CHAPTER

14

Dendrimers in the management of Alzheimer's disease

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1. Introduction

A type of three-dimensional, nanoscale, artificial macromolecules known as dendrimers has a well-defined globular architecture as per Fig. 14.1A [1]. The monodispersity, biocompatibility, and good biodegradability of dendrimers make them special [2]. A typical dendrimer molecule has three fundamental parts: a central core made up of an atom or molecule with at least two identical functional groups; branching units that come from the central core; and various peripheral surface functions [3]. As a result, dendrimers are made up of repeating branching shells; each branching shell is referred to as a generation as per Fig. 14.1B [4,5]. Dendrimers have been effectively used in preclinical delivery of several medicinal compounds with various pharmacological properties, including antiinflammatory, hypolipidemic, antibacterial, antifungal, and anticancer [6].

Psychiatric conditions, other neurodegenerative illnesses, such as fronto-temporal dementia other disorder that cause dementia [7]. When chronic (lasting for months), these encephalopathies are considered in the differential diagnosis of dementia [8]. Toxic-metabolic encephalopathies are the outcome of several insults that might impair cognitive function [9]. The biological cause of dementia is chronic disease that modifies or destroys brain-related nerve cells and synapses [10]. For the most prevalent neurodegenerative diseases of aging that lead to dementia, there are currently no viable treatments to prevent or reverse the underlying disease process [11]. The number of elderly people suffering from dementia is increasing, as are the resources and expenditures connected with their care [12]. This is due to longer life expectancies. People of all ages are susceptible to dementia. A person must have had a higher level of intellectual ability before a drop in order to be classified as demented, per definition. More dementias strike people who have lived long enough to acquire intellectual skills before losing those [13]. Because diagnosing and documenting dementia cases in the poor world is challenging, it is challenging to acquire estimates of the incidence and prevalence of dementia globally [14]. The illnesses that are classified as dementia and the types of dementia that should be included in these surveys also differ between studies [15]. Numerous textbooks claim that degenerative dementias are the most frequent causes of dementia, with AD being the most prevalent in that category. Following AD, considerable percentages of dementia cases (perhaps 10%–20%) are caused by disorders like frontal lobe dementia and dementia of the Lewy body type [16]. But it is becoming better acknowledged that people with so-called vascular dementia frequently experience a mixed overlapping pattern with AD [17].

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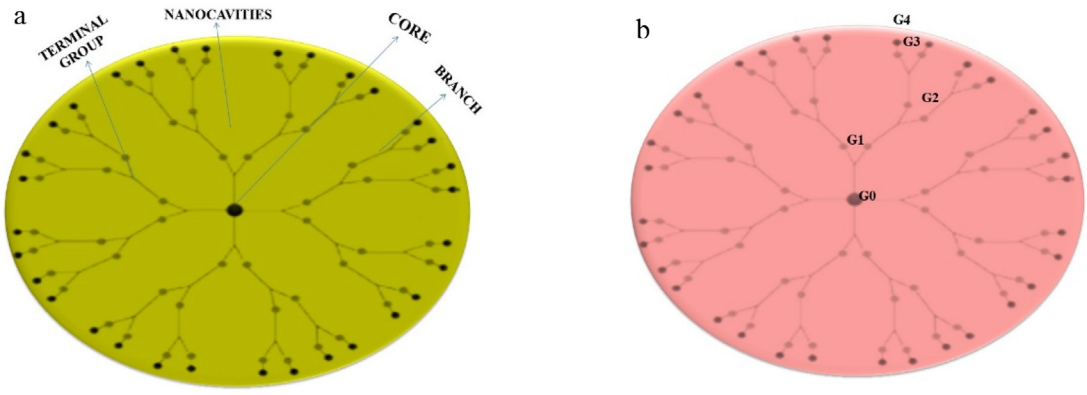

FIG. 14.1 Parts of dendrimers (A) and generation of dendrimers (B).

Additionally, vascular abnormalities including amyloid angiopathy and arteriosclerosis are frequently seen in AD patients [18]. Independent life is impacted by the dementia syndrome of cognitive impairment or cognitive decline [19]. Every 4 s, a new case of dementia is reported globally [20].

2. Synthesis method of dendrimers

The creation of novel dendrimers with unique features for use in biomedical applications is of great interest. A variety of PEPE dendrimers with various topologies were made generations. For instance, Dhanikula and Hildgen combined convergent and divergent strategies to synthesize a new polyester-co-polyether (PEPE) dendrimer with a hydrophilic interior/cavity. Novel dendrimers have been designed with biocompatibility, amphiphilicity, and biodegradability as their primary design objectives for drug delivery applications. To address these different techniques such as, synthetic techniques, natural procedures, mechanical procedures, and dynamic procedures have all been used in the development of medicinal dosage forms [1,21]. Because of its affordability, absence of significant environmental hazards, and biological reduction, the natural synthesis of nanoparticles (NPs) was selected an appealing alternative to chemical methods [22,23]. The distribution of the drug is the most crucial component of any dosage unit, and different polypeptide molecules are utilized to ensure focused on effective drug administration at the right site [24]. By applying various dendritic patterns to the surface, the dendritic approach enhances pharmacological activity, enabling a specific functional molecule to significantly outperform the sum of its individual entries. The synergistic effect produced by dendrites is what causes the increased activity [25]. The main purpose of dendrimers is to change and improve bioavailability in pharmaceuticals by changing pharmacokinetic with pharmacological characteristics of active pharmaceutical ingredient (API) [26]. Dendrimers are an attractive family of nano-vectors for brain targeting and monodisperse in comparison to conventional polymer nanovehicles. Dendrimers' unique structural characteristics also give them the flexibility to carry medicinal medicines via covalent conjugation or electrostatic adsorption [27]. Drug molecules may be trapped inside the cavities of a dendrimer due to the open nature [3,5]. Lower generation is typically patulous and amorphous [4]. Hydrophobic and hydrogen bond interactions, electrostatic interaction, straightforward physical entrapment, and encapsulation are all types of interactions [6]. Due to these interactions, dendrimers may be able to integrate irregular or insoluble medicines, improving their water solubility and bioavailability while also regulating their release [28]. However, significant drug solubility results from steric restriction and electrostatic attachment [29]. While the core was made of the biocompatible component ane tetra carboxylic acid and aspartic acid and it was demonstrated that the dendrimers successfully surround guest molecules with loadings of 15.80 and 6.47% weight-for-weight for rhodamine and beta-carotene, respectively (models of hydrophilic and hydrophobic substances). P-amino benzoic acid and polyethyleneglycol (PEG) are frequently combined to generate the conjugation through amide or ester bonds [30].

3. Types of dendrimers

According to generation of dendrimers, small generation (G) dendrimers (G0-G3) are open and asymmetric in structure, while G4-G6 intermediate generation dendrimers are semirigid structures that can interact with other molecules, and major generation dendrimers (G7−G10) are tough spheres with significant surface steric hindrance [31]. Types of dendrimers according to structure are discussed here.

3.1 Polyamidoamine dendrimers

PAMAM (polyamidoamine) dendrimers 3, 4, and 5 have been proven in studies to prevent the development of amyloid deposits. Compared to the current forms of aggregated proteins, they have hydrolytic characteristics. None of the forms of A resistant to hydrolysis when the dendrimers nanomolecules were added to the cells. This finding suggests that PAMAM dendrimers aren't just amyloid aggregation inhibitors but also have the potential to eliminate the harmful forms of accumulated proteins that are currently present. Dendrimer-peptide ratio-dependent PAMAM dendrimer interference with A [1−28] fibril production is efficient. The creation of fibrils appears to be generally accelerated by low dendrimer-peptide ratios, whereas high dendrimer-peptide ratios appear to slow the polymerization reaction hence reduce the number of fibrils created [32]. The PAMAM dendrimers may lower the effective concentration of amyloid formations in one of three ways: Dendrimers bind peptides increasing dendrimers, locks the free fibrils ends and speeds up the dissolution of the fibrils, respectively [31].

Preformed amyloid fibrils can be broken up into nonfibrillar forms by PAMAM dendrimers. The process of amyloid aggregation is more severely inhibited and PAMAM dendrimers are more effective at rupturing already-formed fibrils when dendrimer generation is higher [32]. Examples of PAMAM dendrimers in Alzheimer's disease are Tacrine (AChE (Acetyl-cholinesterase) inhibitor) loaded PAMAM dendrimers [33], Memantine hydrochloride (NMDA (N-methyl-D-aspartate) inhibitor) loaded PAMAM dendrimers [34], Rivastigmine dendrimers [35], etc.

3.2 Poly(propylene imine) dendrimers

It investigated how the maltose groups via surface modification and amyloid A (1−40) interacted with fourth the fifth generations of poly(propylene imine) (PPI) dendrimers. PPI- G5- Mal prevents the granular development and amorphous protein structures, while lesser toxicity with PPI-G4-Mal of amyloid by fibril protein clumps [31]. In vitro, the neurotoxicity of Aβ was lessened by PPI glycodendrimers with a cationic or an electroneutral maltose shell. On I intranasal administration, these two PPI dendrimers enter the brain and lessen the overall load of Aβ in transgenic AD mice [36].

3.3 Cationic phosphorus dendrimers

Additionally, it was shown that the thermodynamic equilibrium of the Aβ fibrils and the aggregation of proteins are both efficiently disrupted by the cationic phosphorus dendrimers (CPDs) [37]. Cationic phosphorous dendrimers were effective at preventing MAP-Tau from aggregating, which is important for maintaining the stability of the microtubule cell shape. It has been demonstrated that CPDs prevent amyloidogenic proteins from aggregating even at extremely low molar concentrations [31]. Depending on the concentrations utilized, CPDs have been reported to either accelerate or prevent the creation of fibrils from Aβ1-28 and to prevent the Tau protein aggregation. The potential of CPDs to lessen the toxicity associated with refibrillar Aβ1-28 forms was the most notable impact that was noticed after the addition of CPDs. To present, no studies have been done to determine if CPDs have (a) any effect on AChE activity, (b) antioxidant activity, or (c) antiinflammatory effects [38]. The highest dendrimer concentration inhibited AChE activity, suggesting possible real-time conversations between the CPDs and the enzyme. Lineweaver−Burk plots were used to categorize the type of inhibition as partially noncompetitive. It is impossible to classify cationic phosphorus dendrimers as conventional AChE activity inhibitors. They differ structurally from ACh, the enzyme's substrate. CPDs interact indirectly with the enzymes with catalytic pockets by altering the protein's conformation, changing the plasma membrane's properties, or interacting with other membrane-associated proteins [39]. Based on the IC_{50}, which was calculated from the cell survival curve, the cytotoxicity of the CPDs was assessed. Because of their interactions with cell components, cationic dendrimers demonstrate substantial toxicity both in vitro and in vivo. By interacting with negatively charged phospholipids, they create nanopores in the cell membrane and alter the shape of the membrane. Because the bulk of surface groups are not ionized in physiological pH, the toxicity of phosphorus dendrimers is restricted [40].

3.4 Carbosilane dendrimers

Hyperbranched macromolecules called carbosilane dendrimers are one specific dendrimer structure used to transport medicines and nucleic acids. Their terminal groups can be either cationic or anionic, and their structure is described by their generation number. Compounds having silicon and carbon in their molecular skeleton are

known as carbosilanes. Different forms of carbosilane dendrimers with hydrophobic scaffolds and good thermal stability have been created using silicon chemistry. Drugs are encapsulated in the interior layers, reducing toxicity, and controlling drug release. The external layer is made up of functional groups that are ideal for drug conjugation. Therefore, dendrimers are useful as drug delivery vehicles due to their unique features. The potential of carbosilane dendrimers to treat or prevent Parkinson's disease-related processes was studied [41]. One study examined whether carbosilane dendrimers might shield cells from rotenone-induced damage and stop the production of α-synuclein fibrils using the mouse hippocampal cell line (MHippOE-18) (ASN). By interacting with the soluble protein, carbosilane dendrimers may limit the production of ASN fibrils [42].

3.5 PLL dendrimers

Better biocompatibility, safer administration, negligible enzyme degradation, and gene transport usage of PLL dendrimers makes them superior as compared to PAMAM and PPI dendrimers. A G3 PLL dendrimer was found to be able to protect cells from Aβ toxicity in a study in a neuroblastoma cell line. In vivo studies using rats after unilateral intracerebroventricular injection also revealed the presence of these dendrimers in various brain cell populations, including the hippocampal and cortical neurons. Since the dendrimers' widespread localization should allow them to access the dispersed Aβ brain accumulation, they should be able to disrupt the amyloidogenic cascade and lessen the pathological process in Alzheimer's disease [36,43].

3.6 Triazine dendrimers

It is possible to design and easily make triazine dendrimers with orthogonally functional surfaces that make it easier to modify them after they have been made, such as by attaching drugs, PEGylating them, or adding ligands or reporting groups. In his review article, the author provided detailed explanations of how triazine dendrimers are applied in the treatment of cancer, iron overload, septic shock, and diabetes [33,43].

3.7 Glycodendrimers

Dendrimers' inherent toxicity is a significant problem regarding their possible biological applications. A final evaluation of dendrimers as possible antiamyloidogenic drugs is hindered by two significant drawbacks: (a) PAMAM, phosphorous, and PPI dendrimers are found to be too toxic to many of the cell lines used in amyloid toxicity assays because of their ability to create extremely potent electrostatic interactions between the positively charged dendrimer surface and the negatively charged components found on the surface of cell membranes and (b) the peptides (Aβ(1−28) and PrP185-208) employed are not harmful to these cells by themselves [32].

Macromolecular interactions are founded on the formation of weakly interacting, such as hydrogen bonds, which is even more significant than strong electrostatic bonding. The goal is to modify the surface by replacing the positive charge with hydrogen-bond-forming oligosaccharide units, such as glycodendrimers, to create biocompatible dendrimers with antiamyloid action [32,44]. A low amount of cell toxicity has been determined for several cell lines, and these sugar-decorated dendrimers have demonstrated promising features as antiamyloidogenic drugs in comparison to positively charged dendrimers for Alzheimer's disease [44].

4. Method of drug loading in dendrimers

The divergent approach is used to create dendrimers, and in the case of polyamidoamine dendrimers (PAMAMs), it involved layering monomers on top of a multifunctional center. It was a series of Michael's additions according of chemical reaction [31]. Drug molecules can be incorporated into dendrimers' interior pockets or anchored to their surface groups.

4.1 Conventional method of drug loading in dendrimers

Drug and dendrimers were mixed in methanol at different D-to-drug molar ratios. Controls included the preparation of pure dendrimer or drug solutions in methanol. At 28°C, all the samples were homogenized for 24 h. The methanol was subsequently evaporated at 25°C using a Concentrator. To separate the insoluble drug, the

resulting residues were dissolved in PBS 7.4 (10 mM) and kept for centrifugation for 5 min at 10,000 RPM [33]. Igartúa et al. in 2020 loaded tacrine drug molecule in PAMAM dendrimers using above this conventional method which showed remarkable increase in solubility of tacrine [33].

4.2 Conjugation method of dendrimers with protein followed by drug loading

To create a conjugate, the protein was chemically bound to dendrimers via surface amine functions. Firstly, dendrimer was added dropwise to the triethanolamine HCl buffer containing protein and dimethyl-subrimidate mixture. The reaction mixture was allowed to stir for 2 h at room temperature. NMR spectroscopy was used to track the synthesis at every stage. The produced dendrimer-protein conjugate was dissolved in deionized water. Drop by drop, the drug sample was poured into the solution and stirred for 24 h at room temperature. Dialysis was used twice in deionized water to remove extra and unreacted medication [34]. This type of conjugation can be characterized using NMR and FTIR spectroscopic techniques.

5. Properties of dendrimers

5.1 Physical properties

Dendrimers are Spherical in shape, macromolecular, monodisperse, highly branched and tightly packed structure. So, they have excellent physical and chemical properties. They have low viscosity and polydispersity with controlled nano-size as compared to other polymeric structures. Their intrinsic viscosity increases with their molecular weight [45].

5.2 Solubility and compatibility

Surface groups on their structure are responsible for solubility of dendrimers. Number of hydrophilic groups at chain-ends leads to high solubility and high reactivity for drug loading. But, hydrophobic molecules at end-groups containing dendrimers are having solubility in nonpolar solvents. Drug molecules can entrap in dendrimers' globular structure and internal cavities [46].

5.3 Pharmacokinetic properties

Biomedical application such as drug delivery, in imaging, photodynamic therapy etc. of dendrimers are based on their excellent pharmacokinetic properties with modification in peripheral groups with antibodies, peptides and other macromolecules [47].

5.4 Covalent conjugation properties

Dendrimers have excellent characteristic to couple with small molecules with covalent conjugation, that results in greater pharmacological properties to target cells and tissues for activation of drug at the site of action [45,47].

5.5 Polyvalency

Polyvalency means chemical structure have more than one valance for multiple chemical bonds. It plays an important role in functionalization of dendrimers with other molecules to send them at biological receptor site in proper concentration to get better effect of drug [45,47].

5.6 Self-assembling dendrimers

One of the attractive properties of dendrimers is self-assembling structure due to intermolecular forces of their structure. End-groups of dendrimers are spontaneously structured for the formation of branched dendrimers [45,47].

5.7 Electrostatic interactions

Availability of end-groups on the surface of dendrimers make them electrostatically strong to attract opposite charged molecules with them. This property is also applied for drug loading onto the dendrimers. For e.g., methylene blue loading on surface of dendrimers, copper complexes with dendrimers etc [45,47].

6. Characterization of dendrimers

6.1 Drug-dendrimer interaction study

By using Fourier transform infrared (FTIR) spectroscopy, the specific kinds of interactions that have been formed between drugs and dendrimers were investigated. Dendrimer-drug complex lyophilized solid-state samples were used to generate FTIR spectra [32,48]. Types and generation of dendrimers, pH and temperature of medium influences the drug-dendrimer interactions. Dendrimer affinity to bind with drug also responsible for their hydrophobic or electrostatic interaction. Positive and negative charge at terminal groups help poorly soluble drugs to bind with dendrimers by hydrophobic interactions and results in higher water solubility. FTIR spectra of drug-dendrimer complex indicates absence of some absorption bands, and some other bands was displaced. That could result in drug entrapment in hydrophobic pockets of dendrimers.

6.2 Quantification of the drug in dendrimers

The absorbance of the unentrapped drug using a UV-Vis spectrophotometer in PBS 7.4 (10 mM) was measured to quantify it. The absorbance at a maximum concentration in drug solutions at various concentrations was measured. Through linear regression in a concentration range, the equation for the calibration curve was produced. The absorbance found in the suspensions following the dendrimer-drug combination procedure can be attributed to the drug because dendrimers should exhibit weak absorbance at the drug wavelength. As a result, the calibration curve and sample absorbance were correlated, and the total drug was calculated [49,50]. The RP-HPLC method is also used for drug quantification. A calibration curve was used to monitor and assess the drug's retention time.

6.3 In vitro drug release study

In vitro drug release profile from drug-dendrimer complex is performed using the microdialysis technique or any other diffusion techniques [48,51]. In vitro drug release investigation was done using membrane dialysis (5 kDa MWCO range dialysis bag) method in PBS pH 7.4 media at 100 rpm. Aliquots of 0.5 mL sample at a time intervals of 0, 0.25, 0.5, 1, 2, 4, 48 h were collected from receptor compartment. Ideal sink condition was maintained by refilling fresh media with an identical quantity at each preset time interval. The pure drug and drug-dendrimer complex in-vitro cumulative drug release profiles were examined. Withdrawn aliquots were evaluated using this method. It was discovered that the pure drug released more quickly than the dendrimer-drug conjugates [34]. Improved drug release profile from drug-dendrimer complex was found due to drug entrapment in internal cavities with electrostatic interaction.

6.4 Conjugation evaluation

The PAMAM-Lf (Lactoferrin) conjugation was validated by the 1H NMR spectra, and the dendrimers and lactoferrin protons interaction was also indirectly confirmed by the correlation spectroscopic technique. When the protons of PAMAM and Lf were nearby after being chemically coupled, homonuclear interaction was seen using homonuclear spectroscopy [34,48].

The increased skewness of the dendrimer-lactoferrin conjugate suggested height more than average, and the higher Kurtosis mark in the AFM (atomic force microscopy) data further confirmed the conjugation [34].

6.5 Mean particle size, zeta potential, and distribution

Using dynamic light scattering, the dendrimer-drug complex was physically characterized, including the mean particle size, zeta potential, and size distribution determination. Concentration of each sample should be 0.2 mg/mL

in deionized water. Each measurement was made in triplicate at a temperature of 25°C and a 90 degrees angle [52]. Due to drug loading on dendrimers, conjugates exhibit the increased value of zeta potential at larger sizes. The surface charge of a dendrimer-drug complexes influences its interactions with various biological components, its distribution, access, and entry into target cells. It depends on the mixing molar stoichiometry of drug to dendrimer.

6.6 Entrapment efficiency

As they are macromolecules with internal cavities, dendrimer surface modification increases encapsulation efficiency, allowing for a higher percentage of the medicine to be contained [48].

%Entrapment efficiency was obtained using Eq. (14.1).

$$\%EE = \frac{Weight\ of\ drug\ in\ dendrimer}{Weight\ of\ fed\ dendrimer} \times 100 \tag{14.1}$$

Loading efficiency of dendrimers largely depends on types and generation of dendrimers. As the generation deviates from 2 to 4 to 6, their internal cavities are increased, which would be responsible for packing of drug in it.

6.7 Stability study

The stability of the dendrimer-drug complex was examined at 4, 25, and 37°C for 60 days. To do this, UV-Vis absorbance was used to calculate the amount of drug in both the dendrimer-drug complexes and the free drug control [53].

6.8 Toxicity profiles of dendrimer-drug Coadministration

According to a literature review, three separate models such as human RBCs for ex-vivo, cell-culture for in-vitro toxicity profile, and zebrafish larvae for in-vivo toxicity profile were used to examine the free drug, free dendrimers, and dendrimer-drug complex administration [54,55].

6.8.1 In-vitro toxicity study

The neuroblastoma line Neuro-2a from fast-growing mouse has been used in Alzheimer's disease for in-vitro toxicity [48]. In this cell line, it was investigated how dendrimer, drug, and dendrimer-drug complexes affected viability using staining with crystal violet. Metabolic activity is measured using MTT assay, and membrane status using neutral red uptake [33,56]. In a humidity chamber with a 5% CO_2 environment, the cells were cultured at 37°C with a 10% antibiotic solution in a flat bottom 96-well microplate with 10% FBS at a 10×10^3 cells/well density. 100 L of 10-fold-serial dilutions of the drug, the dendrimer, and the dendrimer-drug complex, all prepared in culture media, were added to the media after the first 24 h of growth. Depending on the colorimetric technique being employed, the treatments were withdrawn after 4 or 24 h and replaced by various reagent solutions [33]. Crystal violet staining was applied for viability investigations [57].

6.8.2 Ex-vivo toxicity study

Ex vivo toxicity study was observed using exposed human RBCs for hemolysis and morphological alterations. Human RBCs were treated at 37°C with dendrimer, drug, and dendrimer-drug complex, PBS 7.4 (10 mM) as a negative control and positive control 2% w/v SDS for 4 and 24 h. Utilizing UV-Vis absorbance to measure hemoglobin release, hemolysis was identified. Giemsa staining and optical microscopy were used to analyze the morphological alterations in the cells. Before taking part in the study, the participant (a healthy donor) provided written informed assent to the experimental protocol [33,58]. Many researchers observed that dendrimers were not produced any significant change in properties of red blood cells on interaction with dendrimers and proved that dendrimers are safe and having negligible toxicity.

6.8.3 In vivo toxicity study

Zebrafish larvae is used as a for evaluation of viability and morphological alterations and neurotoxicity, cardiotoxicity, and hepatotoxicity of the dendrimer-drug coadministration. The Institutional Ethics Committees authorized the zebrafish methods to carry out experiments strictly in compliance in regulation to National Institutes of Health for caring and maintaining animals [59]. Larvae were viewed under a stereomicroscope for viability investigations,

and announced dead if no heartbeat was seen. The percentage of living larvae relative to the total number of larvae for each treatment was used to measure viability [33,59]. Neurotoxicity studies observed in larvae by changes in the locomotor activity since they could represent cerebral damage, morphological or fatal consequence, caused by various treatments. An analog-to-digital converter system with multiple channels was used to measure the spontaneous movement [59]. Changes in larval heart rates were examined for cardiotoxicity studies because they may indicate both heart damage and a morphological or fatal effect brought on by various treatments. By counting the number of heartbeats every 15 s while seeing them through a stereomicroscope, the larvae heart rate was calculated and expressed as a % heart rate in the negative control [33]. The larvae were photographed and evaluated according to the severity of abnormalities for morphological alterations studies. Larvae's mean toxicity score for each treatment was calculated. The treated larvae livers were examined for hepatotoxicity investigations. Zebrafish liver in a healthy state is transparent, but after being exposed to hepatotoxic medicines, it darkened, indicating degeneration and/or necrosis [60].

6.8.4 Erythrocytic toxicity study

Hemolysis on human blood cells and erythrocytic toxicity behavior can both be described in terms of dendrimer-drug complexes. The hemotoxicity is caused by the cationic character of the dendrimer. 5 mL Human blood was centrifuged at 1350 rpm to separate the RBCs, and then resuspended in normal saline. Drug and dendrimer-drug complex samples (20 ppm) were made in normal saline, and each sample (1 mL) was introduced to RBCs with continuous stirring of an hour. After centrifugation, supernatants were collected and examined in a UV-vis spectrophotometer after 1 h with the proper saline dilution. Distilled water was entirely hemolytic (blank) [34].

6.9 In vivo pharmacokinetics

Pharmacokinetic research was done using Sprague−Dawley rat's model. The Institutional Animal Ethics Committee approved the in vivo study protocols. Animals should be on fasted state before testing. Blood samples (0.2 mL) were collected in tubes containing heparin between 0 and 24 h from retro-orbital plexus with predetermined time intervals for plasma drug level tests. Centrifugation at 6000 rpm for 20 min at 4°C was used to separate the blood cells, which were then further deproteinized using acetonitrile. HPLC was used to quantify the medication. Rats from each group were sacrificed for the biodistribution investigation after a predetermined amount of medication administration, and the visceral organs were collected and homogenized, and then acetonitrile was used to deproteinize them. HPLC was utilized to measure the medication. Before analysis, deproteinized materials were centrifuged and filtered [34]. According to the study, the dendrimer-mementine complex had a bioavailability of mementine that was 10 to 1.4 times more than that of pure medication. Because of the intrinsic conjugation with dendrimers, the drug's pharmacokinetic behavior was generally enhanced. Using HPLC, the biodistribution of drug and drug-dendrimer complexes in several visceral organs was carried out to assess the effectiveness of the dendrimers' targeting, notably in the brain. Although dendrimer-drug conjugates have higher brain absorption and a significantly longer residence time in the brain than smaller drug molecules, presumably because of enhanced pharmacokinetics, this leads to an improved half-life of the medication in vivo [34].

7. Animal models: In vitro and in vivo studies

7.1 In vitro models

7.1.1 Primary cell line

It may be obtained from a transgenic human or animal. Amyloidal beta oligomers' effects on neuron function and death have been widely studied using immortal rat hippocampus cell lines and primary cortical cultures. Primary cultures can be transfected with APP (amyloid precursor protein) as well, and they can provide insightful data on the function of amyloid beta oligomers [61].

7.1.2 Embryonic rat spinal cord cell line

This cell line mainly used to evaluate therapeutic effects of neural stem cells transplantation into rats for management of AD. Cells collected from embryonic rat spinal cord and ganglia cultivated on collagen-coated glass, showed their capacity for organotypic differentiation and bioelectric properties suited for electrophysiological research. Transplanted motor neurons can form functional connections with the adult mammalian nervous system [62].

7.1.3 Pluripotent stem cells

Same structured genetic variants as per pathogenesis are present in pluripotent stem cell generated from patients with a source of neurons in a defined microenvironment. Primary neurons generated from wild-type mice when treated with Abeta-1-42 in the presence of the dendrimers to see if it may lessen the neurotoxicity of A oligomers, due which dementia decreases. Histidine-maltose PPI dendrimers dramatically decreased the neurotoxicity of soluble A oligomers, as shown by a cell survival assay (colony formation assay, involves growing cell colonies in tissue culture dishes for two to 3 weeks after exposing the cells to a chemical compound) [62,63].

7.2 In vivo models

7.2.1 Sprague–Dawley rat model

Dendrimer plasma drug level tests in Sprague–Dawley rats can be carried out to assess the viability of Donepezil delivery by intravenous route. This model offers good technological opportunities for neurosurgical manipulations, neuroimaging, histopathology, electrophysiological recordings, or serial sampling of cerebrospinal fluid in neuroscience. Attenuation capacity of memory and learning, lipid oxidation suppression through antioxidant content regulation (SOD, GSH, CAT) and lactate dehydrogenase, nitric oxide, acetylcholinesterase inhibition can be evaluated as protein activity using this model [64–66].

7.2.2 Senescence-accelerated mouse (SAM) model

The senescence accelerated prone mouse (SAMP8) is a mouse strain that is used to study the effects of age on AD development. It was developed through phenotypic selection from a common genetic pool of AKR/J strain of mice by Takeda. Multiple therapy options have been investigated in these mice using the SAMP8 strain, which is consistent with the idea that SAMP8 cognitive abnormalities are related to Aβ (Amyloid-β) -associated oxidative stress [67–70].

7.2.3 Scopolamine induced AD model

Scopolamine-induced amnesia is the pharmacological model of AD that is most frequently employed. The most popular methods for creating such models include focal neurotoxic, electrolytic, or mechanical lesions of the cholinergic centers of basal forebrain and more widespread lesions of its cholinergic neurons. Memory problems associated with Alzheimer's disease may also be (partially) replicated by deliberately damaging brain regions or neural networks involved in various aspects of learning and memory. This model can be use to study many areas of brain including cholinergic neurons. Various memory problems which associated with Alzheimer disease can be study by using this model [71–73].

7.2.4 Caenorhabditiselegans model

A free-living nematode with a length of about 1 mm, caenorhabditiselegans, possesses several traits that make it an effective model organism. Since the nematodes are transparent, it is possible to analyze gene expression and embryonic development in living animals. Ab1-42 enhanced the development of amyloid-immunoreactive inclusions when expressed. A portion of these deposits also bind the Aβ -specific pigment thioflavin S, demonstrating the formation of amyloid fibrils like those in human AD. This model is used to study genetics and amyloid expressions. This model is well-suited in evaluation of various molecular mechanisms to elicit AD therapeutics effects [74].

7.2.5 D. Rerio (Zebra fish) model

The zebrafish, also known as the daniorerio, is a 3–5 cm freshwater tropical fish that has been a leading model for research on vertebrate development. It has high number of eggs, faster generation, and transparent embryos outward development. *Danio rerio* is ideal for large-scale genetic screening where identification of the gene underlying these disorders were done by phenotypic flaws. For transient gene knockdown in zebrafish, the most used method is morpholino antisense oligonucleotide injection. The zebrafish with relatively simple nervous system allows easy neurons imaging and creates the possibility to visualize specific neuronal proteins. Screening of varied dendrimers such as PAMAM, PPI, PLL, and carbosilane for effective management of Alzheimer disease can be done using this model [74].

7.2.6 Transgenic drosophila models

A potent genetic model for examining human neurological and neurodegenerative illnesses is Drosophila. A functional in vivo model using Drosophila has been utilized to look for genetic and pharmacological influences on -secretase activity. The γ -secretase complex, which is in charge of cleaving multiple transmembrane proteins intramembrane, is fully assembled in the drosophila. A definite APP (amyloid precursor protein) homolog and an APP-like protein exist in Drosophila. Drosophila model can be used to study various cellular pathways. At the level of cellular pathology, extensive neuron loss and two characteristic hallmarks, senile plaques (SPs) and neurofibrillary tangles (NFTs), are observed in the AD brain. Senile plaques are extracellularly deposited protein aggregates that are referred to as amyloid deposits. This potent model to study neurodegenerative disease have shown significant effect on various factors on amyloid formation, key element in Alzheimer disease. Evaluation of genetic and environmental risk factors of neurodegenerative disease can be done systematically with this model [74].

8. Applications in treatment of Alzheimer's disease

In contrast to liposomes, PAMAM, PPI, PEPE, and peptide dendrimers are having a small size range (1−15 nm) and a high-water solubility, making them a unique drug delivery mechanism [75]. In 1978, Fritz Vogtle reveals hyperbranched fragments; in the early 1980s, Donald Tomalia and his helper; and in the same year, but separately, George R. Newkome. Arborols, from the Latin for "trees," are the name given to the second class of synthesized macromolecules. Although this word is less well-known than "dendrimers," dendrimers are occasionally referred to as "cascade molecules". Drug solubility is a key factor in the blood-brain barrier (BBB) transit of endogenous substances. Due to their inadequate solubility, absorption, distribution, metabolism, excretion, and other unachieved physical qualities, the newer medications (drugs in the preclinical phase) are experiencing difficulties that adversely affect their pharmacokinetics and therapeutic efficacy [76]. This restricts the advancement of novel neurotherapeutics. Genes and about 98% of big protein molecules with molecular weights more than 400−600 Da are unable to penetrate the BBB. The conveyed therapeutic candidate's lipophilicity attests to its successful crossing of the BBB. Most active pharmaceutical ingredients (APIs) are insufficiently soluble in water, which hinders the development of new therapeutic strategies [1]. More than 40%, molecules identified as issue of aqueous solubility and systemic bioavailability. This lead to the additional research in pharmaceutical field during the development of formulations [77,78]. Despite the undisputed use of NPs to enhance drug delivery to the brain via multiple modes of administration, particles larger than 250−300 nm in size indicate ineffective drug delivery in the brain due to poor penetration transport across intracellular and paracellular areas [79]. These drawbacks can be resolved by using nanocarriers, which result in effective drug transport across BBB. Due to their desirable size (less than 10 nm), dendrimers were chosen for this study because they served as effective drug carriers for the CNS delivery of medicines with low hydrophilicity. Dendrimers are created through an interactive, step-by-step synthesis that has a core in their distinctive nanostructure [80]. The engineering of "important nanoscale design parameters" and nanoparticles has been driven by the exclusive enormous range and surface qualities. Cells and connections in the nervous system that are essential for movement, coordination, strength, sensation, and cognition are gradually damaged because of neurodegenerative illnesses. The two neurodegenerative illnesses with the highest prevalence are Alzheimer's. The term "dementia" is specifically used to describe the brain's diminished functionality. Alzheimer's disease is characterized by problems with thinking, remembering, and making decisions; these problems can differ from person to person. Dendrimers are innovative nanotechnology for drug delivery across blood brain barrier [81]. Enhancing stability, BBB delivery, targeted delivery, dummy and carrier for formulations, nanoparticles, nanodrugs, hydrogel for ocular drug administration, transdermal drug delivery, Medical Applications (drug delivery in the body that is directly related to pharmaceutical therapy and also deals with diagnostic perspectives like gene therapy, cancerous drugs, magnetic resonance imaging contrast agents, etc.), Other Applications (like in the field of cosmetics for controlled release of film forming agents and also extend shelf life of the cosmetics product). For dendrimers in hair care, skin care, and nail care products, L'Oreal Unilever and The Dow Chemical Company hold patents [82].

Few intrusive techniques, such the direct administration of drug-loaded nanocarriers or reversible rupture of the BBB, are successful but have issues with patient compliance and other issues. Dendrimers have drawn a lot of attention from researchers among the noninvasive methods for systemic drug delivery across the BBB. Dendrimers are 3D branched nanostructures having several functional groups that can be altered to have a specific function. They have several noteworthy benefits, including biocompatibility, drug loading ability, and nanostructure. BBB penetration is easy with ligand conjugated dendrimers and improve the uptake of medicines in the brain's target tissues.

Dendrimers have two primary mechanisms for preventing the production of amyloid fibrils and dissociating aggregates that have already formed as part of their antiamyloid action. Dendrimers, on the other hand, are most likely the most promising substances that have been linked to antiamyloid action [83]. Temozolamide (TMZ) distribution in the brain can be enhanced by using chitosan-coated polyamidoamine (PAMAM) dendrimers. The bioavailability of TMZ in the brain in vivo was increased by twofold following intraperitoneal administration utilizing a chitosan-PAMAM dendrimer-based formulation [81]. As promising multivalent substances against *Pseudomonas aeruginosa*, "onion peel" glycodendrimers have been identified. Additional "onion peel" glycodendrimer uses that are being researched include those as galectin-antibodies, vaccine delivery systems, and drug-targeting nanoparticles. Researchers have combined dendrimers with sugars and peptides to create glycopeptide dendrimers [84]. The first glycopeptide dendrimers were created using different solid-phase peptide Fmoc chemistry with L-lysine as the amino acid. Sialic acid was the first sugar to be conjugated, and it provided positive results against the hemagglutinin of the flu virus. Furthermore, these dendrimers have been studied for a various purpose such as antibacterial (for gastrointestinal, pulmonary, or urinary tract disorders), antiinflammatory, and vaccine development for HIV and influenza infections. Through the main mechanisms of endocytosis and passive diffusion, dendrimers have been employed to improve medication targeting and cellular uptake. As the primary internalization mechanism, endocytosis has also been shown to exist. The goal of dendrimer systems is to penetrate biological membranes like mucosa, epithelium, and endothelium to diffuse through the plasmatic membrane and reach the organelle. To internalize particles (phagocytosis), macromolecules, or fluids (pinocytosis), eukaryotic cells engage in a process called endocytosis. Most cells have pinocytosis, which can be mediated by receptors such as clathrin, caveolae, and micropinocytosis. Investigations revealed that internalization of cationic PAMAM dendrimers occurs and assert that the medicine is retained in cells, tissues, and/or organs more effectively when complexed to the dendrimer through electrostatic or hydrophobic interactions. With a similar drug release profile, the therapeutic effectiveness of the medication may be seen. Drugs covalently attached to dendrimers, such as dendrimer prodrugs, may demonstrate greater cell retention in addition to delayed release and extended blood circulation periods. The production of -amyloid peptides linked to Alzheimer's disease may be inhibited by maltose and maltotriose-modified PPI glycodendrimers. As drug carriers, antiamyloidogenic, and antiprionic agents, the corresponding substances have been studied in this context. Low intrinsic toxicity has been shown for PPI dendrimers coated with maltose. Maltosylated PPI dendrimers are shown to lessen the toxicity of the -amyloid peptide, linked to AD [85].

9. Clinical trials

Advances in amyloid therapy have been made since the monoclonal antibody aducanumab was approved. The pipeline for AD drugs is well-represented by therapies for tau abnormalities, and synaptic dysfunction in addition to other amyloid approaches. Notwithstanding the difficulties brought on by the present pandemic, there have been a few more clinical trials. Clinical trials are becoming increasingly informed by biomarkers in diagnosis as outcomes. More sensitivity to the effectiveness of the treatment is provided by new clinical outcome measures, particularly composite scales, and scores. Given the significant time sacrifices individuals and their families make during trial participation, the discovery of drugs for AD depends on strong partnerships with these groups [86]. There were 143 medicines being tested for AD in 172 clinical studies in 2022s. The pipeline contained 30 drugs in 31 Phase 1 trials, 82 agents in 94 Phase 2 trials, and 31 agents in 47 Phase 3 trials. Neuropsychiatric drugs make up 6.9% of the agents in trials, while disease-modifying therapies make up 83.2% of the treatment. Symptomatic cognitive enhancing therapies make up 9.8% of the agents in studies. Agents in studies cover a wide range of pharmacological targets. 37% of the compounds in development are modified versions of medications already approved for other uses. To meet recruitment standards, a total of 50,575 participants are required. DMTs are the most frequently studied drugs (119 drugs; 83.2% of all drugs in trials); 24 drugs (16.8%) are symptomatic drugs, including 14 drugs (9.8% of all drugs in trials) that aim to improve cognitive function and 10 drugs that aim to treat neuropsychiatric and behavioral symptoms. 40 of the DMTs (or 33.6% of the total) are biologics, while 79 are small molecules (or 66.4% of the total). Twenty (16.8%) DMTs have amyloid as their main mechanistic target, followed by 10.9% tau, 19.3% inflammation, 16% synaptic neuroprotection. When solely DMTs are considered, there are 67.8% in Phase 3 agents, 86.6% in Phase 2 medications, and 90% in Phase 1 agents [87]. New 53 repurposed agents are currently being developed. Cholinesterase inhibitors and the N-methyl-D-aspartate receptor antagonist able to reduce the dementia symptoms and they cannot halt or reverse the course of the illness [88].

The US FDA has approved the first DMT for the treatment of AD, and its name is aducanumab. The other DMT, edaravone, is used to treat amyotrophic lateral sclerosis, and it is the second DMT that has been licensed in the US for

any neurodegenerative condition. A quick regulatory process based on evidence of amyloid plaque reduction that was thought to reasonably anticipate clinical benefit was utilized to approve aducanumab. Other antiamyloid plaque lowering antibodies that the FDA is now reviewing could follow the accelerated method (donanemab and lecanemab) [89]. The gantenerumab studies request for marketing clearance on clinical results may follow [90]. Future research may qualify more biomarkers as surrogate outcomes that predict clinical benefit. Drug development is aided by surrogate results. The USFDA-approved acetylcholinesterase inhibitor (AChE) medication used to treat mild-to-moderate AD is called donepezil (DZ) [91]. After glucuronidation and excretion in the urine, DZ is metabolized by the CYP 450 isoenzymes 2D6 and 3A4 into four key metabolites. Researchers have recently shown a great deal of interest in the idea of medications more effectively entering the brain when they are able to penetrate the blood-brain barrier [92]. The goal of the current method is to test the hypothesis that the conjugation strategy can improve donepezil (DZ) transport to the brain when used with polyamidoamine (PAMAM) dendrimers. Tacrine (TAC) led to hepatotoxicity on oral administration [93]. Both drug delivery systems and standalone nanodrugs can be made using DG4.0 and DG4.5 PAMAM dendrimer [59].

10. Patents

There are several drugs and dosage forms patented for the treatment and management of Alzheimer's disease including donepezil, memantine, rivastigmine, etc. Various dendrimers application works patented worldwide have been summarized in Table 14.1.

TABLE 14.1 Patents on dendrimers in treatment of Alzheimer's disease.

Publication date	Jurisdiction/ Application date and number	Topic	Summary of work	Patent-legal status (as on 17-11-2022)	References
16-11-2017	WO 2017/195131 A1 10-05-2017	Prion protein-dendrimer conjugates	The invention relates to PrP-derived various peptide fragments use as a medicament in the treatment of Aβ amyloidogenesis-related pathologies and/or Aβ-related toxicity, in particular of Alzheimer disease (AD), to a conjugate comprising PrP-derived peptide fragments with them and a carrier molecule, wherein the carrier molecule is a pharmacologically admissible molecule, preferably a low-generation dendrimer such as PAMAM dendrimer, and a method of manufacturing the above conjugate.	Application-active	[94]
28-03-2019	US 2019/0092837 A1 10-05-2017			Application-pending	[95]
27-10-2020	US 10815292 B2 10-05-2017			Granted-active	[96]
15-09-2021	EP 3468577 B1 10-05-2017			Granted-active	[97]
10-09-2019	US 201715832059 A 05-12-2017	Dendrimer-resveratrol complex	Dendrimer-resveratrol complex with its methods explained here. The methods used in treating various	Granted-active	[98]
21-07-2016	US 201414913272 A 21-08-2014			Application-active	[99]

TABLE 14.1 Patents on dendrimers in treatment of Alzheimer's disease.—cont'd

Publication date	Jurisdiction/ Application date and number	Topic	Summary of work	Patent-legal status (as on 17-11-2022)	References
02-09-2021	US 202117325088 A 19-05-2021		disease including AD and various other CNS diseases. The formulation applied topically to the skin or mucous membranes for treatment purpose.	Application-pending	[100]
26-02-2015	US 2014/0052105 W 21-08-2014			Application-pending	[101]
24-02-2015	CA 2777682 A 07-10-2010	Synthesis of PAMAM dendrimer	New method of synthesis of polyamidoamine (PAMAM) dendrimers that can be used as carrier for molecules for diagnosis and treatment of various diseases.	Granted- INACTIVE	[102]
19-04-2017	CN 201580043659 A 13-08-2015	PAMAM dendrimer for targeting brain	Dendrimers of PAMAM hydroxyl-terminated dendrimers covalently linked active molecule. This composite can cross the BBB and used to target or diagnosis purpose for CNS disease. The composite can be versatile in the way to bind two drugs to utilized for simultaneous delivery.	Application-pending	[103]
07-12-2017	US 201715619737 A 12-06-2017	Dendrimers with CRISPR systems for genome editing as to hematopoietic stem cells (HSCs)	The system created for delivery, engineering, and optimization of system as well as methods. It can target the site accurately and reduce-avoid any toxicity. It can provide vectors and vector systems some of which encode one or more components of a CRISPR complex.	Application-discontinued	[104]

11. Conclusion

The dendrimers can increase transfection efficiency in the CNS; AD and may also reduce the toxicity. The development and designing of dendrimers can be done using various excipients that can also facilitated the targeting and desire release properties in AD. The literature and patents proves dendrimers as successful carriers during experiment in vivo as well as in vitro.

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