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Review Article

# AN UPDATED REVIEW ON THE APPLICATION OF DENDRIMERS AS SUCCESSFUL NANOCARRIERS FOR BRAIN DELIVERY OF THERAPEUTIC MOIETIES

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**ABSTRACT**

It's been nearly 100 y of effort to study the organization and role of the blood brain-barrier and still, we strive to find better techniques to overcome this barrier to deliver the drugs to the brain effectively with reduced systemic side effects. The advances in nanotechnology have given newer horizons in achieving this goal since the nano-scaled systems can modify an existing drug to have a high degree of sensitivity to the physiological conditions and specificity to reach the target organ. Among the various nanocarriers, dendrimers owing to their unique physical and chemical characteristics, represent a potential therapeutic tool in biomedical and pharmaceutical science. Dendrimers, an established polymeric nanocarrier system of the time, can deliver both drugs and genetic material and are being extensively studied to target the brain. The surface modification of dendrimers can reduce their innate toxicity problems and increase the therapeutic efficacy of brain disorders. This review article is an attempt to update on the potential of dendrimers explored in the past five years as a drug delivery avenue that can be considered as a promising solution in the management of a wide range of disorders affecting the central nervous system, including neoplastic, degenerative, and ischemic conditions. The following search criteria were used to expand the review article with the keywords dendrimers, novel drug delivery, nanoparticles, site-specific drug delivery etc.

**Keywords:** Brain disorders, Blood-brain barrier, Nanotechnology, Dendrimers, Targeted delivery


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## INTRODUCTION

The diseases affecting the brain are numerous, including Alzheimer's disease, Huntington's disease, Parkinson's disease, head trauma, amyotrophic lateral sclerosis (ALS), brain cancer, epilepsy, and stroke. The prevalence of these diseases is increasing at an alarming rate. It accounts for 11% of the total world population, which represents over 1.5 billion people globally. The threat worsens as the global burden of CNS disorder is expected to increase to 14.7% by 2020 with the change in population demographics [1].

The treatment of brain disorders persists as a daunting challenge amidst the advances in medical technology because of the natural barrier in the brain. The barrier exists at three interfaces namely the blood-brain barrier, which occurs at the blood vessels of the brain, the blood-cerebrospinal fluid barrier at the choroid plexus, and the blood arachnoid layer of the meninges. These barriers are absolutely a boon to keep the brain and nervous system healthy by strictly prohibiting the entry of any unwanted molecules but a bane when it comes to preventing the therapeutic agents to enter the brain and act in favor of treating the ailment [2].

Nanotechnology offers great promise for revolutionizing medical imaging, diagnostics, and therapeutics [3]. The design and development of innovative nanocarriers made of lipids or polymers as drug delivery systems have been the main input of nanotechnology in pharmaceutics, which helps to carry the drug introduced through various invasive or noninvasive routes in the body in a controlled manner from the site of administration to the therapeutic target [4].

The various nanocarriers used for CNS delivery include polymeric nanoparticles, Solid Lipid Nanoparticles, liposomes, micelles, nanoemulsions, nanogels, nanosuspensions, dendrimers, Carbon Nanotubes and fullerenes. Among them, dendrimers owing to their unique characteristics represent a potential therapeutic tool in biomedical and pharmaceutical science. Low dispersity, high functionality, high penetration ability, high density, and peripheral functional group reactivity are some of the featured advantages [5]. All features of the dendrimer nanoparticle are modifiable for delivery purposes, including the core, the branching pattern, the surface characteristics, and the binding and releasing characteristics to target ligands [6]. This review will mainly focus on the recent therapeutic advances using dendrimers for brain delivery of drugs and nucleic acids for the various CNS disorders.

### Challenges of drug delivery to the brain

The brain is a highly sensitive organ with a well-defined neuronal homeostatic environment, which is crucial for maintaining the functionality of the whole body. The brain barriers, including the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (B-CSFB) along with its neurovascular unit (NVU) protect the brain from potentially harmful blood-borne compounds. Like any other chemical entity, the drug molecules are also foreign substances for these barriers and hence prevent its entry into the brain tissues, which makes the treatment of CNS disorders an unresolvable puzzle despite the enormous advances in CNS research [7].

The factors affecting the restricted entry of the drug moieties into the brain can be classified into two.

1. The anatomical features of the brain barriers.

2. The drug-related properties and metabolic interactions in the body [8].

### Brain barriers

The BBB is an inimitable physiological barrier that firmly isolates the brain from the circulating blood and some of the following factors determine its reluctance to allow the entry of CNS drugs [9].

- The BBB provides strong resistance to the movement of ions, with trans endothelial electrical resistance (TEER) around 1500 Ω $cm^{2,}$ which is about 100 times higher than that for peripheral microvessels (3-30Ω$cm^{2)}$ [10]. This causes a "passive" physical barrier which is due to reduced aqueous-based paracellular diffusion mechanisms which are found in other body parts [11].

- The presence of efflux pumps and the additional enzymatic aspects in the BBB which serves to protect the brain, presents an active barrier [12].

• The BBB Endothelial cells are characterized by increased mitochondrial content, minimal pinocytotic activity, and lack of fenestrations [13]. The restricted paracellular permeability of the capillary endothelial cell is attributed to the two intercellular molecular binding systems-the junction and the tight junction [14].

The B-CSFB is another protective shield that prevents the entry of molecules from the blood to the brain parenchyma containing interstitial fluid and CSF which is found at the arachnoid membrane and choroid plexus. The B-CSFB owing to the choroid plexus passively regulates the passage of drugs into CSF by the presence of tight junctions and actively by its organic acid efflux transporters and at arachnoid membrane, this barrier is passively impermeable to hydrophilic substances to a great extent [15].

**Drug-related factors**

Not only are the specialized anatomy of the brain parenchyma and barrier properties the sole reason hampering the adequate distribution of CNS drugs into the brain, but the physiological mechanisms and the specific drug properties also determine the concentrations of a drug within a specific region of the CNS [16]. A drug administered into the body has to undergo all the basic pharmacokinetic mechanisms such as transport from the site of administration into the systemic circulation (except for compounds administered intravenously), the drug then distributes across the body, further subjected to metabolism/biotransformation by different enzymes and finally eliminated from the body. A drug should possess optimum lipophilicity and solubility to be well absorbed into the body [17, 18]. But on increasing the lipophilicity of the drug tending to increase the permeability across the membranes it leads to a higher rate of metabolic clearance and stronger binding to plasma proteins [19]. The optimum lipophilicity required to facilitate maximum bioavailability needs to be carefully determined in the drug delivery process, particularly for CNS drugs in which the lipophilicity of the drug is the major governing factor that allows them to cross the BBB. The size of the drug and its charge are also prime factors where molecular weight greater than 400-500 Da do not cross the BBB (with some exceptions) in pharmacologically significant amounts [20].

To overcome these challenges, currently, there are various strategies of drug delivery to the brain. It is well classified with examples of application in table 1.

**Table 1: Different strategies of drug delivery to the brain**

| S. No. | Drug delivery technique | Different type of strategies | Examples | References |
|---|---|---|---|---|
| I) | Chemical delivery system | ▪ Lipid-mediated transport (Lipidization of small molecules) | - Diacetylated form of morphine | [21] |
| | | ▪ Prodrug Approach | - Zidovudine (AZT), ganciclovir, benzylbenicillin estradiol. | [22-27] |
| | | ▪ Lock-In System | - 1,4-dihydrotrigonelline-Trigonelline system pyridinium salt redox system | [28, 29] |
| | | ▪ Adsorptive-Mediated Transcytosis | - Aclarubicin-Loaded Cbsa-Np For Glioma Chemotherapy | [30] |
| | | ▪ Carrier-Mediated Transport | - L-DOPA, Gabapentin, Mephalan via LAT1 neutral amino acid carrier | [31] |
| II) | Biological delivery systems | ▪ Receptor-Mediated Transport | - Transferrin Receptor (TfR): Human TfR fused to iduronate 2-sulfatase (IDS): JR-141<br>- Insulin receptor (IR): HIRMAb complexed with *N*-sulfoglucosamine sulfohydrolase (SGSH): HIRMAb-SGSH.<br>- Low-Density Lipoprotein Receptor (LDLR): Angiopep-2 combined with antitumor drug paclitaxel | [32] |
| | | ▪ Active Efflux Transport | - In combination with 3rd generation Pgp inhibitors like tariquidar, zosuquidar, laniquidar | [33] |
| | | ▪ Peptide Vector Strategies | - ANG1005, p97, VH0445 | [34] |
| III) | Disruption of the blood brain barrier(BBB) | ▪ Osmotic disruption/Hyperosmotic Shock | - Mannitol, arabinose<br>- hyperosmolar solutions of lactamide, saline, urea | [35, 36] |
| | | ▪ Biochemical disruption by administration of Vasoactive Substances | - Arachidonic acid, Leukotriene, Bradykinin, Histamine, Serotonin, Polyamines. | [37] |
| | | ▪ BBB Disruption by Alkylglycerols | 1-O-pentylglycerol, 2-*O*-hexyldiglycerol | [38] |
| IV) | Molecular trojan horses for brain drug delivery | ▪ Genetically engineered proteins (peptidomimetics) | - To carry peptides like BDNF, FGF2,VIP and plasmid DNAs like luciferase gene, TH gene, antisense gene | [39] |
| V) | Novel Drug Delivery Approach | ▪ Via Catheters and Pumps. | | [40] |
| | | ▪ Microspheres | - Resperidol® consta, Parlodel® LAR | [41] |
| | | ▪ Biodegradable Wafers | - Intracranial placement of Gliadel wafer | [42] |
| | | ▪ From colloidal drug carrier systems | | [43, 44] |
| | | ▪ From Microchips | | [45] |
| VI) | Other alternative Routes/Methods. | ▪ Intranasal Drug Delivery | - Stadol NS®, Stimate NS, Syneral® Nasal Solution, Zomig Nasal Spray | [46] |
| | | ▪ Convection-Enhanced Diffusion. | - Delivery of GDNF in Parkinson's disease, molecularly targeted recombinant chimera cytotoxic fusion proteins in anti-GBM therapy | [47] |
| | | ▪ Intrathecal/Intraventricular drug delivery | | [48] |

All these approaches for delivering drugs to the brain have shown promising results from the respective studies done but have their own limitations. For example, the invasive techniques including disrupting the blood-brain barrier by using hypertonic solutions of mannitol, arabinose, urea, or synthetic analog of Bradykinin, namely RMP7, or creating a temporary increase in the vascular permeability with agents such as histamine and vasoactive peptides allow direct entry of the drug into the brain; it is accompanied by major side

effects such as damage to the neurons, inflammatory reactions, etc. which restricts the use of the invasive technique as an unsafe method to CNS disorder treatment [49]. Intraventricular route of administration can provide only some degree of penetration to the parenchyma and compare to the target cells; there is more of ependymal cell uptake in the ventricle [50]. Other convection technologies like a focused ultrasound of low intensity in combination with microbubbles or implants can provide localised delivery of drugs but it becomes inefficient to treat disorders that affects multiple areas of the brain, which is the case with most of the CNS disorders [51]. Thus finding a drug delivery system that can deliver the drugs to the target cells precisely and adequately is the need of the hour.

From the past few years, various nanosystems have been investigated for meeting the need for effective therapies for neurological diseases. The vast research happening in the field of nanotechnology has led to a broader understanding of the mechanism of nanoparticle uptake to the brain [52]. Among the various nanomaterials so far developed, dendrimers show a huge potential as an excellent nanocarrier owing to their well-defined, globular, highly branched and controllable nanostructural features, which are unique to them and the terminal groups present can be functionalized with different ligands having multivalency similar to the chemical groups present in different biological systems [53]. In addition to being explored as promising carriers of chemical drugs, therapeutic nucleic acids, proteins and peptides, they are also functional as macromolecular contrast agents and biosensor platforms for CNS therapies, imaging, and diagnosis [54]. All these possibilities offered by dendrimers upraise them to be smart nanocarriers in the future endeavors in drug delivery systems [55].

**Dendrimers**

Dendrimers are three-dimensional polymeric materials composed of repeatedly branched monomeric units called dendrons, which coalesce to form a highly symmetrical structure [56]. The dendrons are single chemically distinct groups attached by chemical bonds to the center of the dendrimer [57]. The term dendrimer originated from the Greek word “dendron” which means a tree-like structure and “meros” which means part [58]. Dendrimers consists of a central core to which repeated branching cycles, commonly referred to as generations, are added. Each generation is assigned a generation number indicating the number of branching reactions performed onto the core molecule [59]. The ends of these branches form the multivalent surface, which can be specialized for specific functions. Fig. 1 shows the basic structure of a dendrimer with three generations.

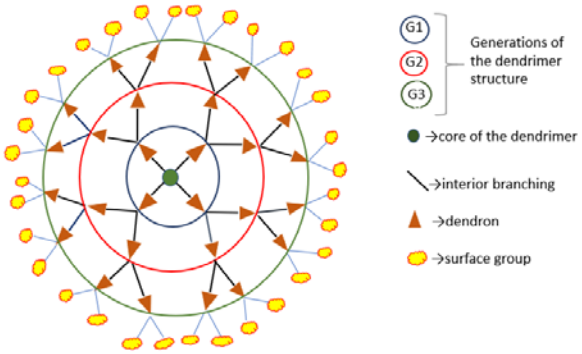


**Fig. 1: The basic structure of a dendrimer with three generations [60]**

The surface functionalization of dendrimers is usually carried out to reduce its rapid clearance by the reticuloendothelial system and its toxicity issues caused by the interaction between the amine-terminated groups and the cell membrane. Surface functionalization improves the transfection efficiency and the specificity of dendrimers. The increased biocompatibility and controlled release behavior based on stimuli responsiveness of functionalized dendrimers enhance its therapeutic efficiency [61]. A relevant finding was done by Vidal F *et al.* (2016) about the correlation of surface characteristics and their neuronal internalization process and its rate of internalization. They studied on four different variations of surface functionalization of a generation 4 PAMAM dendrimer (G4) as follows: an unmodified group, 50% amino surface groups modified with neutral polyethylene glycol groups (PP50), 30% amino groups with anionic acrylate groups (PAc) and 25% with folic groups (PFO). From the confocal microscopy studies, it was understood that only the unmodified and PFO dendrimers are uptaken by the neuronal hippocampus cells accounted for the change in surface charge density of PP50 and PAc modified dendrimers leading to the complete blockade of its cellular internalization. The surface modification also affects the internalization pathway, which was studied from colocalization analysis. Thus, the surface modification should be done based on thorough knowledge as it influences the neuronal uptake following its release from the nanocarrier [62].

Large-sized dendrimers are preferred for the fact that they can increase the dendrimer accumulation in the brain by slowing down the clearance rate and prolonging systemic circulation without losing their inborn capacity to target the stimulated microglia and damaged neurons. Zhang F *et al.* studied the influence of the size of dendrimer on brain uptake by investigating the pharmacokinetic distribution in a clinically-significant canine model of HCA-triggered brain damage. They found out that the Generation 6 (G6) of Polyamidoamine (PAMAM) dendrimers with-OH ending groups had a hydrodynamic diameter around 6.7 nm that lied on the verge of the range of renal filtration and was approximately 1.5-fold greater than G4 dendrimers. Both dendrimers had comparable surface characteristics (neutral and hydrophilic) and they were not likely to attach to plasma serum proteins. However, the slight increase in the size of G6 dendrimers could potentially increase the circulation period after its systemic administration without changing the most important renal clearance pathway [63].

Dendrimers have a multifunctional capability ranging from enhancing solubility, dissolution, gastrointestinal tract (GIT) permeability, stability [64] to promoting better bioavailability, allows multiple drug entrapment, and controlled delivery [64-67].

There are three main sites for drug entrapment in the dendrimer architecture, which is explained in table 2.

**Table 2: Different sites for drug entrapment in the dendrimer**

| Site | | Mechanism | References |
|---|---|---|---|
| 1) | Void spaces | Entrapment of molecules | [68] |
| 2) | Branching points | Hydrogen bonding | [68] |
| 3) | Surface groups | Charge-charge interactions | [68] |

The science of dendrimers can be considered as a combination of molecular and polymer chemistry. The step-by-step-controlled reactions of synthesis makes it a part of molecular chemistry, whereas the repetitive structure of dendrons (monomers) shows the role of it in polymer chemistry [69, 70]. Dendrimers can be synthesized by the following methods:

1. Divergent approach: This is one of the earliest methods where the synthesis begins from the core and extend towards the periphery by the repetition of the two basic reactions a) coupling of the monomer.

b) Transforming the monomer end group to form a new coupling site for the next monomer [71].

2. Convergent approach: This method involves the synthesis of the dendrimer from the periphery to its core by the one to one coupling of the dendritic segments. This was first described by Hawker and Frechet in 1989–1990 [72].

3. Double exponential method: This process involves the accumulation with a single trifunctional monomer in the form $Am(Bm)_2$, which has orthogonally protected functional groups. The number of repeat units per dendrimer is in accordance to a double exponential function in terms of generation, n. There will be a selective removal of the protecting groups on Am in one portion and on Bm in the second portion during the repetitive process, and the two monoprotected intermediates are subsequently coupled in a proper stoichiometric ratio [73].

4. Hyper core/Hyper monomer approach: In this method, the first step is to synthesis the hyper monomer with low-generation dendrons having protected terminal groups by the convergent approach. These monomers are joined to a multifunctional core via their focal point which is called a hyper-core and the terminal groups of the resultant low-generation dendrimer are deprotected. Finally, all the hyper monomers, which are either indistinguishable to the preceding one or different, are reacted with this hyper-core to result in the formation of an anticipated higher generation dendrimer [74, 75].

5. Orthogonal coupling strategy: It is a rapid method of synthesis that can exclude the deprotection steps or the intermediate activation steps leading to an accelerated growth of dendrimers. It involves the chemoselective reaction between the monomers $AB_n$ and $CD_n$, resulting in the doubling of the end groups with a reduced number of reaction steps. Here an orthogonal model of reaction happens between 'A and D' and 'C and B' specifically [76].

6. Click chemistry: Various click reactions including Copper-Assisted Azide-Alkyne Cycloaddition (CuAAC), Thiol-ene and Thiol-yne Click Reactions (TEC TYC), and Diels-Alder (DA) Reaction have been used for dendrimer synthesis provided some of the problems inherent to these methods like the catalyst load might not be enough which has to be optimized precisely or the dendrimer might complex with the metallic portion of the catalyst has to be taken care of. Some novel methods like the Janus method, onion peel strategy etc. are also being developed. Click chemistry application using an effective coupling agent to conjugate the drug with the PEGylated PAMAM dendrimer to improve the polymer-drug coupling efficiency has been reported by Olga Yu. Zolotarskaya *et al*. (2015) [77, 78].

7. Lego chemistry: It is a direct method of synthesis involving two-branched monomers in which each quantitative step gives a corresponding generation (5 steps will give a fifth-generation G5 dendrimer). The reactions will produce only eco-friendly by-products like sodium chloride, water, nitrogen, etc. Moreover, it is an economic as well as time-saving method [79].

Dendrimers have potential applications as drug delivery agents in CNS, oral, nasal, pulmonary, topical and transdermal drug delivery, gene delivery, vaccine delivery, as ophthalmic vehicles, in cancer treatment for targeting imaging as well as therapy such as photodynamic therapy (PDT), boron neutron capture therapy (BNCT), Gadolinium based (Gd) neutron capture therapy (GdNCT). They can also work as a useful tool in the area of diagnosis as molecular probes, X-ray contrast agents, MRI contrast agents, etc. Even though at the stage of infancy, dendrimers are expanding their applications to the biomedical field by its usage in tissue engineering, cell repair, blood substitution and cosmetics and personal care applications [80-82]. It is found that dendrimers are the single category of synthetic macromolecules that can be used as polymeric scaffolds to achieve biomimetic functions. Studies has been reported to show their mimicking capability of the surface structure of proteins requires in angiogenesis inhibition for its usage in antitumor systems, in biomimetic regeneration of Hydroxyapatite, which mimics the organic matrices induced biomineralization process in developing enamel and enhances the binding strength at the remineralization interface[83] and the collagen mimetic dendrimers [83-85].

There are many excellent reviews on dendrimers as CNS delivering agents of drug molecules and other therapeutic agents. In this article, we focus on the latest pharmaceutical application of dendrimers for the transport of drugs, nucleic acids, and proteins/peptides to the brain system that has happened in the years since 2014 [86-88].

**Recent studies on various CNS application of dendrimers**

**1. Brain-specific targeting potential**

Targeting the drug to the site of action and minimizing its distribution to the rest of the body is the prime objective of any drug delivery system. This will reduce the required dose to get the same therapeutic action and in turn reduces the side effects. Targeting can be achieved via several means. The most acceptable and successful method is true to conjugate a specific ligand that has the binding capacity to the receptors and transporters present on the membrane to be crossed at the site of action [89, 90].

On the understanding that the generation 4 polyamidoamine (PAMAM) dendrimers with hydroxyl terminal group (D4-OH) can penetrate the injured BBB and target the activated glia, A Sharma, JE Porterfield *et al.* (2018) was interested to know whether conjugating the targeting ligands would increase the uptake of dendrimers by the brain and other organs. Their study was based on the conjugation of mannose to the surface of multifunctional D4-OH since mannose receptors are typically over-expressed on injured microglia. The method for synthesis was orthogonal Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC) click chemistry, which was very efficient and required lesser atoms. The *in vitro* evaluation of the effect of mannose conjugation as a targeting ligand changed the dendrimer internalization process significantly, thus suggesting receptor-mediated endocytosis of mannose is preferable to non-specific fluid-phase endocytosis. The *in vivo* studies included the CNS uptake and of targeted and non-targeted biodistribution of fluorescently labelled dendrimers in a model of rabbit with maternal intrauterine inflammation-induced with cerebral palsy using fluorescence spectroscopy and confocal microscopy for quantification. Without any reduction in the quantity of dendrimer supplied to the injured glia in the brain, the distribution of the mannose conjugated dendrimer was varied throughout the body of the animal model, indicating that mannose conjugation can change the interaction of the dendrimer with all the body cells without affecting the inherent targeting ability to the inflammatory sites in the brain [91, 92].

Another recent study done for increasing the targetability of drugs towards the brain using dendrimers is the conjugation of PAMAM dendrimers with minocycline, a drug proven potent for neurological diseases. Minocycline has the inherent ability to penetrate the BBB but is required in large doses to attain the therapeutic concentration in the brain resulting in peripheral side effects. The innate stability of minocycline prevents it from chemical modifications. Thus to reduce the dose requirement by site-specific targeting, R Sharma, SY Kim *et al.* (2017) designed a drug dendrimer conjugate, namely hydroxyl-G6 PAMAM dendrimer–9-amino-minocycline conjugate (D-mino) and characterized it is *in vitro* efficiency and *in vivo* aiming capability. The poly(amidoamine) (PAMAM) generation-6 (G6) dendrimers with hydroxyl terminal groups was selected based on the finding that it can stay long in the circulation and can cross the

impaired BBB and they conjugated 9-amino-minocycline (mino) to the surface of the dendrimer through enzyme responsive linkages using a blend of mild copper-catalyzed azide-alkyne click reaction (CuAAC) and microwave energy. The *in vitro* release studies showed that there is no drug release at the physiological pH but a sustained release up to 8 d after an initial low release (below 10% till 48 h) at pH 5.5 which simulates the lysosomal pH wherein the conjugate is taken up. Confocal microscopy and flow cytometry results presented a better cellular uptake of D-mino and further evaluation of anti-inflammatory and antioxidant activity in lipo-polysaccharides-activated murine microglial cells showed reduced production of inflammatory cytokine tumor necrosis factor α (TNF-α) and a significant reduction in oxidative stress by subsiding nitric oxide generation compared to the free drug. The invivo imaging studies with fluorescently labeled dendrimer conjugate (Cy5–D-mino) by systematic intravenous administration to suitable cerebral palsy induced rabbit kits and sacrificed after 24 h indicated the effective distribution of the dendrimer drug conjugate in the periventricular white matter areas of the corpus callosum and periventricular region which were most affected areas of the brain injury model with significant microglial activation. The satisfactory results of the study proved the potential of PAMAM dendrimers for effective drug targeting in neuroinflammatory conditions [93, 94].

H. K Patel *et al.* (2016) evaluated a comparative targeting potential of different ligands by developing dendritic nanoconjugates for the delivery of paclitaxel (PTX) to the cancerous site of the brain. They studied three ligands, namely Concanavalin A, Sialic acid, and glucosamine, which were separately conjugated to the paclitaxel loaded 5.0G poly(propyleneimine) (PPI) dendrimers. This was done based on the point that receptors of sialic acid and GLUTs transporters were overexpressed in BBB, which can be utilized as targeting possibilities [95]. The blood circulation time of the ligand conjugated dendritic nanoparticles and their half-lives were extended when compared to free PTX and PTX-PPI. The biodistribution studies gave significantly high brain concentration of paclitaxel from sialic acid anchored dendrimers (SPPI) than glucosamine anchored dendrimers (GPPI) and very negligible quantity by concanavalin A (ConA) anchored dendrimers (CPPI) but all of them could outstand the free drug and drug-loaded dendrimer. This accounts for the blocking of P-gp efflux system, which remained a major hurdle for the entry of PTX to the brain cells. This in turn increased the bioavailability of the drug in the CNS and all the three ligands in the order SPPI>GPPI>CPPI are efficient to bring an improved therapeutic outcome in treating brain cancer with paclitaxel [96].

**2. Increasing the brain permeability of drugs via cationization with dendrimers**

Most of the drugs are reported to be poorly bioavailable in the brain due to its low permeability across the blood-brain barrier. Incorporating such small therapeutic molecules into the nanoparticles by employing adsorption, covalent bondage, or encapsulation can improve the brain concentration of drugs to a great extent [92]. Such an attempt of improving the permeability of the drug Citicholine which is found effective for the treatment of stroke by cationization of the drug-loaded bovine serum Albumin nanoparticles with G2 PAMAM dendrimers termed as dendrimer amplified albumin nanoparticles (dAA) was done by Pradhan D *et al.* (2019). The advantages of using PAMAM dendrimers over other cationization agents like ethylenediamine, hexamethylenediamine, PEI are that only a small amount of dendrimer is required for cationization as it has sufficient cationic amine groups in a single molecule and thereby reduce toxicity issues too. The permeability study using the *in vitro* BBB model was made of bEnd.3 cells proved that the rate of permeation was increased to thrice the rate of non-cationized albumin nanoparticles, which is accounted for the electrostatic interaction between the brain endothelial surface and the cationic groups in the dendrimer surface leading to cellular absorptive endocytosis. The drug release studies also showed an added advantage of the dAA nanoparticles that there is an increase in the release, which is pH-dependent. There is a slightly acidic pH at the disease site and at this reduced pH, the dendrimer grafted albumin nanoparticles could show increased cumulative percentage drug release at pH 5 than at normal physiologic pH 7.4 whereas the albumin nanoparticles showed the same release irrespective of the change in pH. Besides, unlike the commonly used cationization agents, there is no toxicity factor added to the nanoparticle which has been proved successfully through the MTT assay [97].

**3. Application in foetal neurotherapy**

Certain infections like bacterial vaginosis, chlamydia, cytomegalovirus, syphilis, etc affecting women during their pregnancy can lead to cause life-threatening diseases to the newborns. It is better to control such infections at the foetal stage itself as early as possible by treating prenatally. Such a study was reported by using the PAMAM dendrimers by Zhang F *et al.* in maternal intrauterine inflammation-induced rabbit model with Cerebral Palsy. It is a chronic childhood condition that has the intrauterine infection as a major risk factor. They used neutral and anionic surface-modified PAMAM dendrimers using hydroxyl (D-OH) and carboxyl (D-COOH) terminal groups respectively and injected the dendrimer solutions intra-amniotically to study its placental barrier crossing and microglial targeting to reduce the neuroinflammation. The distribution studies showed that both D-OH and D-COOH enter fetal circulation while D-OH has better brain accumulation owing to its neutral surface charge density and D-COOH was constrained to the blood vessels. This study encourages to consider intra amniotic delivery of dendrimers as an effective targeting of fetal inflammation and associated neurological conditions. These findings give a futuristic hope for a translational therapy based on dendrimers to treat pediatric brain injuries [98].

**4. Reducing the invasiveness of drug administration for CNS delivery**

The success of any CNS treatment is the reachability of the drug to the brain in its minimum dose with the least side effects. Being invasive might ensure the direct delivery of the drug to the site but the risks associated are beyond the benefits. The application of dendrimers to minimize the invasiveness was one of the major aims of the study conducted by B. Srinageshwar *et al.* (2017) by administration via the carotid artery in experimental animals like mice. This team also investigated a novel approach of using mixed surface dendrimers to reduce the toxicity issue which is one of the main drawbacks of conventional cationic dendrimers which refrain them from reaching the clinical studies. From the *in vitro* experiments, they could confirm the uptake of both the cationic amine dendrimers (G1-$NH_2$ and G4-$NH_2$) and the mixed surface dendrimers (G1-90/10 and G4-90/10) using primary cortical culture. The results offer the novel dendrimers to be promising alternatives as carrier systems with much-reduced toxicity. For *in vivo* experiments, there were two methods of administration (1) transplanting the G4-90/10 into mice through the invasive intracranial injections into the striatum; and (2) less invasive dendrimer administration by intracarotid injection through the internal carotid artery. After analyzing the brains of the sacrificed animals 24-h and 1-week post-transplantations, it was confirmed that the G4-90/10 can cross the physiological barrier BBB when introduced through the carotid artery and confined within neurons and glial cells and was found to move through the corpus callosum a week after the intracranial injection. It was shown that the traveling cells were the glial cells that were infected with dendrimers by immunohistochemistry. Overall, the outcomes suggested that the mixed surface dendrimers can be used as a slightly invasive method to transport biomolecules for managing neurological disorders [99].

**5. For protein and peptide delivery**

The delivery of therapeutic protein and peptide molecules for the treatment of many neurodegenerative diseases remains extremely challenging because of its rapid clearance from serum and very limited permeability to the brain. Even though BBB bypassing routes like intracerebroventricular (i. c. v.), intraparenchymal, intranasal (i. n.) or intrathecal (i. t.) have been proposed as direct delivery methods to the brain they show little positive results in practice. In the efforts to develop effective delivery systems, stands up the dendrimer approach. Pierpaolo Moscariello *et al.* (2018) designed a dendronized streptavidin (DSA) which was structurally identical to endogenous proteins. Fig. 2 shows the PAMAM dendrimers of two generations (G2 and G3) with different positive charges were attached to the streptavidin core through biotin click chemistry [100].

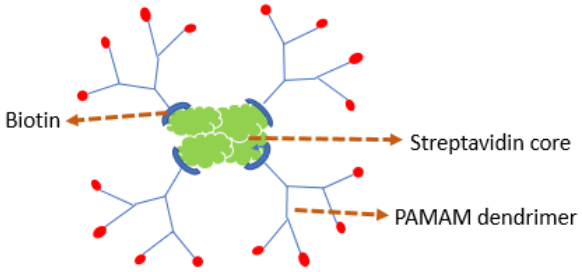


**Fig. 2: The PAMAM dendrimers attached to the streptavidin core through biotin [101]**

The study successfully demonstrated the improved ability of the protein molecule to cross the BBB by transcytosis via the endosomal pathway with high biocompatibility proving a new flexible nanoplatforms for the delivery of biopharmaceuticals can be hopefully developed [102].

### 6. For Gene/Nucleic acid delivery

#### APOPTIN gene delivery

In the case of HIV-1 infections, gene-based therapy is found to be more cure effective than the antiretroviral drugs and its combinatorial therapy owing to the silencing of gene expression of the viral or host mRNA target using RNA interference. The delivery of RNAi therapeutics to the brain to attain the treatment goal is very challenging, obviously due to the difficulty to cross the BBB. siRNA is a promising strategy of therapy as it is synthesized chemically, has targeting specificity, and can be easily subjected to changes with the mutation of the virus without loss of potency. To find out an effective platform for the delivery of siRNA for the treatment of HIV-1 infection, M. J. Serramia et. al (2014) studied the possibility of using dendrimers for site-specific targeting. They could successfully prove that carbosilane dendriplexes of generation 2 made cationic in nature can improve the brain concentration of siRNA protected in the dendrimer by the *in vivo* imaging studies of the fluorescent labelled dendrimer administered as a retro-orbital injection to BALB/c mice. The cellular uptake of dendriplex was found to be double than the random siRNA by the human astrocytes and thereby, increased reduction of viral infectivity by blocking selected protein synthesis was attained [103-105].

#### SRL peptide for targeted gene delivery to the brain (2015)

Application of peptides having an affinity towards the over-expressed molecules at the target site has been explored by Zhao J *et al.* in the work in which they conjugated the PEGylated PAMAM dendrimer with the peptide CREKA to promote improved residence time of the drug at the tumor site in the brain. This peptide molecule has an affinity towards the fibrin molecules which are overproduced in the brains of patients affected by Glioblastoma multiforme, one of the most life-threatening brain tumors [106]. The PAMAM dendrimers, when reduced in size, achieve the blood-brain barrier crossing ability and when further PEGylated, it can remain in the systemic circulation for a prolonged period with reduced cytotoxicity [107]. A similar work was reported by Jiang Y et. al but by using a different peptide, namely Pep-1, which can cross the tumor barrier through endocytosis mediated by interleukin 13 receptor α2 (IL-13Rα2). The anticancer action of arsenic trioxide was improved almost four times compared to drug solution when a peptide RGDyC based on Arg-Gly-Asp amino acid sequence was conjugated to the drug-loaded modified PEG-PAMAM dendrimer. This accounts for the fact that the dendrimers, when conjugated with tumor homing peptides it can increase the effective brain concentration of the dendrimer resulting in improved therapeutic outcome [108, 109].

## CONCLUSION

There is a remarkable rise in the proportion of the population around the world being affected by various CNS disorders every year, especially with the aging population due to the increased life expectancy. In addition, the financial burden of the currently available treatments has stressed the need for effective drug delivery to the brain. The advances in nanotechnology presents the amazing potential of dendrimers as nanocarriers for brain delivery and some of the recent impeccable works done in this direction has been discussed in this article. Dendrimers become superior to other nanocarriers since they can permeate the BBB and be accessible to the brain following systemic circulation due to its unique characteristics. Their controllable nanosize, flexibility in designing numerous molecules via surface functionalization, thereby increasing targetability to the brain, ability to protect and deliver biomolecules like proteins, peptides, nucleotides, and genes promoting them as effective therapeutic and theranostic agents are the significant advantages of the dendrimers giving enormous scope for research in this area. However, a more extensive evaluation of the *in vivo* distribution and safety profile of dendrimers has to be done for bringing forth their reliability as nanocarriers. Despite all these recognized applications, the usage of dendrimers for CNS delivery is static in its embryonic stage and to date, none of the promoted dendrimer formulations has been reported for CNS therapy. Moreover, dendrimer synthesis is laborious even though they are commercially available nowadays which are ready for tuning to any desired feature and the balance between toxicity and biodegradability is highly dependent on the scaffold. Better biocompatibility, newer linker strategies, and wider options to transport biologicals and small molecules using dendrimers are the studies in the pipeline and the evolution of dendrimers as the next generation smart nanocarriers for CNS delivery is eagerly awaited for attaining future goals like the improved modulation of synaptic activity to provide higher neuronal functions in related pathologies such as epilepsy, schizophrenia, neurodegenerative diseases, and drug abuse addiction.

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## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICTS OF INTERESTS

Declared none

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