

Targeted Brain Delivery of Bioactive Molecules Using Nanocarriers

Gajbhiye KR¹, Gajbhiye V² and Soni V^{1*}¹Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar, Madhya Pradesh, India²Center for Nanobioscience, Agharkar Research Institute, Pune, India

Abstract

Delivery of the bioactives to the brain is the utmost challenging task to cope with brain diseases. Brain is protected with blood brain barrier, blood-CSF barrier and efflux systems, which controls the entry of body as well as foreign compounds to access the brain cells. Only nutrients, which are essential for normal metabolism, can enter into the brain. In the shadow of this fact new strategies are being investigated to facilitate the entry of administered therapeutic compound into the brain. Active targeting is an evolving approach, which uses ligand and suitable carrier for the site-specific delivery and is being recently achieved by the use of nanocarriers. These nanocarriers are nano-sized systems, which act as a cargo for the encapsulated drugs. At the same time, endo- or exogenous ligand can be attached to these nanocarriers to recognize specific receptors on brain capillary endothelium leading to delivery of drug in the vicinity of brain cells. Their potential is under immense investigation to increase the therapeutics outcome in the treatment of brain related problems. This review deals with the recent advances in nanocarriers based novel strategies for effective brain specific delivery.

Keywords: Brain diseases; Targeting; Nanocarriers; Cancer; Alzheimer's; Parkinsonism; Epilepsy; AIDS

Introduction

The brain is the center of the nervous system and an integral part of the body acting as a major regulating and conveying organ to maintain the body's homeostasis in response to changes in both the external and internal surroundings. Brain consists of approximately 100 billion neurons and 1 trillion glial cells [1]. The connection between two neurons is known as synapse, which are responsible for flow of information in the form of tiny chemical pulses released by one neuron and taken up by the meeting neuron. Different types and strengths of signals move constantly through the brain's circuits, creating the cellular basis of memories, thoughts, and skills [2].

Foremost confront- The Blood-brain barrier

The Blood-brain barrier (BBB) is a flow barrier necessary for the normal function of the central nervous system [3]. It guards the brain from substances which are neurotoxic in physiological concentrations (e.g. potassium, glycine, glutamate etc) [4]. The endothelial cells of BBB have no fenestrations, and have wide-ranging tight junctions and meager pinocytotic vesicular transportation [3]. The tight junctions of endothelial cells are credit-worthy for the limited paracellular flux of hydrophilic molecules across the BBB [5]. In contrast to this, small lipophilic substances diffuse without restraint across plasma membranes alongside their concentration gradient [6,7]. The endothelial cells are bordered by a basal lamina and with the end-feet of astrocytic glial cells. In places, pericytes are engrafted in the basal lamina between endothelial cell and astrocyte, making particularly close contacts with the endothelial cells. A number of other cell types may be present in the perivascular space, including perivascular macrophages [8]. Terminals from a number of neuronal populations may also end on or near the vessels, which further limit the entry of bioactives. Blood-CSF barrier is another such barrier which hurdles entry of drug molecules in the vicinity of brain cells [9].

Problem behind problem- Efflux systems

Delivery of many compounds across the BBB is heavily dependent on numerous characters of the bioactive molecules such as its lipophilicity. It is believed that more lipophilic compounds of lower

molecular weight can penetrate easily through BBB [10]. However, many drugs [e.g. Vinblastin and Zidovudine (AZT)] do not stay in the brain cells for appropriate time due to the fact that these molecules are substrate for the efflux transporters present on the brain cells [11]. These transporters are responsible for the expulsion of the substrate molecules [12]. Several transporter protein families have been identified, which act as an efflux transporter of the molecules [13]. P-glycoprotein (P-gp) is a phosphorylated glycoprotein with an apparent molecular weight of 170 kDa, was the first drug efflux transporters to be described, followed by several others [14]. Efflux transporters are expressed on BBB and blood-CSF barrier and diminish the activity of many drugs [15].

Brain related diseases

Brain operates promptly and automatically in healthy status. However, when problems occur, the results can be shocking. CNS related problems can be largely divided into those with prominent neurodegeneration and those without such change. Neurodegenerative disorders embrace chronic neurodegenerative disorders that lead to dementia, Alzheimer's disease, frontotemporal lobar degeneration, multi-infarct dementia, disorders of movement, Parkinson's disease and Huntington's disease etc [16]. CNS disorders without evident neurodegenerative pathology embodies a heterogeneous group of disorders including anxiety, depression, schizophrenia, epilepsy, insomnia, autism, brain tumors (most fatal) and many others.

Approaches for treating brain diseases

Treatment of brain diseases was never easy. However, when obligation arrives, some conventional and non-conventional strategies

***Corresponding author:** Soni V, Department of Pharmaceutical Sciences, School of Engineering and Technology, Dr. H. S. Gour University, Sagar (MP) 470 003, India, Tel: +917582-265457; E-mail: drvandanasoni@gmail.com

Received March 05, 2015; Accepted March 30, 2015; Published April 04, 2015

Citation: Gajbhiye KR, Gajbhiye V, Soni V (2015) Targeted Brain Delivery of Bioactive Molecules Using Nanocarriers. J Bioequiv Availab 7: 112-122. doi:10.4172/jbb.1000224

Copyright: © 2015 Gajbhiye KR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

are used. These strategies can broadly be divided into (i) surgical, (ii) non-surgical, (iii) drug modification and (iv) active targeting.

Surgical or invasive approaches: These are physical based techniques that include the use of Intracerebro-ventricular infusion, Convection-enhanced delivery, and Polymer or microchip systems, which directly release therapeutics after implantation in the CNS. Invasive approaches may involve delivery of drugs to the brain cells by mechanically violating BBB [17,18].

Non-surgical strategies: In this approach the drug substances are directly delivered to CNS through opening of TJs by the use of certain chemicals and energy. Chemically, BBB can be disordered via such substances, which have high osmotic pressure [19]. Due to elevated osmotic pressure breaching of tight junctions takes place, leading to the contraction of endothelial cells, by which disarrangement of extracellular proteins occurs and, at last, entry of drug takes place paracellularly [19-23]. Disruption of BBB can also be accomplished by application of ultrasound [24]. However with these approaches, chemotherapeutic agents also acquire in the healthy tissue, causing adverse effects.

Drug modification: The potential to cross the BBB passively depends on the molecular size (< 500 Da), charge (low hydrogen bonding capabilities) and lipophilicity (more the lipophilic, better the transport) [25]. In this chemical approach the drug molecule is tailored to render it more lipophilic in order to ameliorate its penetration into the brain by passive diffusion. Thus prodrugs and lipophilic analogs of parent drug molecules were developed to deliver them inside the brain against CNS disorders. [26,27].

Active targeting: The field of novel drug delivery has fully emerged and came into existence as an ideal approach for drug targeting to the brain [28]. It corresponds to a promising non-invasive approach for better drug delivery to the brain through exploitation of the various influx transport systems depicted within the cerebral endothelial, including carrier-mediated transports (CMT), receptor-mediated endocytosis (RME) and adsorptive-mediated endocytosis (AME) [29]. Earlier, such drug molecules were developed to mimic essential physiological substances, which easily cross BBB [30]. However, nowadays nano-sized carriers are being used widely and under investigation with or without coating or coupling with ligands to deliver the drugs using these transport systems [31]. These carriers are widely consented and appreciated due to their controlled drug release profile as well as due to their special targeting mechanism [32].

There are many reviews published in last decade which shed light on the use of nanocarriers for brain targeting [33,34]. In this review we are focusing on recent brain targeted strategies which have been developed using liposomes, nanoparticles (NPs) and dendrimers since 2005 for various brain diseases like brain tumors, Alzheimer's disease, Parkinson's disease, epilepsy and AIDS.

Brain tumors

A brain tumor is a mass of uncontrolled cancerous cells growing in the brain, which may be of two types; primary and metastatic [35]. Brain tumors compress normal brain tissue, and symptoms can result from that pressure. A person can experience different kinds of symptoms depending on where in the brain a tumor is located [36]. Brain tumors are one of the 10 major causes of death by cancer. Prevalence of gliomas is approximately 5 to 10 per 100,000 populations [37]. Despite aggressive surgery, radiotherapy, and chemotherapy, the average 1 year survival has not amplified in past three decades. This is

due to the fact that brain tumors, upon diagnosis, are usually already 30 to 60 cm³ in volume, approximately 3-6×10¹⁰ cells.

Alzheimer's disease

Alzheimer's disease (AD) is a widespread form of adult onset dementia [38]. It results in regularly worsening difficulty in recalling new information because of the disruption of brain cells involved in forming new memories [39]. As and as damage spreads, individuals also experience confusion, muddled thinking, impaired decision, trouble expressing themselves, and perplexity to time, space, and location. In AD, flow of information at the synapses begins to fail, synapses begin to decline in number, and eventually brain cells die [40]. Amyloid hypothesis states that a protein fragment called β -amyloid (A-beta; a heterogeneous 39-43 amino acid peptide) plays major role. Problem arises when there is overproduction of A-beta or reduction in the brain's ability to dispose it off [41]. Other abnormalities seen in Alzheimer's brain tissue include inflammation and oxidative harm due to very reactive oxygen-containing products of cellular metabolism. Currently, inhibition of β - and γ -secretases responsible for A-beta formation as well as A-beta immunization to reduce A-beta plaques are proposed as potential treatments for AD [42].

Parkinson's disease

Parkinson's disease (PD) also called primary Parkinsonism, was first described by James Parkinson in 1817 [43]. It is the second most common neurodegenerative disorder, after AD, affecting about 1% of adults older than 60 years. Pathologically it is due to the loss of mid brain dopaminergic neurons of the substantia nigra, pars compacta, and of their terminals in the striatum [44]. Over and above the well-known reductions in dopamine, there is convergent evidence for early alterations in cholinergic neurotransmission in PD [45]. The clinical diagnosis of PD preferentially and profoundly relies on history, physical examination, and improvement in sign and symptoms with dopaminergic treatment [46].

Epilepsy

Epilepsy is the most common serious neurological disorder, with an occurrence of 0.5-1% in the general population [47]. It is characterized by repeated, spontaneous brain seizures [48] due to the episodic bursts of electrical activity in certain neurons, which may extend to the entire brain [49]. Such abnormal neuronal activity may have significant blow on the normal cognitive processes and activities of the affected individuals. It has a focal origin in the brain and indications or symptoms depend on the site of the focus, parts wherein the discharges reach and postictal depression to these regions [50]. For example, temporal lobe epilepsy (TLE) can cause memory impairment, and focal epilepsy relating the language-dominant hemisphere may induce word finding and naming difficulties. While certain epilepsy syndromes are related with severe cognitive or behavioral problems [51].

Acquired Immunodeficiency Syndrome (AIDS)

Human immunodeficiency virus (HIV) is a lentivirus (family: Retroviridae) responsible for acquired immunodeficiency syndrome (AIDS) [52]. The CNS serves as one of the chief anatomical reservoirs for the replicating HIV-1 virus. It spreads to the CNS at an early stage of infection and re-infects the peripheral tissues leading to the re-activation of the infection [53]. Infection of the CNS can be proven in about 80% of the HIV-positive individuals and occurs most probably via infected immune cells such as CD4⁺ T lymphocytes, dendritic cells, monocytes and macrophages. These all are cellular reservoirs

of HIV-1 that go across the BBB [54,55]. HIV infection in the CNS leads to the development of asymptomatic neurocognitive impairment, HIV-associated mild neurocognitive disorder, and eventually HIV-associated dementia (HAD) or AIDS dementia complex that comes out as a clinical syndrome of cognitive, motor and behavioural impairment [56]. Due to the CNS infection several neuropathological consequences may also be seen whose characteristics are atrophy, multinucleated giant cells (suggestive of virus-induced fusion of microglia and/or macrophages), reactive astrogliosis, microgliosis, loss of neurons and inflammatory infiltrates full of lymphocytes and macrophages. Altogether these inflammatory and degenerative neuropathological events are termed HIV-1-associated encephalitis [57].

Treatment strategies for brain diseases using nanoparticles

NPs are particulate carriers made of polymers or inorganic material having size range between 10 and 1000 nm. Drugs can be loaded on NPs in the form of a solid solution, dispersion, adsorbed to the surface or can be chemically attached. The possible uses of this nanocarrier in the treatment of brain diseases are being evaluated continuously. Researchers have incorporated many drugs into the NPs for brain diseases and these systems have emerged as one of the most promising approach to deliver the drug across the BBB.

NPs in treatment of brain tumors: Therapeutic benefit in glial tumors is often limited due to low permeability of delivery systems across the BBB, drug resistance and poor access into the tumor. Paclitaxel (PTX) is not effective for treatment of brain cancers due to efflux by P-gp. However, the effect of loading PTX into the poly(lactico-glycolide) (PLGA) NPs (PTX-PLGA-NPs), transferrin (Tf) anchored PLGA NPs (Tf-PTX-PLGA-NPs) and pluronic-P85 coated PLGA NPs (P85-PTX-PLGA-NPs) was evaluated by Shah et al. (2009). This study revealed that there was noteworthy increase in cytotoxicity in the order of Tf-PTX-PLGA-NPs > P85-PTX-PLGA-NPs > PTX-PLGA-NPs in comparison to free drug solution [58]. Likewise, coating of glutathione on PLGA NPs produced similar effect and higher cellular uptake of the NP was demonstrated in RG2 cells. The coated NP (targeted) showed significantly higher cytotoxicity in RG2 cells compared with uncoated NP (non-targeted). Moreover, *in vivo* brain uptake study in mice showed higher brain uptake of these NPs [59].

Another class of NPs fabricated from poly(butyl cyanoacrylate) (PBCA) and coated with tween 80 (T80) enhanced cytotoxic effect of doxorubicin (DOX) towards rat glioma cells expressing P-gp. Additionally, the influence of surfactants on the cytotoxic effect was investigated at different DOX concentrations. Results revealed that the presence of T80 on the NPs significantly improved the cytotoxicity [60]. Kreuter and Gelperina also worked on PBCA and PLGA NPs coated with T80 and poloxamer 188. They reported that these NPs enable the transport of anti-cancer molecules across the BBB. Intravenous (i.v.) injection of these NPs loaded with DOX significantly increased the survival times and also resulted in complete tumor remission in 20-40% of the rats [61].

Non only polymeric, but inorganic NPs have also been utilized for brain tumor treatment. Iron compounds like Fe_3O_4 poss magnetic properties, which was thought to be useful to guide the nanocarriers. These magnetic NPs have shown potential results in targeting brain tumors when magnetic field was applied to home the NPs at specific location in the brain. Magnetic NPs along with magnetic field applied resulted in 9.6 fold higher NP accumulation in the tumor tissue of animals than that accumulated in the contralateral brain tissue [62]. Similar work was carried out with superparamagnetic iron oxide

NPs (SPIO) without any magnetic field. Accumulation of magnetic NPs were observed in glioma cells, when SPIO NP-fluorescein isothiocyanate-chlorotoxin (SPIOFC) and SPIO NP-fluorescein isothiocyanate (SPIOF) were cultured with human U251-MG and rat C6 glioma cells, respectively. The accumulation was noticeably found to be higher in cells cultured with SPIOFC. Moreover, there was preferred accumulation of iron in the glioma cells as compared to the neural cells, clearly stating that SPIOFC is suitable for the specific targeting of glioma cells [63]. Combination of superparamagnetic NPs with thermotherapy was also tried for management of brain tumors, wherein the iron oxide NPs coated with dextran or aminosilane were used. In this study, the intratumoral injection of aminosilane coated NPs emerged superior (4.5-fold higher survival over controls) than dextran coated NPs. Their tumoral deposition was found to be stable, permitting for serialized thermotherapy treatments without repeated injection. Histological and immunohistochemical assessment after treatment displayed large necrotic areas close to particle deposits, a reduced propagation rate and a reactive astrogliosis adjoining to the tumor [64].

Vascular endothelial growth factor (VEGF) up-regulation is trademark of tumor cells and responsible for tumor's rich vasculature. The down-regulation of VEGF was thought to exert stringent effect on tumor cells. Cellular VEGF expression was significantly reduced ($p < 0.01$) when VEGF antisense oligonucleotides was targeted via solid lipid NPs (SLNs) to C6 glioma cells. However, expression remained stable after free VEGF antisense oligonucleotides treatment (without NPs). Similar results were obtained in *in vivo* study in which a pronounced VEGF reduction was obtained in central and peripheral tumor regions of rats treated with SLNs carrying VEGF antisense oligonucleotides, while results were not considerable after free VEGF antisense oligonucleotides treatment (without NPs). Both *in vitro* and *in vivo* rat glioma models demonstrated potential in reducing VEGF expression via VEGF antisense oligonucleotides containing SLNs [65].

NPs in treatment of Alzheimer's disease: These nanocarriers have been explored for AD therapy too. Owing to the severity of AD and knowing the fact that delivery of bioactive molecule for treatment of AD to the brain is one of the most challenging hurdle, strategies to deliver drugs by the use of nanocarriers may be beneficial. Rivastigmine, an anti-cholinergic drug which is used to treat AD was delivered across BBB using PBCA NPs. The drug was administered as a free drug, loaded into PBCA NPs, and loaded into T80 coated NPs. The concentrations of rivastigmine found in liver, spleen, lungs and kidneys were not changed much. However, rivastigmine concentration in brain was significantly increased, in case of PBCA NPs and PBCA NPs coated with T80 in comparison with the free drug. PBCA NPs coated with 1% T80 increased the concentrations by 3.82 fold in the brain as compared to free drug ($p < 0.001$) [66]. Normalized or elevated copper (Cu) levels have been reported to confine or even abolish AD-related pathological plaques and exercise a desirable amyloid-modifying effect. Two different polymeric nanocarrier systems, CS-NPs (core-shell NPs) and CMS-NPs (core-multishell NPs), were reported to transport Cu across the plasma membrane of yeast or higher eukaryotic cells. Intracellular Cu levels were increased up to 3-fold above normal levels with a sub lethal dose of carriers. Both types of carriers released their bound client molecules into the cytosolic section. Particularly, CS-NPs reduced A-beta levels and targeted intracellular organelles more proficiently than CMS-NPs. But overall both nanocarriers were found to transport Cu across cellular membranes, thus increased the levels of bioavailable Cu, and altered A-beta accumulation [67].

As discussed previously that AD may also originate from neurodegeneration occurred by oxidative stress and dysfunctional mitochondria. Thus, use of antioxidants may be a critical strategy for neuroprotection. Ferulic acid (FA) exerts antioxidant action on different paths, which are responsible for degeneration of recombinant β -amyloid peptide (rA β 42) treated cells. Hence, SLNs were used to improve its delivery. Studies on neuroblastoma cells and rA β 42 oligomers confirmed that free and SLNs-loaded FA recovered cell viability. Furthermore, treatment via FA loaded SLNs, reduced reactive oxygen species (ROS) production, re-established mitochondrial membrane potential and abridged cytochrome c release and intrinsic pathway apoptosis activation [68]. Similarly, SH-SY-5Y human neuroblastoma cells were exposed to A-beta for 2 hr and cultures were treated with 0.15 mg/L of vitamin E (as alpha-tocopherol), with or without encapsulation into nanospheres. The treatment was given either concurrently with the addition of A-beta or after 60 min. The researchers found that free vitamin E only protect differentiated cells against ROS when it was applied prior to, or simultaneously with, A-beta. On the contrary, nanosphere encapsulated vitamin E, when applied 1 hr after A-beta was as effective as the application of an equivalent concentration of non-encapsulated vitamin E concurrently with A-beta [69]. Like antioxidants, neuroprotection from AD can also be achieved by subfragments of A-beta. Systemic delivery of intramembranous fragments of A-beta was compared with antigen loaded chitosan NPs. Fluorescence microscopy revealed that not only brain uptake efficiency of nano-antigen was increased (80.6% compared to 20.6% of free antigen) but immunogenicity was also enhanced by using NPs. Here, chitosan NPs served as a cargo for A-beta to permeate through BBB [70].

NPs in treatment of Parkinson's disease: Non-viral gene therapy of chronic degenerative diseases such as PD is a great challenge because of the low transfection efficiency of non-viral gene vectors. To counter this problem lactoferrin (Lf) modified NPs were formulated and examined in the 6-OHDA-lesioned PD model by regimen of multiple dosing intravenous administrations. The results of the neuroprotective evaluation showed that by increasing the number of injections of Lf-modified NPs loaded with human glial cell line-derived neurotrophic factor gene (hGDNF) locomotor activity was improved, there was reduced dopaminergic neuronal loss and improved monoamine neurotransmitter levels in PD rats. Five injections of Lf-modified NPs loading hGDNF displayed much more potent neuroprotection than a single injection, signifying the effectiveness and feasibility of multiple dosing administrations [71]. These researchers again applied the similar delivery system with the same protein but this time in the rotenone-induced chronic rat model of PD and they found that the multiple injections of Lf-modified NPs produced higher GDNF expression and this gene expression was retained for a longer time than the one with a single injection [72]. Neurodegeneration may also occur due to oxidative insult and this may lead to neurodegenerative disease like PD. If the molecules responsible for this oxidative insult can be removed from the surrounding of the neuron than the disease may well be prevented to get worsened. Transport and uptake of antioxidant to scavenge the reactive molecules may be better achieved by the use of the nanocarriers and this was shown by the studies carried out by Carroll et al. (2010). These researchers incorporated Tempol (free radical scavenger which is proved to prevent oxidative insult) inside the PLGA NPs, which were further conjugated to Tf antibody (OX 26). MTT assay revealed that the OX 26 conjugated NPs having Tempol were more effective than non-conjugated NPs or free Tempol solution in preventing cell death. Thus the authors concluded that Tf conjugated

NPs having antioxidant may be useful in preventing neurodegenerative disease [73].

Bioactive molecules employed for epilepsy are generally short acting. Thyrotropin-releasing hormone (TRH; Protirelin) is well known for its anti-convulsant effect but it undergoes extensive tissue metabolism. BBB also confines its permeation into brain, which limits its duration of action. Therefore, the approach to deliver this neuropeptide direct from nose to brain by incorporating it into NPs is a promising mode of therapy to improve its availability into brain. Kindling (chronic, electrically-induced) model of temporal lobe epilepsy was exploited to deliver the neuropeptide via NPs. It was found that TRH-loaded copolymer microdisks rooted in a seizure focus can attenuate kindling development in terms of behavioral stage, after discharge duration (ADD), and clonus duration. Researchers found that intranasal administration of unprotected TRH analogue can intensely suppress fully kindled seizures in a concentration-dependent manner in terms of ADD and seizure stage. Whereas intranasal administration of polylactide NPs (PLA-NPs) with TRH (TRH-PLA-NPs) can impede kindling development in terms of behavioral stage, ADD, and clonus duration. Collectively, the achieved data from the studies provided proof of perception for intranasal delivery of TRH-NPs as a suitable means to suppress seizures and possibly epileptogenesis [74]. Veronesi et al. (2009) provided additional data showing TRH-NPs induced protection against glutamate toxicity *in vitro*, and suggested that intranasal delivery of TRH-NPs are capable of restraining a number of seizure characteristics in the rat kindling model of temporal lobe epilepsy. Neuronal death caused by glutamate was reduced significantly by TRH-NPs. *In vivo* studies on male Sprague-Dawley rats demonstrated that intranasal application of TRH-NPs led to a significant reduction in seizure ADD as kindling advanced. Whereas, the number of stimulations necessitated reaching stage V seizures and to become permanently kindled was significantly greater in TRH-NP-treated subjects. Furthermore, delay to clonus was prolonged, while clonus duration was reduced; signifying a less severe seizure in TRH-NPs treated subjects [75].

NPs in treatment of epilepsy: Epilepsy is a neuroexcitation disorder and targeting of anti-neuroexcitation peptide (ANEP) as thought to be promising approach to treat it. Wanga et al. (2010) [76] investigated targeting of N-Trimethyl chitosan chloride (TMC) NPs loaded with ANEP. In this study male KunMing mice were used and treated with fluorescein iso thio cystate (FITC) solution, (FITC)-ANEP solution and FITC-ANEP-TMC/NPs. The ANEP was distributed rapidly into the brain and the highest concentration appeared at 30 min after administration of FITC-ANEP solution. On the other hand, in animals of FITC-ANEP-TMC/NPs treated group, ANEP appeared in the brain 1 hr after injection and remained there for 3 hr. The results further illustrated that the targetability of ANEP to brain was significantly increased by TMC NPs. Magnetic nanoparticles (MNPs) were also investigated to target epileptogenic tissue. Alphamethyl tryptophan (AMT) was covalently attached to MNPs as MRI agent and these MNPs were injected in rodent model of temporal lobe epilepsy. The AMT-MNPs or plain MNP was administered intravenously during the acute stage 3 days after status epilepticus, and AMT-MNPs in another animals during the chronic stage. The result showed that AMT-MNPs crossed the BBB and were present intra-parenchymal. In the acute condition, AMT-MNPs appeared to concentrate to both hippocampi, while plain MNPs identified only unilateral, inflammatory regions. In the chronic condition, AMT-MNPs uptake occurred in spontaneous seizures, and localization was found precisely in the epileptic regions [77].

NPs in treatment of HIV infection in brain: Transport of the anti-HIV agents across BBB is a key factor in the therapy of the AIDS related neuropathological events. Many strategies have been investigated to deliver the antiviral drugs across the BBB and to impede the infection. Kuo and Chen (2006) [78] studied the effect of PBCA and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) NPs on the permeability of AZT and lamivudine across the *in vitro* BBB model. The result obtained from their studies noticeably demonstrated that while employing PBCA NPs, AZT and lamivudine were permeated across the BBB approximately 8-20 and 10-18 times more, respectively. By the application of MMA-SPM NPs, approximately 100% augmentation in the BBB permeability of the two drugs was observed. Additionally, these co-workers also studied the effect of ethanol on permeability of these preparations and they found that in the presence of 0.5% ethanol, 4-12% improvement in the BBB permeability of the two drugs was observed using NPs. Similar study was carried out by Kuo and Su in year 2007. They compared the effect of PBCA, MMA-SPM and SLN on the permeability of stavudine (D4T), delavirdine (DLV), and saquinavir (SQV) across the BBB. For this purpose, the *in vitro* BBB model was composed of a culture of human BMECs (HBMECs) on a microporous polycarbonate (PC) membrane. Permeability of D4T-, DLV-, and SQV-carrying PBCA NPs, MMA-SPM NPs, and SLNs was anticipated by the drug concentration in receiver chamber of the transport system. They found that the permeability of the three drugs improved by about 12-16 folds on PBCA, 3-7 folds on MMA-SPM, and 4-11 folds in SLNs. For DLV and SQV, the order of permeability improvement was PBCA > SLNs > MMA-SPM and for D4T the order was PBCA > MMA-SPM > SLNs [79]. SLNs were also exploited to improve the transport of HIV Protease Inhibitor (PI), Atazanavir through human brain microvessel endothelial cell line (hCMEC/D3) which is representative of the BBB. The studies revealed that delivery of atazanavir by SLNs led to a significantly much more accumulation by the endothelial cell monolayer as compared to the drug aqueous solution. Additionally, release of Rhodamine-123 (a fluorescent probe) by SLNs also resulted in a higher cellular accumulation [80]. Delivery of another PI, Ritonavir, by Poly (L-lactide) (PLA) NPs was studied by attaching trans-activating transcriptional (TAT) peptide to NPs. They hypothesized that encapsulation in NPs would hinder the effect of P-gp at the same time TAT would boost the uptake across BBB model membrane. In their *in vitro* study they chose Madine Darby canine kidney cells over-expressing P-gp (MDCK-MDR1) and non-P-gp-expressing MDCK wild type (MDCK-wt) because MDCK cells are capable of forming TJs, which, together with the expression of P-gp, makes these cells a suitable model for BBB transport and permeability studies. *In vitro* studies demonstrated that the cumulative transport of free ritonavir from the apical to basolateral sides of diffusion chambers was noticeably inferior as compared to the drug encapsulated unconjugated or TAT-conjugated NPs in the MDCK-MDR1 cell line. For *in vivo* studies P-gp intact (wild type) mice were utilized as an animal model. The result of *in vivo* studies showed higher drug level in the brain up to 3 hr after the administration in case of free drug as compared to ritonavir-loaded unconjugated NPs or TAT conjugated NPs. But later on, the levels were higher in animals which received drug-loaded NPs, particularly in the animals which received TAT-conjugated NPs than in animals which received free drug. The result suggested 800-fold elevated brain ritonavir level with TAT-conjugated NPs than that with ritonavir solution and near about 7 fold higher than the concentration with unconjugated NPs [81].

Tf Anchored Albumin NPs were also investigated to deliver the water soluble drug AZT across the BBB. Albumin NPs were PEGylated

(PEG coating) and Tf was anchored to this system for brain targeting. *In vitro* fluorescence study suggested increased uptake of Tf-anchored NPs in the brain tissues as compared to non-targeted NPs. Furthermore, *in vivo* studies showed significant improvement in brain localization of AZT for Tf anchored PEGylated albumin NPs (Tf-PEG-NPs) [82]. The effect of the route of administration to transport the anti-HIV drug to the brain was studied and for this purpose didanosine (DDI) was incorporated in chitosan NPs. DDI in chitosan NPs was given intranasal and simultaneously in other rats the free drug solution was administered intranasal and i.v. It was found that the brain/plasma, olfactory bulb/plasma and CSF/plasma drug concentration ratios were significantly higher for intranasal administration of DDI NPs or solution than those from i.v. administration of free DDI solution. Thus, the study concluded that both the intranasal route of administration and formulation of DDI in chitosan nanoparticles increased delivery of DDI to CSF and brain [83].

Treatment strategies for brain diseases using liposomes:

Liposomes are vesicles in which an aqueous volume is completely enclosed by a membrane framed of lipid molecule. Liposomal carriers have a strong effect on pharmacokinetics and tissue distribution of incorporated drugs. Stealth, magnetic and cationic liposomes have been extensively employed for the management of brain tumors. Numerous drugs have been investigated with these carriers with or without ligands attached to them. In fact, these systems have also been tried out clinically in the patients with malignant gliomas. In the same context, sulphatide (a glycosphingolipid well-known to bind extracellular matrix glycoproteins and highly up-regulated in many tumors) ligand was exploited to carry liposomes to the cytoplasm of brain cancer cells (U-87MG and CCF-STTG1 glioblastoma cell lines). DOX was readily delivered into the cell nuclei to exert cytotoxicity when delivered via sulphatide conjugated liposomes. Use of sulphatide conjugated liposomes loaded with DOX for treatment of tumor-bearing nude mice exhibited much improved therapeutic effects as compared to free drug or PEG grafted liposomes [84]. Like sulphatide glycosphingolipid, human glioblastoma tumor expresses interleukin 13 (IL-13) and this property was exploited to deliver cytotoxic agent into the tumor cells via liposomes. Intraperitoneal injections of DOX encapsulated in IL-13 conjugated liposomes resulted 5-fold cutback in the intracranial tumor volume of mice and their survival period was also elongated (>200 days after tumor implantation), whereas, life span was not more than 35 days for unconjugated liposomes with the same DOX concentration and there was no evidence of tumor size reduction. This study also illustrated that the nanovesicles do not harm the endothelial cells yet maintain their toxicity to astrocytoma cells [85]. Furthermore, liposomes have also been investigated to transport drug across the BBB and at the same time blocking the multidrug resistance (MDR) effect to triumph over the poor penetration of drug into the tumor tissue. In this dual targeted strategy, tamoxifen was integrated into the lipid bilayer membrane of liposomes and wheat germ agglutinin (WGA) was attached to the surface and topotecan was loaded inside this system. When this system was evaluated for *in vivo* activity into rats bearing C6 glioma cells, it was evident that there was improvement of great magnitude in the overall survival of the brain tumor-bearing rats compared with free topotecan and topotecan liposomes. Researchers suggested that tamoxifen exhibited hindering effect on efflux MDR proteins in the BBB and brain tumors, while WGA served to augment endocytosis via BBB [86].

Similar efforts were also made with daunorubicin liposomes where p-aminophenyl- α -D-mannopyranoside (MAN) and Tf were used as a ligand for dual targeting. *In vitro* and *in vivo* studies on this system

revealed that after one week treatment of the rats bearing C6 glioma cells, the median survival time of rats treated with daunorubicin liposome tailored with MAN and Tf (22 days) was appreciably longer as compared to the rats treated with physiological saline (13 days), free daunorubicin (17 days), daunorubicin liposomes (18 days), daunorubicin liposomes tailored with MAN (19 days) and daunorubicin liposomes tailored with Tf (18 days). The authors suggested that MAN may have played a key role in conveying the liposomes across the BBB and Tf played main role in targeting brain glioma cells [87].

In the present era where AD treatment is under immense investigation, nanocarriers is gaining attraction among the researchers as possible payloads to deliver the anti-Alzheimer's drug at the right spot as well as to understand the correct mechanism of A-beta formation and its neurotoxicity. The potential of liposomes to deliver drugs crossways BBB was tested in wistar rats after administering intranasal rivastigmine liposomes. The study also compared the oral and nasal administration of free drug. It was marked that rivastigmine concentration was elevated in plasma after intranasal administration of liposomal preparation (5 times than orally administered rivastigmine and nearly 3 times as compared to free rivastigmine administered intranasally). Brain concentrations of rivastigmine were also studied after administration of free drug orally, intranasally and intranasal administration by liposomal preparation which was found to be 5.6 times higher in the brain than oral administration [88]. Intranasal delivery of liposomal drug formulation for AD treatment was recently explored by Phachonpai et al. (2010) designed a study to examine the effect of nasal administration of quercetin liposomes on neurodegeneration in the animal model of AD. For this purpose wistar rats were administered with AF64A (which demolishes the cholinergic system, as well increases oxidative stress in all area of hippocampus). In the biochemical assays, activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and malondialdehyde (MDA) level were determined. The results revealed that quercetin liposomes attenuated the deterioration of neurons and cholinergic neurons in hippocampus. In addition to this, the elevation of SOD, CAT and GPx activities and the lessening of MDA in hippocampus were also observed. They concluded that nasal administration of quercetin liposomes may be the potential novel therapeutic tactic against AD [89].

Liposome based vaccine for AD has also been investigated wherein tetra-palmitoylated amyloid 1-15 peptide (palmA β 1-15), or amyloid 1-16 peptide (PEG-A β 1-16) was linked to a PEG at each end. These conjugates were engrafted within a liposome membrane and were inoculated in double transgenic mice which drawn out fast immune responses. PalmA β 1-15 liposomal vaccine evoked an immune response that restored the memory defect of the mice while that of PEG-A β 1-16 had no such effect. CD and NMR studies revealed predominantly β -sheet conformation of palmA β 1-15 and random coil of PEG-A β 1-16. From these findings it was concluded that the association of peptides with liposomes induced a variation of the immunogenic structures and thereby different immunogenicities were observed [90].

Transvascular gene therapy of PD is a relatively new approach to the gene therapy of PD and engrosses the distribution of a therapeutic gene to brain after an intravenous administration and transfer across the BBB. DNA was encapsulated in liposomes and these liposomes were attached to PEG (2000 Da) and after that monoclonal antibody (MAb) was attached to this surrounding PEG [PEGylated immunoliposome (PIL)] which directed this system against Tf receptors (TfR), expressed on BBB and neuronal membrane. In the 6-hydroxydopamine rat model

of experimental PD, striatal tyrosine hydroxylase (TH) activity was wholly normalized after an intravenous administration of TfRMAb-targeted PILs carrying a TH expression plasmid [91]. Gene therapy with the liposomes targeted with MAb for management of PD has also been tested. Rats with experimental PD were administered with intravenous glial-derived neurotrophic factor (GDNF) plasmid DNA (pTHproGDNF) using Trojan horse liposomes (THLs) directed with a monoclonal antibody (MAb) to the rat TfR. Three assays of neurobehavior of rats were tested and terminal striatal TH enzyme activity was assessed in which the result clearly demonstrated that Apomorphine-induced contralateral rotation was reduced to 87%; amphetamine-induced ipsilateral rotation was reduced to 90% and whisker-induced forelimb placement abnormalities were reduced to 77% with THL gene therapy. Result showed complete inhibition of the neurotoxin effects accomplished by multiple intravenous dosing of GDNF plasmid DNA gene therapy, using receptor-targeted THLs [92]. Amino acids are biological products which are used by brain also and can easily cross BBB. Hence, they have been tested with liposomes for delivery of dopamine HCl to the brain exploiting RMT. Liposomes were coupled to glutamate stearylamine. *In vivo* studies revealed that dopamine HCl can be effectively delivered to brain by glutamate-coupled liposomes, and results evidently indicated the superiority of the coupled liposomal formulation over the uncoupled formulation [93]. L-DOPA (LD) which is widely used to treat PD is metabolized in peripheral nervous system to such an extent that it negates the effects of LD administration but if it can be so administered that before reaching central nervous system it does not encounter peripheral nervous system then it can be very beneficial in PD. Chlorotoxin [(CITx) (a 36-amino acid peptide)] modified stealth liposomes (CITx-LS) encapsulating LD produced such favorable effect. CITx modification highly facilitated the uptake of liposomes by brain microvascular endothelial cells *in vitro*. After intraperitoneal injection to mice, the formulation significantly increased the distribution of dopamine and dihydroxyphenyl acetic acid, the metabolites of LD, in the substantia nigra and striata. In the methyl-phenyl-tetrahydropyridine (MPTP)-induced PD mice model, LD-loaded CITx-LS significantly attenuated the behavioral disorders and reduced the MPTP-induced loss of tyrosine hydroxylase-positive dopaminergic neurons [94]. The clinical application of a dopamine receptor agonist-apomorphine, is limited for treating Parkinson's disease, by its instability and the need for frequent injections. But encapsulating it within liposomes to protect it from degradation and for enhanced permeability across the BBB proved to be a promising strategy for targeting apomorphine to the brain. Loading of apomorphine into liposomes slowed the release behavior compared to the drug in an aqueous solution. In comparison to free drug, apomorphine in PEGylated liposomes demonstrated greater stability in plasma. The *in vivo* brain uptake of PEGylated liposomes after an intravenous bolus injection into rats showed that the uptake of PEGylated liposomes into the brain was rapid and prolonged [95]. Prodrug approach has also been employed using liposomal technology. Targeting of prodrugs via liposomes for treatment of PD was achieved when maleic and fumaric diamides preparation of (O,O-diacetyl)-L-Dopa-methylester were given as a liposome formulation, to eliminate fluctuations of LD plasma levels and low bioavailability. It was found that prodrug was able to induce sustained delivery of dopamine (DA) in rat striatal dialysate respecting equimolar intraperitoneal (i.p.) administration of LD. Additionally; neostriatum DA concentration after administration of the synthesized prodrugs against prodrugs in liposomal formulations was compared. The results recommended that cis dimeric prodrug can improve the release of DA in rat brain [96].

Liposomal formulations offer advantages of enhanced delivery to target site along with dose reduction, thereby curtailing the concentration-related adverse effects. The delivery of amiloride hydrochloride (a generally used diuretic which recently has been found to be effective in the treatment of epilepsy) into the brain via liposomes was evaluated by Ali et al. (2007). The effect of free and liposomal entrapped amiloride was observed in male albino Swiss strain mice by the increasing current electroshock (ICES) test, pentylenetetrazole (PTZ)-induced seizures and pentylenetetrazole induced status epilepticus (SE). The studies revealed that there was improved anticonvulsant action with liposome-entrapped amiloride as compared to free amiloride in all three-seizure models. Furthermore, treatment with liposome-entrapped amiloride (0.35 mg/kg) brought out significant increase in seizure threshold and significantly extended the latency to myoclonic jerks and clonic generalized seizures as compared to liposome or free amiloride treated groups [97].

Treatment strategies for brain diseases using dendrimers: Ideally dendrimers are perfect mono-dispersed macromolecules with regular and remarkably branched three-dimensional architecture [98,99]. Novel properties of dendrimers subjected them for immense exploration among the researchers, which has led to their possible use in different pharmaceutical field like drug delivery [100-103], gene delivery [104-107], solubility enhancement [108], transfection [109] etc. Brain delivery of bioactive molecule for different brain diseases via dendrimers is very promising emerging field which has shown some very exiting results for treatment of these diseases. Polyether-copolyester (PEPE) dendrimers were evaluated as drug carriers for the treatment of gliomas by Dhanikula et al. in 2008. D-glucosamine was conjugated to these dendrimers as a ligand for enhancing BBB permeability and tumor targeting of methotrexate (MTX). The effectiveness of this bioconjugate against U87 MG and U 343 MGa cells brought out the results that glucosylated dendrimers were endocytosed in significantly elevated amounts than non-glucosylated dendrimers by both the cell lines. *In vitro* BBB model showed three to five times more amount of MTX across the BBB after loading in the dendrimers. Similar elevated activity was also found even in MTX-resistant cells [110]. MTX delivery to brain tumors cells has also been achieved via PAMAM dendrimers with the use of monoclonal antibody, Cetuximab [(which binds to endothelial growth factor receptor (EGFR) on tumors)]. The conjugate caused significant reduction in tumor volume of F98_{EGFR} gliomas [111]. 5-fluorouracil (5-FU) together with antisense-miR-21 oligonucleotide (as-miR-21) was targeted to brain tumor via PAMAM dendrimer. The study revealed that the cytotoxicity of 5-FU was improved significantly when co-delivered with as-miR-21 and there was dramatically increased apoptosis of U251 cells. Migration ability of the tumor cells was also decreased [112].

Boron neutron capture therapy (BNCT) is also being used to treat tumors. Studies were carried out to evaluate a boronated EGFRvIII (which is a mutant isoform of EGFR)-specific monoclonal antibody, L8A4, conjugated with PAMAM dendrimers for BNCT of the receptor-positive rat glioma (F98_{npEGFRvIII}; which is a mutant receptor). *In vivo* studies revealed that after 24 hr the amounts kept by receptor-positive gliomas were 60.1% following i.t. injection as compared to 14.6% ID/g by receptor-negative tumors [113]. Yang et al. (2009) have summarized the molecular targeting studies of EGFR or EGFRvIII. Rats with F98 rat gliomas [(expressing either wildtype (F98EGFR) or mutant receptors (F98npEGFRvIII)] were treated with intracerebral (either intratumoral or by CED) injection [(alone or in combination with i.v. boronophenylalanine (BPA)] of cetuximab (IMC-C225) and L8A4 (which recognize wildtype EGFR and EGFRvIII, respectively).

These monoclonal antibodies (mAbs) were heavily boronated using PAMAM dendrimers, previously. Following this, BNCT was initiated. It was found that the best survival data were obtained in rats bearing F98_{npEGFRvIII} gliomas that had received CED of BD-L8A4 either alone or in combination with i.v. boronophenylalanine (BPA). Furthermore, in the rats with composite tumors they found that EGFR targeting vehicles are useful, but not stand-alone boron delivery agents because of the heterogeneity of receptor expression in brain tumors [114].

The scientist community is considering RNA dependent gene silencing i.e. RNA interference (RNAi) as the most promising discovery of the decade because it has the potential to change the way in which diseases are treated currently. siRNA therapeutics is at the front position of the effort to create RNAi-based therapies. The intracellular delivery of these biological molecules is being examined continuously. Acetylated PAMAM dendrimers were assessed for the cellular delivery of siRNA to U87 malignant glioma cells. The complex of acetylated dendrimers was made with siRNA, and physical properties of the complexes were studied. Study pointed out that primary amine acetylation of PAMAM dendrimers reduced their cytotoxicity to U87 cells because of step forwarded release of siRNA from dendrimer/siRNA complexes. However, a small fraction (approximately 20%) of primary amines of PAMAM could be modified, which would not affect the delivery of siRNA from PAMAM, but higher extent of amine neutralization reduced the gene silencing efficiency of PAMAM/siRNA delivery vectors [115]. PAMAM dendrimers has also been used to deliver taxol with miR-21 (an oncogene) inhibitor. Delivery of miR-21 inhibitor (loaded with PAMAM dendrimers) with taxol to human glioblastoma U251 and LN229 cells revealed that IC₅₀ values were vividly decreased than those treated with taxol alone. Furthermore, the miR-21 inhibitor significantly enhanced apoptosis in both U251 cells and LN229 cells, and cell invasiveness was damaged [116].

Gajbhiye et al. (2011) developed polysorbate 80 (P80) anchored poly(propyleneimine) (PPI) dendritic nanoconjugate and evaluated targeting potential of anti-cancer drug, docetaxel (DTX) to the brain tumor [99]. *In vitro* cytotoxicity studies on U87MG human glioblastoma cell line suggested that free DTX and DTX loaded plain PPI dendrimers were more cytotoxic to the brain tumor cells as compared to DTX-P80-PPI dendrimers. This might be due to the direct contact of free DTX and DTX-PPI to the brain tumor cells. Conversely, the *in vivo* anti-cancer activity in brain tumor bearing rats revealed that DTX loaded P80 conjugated dendrimers reduced the tumor volume extremely significantly ($p < 0.0001$; more than 50%) as compared to free DTX and DTX-PPI. The median survival time for brain tumor bearing rats treated with DTX-P80-PPI dendrimers (42 days) was extended very significantly as compared to DTX-PPI (23 days; $p < 0.001$) and free DTX (18 days; $p < 0.001$). Higher BBB crossing by P80 anchoring might have furnished for enhanced delivery of DTX to tumor. However, free DTX and DTX-PPI fails to cross BBB as compared to DTX-P80-PPI. Gamma scintigraphy and biodistribution studies further confirmed the targeting efficiency and higher biodistribution of ligand conjugated dendrimer into the brain. The results concluded that the developed nanoconjugate has potential to deliver significantly higher amount of drug to brain tumor for improved therapeutic outcome [99]. Patel et al. (2012) explored use of thiamine (Tm) conjugated PPI dendrimers for enhanced delivery of PTX across BBB. Cytotoxicity studies of free PTX, PTX-PPI and PTX-Tm-PPI dendrimers on IMR-32 human neuroblastoma cells demonstrated higher cytotoxicity of PTX-Tm-PPI than plain PTX and PTX-PPI. Biodistribution studies in Sprague Dawley strain further confirmed higher targeting potential of PTX-Tm-PPI into the brain than free PTX and PTX-PPI [117]. Wang et

al. (2014) developed iRGD (Internalizing RGD) conjugated PEG-PAMAM-cis-aconityl-DOX (iRGD-PPCD) conjugates for increased tumor penetration using neuropilin-1 receptors. C6 glioma spheroid penetration studies showed that iRGD functionalized conjugates demonstrated higher penetrating ability as compared to cyclic RGD anchored conjugates. The median survival time for iRGD and RGD functionalized dendritic conjugates in tumor induced animals were 57.5 and 43.5 days respectively. The result indicated that iRGD mediated targeting can significantly improve therapeutic outcome [118].

Amongst the novel carriers dendrimers are gaining attraction as a possible carrier of drugs for the treatment of AD. Branched polyamines shown to purge prion protein PrP^{Sc} and it was hypothesized that these systems may be useful for other neurodegenerative diseases also [119]. A-beta binds with relatively higher affinity to clustered sialic acid residues on cell surfaces and removal of these cell surface sialic acids may weaken A-beta toxicity. Influenced by this finding Patel et al. (2006) framed sialic acid conjugated dendrimeric polymers and assessed the ability of these sialic acid conjugated dendrimers to prohibit A-beta toxicity. After the analysis of viability of neuroblastoma cells and analysis of the effects of soluble and clustered sialic acid mimics on A-beta cell toxicity, it was concluded that attenuation of A-beta induced toxicity by soluble sialic acid was effectual only at high sialic acid concentrations and low A-beta concentration. On the contrary the sialic acid conjugated dendrimeric polymers were able to attenuate A-beta toxicity at micromolar concentrations, or almost three orders of magnitude lower concentrations than the soluble sialic acid. Furthermore, they also found that the toxicity prevention properties of the sialic acid modified dendrimers were a function of dendrimer size [120].

Dendrimers have been thought to interact with amyloid proteins and studies have also been carried out to find this interaction to attenuate the formation of these amyloid proteins by dendrimers. The effect of polypropyleneimine (PPI) dendrimers on the formation of amyloid fibrils as a function of pH was evaluated in order to gain insight in the aggregation mechanism and its inhibition. Amyloid fibrils from prion peptide PrP and Alzheimer's peptide A-beta were produced *in vitro*, and their formation was observed using the dye thioflavin T whose fluorescence is dependent on the formation of amyloid aggregates. The results unveiled that the level of protonation (and hence pH) of Histidine, Glutamic acid, and Aspartic acid residues is crucial for the final effect, especially at low dendrimer concentration when their inhibiting capacity depends on the pH. At the highest concentrations, dendrimers were most effective against fibril formations for both Prion and Alzheimer's peptides [121]. In the same context, to detect the influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases Klajnert et al. used Thioflavin T dye on the formation of amyloid aggregates, for A-beta and Prp in the absence and presence of heparin at pH 5.5. The presence of heparin in the medium (0.041 mg/ml) at pH 5.5 shortened the lag time of the aggregation process. However, the presence of the PAMAM 3.0G dendrimer (5 μM) entirely inhibited aggregation in the monitored time interval. Furthermore, the effect of dendrimer concentration was checked in the presence of heparin for both the peptides. The observed fluorescence changes revealed that at certain low concentrations of dendrimers the aggregation process was expedited whereas higher concentrations do decelerated it down. The final concentration (fluorescence) of A-beta aggregates not seemed to be considerably affected by the presence of dendrimers, but these dendrimers clearly lowered it in the case of PrP [122].

These researchers further studied the influence of dendrimer's structure on its activity against amyloid fibril formation. They employed the third, fourth, and fifth generation of polyamidoamine dendrimers (PAMAM G 3.0, PAMAM G 4.0, and PAMAM G 5.0) with the purpose to study how dendrimers structure and size determine their effect on amyloid formation. As a continuation of their preceding studies they choose Alzheimer's peptide Ab 1-28 and a segment of prion protein PrP 185-208. In the case of Ab 1-28 the duration of the nucleation reaction [formation of non-fibrillar structures (nuclei) by proteins] did not change much, compared to the control, in the presence of PAMAM dendrimers. At low dendrimer concentration (0.01 μmol/l) there was no effect on the elongation rate (formation of fibrillar β-sheet structures from nuclei). PAMAM G 3.0 dendrimers, however clearly slowed down the elongation reaction when present at 0.1 and 1 μmol/l, as well as significantly lessened the amount of final fibrils formed. The effect of PAMAM G 4.0, and PAMAM G 5.0 at 0.1 μmol/l on the elongation rate was much less marked than for PAMAM G 3.0, although at 1 μmol PAMAM G 4.0 and PAMAM G 5.0 entirely inhibited the formation of fibers in the examined time interval. Thus they concluded that low generation PAMAM dendrimers had a clear influence on the elongation rate of Ab 1-28 aggregation [an effect which was less manifested for higher generations (4.0 and 5.0)]. However, for all generations studied there was a clear effect on the final concentration of fibrils: the higher the concentration of dendrimer and the higher the generation, the lesser the amount of fibrils formed [123].

Alpha-synuclein is a protein which is observed in the neurons in PD and its intracellular transfer is thought to be involved in the spread of PD to more and more neurons. Rekas et al. (2009) [124] observed the effect of PAMAM dendrimers (generations 3.0, 4.0 and 5.0) on the fibrillation of alpha-synuclein and found that PAMAM dendrimers suppressed the fibrillation and this effect was increased with both PAMAM concentration and generation number. They also found that as the PAMAM concentration increased there were structural changes in the formed aggregates of alpha-synuclein from cylindrical to dense three-dimensional ones. PAMAM also effectively advanced the breaking down of pre-existing fibrils of alpha-synuclein.

Dendrimers have the potential to be used as a versatile carrier for drug delivery and non-viral vector for gene therapy against HIV infection [125,126]. Gonzalo et al. (2010) examined the ability of dendrimer 2G-[Si{O(CH₂)₂N(Me)₂⁺(CH₂)₂NMe₃⁺(I₂)₂}]₈ (NN16) to transfect a wide range of cell types, as well as the possible biomedical use in direct or indirect suppression of HIV replication. They observed cells implicated in HIV infection such as primary peripheral blood mononuclear cells (PBMC) and immortalized suspension cells (lymphocytes), primary macrophages and immortalized adherent cells (astrocytes and trophoblasts) and dendritic cells. Dendrimers were used to deliver antisense oligonucleotides and small interfering RNA (siRNA) and their transfection ability and gene knockdown was inspected in this study. Imaging of cellular intake illustrated high transfection efficiency of genetic material in all cells examined. The dendrimers complexed with siRNA displayed therapeutic potential by distinctively inhibiting cyclooxygenase-2 gene expression in HIV-infected nervous system cells [126]. Recently, Serramia et al. (2015) studied the potential of carbosilane dendrimers for delivery of siRNA against HIV-1 Nef in human primary astrocytes to interfere HIV-1 infectivity. The result demonstrated high Nef silencing with reducing HIV-1 infectivity in dendriplexes treated astrocytes than control or siRandom treated astrocytes. Biodistribution studies in BALB/c mice showed that dendrimer were able to transport siRNA into the brain [127].

Conclusion

The concept of active targeting was started in 1978. Till now this is the most advanced technology applied for the maintenance of the brain diseases in which invasive approaches can be minimized. Studies included in this review clarifies that the nanocarriers used for brain targeting, after proper modification of the surface can easily penetrate through blood capillary endothelial cells. Furthermore, conjugation of the endogenous or exogenous site-specific ligands to these nanocarriers can navigate these systems across BBB, resulting in the augmented drug concentration in the brain. Thus, they can reduce the dose and ultimately adverse effects of the bioactive molecules. However, these systems need to be further tested for many aspects particularly safety and efficacy before their clinical use. A lots of diseases related to the brain can be benefited from the use of these nanocarriers but among the disease discussed above the main emphasis till now is on brain tumors; and diseases like PD, epilepsy and AIDS are far behind in the queue and need more attention in the future. Literature also suggest that the main emphasis has been given to cross the BBB and not to target the bioactive molecule to the specific cells in the brain. The cellular level brain targeting (targeting to specific cells responsible for brain ailments) may be most expanding horizon in the future of brain targeting meadow.

References

- Nolte J (2002) *The Human Brain: An Introduction to its functional anatomy*. (2ndEdn) Mosby Inc, St. Louis, MO.
- Alzheimer's Association (2008) 2008 Alzheimer's disease facts and figures. *Alzheimers Dement* 4: 110-133.
- Ballabh P, Braun A, Nedergaard M (2004) The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 16: 1-13.
- Gururangan S, Friedman HS (2002) Innovations in design and delivery of chemotherapy for brain tumors. *Neuroimaging Clin N Am* 12: 583-597.
- Minagar A, Jy W, Jimenez JJ, Alexander JS (2006) Multiple sclerosis as a vascular disease. *Neurol Res* 28: 230-235.
- Banks WA (2009) Characteristics of compounds that cross the blood-brain barrier. *BMC Neurol* 9 Suppl 1: S3.
- Gabathuler R (2010) Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. *Neurobiol Dis* 37: 48-57.
- Begley DJ, Brightman MW (2003) Structural and functional aspects of the blood-brain barrier. *Prog Drug Res* 61: 39-78.
- Engelhardt B, Sorokin L (2009) The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Semin Immunopathol* 31: 497-511.
- Nau R, Sorgel F, Eiffert H (2010) Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin Microbiol Rev* 23: 858-883.
- Sun H, Dai H, Shaik N, Elmquist WF (2003) Drug efflux transporters in the CNS. *Adv Drug Deliv Rev* 55: 83-105.
- Mikitsch JL, ChackoAM (2014) Pathways for small molecule delivery to the central nervous system across the blood-brain barrier. *Perspect Medicin Chem* 6: 11-24.
- Loscher W, Potschka H (2005) Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *Neuro Rx* 2: 86-98.
- Idriss HT, Hannun YA, Boulpaep E, Basavappa S (2000) Regulation of volume-activated chloride channels by P-glycoprotein: phosphorylation has the final say. *J Physiol* 524: 629-636.
- Loscher W, Potschka H (2005) Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol* 76: 22-76.
- Zhang L, Sheng R, Qin Z (2009) The lysosome and neurodegenerative diseases. *ActaBiochimBiophys Sin (Shanghai)* 41: 437-445.
- Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, et al. (1994) Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci USA* 91: 2076-2080.
- Fung LK, Ewend MG, Sills A, Sipos EP, Thompson R, et al. (1998) Pharmacokinetics of interstitial delivery of carmustine, 4-hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer implant in the monkey brain. *Cancer Res* 58: 672-684.
- Pardridge WM (2007) Blood-brain barrier delivery. *Drug Discov Today* 12: 54-61.
- Rapoport SI (2000) Osmotic opening of the blood-brain barrier: principles, mechanism, and therapeutic applications. *Cell Mol Neurobiol* 20: 217-230.
- Begley DJ (2004) Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. *Pharmacol Ther* 104: 29-45.
- Bortongan CV, Emerich DF (2003) Facilitation of drug entry into the CNS via transient permeation of blood brain barrier: laboratory and preliminary clinical evidence from bradykinin receptor agonist, Cereport. *Brain Res Bull* 60: 297-306.
- Black KL, Ningaraj NS (2004) Modulation of brain tumor capillaries for enhanced drug delivery selectively to brain tumor. *Cancer Control* 11: 165-173.
- Hynynen K (2008) Ultrasound for drug and gene delivery to the brain. *Adv Drug Deliv Rev* 60: 1209-1217.
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46: 3-26.
- Brewster ME, Raghavan K, Pop E, Bodor N (1994) Enhanced delivery of ganciclovir to the brain through the use of redox targeting. *Antimicrob Agents Chemother* 38: 817-823.
- Bradley MO, Webb NL, Anthony FH, Devanesan P, Witman PA, et al. (2001) Tumor targeting by covalent conjugation of a natural fatty acid to paclitaxel. *Clin Cancer Res* 7: 3229-3238.
- Stenehjem DD, Hartz AM, Bauer B, Anderson GW (2009) Novel and emerging strategies in drug delivery for overcoming the blood-brain barrier. *Future Med Chem* 1: 1623-1641.
- Bhaskar S, Tian F, Stoeger T, Kreyling W, Fuente JM, et al. (2010) Multifunctional Nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. *Particle Fibre Toxicol* 7:3.
- Cornford EM, Young D, Paxton JW, Finlay GJ, Wilson WR, et al. (1992) Melphalan penetration of the blood-brain barrier via the neutral amino acid transporter in tumor-bearing brain. *Cancer Res* 52: 138-143.
- Tiwari AK, Gajbhiye V, Sharma R, Jain NK (2010) Carrier mediated protein and peptide stabilization. *Drug Deliv* 17: 605-616.
- Gajbhiye V, Jain NK (2011) Novel carriers for controlled site specific delivery of anti-inflammatory agents. *Anti-Inflamm Anti-Allergy Age Med Chem* 10: 166-179.
- Beduneau A, Saulnier P, Benoit JP (2007) Active targeting of brain tumors using nanocarriers. *Biomaterials* 28: 4947-4967.
- Tzeng SY, Green JJ (2013) Therapeutic nanomedicine for brain cancer. *Ther Deliv* 4: 687-704.
- Athira VS, Dhas AJ (2015) A survey on detection and segmentation of brain tumors in MR images. *Int J Eng Res* 3: 32-36.
- Armstrong TS, Mendoza T, Gning I, Coco C, Cohen MZ, et al. (2006) Validation of the M.D. Anderson Symptom Inventory Brain Tumor Module (MDASI-BT). *J Neurooncol* 80: 27-35.
- Behin A, Hoang-Xuan K, Carpentier AF, Delattre JY (2003) Primary brain tumours in adults. *Lancet* 361: 323-331.
- van der Flier WM, Scheltens P (2005) Epidemiology and risk factors of dementia. *J Neurol Neurosurg Psychiatry* 76 Suppl 5: v2-7.
- Souchay C, Moulin CJ (2009) Memory and consciousness in Alzheimer's disease. *Curr Alzheimer Res* 6: 186-195.
- Brickman AM, Small SA, Fleisher A (2009) Pinpointing synaptic loss caused by Alzheimer's disease with fMRI. *Behav Neurol* 21: 93-100.
- Selkoe DJ (1996) Amyloid beta-protein and the genetics of Alzheimer's disease. *J Biol Chem* 271: 18295-18298.

42. Dovey HF, John V, Anderson JP, Chen LZ, de Saint Andrieu P, et al. (2001) Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. *J Neurochem* 76: 173-181.
43. Parkinson J (2002) An essay on the shaking palsy. 1817. *J Neuropsychiatry Clin Neurosci* 14: 223-236.
44. Granado N, Ares-Santos S, Moratalla R (2013) Methamphetamine and Parkinson's disease. *Parkinsons Dis* 2013: 308052.
45. Bohnen NI, Albin RL (2011) The cholinergic system and Parkinson disease. *Behav Brain Res* 221: 564-573.
46. Samii A, Nutt JG, Ransom BR (2004) Parkinson's disease. *Lancet* 363: 1783-1793.
47. Mc Corry D, Chadwick D, Marson A (2004) Current drug treatment of epilepsy in adults. *Lancet Neurol* 3: 729-735.
48. Aroniadou-Anderjaska V, Fritsch B, Qashu F, Braga MF (2008) Pathology and pathophysiology of the amygdala in epileptogenesis and epilepsy. *Epilepsy Res* 78: 102-116.
49. Motamedi G, Meador K (2003) Epilepsy and cognition. *Epilep Behav* 4: 25-38.
50. Tripathi KD (2013) Essentials of medical pharmacology (7thEdn). Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, India.
51. Jokeit H, Ebner A (2002) Effects of chronic epilepsy on intellectual functions. *Prog Brain Res* 135: 455-463.
52. Freed EO (2001) HIV-1 replication. *Somat Cell Mol Genet* 26: 13-33.
53. Banks WA, Freed EO, Wolf KM, Robinson SM, Franko M, et al. (2001) Transport of human immunodeficiency virus type 1 pseudoviruses across the blood-brain barrier: role of envelope proteins and adsorptive endocytosis. *J Virol* 75: 4681-4691.
54. Valcour V, Paul R (2006) HIV infection and dementia in older adults. *Clin Infect Dis* 42: 1449-1454.
55. McArthur JC (2004) HIV dementia: an evolving disease. *J Neuroimmunol* 157: 3-10.
56. Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, et al. (2007) Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 69: 1789-1799.
57. Ghafouri M1, Amini S, Khalili K, Sawaya BE (2006) HIV-1 associated dementia: symptoms and causes. See comment in PubMed Commons below *Retrovirology* 3: 28.
58. Shah N, Chaudhari K, Dantuluri P, Murthy RS, Das S (2009) Paclitaxel-loaded PLGA nanoparticles surface modified with transferrin and Pluronic((R))P85, an in vitro cell line and in vivo biodistribution studies on rat model. *J Drug Target* 17: 533-542.
59. Geldenhuys W, Mbimba T, Bui T, Harrison K, Sutariya V (2011) Brain-targeted delivery of paclitaxel using glutathione-coated nanoparticles for brain cancers. *J Drug Target* 19: 837-845.
60. De Juan BS, Von Briesen H, Gelperina SE, Kreuter J (2006) Cytotoxicity of doxorubicin bound to poly(butyl cyanoacrylate) nanoparticles in rat glioma cell lines using different assays. *J Drug Target* 14: 614-622.
61. Kreuter J, Gelperina S (2008) Use of nanoparticles for cerebral cancer. *Tumori* 94: 271-277.
62. Chertok B, David AE, Huang Y, Yang VC (2007) Glioma selectivity of magnetically targeted nanoparticles: a role of abnormal tumor hydrodynamics. *J Control Release* 122: 315-323.
63. Meng XX, Wan JQ, Jing M, Zhao SG, Cai W, et al. (2007) Specific targeting of gliomas with multifunctional superparamagnetic iron oxide nanoparticle optical and magnetic resonance imaging contrast agents. *ActaPharmacolSinica* 28: 2019-2026.
64. Jordan A, Scholz R, Maier-Hauff K, van Landeghem FK, Waldoefer N, et al. (2006) The effect of thermotherapy using magnetic nanoparticles on rat malignant glioma. *J Neurooncol* 78: 7-14.
65. Brioschi AM, Calderoni S, Pradotto LG, Guido M, Strada A, et al. (2009) Solid lipid nanoparticles carrying oligonucleotides inhibit vascular endothelial growth factor expression in rat glioma models. *J Nanoneurosci* 1: 65-74.
66. Wilson B, Samanta MK, Santhi K, Kumara KPS, Paramakrishnan N, et al. (2008) Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease. *Brain Res* 1200: 159-168.
67. Treiber C, Quadir MA, Voigt P, Radowski M, Xu S, et al. (2009) Cellular copper import by nanocarrier systems, intracellular availability, and effects on amyloid beta peptide secretion. *Biochem* 48: 4273-4284.
68. Picone P, Bondi ML, Montana G, Bruno A, Pitarresi G, et al. (2009) Ferulic acid inhibits oxidative stress and cell death induced by Ab oligomers: improved delivery by solid lipid nanoparticles. *Free Radic Res* 43: 1133-1145.
69. Shea TB, Ortiz D, Nicolosi RJ, Kumar R, Watterson AC (2005) Nanosphere-mediated delivery of vitamin E increases its efficacy against oxidative stress resulting from exposure to amyloid beta. *J Alzheimers Dis* 7: 297-301.
70. Songjiang Z, Lixiang W (2009) Amyloid-beta associated with chitosan nanocarrier has favorable immunogenicity and permeates the BBB. *AAPS Pharm Sci Tech* 10: 900-905.
71. Huang R, Han L, Li J, Ren F, Ke W, et al. (2009) Neuroprotection in a 6-hydroxydopamine-lesioned Parkinson model using lactoferrin-modified nanoparticles. *J Gene Med* 11: 754-763.
72. Huang R, Ke W, Liu Y, Wu D, Feng L, et al. (2010) Gene therapy using lactoferrin-modified nanoparticles in a rotenone-induced chronic Parkinson model. *J NeuroSci* 290: 123-130.
73. Carroll RT, Bhatia D, Geldenhuys W, Bhatia R, Miladore N, et al. (2010) Brain-targeted delivery of Tempol-loaded nanoparticles for neurological disorders. *J Drug Target* 18: 665-674.
74. Kubek MJ, Domb AJ, Veronesi MC (2009) Attenuation of kindled seizures by intranasal delivery of neuropeptide-loaded nanoparticles. *Neurotherapeutics* 6: 359-371.
75. Veronesi MC, Aldouby Y, Domb AJ, Kubek MJ (2009) Thyrotropin-releasing hormone D,L polylactide nanoparticles (TRH-NPs) protect against glutamate toxicity in vitro and kindling development in vivo. *Brain Res* 1303: 151-160.
76. Wanga S, Jiang T, Ma M, Hu Y, Zhang J (2010) Preparation and evaluation of anti-neuroexcitation peptide (ANEP) loaded N-trimethyl chitosan chloride nanoparticles for brain-targeting. *Int J Pharm* 386: 249-255.
77. Akhtari M, Bragin A, Cohen M, Moats R, Brenker F, et al. (2008) Functionalized magnetonanoparticles for MRI diagnosis and localization in epilepsy. *Epilepsia* 49: 1419-1430.
78. Kuo YC, Chen HH (2006) Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood-brain barrier. *Int J Pharm* 327: 160-169.
79. Kuo YC, Su FL (2007) Transport of stavudine, delavirdine, and saquinavir across the blood-brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate, and solid lipid nanoparticles. *Int J Pharm* 340: 143-152.
80. Chattopadhyay N, Zastre J, Wong HL, Wu XY, Bendayan R (2008) Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line. *Pharm Res* 25: 2262-2271.
81. Rao KS, Reddy MK, Horning JL, Labhasetwar V (2008) TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. *Biomaterials* 29: 4429-4438.
82. Mishra V, Mahor S, Rawat A, Gupta PN, Dubey P, et al. (2006) Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles. *J Drug Target* 14: 45-53.
83. Al-Ghananeem AM, Saeed H, Florence R, Yokel RA, Malkawi AH (2010) Intranasal drug delivery of didanosine-loaded chitosan nanoparticles for brain targeting; an attractive route against infections caused by AIDS viruses. *J Drug Target* 18: 381-388.
84. Shao K, Hou Q, Duan W, Go ML, Wong KP, et al. (2006) Intracellular drug delivery by sulfatide-mediated liposomes to gliomas. *J Control Release* 115: 150-157.
85. Madhankumar AB, Slagle-Webb B, Wang X, Yang QX, Antonetti DA, et al. (2009) Efficacy of interleukin-13 receptor-targeted liposomal doxorubicin in the intracranial brain tumor model. *Mol Cancer Ther* 8: 648-654.
86. Du J, Lu W, Ying X, Liu W, Du P, et al. (2009) Dual-Targeting topotecan liposomes modified with tamoxifen and wheat germ agglutinin significantly

- improve drug transport across the blood-brain barrier and survival of brain tumor-bearing animals. *Mol Pharm* 6: 905-917.
87. Ying X, Wen H, Lu WL, Du J, Guo J, et al. (2010) Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma in animals. *J Control Release* 141: 183-192.
88. Arumugam K, Subramanian GS, Mallayasamy SR, Averineni RK, Reddy MS, et al. (2008) A study of rivastigmine liposomes for delivery into the brain through intranasal route. *Acta Pharm* 58: 287-297.
89. Phachonpai W, Wattanathorn J, Muchimapura S, Tong-Un T, Preechagoon D (2010) Neuroprotective effect of quercetin encapsulated liposomes: a novel therapeutic strategy against alzheimer's disease. *Am J App Sci* 7: 480-485.
90. Muhs A, Hickman DT, Pihlgren M, Chuard N, Giriens V, et al. (2007) Liposomal vaccines with conformation-specific amyloid peptide antigens define immune response and efficacy in APP transgenic mice. *ProcNatAcadSci U S A* 104: 9810-9815.
91. Pardridge WM (2005) Tyrosine hydroxylase replacement in experimental Parkinson's disease with transvascular gene therapy. *Neuro Rx* 2: 129-138.
92. Zhang Y, Pardridge WM (2009) Near complete rescue of experimental Parkinson's disease with intravenous, non-viral GDNF gene therapy. *Pharm Res* 26: 1059-1063.
93. Khare P, Jain A, Jain NK, Soni V, Jain SK (2009) Glutamate-conjugated liposomes of dopamine hydrochloride for effective management of parkinsonism's. *PDA J Pharm Sci Technol* 63: 372-379.
94. Xiang Y, Wu Q, Liang L, Wang X, Wang J, et al. (2012) Chlorotoxin-modified stealth liposomes encapsulating levodopa for the targeting delivery against Parkinson's disease in the MPTP-induced mice model. *J Drug Target* 20: 67-75.
95. Hsu SA, Al-Suwayeh S, Chen C, Chi C, Fang J (2011) PEGylated liposomes incorporated with nonionic surfactants as an apomorphine delivery system targeting the brain: in vitro release and in vivo real-time imaging. *CurrNanosci* 7: 191-199.
96. Di Stefano A, Sozio P, Iannitelli A, Marianecchi C, Santucci E, et al. (2006) Maleic- and fumaric-diamides of (O,O-diacetyl)-L-Dopa-methylester as anti-Parkinson prodrugs in liposomal formulation. *J Drug Target* 14: 652-661.
97. Ali A, Kolappa Pillai K, Jalees Ahmad F, Dua Y, Iqbal Khan Z, et al. (2007) Comparative efficacy of liposome-entrapped amiloride and free amiloride in animal models of seizures and serum potassium in mice. *Eur Neuropsychopharmacol* 17: 227-229.
98. Gajbhiye V, Kumar PV, Tekade RK, Jain NK (2007) Pharmaceutical and biomedical potential of PEGylated dendrimers. *Curr Pharm Design* 13: 415-429
99. Gajbhiye V, Jain NK (2011) The treatment of Glioblastoma Xenografts by surfactant conjugated dendritic nanoconjugates. *Biomaterials* 32: 6213-6225.
100. Gajbhiye V, Kumar PV, Sharma A, Jain NK (2008) Novel PEGylated PPI dendritic nanostructures for sustained delivery of anti-inflammatory agent. *Curr Nanosci* 4: 267-277.
101. Gajbhiye V, Kumar PV, Sharma A, Agarwal A, Asthana A, et al. (2008) Dendritic nanoarchitectures mediated transdermal and oral delivery of bioactives. *Indian J Pharm Sci* 70: 431-439.
102. Kurmi BD, Gajbhiye V, Kayat J, Jain NK (2011) Lactoferrin-conjugated dendritic nanoconstructs for lung targeting of methotrexate. *J Pharm Sci* 100: 2311-2320.
103. Gajbhiye V, Escalante L, Chen G, Laperle A, Zheng Q, et al. (2014) Drug-loaded nanoparticles induce gene expression in human pluripotent stem cell derivatives. *Nanoscale* 6: 521-531.
104. Gajbhiye V, Gong S (2013) Lectin functionalized nanocarriers for gene delivery. *Biotechnol Adv* 31: 552-562.
105. Somani S, Blatchford DR, Millington O, Stevenson ML, Dufes C (2014) Transferrin-bearing polypropyleniminedendrimer for targeted gene delivery to the brain. *J Control Release* 188: 78-86.
106. Dutta T, Aghase HP, Vijayarajkumar P, Jain NK (2006) Dendrosome-based gene delivery. *J ExpNanosci* 1: 235-248.
107. Nishiyama N, Iriyama A, Jang WD, Miyata K, Itaka K, et al. (2005) Light-induced gene transfer from packaged DNA enveloped in a dendritic photosensitizer. *Nat Mater* 4: 934-941.
108. Sideratou Z, Tsiourvas D, Paleos CM (2001) Solubilization and release properties of PEGylated diaminobutane poly(propylene imine) dendrimers. *J Colloid Int Sci* 242: 272-276.
109. Kesharwani P, Gajbhiye V, Jain NK (2012) A review of nanocarriers for the delivery of small interfering RNA. *Biomaterials* 33: 7138-7150.
110. Dhanikula RS, Argaw A, Bouchard JF, Hildgen P (2008) Methotrexate loaded polyether-copolyester dendrimers for the treatment of gliomas: enhanced efficacy and intratumoral transport capability. *Mol Pharm* 5: 105-116.
111. Wu G, Barth RF, Yang W, Kawabata S, Zhang L, et al. (2006) Targeted delivery of methotrexate to epidermal growth factor receptor-positive brain tumors by means of cetuximab (IMC-C225) dendrimer bio conjugates. *Mol Cancer Ther* 5: 52-59.
112. Ren Y, Kang CS, Yuan XB, Zhou X, Xu P, et al. (2010) Co-delivery of as-miR-21 and 5-FU by poly(amidoamine) dendrimer attenuates human glioma cell growth in vitro. *J BiomaterSciPolym Ed* 21: 303-314.
113. Yang W, Barth RF, Wu G, Kawabata S, Sferra TJ, et al. (2006) Molecular targeting and treatment of EGFRvIII-positive gliomas using boronated monoclonal antibody L8A4. *Clin Cancer Res* 12: 3792-3802.
114. Yang W, Barth RF, Wu G, Tjarks W, Binns P, et al. (2009) Boron neutron capture therapy of EGFR or EGFRvIII positive gliomas using either boronated monoclonal antibodies or epidermal growth factor as molecular targeting agents. *Appl Radiat Isot* 67: S328-S331.
115. Waite CL, Sparks SM, Uhrich KE, Roth CM (2009) Acetylation of PAMAM dendrimers for cellular delivery of siRNA. *BMC Biotechnol* 9: 38.
116. Ren Y, Kang CS, Yuan XB, Han L, Wang GX, et al. (2010) MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. *BMC Cancer* 10: 27.
117. Patel SK, Gajbhiye V, Jain NK (2012) Synthesis, characterization and brain targeting potential of paclitaxel loaded thiamine-PPI nanoconjugates. *J Drug Target* 20: 841-849.
118. Wang K, Zhang X, Liu Y, Liu C, Jiang B, et al. (2014) Tumor penetrability and anti-angiogenesis using iRGD-mediated delivery of doxorubicin-polymer conjugates. *Biomaterials* 35: 8735-8747.
119. Supattapone S, Nguyen HO, Cohen FE, Prusiner SB, Scott MR (1999) Elimination of prions by branched polyamines and implications for therapeutics. *Proc Natl Acad Sci U S A* 96: 14529-14534.
120. Patel D, Henry J, Good T (2006) Attenuation of beta-amyloid induced toxicity by sialic acid-conjugated dendritic polymers. *Biochim Biophys Acta* 1760: 1802-1809.
121. Klajnert B, Cladera J, Bryszewska M (2006) Molecular interactions of dendrimers with amyloid peptides: pH dependence. See comment in PubMed Commons below *Biomacromolecules* 7: 2186-2191.
122. Klajnert B, Cortijo-Arellano M, Bryszewska M, Cladera J (2006) Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases. *Biochem Biophys Res Commun* 339: 577-582.
123. Klajnert B, Cortijo-Arellano M, Cladera J, Bryszewska M (2006) Influence of dendrimer's structure on its activity against amyloid fibril formation. *Biochem Biophys Res Commun* 345: 21-28.
124. Rekas A, Lo V, Gadd GE, Cappai R, Yun SI (2009) PAMAM dendrimers as potential agents against fibrillation of alpha-synuclein, a Parkinson's disease-related protein. *Macromol Biosci* 9: 230-238.
125. Gajbhiye V, Ganesh N, Barve J, Jain NK (2013) Synthesis, characterization and targeting potential of zidovudine loaded sialic acid conjugated-mannosylated poly(propyleneimine) dendrimers. *Eur J Pharm Sci* 48: 668-679.
126. Gonzalo T, Clemente MI, Chonco L, Weber ND, Iaz L, et al. (2010) Gene therapy in HIV-infected cells to decrease viral impact by using an alternative delivery method. *Chem Med Chem* 5: 921-929.
127. Serramia MJ, Alvarez S, Fuentes-Paniagua E, Clemente MI, Sanchez-Nieves J, et al. (2015) In vivo delivery of siRNA to the brain by carbosilane dendrimer. *J Control Release* 200: 60-70.