticles it is found that composition of lipid and surfactant plays an important role in toxicity. So optimization and regulation of composition is a key factor in reducing toxicity [136]. In order to have less neurotoxicity, a proper evaluation of physicochemical characteristics such the morphology, surface area, surface charge, coating, purity, material solubility, and the materials used for formulation should be considered. Other parameters, such as the dosage, administration route, concentration of the drug in the target organ, duration of action, and the degradation time of the biodegradable materials are most important and fundamental problems to be considered in the evaluation of nanoparticle neurotoxicology [131, 137]. A list of neurotoxicity study of different nanoparticles is shown in Table 15.2.

Table 15.2 Neurotoxicity study of nanoparticles

Types of nanoparticles	Neurotoxicity study	Results	References
Zinc oxide nanoparticles	Zinc oxide nanoparticles induced neurotoxicity in different age group C57BL/6 J mice	Neurotoxicity in old aged mice is due to the suppression of hippocampal cAMP response element binding protein (CREB), phosphorylated CREB, synapsin I, and cAMP	[138]
Iron oxide nanoparticles	Toxicity assessment of iron oxide nanoparticles in Zebrafish (Danio rerio) early life stages	≥10 mg/L of iron oxide nanoparticles induced developmental toxicity in Zebra fish embryos, causing mortality, hatching delay and malformation	[139]

(continued)

Table 15.2 (continued)

Types of nanoparticles	Neurotoxicity study	Results	References
Superparamagnetic iron oxide nanoparticles (SPIONs)	Characterization of superparamagnetic iron oxide nanoparticle-induced apoptosis in dopaminergic neuronal PC12 cells and mouse hippocampus and striatum	It showed a dose-dependent cytotoxic in PC12 cells at 60–200 μg/mL but not at 10–50 μg/mL, reduced cell viability, decreased the PC12 cells capacity to extend neuritis in response to nerve growth factor (NGF), increased PC12 cell apoptosis. Also decreased the TH^+ fiber density in both the dorsal striatum and the hippocampus	[140]
Oleic acid-coated iron oxide nanoparticles	Neurotoxicity assessment of oleic acid-coated iron oxide nanoparticles in human neuronal cells SH-SY5Y cells	Moderate cytotoxicity related to cell membrane impairment, cell cycle disruption and cell death induction, especially notable in serum-free medium	[141]
Iron oxide nanoparticles	Study based on iron oxide nanoparticles induces cell cycle-dependent neuronal apoptosis in mice	The result showed an increased level of oxidants, β amyloid accumulation, reduced level of cdk5, which indicates cell apoptosis and DNA damage due to overexpression of RNA Pol II and PARP cleavage	[142]
Silica-coated iron oxide nanoparticles	Study of the cytotoxicity and genotoxicity of silica-coated ION was evaluated on human A172 glioblastoma cells	The result showed certain cytotoxicity, related to cell cycle disruption and cell death. Scarce genotoxic effects and no alteration of the DNA repair process were observed	[143]
Iron oxide nanoparticles	Toxicity evaluation of magnetic iron oxide nanoparticles with egg albumin and subsequent toxicity on chicken embryo	Histology of brain tissue revealed degeneration of neurons (50–60%) at 10–100 μg/ml dose range of IONs	[144]

6 Conclusion

Nanoparticles have got significant potential in targeting therapeutics to the brain in the treatment of several diseases such as Alzheimer disease, cancer, Parkinson disease, epilepsy, and HIV encephalopathy. Till now several drugs are targeted to the brain based on several strategies as discussed. A prior challenge in drug targeting to the brain is the avoidance of opsonization and phagocytosis in blood, which can now be easily avoided by surface-functionalized nanoparticles, facilitating an efficient drug delivery to the brain. Still, neural cell death associated with oxidative

stress including neurodegenerative diseases may occur due to ROS released by nanoparticles in neurons. Thus, it is essential to carry out neurotoxicity or cytotoxicity studies for nanoformulations. However, results obtained in animal studies may not be extrapolated to human beings due to differences in physiological conditions of the body, genetic factors, and differences in transport mechanisms. Thus, studies dealing with pharmacokinetic and pharmacodynamic effects of nanoparticles on the physiological conditions of the body have to be considered for efficient drug targeting to the brain with reduced neurotoxicity. Reduction of neurotoxicity can be achieved by proper evaluation of the physicochemical characteristics of nanoparticles as well as their pharmacokinetic and pharmacodynamic parameters.

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