TITLE

Applications of dendrimers for brain delivery and cancer therapy

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ABSTRACT

Dendrimers are emerging as potential non-viral vectors for efficiently delivering drugs and nucleic acids to the brain and cancer cells. These polymers are highly branched, three-dimensional macromolecules with modifiable surface functionalities and available internal cavities that make them attractive as delivery systems for drug and gene delivery applications. This review highlights the recent therapeutic advances resulting from the use of dendrimers for brain targeting and cancer treatment.

KEYWORDS

Dendrimer; cancer therapy; brain delivery; gene therapy; tumor targeting

INTRODUCTION

Treatment of brain diseases and cancer represent major therapeutic challenges in modern medicine. Cerebral diseases such as Alzheimer's and Parkinson's diseases affect a large percentage of the world's population and hardly respond to intravenously administered, small molecule treatment [1,2]. In addition, cancer remains one of the leading causes of mortality, accounting for 8.2 million deaths in 2012.

Recent advances in multi-disciplinary research have led to the discovery of numerous promising drugs against cerebral diseases and cancer. However, most of these drugs fail to specifically reach the pathological site, resulting in secondary effects on healthy tissues. In order to remediate this problem, it is therefore crucial to be able to deliver these drug candidates specifically to their site of action.

In this context, dendrimers are emerging as potential non-viral vectors for efficiently delivering drugs and nucleic acids to the brain and cancer cells. They are polymeric molecules with perfectly branched multiple monomers that emerge radially from a central core similar to a tree (*dendron* in Greek) [3]. Their modifiable surface functionalities and available internal cavities make them attractive as delivery systems for drug and gene delivery applications. (For comparison of the advantages and inconvenients of dendrimers with other delivery systems, please refer to reference [4]). These symmetrical molecules can be synthesized to a definite size in a reproducible manner to form spherical macromolecules, as initially described by the Vögtle group in the late 1970s [5], the Tomalia group and the Newkome group in the 1980s [3,6-8]. The dendrimers can be synthesized by mainly two methods, the Tomalia-type divergent synthesis, in which the dendrimer is formed in a step-wise manner from the core to the periphery [9] and the Fréchet-type convergent synthesis,

in which the dendrons are synthesized first and then anchored to a multi-functional core [10].

Dendrimer can be divided into three structural domains:

• a multivalent surface which has been largely exploited by many investigators as a means to achieve the conjugation of targeting moieties and the binding of drugs or nucleic acids for therapeutic applications.

• dendrons delimitating void spaces shielded by the surface. This domain has been used for the encapsulation of various chemically sensitive drugs.

• the core which allowed the attachment of the dendrons [11] (Figure 1).

Each of these three parts can be tailored for a desired function of the dendrimers such as drug delivery, molecular sensors, enzyme mimics and bioimaging (Figure 2). This review will mainly focus on the recent therapeutic advances made using dendrimers for brain targeting and cancer therapy. (For further reading about therapeutic, imaging and diagnostic applications of dendrimers, please refer to the reviews [12-14]).

1. DENDRIMERS FOR BRAIN DELIVERY

Diseases of the central nervous system (CNS), such as Alzheimer's disease, Huntington's disease, Parkinson's disease and brain cancer currently stand for 11 % of the global burden of disease. The number of new drugs approved for the treatment of CNS disorders remains very low due to their inability to cross the blood-brain barrier (BBB), a key regulating site for drug access to the brain which acts as an entrance gateway for various nutrients to the brain while protecting it from potentially toxic compounds [1-2]. The barrier function of BBB is a combination of physical barrier, transport barrier and metabolic barrier [15]. The low and selective permeability of BBB can be credited to its unique biological properties:

• the lack of fenestrations, vesicular transport and pinocytosis in the endothelial cells [16,17].

• the physical barrier due to the presence of tight junctions between adjacent endothelial cells [18].

• the transport barriers resulting from the expression of various transporters including Glucose transporter 1 (GLUT-1), L-type amino acid transporter (LAT1), transferrin receptors (TfR), insulin receptors, low density lipoprotein receptor- related proteins (LRP1 and LRP2), and ATP family of efflux transporters such as p-glycoprotein (P-gp) and the multidrug resistance-related proteins (MRP). These transporters and receptors expressed on the capillary endothelial cells of the BBB only carry specific molecules to / from the brain [19].

• the enzymatic barrier of degrading enzymes localized in endothelial cells.

Although the multi-tasking BBB plays an essential role in the development of CNS as a complex integrated network, it poses a major problem for treatment strategies that

require the delivery of drugs and nucleic acids to the brain for the treatment of CNS disorders. This issue can however be overcome by exploiting the specific transport systems expressed on the BBB for the transport of therapeutics to the brain. Various dendrimers based on this delivery strategy have been developed and preclinically evaluated. Among other nanomaterials, they appear to be particularly promising in this application due to the various advantages they offer, such as monodispersity, lack of immunogenicity, permeation through biological barriers, improved drug stability and maintenance of drug levels in the therapeutically desirable range [11].

1.1 Dendrimers for transferrin receptor targeting

Out of all the tissues in the body, the expression of the TfRs is mainly found on the brain capillary endothelium which forms the BBB [20]. TfR is responsible for the transport and distribution of iron in the body through an iron-binding glycoprotein, transferrin (Tf). Since the last two decades, Tf has been extensively investigated as a targeting ligand for the drug and gene delivery systems to transport therapeutics to the brain [21].

Due to their unique physico-chemical properties, dendrimers have been shown to be promising candidates for brain delivery. Polyamidoamide (PAMAM) dendrimer (Figure 3) is the most researched candidate for the delivery of the therapeutics to brain via TfR targeting. In a study by Huang and colleagues [22], PAMAM (G5) was conjugated to Tf through a bifunctional polyethyleneglycol (PEG) spacer and complexed to a plasmid DNA encoding green fluorescent protein (GFP). After intravenous administration to mice, PAMAM-PEG-Tf/DNA was found to be able to cross the BBB, as demonstrated by a body distribution study of ^{125}I-labelled dendrimers. Qualitative analysis demonstrated GFP expression in several brain

areas such as the cortical layer, hippocampus, *caudate putamen*, *substantia nigra* and the 4th ventricle. This gene expression was about 2-fold higher compared to the PAMAM/DNA and PAMAM-PEG/DNA complexes.

Following the discovery that wheat germ agglutinin (WGA) was a promising ligand for increasing drug uptake to the brain [23], and binding to cancer cells [24], a dual targeting nanocarrier, PEGylated PAMAM (G4) conjugated to Tf and WGA on the periphery and loading doxorubicin (DOX) in its interior was synthesized. Due to its dual targeting effect, this delivery system led to an increased transport ratio of 13.5 % in an *in vitro* model of BBB, compared to 8 % for PAMAM-PEG-WGA, 7 % for PAMAM-PEG-Tf and 5% for free DOX. Moreover, this dual targeted dendrimer-based therapeutic system decreased the viability of C6 glioma cells in a brain microvascular endothelial cells (BMVEC)/C6 glioma co-culture model. The viability of C6 glioma cells was 14.5 % in comparison with 21.3 % for PAMAM-PEG-Tf-DOX, 23.7 % for PAMAM-PEG-WGA-DOX and 22.4 % for free DOX [25].

Multidrug resistance proteins, consisting of a family of ATP-binding cassette (ABC) proteins such as P-glycoprotein (P-gp), are expressed not only on the endothelial cells of the BBB but also on glioma cells. They function as efflux transporters, restricting drug transport across the BBB and prohibiting cellular uptake by glioma cells [26,27]. Tamoxifen (TAM), an estrogen receptor antagonist, has the ability to inhibit the multidrug resistance proteins and thus improve BBB transport [28,29]. Based on this, Li *et al*. [30] synthesized a pH-sensitive nanocarrier, consisting of PAMAM (G4) conjugated to Tf on the exterior and encapsulating TAM with a loading efficacy of 27 drug molecules per dendrimer molecule. DOX was also peripherally linked to the peripheral amino groups of PAMAM through an acid labile hydrazone bond, with a conjugation efficacy of 7-8 doxorubicin molecules per dendrimer

molecule. In an *in vitro* model of BBB, PAMAM-DOX-PEG-Tf-TAM resulted in an increased DOX transport ratio of 6.1%, compared to 4.9% for PAMAM-DOX-PEG-Tf and 4.6% for PAMAM-DOX-PEG. This targeted dendrimer-based therapeutic system also decreased the viability of C6 cells in a BMVEC/C6 glioma co-culture model. The viability of C6 glioma cells was 68.8% for PAMAM-DOX-PEG-Tf-TAM, reduced compared to that observed with other treatments (76.1% for PAMAM-DOX-PEG-Tf and 83.9 % for PAMAM-DOX-PEG).

Another dendrimer, polypropylenimine (PPI) (Figure 3), is a promising alternative to PAMAM for gene delivery to the brain and cancer cells. Initial studies by Kabanov and colleagues [31] demonstrated that this dendrimer binds to DNA via electrostatic interactions involving only the peripheral amine groups, allowing the tertiary amine groups present inside the dendrimer to act as “proton sponge” in the endosome. In a recent study, we demonstrated that the intravenous injection of transferrin-bearing PPI dendriplex more than doubled the gene expression in the brain compared to the unmodified dendriplex, while decreasing the non-specific gene expression in the lung. Gene expression was at least 3-fold higher in the brain than in any tested peripheral organs and was at its highest 24h following the injection of the treatments. These results suggest that transferrin-bearing polypropylenimine dendrimer is a highly promising gene delivery system to the brain [32].

1.2. Dendrimer-based low density lipoprotein receptor-related proteins (LRP1 & LRP2) targeting

Low density lipoprotein receptor-related proteins (LRP1 and LRP2) are multi-functional scavenger receptors expressed on the BBB. They have an ability to bind to

a range of molecules, including proteinases, proteinase-inhibitor complexes and lipoprotein lipase-enriched lipoproteins, and make them cross the BBB [33].

Lactoferrin (Lf) is a single chain cationic iron-binding protein belonging to the Tf family. Lf is capable of crossing the BBB through binding to LRP receptors [34]. Taking advantage of this ability, Huang and colleagues [35] synthesized a non-viral brain-targeted gene delivery system consisting of PAMAM (G5) conjugated to Lf via a PEG spacer and complexed to a plasmid DNA encoding green fluorescent protein (GFP). Following intravenous administration of PAMAM-PEG-Lf/DNA in mice, GFP expression was observed in the cortical layer, hippocampus, *caudate putamen*, *substantia nigra* and fourth ventricle of the animals. This gene expression was 5.2-fold higher compared to that of PAMAM-PEG/DNA and PAMAM/DNA. In addition, the authors demonstrated that both LRP receptor and adsorptive-mediated mechanisms contributed to the higher uptake of the PAMAM-PEG-Lf/DNA in the brain capillary endothelial cells [36].

Angiopeps, a family of peptides derived from Kunitz domains of the drug aprotinin and other proteins, are highly effective brain targeting ligands. They are transported across the BBB through LRP1-mediated transcytosis [37]. Angiopep-2 was conjugated to PAMAM through a specific reaction with the terminal N-hydroxysuccinimidyl of the bifunctional PEG derivative. After intravenous administration of PAMAM-PEG-Angiopep complexed with a plasmid DNA encoding GFP, the brain uptake of DNA was up to 8.4-fold higher than with PAMAM/DNA in mice. Gene expression in brain was observed in the cortical layer, *caudate putamen*, hippocampus and *substantia nigra*. Both LRP-mediated endocytosis and adsorptive endocytosis contributed to the mechanism of cellular uptake of PAMAM-PEG-Angiopep [38]. In another study, Angiopep was conjugated to the dendrigraft poly-L-

lysine (DGL) (G3) via a PEG linkage and complexed to a plasmid DNA encoding Human Glial-Derived Neurotrophic Factor (*h*GDNF), to evaluate its neuroprotective effect in a Parkinson's disease model. The DGL-PEG-Angiopep/*h*GDNF not only exhibited a higher cellular DNA uptake and gene expression in brain cells compared to the unmodified DGL, but also improved the locomotor activity and recovery of the dopaminergic neurons [39].

1.3. Dendrimer based glucose transporter (GLUT-1) targeting

Glucose transporter GLUT-1 is abundantly expressed on the blood-brain barrier for transporting optimal levels of glucose to the brain for its normal functioning [40]. Based on this, Dhanikula and colleagues [41] synthesized polyether-copolyester (PEPE) dendrimers (G2) conjugated to D-glucosamine via carbamate linkage and loaded with up to 20.8% w/w methotrexate (MTX) for the treatment of gliomas. As GLUT-1 is also expressed on brain tumors, the glycosylation of the MTX-loaded PEPE dendrimers not only enhanced the drug transport across the BBB compared to the free MTX but also allow the of high amounts of MTX in the central necrotic regions of the avascular brain tumor spheroids.

1.4. Other dendrimer based targeting strategies

Leptin, a 146 amino acid polypeptide, is secreted in the blood by the adipocytes in response to the food intake and acts to regulate appetite and retard weight gain. Leptin receptors are present on the luminal side of the brain microvessels and on the choroid plexus [42]. Exogenous leptin cannot be used as a targeting ligand for brain uptake, as the leptin receptors are saturated at very low concentrations (0.15-5 ng/ml) of the endogenous leptin. However peptide fragments derived from leptin contain

important sequences for leptin receptor binding on the BBB. Amongst them, the sequence 61-90 (leptin 30) is taken up by the brain and shows equivalent brain concentration as its parent molecule leptin [43]. Based on this result, the dendrigraft poly-L-lysine (DGL) was conjugated to leptin 30 through a PEG spacer and evaluated for gene delivery to the brain. After intravenous administration of DGL-PEG-Leptin30 complexed to a plasmid DNA, the gene expression was higher than that of DGL-PEG/DNA and DGL/DNA. It was observed mainly in cortical layer, hippocampus, *caudate putamen* and *substantia nigra* [44].

Magnetic delivery systems have been widely investigated for the targeted delivery of therapeutics. Han and colleagues [45] synthesized a novel brain-targeted bi-functional gene delivery system based on natural magnetic particles, called magnetosomes (MS), and combining the brain delivery effects of an external magnetic field and a cell-penetrating peptide. To this end, they conjugated MS to PAMAM and to a cell penetrating peptide, the transactivating transcriptional activator (Tat) protein. After intravenous administration of radiolabelled Tat-MS-PAMAM and MS-PAMAM, followed by the application of an external magnetic field for 25 min, Tat-MS-PAMAM exhibited a 2-fold brain uptake increase compared to MS-PAMAM. Without magnetic field, this brain uptake fell by 1.88-fold. However, it was not possible to evaluate the direct role played by TAT grafting and the magnetosomes in this brain delivery increase, due to the absence of PAMAM-DNA testing in this study.

The use of overexpressed receptors/transporters on the BBB to facilitate the uptake of DNA or drugs to the brain can however be limited by the non-specific presence of receptors/ transporters on other tissues. For example, Tf can recognize both TfR1 and TfR2, but has a much higher affinity for TfR1 (25-fold higher) [46]. TfR1 is

expressed at low levels in most human tissues, but also at high levels on the vascular endothelium of brain capillaries [20]. In addition, its levels of expression on cancer cells are up to 100-fold higher than those on normal cells [47], making this receptor a promising target for the delivery of therapeutics to the brain and cancer cells. In comparison, TfR2 is mostly expressed on hepatocytes and on a wide range of tissues but at very low levels [46]. Although the risk of non-specific delivery of therapeutics to the liver and other normal tissues exists, it is therefore very limited due to the higher affinity of Tf for TfR1 receptor.

2. DENDRIMERS FOR CANCER THERAPY

1.1. Drug delivery

Dendrimer-based drug delivery systems have been developed in order to improve the biodistribution of a drug in the body and to allow the controlled release of the drug at its target site. Their high aqueous solubility, low toxicity, compact globular shape and controlled surface functionalities made them ideal carriers for anti-cancer drugs [11]. Dendrimers can also facilitate passive targeting of anti-cancer drugs to tumor tissue. This selective accumulation of macromolecules in tumors, termed “enhanced permeability and retention (EPR) effect”, is the consequence of the combination of reduced lymphatic drainage and increased permeability of tumor vasculature to macromolecules [48].

Dendrimers can either non-covalently encapsulate drugs in the void spaces within the dendritic structure, or carry them via covalent conjugation to the surface groups.

1.1.1. Encapsulation of drugs within the dendrimers

The internal cavity of the dendrimers could be used for the encapsulation of anti-cancer drugs, offering the advantage of subsequent controlled release of the drug to the tumors. Another less frequently used encapsulation strategy is to entrap anti-cancer drugs by micellar formulation of dendrimers.

Small hydrophobic anti-cancer drugs are generally entrapped in the dendrimers. Their solubility and toxicity against cancer cells have been shown to be increased by encapsulation within dendrimers (G4 to G6) [14].

Various anti-cancer drugs have been studied as dendrimer cargos, as described below.

• Camptothecin

Camptothecin has its anti-cancer efficacy limited by its low water solubility and secondary effects on normal tissue, including bladder inflammation. To overcome this limitation, 10-hydroxycamptothecin has been encapsulated in a poly (glycerol succinic acid) (G4) dendrimer at a concentration of 120 µM, which corresponded to a 20-fold increase in drug solubility in water compared to that of the unencapsulated drug (6 µM) [49]. Treatment of a panel of cancer cells with the drug-loaded dendrimer resulted in a decrease of IC_{50} compared to free drug in DMSO for all the cell lines tested, by up to 7.1-fold in MCF-7 breast carcinoma [50]. This improved anti-cancer effect resulted from the faster internalization of the drug encapsulated in the dendrimer, with intracellular concentration 16-fold higher than the free drug after 2 h treatment incubation. Retention time of the drug carried by dendrimers was also longer compared to free drug in solution (respectively 50% and 35% 10 h after the start of the treatment) [50].

• Cisplatin

Another drug, cisplatin, also has its anti-cancer therapeutic effect limited by its poor water solubility as well as the development of drug resistance by some cancer cell lines. The encapsulation of cisplatin in PAMAM dendrimer (dendrimer-Pt : 20-25 wt % platinum) resulted in an increase accumulation of the drug in B16F10 melanomas as a result of the passive targeting of tumor tissues due to the EPR effect and decreased the toxicity on normal tissues by 3-to 15-fold compared to the free drug in solution, in a murine model [51].

• Doxorubicin

To increase drug solubility in water and decrease its secondary effects in healthy tissues, doxorubicin was encapsulated in PEGylated PAMAM dendrimers (G3 and 4). The authors of this study demonstrated that the dendrimer size and PEG chain length have a major impact on the encapsulation efficacy of the drug, with PAMAM dendrimer (G4) and PEG $_{2000}$ leading to the highest drug encapsulation of 6.5 doxorubicin molecules per dendrimer molecule [52].

In another study, PAMAM dendrimer (G4) loaded with doxorubicin at a doxorubicin to PAMAM molar ratio of 3.56 ± 0.04 was then encapsulated in liposomes. This "PAMAM-doxorubicin in liposomes" formulation presents the advantage of modulating the release and the *in vivo* stability of doxorubicin, which would otherwise leak very rapidly out of the liposomes. It resulted in an even slower release of the drug from the delivery system, which was necessary to increase its therapeutic index and reduce its side effects on healthy cells. This formulation was shown to be efficacious against various cancer cell lines, including DU145 human prostate carcinoma and MCF-7 human breast carcinoma [53].

• Etoposide

Etoposide, an inhibitor of the enzyme topoisomerase II, is poorly water soluble. In order to remediate to this issue, this drug was loaded in micelles made of PAMAM (G2) block co-polymer containing poly (γ-caprolactone) and PEG [54]. These polymeric micelles have been developed to overcome the stability limitations generally encountered with the classical micelle structures that may suddenly dissociate and cause serious toxicity issues. The covalent binding of the lipophilic

poly (γ-caprolactone) and the PAMAM dendrimer as the core was shown to increase the stability of the polymeric micelle.

Etoposide was loaded in dendrimer-based polymeric micelles at a loading capacity of up to 22% (w/w). *In vitro*, the treatment of porcine kidney epithelial cells (LLC-PK) with this formulation showed a comparable cytotoxicity to that of the drug solution, which demonstrated that the drug was released from the polymer micelles and was able to exert its cytotoxic effects on cells [54].

• 5-fluorouracil

To improve its water solubility, the pyrimidine analogue 5-fluorouracil was encapsulated in PEGylated PAMAM (G4), improving the drug loading by 12-fold compared to that achieved with non-PEGylated dendrimer. Non-PEGylated PAMAM dendrimers suffer from drug leakage due to the relative unshielding of the void spaces containing the drug and hemolytic toxicity due to the presence of NH_2 groups on their surface. The coating on the surface of PAMAM dendrimers with PEG can therefore increase drug loading and overcome hemolytic toxicity. As a result, 5-fluorouracil displayed a sustained release from the delivery system over 6 days [55].

• Methotrexate

To improve its bioavailability and its poor water solubility, methotrexate has been encapsulated in PEGylated PAMAM (G3 and G4) dendrimers. As for doxorubicin, the encapsulation efficiency of this drug was the highest with PAMAM (G4) and PEG $_{2000}$, resulting in the encapsulation of 26 methotrexate molecules per dendrimer molecule [52].

In another study, methotrexate has also been encapsulated in melamine-based dendrimers. These types of dendrimers have been shown to be able to solubilize hydrophobic drugs and to reduce their organ toxicity without altering their therapeutic efficacy. The intraperitoneal injection of the known hepatotoxic drug methotrexate loaded in melamine-based dendrimers resulted in a decrease of hepatotoxicity in mice, with a 27% decrease of the levels of alanine transaminase compared to those obtained with the free drug [56].

• Paclitaxel

Like the drugs mentioned above, the therapeutic use of the mitotic inhibitor paclitaxel is limited by its poor solubility in water. This problem has been overcome by encapsulation of the drug in polyglycerol dendrimers, which led to a drug water solubility 400-fold higher than that of the free drug [57].

However, the “encapsulation of drugs within the dendrimer” approach is limited by the very small capacity of the void spaces within the dendrimers and the difficulty to effectively control the release of the encapsulated drugs. An alternative delivery approach for larger molecules is therefore to conjugate then to the surface of the dendrimers.

1.1.2. Conjugation of drugs to the dendrimers

Anti-cancer drugs can be covalently conjugated to the peripheral groups of the dendrimer. Owing to the multifunctional architecture of the dendrimer, many drug molecules can be attached to one dendrimer, as well as the targeting moieties for

enhanced tumor-delivery specificity. The release of the drug is controlled by the degradable chemical bonds linking the drug and the dendrimer.

Various anti-cancer drugs have been studied as dendrimer-drug conjugates, as described below. This review will focus on the *in vivo* therapeutic data obtained with these conjugates.

• Cisplatin

Cisplatin was conjugated to PAMAM dendrimer (G3.5) (dendrimer-cisplatin: 20-25 wt % platinum), which resulted in increased water solubility and slow release of the drug [51,58]. The intravenous administration of the conjugate to mice bearing subcutaneous B16F10 tumors led to selective accumulation in tumors and anti-tumor efficacy, unlike the free drug solution. The conjugate also showed therapeutic efficacy against all the tumors tested, including a platinum-resistant tumor model.

• Doxorubicin

Doxorubicin was conjugated to one side of a 2,2-bis (hydroxymethyl) propionic acid dendrimer (G3), the other side of the dendrimer being PEGylated, to form an asymmetric bow-tie dendrimer containing 8-10 wt % doxorubicin [59]. The acyl hydrazine linkage used to conjugate the drug to the dendrimer was pH sensitive, thus releasing the drug after it reached its target. A single intravenous administration of this doxorubicin conjugate in mice bearing subcutaneous doxorubicin-insensitive C-26 colon carcinoma tumors resulted in complete tumor regression and survival of all mice over 60 days. By contrast, no complete tumor regression was observed following treatment with the drug solution.

• Methotrexate

Methotrexate has been conjugated to folic acid- and fluorescein isothiocyanate-bearing PAMAM dendrimer (G5). This conjugate provided tumor targeting and inhibited cell growth of KB cells overexpressing folic acid receptors. By contrast, the methotrexate-dendrimer conjugate without folic acid did not succeed in inhibiting the growth of these cancer cells [60].

In addition, methotrexate was covalently linked to PAMAM (G5) as well, but this dendrimer was conjugated to cetuximab instead of folic acid [61]. Cetuximab is a monoclonal antibody which exerts its therapeutic effect as an epidermal growth factor inhibitor. Cetuximab-bearing conjugate containing 12.6 molecules of methotrexate per unit of dendrimer was tested on rats bearing brain implants of EGFR-expressing F98 rat glioma cell line, but unfortunately did not lead to a therapeutic improvement compared to the free drug. The median survival times of the rats receiving the conjugate, the monoclonal antibody alone or the free drug were respectively of 15, 17 and 19.5 days, which was not statistically different [61]. This may be due to the fact that the conjugation of the drug to PAMAM and a monoclonal antibody may have decreased the binding affinity of the drug for the dihydrofolate reductase, resulting in a loss of the antifolate activity of the drug. Another possibility is that the drug was not released from the conjugate and could not exert its therapeutic effect [61].

1.2. Gene delivery

The use of dendrimers as delivery systems for nucleic acids has been widely investigated for the treatment of cancers. In this review, we will focus on the dendrimers used *in vivo* following intratumoral or intravenous administration, for which therapeutic effects have been obtained.

1.2.1. Intratumoral administration

PAMAM dendrimers have become widely used as non-viral carriers for gene delivery. Superfect® PAMAM dendrimer complexed to plasmid DNA encoding herpes simplex virus thymidine kinase (HSV-tk) at a dendrimer: DNA weight ratio of 3:1 resulted in growth delay [62]. This effect was even accentuated by the fact that the plasmid contained Epstein-Barr virus sequences conferring the capability to replicate and stay in the nucleus of the transfected cells.

Another study involved the same Superfect® PAMAM dendrimer, but this time complexed to a 36-mer oligonucleotide, which itself complexed the therapeutic plasmid DNA encoding the anti-angiogenic peptide angiostatin or the tissue inhibitor of metalloproteinase (TIMP)-2 [63]. The intratumoral administration of this dendrimer/plasmid/oligonucleotide complex led to growth delay of the tumor.

In addition, Bai and colleagues [64] developed an arginine-bearing PAMAM dendrimer to deliver plasmid DNA encoding human interferon beta to mice bearing U87MG brain tumor. Mice treated with intratumoral administration of the dendriplex showed a decrease of their tumors and induction of apoptosis compared to control animals.

1.2.2. Intravenous administration

• Polyamidoamine (PAMAM) dendrimer

Wu and colleagues recently developed a PAMAM dendrimer (G5) with a triethanolamine core, able to interact with siRNA to protect it from degradation and facilitate gene delivery to the cells [65]. This dendriplex was able to deliver siRNA to prostate tumors, thus resulting in gene silencing of the heat shock protein Hsp27 and in anti-cancer effects against the tumors [66].

In another study, Superfect® PAMAM (G5) was complexed to Epstein-Barr virus-based plasmid vectors carrying the herpes simplex virus-1 thymidine kinase gene for suicide gene therapy against cancer in mice [62]. The intravenous administration of this complex led to suppression of tumor growth and prolonged survival times of the tumor-bearing mice.

PAMAM dendrimer has been used for co-administration of a therapeutic DNA and an anti-cancer drug. This was the case in a study by Han and colleagues, where a PEGylated PAMAM carrying doxorubicin was complexed to a plasmid DNA encoding human tumor necrosis factor-related apoptosis–inducing ligand (TRAIL) and conjugated to the peptide HAIYPRH (T7), a transferrin receptor-specific peptide [67]. The peptide-conjugated dendriplex led to an enhanced cellular uptake of DNA and accumulation in tumor than the non-targeted complex, in the human liver cancer Bel-7402 cells overexpressing Tf receptors. This synergistic treatment resulted in a tumor decrease in mice compared to the non-modified dendrimer [67].

PAMAM dendrimer was also chosen as delivery system for the treatment of glioma following intravenous injection. To this end, the PEGylated dendrimer was conjugated to Angiopep-2, which can target the low density lipoprotein receptor-related protein 1 (LRP-1) overexpressed on brain capillary endothelial cells and on

glioma cells. Angiopep-2-bearing dendrimer was then complexed to a plasmid DNA encoding TRAIL, able to specifically induce cell apoptosis of brain tumors without secondary effects to normal cells [68]. The median survival time of the mice following intravenous administration of the Angiopep-bearing dendriplex and temozolomide was respectively 61 and 49 days, which demonstrated the ability of the dendriplex to target the glioma after intravenous administration [68].

PAMAM dendrimer can also be used in conjunction of magnetic nanoparticles for gene therapy of brain tumors. Han and colleagues conjugated the PAMAM dendrimer to magnetic nanoparticles and Tat peptides [69]. This conjugate was then complexed to small interfering RNA expression plasmid able to downregulate the EGF receptor gene. The intravenous administration of this conjugate to mice bearing subcutaneous U251 tumors downregulated the expression of oncoproteins and slowed the growth rate of the tumors compared to controls [69].

• Polypropylenimine (PPI) dendrimer

siRNA/PPI complexes were successively caged with a dithiol-containing cross-linker, PEG and then conjugated with a synthetic analogue of luteinizing hormone releasing hormone (LHRH) peptide to deliver the siRNA specifically to LHRH receptor-positive cancer cells [70]. The administration of LHRH-bearing PPI complex resulted in an increased siRNA accumulation in tumors, internalization by cancer cells and gene silencing.

LHRH peptide was also used for the cancer specific targeting of PPI dendrimer (G5) complexed to superparamagnetic iron oxide nanoparticles (SPION) and siRNA [71]. SPION have been widely studied as magnetic resonance imaging (MRI) contrast

agents. Their integration to this therapeutic system was intended to allow MRI visualization of the disease progression and therapeutic response.

This targeted delivery system enhanced the anti-cancer efficacy of the drug cisplatin, by suppressing the anti-apoptotic defense by siRNA targeted to BCL2 mRNA in mice bearing lung cancer xenografts. A result of this treatment was a tumor volume decrease by 75% in comparison with controls.

Dufès and colleagues have demonstrated that the intravenous administration of PPI (G3) complexed to plasmid DNA encoding tumor necrosis factor (TNF)α under control of a tumor-specific promoter resulted in regression of subcutaneous A431 epidermoid carcinoma and survival of 100% of the mice [72,73]. This treatment appeared to be well tolerated, as there was no weight loss compared to controls.

More recently, Dufès and colleagues demonstrated that a Tf-bearing PPI dendriplex resulted in gene expression mainly in the tumors after intravenous administration [74]. As a consequence of this improved distribution, the intravenous administration of the Tf-bearing PPI dendriplex encoding TNFα led to a rapid and sustained tumor regression over one month (90% complete response, 10% partial response on A431 human epidermoid tumors). It also resulted in tumor suppression for 60% of PC-3 and 50% of DU145 prostate tumors [75]. In a parallel study by the same group, Tf-bearing PPI dendriplex encoding p73, a member of the p53 family of transcription factors, led to complete tumor suppression for 10% of A431 and B16-F10 tumors and long-term survival of the animals [76]. These treatments were well tolerated by the animals, demonstrating that Tf-bearing PPI is a promising delivery system for cancer therapy.

CONCLUSIONS & FUTURE PERSPECTIVE

Dendrimer-based delivery systems have demonstrated to be highly promising carriers of therapeutic genes and drugs to the brain and cancer cells. Until recently, the full exploitation of this approach was hampered due to specific delivery and safety issues. This problem is now being overcome by the use of targeted strategies allowing intravenous delivery of these nanomedicines. Moreover, intensive study on dendrimers has provided critical technological advances that will benefit the design of "next generation" dendrimers. Dendritic nanotechnology enables the synthesis of well-defined, globular structures incorporating specific chemical groups tailored for increasing the safe and effective delivery of a nucleic acid or a drug to its site of action, in a controlled way. For example, the further development of dendrimers with environmentally sensitive linkages should improve the drug release from the dendrimer. Moreover, the exploitation of new targeting strategies should optimize the delivery of nucleic acids and drugs to the brain or tumors, and therefore improve their therapeutic efficacy. In addition, the bioactive agents can be entrapped within the dendrimer structure, conjugated or complexed to the dendrimer surface, which allows a precise tailoring of the properties of the carriers to the specific needs of their cargos in terms of solubility, protection against degradation, release and delivery to the site of action. More generally, dendrimers are likely to be used in therapeutic strategies combining targeting, imaging, diagnostics and therapy, due to their multifunctional architecture. Clinical trials using dendrimers in cancer therapy are still pending, but dendrimers have already been successfully introduced in the clinic as anti-viral agents. Continued research in this area should therefore enable the preparation of highly specific, highly efficacious dendrimer-based nanomedicines towards the treatment of cancer and brain diseases in the clinic.

EXECUTIVE SUMMARY

Rationale for using dendrimers for brain delivery and cancer therapy:

- The use of promising novel therapeutics is limited by their inability to specifically reach the brain and tumors after intravenous administration, resulting in toxicity to healthy tissues.
- Dendrimers are emerging as potential non-viral vectors for efficiently delivering drugs and nucleic acids to the brain and cancer cells.
- They are well-defined, globular structures incorporating specific chemical groups tailored for increasing the safe and effective delivery of a nucleic acid or a drug to its site of action, in a controlled way.
- The bioactive agents can be entrapped within the dendrimer structure, conjugated or complexed to the dendrimer surface, which allows a precise tailoring of the properties of the carriers to the specific needs of their cargos in terms of solubility, protection against degradation, release and delivery to the site of action.

Applications of dendrimers for brain delivery:

- Dendrimers can deliver drugs and nucleic acids to the brain by exploiting the specific transport systems expressed on the blood-brain barrier
- Various dendrimers targeting the transferrin receptor, the low density lipoprotein receptor-related proteins, the glucose transporter GLUT-1 and the leptin receptors have been developed.
- Brain uptake of drugs and nucleic acids carried by targeted dendrimers was increased compared to non-targeted dendrimers.

Applications of dendrimers for cancer therapy:

- Dendrimers can either non-covalently encapsulate anti-cancer drugs in the void spaces within the dendritic structure, or carry them via covalent conjugation to the surface groups. A wide range of anti-cancer drugs have been studied as dendrimer cargos.
- Water solubility and therapeutic efficacy against tumors have been enhanced for the anti-cancer drugs carried by dendrimers.
- Dendrimers have been used for the delivery of siRNA, plasmid DNA, alone or associated to anti-cancer drugs, to the tumors following intratumoral or intravenous administration.
- Tumor regression has been obtained following intravenous administration of therapeutic nucleic acids carried by targeted dendrimers.

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REFERENCE ANNOTATIONS

Publications of special note have been highlighted as:

** of considerable interest

** 3 Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, *et al*.: A new class of polymers: Starburst-dendritic macromolecules. *Polym. J.* 17, 117-132 (1985).

One of the first publications about dendrimers by the pioneers in the field.

** 5 Buhleier E, Wehner W, Vögtle F: “Cascade" and "Nonskid-chain-like" syntheses of molecular cavity topologies. *Synthesis* 2, 155-158 (1978).

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** 22 Huang RQ, Qu YH, Ke WL, Zhu JH, Pei YY, Jiang C: Efficient gene delivery targeted to the brain using a transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. *FASEB J.* 21, 1117–1125 (2007).

Demonstrates that transferrin-bearing PEGylated PAMAM dendriplex can cross the blood-brain barrier following intravenous administration.

** 32 Somani S, Blatchford DR, Millington O, Dufès C: Transferrin-bearing polypropylenimine dendrimer for targeted gene delivery to the brain. *J. Control. Release* doi: 10.1016/j.jconrel.2014.06.006 (2014)

Demonstrates that the intravenous injection of transferrin-bearing PPI dendriplex more than doubled the gene expression in the brain compared to the unmodified dendriplex, while decreasing the non-specific gene expression in the lung.

** 51 Malik N, Evagorou EG, Duncan R: Dendrimer-platinate: a novel approach to cancer chemotherapy. *Anticancer Drugs* 10, 767–776 (1999).

Demonstrates that the conjugation of cisplatin to PAMAM dendrimer results in increased water solubility and slow release of the drug. The intravenous administration of the conjugate to mice bearing subcutaneous B16F10 tumors led to selective accumulation in tumors and anti-tumor efficacy.

** 74 Koppu S, Oh YJ, Edrada-Ebel R, *et al*.: Tumor regression after systemic administration of a novel tumor-targeted gene delivery system carrying a therapeutic plasmid DNA. *J. Control. Release* 143, 215-221 (2010).

Demonstrates for the first time that the intravenous administration of transferrin-bearing DAB dendriplex encoding TNFα leads to tumor suppression of 90% of A431 human epidermoid carcinoma tumors in mice.

FIGURE LEGENDS

Figure 1. Schematic 2D representation of a dendrimer.

Figure 2. Schematic representation of dendrimer-drug/nucleic acid delivery systems.

Figure 3. Chemical structure of polyamidoamine (PAMAM) (G3) and polypropylenimine (PPI) (G3) dendrimers.

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Figure 1

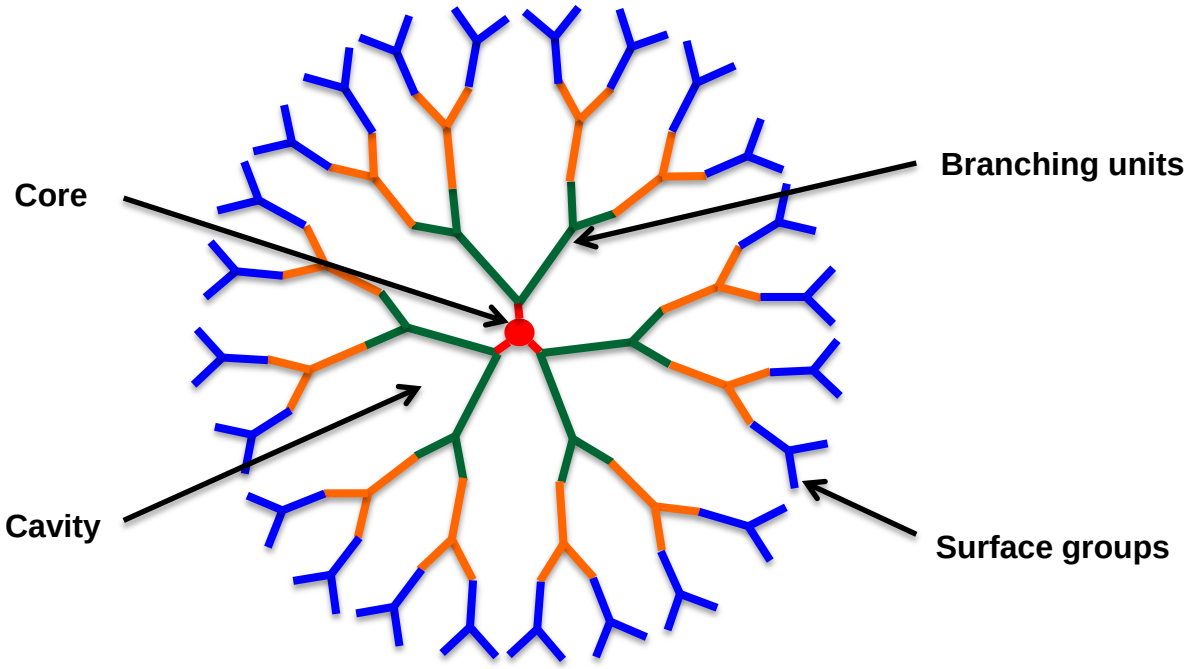
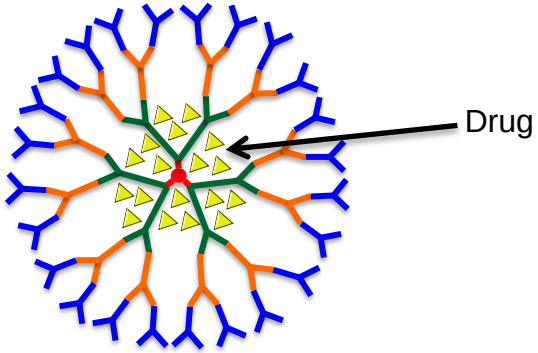
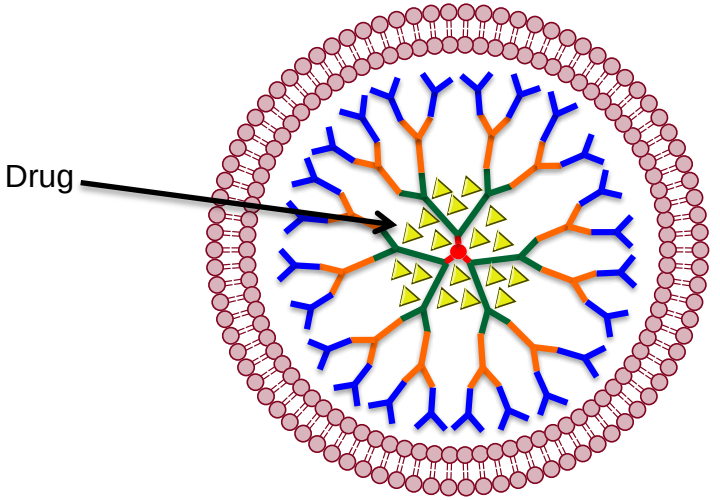


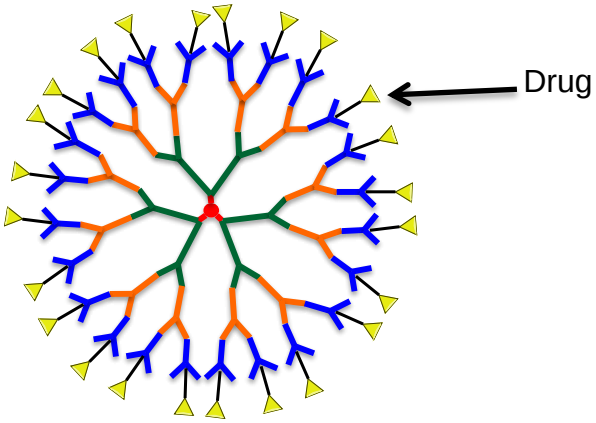
Figure 2



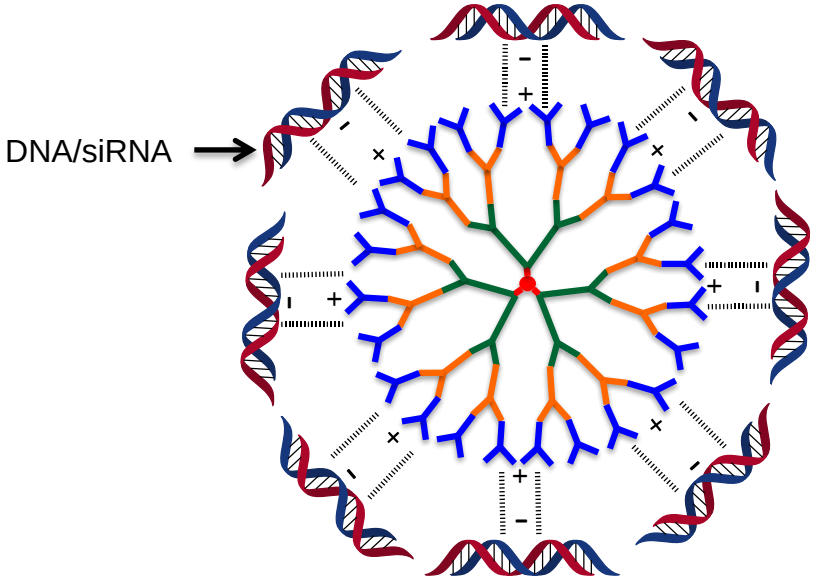
Drug encapsulated dendrimer



Liposome encapsulating drug encapsulated dendrimer

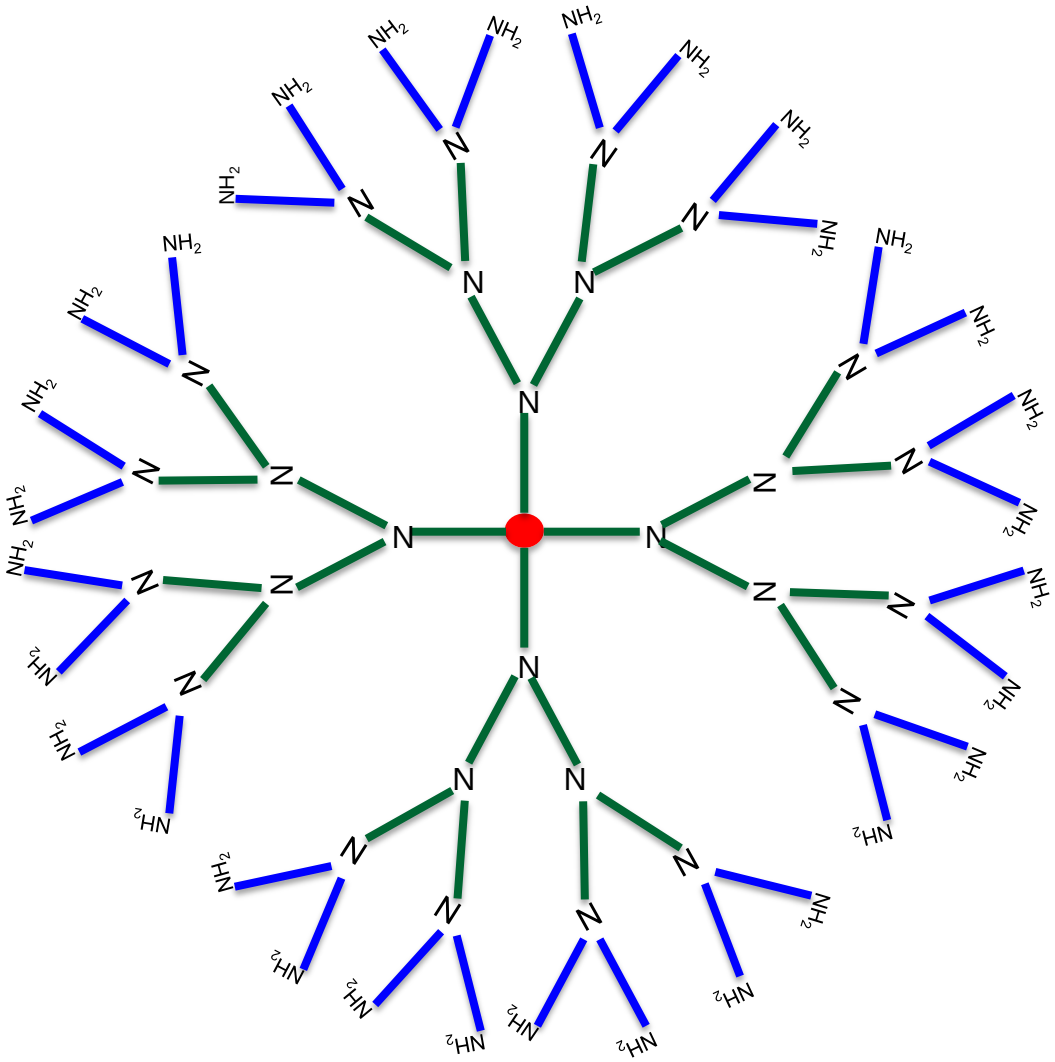


Drug conjugated to dendrimer by covalent linkage



DNA/siRNA complexed to dendrimer via electrostatic interactions

Figure 3



PAMAM dendrimer

= $N\text{-}(CH_2)_2\text{-}N$

N — = $N\text{-}(CH_2)_2\text{-}CO\text{-}NH\text{-}(CH_2)_2\text{-}N$

N — = $N\text{-}(CH_2)_2\text{-}CO\text{-}NH\text{-}(CH_2)_2\text{-}NH_2$

PPI dendrimer

= $N\text{-}(CH_2)_4\text{-}N$

N — = $N\text{-}(CH_2)_3\text{-}N$

N — = $N\text{-}(CH_2)_3\text{-}NH_2$